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Cardiovascular responses to biogenic amines and adrenergic receptor characterization in the fetal guinea pig

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LIST OF ABBREVIATIONS

ED_{50}	drug concentration resulting in 50% of the maximal response
pD_2	negative logarithm of the ED_{50}
pA_2	negative logarithm of the concentration of antagonist required to give a twofold shift in the dose response curve (a dose ratio of 2)
K_B	dissociation constant of a receptor for a specified antagonist

LITERATURE REVIEW

As shown by history, ignorance of a compound's effects on the fetus or extrapolation of adult responses to the fetus can prove disastrous as illustrated by the thalidomide tragedy (Mellin and Katzenstein, 1962). Presently, sudden infant death syndrome (Beckwith, 1975) is drawing much attention in hopes of solving this very elusive and fatal syndrome. Before answers to problems such as sudden infant death syndrome can be solved, fundamental knowledge about the function of the fetal cardiovascular system must be obtained.

At present, primarily sheep are used for conducting in vivo experiments in fetal physiology and pharmacology. Fetal lambs are desirable because of their advanced maturity and size near the end of gestation. However the ovine breeding cycle is such that experiments can usually only be conducted for a few months of the year and pregnant ewes are expensive. Studies using sheep have indicated a difference in the magnitude of responsiveness to vasopressor agents between the adult and fetus (Dawes et al., 1956; Assali et al., 1977).

The guinea pig, like the lamb, is quite mature at birth. However unlike the lambs, guinea pigs can be bred throughout the year and give birth to multiple young. The guinea pig placenta is hemo-endothelial which is very similar to the hemochorial type found in primates. Sheep, unlike primates and rodents, have a syndesmochorial placenta. Another advantage of the guinea pig is that they are a relatively inexpensive laboratory animal. Dornhorst and Young (1952) found a decreased sensitivity to norepinephrine in the fetal guinea pig when compared to the

mother, but the cause for this difference has not been extensively investigated.

The study of fetal pharmacodynamics or physiology presents many unusual problems. The fetal cardiovascular system may have some or possibly all of the homeostatic mechanisms of an adult, depending on the gestational age and species under investigation. However, the fetus is not interacting with the same environment as the adult, and some of the vital organs present in the adult are not yet functional in the fetus. Yet, certain systems such as the umbilical-placental circulation are present in the fetus but not in the adult. The umbilical-placental circulation allows for the interaction of the fetus with the adult. The fetal umbilical-placental unit along with nonuniform maturation of various fetal organs and physiological systems brings about many hemodynamic responses in the fetus which are not comparable in the adult.

Vasopressin when given to pregnant cats caused a decrease in fetal arterial blood pressure and initiated uterine contractions. The uterine contractions during the period of increased maternal arterial blood pressure resulted in a transient increase in fetal blood pressure which was followed by a decrease in fetal blood pressure (Clark, 1932).

Clamping the umbilical cord of rabbits before day eleven of gestation caused a decrease in fetal heart rate which was not altered by decapitation. At day twenty, clamping the umbilical cord resulted in a triphasic heart rate response; a decrease followed by an increase which was followed by a decrease in fetal heart rate. Up to day thirty-six, a gradual change in the triphasic response occurred. The transient increase in fetal heart rate disappeared leaving only the decrease in

fetal heart rate after clamping the umbilical cord (Bauer, 1938). These early studies indicated that fetal responses to stimuli differed from those of an adult. It was proposed that changing neural tone of the fetus was the reason for the unusual responses.

Over the years, the investigative techniques for studying fetal cardiovascular responses have been refined. Experimentation on the fetal cardiovascular system can be broken down into two broad classes according to the techniques used. The first and oldest class is that of acute preparations in which the fetus is partially or totally removed from the uterus, leaving the umbilical circulation intact. The required manipulations of the fetus are performed and the parameters of interest measured. These experiments last only a few hours, after which the animal is terminated. The second class of experiments are of a chronic nature. For this type, the necessary fetal manipulations are made under surgical anesthesia, and the fetus is placed back in the uterus. The pregnant animal is sutured and allowed to recover. The fetal cannulas are exposed for monitoring in utero fetal cardiovascular parameters (Assali et al., 1974; Nuwayhid et al., 1975).

There is disagreement over which of the above mentioned techniques is better. Investigators have shown that during complete exteriorization there is a decrease in umbilical blood flow while fetal blood gases remain unchanged (Heymann and Rudolph, 1967). Others contend that, in an acute experiment although maternal cardiovascular parameters may not be the same as that of an unanesthetized adult, this does not invalidate acute preparations. It has also been shown that there is no significant difference between the fetal cardiovascular parameters in acute and chronic

experiments (Assali et al., 1974). A factor to be considered when evaluating chronic versus acute fetal experiments is that with chronic experiments invasive techniques are still being used and the fetal cardiovascular system does not return to a basal level until several days postoperative (Kirkpatrick et al., 1976). Another disadvantage of chronic preparations is that the number of failures is large (Assali et al., 1974).

If the decision is made to perform acute experiments then the effects of the anesthetic being used must be considered. Deep pentobarbital or halothane anesthesia abolishes all cardiovascular responses to afferent vagal stimulation (Assali et al., 1974). The use of these anesthetics would not be advisable when studying the fetal cardiovascular system. Finally when considering the results of fetal experiments, one must keep in mind the physiological changes that occur to the adult animal during pregnancy (Assali et al., 1974).

Anatomy of the Fetal Cardiovascular System

In order to properly study the effects of drugs on the fetal cardiovascular system, it is necessary to have an understanding of fetal anatomy and physiology. There are entire books on each of these areas. In this review, I will limit myself to the factors directly related to my project.

Four gross anatomical features of the fetal cardiovascular system need to be considered. The first and most obvious is the presence of the umbilical-placental circulation. This is a low resistance circuit in parallel with the fetal systemic circulation. It provides for the fetus, proper gas exchange, waste removal, and a supply of nutrients.

The umbilical-placental circulation receives a major portion of the fetal cardiac output and, therefore, may greatly influence the fetal response to vasoactive compounds. Reliable dose response curves to norepinephrine could not be obtained from perfused guinea pig placentas (Panigel, 1962). A second anatomical difference in the fetal cardiovascular system from that of the adult is the ductus arteriosus. It allows oxygenated blood returning from the placenta to be shunted from the pulmonary artery into the ascending aorta, thus bypassing the nonfunctional fetal lungs. A third variation, the foramen ovale, allows blood to be moved from the right atria directly to the left atria, again bypassing the lungs. The fourth major anatomical difference is the ductus venosus. It is another low resistance shunt allowing the majority of oxygenated blood returning from the placenta to pass directly through the liver thereby avoiding the hepatic sinusoidal system. Therefore the fetal cardiovascular system will be exposed to a greater amount of any chemical administered because it is not exposed to catabolism by the hepatic microsomal system (Mirkin and Singh, 1972) or the lungs (Said, 1973). Located along the ductus venosus is a sphincter which serves to prevent overload of the heart and to maintain placental pressure thereby preventing collapse of the fetal villi (Bonica, 1967).

In addition to gross anatomical differences, one must be aware of the histological differences. Rudolph and Heymann (1968) stated in a review article that the pulmonary blood vessels contain small amounts of smooth muscle during the first half of gestation, after which lumen size increases with wall thickness due to smooth muscle cell growth. This cell growth may result from increased blood pressure which has been shown

to stimulate vascular smooth muscle development (Levin et al., 1978). Regarding myocardial differences, the number of myofibrils is less in the fetus than in the adult, and the fetal myocardium has a greater percentage of noncontractile mass than does the adult (Friedman, 1973, as cited by Rudolph and Heymann, 1974). Work on the human heart has shown that the sino-atrial node contains a large number of pacemaker cells which decrease in number with increasing gestational age. The atrioventricular node and the bundle of His are poorly defined, and the action potentials of the fetal myocardium show widespread pacemaker activity which, in adults, is found only in the sino-atrial and Purkinje fibers (Coltart, Spilker, and Meldrum, 1971).

Neural innervation of the fetal myocardium from a developmental standpoint is species specific. The fetal guinea pig heart has complete sympathetic and parasympathetic innervation at day thirty of a sixty-five day gestational period (Hoar and Hall, 1970; Pappano, 1977). It should be noted that the physical presence of neurons does not mean that they are functional. This is illustrated by the fact that, although parasympathetic innervation of the heart precedes sympathetic, the sympathetic tone exceeds parasympathetic tone throughout gestation (Nuwayhid et al., 1975; Rudolph and Heymann, 1974; Assali et al., 1977).

A possible alternative to neural control has been proposed for the fetal myocardium and large arteries of the neonatal rabbit, which contain small intensely fluorescent cells (Papka, 1975). These cells contain dense core vesicles and are acetylcholinesterase positive. Some neural enlargements occur near these small intensely fluorescent cells. These enlargements are also acetylcholinesterase positive. It has been suggested

that these small intensely fluorescent cells may release catecholamines or even acetylcholine prior to neural innervation, thereby acting as a local regulating mechanism. Regarding the systemic circulation, it appears that its innervation lags behind that of the myocardium (Wyse et al., 1976; Harris and Van Petten, 1978) with development continuing postpartum.

Fetal Cardiovascular Parameters and Function

Having presented the major anatomical differences between the fetus and adult, it is necessary to discuss the functional differences. It has been found that as gestation progresses there is a gradual increase in fetal blood pressure. During the last trimester, the mean fetal blood pressure in lambs increased from 46 mm Hg to 67 mm Hg (Joelsson et al., 1972; Assali et al., 1977). This value compares to an adult sheep value of 93 mm Hg (Biological Handbooks, Respiration and Circulation, 1971). Regarding the gradual rise in fetal blood pressure as gestation progresses, it is of interest that vagotomy in near term lambs has no effect on pulmonary resistance whereas sympathectomy results in pulmonary vasodilation (Rudolph and Heymann, 1968). Perhaps the sympathetic autonomic nervous system exerts an increasing amount of tone on the peripheral and pulmonary circulations as gestation progresses.

Just as circulatory shunts affect fetal blood pressure, blood flow to the various organs differs from the adult and also shows major change throughout gestation. In utero alterations of specific organ perfusion result from redistribution rather than changes in cardiac output. For example, as gestation progresses the percentage of cardiac output

distributed to the lungs gradually increases while that to the placenta decreases. This change may result from either an increase in placental resistance or a decrease in pulmonary resistance. At approximately 100 days gestation in the lamb, the pulmonary flow suddenly increases. Surface active materials appear in the lungs at this same time, and this suggests that the increased flow results from increased metabolic needs. A rapid increase in gut blood flow occurs at approximately 120 days of gestation. This too may be the result of increased metabolic needs. Throughout gestation an increase in blood flow to the brain occurs. This increase is gradual and is most likely the result of blood vessel proliferation (Rudolph and Heymann, 1970).

The fetal blood gases vary greatly from those of an adult. Normal values for fetal sheep are PaO_2 = 24 mm Hg, $PaCO_2$ = 45 mm Hg, and pHa = 7.330 (Dawes, 1968). These compare to maternal values of PaO_2 = 82 mm Hg, $PaCO_2$ = 36 mm Hg, and pHa = 7.440. Perhaps this difference in blood gases and pH plays a role in the responsiveness of maternal and fetal tissues to stimuli.

With basal fetal cardiovascular parameters changing throughout gestation, one expects the cardiovascular reactions to vary depending upon the stage of fetal development. Of major concern with regard to the cardiovascular reactivity is the functional development of the autonomic nervous system. In premature and immature lambs, no parasympathetic tone is present, and the mature fetal lamb has only a small degree of parasympathetic tone. In all three of the above fetal age groups, however, the parasympathetic system was capable, if stimulated, of exerting an influence on the cardiovascular system (Nuwayhid et al., 1975; Assali

et al., 1977).

Activation of adrenergic receptors is present in the lamb as early as day 60 of gestation. Whether this receptor activity is the result of transmitter release from nerves or small intensely fluorescent cells is unknown. There is some evidence to indicate that receptor activation increases with gestational age, as shown by changes in fetal blood pressure and heart rate when alpha and beta-adrenergic blockers were administered to the fetus (Nuwayhid et al., 1975). However, these data were obtained from experiments in which the fetus may not have been totally recovered from the trauma of the surgical procedure. In the fetal lamb, the release of catecholamines from the adrenal medulla in response to asphyxia is greater than that resulting solely from splanchnic nerve stimulation. As gestation progresses, the role of splanchnic stimulation in the adrenal medulla response to asphyxia increases in comparison to the direct effect of asphyxia on the adrenal medulla. In young fetal lambs and other animals which are quite neurologically immature at birth, it appears that circulating catecholamines resulting from the direct effect of hypoxia on the adrenal medulla play a more prominent role in cardiovascular control than in the adult. In the fetal lamb at 125 days of gestation, norepinephrine is the primary catecholamine released from the adrenal medulla. With increasing gestational age, epinephrine becomes the more prominent catecholamine being released from the adrenal medulla (Comline and Silver, 1961).

The baroreceptors are a primary mechanism for providing cardiovascular control in the adult. The gestational age at which baroreceptors provide control of the fetal cardiovascular system and the degree of

control exerted is still disputed. Some investigators have found that baroreceptor activity is present at 0.55 of gestation in fetal lambs while others contend that baroreceptor reflexes develop from 0.68 of gestation on (Boddy, 1976).

Working in conjunction with the baroreceptors are the chemoreceptors. The fetal responses to asphyxia and hypoxia are complex and variable and will not be discussed in depth. The chemoreceptors of the carotid body appear to play a minor role when compared to the aortic body chemoreceptors in the fetal lamb. It is felt that the latter is supplied by blood from the pulmonary trunk rather than the aorta. This exposes the receptors to a higher CO_2 concentration thus making them more sensitive to changes in the fetal physiological condition (Dawes, 1968). The response of fetal lambs to asphyxia is in part a pulmonary vasoconstriction. This vasoconstriction is abolished by sympathectomy and adrenalectomy in fetuses greater than 100 days gestational age. However, in fetuses of 90 days age and less, the vasoconstriction persists after sympathectomy. In the older age group of fetal lambs, in addition to the pulmonary vasoconstriction, a hindlimb vasoconstriction takes place (Born et al., 1956; Dawes, 1968). The vasoconstriction of these vascular beds increases placental blood flow by 30-50 percent while coronary and cerebral blood flow increases 8-10 times. Thus a shifting of blood flow to the vital organs, excluding the lungs, occurs during asphyxia (Dawes, 1966; Campbell et al., 1967).

The peculiarity of the fetal cardiovascular system's reactivity is illustrated by the pulmonary vasculature's response to elevated intracranial pressure. In neonatal goats, an increased intracranial pressure resulted in pulmonary hypertension. However in fetal goats, the same

magnitude of increased intracranial pressure elicited pulmonary vasodilation (Hessler and Cassin, 1977). It was proposed that, because the pulmonary vasculature of the fetus is already constricted, sympathetic activation in response to increased intracranial pressure could result only in vasodilation. However, the pulmonary response to asphyxia as described previously does not lend credence to this hypothesis. A slight pulmonary vasodilation has been noted in adults in response to elevated intracranial pressure but not to the extent reported in the fetus (Lloyd, 1973).

Fetal Cardiac Function

In the fetal heart, both the conductive system and myocardium have been found to be functionally different from the adult. The fetus has a cardiac output which is approximately two times that of an adult (Rudolph and Heymann, 1974). This high cardiac output provides a safety margin for the fetus, in light of the fact that the oxygen tension of the fetal blood is low compared to the adult (Dawes, 1968). It must also be remembered that fetal hemoglobin has a higher affinity for oxygen than maternal hemoglobin. Therefore, some propose that a greater blood flow is needed to provide an adequate oxygen supply to the tissues (Klophenstein and Rudolph, 1978).

Cardiac output can be changed by altering either heart rate or stroke volume. Initially it was thought that lamb fetal heart rate was constant throughout gestation (Reynolds, 1954). However with the refinement of techniques, more recent studies have shown that as gestation progresses the fetal heart rate gradually decreases. During the last trimester of pregnancy, the heart rate in lambs decreased from an average of 200 to

170 beats per minute (Joelsson et al., 1972; Assali et al., 1977; Klophenstein and Rudolph, 1978). This compares with an adult heart rate of 75 beats per minute (Biological Handbooks, Respiration and Circulation, 1971). A decreasing heart rate points towards an increasing influence of the parasympathetic nervous system or to a decreasing sympathetic tone on the heart. Atropine and propranolol altered cardiovascular parameters to the same extent in both neonatal and adult sheep. Therefore, this decrease in fetal heart rate which occurs during gestation appears to be a result of intrinsic changes of the myocardial pacemaker and conduction system (Assali et al., 1977; Klophenstein and Rudolph, 1978). In vitro, human atria up to 20 weeks of gestational age show a decreasing basal rate with increasing gestational age. Atropine had no effect on resting rate nor did propranolol or cocaine. Stretching, which increases the rate of sino-atrial node firing in the adult, had no effect on the fetal sino-atrial node firing rate (Walker, 1974). Therefore, the continuous decrease in rate prior to 20 weeks in the human fetal heart must result from intrinsic changes in resting membrane potential, possibly via the sodium pump. It has also been suggested that the decrease in spontaneity of the sino-atrial node may be related to increased cell cluster size of the sino-atrial node (DeHaan and Sachs, 1973, as cited by Walker, 1974).

Concerning the metabolism of the fetal myocardium, Su and Friedman (1973) found that, under hypoxic conditions, adult sheep hearts in vitro ceased functioning after approximately 10 minutes while fetal hearts continued to beat for 1-2 hours. In contrast, with the blocking of glycolysis under otherwise normal conditions, the fetal heart failed faster than the adult heart. Similar results were obtained using adult

and fetal rat hearts (Shepard et al., 1969). This decreased tolerance to hypoxia was correlated to an increase in enzymes involved in oxidative phosphorylation. Others found the amount of glycolytic enzymes in neonatal and fetal guinea pigs to be more than that of the adult (Barrie and Harris, 1977). These data indicate that the fetal heart is more dependent upon anaerobic glycolysis than oxidative phosphorylation for energy.

When comparing the mechanical properties of fetal and maternal myocardium, one finds obvious differences. The fetal and neonatal heart are less compliant and maintain higher resting tensions at any point along the length-tension curve (Friedman, 1973, cited in Rudolph and Heymann, 1974). Previously it was contended that Frank-Starling's Law of the heart was not important in the fetus and that cardiac output could only be increased by increasing the rate (Heymann and Rudolph, 1973). However, Kirkpatrick et al. (1976) have shown that myocardial fiber length is an important control mechanism in the fetus in utero in that the fetus can increase its stroke volume over a range of 2.5-8.0 mm Hg before failure occurs. The overall lower performance of the fetal heart could be the result of fewer sarcomeres or a possible disturbance of the excitation contraction coupling mechanism (Friedman, 1973, cited by Rudolph and Heymann, 1974). Contrasting this lower fetal cardiac performance is the fact that pressure induced enlargements of the left ventricle during fetal growth did not affect the neonates' ability to respond to isoproterenol or to compensate for overloading. This is unlike pressure induced enlargements of adult hearts which result in a compromise of cardiac function. These enlargements also diminished myocardial DNA of the adult

but the myocardial DNA content of the neonate was increased (Dowell and McManus, 1978).

Bradycardia, whatever the cause, can be classified into two categories. The first type is of vagal origin and occurs in the fetus during hypoxia or grunting. Vagal mediated bradycardia is associated with increased vascular resistance and excitation of baroreceptors. The second type of bradycardia can be called terminal bradycardia and is not vagally mediated since the effect is not prevented by atropine. Systemic hypertension is not the underlying cause of this second type of bradycardia (Goodlin and Haesslein, 1977). The duration of the bradycardia is important because during fetal bradycardia the systolic time interval does not increase. This leads to decreased cardiac output which can be detrimental if prolonged. Investigations of fetal bradycardia in fetal guinea pigs and lambs showed that with severe hypoxia there occurred a sudden onset bradycardia which resulted from a Type II A-V block in the lamb. In the guinea pig, changes in the S-T segment of the fetal ECG were seen prior to bradycardia. Isoproterenol caused changes in the ECG similar to those resulting from hypoxia. Both isoproterenol and mild hypoxia related changes were prevented by propranolol (Rosen, 1976). Changes in the ECG which precede bradycardia indicate direct myocardial depression. Additional evidence supporting the idea of direct myocardial depression during hypoxia is the fact that the bradycardia is correlated with depletion of fetal liver, brain, and cardiac glycogen stores. Also, metabolic but not respiratory acidosis was associated with fetal ECG changes.

Fetal Cardiovascular Response to Drugs

Throughout gestation the fetus may be exposed to a number of exogenous compounds, perhaps as a treatment for a maternal or fetal disorder or to assist in parturition. In these instances of treatment, to ensure the health of both mother and fetus, the following considerations must be made: drug action in the mother along with metabolism and elimination, placental crossing, fetal distribution, and fetal responsiveness (Van Petten, 1975). The inability to predict drug action on the fetal cardiovascular system has been demonstrated. When chlorpromazine or pentobarbital were administered to pregnant goats, the maximal blood concentration obtained by the fetus was one-half that of the mother. Chlorpromazine caused an increase in maternal heart rate while pentobarbital, contrary to what one would predict, resulted in a decreased maternal heart rate and both of the drugs caused an increase in fetal heart rate. Phenylbutazone also crosses the placenta causing fetal ECG changes and renal damage (Boulos et al., 1971). Maternally administered cardiac glycosides have been shown to attain fetal cardiac concentrations up to six times those of simultaneously measured maternal cardiac concentrations when expressed on a per gram of cardiac tissue basis (Mirkin and Singh, 1972). Sharpe (1974) found that indomethacin given to pregnant rats caused a decrease in the diameter of the ductus arteriosus. Abnormal blood gases were measured in these resultant neonates when compared to control animals.

