The effects of elevations in ambient CO concentration on the near-term sow and prenatal piglet

by

Mark Anthony Dominick

A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Department: Veterinary Pathology
Major: Veterinary Pathology
(Veterinary Toxicology)

Iowa State University
Ames, Iowa

1982

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The trend toward an increase in livestock confinement operations in the United States has introduced potential health problems associated with animal exposure to toxic gases. One potential problem has been exposure to high atmospheric carbon monoxide (CO) concentrations in swine farrowing houses. The rise in CO levels within the farrowing facility has been attributed primarily to incomplete fuel combustion in gas heaters coupled with inadequate ventilation during winter months. Ventilation in farrowing facilities often has been reduced during winter months to conserve energy and thereby reduce operating costs.

The adverse animal health effects from elevations in ambient CO have been particularly evident in the swine confinement industry. Several field case reports revealed an increased incidence of stillborn full-term piglets where elevations in atmospheric CO were recorded (Carson and Donham, 1978; Carson et al., 1980; Keller, 1976; Stadler, 1973; Wood, 1978, 1979). The stillbirths occurred after the placement of near-term sows into conventional farrowing houses where supplemental heat was provided. Upon further investigation, it was found that malfunctioning of gas fired heaters and inadequate ventilation were responsible for the elevated atmospheric levels of CO. The CO levels recorded in the farrowing houses varied from 120-170 parts per million (ppm) in these field cases. Complete post-mortem examination of stillborn piglets failed to establish an infectious cause for the increased incidence of stillbirths. Improvement of ventilation and cleaning of the gas burners were reported to alleviate the problem.

The diagnosis of CO induced stillbirths in confined domestic swine has been based upon documentation of elevations in atmospheric CO and maternal carboxyhemoglobin (COHb), and the elimination of infectious, nutritional, metabolic or other toxic causes for stillbirth. Little to no experimental work has documented an increased incidence of stillbirths in sows exposed to low concentrations of atmospheric CO. In addition, examination of the stillborn piglet may provide further evidence of CO intoxication.

Reliable methods to determine blood COHb concentrations are essential for a laboratory confirmation of field cases of CO-intoxication in pigs. The mode, temperature and duration of blood storage prior to analysis for blood COHb content are all important considerations in establishing the reliability of the blood COHb values determined in the laboratory. Therefore, the effects of time and temperature on the stability of blood COHb concentrations need to be examined.

The primary objectives of this investigation were as follows:

1) To determine the effects of elevated ambient CO concentrations on the near term sow and the prenatal piglet.
2) To examine stillborn piglets for chemical and morphological evidence of CO intoxication.
3) To evaluate the effects of temperature and time on the stability of blood COHb concentrations.

LITERATURE REVIEW

Physical and Chemical Properties of Carbon Monoxide

With the exception of carbon dioxide, CO is the most abundant air pollutant present in the lower atmosphere. CO is an imperceptible gas most commonly derived from the incomplete combustion of carbonaceous materials burned in a limited supply of oxygen. CO is a diatomic molecule with a molecular weight of 28.01 grams/mole. Its specific gravity is .967 relative to air.

Under normal conditions, CO is quite stable, chemically inert and highly diffusible. CO is similar to oxygen in size, molecular weight and diffusing capacity. It acts chiefly as a ligand, binding selectively to metals to form metal carbonyls (Antonini, 1967; Coughey, 1970).

In biological systems, CO binding to hemoglobin (Hb) is dependent upon the availability of ferrous iron or upon its ability to displace oxygen as a ligand. CO is known to bind reversibly with the ferrous iron complex of protoporphyrin IX in Hb resulting in the formation of COHb (Antonini, 1967). The solubility of CO in body fluids is low even under conditions of elevated arterial COHb concentration.

Uptake of Carbon Monoxide

Once CO is inhaled, it readily diffuses from the alveoli into the pulmonary capillaries where it binds rapidly to Hb (Root, 1965). In man, the uptake of CO and subsequent rise of pulmonary arterial COHb is initially linear; however, COHb rises more slowly as the partial pressure of CO in the pulmonary arterial blood approaches that of the

alveolar air (Lilienthal, 1950). When a mammal breathes air containing a known concentration of CO for several hours, a state of equilibrium is reached with respect to CO in which the partial pressure of CO (P_{CO}) in the blood of the pulmonary capillaries equals that in the alveolar and ambient air (Bartlett, 1968). As long as the animal continues to breathe air with the same P_{CO}, no net uptake or excretion of CO occurs.

The CO diffusing capacity of the lungs will increase with increasing work load until maximal oxygen consumption is reached (Johnson et al., 1965; Newman et al., 1962). Johnson and his associates (1965) also reported that pulmonary CO diffusing capacity will not increase further as exercise work load increases to a point beyond that causing maximal oxygen consumption.

The concentration of blood COHb is markedly dependent on ventilation rate, the percentage of CO in the inspired air and the exposure time to CO (Forbes et al., 1945; Root, 1965). Therefore, the same blood COHb content could be attained by two animals exposed to different atmospheric levels of CO provided the workload and ventilation rate were different between the two. It is recognized that the rate at which inhaled CO reacts with oxygenated blood may differ from the pulmonary diffusion capacity for CO because of changes in body temperature, differences in animal species or the presence of iron-deficiency anemias (Burrow and Niden, 1963; Otis and Jade, 1957; Power et al., 1969).

Coburn and his coworkers (1965) determined a method to predict blood COHb levels resulting from CO exposures in man. They found that with 50, 100 and 500 parts per million (ppm) CO in the atmosphere, the

resultant COHb values were 7, 14 and 44% saturation, respectively. A CO inhaled concentration of 10,000 ppm (1%) should produce a 94% saturation of Hb as COHb in man.

Reaction of Carbon Monoxide with Hemoproteins

The combination of CO with available Hb depends on the partial pressures of CO and O_2 since the two gases compete for the same reactive sites on the Hb molecule (Douglas et al., 1912). This relationship is described in the Haldane equation:

$$\frac{[COHb]}{[O_2Hb]} = M \times \frac{P_{CO}}{P_{O_2}}$$

In this equation, [COHb] and [O_2Hb] represent the concentrations of COHb and oxyhemoglobin (O_2Hb) in saturation percent. M is relative affinity constant and P_{CO} and P_{O_2} represent the partial pressures of CO and oxygen in the blood. M has been found to vary from 200-300 in man (Killick, 1940; Roughton and Darling, 1944). This compares to a value of 150 for the relative affinity constant in miniature pigs (Klimisch et al., 1975). The high values of M reflects a relatively stronger affinity of CO for Hb.

Haldane (1912-1913) found that CO binding to Hb results in a leftward shift of the O_2Hb dissociation curve and a change in its shape from sigmoid to more hyperbolic. This results in a displacement of O_2 from Hb in the arterial blood and interferes with O_2 release to the tissues. The interaction of CO with Hb alters the affinity of Hb for oxygen on its remaining binding sites. The O_2 once bound is given

up to the tissues less readily than normally. A recognized consequence of this effect is that a given degree of carboxyhemoglobinemia produces a more severe tissue hypoxia than a comparable degree of simple anemia (Killick, 1940).

On the basis of more recent data, it appears that CO binds to compounds other than Hb. It is known that CO binds *in vitro* to the hemoproteins myoglobin and cytochrome oxidase (Coburn, 1970; Coburn, 1979; Coburn and Mayers, 1971). Coburn suggests that CO binding to cytochrome oxidase is unlikely to be an *in vivo* mechanism of CO toxicity. Utilizing a rabbit aorta organ bath with a constant P_{O_2}, he demonstrated that CO at a tension 1000 times greater than seen *in vivo* had only a small effect on oxygen uptake by cytochrome oxidase.

Whether CO binding to myoglobin represents a mechanism of CO toxicity depends on the function of myoglobin and how critical myoglobin is to cell oxygenation (Coburn and Mayers, 1971). Wittenberg (1966) suggested that myoglobin may facilitate O_2 diffusion within the cytoplasm or that myoglobin may provide stores of accessible O_2 in the proximity of mitochondria.

In cases of CO intoxication in man, myoglobin accounts for about 20% of the body's CO capacity (Luomanmäki, 1966). The relative affinity constant (M) from the Haldane equation has a numerical value of 40 suggesting that the relative affinity of CO over O_2 for myoglobin is less than that for Hb (Rossi-Fanelli and Antonini, 1958). The sensitivity of the myocardium to CO is well-documented although the exact mechanism of toxicity has not been determined (Ehrich et al.,

1944; Penny et al., 1980). Whether binding of myoglobin by CO is a mechanism of cardiac toxicity is as yet undetermined.

Carbon Monoxide Elimination

CO is eliminated primarily via the lungs with a small percentage oxidized to CO_2 in man (Root, 1965). Elimination is most rapid when the P_{CO} gradient between blood and alveolar air is maximal and begins to slow as the P_{CO} of alveolar air approaches the P_{CO} of the pulmonary capillary.

The rate of CO elimination from the body is complex and appears to depend on the concentration of CO exposure, the length of the period of exposure and whether the exposure was continuous or discontinuous (Coburn et al., 1965; Peterson and Stewart, 1970). Several studies revealed a calculated CO half-life in blood ranging from 1.5 to 5 hours in man (Forbes et al., 1945; Pace et al., 1950; Peterson and Stewart, 1970; Roughton and Root, 1945).

Following brief CO inhalation, the kinetics of early COHb decline may reflect redistribution of CO from the blood compartment to myoglobin, cytochrome enzymes and to the splenic pool (Wagner et al., 1975). Wagner and his associates (1975) found that administration of 1-6% CO to dogs for 3 minutes resulted in an elimination curve that was biphasic with the initial curve exponential followed by an elimination curve that was linear. They determined a CO half-life of 190 minutes in dogs with COHb levels between 5-16% and a CO half-life of 134 minutes when COHb levels were 20-43%.

Donham et al. (ca. 1982) exposed mini-pigs to various atmospheric levels of CO for eight hours and plotted uptake and elimination curves by recording COHb every 30 minutes subsequent to exposure. They found that CO elimination was relatively rapid in the pig with one-half of peak levels of COHb remaining approximately 1.5 hours post exposure.

Transplacental Diffusion of Carbon Monoxide

Placental diffusing capacity of a gas is defined as the volume of gas (milliliters) diffusing across the placental membrane per unit of time (minutes) per unit of partial pressure difference between maternal and fetal blood (Torr) (Bartels and Moll, 1962). Placental diffusing capacity thus represents a measure of gas exchange between maternal and fetal circulation. However, transplacental movement of CO is dependent not only on diffusion across the placental tissues, but diffusion across the erythrocyte membranes and CO reaction rates with Hb (Longo et al., 1967). Classically, passive diffusion of CO between maternal and fetal blood has been considered the method of placental CO exchange. However, several investigators have suggested carrier facilitated transport as a method of CO movement across placental membranes (Bissonnette et al., 1977; Burns and Gurtner, 1973). Burns and Gurtner (1973) have suggested that cytochrome P_{450} facilitates placental O_2 and CO diffusion. Longo and Ching (1977) compared placental diffusion in pregnant ewes before and after administration of drugs known to bind cytochrome P_{450}. Each drug was administered in doses several times the amount considered pharmacologically active. They

found binding of cytochrome P_{450} had little effect on placental diffusion of CO.

Several factors affect the movement of CO across the placenta. These factors include the duration of gestation, fetal weight, placental weight, maternal COHb concentration, O_2 tensions in maternal and fetal blood and fetal hemoglobin concentrations. Bissonnette and Wickham (1977) reported that in guinea pigs, a significant increase in placental diffusing capacity for CO occurs with advancing gestational age. This increase appeared to be independent of placental weight but instead associated with increased fetal weight. Longo and Ching (1977) found that the diffusing capacity of the placenta in sheep also increased during the course of gestation but that the value was relatively constant when adjusted for fetal weight.

The rate limiting step influencing the movement of CO across the placenta may be the chemical reaction rate of CO with maternal and fetal Hb. Kruhoffer (1954) demonstrated that while air is breathed by man approximately one-half of the total resistance to CO exchange is due to the chemical reaction rates of CO with intracellular Hb. Since CO must pass through two vascular beds in the placenta, the major resistance to CO diffusion may not be placental membranes but instead the chemical reaction rates of CO with Hb.

General Physiologic Effects of Carbon Monoxide on the Fetus

The diffusion of CO into the fetal circulation leads to an increase in fetal COHb levels. The level of fetal COHb attained will depend upon

the maternal COHb level, the rate of endogenous production of CO in the fetus, and the relative affinity of fetal Hb for CO compared to O_2 (Longo, 1970). Fetal Hb has an intrinsically higher affinity for O_2 than maternal Hb does in most mammals (Bartels and Bauer, 1969). It seems plausible then that the same relationship may exist with CO.

There are several possible mechanisms to explain the effects of CO on the fetus. Forster (1970) believed the prime factor responsible for the toxic effects of CO exposure to pregnant mammals is likely the hypoxia associated with elevated COHb. By competing with O_2 for Hb, CO decreases the capacity of blood to carry O_2 while increasing the affinity of Hb for bound O_2. This in turn shifts the O_2 dissociation curve to the left which means that the O_2 tension of the blood must decrease to lower than normal values before a given amount of O_2 will be released from Hb (Longo, 1970). This response to CO occurs in maternal and fetal blood; however, the consequences may be more drastic for the fetus since the P_{O_2} in blood supplying fetal tissues is normally relatively low (Longo, 1976). The shift to the left of the maternal O_2 dissociation curve will tend to decrease the normal partial pressure gradient for O_2 from maternal to fetal blood across the placenta. This will also have the effect of lowering fetal P_{O_2} (Longo, 1976; Longo and Hill, 1977).

Longo and Hill (1977) documented changes in maternal and fetal sheep COHb concentrations during CO uptake and elimination after ewes were exposed to several ambient CO concentrations. Following CO exposure, the maternal COHb concentration increased rapidly during the

first 2-3 hours. COHb values continued to increase until reaching a constant value at 6-8 hours post-exposure. They found that fetal COHb concentrations did not rise in phase with maternal values. During the first hour of exposure, fetal COHb changed only slightly. In the next 4-5 hours, it increased at a relatively slow rate as compared to the rise in maternal COHb. Following this phase, fetal COHb continued to rise over the next 24 hours. Final steady-state values for COHb were not reached until 36-48 hours post-exposure. At equilibrium, the COHb concentration of the fetus exceeded the COHb concentration of the mother by 58%. During this experiment, 57% of the fetuses died when fetal COHb concentrations exceeded 15% for 30 minutes or longer. The cause of death was reported as interference with fetal tissue oxygenation. No ill effects were noted in the ewes.

Adult animals subjected to CO-induced hypoxia react with increases in cardiac output which should lead to increases in coronary blood flow and tissue blood flow (Ayers et al., 1970). This compensatory adjustment does not appear to be initiated by the fetus in utero to any extent during exposure to CO. Longo and Hill (1977) noted that the decreases in fetal oxygen tension during CO exposure resembled decreases that would be predicted if no increase in tissue blood flow had occurred. Since fetal cardiac output normally equals 2-3 times that of the adult on a per weight basis, the fetus probably cannot increase its cardiac output significantly (Power and Longo, 1975). It is likely that the fetus operates normally at peak cardiac function.

The work by Longo and Hill (1977) has significant physiologic implications. Their work with pregnant sheep suggested the following:

1. Fetal COHb concentration changes relatively slowly compared to the rate of change of the COHb content of the mother.
2. Since maternal Hb has a high affinity for CO, the partial pressure of CO in maternal blood remains low despite a high maternal COHb concentration.
3. A significant rise in maternal blood P_{CO} must occur in order to establish a CO gradient that will drive a substantial amount of CO across the placenta into the fetal circulation.
4. Under steady-state conditions, the COHb concentration of the fetus exceeds that of the mother due to the increased affinity of fetal blood for O_2 and CO when compared to maternal blood.
5. It seems likely the CO-induced hypoxic and ischemic effects on the fetus may be greater than those effects on the mother considering the inability of the fetus to compensate for decreased blood P_{O_2} through increasing cardiac output.

Carbon Monoxide-Related Fetal Brain Lesions

The vast majority of fetal studies documenting gross and histopathologic evidence of CO-related neurotoxicity have been case reports of acute human fetal exposure (Longo, 1970). Incidents of suicide and accidental exposures of pregnant women have produced degenerative and destructive lesions within the brains of the fetuses. Fetal brain lesions involving basal ganglia, the cerebral cortex and the centrum semiovale are common findings (Ginsberg and Myers, 1976). The fetal brain lesions described are similar morphologically and anatomically to lesions

described in cases of acute exposure to human adults (Brucker, 1967; Lapresle and Fardeau, 1967; Stewart, 1920). In many cases, the fetus survived delivery and exhibited neurological abnormalities such as hypotonia, areflexia, involuntary movements and persistent seizures (Longo, 1970). Yet, in other cases of human fetal exposure, no gross neuropathologic abnormalities could be seen.

Ginsberg and Myers (1976) have examined the effects of acute CO exposure on the fetus utilizing late-term pregnant Rhesus monkeys. Arterial catheters, electrocardiographic and electroencephalographic leads were inserted into the fetuses in order to monitor fetal response following acute CO exposure of the mother. The mothers were then exposed to 1000-3000 ppm inspired CO for 1-3 hours. Maternal COHb levels rose to 50-60% saturation of Hb during exposure without external signs of CO intoxication in the mother. The fetuses developed severe hypoxemia in response to depressed maternal arterial O_2 content. Following CO exposure, the fetuses were delivered and revived. Fifty percent of the fetuses delivered revealed neuropathologic abnormalities. Post-mortem examination of the brains from the affected neonates revealed marked swelling and extensive hemorrhagic necrosis of the cerebral cortex and basal ganglia.

The investigators concluded that the fetal brain has a high threshold to hypoxemia and ischemia but is susceptible to extensive injury once a critical level of deoxygenation is exceeded. The fetal brain injury observed in this study was thought to be the direct result of fetal hypoxemia and decreased cerebral perfusion giving rise to extensive cerebral edema which further reduces cerebral perfusion.

Chronic prenatal low level CO exposures in pregnant rats have been investigated by Garvey and Longo (1978). They found that when pregnant rats were continuously exposed to 90 ppm CO, fetal brain weight increased 14% over normal by 20 days gestation. The increase in brain weight was attributed to an increased water content of the brain. No histopathologic examination of fetal brain tissue was attempted in their investigation and no significant pathologic changes in other fetal tissue was described.

Pathogenesis of Carbon Monoxide Induced Neurotoxicity

The details of the pathogenesis of CO-related neurotoxicity are not completely understood. Tissue hypoxia appears to be a basis for the toxic effects of CO; however, the neuropathologic appearance of CO intoxication suggests a concomitant impairment of cerebral perfusion (Ginsberg, 1980).

Early in the course of a hypoxic insult, vasodilation occurs in order to increase cerebral blood flow and reduce brain hypoxia (Kogure et al., 1970). However, the myocardium will not tolerate hypoxia and the resultant lactic acidosis (Scheuer, 1967). As the state of hypoxia continues, myocardial depression occurs (Siesjo and Plum, 1973). The myocardial depression leads to systemic hypotension and/or cardiac arrhythmias that, in turn, reduce cerebral perfusion. This leads to cerebral oligemia (i.e., incomplete cerebral ischemia) superimposed upon tissue hypoxia. Cerebral oligemia hinders the delivery of glucose and the removal of metabolic wastes from susceptible brain tissue (Siesjo, 1973). With increasing hypoperfusion and oligemia, areas of cerebral cortex lying

within arterial boundary zones may become selectively hypoperfused (Brierly et al., 1969).

Lesions in the globi pallidi are common in cases of acute CO intoxication in man where an apparent recovery from the exposure is followed by a sudden onset of neurological deficits leading to death. Evidence exists suggesting that oligemia may be required for the production of lesions in the globus pallidus. Necrosis of the globus pallidus has been observed due to extensive hypoperfusion without accompanying hypoxemia (Brierly et al., 1969). Injection studies in man suggest the anterior dorsal portion of the globus pallidus may lie within a poorly vascularized arterial border zone rendering it highly susceptible to hypoperfusion and hypoxia (Salamon, 1971, cited by Ginsberg (1980)).

CO-induced hypoxia alone does not appear to account for the white matter lesions found with CO intoxication (Ginsberg et al., 1974). Since gray matter is generally regarded as more sensitive to hypoxia than white matter, the general lack of gray matter lesions in cases of CO-induced encephalopathy suggests hypoxia alone is not responsible for CO-related neuropathologic lesions. Other factors apparently are involved in production of the lesions. Ginsberg et al. (1974) suggested that a degree of hypoxia well-tolerated by a normally perfused brain might cause regional tissue necrosis if complicated by systemic hypoperfusion and cerebral oligemia.

MATERIALS AND METHODS

Experimental Animals

Thirteen Pittman-Moore mini-pigs[1] and five domestic swine were utilized in this investigation. The thirteen mini-pigs included twelve sexually mature sows and one boar. The domestic pigs included four adult cross-bred sows and one Hampshire boar. Paired serum samples were collected from each animal and examined for serum neutralizing antiboby titers to Leptospira sp., Brucella sp., pseudorabies virus, parvo virus, and Eperythrozoon suis. All animals had antibody titers to parvo virus. No significant antibody titers were demonstrated against the other agents.

Housing and Husbandry

The mini-pigs and domestic sows were housed separately in isolated rooms. Each sow was randomly assigned to a pen measuring 1.5m x 3m. One-half of the cement floor in each pen was covered with 10cm of wood chip bedding to reduce the potential for development of lameness problems.

The temperature within the rooms was thermostatically controlled and maintained between 18-21 degrees Centigrade (C). Lights were turned off for approximately twelve hours per day to establish a regulated diurnal cycle. Sows were given water ad libitum and fed

---

[1]Institute of Agricultural Medicine, Iowa City, Iowa.

1.35 kg/day of a custom mixed gestation-lactation ration[1].

Sows were observed daily for external signs of estrus including relaxed, edematous vulva, increases in vaginal mucus and changes in behavior (Roberts, 1971). Upon detection of estrus, sows were pen bred and the date of breeding recorded. The sows were observed for return of estrus and rebred when necessary. When breeding was successful and no return of estrus occurred, the farrowing date was calculated using an average gestation length of 114 days (Roberts, 1971).

Environmental Chamber Design

An environmental chamber measuring 1.2m x 1.2m x 1.8m was constructed out of 1.25cm thick plywood. Three 38cm x 38cm plexiglass windows were strategically placed on three surfaces of the chamber to facilitate observation of the contained sow. The chamber was equipped with an automatic waterer and a 5,000 BTU window air conditioner. The air conditioner was added to the system to regulate temperature and control humidity.

A farrowing crate equipped with wheels for rolling it into and out ot the chamber was constructed of 1.25cm thick plywood and commercially available 64mm diameter steel-rod livestock panels. The farrowing crate measured 1.1m x 1.5m at the base, 0.6m x 1.5m at the top and was approximately 1m high. The wide base allowed the animal to lie down within the crate and the narrow top prevented the sow from turning around once the crate was placed in the environmental chamber.

The atmosphere of the chamber was created by mixing CO with

---

[1]Cargill-Nutrena, Feed Division, Rowan, Iowa.

compressed air and metering the resultant mixture into the chamber. Figure 1 is a schematic drawing of the gas mixing and delivery system for the environmental chamber. A CO gas cylinder[1] containing 175 cubic feet of 99.5% pure CO was connected to a standard gas regulator valve. A needle control valve placed in line from the CO cylinder was followed by a low volume Gilmont flowmeter (0-100 milliliter/minute)[2]. An in-house source of compressed air was channelled via 1.25cm diameter polyvinyl chloride (PVC) tubing[3] through an anhydrous calcium sulfate chip filter[4] to remove excess moisture.

The volume of compressed air entering the chamber was monitored by a standard oxygen flow meter in line from the source of compressed air. The CO and air were mixed at a T-connector and channelled by a single 2m line into the environmental chamber. Both the CO and air lines were constructed from 1.25cm diameter PVC tubing. The regulator on the compressed CO cylinder was held at 2 Kg pressure per square cm. The volume of air entering the chamber was set at 15 liters/minute while the volume of CO entering the chamber was varied according to the CO exposure level desired.

The CO concentration within the environmental chamber was monitored using a direct indicating CO detector[5] connected to the chamber

---

[1]Matheson, Joliet, Illinois.

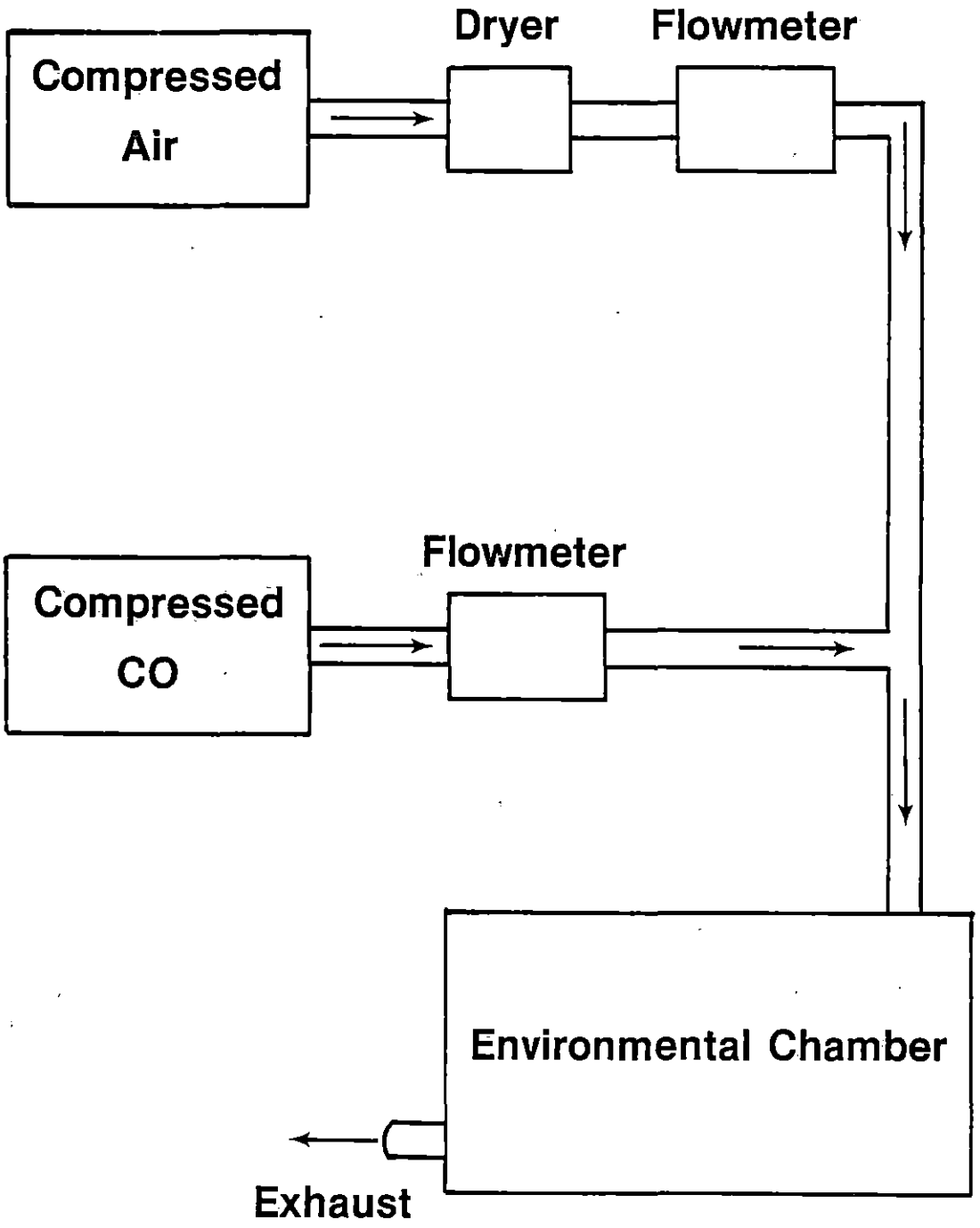
[2]American Scientific Products, Minneapolis, Minnesota.