Interpreting the hemodynamic responses of the fetus to drugs requires an understanding of developmental anatomy, physiology, and pharmacology. The explanation for the lack of parasympathetic tone to the heart as

mentioned previously is disputed. Acetylcholinesterases have been found in the chick embryo heart preceding vagal innervation, and electrical stimulation of these hearts causes the release of acetylcholine, thereby affecting cardiac automaticity (Pappano, 1977). In mouse, rat, and human cardiac tissue, acetylcholine also exerted a negative chronotropic effect before neural innervation. In the mouse atria, the effect of a given concentration of acetylcholine increased as gestational age increased (Wildenthal, 1973; Gennser and Nilsson, 1970; Robkin et al., 1976). Investigators (Friedman, 1972) have also found that the acetylcholine dose response curves for fetal lamb hearts were similar to those of adult hearts. Regarding the vascular response to acetylcholine, pulmonary vasodilation is seen, but little effect on the systemic vasculature occurs, and the acetylcholine ED_{50} for a given type of blood vessel remains unchanged as gestation progresses (Dawes, 1966; Dawes, 1968; Rudolph and Heymann, 1968; Nuwayhid et al., 1975).

As with acetylcholine, prior to innervation of the rat and human heart adrenergic compounds elicit a response, thereby demonstrating that adrenergic receptors are present and functional (Gennser and Nilsson, 1970; Robkin et al., 1976). Electrical stimulation of isolated human atria prompted norepinephrine release from the autonomic ground plexus, and nicotine elicited positive inotropic responses when electrical stimulation failed (Walker, 1975). Perhaps the neurotransmitter(s) affecting the heart in this latter instance originate(s) from the small intensely fluorescent cells, described previously in this review.

For proper neural function, there must be an adequate supply of transmitter present. In human hearts from fetuses 11-23 cm in length,

norepinephrine synthesis occurred at the same rate as in the adult (Gennser and Studnitz, 1975), and within hours after birth the norepinephrine concentration per unit weight increased throughout the body (Roffi and Motelica-Heino, 1975). Perhaps this results from an increased metabolic rate accompanying the increased availability of oxygen at birth. The tyrosine hydroxylase activity, which is of primary importance in norepinephrine synthesis, was less in fetal lambs than neonates (Friedman, 1972). In conjunction with this decreased tyrosine hydroxylase activity, the norepinephrine stores in the heart were lower in the fetus and this was associated with a paucity of sympathetic innervation. This paucity of innervation was proposed as the cause of the increased sensitivity of the fetal lamb myocardium to norepinephrine when compared to the mother in vitro, presumably due to a lack of uptake (Friedman, 1972; Euler, 1972). From work on mouse atria (Wildenthal, 1973) it appears that, although the magnitude of chronotropic response increases from 15-16 days gestation on, the sensitivity to norepinephrine remained the same, i.e., the ED_{50} values were unchanged. These in vitro studies indicate a similarity between maternal and fetal sensitivity to norepinephrine in these species. However, the administration of catecholamines in vivo shows that the fetus is less responsive than the mother with respect to blood pressure changes (Assali et al., 1977). Despite the decreased magnitude of the fetal response in in vivo experiments in which fetal weight was estimated, the threshold dose for norepinephrine in the fetus and the mother was the same and the ED_{50} value did not change as gestation progressed (Nuwayhid et al., 1975; Priviteria et al., 1968). Some drug responses in the fetus are altered from those of the adult, and it does not appear to be an

instance of decreased fetal sensitivity. Arrhythmias and cardiac arrest are often seen at low doses of isoproterenol in the fetus but not the mother. Propranolol, a beta-adrenergic antagonist, shifted the dose response curves for isoproterenol in the pregnant ewe to the right by an equivalent degree in both the ewe and fetus. The blockade by propranolol in the fetus was however 2-3 times the duration of that in the ewe whereas the maximal propranolol concentration in the fetus was only 5 percent of that present in the ewe (Truelove et al., 1973; Van Petten and Willes, 1970). Vasopressors and vasodepressors are not the only drugs to which the fetus exhibits an altered responsiveness in vivo. Berman et al. (1977) found digoxin to be less potent in the fetus than in the adult.

Isolated Tissue Studies

In spite of the fact that in vitro studies have indicated similar sensitivities to parasympathomimetic and sympathomimetic compounds, in vivo experiments suggest the presence of both qualitative and quantitative differences between fetal and maternal responses to vasoactive drugs. The cause of these differences is unresolved. Some feel that the presence of the low resistance umbilical-placental circulation is the cause of these differences (Assali et al., 1977; Dawes, 1968). However, an immaturity of a portion of the neuroeffector mechanism is another possible explanation. One portion of the neuroeffector mechanism which can be examined is the receptor. When seeking to characterize receptors, one should use in vitro techniques since in vivo experiments present many variables which cannot be controlled (Furchgott, 1972; Furchgott, 1967).

Initially, adrenergic receptors were classified into alpha (α) and beta (β) subgroups (Alquist, 1948). Later the beta-adrenergic receptors were subclassified into $beta_1$, those affecting the heart and lypolysis, and $beta_2$, those associated with vascular and bronchial relaxation (Lands, 1967). Presently, evidence is mounting for the subclassification of alpha-adrenergic receptors into $alpha_1$ and $alpha_2$ (Wikberg, 1978). $Alpha_1$-adrenergic receptors are associated with vasoconstriction, and $alpha_2$-adrenergic receptors are associated with the central nervous system and renin release from the kidneys. With the recent realization that subunits of adrenergic-receptors exist, it seems possible that in the fetus the distinction between receptor types has not clearly developed and their affinity for adrenergic drugs may differ from that of the adult.

Barret et al. (1972) demonstrated that stimulation of $beta_1$-adrenergic receptors was effective as early as 0.4 gestational period in the fetal lamb while responses to alpha-adrenergic stimulation first appeared at a gestational age of 0.5. Others have shown that $beta_1$-adrenergic receptors of the fetal rat heart bind norepinephrine and activate adenyl cyclase but no inotropic or chronotropic responses are seen (Martin et al., 1973). Looking at this evidence, the difference between maternal and fetal heart reactivity seems to lie at some point beyond receptor activation, perhaps an alteration in the calcium pools, an absence of enzymes associated with contraction, or various physical arrangements of contractile proteins in cardiac muscle.

To complement the cardiac studies, work on isolated fetal blood vessels has been carried out. The information regarding the mechanics of smooth muscle contraction is in itself limited (Dorbin and Canfield,

1973). It is known that the response to norepinephrine varies in relation to the strain upon the vessel prior to excitation (Dorbin, 1973). This is due to the fact that the passive tissue elements determine the length of the contractile elements, thus altering vessel reactivity. Also, if the collagen:elastin ratios of various vessels differ, regardless of the fact that the total amount of connective tissue may be the same, the mechanics of contraction of the vessels will differ (Cox, 1975).

Despite the fact that much remains to be determined regarding the mechanics of smooth muscle contraction, vital information regarding receptor mechanisms can be obtained from isolated tissue studies if the following guidelines are adhered to. First is the acceptance of Clark's theory (Goodman and Gilman, 1975) that the magnitude of a drug response is proportional to the number of receptors occupied. Second, that the conditions set forth by Furchgott (1972) for conducting isolated tissue experiments are met. These are discussed in detail in the Methods and Materials section.

Using isolated tissue techniques, the $beta_2$-adrenergic receptors of newborn rabbits were studied by measuring their ability to relax strips of aorta. Sodium nitrite relaxed vessels of all age groups equally whereas isoproterenol was less effective in animals less than 10 days of age than in adults (Park et al., 1976). The pA_2 values which characterize the receptors were the same for neonates and adults. This information indicates that some site beyond the point of receptor activation is altered in neonatal blood vessels. This reflects the situation existing in fetal rat myocardium (Martin et al., 1973) mentioned earlier in this literature review. In other animal studies, no difference was found in

the pA_2 value for alpha-adrenergic receptors in fetal lambs nor the ED_{50} of norepinephrine as gestation progressed (Wyse et al., 1976). With respect to the muscarinic receptors, the pD_2 values for acetylcholine in the guinea pig intestine are the same for mother and fetus (Boreus and McMurphy, 1971). Information such as this indicates that the receptor affinity for cholinergic and adrenergic compounds is the same in both the adult and fetus. This does not, however, eliminate the possibility of an increase in the number of receptors as tissue growth occurs.

Studies on the sequential development of the entire neuroeffector mechanism in fetal lambs were performed by Su et al. (1977). This investigation revealed that enzymatic mechanisms for degradation of norepinephrine were present at early stages of gestation. This was followed in sequence by alpha-adrenergic receptor development, neuronal transmitter uptake, and finally neuronal release of transmitter.

Despite the descrepancies in some of the results and the nonstandardized techniques used for in vivo experiments, certain points are evident: 1) the fetal cardiovascular response to a vasoactive drug differs from that of the adult, and 2) this difference lies in part at some point beyond receptor activation.

The preceding literature review presented some of the major anatomical and physiological differences between the fetal and adult cardiovascular systems. It appears as though the overall differences in reactivity of the fetal and maternal cardiovascular systems are partially based on a difference at the cellular level. This allows one to hypothesize that the low resistance shunts of the fetal cardiovascular system may not be

the primary cause for the altered sensitivity of the fetus to drugs as has been proposed (Assali et al., 1977; Dawes, 1968).

Statement of the Problem

From the information in this literature review, it is evident that there is a large amount of uncertainty in the field of fetal pharmacodynamics. With this is mind, the following goals were established: 1) to develop and use the guinea pig model for studying fetal pharmacodynamics since it is a low cost, readily available laboratory animal with a breeding cycle that permits experimentation throughout the year, 2) to use this model to study fetal and maternal responses to biogenic amines, and 3) to use isolated tissues (and appropriate techniques) from the mother and fetus to better define the difference in responsiveness between mother and fetus observed in the in vivo experiments.

METHODS AND MATERIALS

Breeding

Guinea pigs (Cavia cabaya) were obtained through Laboratory Animal Resources at Iowa State University where their physical condition was examined. Animals of ill health were not accepted. As this project progressed an average of two timed pregnant guinea pigs a week was necessary, and this necessitated setting up a breeding program. This required a breeding stock of approximately 15-20 females weighing at least 500gms along with three mature males. To maintain an adequate number of animals in the colony, each week the newly bred females were replaced with nonpregnant females. The guinea pigs were housed separately to limit the spread of disease.

To obtain timed pregnancies, it was necessary to determine when the animal was in estrous. In guinea pigs this can be done by examining the membrane covering the vaginal opening which is easily visible when the animal is in dorsal recumbency. This membrane ruptures just prior to estrous and will remain open for 1-3 days (Elvidge, 1972; Manning and Wagner, 1976). The membrane starts reforming after ovulation has occurred.

The vaginal membranes of the nonpregnant guinea pigs were checked daily for a period of three weeks after their arrival at the colony. This allowed the animal to complete one sexual cycle and the date of membrane disappearance was determined. With this information, one can predict the next ovulation date to be an average of 16 days from the observed breaking of the vaginal membrane. Two days prior to the predicted

ovulation date the female was placed in a cage with a male. During this breeding period, daily checks were made for the presence of the membrane. After the membrane broke, mating was indicated by the presence of a white vaginal mucous plug (1-2 cm in length, 0.5 cm in diameter) in the bottom of the cage (Manning and Wagner, 1976). In this study, the day that the plug appeared was designated as day one of gestation. After a successful breeding, the female was separated from the male and daily examination was no longer performed in order to minimize excitement and decrease the chance of abortion. The gestation period for a guinea pig averages 68 days (Manning and Wagner, 1976), and pregnancy can be confirmed by palpation at 25 days (Elvidge, 1972; Matthews and Jackson, 1977). Females were utilized for *in vivo* experiments on day 60 $\pm$ 2 of gestation.

When setting up a breeding program, one must consider that some females may abort and some die from disease. It is therefore convenient to have contacts with an external supplier of timed pregnancies that can supply you during a period in which there are no pregnant females near term in the colony. If a supplier can provide pregnant guinea pigs with no breeding dates, palpation of the fetal head plus spreading of the maternal pubic symphysis can be used to estimate the last week of gestation (Manning and Wagner, 1976).

In Vivo Experiments

Anesthesia

For conducting the *in vivo* experiments, two methods of anesthesia were used. Pentobarbital sodium (20 mg/ug) given intrathoracically with supplemental doses (5 mg/kg) was used in six animals. For the remaining

experiments, anesthesia was induced with sodium thiopental (20 mg/kg) given intrathoracically with additional doses (5 mg/kg) administered to obtain the desired depth of anesthesia. After being anesthetized, the pregnant female was tied in dorsal recumbency on a thermal pad adjusted to approximately 103° F. Anesthesia was maintained with methoxyflurane administered with a small animal anesthesia apparatus constructed in our laboratory (Luschei and Mehaffey, 1967) and delivered using compressed room air through a small animal face cone. The animals were allowed to respire on their own and adequate depth of anesthesia was determined using withdrawal reflexes.

Cannulations

A midline incision was made along the ventral aspect of the neck, with lidocaine used along all lines of the incision. A traecheostomy was performed using polyethylene #240 tubing. The anesthetic gas mixture, which was previously being administered through a face mask was made to pass over the end of the endotracheal tube while the animal breathed spontaneously. The maternal common carotid and external jugular blood vessels were cannulated for arterial blood pressure monitoring and administration of the drugs, respectively. Heparinized 0.15 M saline (10 USP units/ml) was used to maintain catheter patency. After these cannulations were completed, arterial blood gas values were obtained for the mother. If necessary, the concentration of anesthetic in the gas mixture was adjusted to prevent severe hypoxia.

After locating a fetus which was in a suitable position, by palpation of the fetal head a transverse incision of approximately 5 cm in length

was made through the maternal abdominal wall and peritoneum using a cautery unit. A circular operating field was secured by suturing the skin and muscle surrounding the incision to a plastic coated oval lead ring which was attached to an upright ringstand (Figure 1). The lead ring could be adjusted to accommodate incisions of various sizes and locations.

The cautery unit was then used to make an incision in the uterus parallel to the major uterine blood vessels to expose the fetal head. The edges of the uterine incision were sutured to the maternal abdominal wall and the fetus gently lifted part way out to expose the ventral aspect of the neck. The amniotic membrane was cut from around the neck region leaving the head covered by the membrane. A suture was passed under the upper incisors of the fetus taking care not to break the amniotic membrane. This suture was tied to the aforementioned ringstand and secured the position of the fetus with the exposed ventral aspect of the neck perpendicular to the horizontal operating plane. A one cm midline incision was made along the ventral aspect of the fetal neck using a cautery unit. The common carotid artery and external jugular vein were then gently separated from the surrounding tissue using microsurgical forceps. Suture (6-0) was passed under each blood vessel while gently lifting the blood vessel. This prevented rotation of the vessel as the suture was passed underneath.

Cannulation was performed by gently lifting the blood vessel with spread forceps to occlude the blood flow. A cut was made with microsurgical scissors through the wall of the blood vessel segment located between the tips of the spread forceps. The cannula was gently inserted

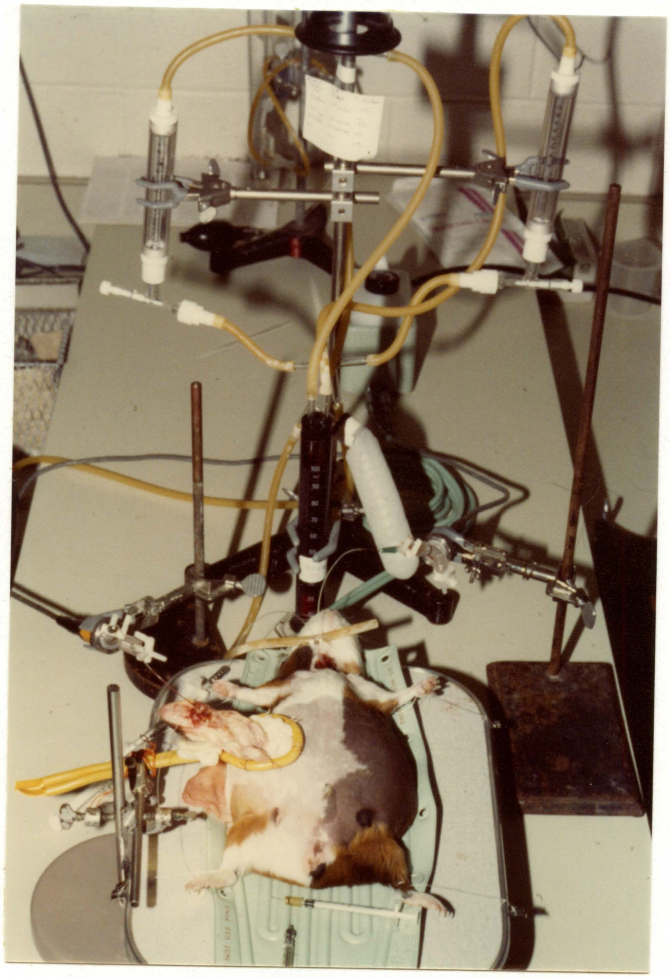


Fig. 1. Experimental setup

into the blood vessel and secured using a surgeons knot. Polyethylene tubing #10 was used to cannulate the fetal jugular vein. The carotid artery cannula was prepared from polyethylene tubing #50 which had been heated in boiling water, stretched, and cut to obtain a tapered beveled end. This facilitated the entry of the cannula into the blood vessel. The carotid and jugular cannulas were used to monitor blood pressure and administer drugs, respectively. The arterial cannula was maintained patent by periodically flushing with heparinized 0.15 M saline (100 USP units/ml).

To minimize the volume of injection required for drug administration to the fetus, the apparatus in Figure 2 was constructed. The maximal volume required to deliver a drug to the fetus with this device was 300 μl.

Blood pressure and heart rate monitoring

Blood pressure and heart rate were recorded with a Beckman R-611 Dynograph recorder. Arterial blood pressure was monitored using a Bell and Howe type 4-327-0121 physiological pressure transducer connected to a type 9853A voltage/pulse pressure coupler of the recorder. To negate any damping effect that the cannulas might have had on the pressure waves, all blood pressures were converted to mean blood pressures according to Riggs (1963) as,

$$\bar{C}p = (Cpt_1 - Cpt_2)/[Ln\ (Cpt_1/Cpt_2)]$$

where $\bar{C}p$ is mean blood pressure, Cpt_1 is systolic blood pressure, and Cpt_2 is diastolic blood pressure. Heart rate was determined using a

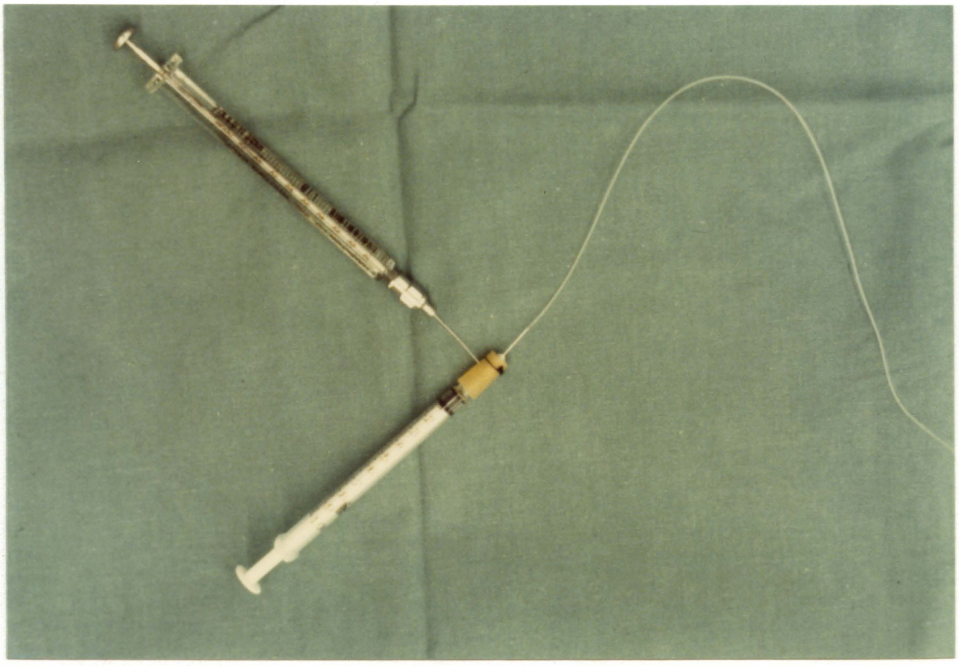


Figure 2. Fetal injection apparatus

type 9857B cardiotachometer coupler triggered by the pulse pressure wave of the arterial pressure recording.

Blood collection and analysis

Anaerobic blood samples were collected in one ml heparinized (100 USP units/ml) plastic syringes. Analysis of arterial oxygen partial pressure (PaO_2), arterial carbon dioxide partial pressure ($PaCO_2$), pH, bicarbonate ion, and base excess was performed using a pH/Blood Gas Analyzer 513 (Instrumental Laboratories).