[3]Tygon^{R}, Fisher Scientific Co., Itasca, Illinois.

[4]Drierite^{R}, Fisher Scientific Co., Itasca, Illinois.

[5]General Electric, Air Craft Equipment Division, Wilmington, Massachusetts.

Figure 1. Diagrammatic presentation of the gas mixing and delivery system for the environmental chamber


Dryer
Flowmeter
Compressed Air
Flowmeter
Compressed CO
Environmental Chamber
Exhaust

by 0.6cm PVC tubing. The temperature within the chamber was monitored using a Porto-Hygro-Therm thermometer[1].

Carboxyhemoglobin Determination

Venous blood COHb, O_2Hb and Hb values were determined spectrophotometrically using a Model IL182 Co-oximeter[2]. From the collection syringe, approximately 1/2 ml. of heparinized blood was introduced into the Co-oximeter for each sample. The procedure was repeated three times for each sample to check for precision in measurement.

Exposure to Carbon Monoxide

Pregnant sows were exposed twenty-two times to atmospheric levels of CO ranging from 150-400 ppm for 48-96 hours. The levels and duration of exposure, and the number of sows exposed are recorded in Table 1. The CO levels listed in the table represent atmospheric CO concentrations within the environmental chamber expressed in ppm. The duration is recorded as the time of exposure in hours. N is the number of sows exposed at each listed concentration of atmospheric CO.

A 5 ml. blood sample was collected from the anterior vena cava of each pregnant sow prior to placement in the environmental chamber. The blood samples were analyzed for percent COHb and the results recorded to furnish baseline COHb values prior to CO exposure. Pregnant

---

[1]Jersey Technical Electronics, Inc., Middlesex, New Jersey.

[2]Instrumentation Laboratory, Inc., Lexington, Massachusetts.

Table 1. Experimental treatments

CO level	Duration	N
150 ppm	48 hours	2
150	72	1
200	48	4
200	72	2
250	48	2
250	72	2
250	96	1
300	48	3
300	96	1
350	48	2
350	72	1
400	96	1

sows at 108-110 days gestation were then loaded into the mobile farrowing crate and placed into the environmental chamber.

Once the sow was contained, records of atmospheric CO levels within the environmental chamber were made each hour utilizing a direct reading CO detector. The temperature within the chamber was recorded simultaneously. To assure accuracy and precision in the measurement of chamber CO levels, the CO detector was calibrated daily during the period of CO exposure to the sow. Calibration was accomplished using a standardized CO cylinder containing 179.2 ppm CO.

The sow was removed from the environmental chamber once a day during

exposure so bedding could be changed in the crate and the sow fed. This process exposed the sow to room air for 15 minutes a day during the treatment period; however, a significant decline in maternal COHb was not expected during this brief feeding period.

Upon completion of CO exposure, the sow was removed from the environmental chamber and a 10 ml. blood sample was drawn from the anterior vena cava utilizing a 10 cc plastic syringe attached to a 16 gauge x 9 cm needle. Sodium heparin (10,000 USP units/ml)[1] was used as the anticoagulant in each syringe. A portion of the maternal blood sample was then analyzed for percent COHb. This value was recorded as the post-exposure maternal COHb.

Provided the sow had not initiated parturition within the environmental chamber, she was placed into a farrowing pen and observed several times daily for signs of parturition. All stillborn and live piglets delivered were immediately necropsied.

Three sows were exposed to 250, 300 and 400 ppm atmospheric CO for 96 hours according to procedures outlined above. In addition, the fetuses from these sows were delivered by Cesarean section immediately following removal of the sows from the environmental chamber. The sows were given xylazine (100 mg/ml)[2] at a dose of 2.2 mg/Kg lbs body weight as a sedative and muscle relaxant prior to surgery. Spinal analgesia was obtained with an epidural block at the lumbosacral

---

[1]Elkins-Sinn, Inc., Cherry Hill, New Jersey.

[2]Rompun^{R} Bay Vet, Shawnee, Kansas.

junction utilizing approximately 20 cc of 2% Xylocaine HCL (20 mg/ml)[1].

A paramedium horizontal skin incision approximately 15 cm in length was made along the left abdominal wall immediately dorsal to the line of mammae. The incision was continued through subcutaneous fat, abdominal musculature and peritoneum. The left horn of the gravid uterus was exteriorized and incised. All piglets were removed from the left horn and heparinized blood samples were immediately collected from live piglets by cardiac puncture. The incision in the left horn of the uterus was closed and the right horn exteriorized and incised. Piglets retrieved from the right horn were handled in similar fashion to those removed from the left horn of the uterus. The incisions in the right uterine horn and abdominal wall were closed and the sow placed into a pen for observation.

Blood samples collected from piglets delivered by Cesarean sections were analyzed for percent COHb, percent O_2Hb and Hb content. The piglets were necropsied and fetal tissues were processed for histologic evaluation.

Fetal Necropsy and Tissue Processing

All stillborn and live piglets recovered from sows exposed to CO were necropsied as soon as possible after birth. Live piglets were killed by electrocution prior to necropsy. Each piglet in a litter was examined for gross lesions suggestive of CO intoxication. Samples of lung, liver and stomach contents were collected from stillborn

---

[1] Lidocaine® Astra, Worcester, Massachusetts.

piglets and cultured for bacterial pathogens. Sections of spleen and lung were cultured for pseudorabies virus. Bacteriologic and virologic examinations of tissues were completed to document the absence of an infectious cause for the increases in stillbirth rates anticipated.

Tissues collected for histologic examination included heart, liver, and brain. Tissues were fixed in 10% neutral buffered formalin with the volume of formalin equal to ten times the volume of tissue. Fixation was continued for 30 days before tissue sections were processed for histopathologic examination. Brain sections from piglets in each litter were taken serially through the cerebral cortex, brain stem, cerebellum and medulla. Sections of liver and ventricular myocardium were also submitted for histologic processing.

Tissue processing involved an initial dehydration of sections in graded alcohol solutions. Tissues were then cleared with xylene, infiltrated with paraffin, and embedded in tissue blocks for sectioning. Liver, heart and lung were sectioned at 6 μm while brain was sectioned at 8 μm to reduce the cutting and tearing artifacts noted in brain sectioned cut at 6 μm. Tissue sections were placed on glass slides, passed through xylene to remove paraffin, and through graded alcohol solutions to remove xylene and rehydrate the tissues. All sections were stained with hematoxylin and eosin (H&E). Fetal brain sections with definable lesions on H&E were stained with a Luxol Fast Blue-Cresyl Echt Violet myelin stain.

Blood COHb Stability Studies

Eight sows were exposed to 150 ppm CO for 8 hours. After removal of each sow from the CO inhalation chamber, 60 mls. of blood were collected by venopuncture from the anterior vena cava using a 16 gauge x 9 cm stainless steel needle attached to 60 cc sterile plastic syringe.

Twenty-two 3 cc plastic syringes containing approximately 2.5 ml. of blood each were prepared from the 60 ml. blood sample. All air bubbles and dead space were removed from the syringes containing the blood. A plastic cap was placed on the luer syringe to reduce exposure of the sample with air.

Twenty-one of the 2.5 ml. blood samples were randomly assigned to three temperature treatment groups so that each treatment group would contain seven blood samples. The treatment groups, representing different temperatures for blood storage, were as follows:

Treatment 1 – Room temperature storage (22°C)

Treatment 2 – Chilled blood storage (4.4°C)

Treatment 3 – Frozen blood storage (-17.6°C)

COHb values were determined daily for seven days following removal of the sow from the CO inhalation chamber. Frozen blood samples were thawed prior to COHb analysis. Any difference in COHb values between time zero and day 7 due to temperature or time was determined. A time zero COHb value, determined when the sow was removed from the environmental chamber, served as a basis for comparison.

Statistical Analyses

Maternal COHb levels would be expected to rise with elevations in atmospheric CO within the environmental chamber. The relationship of known atmospheric CO concentrations to the resultant maternal COHb was determined using simple linear regression analysis. The concentrations of atmospheric CO represented the independent variable y. The maternal COHb value recorded at each level of CO exposure represented the dependent variable X. The least squares means were generated from the data to represent the expected maternal COHb value at a known atmospheric CO concentration.

The effects of temperature and time on stability of maternal COHb values were examined by comparing time zero COHb values to COHb values obtained several days later within each treatment group. Any effect attributed to time within a treatment group could be assessed using a t-test for paired comparisons (time 0 and day 7). The differences in maternal COHb attributable to the treatments (temperature) were determined by analysis of variance. Treatment groups were examined at time 0, day 2 and day 7 for differences.

RESULTS

Maternal Exposure to Carbon Monoxide

The COHb concentrations determined prior to and following CO exposure are recorded in Table 2. The CO concentrations listed in Table 2 are measurements in ppm within the environmental chamber. The data in Table 2 suggest a linear relationship between the atmospheric CO concentration and the maternal COHb concentration.

Table 2. Maternal COHb expressed as a % of total hemoglobin prior to and following exposure to elevations in atmospheric CO

Sow identification	% COHb		CO concentrations in air (ppm)
	Pre-exposure	Post-exposure	
94	1.2	14.8	150
90	1.8	13.8	150
88	0.9	15.4	150
99	1.1	16.1	200
96	1.4	23.0	200
87	1.5	22.5	200
92	1.7	23.8	200
93	2.0	18.0	200
89	1.5	19.1	200
93	1.3	24.6	250
94	1.2	25.8	250
Y3	1.8	24.5	250
2	0.7	23.5	250
94	1.0	22.5	250
92	1.4	27.8	300
3	1.5	28.1	300
7	1.6	29.2	300
LNT	1.7	32.0	300
Y1	1.6	31.3	350
88	1.1	30.8	350
5	1.5	29.0	350
90	1.1	34.2	400
Y2	2.0	2.1	Control
88	1.3	1.2	Control

This relationship between the CO concentration in the air and the resultant maternal COHb concentration is depicted in Figure 2. The line in Figure 2 represents the predicted value ($\hat{y}$) for maternal % COHb against concentrations of atmospheric CO. The predicted values for maternal COHb at given concentrations of CO were determined by the method of least squares (Snedecor and Cochran, 1967). The coefficient of determination ($R^2 = .95$) indicated that the sum of squares attributed to the regression of maternal % COHb on atmospheric CO concentration accounted for most of the total sum of squares. A significant linear relationship existed between maternal COHb and atmospheric CO concentration at $p < .0001$ ($F = 415$, $df = 1, 22$).

The duration of CO exposure had no significant effect on maternal COHb concentration at $p < .7825$ ($F = .08$, $df = 2, 18$) when both CO concentration and time were considered as variables affecting maternal % COHb. The effect of time on maternal COHb concentration was considered after 48, 72 and 96 hours of exposure.

Incidence of Stillbirths

The stillbirth rate in each litter delivered by sows exposed to known concentrations of atmospheric CO is presented in Table 3. The maternal COHb concentration determined following exposure to CO is included in Table 3. Between maternal COHb levels of 23.8-31.5%, a high degree of variability existed in stillbirth rates per litter. Stillbirth rates per litter varying from 16.7-100% occurred when sows were exposed to 300 ppm CO while the resultant maternal COHb values

Figure 2. Plot of maternal COHb concentration versus atmospheric concentration of CO. The circles represent observed values of COHb at a known concentration of CO while the triangle represents the predicted value ($\hat{y}$) for COHb at the same CO concentration. The maternal COHb concentration is reported as a percent saturation of total Hb

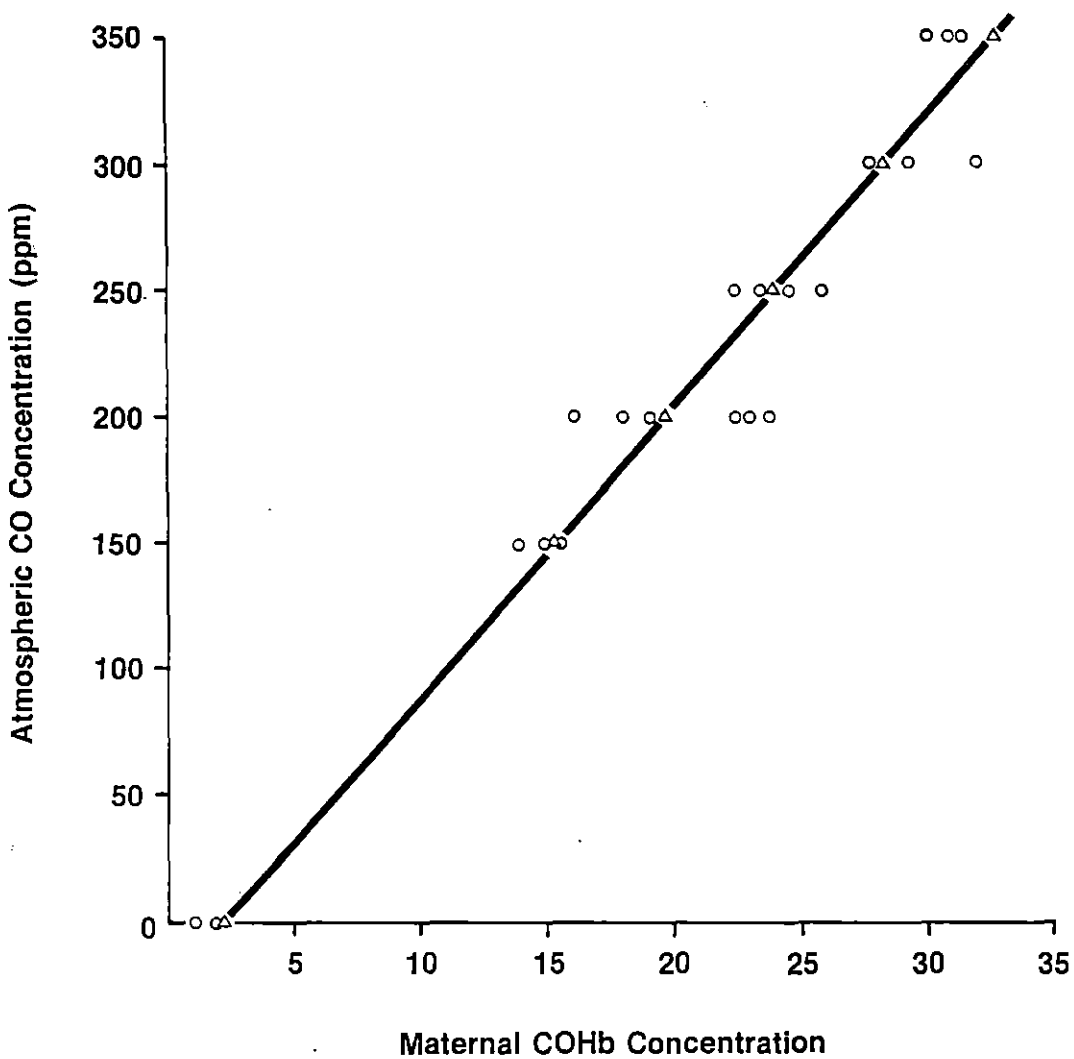

Atmospheric CO Concentration (ppm)
350
300
250
200
150
100
50
0
5
10
15
20
25
30
35
Maternal COHb Concentration

Table 3. Incidence of stillbirths per litter at given concentrations of atmospheric CO and % maternal COHb

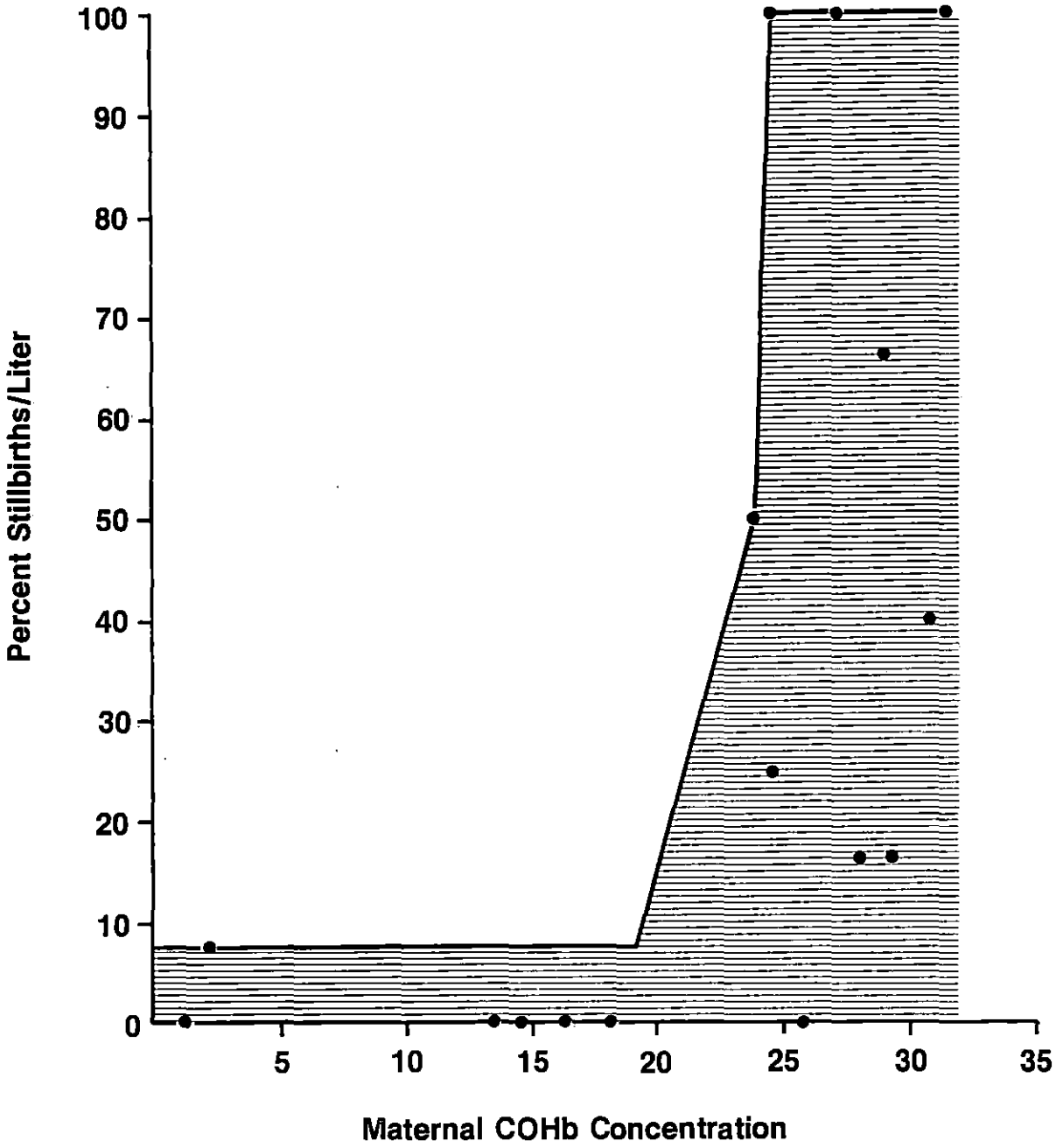
Sow number	CO level (ppm)	% maternal COHb	Percent stillbirths/litter
94	150	13.6	0.0
90	150	14.8	0.0
93	200	18.0	0.0
89	200	16.1	0.0
92	200	23.8	50.0
94	250	25.8	0.0
Y3	250	24.5	25.0
93	250	24.6	100.0
3	300	28.0	16.7
7	300	29.2	16.7
92	300	27.1	100.0
5	350	29.0	66.7
88	350	30.8	40.0
Y1	350	31.5	100.0
86	Control	1.2	0.0
Y2	Control	2.1	7.7

were similar. No stillbirths occurred in sows exposed to 150-200 ppm CO where their resultant COHb concentrations ranged from 13.6-18.0% of total hemoglobin. One of 13 piglets was delivered stillborn by one control sow. No stillbirths were recorded in the remaining control litter.

The variability in stillbirth rates recorded when the maternal COHb concentration ranged from 23-32% is further depicted in Figure 3. Each plotted point represents the stillbirth rate per litter against the measured maternal COHb concentration following removal of the sow from the environmental chamber.

Table 4 presents the percent of total births that occurred as stillbirths at each CO exposure concentration. The total number of sows

Figure 3. Percent stillbirths per litter vs % maternal COHb (note the variability in % stillbirths recorded per litter when % maternal COHb reached levels of 23-32% of total hemoglobin)


Percent Stillbirths/Liter
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
Maternal COHb Concentration

that delivered piglets following exposure to CO is also included in Table 4. An overall increase in stillbirth rate was demonstrated as concentrations of CO increased. The stillbirth rate recorded in the control sows was below the recognized normal stillbirth rate in swine of 6% (Sprecher et al., 1974).

Table 4. Overall incidence of stillbirths in pregnant sows at exposure concentrations of atmospheric CO

Ambient CO level (ppm)	Number of sows	Live births	Stillbirths	% stillbirths
150	2	13	0	0
200	3	14	1	6.7
250	4	15	8	34.8
300	3	15	11	42.3
350	3	5	20	80.0
Control	2	22	1	4.3

Positive correlations between the variables CO exposure level, maternal COHb concentration and stillbirth rate were determined. The correlation coefficients (r) between the CO exposure level and maternal COHb, CO exposure level and stillbirth rate, and the maternal COHb concentration and stillbirth rate were calculated as .978, .576 and .576, respectively. The r for CO exposure level and maternal COHb concentration was highly significant ($p < .0001$). The r between CO exposure level and stillbirth rate and between maternal COHb concentration and stillbirth rate were also significant ($p < .025$).

Cesarean Section Derived Piglets

Full-term piglets from three sows exposed for 96 hours to atmospheric CO levels of 250, 300 and 400 ppm, respectively, were recovered by Cesarean section within one hour after removal of the sow from the environmental chamber. The piglets recovered from sows exposed to 250 and 300 ppm CO were depressed, lethargic and ataxic. All piglets removed from the sow exposed to 400 ppm CO for 96 hours were dead and had moderate intrauterine autolysis. The percent COHb determined in the live piglets recovered as compared to maternal COHb following cessation of CO exposure is recorded in Table 5. The fetal COHb concentration was consistently greater than the maternal COHb concentration at 250 and 300 ppm CO exposure to the sow. Fetal blood COHb concentrations were not determined in the dead fetuses since unclotted blood samples were required for analysis.

Gross Lesions in the Piglet

Nonspecific gross lesions were noted in all stillborn piglets examined from sows exposed to CO concentrations equal to or greater than 250 ppm. Diffuse cherry red discoloration of subcutaneous tissues, muscle and peritoneal and thoracic viscera was found in all stillborn piglets. In addition, moderate to severe intrauterine autolysis occurred in stillborn piglets delivered by sows 3, 5, 88, 90, 92, LNT, Y1 and Y3. Approximately 5-20 cc of a dark red-brown pleural effusion was found in the thoracic cavity of many of the stillborn fetuses necropsied (Figure 4). This lesion was interpreted to be a result

Table 5. Blood COHb concentrations in Cesarean section derived piglets as compared to maternal COHb concentrations following exposure to CO

Maternal CO exposure (ppm)	% maternal COHb	Piglet number[a]	% neonatal blood COHb
250	22.5	1	26.9
		2	24.2
		3	24.2
		4	25.2
300	32.0	1	39.0
		2	38.6
		3	39.2
		4	38.8
		5	33.0
		6	34.0
400	34.2	1-8[b]	NA[c]

[a]Order of removal from uterus during Cesarean section.

[b]Eight piglets recovered dead.

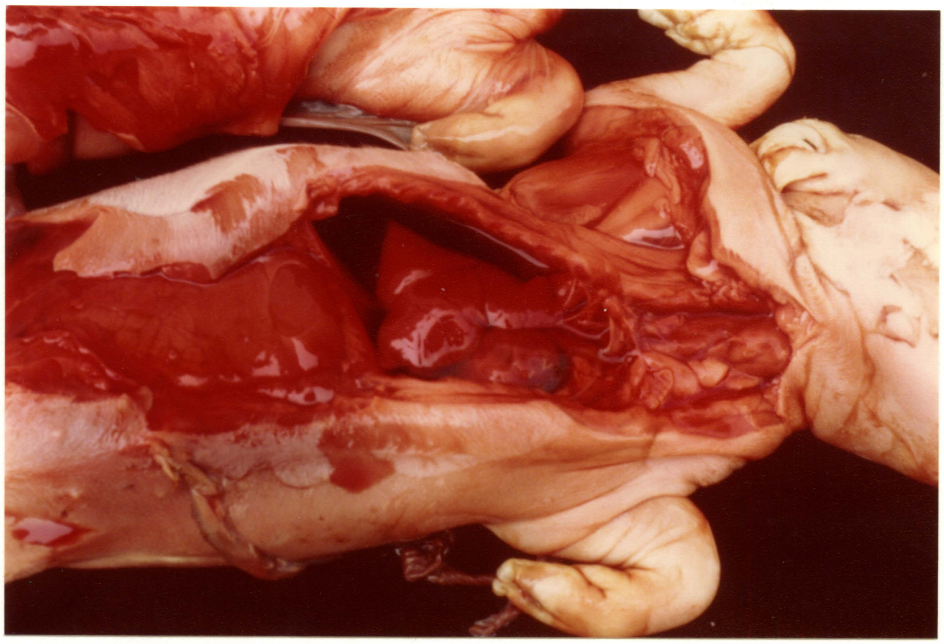
[c]Not available.

of post mortem autolysis and was limited to piglets recovered from sows exposed to concentrations greater than or equal to 300 ppm CO.

Microscopic Lesions in the Piglet

Histopathologic examination of multiple sections of neonatal brain revealed hypoxic-ischemic leukoencephalopathy in piglets from 3 litters out of 14 examined. Brain lesions included multifocal areas of leukoencephalomalacia, spongiosus, fragmentation of myelin sheaths, vacuolar degeneration of the neuropile within the brain stem, and multiple hemorrhages throughout the white matter of the brain. Diffuse vascular congestion

Figure 4. Piglet recovered by Cesarean section from sow exposed to 400 ppm CO for 96 hours. Note the cherry red discoloration of subcutaneous tissues and the large volume of reddish-brown fluid in the thoracic cavity



was found in neonatal brain sections from the majority of CO exposed litters examined.

One stillborn and one live piglet were delivered by sow 92 following 72 hours of CO exposure at 200 ppm. No significant lesions were found on histopathologic examination of brain sections from the live piglet. Microscopic examination of brain sections from the stillborn piglet revealed focal cortical malacia and spongiosus of the hemispheral white and gray matter. The necrotic focus was infiltrated by high numbers of lipid-laden macrophages (gitter cells) and contained high numbers of reactive glial cells (Figure 5). Diffuse venous congestion and vascular proliferation peripheral to the necrotic focus was found in several sections of cerebrum examined.

Two weak, depressed piglets and four stillborn piglets were delivered by sow 5 four days after 48 hours of maternal CO exposure at 350 ppm. Microscopic examination of the stillborn piglets was not attempted due to extensive intrauterine autolysis. On histopathologic examination of brain sections from one of the piglets born alive, there was focal necrosis of the left hemispheral white matter (Figure 6). Within the necrotic area, there were high numbers of gitter cells. Peripheral to the necrotic focus, extensive glial-vascular proliferation, hemorrhage and gitter cell infiltration were observed (Figures 7 and 8). In brain sections from the remaining live piglet, there were diffuse venous congestion and mild vacuolization of the neuropile within the brain stem. No evidence of cortical malacia was observed in sections of brain examined from the second piglet.