Design

The following drugs were administered intravenously to both mother and fetus on a per kg body weight basis: norepinephrine, isoproterenol, acetylcholine, phenylephrine, and atropine. The drugs were administered using physiological saline as the carrier and made up fresh for each experiment from frozen (1 mg base/ml) stock solutions. For each preparation only one of the above drugs was used unless physiological parameters indicated that the animal had not deteriorated to any major extent. The drug being studied was administered first to the fetus progressing from low to high doses (run 1). Following each dose, the blood pressure and heart rate were allowed to return to normal or to stable conditions before administering the next dose. After completing run 1, the same dosages were given but this time progressing from high to low (run 2). This procedure was used to determine if the dosing sequence significantly altered the magnitude of the drug response. After both run 1 and run 2 had been completed in the fetus, the same procedure was performed on the mother. In the fetus, the largest volume of drug administered was

100 µl which was flushed into the animal with 200 µl of physiological saline. When a volume of 300 µl saline was given to the fetus over a 5-10 second period, no effect was seen. The largest volume of drug administered to the mother was one ml followed by one ml of saline. Again, when an equivalent volume of physiological saline was given, the maternal cardiovascular parameters were unaffected.

Prior to each run in both mother and fetus, approximately one ml of maternal blood was obtained to determine gas tensions and pH, in order to assess the status of the preparation. After completing all drug injections, the animals were killed by administering a lethal dose of magnesium sulfate intravenously. During an experiment, the fetal weight was estimated by sight for drug administration. Upon termination, the fetus was removed and weighed for accurate determination of the dosages used.

In Vitro Experiments

To further characterize the relationship between maternal and fetal responses to vasoactive drugs, investigation of the alpha-adrenergic receptors of the thoracic aorta and cardiac beta-adrenergic receptors was undertaken using isolated tissue techniques.

To obtain the tissues, an adult pregnant animal within one week of term was killed by decapitation. The thoracic cavity was opened and the heart and thoracic aorta removed and transferred immediately to a modified Krebs-Henseleit salt solution (Krebs, Appendix A). The Krebs solution was aerated with 95 percent oxygen, 5 percent carbon dioxide. Without delay the maternal abdominal cavity was opened exposing the uterus.

The uterus was cut open, and the hearts and thoracic aortas of two fetuses were removed and transferred to a beaker of oxygenated Krebs solution. Each blood vessel was placed in a petri dish, and a metal rod of approximately 0.5 mm diameter was passed through the lumen. While keeping the blood vessel submerged at all times, microsurgical scissors were used to helically cut each blood vessel into a strip, starting at the cardiac portion and proceeding distally. After each blood vessel had been cut, two strips of approximately one cm each were prepared by cutting the original strip into two parts. To each strip a small loop of suture material was tied around one end to anchor the strip to the tissue holder. The other end of the strip was secured with suture material to an isotonic transducer. Isotonic contractions were registered on a Beckman R-611 Dynograph recorder. Each strip was transferred to a 10 ml isolated tissue bath containing Krebs solution and aerated with 95% O_2:5% CO_2 and maintained at 37° C. The strips were connected to isotonic transducers with a resting tension of one gram for maternal segments and 0.5 grams for the fetal segments. The strips were allowed to equilibrate for at least one hour or until a steady baseline was obtained.

The method chosen to characterize the alpha adrenergic receptors was that of determining the K_B or pA_x (Schild, 1949; Furchgott, 1972) value of the receptor for a specified antagonist. The K_B value is the dissociation constant of the receptor for a specific antagonist and is related to pA_x as follows: when a dose ratio (x) of two is chosen, the negative log of K_B is equal to the pA_2 value. The procedures used to determine pA_2 values of drug receptors were the same as those discussed by Furchgott

(1972). The formula used to calculate the pA_2 values is indicated below:

$$[A']/[A] - 1 = [B]/K_B \qquad \text{(eq. 1; Furchgott, 1972)}$$

where B is the concentration of antagonist to which the tissue is exposed; [A] is the concentration of agonist required to produce a given control response; [A'] is the concentration of agonist required, in the presence of the antagonist, to produce a response equivalent to the control response; and [A']/[A] is the dose ratio. When these three values are known, the K_B can be calculated. Equation 1 can be manipulated to give:

$$\log (\text{dose ratio} - 1) = \log [B] - \log K_B \qquad \text{(eq. 2; Furchgott, 1972)}$$

Equation 2 in turn can be written as:

$$\log (x-1) = (\log 1/K_B) - pA_x \qquad \text{(eq. 3; Schild, 1949)}$$

From equation 3, it can be seen that if x = 2, then

$$-\log K_B = pA_2. \qquad \text{(eq. 4)}$$

Therefore, from an experiment, the K_B can be calculated using equation 1. This can then be converted into a pA_2 value using equation 4.

For a reliable determination of pA_2 values, the following conditions should be satisfied (Furchgott, 1967). 1) The agonist should act directly on the receptor in question and not produce any of its effect by indirectly liberating endogenous catecholamines in the test system. 2) The capacity of other types of receptors in the effector system to be activated by the agonists should be eliminated or made negligible. Thus, if responses

mediated by alpha-adrenergic receptors are being studied, the beta-adrenergic receptors should be blocked; if responses mediated by beta receptors are being measured, the alpha receptors should be blocked. 3) All processes other than passive diffusion which effectively remove any of the agonist from the region of the receptors should be blocked. 4) The agonist concentration in the fluid perfusing the effector system should be known and maintained at a fixed level for a period long enough to obtain the maximum response to the concentration used. 5) An experimental design should be used such that any change in sensitivity of the effector system to the agonist during the course of an experiment is apparent and corrected for. 6) Sufficient data should be obtained for the plotting of accurate and reliable partial or complete concentration-response curves of the agonist.

To meet these requirements set forth by Furchgott (1967), the experiments were carried out in the following manner after the initial equilibration period. All vessels were contracted with a single dose of norepinephrine giving a final bath concentration of 3×10^{-8} M. The vessels were then washed with fresh Krebs solution, and the monoamine oxidase inhibitor iproniazid phosphate was added to give a final bath concentration of 100 μg/ml. After 15 minutes, propranolol (3×10^{-7} M) was added to block the beta-adrenergic receptors. After the tissues had been in contact with the iproniazid for 45 minutes, they were rinsed. Then the propranolol was once again added. At this time, tropolone HCl (3×10^{-5} M) and cocaine HCl (3×10^{-6} M) were added to inhibit catecholamine-o-methyl transferase and neuronal uptake of norepinephrine, respectively. After 15 minutes contact time with cocaine, tropolone,

and propranolol, cumulative doses of norepinephrine were administered in half log increments over the range of 1×10^{-9} M to 1×10^{-6} M. When the final dose of norepinephrine had attained its maximal effect, the tissues were washed with fresh Krebs solution and allowed to relax to baseline.

All agonists were made up fresh in physiological saline for each experiment from stock solutions of 1×10^{-2} M which in turn were made fresh every week and stored in a freezer. The drugs were administered to the baths using lambda pipets to obtain the specified bath concentrations given above. After the tissues reached baseline, the same protocol was repeated but this time omitting the iproniazid. Both segments from one fetal vessel were equilibrated with the desired phentolamine concentration for 90 minutes prior to administering norepinephrine. Segments of the second fetal vessel were time controls, substituting physiological saline for phentolamine. One maternal vessel segment was treated and the other segment was used as a time control. The time control allows one to determine the change in sensitivity of the tissue to the agonist throughout the experiment. This is taken into account when calculating the degree of antagonism by phentolamine.

To obtain dose response curves, the magnitude of the contraction of each segment resulting from 1×10^{-6} M norepinephrine in the first cumulative dose series was used as a maximum, and all other contractions were taken as a percent of this maximal contraction. Dose response curves were constructed by plotting $\log_{10}$ of the drug concentration on the X-axis and percent maximal contraction on the Y-axis. Although the response to 1×10^{-6} M norepinephrine is not the absolute maximal

contraction that can be obtained, this procedure still allows one to work on the linear portion of the dose response curve while avoiding desensitization resulting from high doses of norepinephrine. Thus, the degree of tissue deterioration is minimized.

After plotting the dose response curves, the dose which gave 50 percent contraction (ED_{50}) in the control and treated was used to determine the dose ratio of equation 1. This ratio was corrected for the change in tissue sensitivity which occurred with time by using the dose ratio for the time control.

At the start of this project, plans were made to characterize the atrial beta-adrenergic receptors. The left atria was trimmed free of all other cardiac tissue and suspended in a 10 ml isolated tissue bath, perfused with Krebs, and aerated with 95% O_2:5% CO_2. The experiments were conducted at 32.5° C to maintain the integrity of the tissue (Blinks, 1967). The resting tension for the tissues was between approximately 70-90 percent of that giving maximal contraction for each tissue. The atria were electrically stimulated using either silver or platinum electrodes at a rate of 60 min^{-1} in the initial experiments. This was reduced to 30 min^{-1} in subsequent experiments. The stimulus strength ranged from 10-50 volts. After several experiments during which many combinations of the above parameters were tried, I concluded that the fetal atria deteriorated too rapidly to allow any reliable data to be obtained. This prevented the characterization of the beta-adrenergic cardiac receptors.

Statistical Analysis

In vivo

The basal values for maternal and fetal heart rate and blood pressure were analyzed using the Students t-test (Snedecor and Cochran, 1967). For comparison of the fetal and maternal responsiveness to the drugs tested, the slopes of the blood pressure and heart rate dose response curves were analyzed (Appendix B).

In vitro

For analysis of the data from the in vitro experiments, the ED_{50} and pA_2 values for each experiment were calculated. The means were calculated, and the Students t-test was used for test of significance (Snedecor and Cochran, 1967).

Equipment and Chemicals and Their Manufacturers

Drugs and chemicals

Acetylcholine and sodium heparin	Sigma Chemical Co.
Cocaine HCl	Merck and Co., Inc.
Iproniazid phosphate	Hoffman-Laroche Inc.
Isoproterenol and phenylephrine	Sterling-Winthrop Research Institute
Lidocaine HCl	Interstate Drug Exchange
Methoxyflurane	Pitman-Moore Inc.
Norepinephrine HCl	Calbiochem
Phentolamine	Ciba Chemical Co.
Propranolol	Imperial Chemical Co.
Sodium chloride	Fisher Scientific Co.
Sodium heparin	Interstate Drug Exchange
Sodium thiopental	Diamond Laboratories

Tropolone	Aldrich Chemical Co., Inc.
95% O_2:5% CO_2 gas mixture	Union Carbide Corp.

Equipment

Handilead ring	Instruments for Research & Industry
Isolated tissue baths	Metroware
Isotonic transducers (Model 33-03-981)	Gould Inc.
Lambda pipets	H. E. Pederson
Microsurgical scissors	Roboz Microsurgical Instrument, Inc.
pH/Blood Gas Analyzer 513	Instrumental Laboratories
Physiological pressure transducer (Type 4-327-0121)	Bell and Howe
Polyethylene tubing	Intramedic Corp.
R-611 dynograph recorder and tachograph coupler (Type 9857B)	Beckman Instrument Inc.
Suture material	Ethicon, Inc.
Thermal pad	Gorman Rupp Industries

RESULTS

In Vivo Experiments

Two classes of in vivo experiments were conducted for this project. In one class, pentobarbital sodium was used as the anesthetic. In the other class, the experiments were carried out under methoxyflurane anesthesia. For these two classes of experiments, the basal heart rates and mean arterial blood pressures are presented in Table 1.

Table 1. Mean arterial blood pressure (MABP) and heart rate under pentobarbital and methoxyflurane anesthesia

		Fetal	Maternal
A[a]	MABP (mm Hg)	33.2 ± 0.8[b]*	54.3 ± 1.1*
	Heart rate (min^{-1})	232.5 ± 10.4	257.0 ± 10.2
B[c]	MABP (mm Hg)	28.1 ± 1.1*	43.8 ± 1.6*
	Heart rate (min^{-1})	197.2 ± 5.7*	223.0 ± 8.7*

[a] A (pentobarbital, n = 6).

[b] Mean ± SE.

[c] B (methoxyflurane, n = 15).

* Maternal significantly different than fetal ($p < 0.05$).

Basal fetal and maternal arterial blood pressures under pentobarbital anesthesia were significantly different ($p < 0.05$) while the basal heart rates were similar ($p > 0.05$). Under methoxyflurane, both basal mean

arterial blood pressure and basal heart rate of the fetus were significantly different from those of the mother ($p < 0.05$). The values in Table 1 compare with values found in the literature of 57 mm Hg and 269 min^{-1} for the adult mean arterial blood pressure and heart rate, respectively (Marshall and Hanna, 1956). For term fetal guinea pigs, no values could be found in the literature for arterial blood pressure, but a value of 189 min^{-1} has been reported for heart rate (Biological Handbooks, Respiration and Circulation, 1971).

For each drug tested in vivo, the data are expressed as percent change from basal values for mother and fetus because of the different initial basal values for mother and fetus. After transforming the data into linear functions, regression formulas were derived to describe the responses. These formulas were used to construct the graphs and test for similarity of slope between maternal and fetal responses.

The heart rate and blood pressure responses of the mother and fetus in response to norepinephrine administration under pentobarbital anesthesia are shown in Figures 3 and 4. In all cases, norepinephrine resulted in an increase in heart rate and blood pressure. Both the regressions describing heart rate changes and those describing blood pressure were significantly different between maternal and fetal groups ($p < 0.05$). The slope of the maternal response was greater for both heart rate and blood pressure. Within the fetal group, the regressions describing the fetal heart rate response possessed a common slope ($p > 0.05$) and, likewise, the fetal blood pressure response possessed a common slope among animals ($p < 0.05$). For the regressions describing maternal heart rate responses to norepinephrine, a common slope was not present among

Figure 3. Percent change in mean arterial blood pressure (MABP) in response to norepinephrine (NE) administration under pentobarbital anesthesia, for 6 experiments

A entire fetal dose response curve
B lower portion of fetal dose response curve
C entire maternal dose response curve
I individual 95 percent confidence limits
· observed value
⊙ two points indicated
Δ more than two points indicated
● predicted value

Fetal regression: $y = -82.2 + 106.9\ \sqrt[10]{\text{dose}}$
S.E. slope = 8.4, S.E. intercept = 9.4, coefficent of determination: $(r^2) = 0.67$

Maternal regression: $y = -175.0 + 255.9\ \sqrt[10]{\text{dose}}$
S.E. slope = 25.9, S.E. intercept = 23.8, coefficient of determination: $(r^2) = 0.68$

Control fetal blood pressure: 33.2 ± 0.8 mm Hg

Control maternal blood pressure: 54.4 ± 1.1 mm Hg

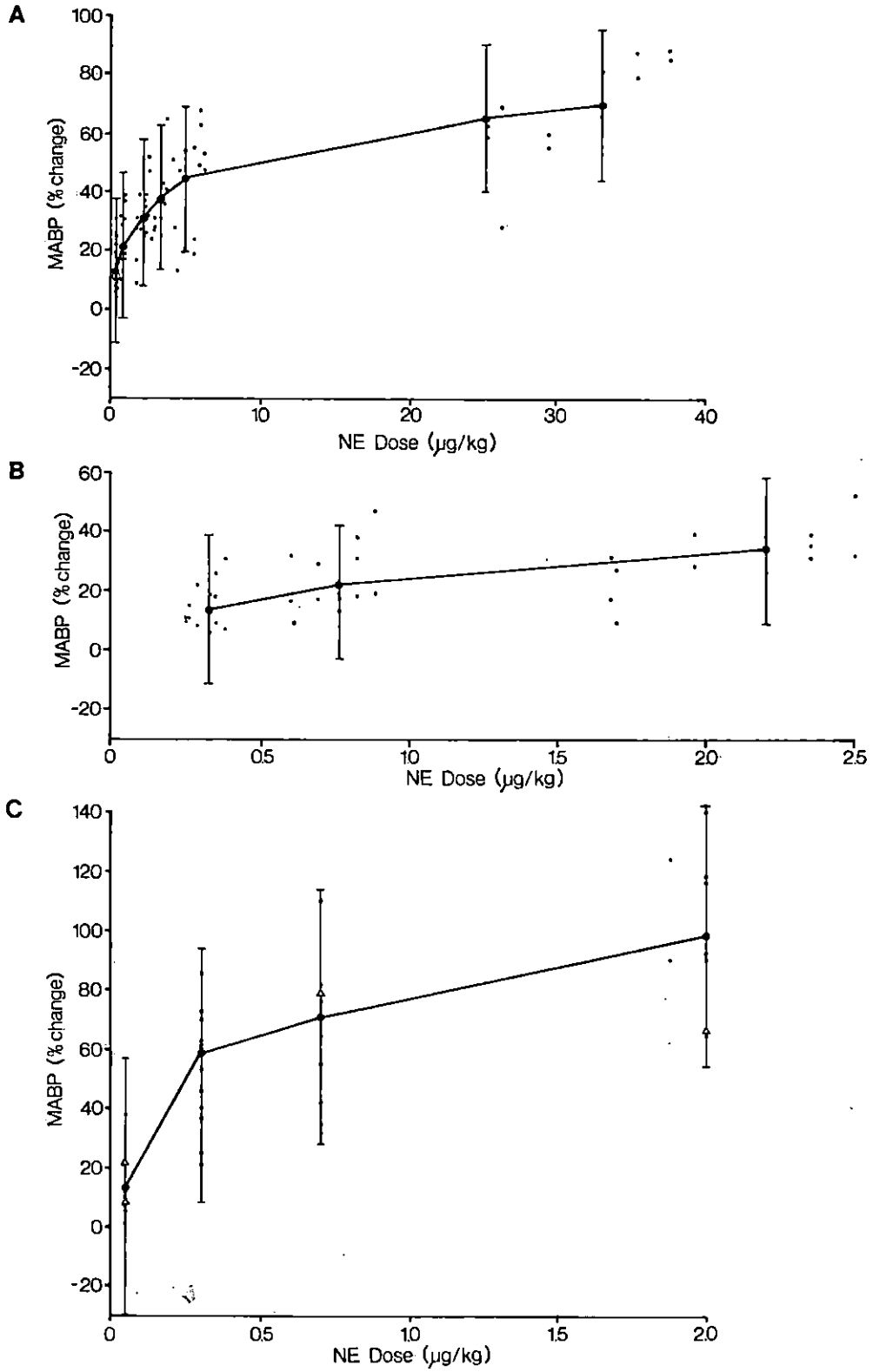

A
MABP (% change)
NE Dose (µg/kg)
B
MABP (% change)
NE Dose (µg/kg)
C
MABP (% change)
NE Dose (µg/kg)

Figure 4. Percent change in heart rate in response to norepinephrine (NE) under pentobarbital anesthesia, for 6 experiments

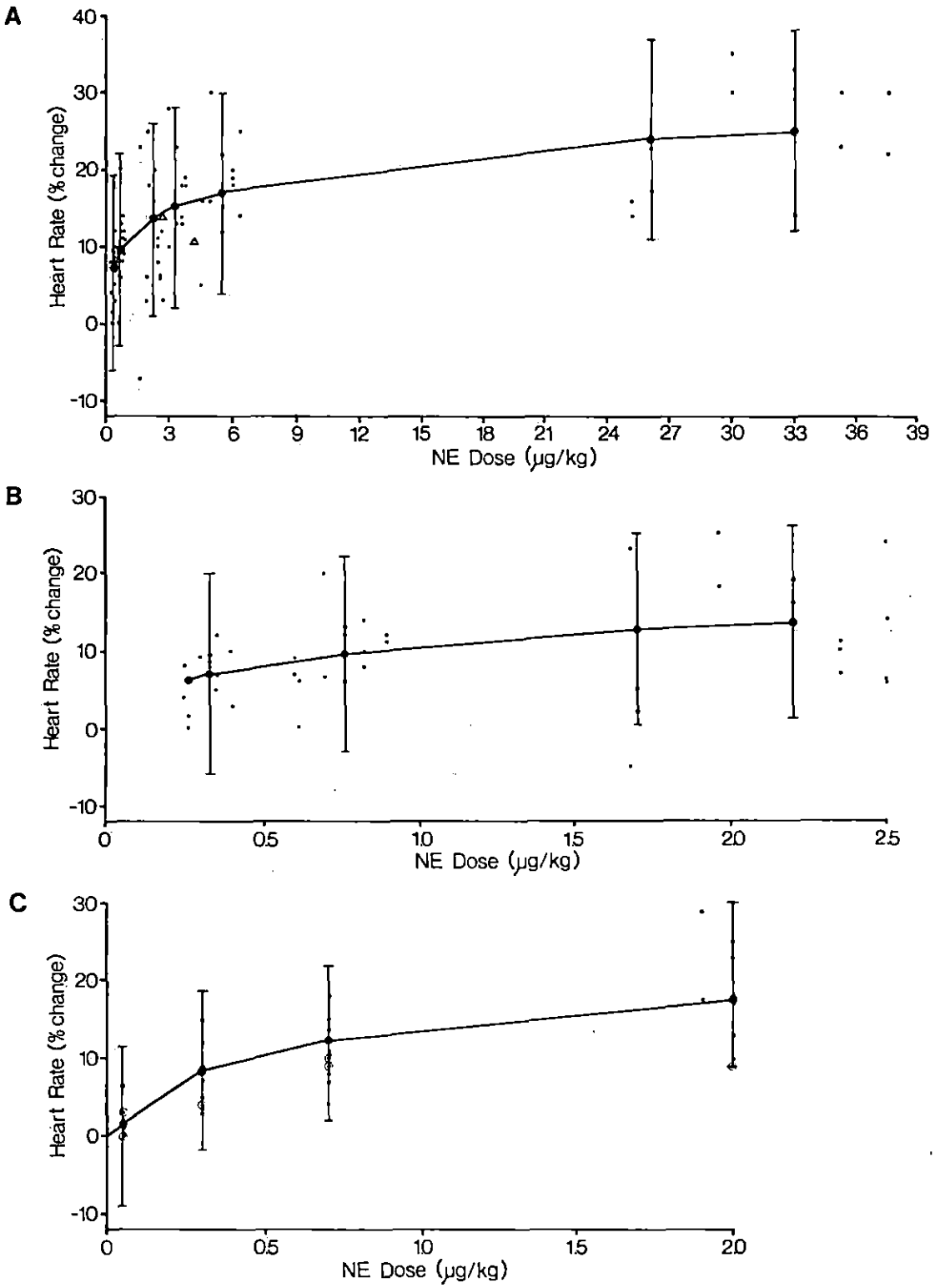
A entire fetal dose response curve
B lower portion of fetal dose response curve
C entire maternal dose response curve
I individual 95 percent confidence limits
· observed value
⊙ two points indicated
Δ more than two points indicated
● predicted

Fetal regression: $y = -24.1 + 34.7\ \sqrt[10]{\text{dose.}}$
S.E. slope = 4.4, S.E. intercept = 4.9, coefficient of determination (r^2) = 0.45

Maternal regression: $y = -34.7 + 48.5\ \sqrt[10]{\text{dose.}}$
S.E. slope = 0.61, S.E. intercept = 5.6, coefficient of determination (r^2) = 0.58

Control fetal heart rate: 232 min^{-1}

Control maternal heart rate: 257 min^{-1}


A
Heart Rate (% change)
NE Dose (µg/kg)
B
Heart Rate (% change)
NE Dose (µg/kg)
C
Heart Rate (% change)
NE Dose (µg/kg)

the animals ($p < 0.05$). While the regressions describing maternal blood pressure did possess a common slope ($p > 0.05$). Each animal had different y intercepts so no analysis could be performed to test for likeness of maternal and fetal intercepts for any of the drugs used.