Six live piglets were recovered by Cesarean section from sow LNT-

Figure 5. Focus of cerebral necrosis and glial cell proliferation. Note the presence of lipid-laden macrophages (gitter cells) within the lesion. Arrow points to a gitter cell

Figure 6. Pale focus of cerebral white matter necrosis in a neonatal piglet brain following maternal CO exposure of 350 ppm for 48 hours

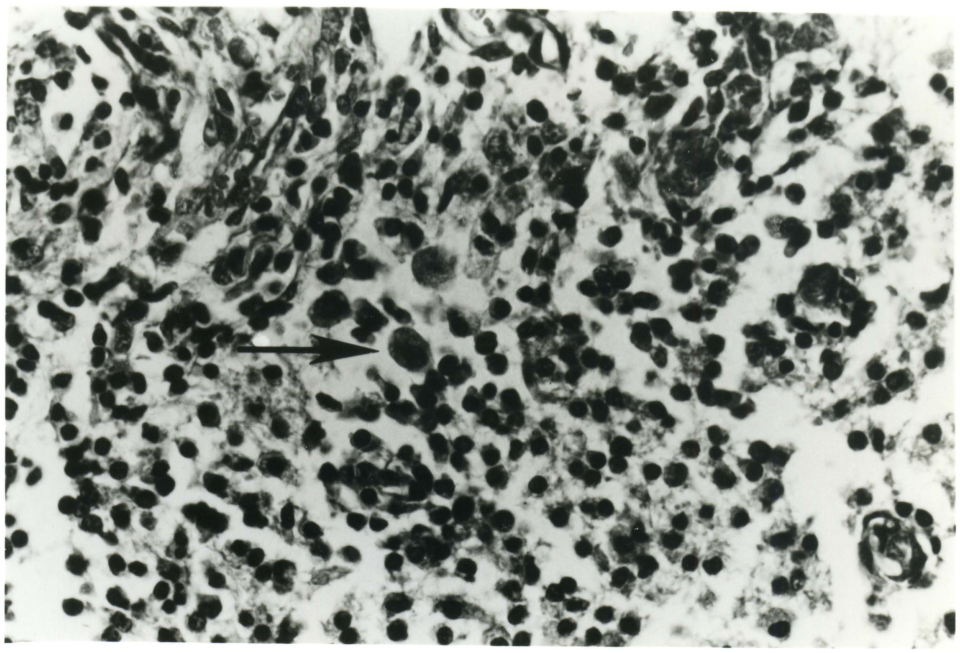
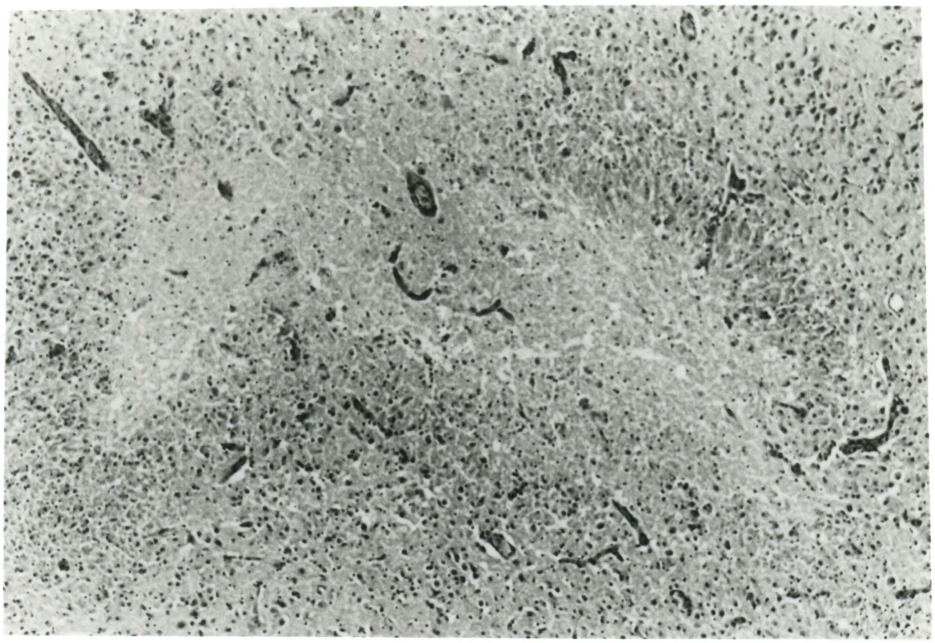
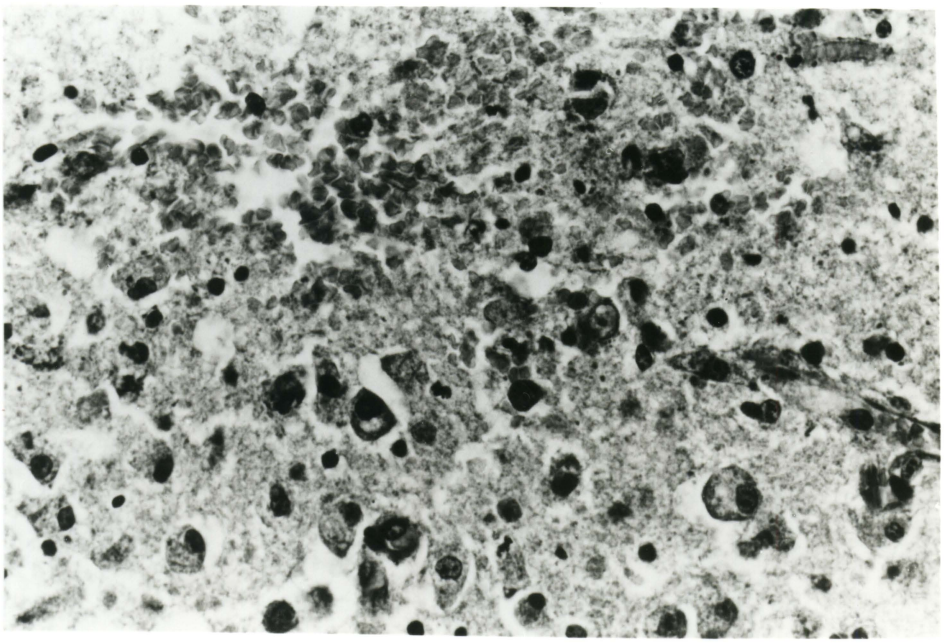


Figure 7. Higher magnification of a necrotic focus from the piglet brain in Figure 6. Note the glial-vascular proliferation peripheral to the necrotic center of the lesion

Figure 8. Higher magnification of Figure 6 demonstrating focal hemorrhage and high numbers of gitter cells within the necrotic area





following 96 hours of maternal CO exposure at 300 ppm. Brain sections from the piglets had multifocal petechial hemorrhages and vacuolization of the neuropile (Figures 9 and 10). Focal hemorrhagic lesions were found throughout the hemispheral white matter, deep cortical white matter and brain stem. The distribution and extent of the lesions noted were similar in all sections of brain examined from piglets in this litter. The cerebellum from one piglet contained a pale, poorly demarcated focus of hemorrhage and edema (Figure 11). Swollen oligocytes and astrocytes were found within the pale, edematous area.

Degenerative changes in myelin appeared in Luxol fast blue stained brain sections of piglets examined from sows 92, 5, and LNT. At low magnification, the intensity of myelin staining was noticeably reduced in brain sections from experimental groups as compared to brain sections from neonatal control piglets. At higher magnification, the myelinated fibers in subcortical white matter appeared disorganized, fragmented and discontinuous in experimental piglets recovered from these sows (Figure 12). No histologic evidence of myelin degeneration was found in other piglets examined from experimental and control sows.

Liver sections from experimental piglets had multiple foci of mononuclear cells characterized by hyperchromatic nuclei with prominent nucleoli, focally high mitotic indices, and a high nuclear to cytoplasmic ratio. The foci of mononuclear cells were considered extramedullary hematopoeitic centers (EMHC) containing primarily erythroblastic cells (Figures 13 and 14). The number of EMHC

Figure 9. Multifocal petechial hemorrhages and vacuolization within the medulla of piglet LNT-1

Figure 10. Multiple hemorrhages within myelinated nerve fiber bundles in the brain stem of piglet LNT-1

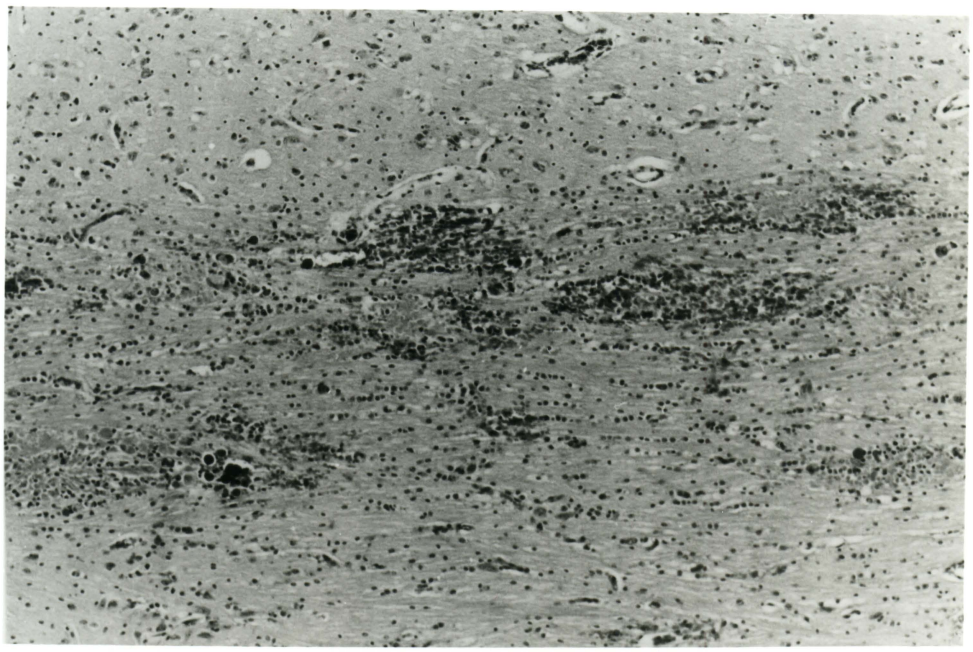
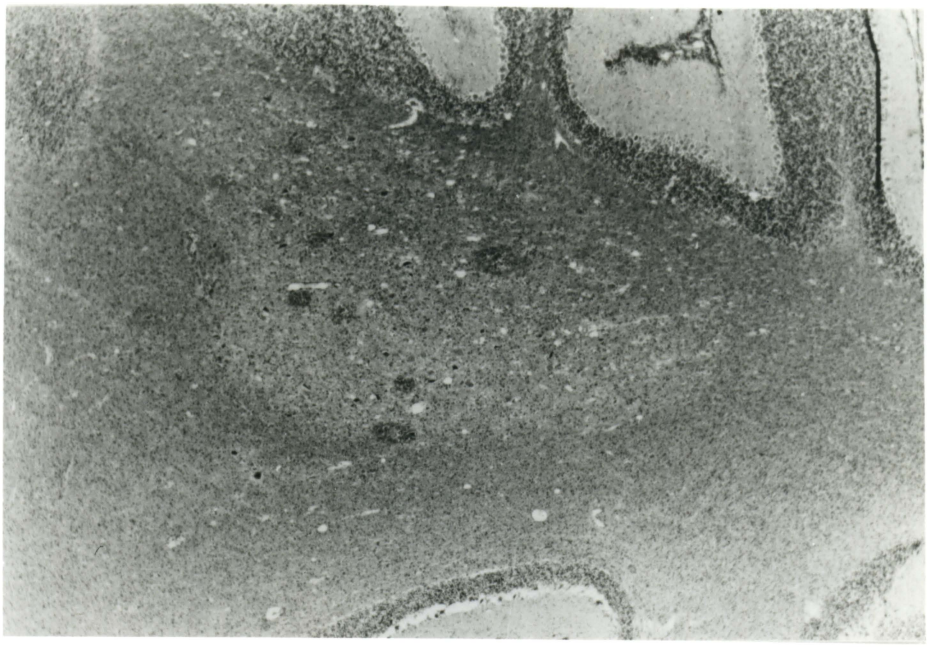


Figure 11. Focus of cerebellar edema and degeneration surrounding multiple petechia

Figure 12. Disruption and fragmentation of myelin sheaths in subcortical white matter



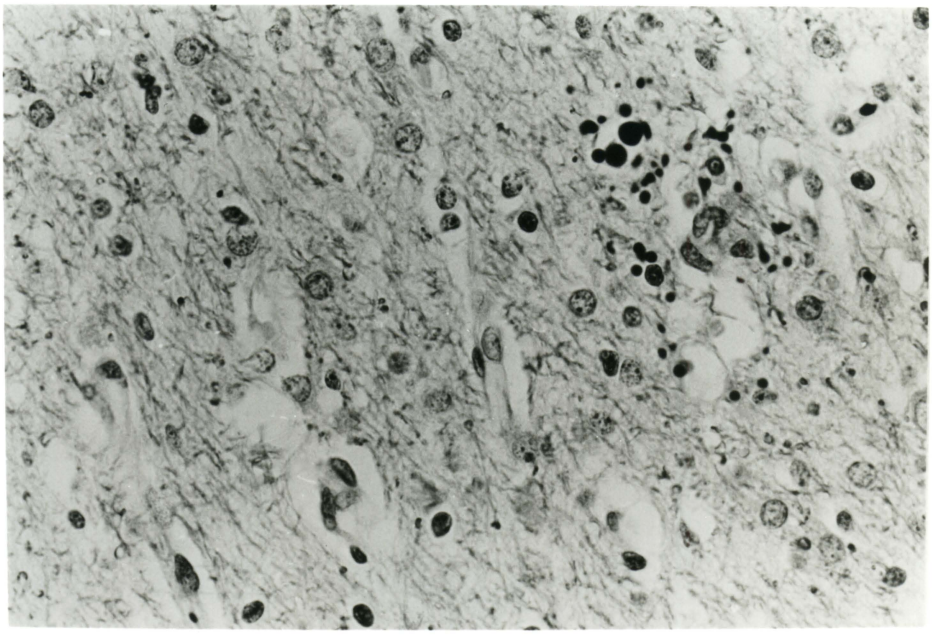
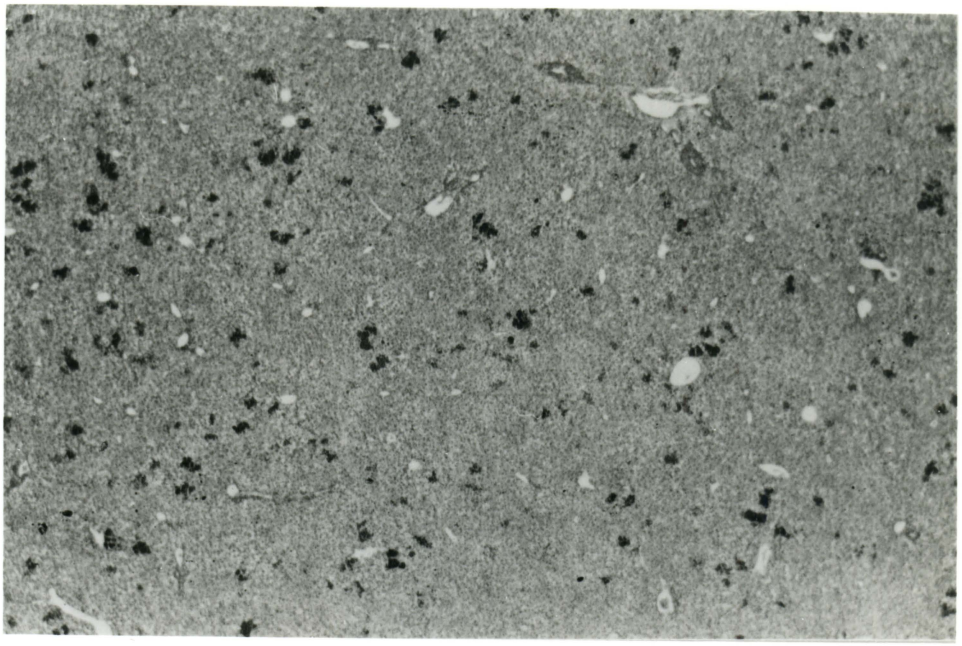
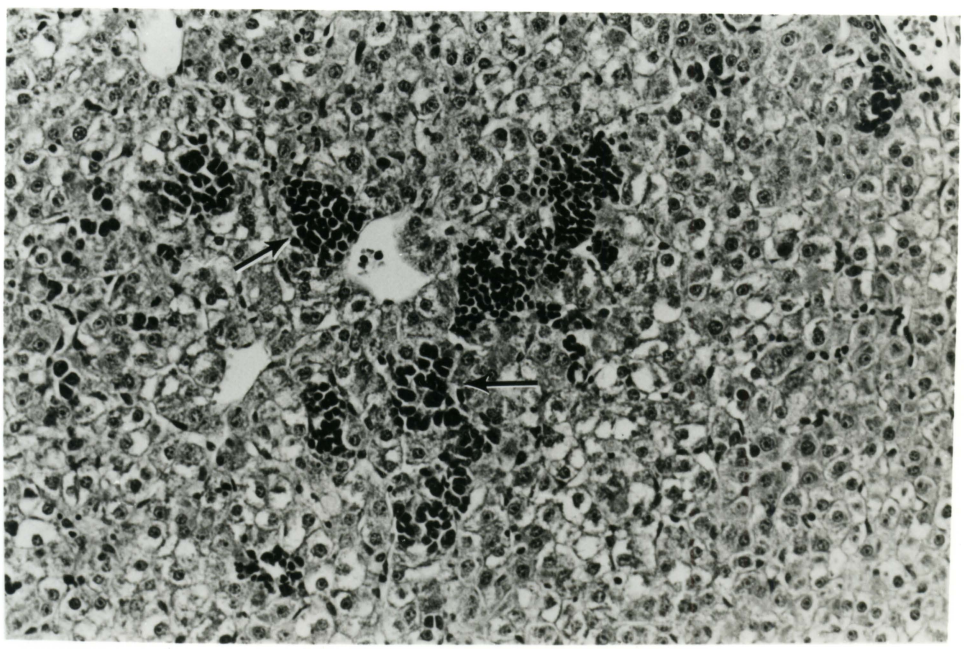


Figure 13. High numbers of extramedullary hematopoeitic centers (EMHC) within liver sections from experimental piglets

Figure 14. Higher magnification of Figure 13 with arrows pointing to clusters of cells resembling rubriblasts





found per high power field was determined and consistently higher numbers were recorded in experimental piglets as compared to control piglets. The significance of this finding was questioned since a limited number of fresh liver sections were available for microscopic examination. In addition, no relationships could be demonstrated between the number of EMHC seen per high power field and the level or duration of maternal CO exposure.

No lesions were seen on histopathologic examination of ventricular myocardium from experimental piglets.

Analysis of Thoracic Fluid

A frequent finding in the stillborn piglets was approximately 5-20 cc of dark red-brown fluid in the thoracic cavity. Analysis of the thoracic fluid for COHb content was attempted whenever an adequate volume of thoracic fluid was available from the stillborn piglets. The results of the analyses are presented in Table 6.

Thoracic fluid was available only in stillborn piglets collected from sows exposed to atmospheric CO concentrations of 300 ppm or greater. The COHb concentration determined in fetal thoracic fluid varied considerably between piglets within the same litter. The thoracic fluid COHb content of stillborn piglet 7-3 was six times higher than the COHb concentration determined for piglets 7-1 and 7-2. The latter two piglets were born alive and weak. A similar condition occurred with piglets 5-3 and 5-4 where thoracic fluid COHb concentrations of 4.1 and 6.3, respectively, were recorded while no detectable amount of COHb was

Table 6. Thoracic fluid % COHb in stillborn piglets as compared to maternal % COHb and level of CO exposure

Level CO exposure (ppm)	Maternal % COHb	Sow-piglet number	Piglet thoracic fluid % COHb
300	29.2	7-1[a]	0.2
		7-2	0.2
		7-3	1.2
		7-4	NA[b]
350	29.0	5-1[c]	0.0
		5-2	0.0
		5-3	4.1
		5-4	6.3
350	31.5	Y1-1[d]	3.0
		Y1-2	1.4
		Y1-3	0.8
		Y1-4	1.3
		Y1-5	1.4
		Y1-6	0.6
		Y1-7	1.0
		Y1-8	1.8
		Y1-9	1.6
		Y1-10	1.3
		Y1-11	1.3
		Y1-12	2.2
		Y1-13	0.4
		Y1-14	NA
350	30.8	88-1	4.2
		88-2	3.0

[a]Piglets 7-1 and 7-2 were born alive and weak.

[b]No thoracic fluid available.

[c]Piglets 5-1 and 5-2 were born alive and weak.

[d]Y1 piglets were retained within the uterus for 17 days following CO exposure.

Table 6. Continued

Level CO exposure (ppm)	Maternal % COHb	Sow-piglet number	Piglet thoracic fluid % COHb
400	34.4	90-1[e]	0
		90-2	9.2
		90-3	8.5
		90-4	8.6
		90-5	10.0
		90-6	10.1
		90-7	7.5
		90-8	8.1

[e]All piglets collected by Cesarean section; 90-1 was a mummified fetus.

found in piglets 5-1 and 5-2.

All 14 piglets delivered by sow Y1 were stillborn. The piglets were severely autolyzed and 17 days beyond term when delivered. Nevertheless, all piglets contained detectable COHb within the thoracic fluid.

Piglets were collected from sow 90 by Cesarean section following 96 hours CO exposure at 400 ppm. All the piglets were found dead in utero. Piglet 90-1 was mummified and presumably dead prior to the sow's exposure to CO. Piglets 90-2 through 90-8 had mild in utero autolysis and gross lesions suggestive of CO intoxication. The thoracic fluid COHb concentrations ranged from 8.1-10.1% within piglets taken from sow 90.

Time and Temperature Effects on Carboxyhemoglobin Stability

The effect of prolonged blood sample storage on the stability of maternal % COHb at three different storage temperatures was examined. The maternal COHb concentration determined in blood samples stored at room temperature (22°C) over a seven-day period is recorded in Table 7. No significant change in maternal % COHb was found to occur over time at room temperature.

Table 7. Maternal % COHb from blood samples stored at room temperature (22°C) over seven days

	Maternal % COHb (150 ppm CO exposure)							
Sow number	Time[a] 0	1	2	3	4	5	6	7
86	13.7	13.6	13.6	14.2	14.2	14.2	14.2	14.2
2	15.2	15.2	15.3	15.6	15.6	15.6	15.6	15.6
5	13.8	13.3	13.4	13.4	13.3	13.2	13.0	13.1
88	14.8	14.6	14.6	14.7	14.7	14.6	14.4	14.4
94	12.8	12.5	12.0	12.6	12.6	12.5	12.6	12.8
90	15.6	15.4	15.5	15.5	15.5	15.6	15.7	15.7
92	14.3	14.2	14.2	14.2	14.3	14.4	14.4	14.2

[a]The number of days since exposure to elevations in atmospheric CO.

The maternal COHb concentration determined from blood samples chilled to 4.4°C is presented in Table 8. Samples were analyzed daily for seven days with no significant change in maternal % COHb demonstratable over time.

Table 8. Maternal % COHb from chilled blood samples stored at 4.4°C over seven days

Sow number	Time[a]	Maternal % COHb (150 ppm CO exposure) 0	1	2	3	4	5	6	7
86		13.7	13.7	13.7	13.6	13.4	13.5	13.6	13.8
2		15.2	15.0	15.4	15.3	15.6	15.4	15.4	15.4
5		13.8	13.3	13.4	13.6	13.4	13.3	13.2	13.2
88		14.8	14.7	14.8	14.7	14.6	14.5	14.2	14.2
94		12.8	12.5	12.4	12.3	11.9	12.1	11.8	12.0
90		15.6	15.0	15.4	15.4	15.3	15.4	15.5	15.5
92		14.3	14.3	14.2	13.8	14.2	14.2	14.2	14.2

[a]The number of days since exposure to elevations in atmospheric CO.

The maternal COHb concentration recorded from blood samples stored over seven days at -17.6°C is presented in Table 9. A significant decline in maternal % COHb occurred in the frozen samples over time at $p < .0001$ ($F = 20.67$, $df = 1, 54$). Under all three temperature treatments, the maternal % COHb determined immediately following CO exposure to the sow was recorded as time 0.

The differences in the stability of maternal COHb concentration due to temperature effects were examined using analysis of variance. Twenty-four hours after CO exposure (time 1), no significant difference in maternal % COHb could be demonstrated due to the temperature treatments. At time 2, differences in maternal % COHb due to the temperature treatments were approaching significance at $p < .089$ ($F = 2.78$,

Table 9. Maternal % COHb from frozen samples stored at -17.6°C over seven days

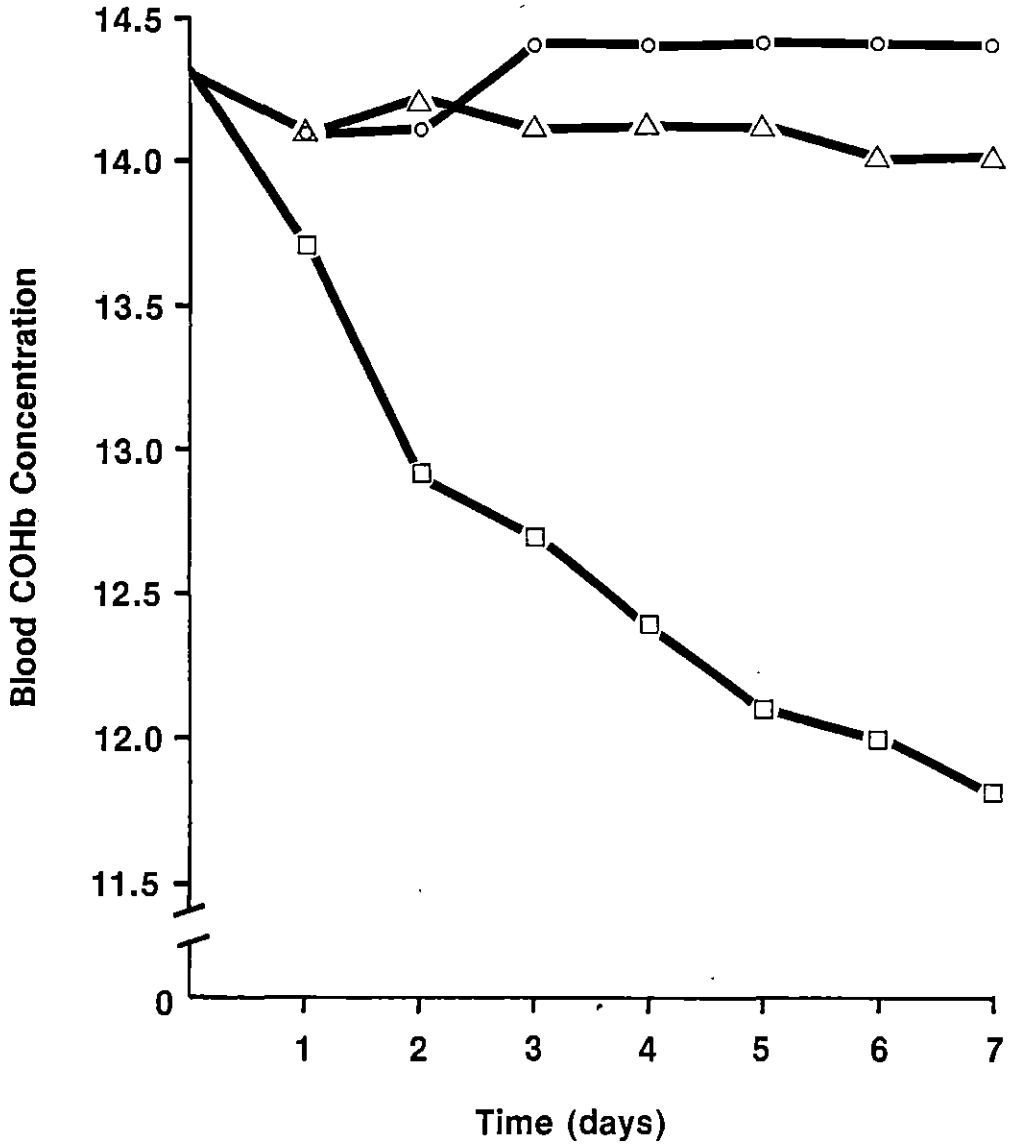
Sow number	Time[a]	Maternal % COHb (150 ppm CO exposure) 0	1	2	3	4	5	6	7
86		13.7	13.8	12.3	11.9	11.8	11.5	11.4	11.6
2		15.2	15.4	14.0	14.7	14.0	13.7	13.6	13.2
5		13.8	12.7	12.2	12.2	11.8	11.5	11.4	11.0
88		14.8	13.7	13.6	13.1	12.7	12.3	11.7	11.6
94		12.8	11.2	10.8	10.7	10.2	9.8	9.8	9.8
90		15.6	15.7	14.7	14.2	14.0	14.0	14.0	13.8
92		14.3	13.2	12.8	12.4	12.1	12.0	11.8	11.7

[a]The number of days since exposure to elevations in atmospheric CO.

df = 2, 18). When the stability of % COHb stored at varied temperatures was considered at day 7, a highly significant difference in % COHb due to treatment effects could be demonstrated at $p < .0023$ ($F = 8.65$, df = 2, 18).