Norepinephrine administration under methoxyflurane anesthesia, as with pentobarbital anesthesia, increased both maternal and fetal heart rate and blood pressure. The recordings and data are presented in Figures 5-7. The slopes describing heart rate and blood pressure responses were both significantly different between mother and fetus ($p < 0.05$). Tests were performed to determine the presence of common slopes within fetal and within maternal heart rate responses. Tests were also done within fetal and within maternal blood pressure responses. Neither blood pressure nor heart rate responses to norepinephrine within the fetal group could be described by a single slope ($p < 0.05$). However, the heart rate and blood pressure responses within the maternal group could each be described using a common slope for each of the parameters ($p > 0.05$). An important point that should be brought out is that, although the magnitude of the response and slopes of the maternal and fetal regressions differ, the dose to produce a threshold effect was similar in both mother and fetus as shown by the plots.

When examining the actual recordings, Figure 7, some important aspects should be noted. First is the transient plateau in the fetal blood pressure response when high doses were administered to the fetus. Second is the fact that, when norepinephrine was given to the mother, a transient fetal bradycardia and hypotension occurred.

Figure 5. Percent change in mean arterial blood pressure (MABP) in response to norepinephrine (NE) administration under methoxyflurane anesthesia, for 3 experiments

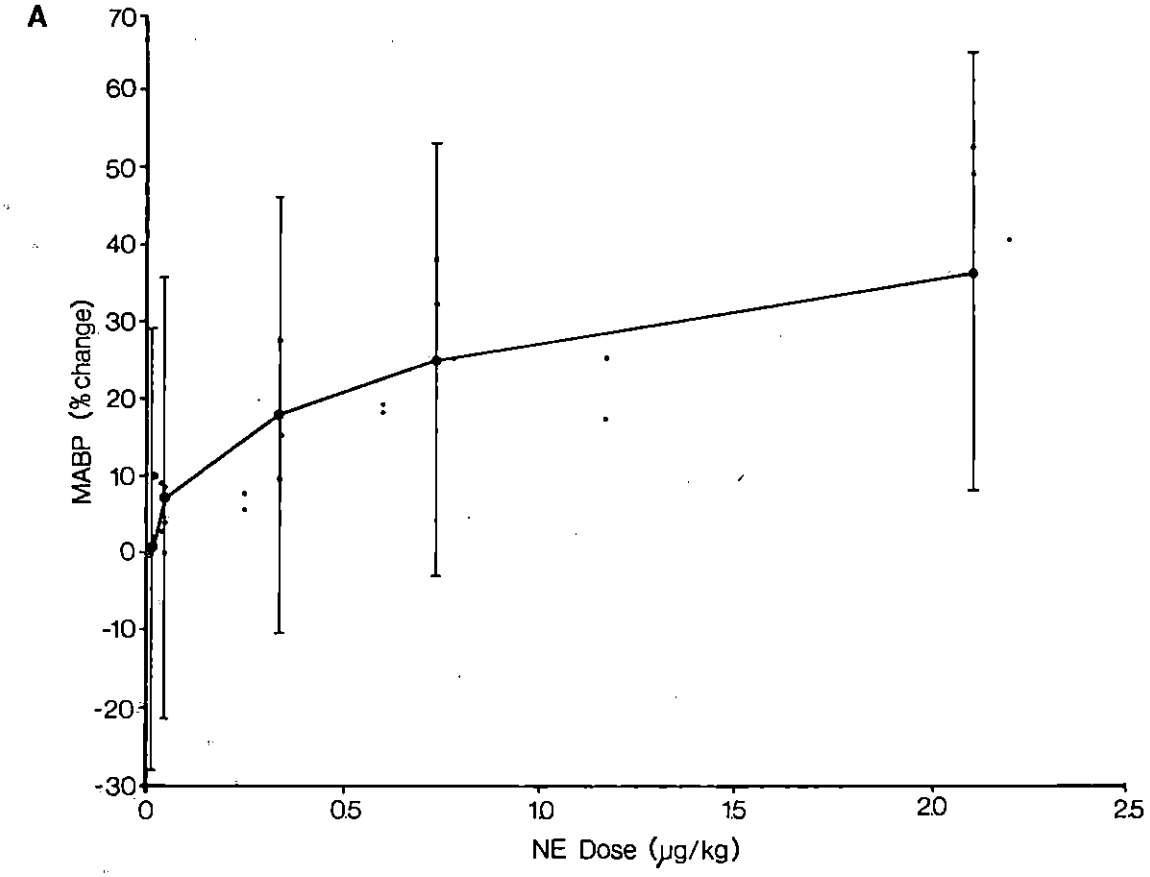
A fetal dose response curve
B maternal dose response curve
I individual 95 percent confidence limits
· observed value
⊙ two points indicated
Δ more than two points indicated
● predicted

Fetal regression: $y = -11.9 + 39.9 \sqrt[4]{\text{dose.}}$
S.E. slope = 3.1, S.E. intercept = 3.9, coefficient of determination (r^2) = 0.79

Maternal regression: $y = -48.7 + 170.7 \sqrt[4]{\text{dose.}}$
S.E. slope = 15.3, S.E. intercept = 11.9, coefficient of determination (r^2) = 0.81

Control fetal blood pressure: 28.1 ± 1.1 mm Hg

Control maternal blood pressure: 43.8 ± 1.6 mm Hg


A
70
60
50
40
30
20
10
0
-10
-20
-30
MABP (% change)
0
0.5
1.0
1.5
2.0
2.5
NE Dose (µg/kg)

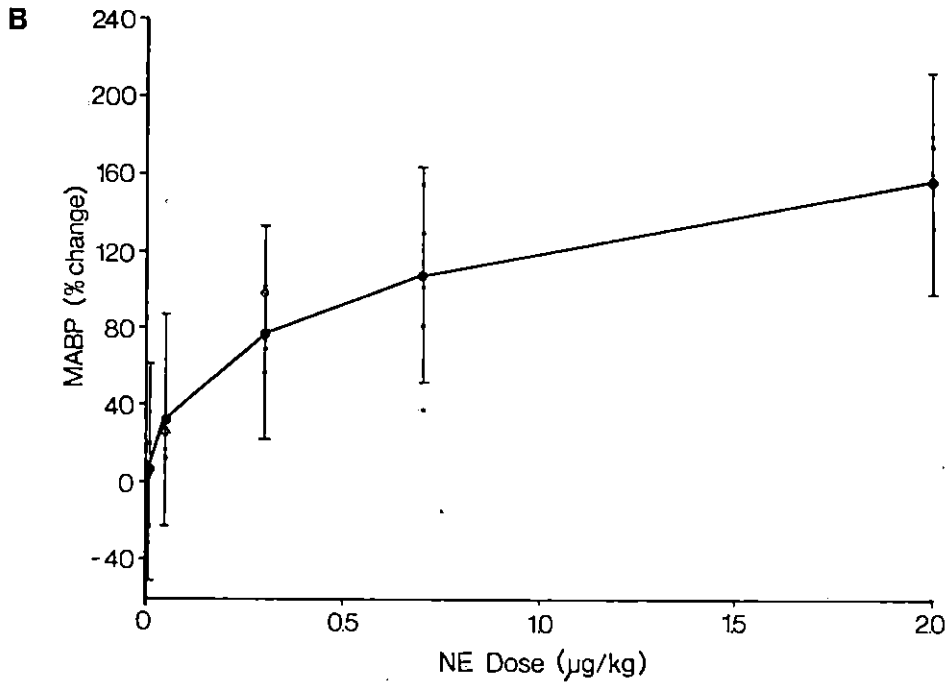

B
240
200
160
120
80
40
0
-40
MABP (% change)
0
0.5
1.0
1.5
2.0
NE Dose (µg/kg)

Figure 6. Percent change in heart rate in response to norepinephrine (NE) administration, under methoxyflurane anesthesia, for 3 experiments

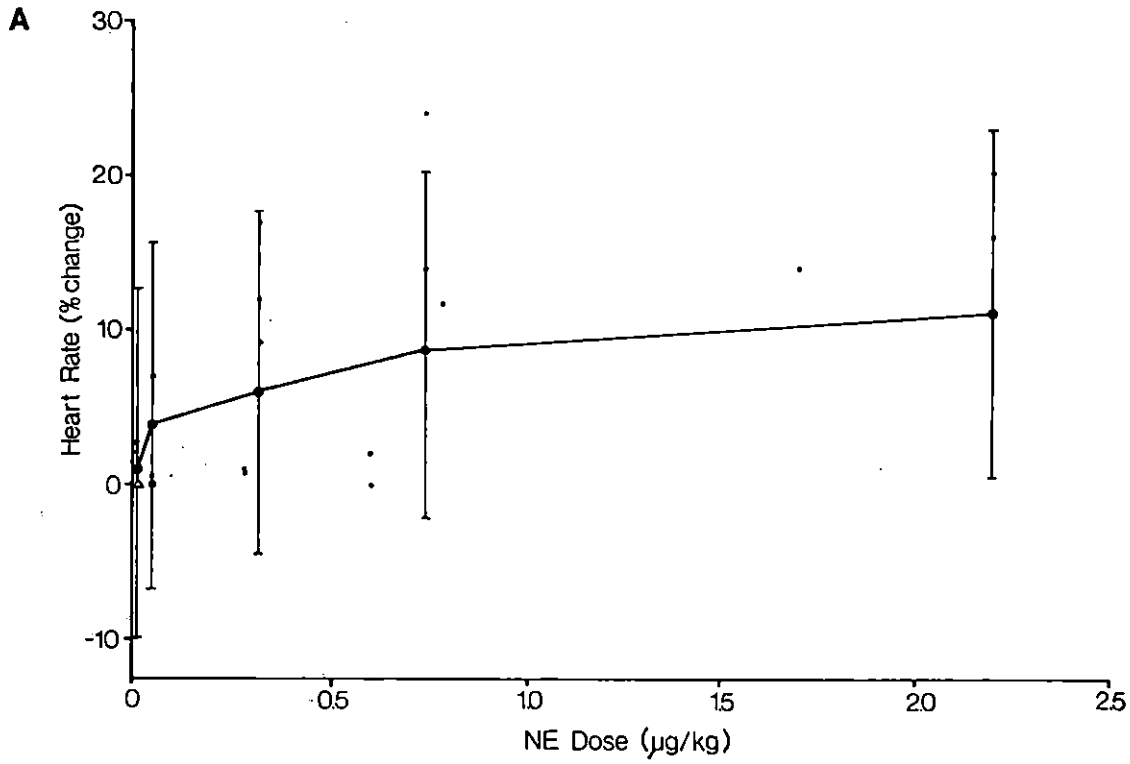
A fetal dose response curve
B maternal dose response curve
I 95 percent confidence limits
· observed value
⊙ two points indicated
Δ more than two points indicated
● predicted

Fetal regression: $y = -1.7 + 11.5 \sqrt[4]{\text{dose.}}$
S.E. slope = 1.3, S.E. intercept = 1.6, coefficient of determination (r^2) = 0.68

Maternal regression: $y = -5.7 + 18.0 \sqrt[4]{\text{dose.}}$
S.E. slope = 2.5, S.E. intercept = 1.9, coefficient of determination (r^2) = 0.66

Control fetal heart rate: 197 min^{-1}

Control maternal heart rate: 223 min^{-1}


A
30
20
10
0
-10
Heart Rate (% change)
0
0.5
1.0
1.5
2.0
2.5
NE Dose (µg/kg)

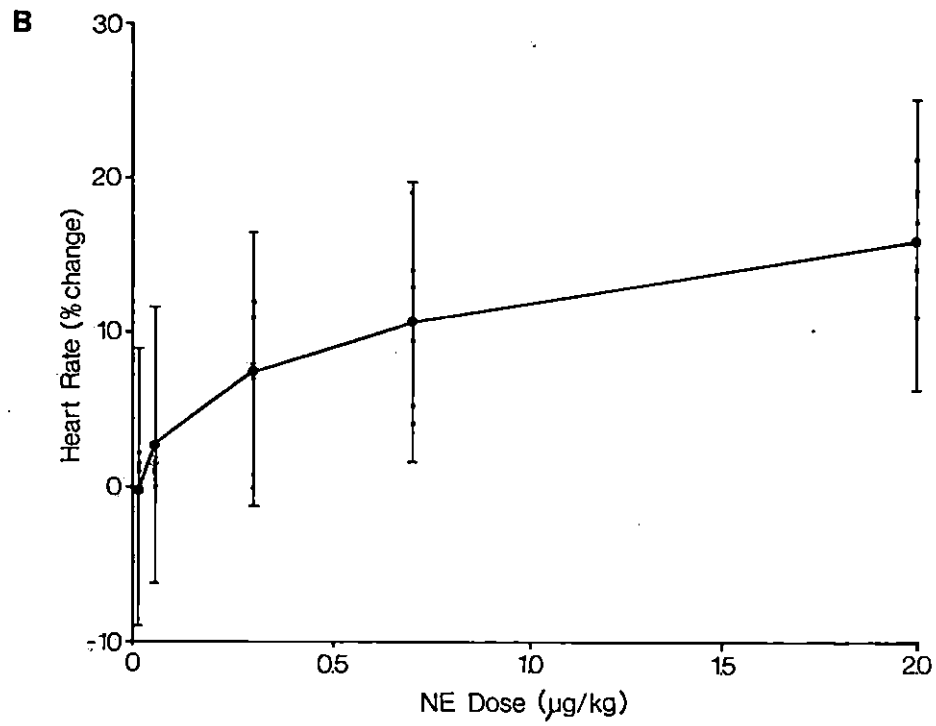

B
30
20
10
0
-10
Heart Rate (% change)
0
0.5
1.0
1.5
2.0
NE Dose (µg/kg)

Figure 7. Recording of blood pressure and heart rate during norepinephrine administration under methoxyflurane anesthesia; arrow indicates time of injection

A fetal administration

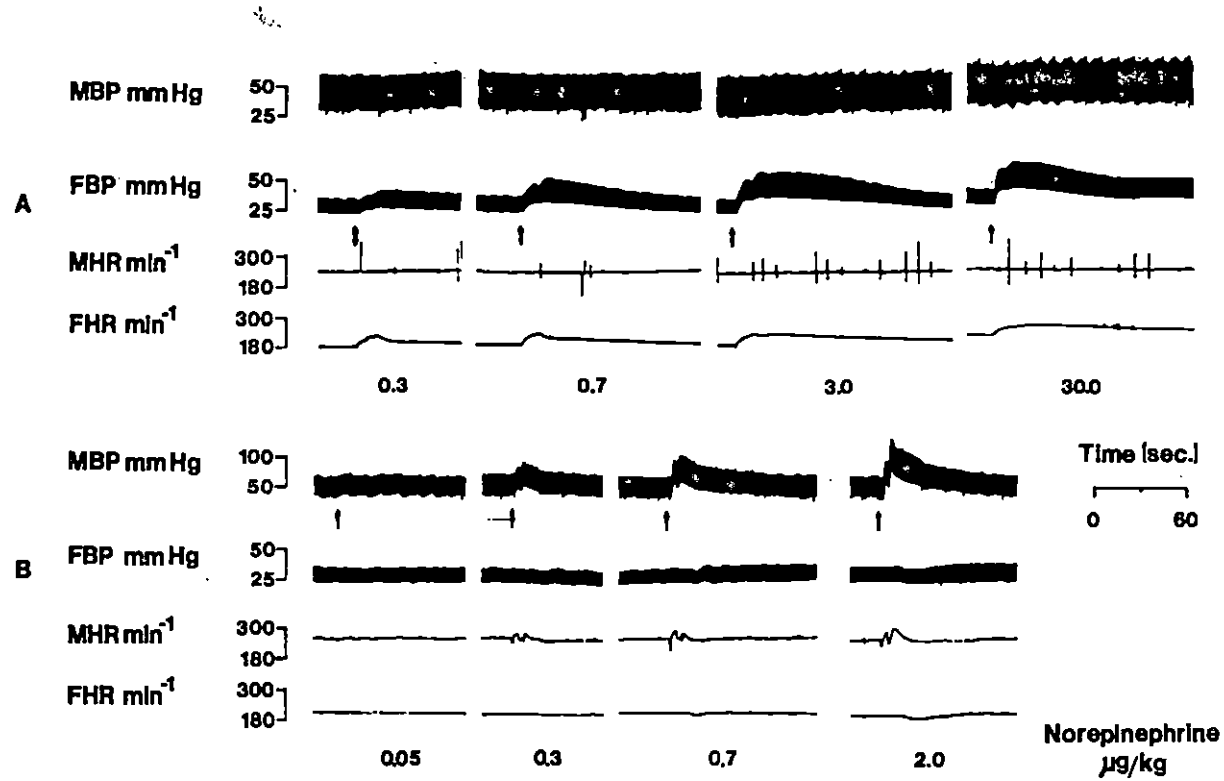
B maternal administration

MBP maternal blood pressure

MHR maternal heart rate

FBP fetal blood pressure

FHR fetal heart rate


A
MBP mmHg
50
25
FBP mmHg
50
25
MHR min-1
300
180
FHR min-1
300
180
0.3
0.7
3.0
30.0
B
MBP mmHg
100
50
FBP mmHg
50
25
MHR min-1
300
180
FHR min-1
300
180
0.05
0.3
0.7
2.0
Norepinephrine µg/kg
Time [sec.]
0
60

The maternal and fetal responses to acetylcholine under methoxyflurane anesthesia are presented in Figures 8-10. In both mother and fetus, a decrease in mean arterial blood pressure and an increase in heart rate resulted from acetylcholine administration. The slope of the regression equation describing maternal blood pressure response was significantly greater than the slope of the regression describing the fetal blood pressure response ($p < 0.05$). A common slope existed within the maternal blood pressure responses to acetylcholine ($p > 0.05$). The fetal blood pressure responses to acetylcholine were not as consistent as the maternal responses. A common slope describing the fetal blood pressure changes was not present ($p < 0.05$). The slope describing the maternal heart rate response to acetylcholine was also significantly greater than that of the fetus ($p < 0.05$). Neither within the fetal group nor within the maternal group were common slopes able to describe the heart rate changes ($p < 0.05$).

The results of the alpha-adrenergic agonist, phenylephrine, are presented in Figures 11-13. Phenylephrine increased blood pressure in both mother and fetus. The slope of the regression equation describing maternal blood pressure response was significantly greater ($p < 0.05$) than the slope describing fetal blood pressure response. A common slope was not present among the regression equations describing the blood pressure changes of the fetuses ($p < 0.05$). Nor did a common slope exist among the regression equations describing the maternal blood pressure changes ($p < 0.05$). Not only were the regression equations describing maternal and fetal heart rate responses to phenylephrine significantly different in magnitude ($p < 0.05$), the slope was negative in the maternal

Figure 8. Percent change in mean arterial blood pressure (MABP) in response to acetylcholine (ACH) administration, under methoxyflurane anesthesia, for 5 experiments

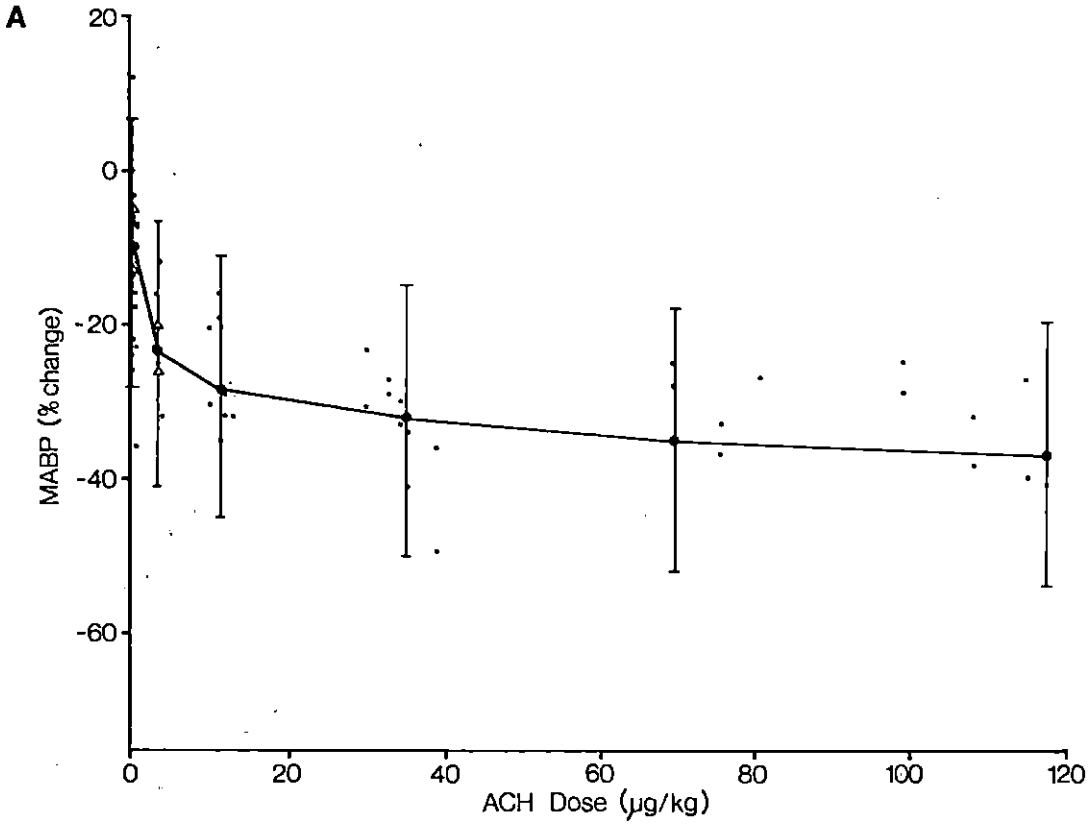
A fetal dose response curve
B maternal dose response curve
I 95 percent confidence limits
· observed value
Δ more than two points indicated
● predicted

Fetal regression: $y = -18.9 - 8.7$ (log dose).
S.E. slope = 8.3, S.E. intercept = 1.1, coefficient of determination (r^2) = 0.61

Maternal regression: $y = -32.8 - 13.4$ (log dose).
S.E. slope = 0.95, S.E. intercept = 1.14, coefficient of determination (r^2) = 0.77

Control fetal blood pressure: 28.1 ± 1.1 mm Hg

Control maternal blood pressure: 43.8 ± 1.6 mm Hg


A
20
0
-20
-40
-60
MABP (% change)
0
20
40
60
80
100
120
ACH Dose (µg/kg)

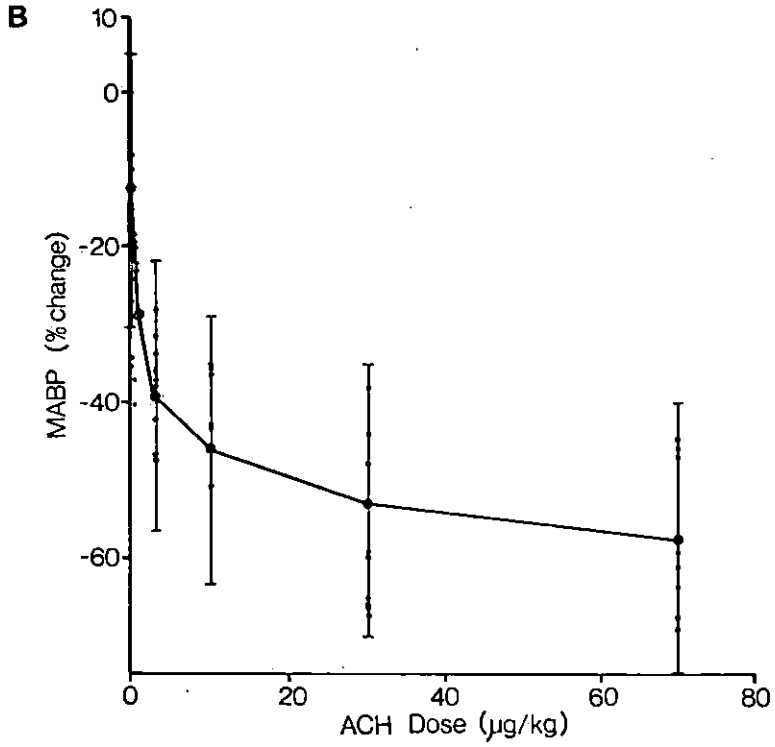

B
10
0
-20
-40
-60
MABP (% change)
0
20
40
60
80
ACH Dose (µg/kg)

Figure 9. Percent change in heart rate in response to acetylcholine (ACH) administration under methoxyflurane anesthesia, for 5 experiments

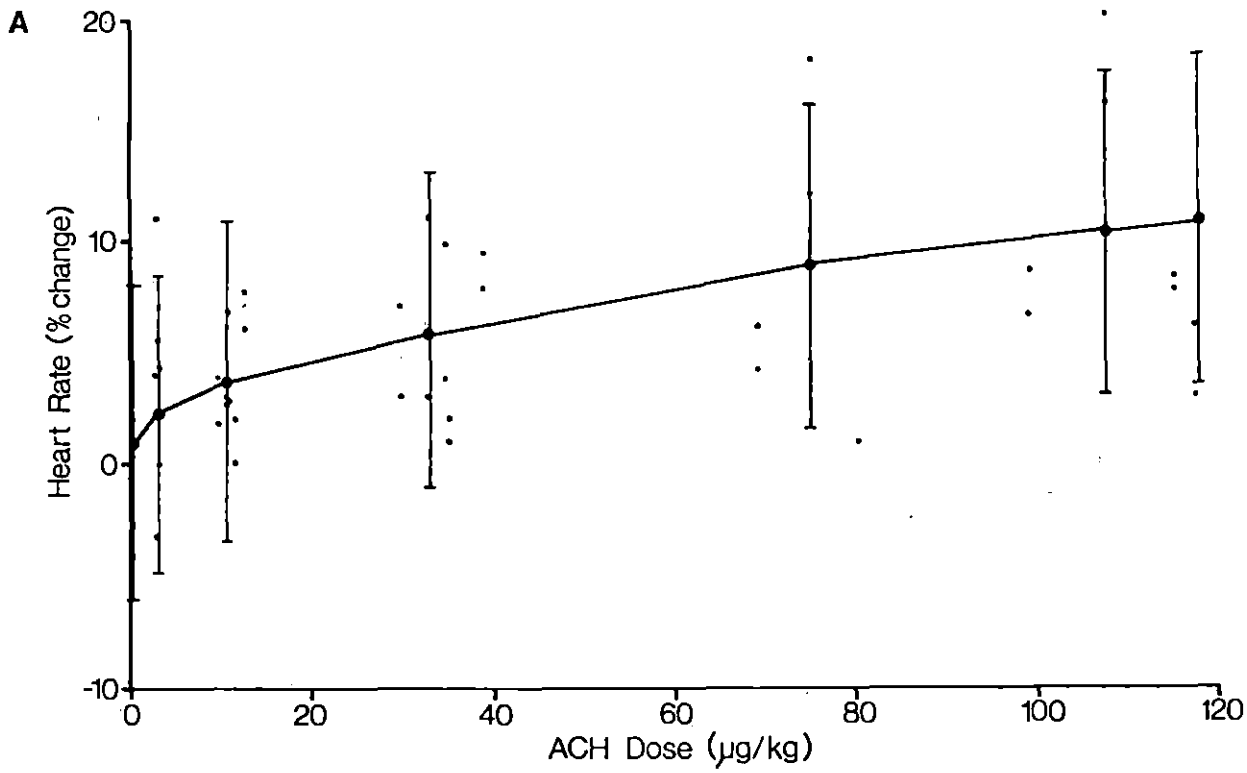
A fetal dose response curve
B maternal dose response curve
I 95 percent confidence limits
· observed value
Δ more than two points indicated
● predicted

Fetal regression: $y = 0.59 + 0.94 \sqrt[2]{dose.}$
S.E. slope = 0.94, S.E. intercept = 0.59, coefficient of determination (r^2) = 0.49

Maternal regression: $y = 0.24 + 1.11 \sqrt[2]{dose.}$
S.E. slope = 0.12, S.E. intercept = 0.45, coefficient of determination (r^2) = 0.61

Control fetal heart rate: 197 min^{-1}

Control maternal heart rate: 233 min^{-1}


A
20
10
0
-10
Heart Rate (% change)
0
20
40
60
80
100
120
ACH Dose (µg/kg)

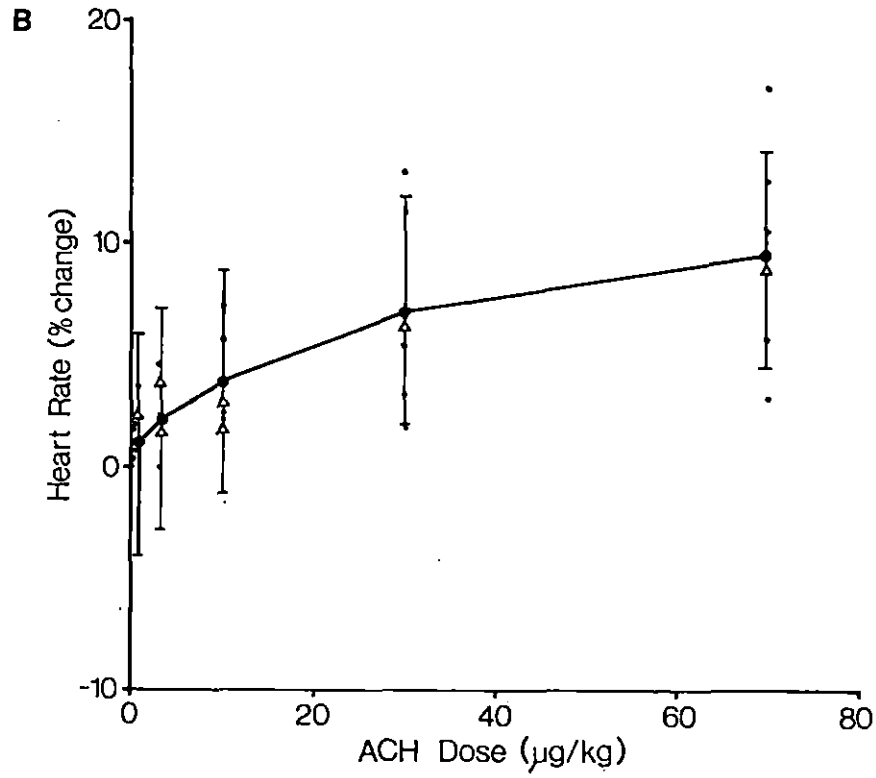

B
20
10
0
-10
Heart Rate (% change)
0
20
40
60
80
ACH Dose (µg/kg)

Figure 10. Recording of blood pressure and heart rate during acetylcholine administration under methoxyflurane anesthesia; arrow indicates time of injection

A	fetal administration
B	maternal administration
MBP	maternal blood pressure
MHR	maternal heart rate
FHR	fetal heart rate
FBP	fetal blood pressure

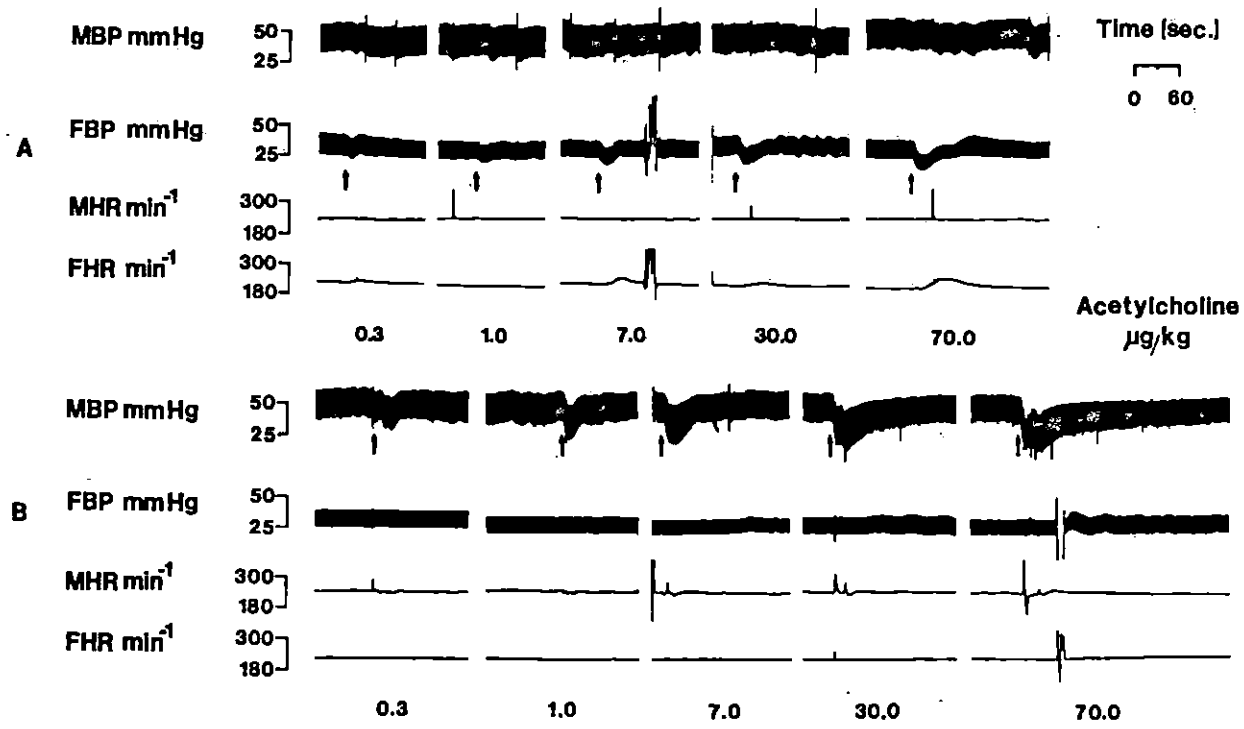

A
MBP mmHg
50
25
FBP mmHg
50
25
MHR min-1
300
180
FHR min-1
300
180
Time (sec.)
0 60
0.3
1.0
7.0
30.0
70.0
Acetylcholine
µg/kg
B
MBP mmHg
50
25
FBP mmHg
50
25
MHR min-1
300
180
FHR min-1
300
180
0.3
1.0
7.0
30.0
70.0

Figure 11. Percent increase in mean arterial blood pressure (MABP) in response to phenylephrine (PE) administration under methoxyflurane anesthesia, for 5 experiments

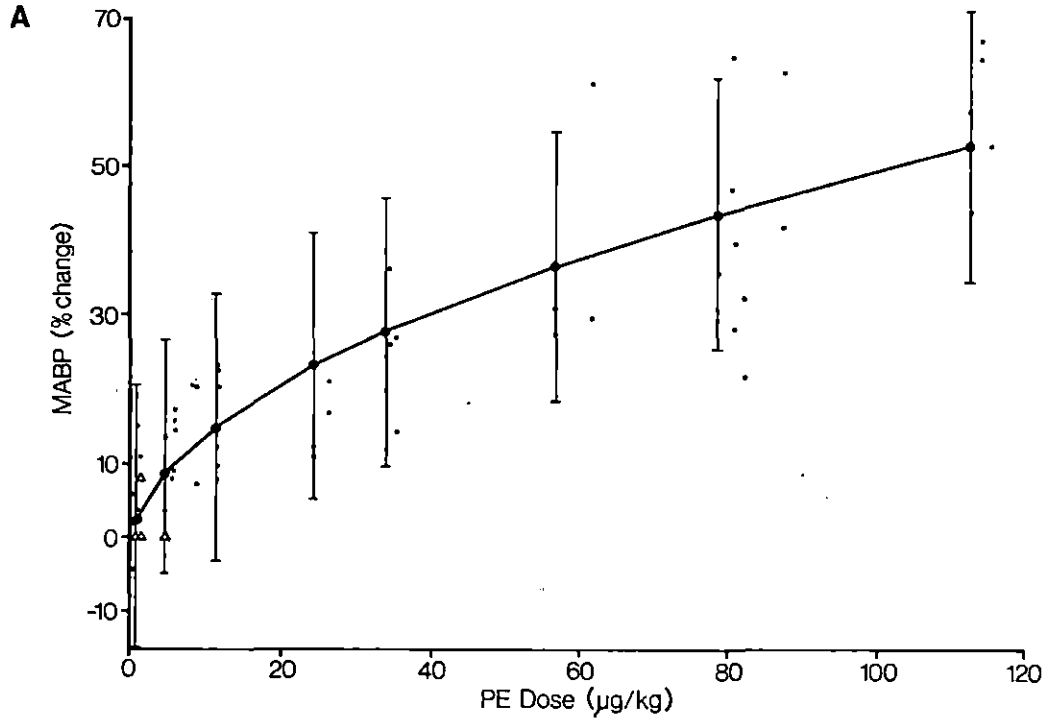
A fetal dose response curve
B maternal dose response curve
I 95 percent confidence limits
· observed value
Δ more than two points indicated
● predicted

Fetal regression: $y = -2.45 + 5.8 \sqrt[2]{\text{dose.}}$
S.E. slope = 5.18, S.E. intercept = 2.45, coefficient of determination (r^2) = 0.85

Maternal regression: $y = 123 + 16.7 \sqrt[2]{\text{dose.}}$
S.E. slope = 1.1, S.E. intercept = 4.9, coefficient of determination (r^2) = 0.79

Control fetal blood pressure: 28.1 ± 1.1 mm Hg

Control maternal blood pressure: 43.8 ± 1.6 mm Hg


A
70
50
30
10
0
-10
MABP (% change)
0
20
40
60
80
100
120
PE Dose (µg/kg)

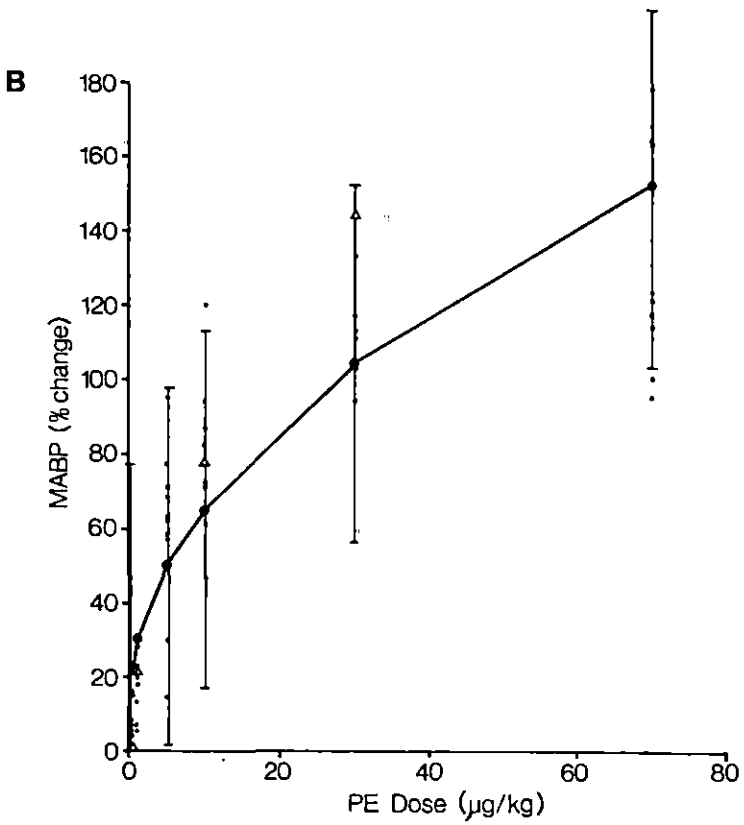

B
180
160
140
120
100
80
60
40
20
0
MABP (% change)
0
20
40
60
80
PE Dose (µg/kg)

Figure 12. Percent change in heart rate in response to phenylephrine (PE) administration under methoxyflurane anesthesia, for 5 experiments