The significant differences in COHb concentrations attributable to temperature treatment effects at day 7 were assumed to be a function of the significant drop in COHb concentrations determined in blood samples stored at -17.6°C. This can be visualized in Figure 15 where mean COHb concentrations for each time period and treatment are presented.

Figure 15. Mean COHb concentrations for each time period and temperature treatment are presented. The circles, triangles and squares represent blood COHb concentrations determined over time in blood samples stored at 22, 4.4 and -17.6°C, respectively


14.5
14.0
13.5
13.0
12.5
12.0
11.5
0
1
2
3
4
5
6
7
Blood COHb Concentration
Time (days)

DISCUSSION AND CONCLUSIONS

A higher incidence of stillbirths was found in pregnant sows exposed to atmospheric CO concentrations at or exceeding 250 ppm when compared to pregnant sows not exposed to CO. Nearly 35% of the piglets were stillborn when full-term sows were exposed to 250 ppm CO for 48 hours or more. The incidence of stillbirths increased as the level of CO exposure was increased with an overall stillbirth rate of 80% recorded in sows exposed to CO concentrations of 350 ppm. All sows exposed to atmospheric concentrations of 150-200 ppm CO delivered normal, healthy piglets with the exception of one sow exposed to 200 ppm CO that delivered one live and one dead piglet. In contrast, field cases of CO-induced stillbirths in swine have been reported at levels between 120-200 ppm CO (Carson et al., 1980; Keller, 1976; Wood, 1978, 1979). In addition, the stillbirth rates recorded were significantly higher than the reported normal stillbirth rate in swine at 6% (Sprecher et al., 1974).

A linear relationship appears to exist between the maternal COHb concentration and the atmospheric CO concentration in the range of 150 to 350 ppm. The predicted value for the maternal COHb concentration at 250 ppm CO exposure was calculated as 23.9%. This is consistent with previous work with miniature pigs kept in an atmosphere containing approximately 250 ppm CO where a concentration of 22% COHb was attained within 8 hours (Klimisch et al., 1975).

A strong correlation exists between the maternal COHb concentration and the incidence of stillbirth in each litter. No stillbirths occurred

in sows exposed to CO for 48-72 hours when the resultant maternal COHb ranged from 13-18% saturation of total Hb. However, CO-induced stillbirths occurred when maternal COHb concentrations exceeded 23% following 48-96 hours of CO exposure to sows at 108-110 days gestation. This suggests that at equilibrium, a fetal COHb concentration high enough to be a factor in hypoxic fetal death is attained when the maternal COHb concentration reaches a critical level of greater than 23%.

Previous work by Longo and Hill (1977) suggests that fetal COHb does not reach a steady state until 36-48 hours post-exposure in sheep. They found that at equilibrium, COHb concentrations in the fetus exceeded concentrations in the mother by 58%. This implied that fetal Hb has a higher affinity for CO than maternal Hb. If similar uptake kinetics occur in swine, fetal COHb concentrations at or exceeding maternal concentrations would be expected after 48 hours exposure.

Fetal Hb appears to have a higher affinity for CO than maternal Hb in swine. Previous research shows that fetal pig Hb has a higher affinity for O_2 than adult pig Hb (Novy et al., 1973). COHb concentrations exceeding maternal COHb concentrations were found in piglets recovered by Cesarean section one hour after CO exposure to the sow. It would seem likely that a partial decline in fetal COHb concentration had already occurred one hour post-exposure. Nevertheless, piglet COHb concentrations exceeded maternal values by 3 to 22%. The live piglets recovered by Cesarean section were weak and lethargic with COHb concentrations ranging from 24.2-39.0% in two of three litters examined. Previous work with pregnant ewes demonstrated fetal COHb concentrations of 15% for 30 minutes killed 57% of the fetuses in the

study (Longo and Hill, 1977). These results suggest that the porcine late-term fetus is less susceptible than the fetal lamb to elevations in fetal COHb concentration and that higher concentrations of fetal COHb are necessary to cause intrauterine hypoxic death of the porcine fetus.

A high degree of variability occurred in the stillbirth rates per litter when maternal COHb concentrations exceeded 23% after 48-96 hours of CO exposure. Contrasting the results from two litters, a maternal COHb concentration of 24.6% correlated with a 100% stillbirth rate, while a maternal COHb concentration of 29.2% resulted in a stillbirth rate of only 16.7%. These differences may be accounted for by maternal and fetal biological variation, differences in placental blood flow and litter size or by differences in the gestation length when CO exposure was initiated. Although all sows were exposed to CO at 108-110 days gestation, the average gestation length in swine is 114 ± 4 days and such variability in the normal gestation length suggests that fetuses may have been exposed to CO at term or several days prior to term.

The stage of fetal maturity during maternal CO exposure may be an essential factor in the pathogenesis of CO-induced stillbirths in swine when fetal COHb concentrations are not sufficiently elevated to cause hypoxic death of the fetus directly. A fetal pituitary-adrenal axis is implicated as the initiator of parturition in swine (First and Bosc, 1979). Presumably, fetal cortisol indirectly causes leutolysis by increasing prostaglandin $f_2\alpha$ concentrations in maternal blood. Prostaglandin $f_2\alpha$ also stimulates the pituitary release of oxytocin, increases plasma relaxin concentrations, and directly increases the contractility

of the myometrium. It has been suggested that the fetus must reach a certain stage of maturity before fetal cortisol can initiate the sequence of events that lead to parturition. It seems plausible that near-term fetuses at the necessary critical stage in maturity and simultaneously subjected to CO-induced hypoxia could release cortisol in response to stress and subsequently initiate parturition.

The porcine fetus is normally subjected to intermittent hypoxia for up to 24 hours prior to delivery once uterine contractions have been initiated (First and Bosc, 1979). Although the fetus normally overcomes this intermittent hypoxia, a fetus concurrently challenged by CO-induced hypoxia may find the additional stress of parturition life-threatening, whereas a fetus subjected to CO-induced hypoxia prior to reaching the critical age necessary for initiation of parturition would not experience the additional hypoxia associated with parturition. If the fetal COHb concentration was restored to normal before parturition ensued, an additional fetal stress from prior maternal exposure to CO may not be a factor. This mechanism may partially account for the recorded variability in stillbirth rates per litter when maternal COHb concentrations were similar.

Moderate to severe intrauterine autolysis occurred when maternal CO exposure led to intrauterine death of all piglets within a litter. Three sows delivered all dead piglets and in each case, piglets were retained for 10-17 days past the calculated due date. The delay in parturition experienced by these sows led to the severe intrauterine autolysis of the piglets observed. Much less autolysis was observed in stillborn piglets when live piglets were delivered in the same litter at the

calculated due date. Presumably, the lack of induction of parturition by viable piglets prevented the delivery of dead piglets at term and prolonged retention occurred.

Common gross lesions of CO intoxication in the piglet were cherry red discoloration of the subcutaneous tissues and thoracic viscera, and the accumulation of a large volume of red-brown thoracic fluid. These lesions were found in the majority of stillborn piglets necropsied in this study. Similar gross lesions have been recorded in field cases of CO-induced stillbirths in swine. The pleural effusion noted in the thoracic cavity of stillborn piglets represented leakage of blood constituents during the autolytic process.

Piglet thoracic fluid appears to be a useful indicator of fetal CO exposure. Analysis of thoracic fluid revealed detectable COHb concentrations ranging from .4-10.1% saturation Hb in CO intoxicated stillborn piglets from five litters. No detectable COHb was found in the thoracic fluid of nonexposed piglets. Thoracic fluid COHb concentrations were lowest in piglets retained in utero the longest beyond term. No correlation between maternal COHb concentrations and piglet thoracic fluid COHb concentrations was made since only a limited number of piglet thoracic fluid samples were available for analysis. The thoracic fluid COHb concentrations recorded in live piglets delivered by sows 7 and 5 were markedly lower than COHb concentrations in the thoracic fluid of stillborn piglets in the same litters. This latter observation suggests that analysis of piglet thoracic fluid for COHb may represent a reliable diagnostic aid for determination of CO poisoning in prenatal piglets.

Prolonged, low level maternal CO exposure during late term gestation may produce hypoxic-ischemic leukoencephalopathy in the fetal and neonatal piglet. Focal leukoencephalomalacia was found in one stillborn piglet only; however, histologic examination of stillborn piglet brains was seldom completed due to extensive post-mortem autolysis. On examination of live piglets from two litters recovered at term by Cesarean section and natural birth, respectively, there were focal leukoencephalomalacia and spongiosus, astrocytic and microglial proliferation, multifocal petechial hemorrhages, and changes suggestive of myelin degeneration in the cortical white matter, brain stem and cerebellum. Similar lesions associated with CO poisoning have been described in man. Lapresle and Fardeau (1967) described three major categories of white matter damage produced by high level CO exposure in man. The first type described involves multifocal necrotic lesions of the deep hemispheral white matter with extensive astrocytic and microglial proliferation. The second category represents extensive necrotic zones in the hemispheral white matter. The third form of leukoencephalopathy involves symmetrical myelin damage with the lesion distribution predominantly in the deep central white matter. The piglets with histological evidence of CO-induced leukoencephalopathy developed necrotic lesions primarily within the hemispheral white matter in this investigation.

Few animal studies have been completed where maternal CO exposure has led to the development of fetal brain lesions. In previous work with pregnant monkeys cerebral edema of the fetal brain, necrosis of the basal ganglia and sparing of the cerebral cortex were seen following a single maternal exposure to 1000-3000 ppm inspired CO over

1-3 hours (Ginsberg and Myers, 1974, 1976). The brain lesions observed were believed the result of fetal hypoxia and cerebral hypoperfusion, giving rise to extensive cerebral edema, further reducing cerebral perfusion. No previous research could be found where fetal brain injury occurred following prolonged low level maternal CO exposure.

The livers of piglets exposed to CO *in utero* contained high numbers of mononuclear cell clusters resembling extramedullary hematopoietic centers containing primarily rubriblasts. Extramedullary hematopoietic centers are normally present in variable numbers in the fetal liver up to the time of birth (Arey, 1965). With a sudden onset of CO-induced fetal hypoxia, release of erythropoietin by the kidney would be a logical sequela. Erythropoietin would stimulate unipotential stem cell duplication and ultimately differentiation into rubriblasts (Duncan and Prasse, 1977). However, mature reticulocytes would not be expected to appear in the blood until 5-6 days following the hypoxic stimulation. Pregnant sows were exposed to CO for 48-96 hours and maximum fetal COHb concentrations were probably not reached for 24-36 hours following maternal exposure. Therefore, the fetal response to hypoxia may have developed sometime after maternal CO exposure began and primarily stem cells and rubriblasts would have been present in extramedullary hematopoietic centers seen histologically in the liver. Similar cells appeared in high numbers in the liver sections examined from experimental piglets. However, absolute identification of the cells as rubriblasts would depend on electron microscopic examination.

Blood sample storage under chilled conditions or at room temperature appears to provide stable COHb concentrations in the blood over time.

The precision in measurement of blood COHb concentrations was excellent when samples were stored at 22°C or 4.4°C and analyzed daily for seven days. Blood samples frozen to -17.6°C and thawed prior to analysis for COHb concentration had a significant drop in % COHb over time. This decline may have been associated with hemolysis of the sample during the freezing/thawing procedure or the result of thermal and enzymatic denaturation of the COHb moiety. Based on these results, blood samples submitted to a diagnostic laboratory for COHb analysis should be shipped at temperatures above freezing to increase the reliability of the results obtained.

SUMMARY

The incidence of stillbirths in near term pregnant sows exposed to prolonged low levels of CO was investigated. Sows at 108-110 days gestation were exposed to atmospheric CO concentrations of 150-400 ppm for 48-96 hours. No clinical manifestations of CO intoxication were observed in the sows. The incidence of stillbirth increased significantly as the level of maternal CO exposure was increased. Stillbirth rates of 6.7, 34.8, 42.3 and 80.0% were recorded at 200, 250, 300 and 350 ppm CO exposure, respectively. This compared to a stillbirth rate of 4.3% in control sows. A highly significant linear relationship was determined between atmospheric CO exposure levels and the resultant maternal COHb concentration ($p < .0001$). The incidence of stillbirth increased significantly above controls when maternal COHb concentrations exceeded 23% saturation of Hb. Fetal Hb appeared to have a higher affinity for CO than maternal Hb. Live piglets collected by Cesarean section following 96 hours of maternal CO exposure had consistently higher COHb concentrations than maternal values. Common gross lesions in CO-intoxicated, stillborn piglets were cherry red discoloration of the subcutaneous tissues and viscera and the accumulation of a large volume of red-brown pleural effusion in the thoracic cavity. Histologically, there was hypoxic-ischemic leukoencephalopathy observed in brain sections from 3 of 14 litters. Lesions included focal leukoencephalomalacia, glial-vascular proliferation, multifocal hemorrhage and vacuolation of the neuropile and myelin degeneration. Thoracic fluid from stillborn piglets had detectable levels of COHb. Analysis

of piglet thoracic fluid for COHb appears to be a useful measure in determining fetal CO exposure. The effects of time and temperature on the stability of COHb concentration in the blood was analyzed. Blood COHb concentrations remained stable over time in blood samples stored at room temperature or chilled to 4.4°C. Storage of blood samples at -17.6°C led to a significant decline in COHb concentrations over time ($p < .005$).