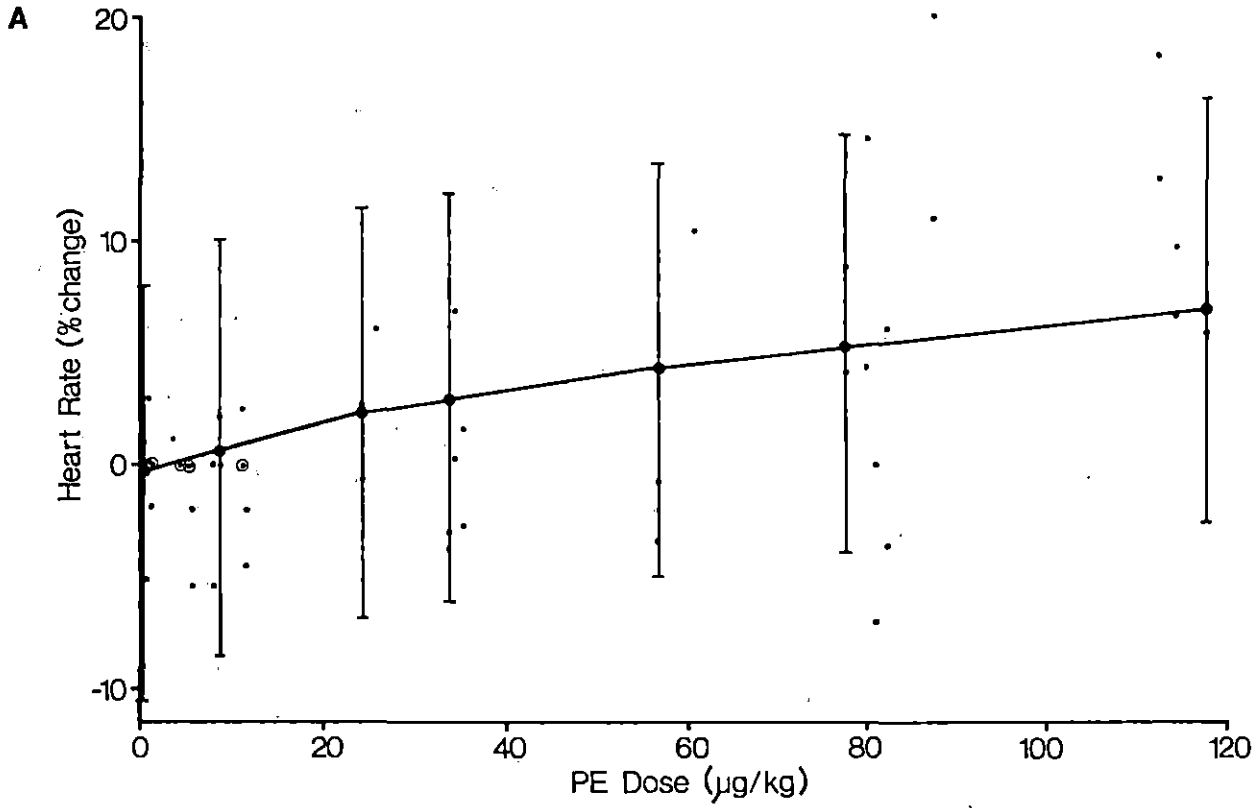
A fetal dose response curve
B maternal dose response curve
I 95 percent confidence limits
· observed value
⊙ two points indicated
Δ more than two points indicated
● predicted

Fetal regression: $y = -1.58 + 0.78 \sqrt[2]{\text{dose.}}$
S.E. slope = 0.16, S.E. intercept = 0.91, coefficient of determination (r^2) = 0.26

Maternal regression: $y = 1.25 - 1.32 \sqrt[2]{\text{dose.}}$
S.E. slope = 0.04, S.E. intercept = 1.72, coefficient of determination (r^2) = 0.16

Control fetal heart rate: 197 min^{-1}

Control maternal heart rate: 223 min^{-1}


A
20
10
0
-10
Heart Rate (% change)
0
20
40
60
80
100
120
PE Dose (µg/kg)

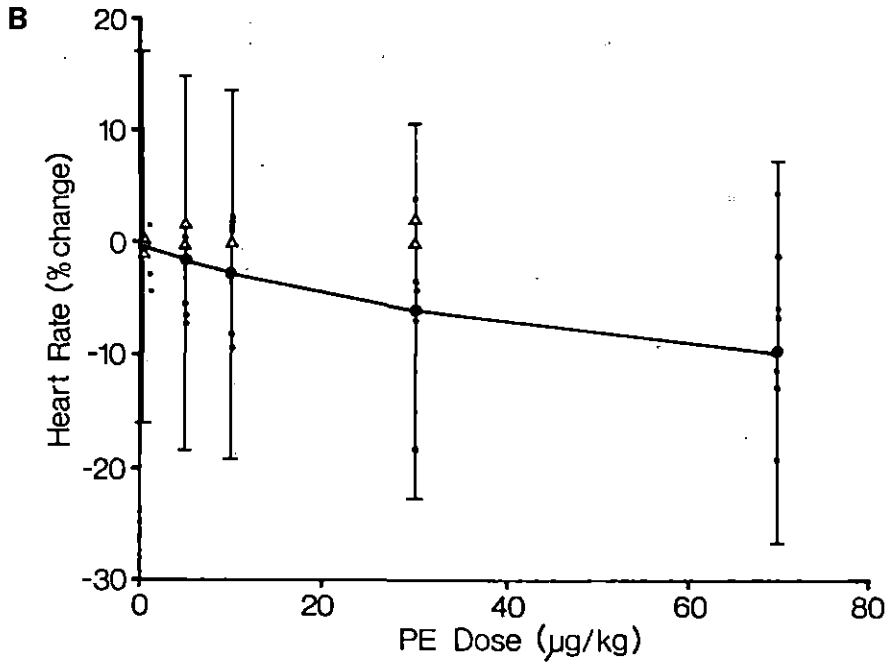
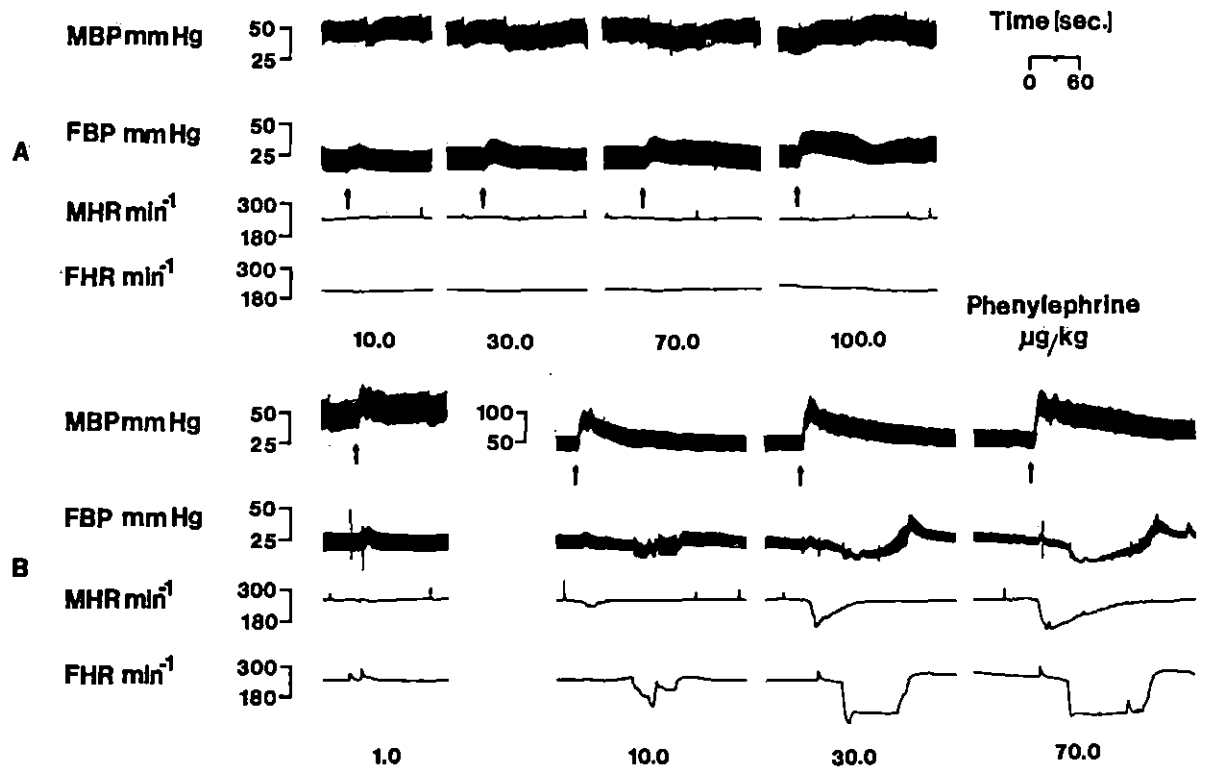

B
20
10
0
-10
-20
-30
Heart Rate (% change)
0
20
40
60
80
PE Dose (µg/kg)

Figure 13. Blood pressure and heart rate recording during phenylephrine administration under methoxyflurane anesthesia; arrow indicates time of injection

A	fetal administration
B	maternal administration
MBP	maternal blood pressure
MHR	maternal heart rate
FBP	fetal blood pressure
FHR	fetal heart rate


A
MBP mm Hg
50
25
FBP mm Hg
50
25
MHR min-1
300
180
FHR min-1
300
180
Time [sec.]
0 60
10.0
30.0
70.0
100.0
Phenylephrine
µg/kg
B
MBP mm Hg
50
25
100
50
FBP mm Hg
50
25
MHR min-1
300
180
FHR min-1
300
180
1.0
10.0
30.0
70.0

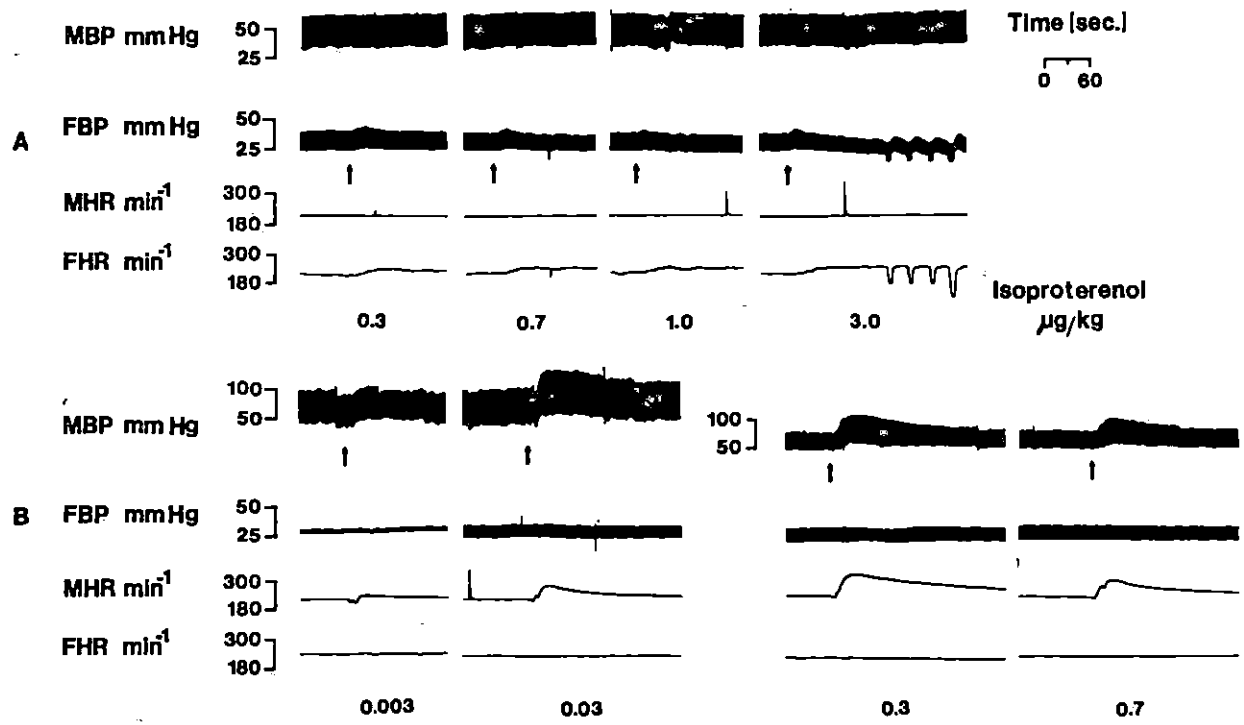
group and positive in the fetal group. When tests for common slopes for the regression equations within the mothers and within the fetal groups were performed, no common slope for either group was found ($p < 0.05$). Inspection of the data indicated that the fetal group could be divided into those which respond to phenylephrine administration with an increase in heart rate and those which respond with a slight decrease in heart rate ($p < 0.05$). Although a common slope for the regressions describing the maternal heart rate responses to phenylephrine was not apparent, there did not appear to be two distinct groups as seen with the fetuses.

The above information regarding the overall heart rate response to phenylephrine should be kept in mind when examining the recordings in Figure 13. Despite the increase in fetal blood pressure when phenylephrine was given directly to the fetus, little reflex fetal bradycardia was seen. In contrast, reflex maternal bradycardia was pronounced when phenylephrine was given to the mother. Following the phenylephrine induced increase in maternal blood pressure, there occurred a fetal bradycardia (Figure 13). This response was not observed when comparable doses were administered directly to the fetus. These events are similar to those mentioned previously regarding norephinephrine (Figure 7).

Responses to isproterenol are shown in Figures 14 and 15. Fetal responses were very inconsistent. Arrhythmias and cardiac arrests were frequent. This is illustrated at the dose of 3.0 μg/kg in Figure 14. A sufficient amount of data regarding heart rate and blood pressure changes in response to isoproterenol could not be collected to allow quantitative analysis. From a qualitative standpoint, it can be seen

Figure 14. Recording of blood pressure and heart rate during isoproterenol administration under methoxyflurane anesthesia; arrow indicates time of injection

A	fetal administration
B	maternal administration
MBP	maternal blood pressure
MHR	maternal heart rate
FBP	fetal blood pressure
FHR	fetal heart rate


MBP mmHg
50
25
Time [sec.]
0 60
A
FBP mmHg
50
25
MHR min-1
300
180
FHR min-1
300
180
0.3
0.7
1.0
3.0
Isoproterenol
µg/kg
MBP mmHg
100
50
100
50
B
FBP mmHg
50
25
MHR min-1
300
180
FHR min-1
300
180
0.003
0.03
0.3
0.7

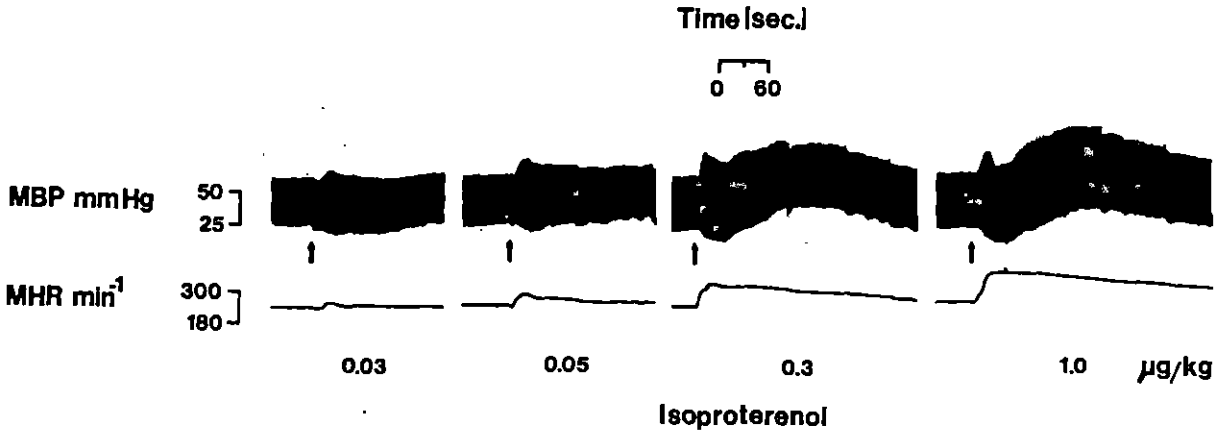


Figure 15. Recording of maternal arterial blood pressure (MBP) and heart rate (MHR) during isoproterenol injection under methoxyflurane anesthesia; arrow indicates time of injection

from the recordings that in some mothers isoproterenol produced a transient decrease in mean arterial blood pressure, Figure 15, while in others only an increase in blood pressure was seen, Figure 14.

None of the drugs used *in vivo* showed a distinct difference in the duration of action between mother and fetus.

The modifying effects of atropine on basal heart rate and mean arterial blood pressure for the mother and fetus are shown in Table 2. The effectiveness of the atropine blockade was determined by its ability to prevent a decrease in blood pressure following the i.v. administration of 5.0 μg/kg of acetylcholine. In both types of anesthesia, a significant increase in basal fetal heart rate was seen after the administration of atropine ($p < 0.05$). The mothers responded to atropine with an increase in basal heart rate but this was not statistically significant ($p > 0.05$). The mean arterial blood pressure for both mother and fetus was not significantly altered by atropine ($p > 0.05$). In addition, the heart rate and blood pressure responses of the mother and fetus to norepinephrine were not significantly ($p > 0.05$) altered by the presence of atropine.

In Vitro Experiments

In order to obtain information which would assist in explaining the cause of decreased fetal responsiveness to biogenic amines *in vivo*, *in vitro* experiments were performed. In four experiments, the tissues were contracted to norepinephrine using a cumulative dose response series (Figure 16). The sensitivity difference between maternal and fetal vessels to norepinephrine, determined from the ED_{50} values (Table 3), was nonsignificant ($p > 0.05$).

Table 2. Effects of atropine (1 mg/kg) on basal mean arterial blood pressure (MABP) and heart rate during pentobarbital and methoxyflurane anesthesia

		Before atropine		After atropine	
		Fetal	Maternal	Fetal	Maternal
Pentobarbital (n = 6)	MABP (mm Hg)	30.0 ± 1.1[a]	55.9 ± 4.5	30.9 ± 1.1	53.5 ± 4.5
	Heart rate (min^{-1})	224.0 ± 3.3	268.5 ± 10.3	254.5 ± 3.3*	276.0 ± 10.3
Methoxyflurane (n = 5)	MABP (mm Hg)	26.9 ± 0.3	44.8 ± 0.9	26.6 ± 0.3	43.9 ± 0.9
	Heart rate (min^{-1})	207.0 ± 0.8	195.0 ± 1.2	217.0 ± 0.8*	198.0 ± 1.2

[a]Mean ± S.E.

*Indicates significant ($p > 0.05$) difference in values before and after atropine.

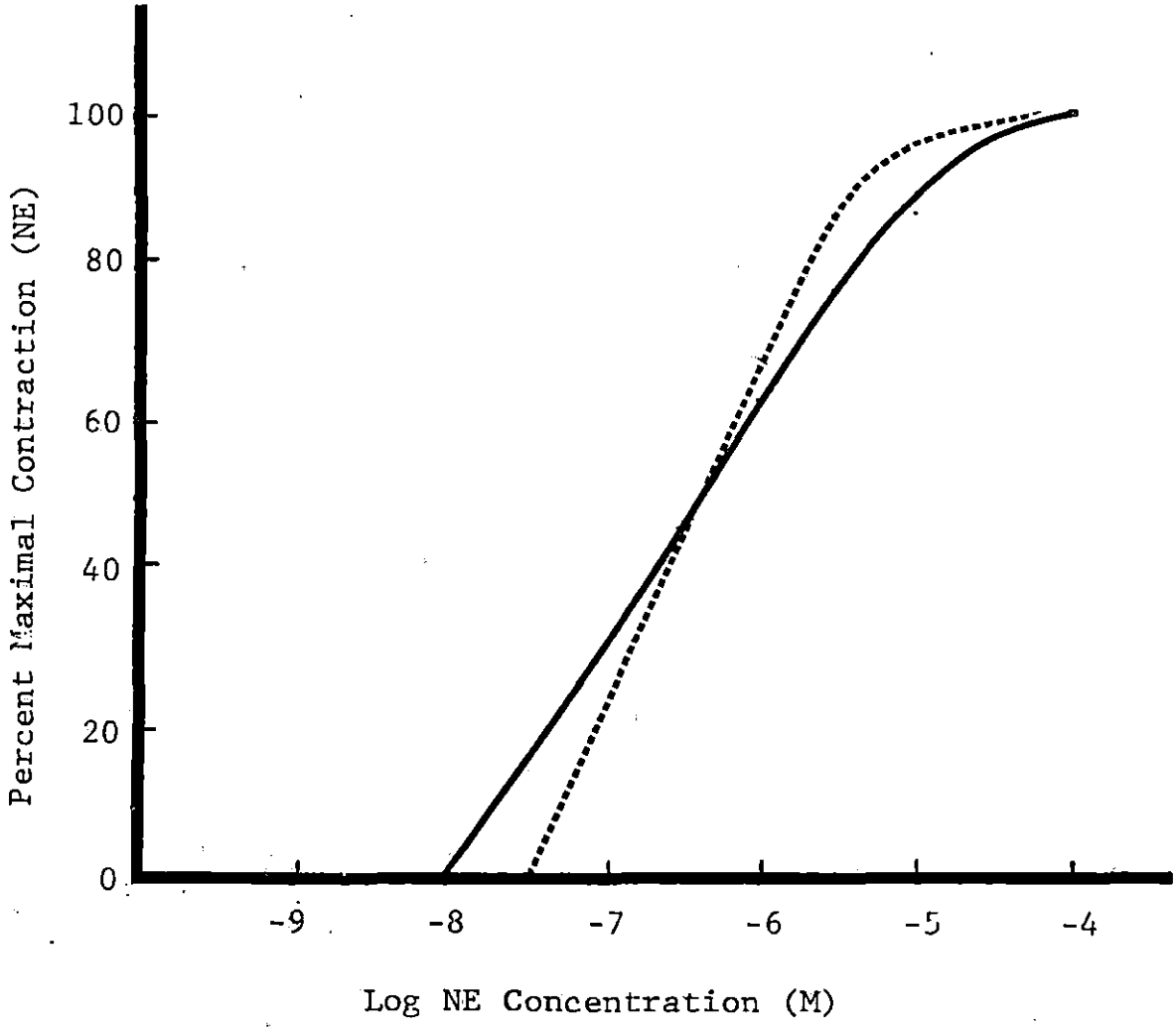
Table 3. Norepinephrine ED_{50} values for the thoracic aorta

Experiment	Fetal	Maternal
1	3.5×10^{-7} M	4.0×10^{-7} M
2	2.2×10^{-7} M	3.1×10^{-7} M
3	5.8×10^{-7} M	2.5×10^{-7} M
4	5.6×10^{-7} M	2.5×10^{-7} M
Mean $\pm$ S.E.	$4.3(\pm 1.8) \times 10^{-7}$ M	$3.0(\pm 0.4) \times 10^{-7}$ M

Phentolamine was used as the alpha-adrenergic antagonist for determining pA_2 values in six experiments. The dose response curves from one experiment are shown in Figures 17 and 18. The curves were constructed using the contraction caused by 1×10^{-6} M norepinephrine as maximal contraction. For the maternal vessels, the pA_2 values were obtained using a phentolamine concentration of 3×10^{-7} M. For the fetal vessels, a phentolamine concentration of 1×10^{-6} M was used.

With the information described previously in the methods section, the pA_2 values were calculated (Table 4). The unpaired students t-test revealed no significant difference ($p > 0.05$) between maternal and fetal pA_2 values.

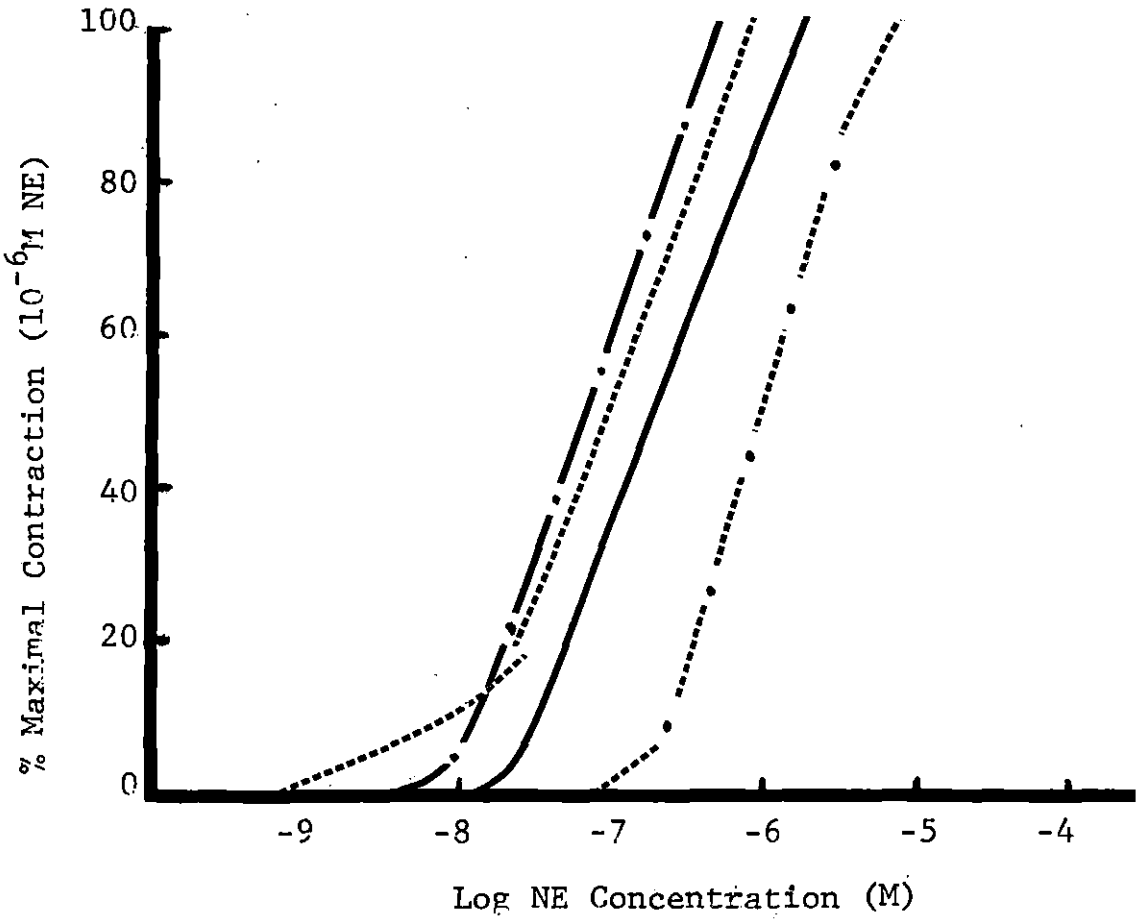
From a qualitative aspect, the following observations were made as a result of the *in vitro* experiments. The fetal vessels generally showed a high level of spontaneous activity during the equilibration period. Throughout the experiments, a difference was observed in the rate and



(——) fetal thoracic aorta

(----) maternal thoracic aorta

Figure 16. Dose response curves of thoracic aorta from one experiment in response to cumulative doses of norepinephrine (NE)



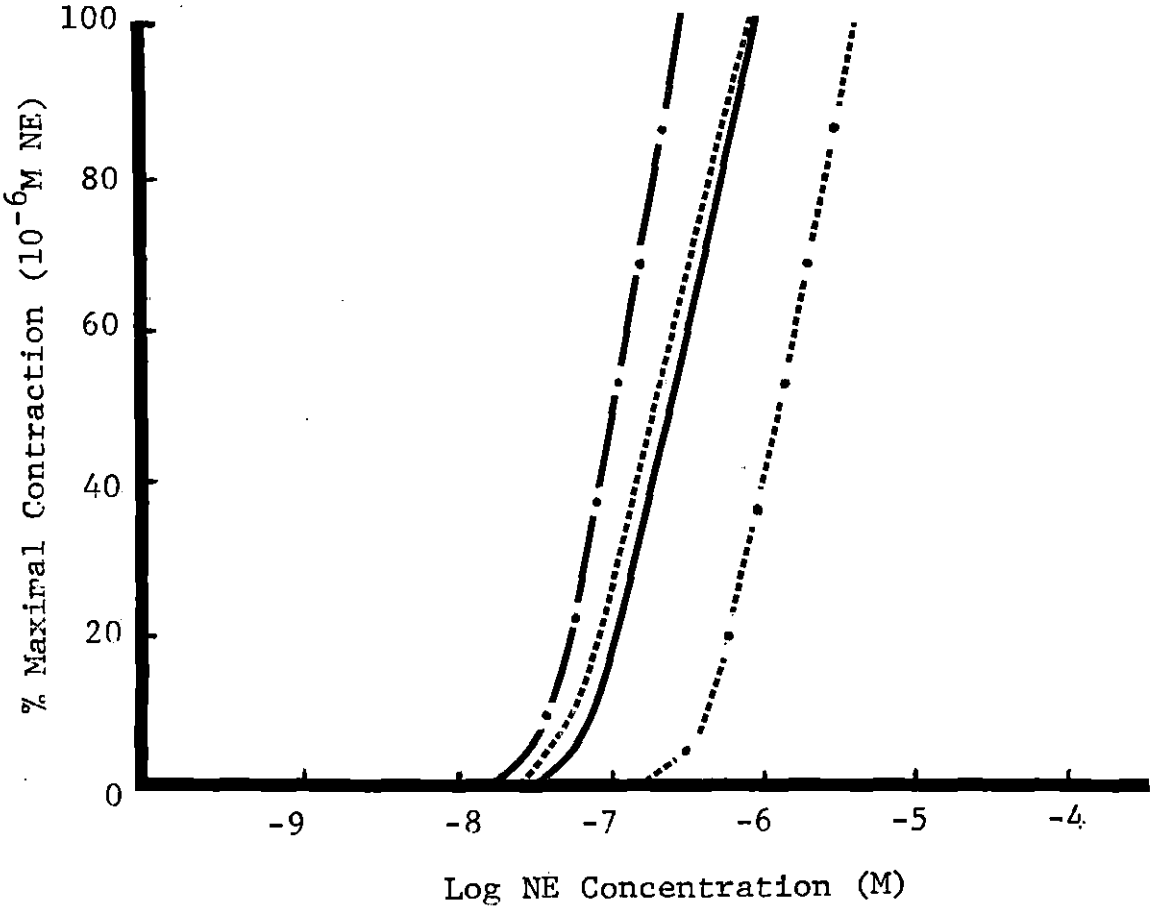
(——) tissue 1 (control)

(——·——·) tissue 1 (time control)

(----) tissue 2 (control)

(---·---·---·) tissue 2 (phentolamine)

Figure 17. Fetal dose response curves to norepinephrine (NE)



(——) tissue 1 (control)

(——·——·) tissue 1 (time control)

(----) tissue 2 (control)

(---·---·---·) tissue 2 (phentolamine)

Figure 18. Maternal dose response curves to norepinephrine (NE)

Table 4. Phentolamine pA_2 values on the thoracic aorta

Experiment	Fetal	Maternal
1	7.8	7.9
2	7.8	7.6
3	7.7	7.3
4	7.5	7.4
5	7.5	7.8
6	7.5	7.8
Mean $\pm$ S.E.	7.6 $\pm$ 0.06	7.6 $\pm$ 0.10

type of contractions elicited by norepinephrine in maternal and fetal vessels. The fetal vessel contracted more slowly than the maternal. In addition, the fetal vessels often contracted in a step-like manner in response to the lower norepinephrine concentrations as opposed to the smooth continuous contractions throughout this concentration range of the maternal vessels. This is shown in Figure 19 which is a tracing from an actual record.

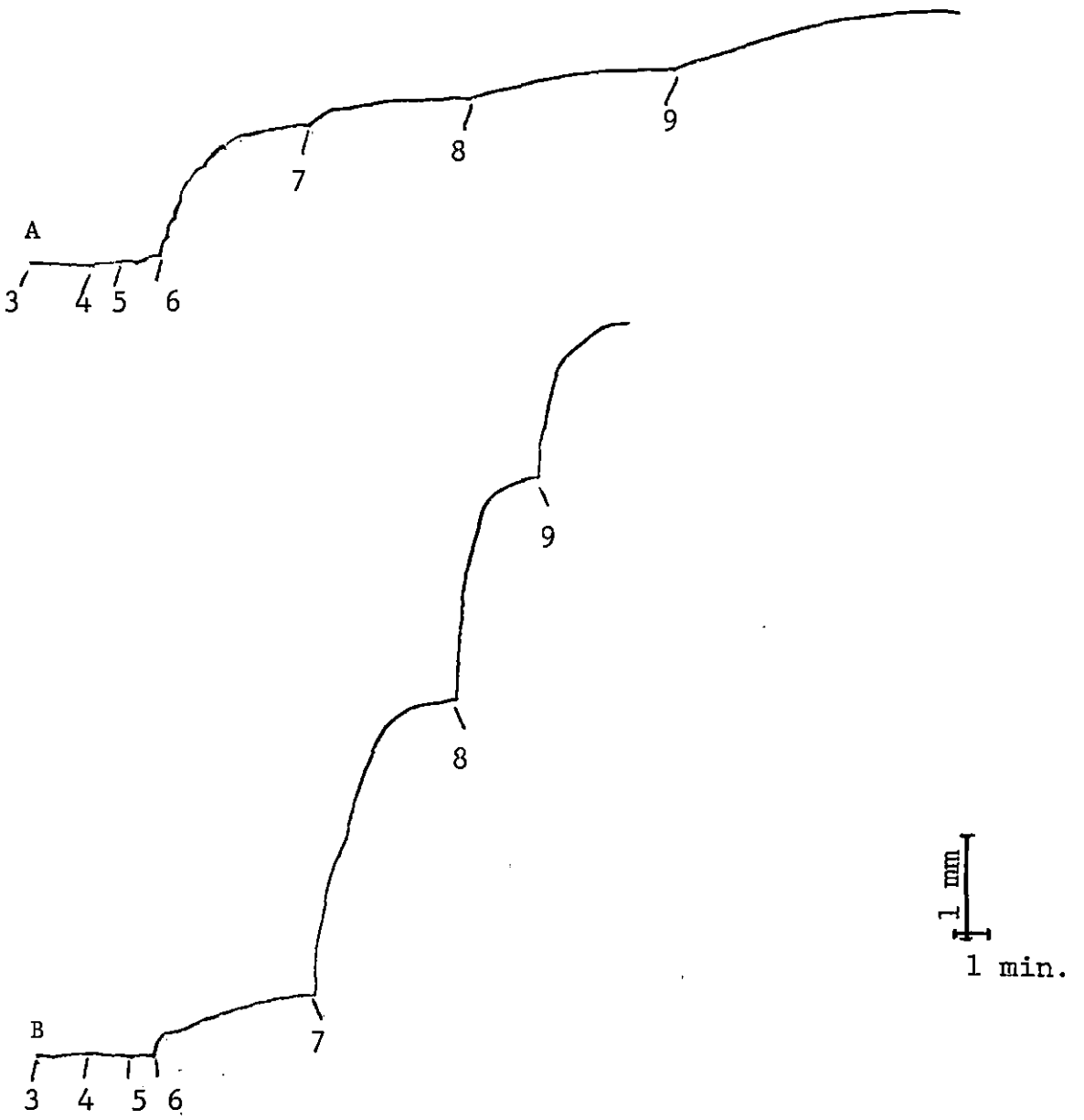


Figure 19. Recording of isotonic contractions of fetal (A) and maternal (B) thoracic aorta to norepinephrine. Code for final bath concentration of norepinephrine: 3 = 1 x 10^{-9} M, 4 = 3 x 10^{-9}, 5 = 1 x 10^{-8}, 6 = 3 x 10^{-8}, 7 = 1 x 10^{-7}, 8 = 3 x 10^{-7}, 9 = 1 x 10^{-6}

DISCUSSION

The proper choice of anesthetic is well-illustrated by the difference in basal cardiovascular parameters resulting from the use of pentobarbital when compared to the values for the same parameters under methoxyflurane anesthesia. The elevated values for basal heart rate and mean arterial blood pressure during pentobarbital when compared to methoxyflurane values are most likely the result of abolishment of central vagal inhibition (Assali et al., 1974). During pentobarbital anesthesia, the basal fetal heart rate and blood pressure were both 18 percent higher than the value obtained during methoxyflurane anesthesia. Likewise, the maternal basal heart rate was 15 percent higher, and basal blood pressure was 24 percent higher during pentobarbital anesthesia as compared to heart rate and blood pressure measured during methoxyflurane anesthesia. The fact that heart rate and blood pressure increases were comparable in the fetus while the maternal blood pressure increase was greater than the heart rate increase suggests that blood pressure control in the fetal guinea pig is different from control of blood pressure in the mother. The developmental lag in peripheral innervation as compared to cardiac innervation in the fetus may be one reason for the altered control of fetal blood pressure in comparison to the maternal blood pressure (Wyse et al., 1976; Harris and Van Petten, 1978). This lack of functional innervation suggests that neural control of blood pressure in the fetus is mediated primarily by changing the heart rate rather than peripheral vasomotor tone. In the mother, where peripheral neural circulatory control is present, the blood pressure may be dually regulated by a change in either

cardiac output and/or peripheral resistance.

The cited literature values for basal heart rate and blood pressure of 269 min^{-1} and 57 mm Hg for maternal and 189 min^{-1} for fetal were obtained during pentobarbital, ether, or local anesthesia using procaine (Marshall and Hanna, 1956; Biological Handbook, Respiration and Circulation, 1971). The data from my pentobarbital experiments agree well with the literature values.

The lower fetal guinea pig heart rate in comparison to the adult is contrary to the data from sheep experiments and values for humans (Joelsson et al., 1972; Assali et al., 1977; Klophenstein and Rudolph, 1978; Schifferli and Caldeyro-Barcia, 1973). It does, however, agree with other data collected on fetal guinea pigs which indicate a heart rate at mid gestation of 165 min^{-1}, a rate of 189 min^{-1} at 50 days gestation, and a rate of 289 min^{-1} in the newborn. Again, pentobarbital was used as the anesthetic in attaining these values cited in the literature (Biological Handbooks, Respiration and Circulation, 1971). Pentobarbital significantly increased ($p < 0.05$) the fetal heart rate in my experiments when compared to the values obtained under methoxyflurane anesthesia. One of the actions of pentobarbital is to attenuate cardiomotor vagal tone whereas methoxyflurane leaves the heart rate unaltered except during deep anesthesia (North et al., 1961). One might suggest that the elevated basal heart rate under pentobarbital when compared to methoxyflurane is a result of elimination, by pentobarbital, of a large amount of the inhibitory cardiac vagal tone normally present in the term guinea pig. Some may consider the lower basal fetal heart rate in comparison to that of the adult to be the result of fetal asphyxia. These experiments were performed with the

animals in dorsal recumbency which could occlude the caval veins, thereby decreasing blood flow to the uterus (Moir, 1976). Although the maternal blood gases and pH were normal, there may have been insufficient blood flow to the placenta resulting in fetal asphyxia. This asphyxia can lead to fetal myocardial depression which results in terminal bradycardia (Rosen, 1976). However, it is unlikely that fetal asphyxia was the cause of the decreased fetal heart rate in my experiments for the following reasons. 1) If caval occlusion was present, the decreased venous return would require an increased maternal heart rate to maintain cardiac output. No increased maternal heart rate occurred in my experiments when compared to the values found in the literature. 2) My preparations lasted for several hours without fetal heart rate or blood pressure showing any signs of deterioration. 3) During initial experiments, fetal electrocardiograms were recorded and no arrhythmias appeared.

The low basal fetal blood pressures in comparison to adult values are in agreement with data from other fetal experiments (Joelsson et al., 1972; Assali et al., 1977). There are several possible reasons for low fetal blood pressures. They are partially the result of low resistance shunts present in the fetal circulatory system and umbilical-placental circulation. In addition, the lack of functional sympathetic innervation to the peripheral vasculature (Wyse et al., 1976; Harris and Van Petten, 1978) thereby decreasing systemic resistance may offer another explanation. Increased quantities of circulatory vasodepressor agents in the fetus but not in the adult is another possibility and is substantiated by the finding that fetal vessels have a greater ability to synthesize the vasodilator prostacyclin (Terragno et al., 1978).

Keeping in mind that fetal cardiac output is approximately twice that of an adult (Rudolph and Heymann, 1974) and that cardiac output is proportional to arterial pressure/peripheral resistance, then whatever factors causing decreased fetal blood pressure must be working by drastically decreasing the resistance of the fetal vascular system.

Along with low fetal blood pressure, a decreased fetal responsiveness compared to maternal responsiveness was seen for all vasoactive biogenic amines tested. In both sets of experiments in which norepinephrine was administered, the decreased fetal responsiveness, i.e., mean arterial blood pressure changes and heart rate changes, agrees with results obtained in sheep experiments (Assali et al., 1977). As a result of these past experiments, it has been proposed that the magnitude of the elevated blood pressure in response to catecholamines was less in the fetus than the adult. The ED_{50} values remained the same throughout gestation (Nuwayhid et al., 1975). This latter statement should not be weighed too heavily, remembering that, in vivo, none of the parameters mentioned by Furchgott (1967) for receptor characterization can be controlled in the intact animal. Secondly in most fetal lamb experiments, the drug dosages are calculated solely by estimating the fetal weight from gestational age (Stephenson, 1958). This estimation reduces the accuracy of the results if one is evaluating the ED_{50} values.

Norepinephrine has both alpha- and $beta_1$-adrenergic receptor activity. Therefore, using solely this drug, it cannot be stated definitively whether the decreased fetal pressor response to norepinephrine seen in my experiments is the result of a decreased cardiac sensitivity alone or whether the vascular reactivity is also less in the fetus. The results from the

phenylephrine experiments serve to answer this question.

The results of the pure alpha-adrenergic agonist, phenylephrine, indicate that decreased fetal responsiveness to catecholamines is partially due to decreased peripheral vascular sensitivity. Fetal pulmonary vessel walls possess decreased amounts of smooth muscle mass in comparison to adult vessels, and hypertension stimulates smooth muscle cell growth (Rudolph and Heymann, 1978; Levin et al., 1978). Perhaps the peripheral vasculature possesses these same characteristics. If so, the lack of peripheral vasoconstriction could be due to a lack of smooth muscle cell mass. At birth, with the elimination of the low resistance shunts, an increased fetal blood pressure results. Perhaps this increased blood pressure stimulates vascular smooth muscle cell growth, thereby increasing the constrictive capabilities of the peripheral vasculature (Rudolph and Heymann, 1978; Levin et al., 1978).

A decreased smooth muscle mass is not the only tenable explanation of decreased peripheral vasoconstriction. Perhaps the decreased fetal arterial oxygen tensions render the vessels incapable of producing a large contractile force when excited. Another possible explanation for the decreased vasoconstriction would be the presence of a circulating vasodepressor in the fetus which would oppose any excitatory effects of the vasoconstrictors administered. A possible candidate for such a compound is prostacyclin, as mentioned earlier in this discussion (Terragno et al., 1978).

My results argue against the notion that decreased fetal responsiveness to catecholamines as seen in my <u>in vivo</u> experiments is the result of altered adrenergic receptor affinity. Both the ED_{50} values and pA_2 values

for the alpha-adrenergic receptor of the thoracic aorta were similar ($p > 0.05$) in the mother and fetus. The pA_2 value for phentolamine interacting with the alpha-adrenergic receptors of 7.6 arrived at from my data, is very similar to that cited in the literature. For the rabbit aorta, using phentolamine as the antagonist and phenylephrine as the agonist, a pA_2 value of 7.83 has been reported (Furchgott, 1970). The similarity of the affinity of the alpha-adrenergic receptor of maternal and fetal guinea pig aorta is supported by data from in vitro experiments conducted using fetal lamb vessels (Wyse et al., 1976).