REFERENCES

Anson, M. L., Barcroft, J., Mirsky, A. E., and Oinuma, S. 1924-1925. On the correlation between the spectra of various hemoglobin and their affinities for oxygen and carbon monoxide. Proceedings of the Royal Society of London SB. 97: 61-83.

Antonini, E. 1967. Hemoglobin and its reactions with ligands. Science 158: 1417-1425.

Arey, L. B. 1965. Developmental Anatomy. A Textbook and Laboratory Manual of Embryology. W. B. Saunders Company, Philadelphia, Pennsylvania.

Ayers, S. M., Giannelli, Jr., S., and Mueller, H. 1970. Myocardial and systemic responses to carboxyhemoglobin. Annals of the New York Academy of Science 174: 268-293.

Bartels, H. W., and Bauer, Ch. 1969. Relation between oxygen dissociation curve and tissue oxygenation. Försvarsmedicin 5: 227-233.

Bartels, H. W., and Moll, J. M. 1962. Physiology of gas exchange in the human placenta. American Journal of Obstetrics and Gynecology 84: 1714-1730.

Bartlett, D. 1968. Pathophysiology of exposure to low concentrations of carbon monoxide. Archives of Environmental Health 16: 719-727.

Bissonnette, J. M., and Wickham, W. K. 1977. Placental diffusing capacity for carbon monoxide in unanesthetized guinea pigs. Respiration Physiology 31: 161-168.

Bissonnette, J. M., Wickham, W. K., and Drummond, W. H. 1977. Placental diffusing capacities at varied carbon monoxide tensions. Journal of Clinical Investigation 59: 1038-1044.

Brierly, J. B., Brown, A. W., Excell, B. J., and Meldrum, B. S. 1969. Brain damage in the rhesus monkey resulting from profound arterial hypotension; I. Its nature, distribution and general physiological correlates. Brain Research 13: 68-100.

Brucker, J. M. 1967. Neuropathological problems posed by carbon monoxide poisoning and anoxia. Progress in Brain Research 24: 75-100.

Burns, B., and Gurtner, G. 1973. A specific carrier for O_2 and CO in the lung and placenta. Drug Metabolism and Disposition 1: 374-379.

Burrow, B., and Niden, A. H. 1963. The effects of anemia and hemorrhagic shock on pulmonary diffusion in the dog lung. Journal of Applied Physiology 18: 123-128.

Carson, T. L., Donham, K. J., Dominick, M. A., Bertram, T. A. 1980. Carbon monoxide induced abortion in swine: An update. American Association of Veterinary Laboratory Diagnosticians 23: 59-66.

Carson, T. L., and Donham, K. J. 1978. Carbon monoxide abortion in Iowa swine. American Association of Veterinary Laboratory Diagnosticians 21: 179-184.

Coburn, R. F. 1970. The carbon monoxide body stores. Annals of the New York Academy of Science 174: 11-22.

Coburn, R. F. 1979. Mechanisms of carbon monoxide toxicity. Preventive Medicine 8: 310-322.

Coburn, R. F., and Mayers, L. B. 1971. Myoglobin O_2 tension determined from measurements of carboxymyoglobin in skeletal muscle. American Journal of Physiology 220: 66-74.

Coburn, R. F., Blakemore, W. S., and Forster, R. E. 1963. Endogenous carbon monoxide production in man. Journal of Clinical Investigation 42: 1172-1178.

Coburn, R. F., Forster, R. E., and Kane, P. B. 1965. Considerations of the physiological variables that determine the blood carboxyhemoglobin concentration in man. Journal of Clinical Investigation 44: 1899-1910.

Coughey, W. S. 1970. Carbon monoxide bonding in hemeproteins. Annals of the New York Academy of Science 174: 148-153.

Curtis, G. W., Algeri, E. J., McBay, A. J., and Ford, R. 1955. The transplacental diffusion of carbon monoxide. The American Medical Association Archives of Pathology 39: 677-690.

Donham, K. J., Carson, T. L., Adrian, B. R., and Gay, W. ca. 1982. The relationship of environmental levels of carbon monoxide and blood levels of carboxyhemoglobin in swine. Journal of the American Veterinary Medical Association.

Douglas, C. G., Haldane, J. S., and Haldane, J. B. S. 1912. The laws of combination of haemoglobin with carbon monoxide and oxygen. Journal of Physiology 44: 275-304.

Duncan, J. R., and Prasse, K. W. 1977. Veterinary Laboratory Medicine. Iowa State University Press, Ames, Iowa.

Ehrich, W. E., Bellet, S., and Lewey, F. H. 1944. Cardiac changes from CO poisoning. American Journal of the Medical Sciences 208: 511-523.

First, N. L., and Bosc, M. J. 1979. Proposed mechanisms controlling parturition and the induction of parturition in swine. Journal of

Animal Science 48 (6): 1407-1421.

Forbes, W. H., Sargent, F., and Roughton, F. J. W. 1945. The rate of carbon monoxide uptake by normal men. American Journal of Physiology 143: 594-608.

Forster, R. E. 1970. Carbon monoxide and the partial pressure of oxygen in tissue. Annals of New York Academy of Science 174: 233-241.

Garvey, D. J., and Longo, L. D. 1978. Chronic low level maternal carbon monoxide exposure and fetal growth and development. Biology of Reproduction 19: 8-14.

Ginsberg, M. D. 1980. Carbon Monoxide. Pages 374-394 in P. S. Spencer and H. H. Schaumburg, eds. Experimental and Clinical Neurotoxicology. Williams and Wilkins, Baltimore, Maryland.

Ginsberg, M. D., and Myers, R. E. 1974. Fetal brain damage following maternal carbon monoxide intoxication: An experimental study. Acta Obstetricia et Gynecologica Scandinavica 53: 309-317.

Ginsberg, M. D., and Myers, R. E. 1976. Fetal brain injury after maternal carbon monoxide intoxication. Clinical and neuropathologic aspects. Neurology 26: 15-23.

Ginsberg, M. D., Myers, R. E., and McDonogh, B. F. 1974. Experimental carbon monoxide encephalopathy in the primate. II. Clinical aspects, neuropathology, and physiologic correlations. Archives of Neurology 30: 209-216.

Goldbaum, L. R., et al. 1977. Studies on the relationship between carboxyhemoglobin concentration and toxicity. Aviation, Space and Environmental Medicine 969-970.

Haldane, J. B. S. 1912-1913. The dissociation of oxyhaemoglobin in human blood during partial CO poisoning. Journal of Physiology 45: xxii-xxiv.

Johnson, R. L., Taylor, H. F., and Lawson, W. H. 1965. Maximal diffusing capacity of the lung. Journal of Clinical Investigation 44 (3): 349-355.

Keller, H. 1976. Higher carbon monoxide content of barn as a cause of swine stillbirths. Schweizer Archiv fuer Tierheilkunde 118: 425-428.

Killick, E. M. 1940. Carbon monoxide anoxemia. Physiology Review 20: 313-344.

Klimisch, H. J., Chevalier, H. J., Harke, H. P., and Dontenwill, W. 1975. Uptake of carbon monoxide in blood of miniature pigs and other mammals. Toxicology 3: 301-310.

Kogure, K., Scheinberg, P., Reinmuth, O. M., Fujishima, M., and Busti, R. 1970. Mechanisms of cerebral vasodilatation in hypoxia. Journal of Applied Physiology 29: 223-229.

Kruhoffer, P. 1954. Studies on the lung diffusion coefficient for carbon monoxide in normal human subjects by means of C14O. Acta Physiologica Scandinavica 32: 106-123.

Lapresle, J., Fardeau, M. 1967. The central nervous system and carbon monoxide poisoning. II. Anatomical study of brain lesions following intoxication with carbon monoxide (22 cases). Progress in Brain Research 24: 31-74.

Lilienthal, Jr., J. L. 1950. Carbon monoxide. Pharmacology Review 2: 324-354.

Longo, L. D. 1970. Carbon monoxide in the pregnant mother and fetus and its exchange across the placenta. Annals of the New York Academy of Science 174: 313-341.

Longo, L. D. 1976. Carbon monoxide: Effects on oxygenation of the fetus in utero. Science 194: 523-525.

Longo, L. D., and Ching, K. S. 1977. Placental diffusing capacity for carbon monoxide and oxygen in unanesthetized sheep. Journal of Applied Physiology 43: 885-893.

Longo, L. D., and Hill, E. P. 1977. Carbon monoxide uptake and elimination in fetal and maternal sheep. American Journal of Physiology 232: 324-330.

Longo, L. D., Power, G. G., and Forster II, R. E. 1967. Respiratory function of the placenta as determined with carbon monoxide in sheep and dogs. Journal of Clinical Investigation 46: 812-828.

Longo, L. D., Power, G. G., and Forster II, R. E. 1969. Placental diffusing capacity for carbon monoxide at varying partial pressures of oxygen. Journal of Applied Physiology 26: 360-370.

Luomanmäki, K. 1966. Studies on the metabolism of carbon monoxide. Annals Medicinae Experimentalis et Biologiae Fenniae 44: 1-55.

Newman, F., Smalley, B. F., and Thomson, M. L. 1962. Effect of exercise, body and lung size on CO diffusion in athletes and nonathletes. Journal of Applied Physiology 17: 649-654.

Novy, M. J., Hoversland, A. S., Dhindsa, D. S., and Metcalfe, J. 1973. Blood oxygen affinity and hemoglobin type in adult, newborn and fetal pigs. Respiration Physiology 19: 1-11.

Otis, A. B., and Jude, J. 1957. Effect of body temperature on pulmonary gas exchange. American Journal of Physiology 188: 355-359.

Pace, N., Strajman, E., and Walker, E. L. 1950. Acceleration of carbon monoxide elimination in man by high pressure oxygen. Science 111: 652-654.

Penny, D. G., Baylerian, M. S., and Fanning, K. E. 1980. Temporary and lasting cardiac effects of pre- and postnatal exposure to carbon monoxide. Toxicology and Applied Pharmacology 53: 271-278.

Peterson, J. E., and Stewart, R. D. 1970. Absorption and elimination of carbon monoxide by inactive young men. Archives Environmental Health 21: 165-171.

Power, G. G., Aoki, V. S., Lawson, W. H., and Gregg, J. B. 1969. Pulmonary diffusing capacity at reduced temperatures. Clinical Research 17: 418. (Abstract)

Power, G. G., and Longo, L. D. 1975. Fetal circulation times and their implications for tissue oxygenation. Gynecology Investigation 6: 342-355.

Roberts, S. J. 1971. Obstetrics and Genital Diseases. S. J. Roberts, Ithaca, New York.

Root, W. S. 1965. Carbon Monoxide. Pages 1087-1098 in W. O. Fenn and H. Rahn, eds. Handbook of Physiology. Volume II. Section 3: Respiration. American Physiological Society, Washington, D.C.

Rossi-Fanelli, A., and Antonini, E. 1958. Studies on the oxygen and carbon monoxide equilibria of human myoglobin. Archives of Biochemistry 77: 478-492.

Roughton, F. J. W., and Darling, R. C. 1944. The effect of carbon monoxide on the oxyhemoglobin dissociation curve. American Journal of Physiology 141: 17-31.

Roughton, F. J. W., and Root, W. S. 1945. The fate of CO in the body during recovery from mild carbon monoxide poisoning in man. American Journal of Physiology 145: 239-252.

Scheuer, J. 1967. Myocardial metabolism in cardiac hypoxia. American Journal of Cardiology 19: 385-392.

Siesjo, B. K., and Plum, F. 1973. Pathophysiology of Anoxic Brain Damage. Page 319 in G. E. Goull, ed. Biology of Brain Dysfunction. Volume I. Plenum Press, New York, New York.

Snedecor, G. W., and Cochran, W. G. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa.

Sprecher, D. J., Leman, A. D., Dziuk, P. J., Cropper, M., and DeDecker, M. 1974. Causes and control of swine stillbirths. Journal of the American Veterinary Medical Association 165: 698-701.

Stadler, E. 1973. Carbon monoxide poisoning in a swine breeding herd. Praktische Tierarzi 54 (8): 337-338.

Stewart, R. M. 1920. A contribution to the histopathology of carbon monoxide poisoning. The Journal of Neurology and Psychopathology 1: 105-116.

Wagner, J. A., Howath, S. M., and Dahms, T. E. 1975. Carbon monoxide elimination. Respiration Physiology 23: 41-47.

Wittenberg, J. B. 1966. Myoglobin-facilitated diffusion of oxygen. Journal of General Physiology 49: 57-74.

Wood, E. N. 1978. The incidence of stillborn piglets associated with high atmospheric carbon monoxide levels. The Pig Journal 3: 117-118.

Wood, E. N. 1979. Increased incidence of stillbirth in piglets associated with high levels of atmospheric carbon monoxide. Veterinary Record 104: 283-284.

ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. Thomas L. Carson, chairman of my graduate committee, for the guidance, support and patience he extended me throughout this investigation and during the preparation of the thesis.

I wish to thank the other members of my graduate committee: Dr. John P. Kluge, chairman of the Department of Veterinary Pathology; Dr. Dwaine S. Bundy; and my special thanks to Dr. Lawrence E. Evans for his surgical assistance in this project.

I would also like to thank Dr. W. E. Lloyd for his guidance and encouragement throughout my professional training in toxicology.

I wish to express my sincere appreciation to Dr. Vaughn A. Seaton, head of the Iowa Veterinary Diagnostic Laboratory, for his support and encouragement during my residency in toxicology.

I wish also to thank Larry Elsken and Jerry Petersen for their technical assistance with this project. Their dedicated, conscientious animal care and assistance in monitoring the sows in the environmental chamber during CO exposure periods is deeply appreciated.

Lastly, my special thanks to my wife, Sally, for her support and encouragement during all of my professional training.