The cause of the decreased fetal sensitivity to acetylcholine with regard to blood pressure and heart rate is unclear. The intestinal muscarinic receptors have been shown to have the same pD_2 values in the adult and fetal guinea pig (Boreus and McMurphy, 1971). This would lead one to speculate that the affinity of the muscarinic receptors of the cardiovascular system are the same for mother and fetus. A quantitative difference between maternal and fetal plasma cholinesterase activity has been demonstrated in sheep with the activity in the fetus being greater (Bell and Van Petten, 1976). If this holds true for the guinea pig, then a greater rate of degradation of acetylcholine by the fetus could explain the decreased sensitivity to acetylcholine. Another possibility is that the fetal vasculature is maximally dilated so that attempts to elicit further dilation are ineffective. The effect of acetylcholine on the heart is one of negative inotropy and negative chronotropy. The positive chronotropy seen in both the mother and fetus in my experiments was most likely not the direct effect of acetylcholine. Higher doses than those used in these experiments would be required to produce direct cardiac

effects which could be monitored. This positive chronotropy could result from a baroreceptor reflex which has been shown to be active in fetal sheep (Rudolph and Heymann, 1973). The functionality of baroreceptors would lend support to the hypothesis of a homeostatic vagal tone in the fetus which was presented earlier in the discussion of anesthetic effects. However, until further experiments are performed, this positive chronotropy seen during acetylcholine administration cannot be definitely attributed to the baroreflex. Other possible explanations could include a catecholamine release from small intensely fluorescent cells (Papka, 1975) or the adrenal medulla.

The heart rate changes seen in the phenylephrine experiments are unusual. There appeared to be either a bradycardia or no effect at all on maternal heart rate. However, in the fetus, either a slight negative chronotropic or a larger positive chronotropic response was observed. Since phenylephrine is classified as a relatively pure acting alpha-adrenergic agonist, no stimulatory cardiac effects are seen in the adult. Yet cardiac excitatory effects appeared in the fetus particularly at higher doses. There is evidence that alpha-adrenergic receptor activation in the guinea pig atria elicits a positive chronotropic response (Govier, 1968). In the fetal guinea pig, perhaps this alpha-adrenergic receptor stimulation plays a more important role than in the adult. It has been shown in fetal rats that $beta_1$-adrenergic receptor activation results in changes in adenyl cyclase activity without inotropic or chronotropic effects (Martin et al., 1973). Perhaps mechanisms beyond receptor binding are not well-developed in the fetus. If there is lack of

development in the receptor effector mechanism, perhaps alpha-adrenergic receptor activation in the fetus results in metabolic changes not occurring in the adult. For example, the positive chronotropic and inotropic responses seen during $beta_1$ stimulation appear to be mediated by increases in cyclic AMP levels (Kukovetz and Poch, 1972). If the receptor effector mechanism is not well-developed in the fetus, then perhaps alpha-adrenergic activation causes a rise in cyclic AMP. This might result in the positive chronotropy seen during fetal administration of phenylephrine. However, biochemical studies are necessary before any definitive statements can be made. Although phenylephrine is classified as a pure alpha-adrenergic agonist, high concentrations of the drug can induce nonspecific beta receptor activation (Furchgott, 1972) thus leading to excitation of the fetal heart. Perhaps this nonspecific receptor activation also occurs in the adult. However, it may be physiologically antagonized by a greater baroreceptor activity in the adult, thereby preventing the manifestation of any cardiac effects.

It is apparent that a high degree of variability exists especially with respect to heart rate responses. So much so that, in some sets of experiments, no common slope for the regression on heart rate changes either within maternal group or within fetal groups could be found ($p < 0.05$). The duration of injection was kept as constant as possible. However, this time factor could introduce variability. With the anatomical shunts present in the heart and major vessels of the fetus, a high percentage of blood returning via the superior vena cava passes through the right side of the heart and into the pulmonary artery. A high proportion of blood returning via the inferior vena cava enters

the right atria and flows through the foramen ovale and out the left ventricle (Dawes, 1968; Joelsson et al., 1972). Therefore, positioning of the jugular cannula and force of injection could alter the amount of drug that the heart is exposed to at a given dose. The lack of common slopes for either maternal or fetal heart rate changes may also result from a difference in the level of baroreceptor activity which is attempting to return the heart rate and blood pressure to normal. This baroreceptor activity as with many reflexes can be depressed by the use of anesthetics. Throughout each experiment, the anesthetic concentration had to be adjusted in an attempt to keep the animal properly anesthetized. For some experiments, perhaps the baroreceptor reflex was depressed to different extents. This would give rise to different degrees of vagal inhibition to the heart for a given increase in blood pressure.

Finally, with respect to the difference in magnitude of heart rate response within groups, is the fact that mouse atria, human ventricular tissue, and fetal sheep show an increasing magnitude of response to norepinephrine and acetylcholine as gestation progresses (Wildenthal, 1973; Nuwayhid et al., 1975; Coltart and Spilker, 1972). Rates of fetal maturation do vary, and a difference of a few days in gestational age may cause a significant difference in the guinea pig from a developmental standpoint. Although the guinea pigs were used at 60 $\pm$ 2 days gestational age, perhaps there was a difference in the level of fetal development among animals thus altering the degree of responsiveness to drugs.

The fact that for some drugs common slopes were not present for heart rate and blood pressure changes within either a fetal or maternal group did not invalidate the fact that the slopes describing the overall

responses were different between mothers and fetuses. The lack of a common slope within groups was generally due to the unusual responses of one or two animals in the group. When tests of significance were done for each separate experiment, a significant difference between mother and fetus almost always appeared, except for the heart rate changes in response to norepinephrine under pentobarbital anesthesia. When the data from all of the animals for a specific drug were pooled, the difference between mother and fetuses generally became more significant.

With the rise in fetal blood pressure resulting from phenylephrine administration, very little or no reflex fetal bradycardia occurred. Comparing this to the acetylcholine effects, it seems to indicate that the fetal baroreceptors are responsive to hypotension but not hypertension. The literature states that the baroreceptors in fetal lambs are functional at 0.55 gestational age (Rudolph and Heymann, 1973). These fetal lamb experiments were conducted on unanesthetized lambs in utero, which could explain the discrepancy between their findings and mine. Another possible cause of this discrepancy is that of interspecies differences.

The fact that the administration of atropine to the preparation resulted in an increase in basal heart rate of the fetus and not the mother may possibly be explained in the following manner. Consider the fact that pentobarbital anesthesia decreases vagal tone (Assali et al., 1974) as mentioned previously in this discussion. Also bear in mind the fact that the fetal plasma concentration of pentobarbital is less than that of the mother (Boulos et al., 1971). In this situation, the fetal vagal cardiac reflexes would not be depressed as much as the maternal reflexes. This would allow for atropine to further depress the fetal

cardiac vagal tone. The atropine effect on the maternal vagal tone would not be as evident because the tone had already been eliminated to a large extent by the pentobarbital. There are two other factors one must consider when interpreting the atropine data. 1) The atropine was first administered to the mother on a per weight basis; this included litter weight. Then the atropine was administered directly to the fetus on a per weight basis. It is known that atropine crosses the placenta (Hellman and Fellisti, 1967). So that in actuality the fetus was getting in excess of the calculated 1 mg/kg dose. 2) Some rodents are known to possess the enzyme atropinesterase which degrades belladonna alkaloids (Goodman and Gilman, 1975). Although no evidence regarding which species possess this enzyme could be located in the literature, perhaps the guinea pig possesses the enzyme. In the rabbit, atropinesterase is not present until postpartum (Sawin and Glick, 1943). Therefore if atropinesterase is only present in the adult guinea pig, atropine would have a longer half-life in the fetus than in the adult. While bearing in mind the effects of pentobarbital on vagal tone, the relative concentrations of pentobarbital and atropine in the mother and fetus, and the possible presence of atropinesterase; one can see how in these experiments atropine could have affected fetal cardiovascular parameters without altering those of the adult. As to why basal heart rate but not basal blood pressure in the fetus was affected by atropine's presence, perhaps the compliance of the fetal vascular beds masked any changes in peripheral sympathetic tone.

The presence of atropine in the preparation would be expected to enhance the blood pressure and heart rate changes produced by norepinephrine . This enhancement normally results from the elimination of the

baroreceptor reflex. However, no enhancement was seen in my experiments either under pentobarbital or methoxyflurane anesthesia. This lack of atropine enhancement may be the result of three factors. It is well-known that, in order to block neural effects with an antagonist, higher concentrations than those necessary to block an exogenous compounds actions are required. Although the effects of the test dose of acetylcholine were blocked by 1 mg/kg of atropine, perhaps this was not a high enough concentration to prevent intrinsic inhibitory vagal influence on the heart. Secondly, one must consider that a significant amount of time elapsed between atropine administration and the norepinephrine injections. Therefore if atropinesterase is present in the guinea pig, a significant amount of atropine degradation would have occurred, thus making the atropine less effective in eliminating vagal influence on the heart. Finally, one cannot discount the possibility of anesthetic depression of cardiovascular reflexes before the administration of atropine. This is an especially strong possibility in the experiments in which pentobarbital was used.

The fact that the placental weight was not taken into consideration when calculating the drug doses may at first appear to be a possible explanation for the decreased fetal sensitivity to the biogenic amines. The placental mass is approximately 9-10 percent of the fetal mass (Eckstein et al., 1955). A 10 percent change in doses would not be expected to alter the dose response curve enough to account for the large difference in the maternal and fetal responsiveness to the drugs tested.

Although no quantitative analysis was performed on the duration of action of the various drugs tested <u>in vivo</u>, there did not appear to be

any distinctive difference between the mother and the fetus in this aspect.

The fetal cardiovascular changes seen during the administration of the vasoconstrictor drugs to the mother should not be overlooked. The possibility of the drugs having crossed the placenta and affecting the fetus is excluded by the facts: 1) when comparable doses were given directly to the fetus, different fetal responses were obtained than when the drug was given to the mother; and 2) studies using H^3-norepinephrine administered to the mother have shown that very little crosses the placenta (Mirkin and Singh, 1972). The most probable explanation for the fetal response to maternal norepinephrine administration is that it is the result of fetal asphyxia. This asphyxia could come about in two ways. The first of which was noticed in experiments using the guinea pig (Dornhorst and Young, 1952). High doses of norepinephrine given to the mother resulted in fetal bradycardia and hypotension. These fetal changes seemed to be correlated with the placenta becoming cyanotic. This cyanosis presumably resulted from the severe peripheral vasoconstriction by norepinephrine, thus decreasing maternal blood flow to the placenta. The second possible way to bring about fetal asphyxia depends on whether sluice flow exists in the guinea pig placenta (Power and Longo, 1973). If sluice flow exists and the blood pressure in the maternal placental sinuses is elevated to exceed that of the fetal villi, the villi will collapse from the excessive surrounding external pressure, preventing proper perfusion of the fetal side of the placenta. This villi collapse would have a twofold effect on the fetus: 1) asphyxia and 2) hypertension, in excess of the pressure in the maternal sinuses, as a result of

damming up of the blood on the fetal side of the placenta.

The fetal responses to asphyxia are variable and depend on the method in which asphyxia is induced (Chernoff and Grabowski, 1971; Bauer, 1938; Martin and Young, 1960; Gaxiano and Freeman, 1977; James, 1976). The fetus may respond with tachycardia and hypertension, as appeared with the maternal administration of the lower dose of phenylephrine. However, if severe asphyxia occurs, depression of the fetal cardiovascular system results and hypotension with bradycardia occurs (Adamsons et al., 1971).

The transient plateau in the rise of fetal blood pressure in response to norepinephrine administration was too consistent to be an injection artifact. The initial rise may be the positive inotropic and chronotropic effect of norepinephrine on the fetal heart and vasoconstriction of the systemic vasculature. The second rise would be the result of norepinephrine's effect on the placental vasculature, causing vasoconstriction (Panigel, 1962; Euler, 1938).

The fetal cardiac arrhythmias and arrests which occurred during isoproterenol administration may indicate that additional caution should be taken during the use of gaseous anesthetics in obstetrics. It is known that methoxyflurane sensitizes the adult myocardium to catecholamines (Goodman and Gilman, 1975). In my experiments, this sensitization seemed to be greater for the fetus than the mother. This is not totally unexpected if one considers the differences in conductive systems and metabolism demonstrated between the fetal and adult myocardium (Shepard et al., 1969; Su and Friedman, 1973; Coltart et al., 1971). Although fetal beta$_1$ cardiac receptors are present and active, the fetal myocardium metabolically functions differently than the adult myocardium. This aspect of

increased frequency of cardiac arrhythmia and arrest in the fetus, in response to isoproterenol administration during methoxyflurane anesthesia, requires further experimentation before definitive statements can be made.

The differences in fetal myocardial metabolism in combination with the high level of metabolic activity of cardiac tissue may partially explain the deterioration of the fetal atria during my in vitro experiments. And because of this, I was not able to characterize the cardiac beta-adrenergic receptors. From the work of others (Wyse et al., 1976; Martin et al., 1973; Park et al., 1976) and the characterization of the alpha-adrenergic receptors of the guinea pig aorta in my experiments, I feel confident in stating that future work will probably indicate a similarity between the fetal and maternal $beta_1$-adrenergic receptor affinity.

The fact that isoproterenol caused an increase in blood pressure in some adults and a transient decrease in others was presented to illustrate the inconsistency of the response to this drug. No explanation for these varied responses will be attempted.

The in vitro experiments using the thoracic aorta showed that the alpha-adrenergic receptors of the mother and fetus have the same affinity in the guinea pig, as judged by the pA_2 and ED_{50} values. Therefore, the difference in sensitivity to vasopressors seen between mother and fetus is not likely based on a difference of the affinity of the alpha-adrenergic receptor to alpha agonists. However, differences between maternal and fetal isometric contractions in response to norepinephrine did occur in vitro, indicating that there is a difference at the cellular level. The

fetal vessels contracted in steps similar to the contractions described in in vitro studies of the ductus arterious (Fay, 1971). However, for my experiments, segments were taken from the distal portion of the fetal thoracic aorta to avoid the complications presented by the special characteristics of the ductus arterious smooth muscle (Cassels, 1973). Perhaps the smooth muscle of the fetal vasculature is phasic as opposed to the tonic type which is found in the aorta of adult animals (Wolf and Werthessen, 1975). Histological studies in addition to electrical recordings during contraction would be required to answer this question. The slowness of fetal contractions could be the result of decreased intracellular stores of Ca^{++}. Therefore, contraction would be dependent on the influx of extracellular Ca^{++}. In smooth muscle, it has been suggested that mitochondria may act as a sequestering organelle for Ca^{++} ions (Wolf and Wethessen, 1975). If this is the case and if fetal vessels depend primarily on anaerobic glycolysis for energy as does the fetal heart (Su and Friedman, 1973; Shepart et al., 1969), the number of mitochondria may be decreased compared to that of an adult. If so, the intracellular Ca^{++} pool would be less in the fetus than the adult. This may give rise to a slower, weaker contraction of the fetal vessels in comparison to the adult vessels. The large amount of spontaneity in the fetal vessels in vitro may have been the result of high oxygen tensions, which are unusual for the fetal vessels. Increased oxygen tension is known to cause contraction of the smooth muscle of the ductus arterious (Fay, 1971). Perhaps this oxygen sensitivity also applies to the smooth muscle of the fetal thoracic aorta.

The alpha-adrenergic receptors of the maternal and fetal guinea pig are similar. However, many events must occur following receptor activation before muscle contraction takes place (Wolf and Werthessen, 1975). The possibility that differences between mother and fetus exist in the chain of events leading to muscle contraction appears strong.

Studies such as the ones performed in this project have established a difference between maternal and fetal sensitivity to vasoactive compounds. However, the cause of this difference still remains to be elucidated. To answer this question of sensitivity difference, many more studies, both in vivo and in vitro, with a strong biochemical basis must be performed. Possible in vitro experiments that could be conducted in the future to help answer some of the questions formed as a result of this study would be: isolated tissue experiments carried out at various oxygen tensions to examine the effects of vascular reactivity; the effect of inhibition of prostaglandin synthesis on vascular reactivity; electrophysiological studies; and Ca^{++} fluxes in fetal smooth muscle using compounds such as verapamil, which selectively block spike discharges and phasic contractions of smooth muscle (Wolf and Werthessen, 1975). Histological studies of the fetal vascular smooth muscle would also prove useful.

Further in vivo studies could be conducted making use of both specific agonists and antagonists. To determine the presence of baroreceptor activity in the fetus, vagotomies could be performed. The use of microspheres or possibly even flow probes for the measurement of blood flow alterations as a result of drug administration in the guinea pig would

also prove useful.

This project has shown that, with the proper amount of dexterity and basic surgical equipment, fetal guinea pigs can be used to conduct acute cardiovascular experiments. The researcher is somewhat limited in the type of experiments that can be performed because of the size of the animal. However, the preparation has some definite advantages, regarding acute preparations, in comparison to sheep. The guinea pig is an inexpensive and readily available laboratory animal, so that large numbers of experiments can be performed in a short period of time, in comparison to sheep experiments. Because of the similarity of the guinea pig placenta to that of humans, it would serve as a very good model for fetal pharmacokinetic studies.

Acute preparations have certain disadvantages when studying the cardiovascular function, but they are still valuable for the initial testing of hypotheses without the expenditure of significant sums of money. In addition, the large n values that one can attain with this preparation should prove valuable. Dornhorst and Young (1952) and Rosen (1976) appear to be the only investigators to have made use of the guinea pig as a model for fetal physiological and pharmacological experiments. With this project, I have shown that more quantitative studies than those done in the past are possible, and the techniques developed here have the potential to help elucidate the function of the fetal cardiovascular system.

SUMMARY

In this project, the techniques were developed for the acute study of pharmacodynamic drugs in the near term fetal guinea pig. The technique was then used to study the effects on maternal and fetal heart rate and blood pressure of the following biogenic amines: norepinephrine, acetylcholine, phenylephrine, and isoproterenol. In addition to these drugs, the effects of atropine on cardiovascular responses to norepinephrine were examined.

Upon completion of the *in vivo* studies, isolated tissue studies were conducted in order to characterize the cardiovascular adrenergic receptors of the maternal and fetal guinea pig.

The combination of these studies lead to the formulation of the following conclusions:

1) Use of the guinea pig as a model for conducting acute *in vivo* fetal cardiovascular experiments is possible.
2) A difference in magnitude of response to biogenic amines with respect to blood pressure and heart rate changes exists between mother and fetus.
3) The affinity of the alpha-adrenergic receptors for phentolamine in maternal and fetal blood vessels is similar.

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APPENDIX A

Composition of modified Krebs-Henseliet solution:

	grams/liter
NaCl	6.74
KCl	0.35
$CaCl_2 \cdot 2H_2O$	0.20
$MgSO_4 \cdot 7H_2O$	0.14
KH_2PO_4	0.16
Na (EDTA)	0.01
$NaHCO_3$	1.86
Glucose (dextrose)	1.42

APPENDIX B[1]

An examination of the data clearly indicated that the relationship between dosage and percentage change of blood pressure and heart rate is nonlinear. Since a linear relationship was desired, a transformation was needed. Lacking a theoretical reason to prefer one particular transformation, four different power transformations were tried: $\log_{10}(\text{dose})$, dose, $\sqrt[4]{\text{dose}}$, and $\sqrt[10]{\text{dose}}$. Although it was previously thought that $\log_{10}(\text{dose})$ would give a linear relationship, it proved to be inadequate for most of the drugs. For instance, with phenylephrine the square root transformation reduced the residual sum of squares by 43% from the fit obtained by $\log_{10}(\text{dose})$ for the fetal percent change in blood pressure. For norepinephrine under pentobarbital anesthesia, norepinephrine under methoxyflurane anesthesia, and phenylephrine under methoxyflurane anesthesia, the same transformation was used for both percentage change in blood pressure and percentage change in heart rate. For acetylcholine under methoxyflurane anesthesia, $\log_{10}(\text{dose})$ worked very well for percentage change blood pressure and square root dose worked very well for percentage change in heart rate. Phenylephrine was particularly interesting for percentage change in blood pressure. For the fetuses, square root dose was much better than $\log_{10}(\text{dose})$ (20-43% reduction in residual sum of squares). But for the mothers, any of the transformations were much better than square root dose (30-35% reduction in the residual sum of squares). In order to show that indeed mother and fetus react

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[1]Michael D. Hale, Statistics Department, Iowa State University, Ames.

differently, a square root transformation was applied to both.[1]

Each drug was analyzed separately. After standardizing to a mean of zero, a regresssion line was fit to each mother, fetus, and mother-fetus pair. The residual sum of squares for mother:, RSSM:, and fetus:, RSSF:, were then subtracted from the residual sum of squares for mother-fetus pair:, RSSP:.

Then the F statistic was:

$$\frac{\sum_{i=1}^{n} (\text{RSSP:} - \text{RSSM:} + \text{RSSF:})/n}{\sum_{i=1}^{n} (\text{RSSM:} + \text{RSSF:})/(T - 4)}$$

with n and T-2n degrees of freedom. Where n is the number of pairs and T is the total number of observations for both fetuses and mothers. The statistic was used to test whether the rate of response was the same for mother and fetus. Similarly, a regression line was fit for each fetus and one common regression line fit to all fetuses. Then,

$$F = \frac{(\text{RSS common} - \sum_{i=1}^{n} \text{RSSF:})/(n - 1)}{\sum_{i=1}^{n} \text{RSSF:}/T_{\text{fetus}} - 2n}$$

was formed to test for a common rate of response for fetuses, likewise for mothers.

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[1] To prove differences statistically, one assumes a common model. If data show the model to be untenable, then one may assert that there is a difference at the level of probability at which the model was found unacceptable.

To obtain confidence bands and predicted response, a common regression line was fit to the understandardized data for the fetuses and for the mothers.