Ethanol Disposition and Effect in Pregnant Sheep and Newborn Lambs

A thesis presented to the University of Manitoba

In partial fulfillment of the requirements for the degree of Master of Science

By

Marianne E. Cumming

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ABSTRACT

Healthy pregnant sheep, gestational age 124 to 142 days, were surgically prepared with indwelling catheters in the maternal and fetal femoral arteries and veins. A 9.5 percent v/v solution of ethanol in 5 percent w/v dextrose in water was infused via the maternal femoral vein at a rate of 15 ml/kg over 2 hours (1.2 g/kg). Simultaneous blood samples were obtained from the maternal and fetal femoral arteries, at 30 minute intervals starting just prior to the infusion and for 5 to 7 hours after the infusion. Peak maternal and fetal ethanol concentrations, both at 2 hours after start of infusion, were 1.8 ± 0.1 and 1.9 ± 0.1 g/L (mean ± S.E.) respectively. Maternal and fetal plasma ethanol clearance rates were 203 ± 13 and 206 ± 5 mg/L/h respectively. Slight increases in maternal and fetal heart rates were observed with maternal ethanol infusion, but no other cardiovascular changes were demonstrated.

Infusion of this same ethanol solution at the same rate into the fetus for 1 hour (0.6 g/kg) resulted in a peak concentration 4 times greater in the fetus than the mother, 4.7 and 1.2 g/L respectively. Fetal death occurred 5 minutes later. Fetal ethanol infusion of 0.5 g/kg over 4 hours also produced a peak concentration 4 times greater in the fetus than in the mother, 1.2 and 0.3 g/L respectively. This difference was minimized using a lower infusion rate of 0.3 g/kg over 3.5 hours. Maternal and fetal peak concentrations were 0.5 and 0.4 g/L respectively. These data may be interpreted as evidence for impaired transfer of ethanol from fetus to mother. Maternal and fetal plasma ethanol clearance rates were similar to those in the maternal infusion studies, 180 ± 11 and 197 mg/L/h respectively. Fetal heart rate was found to decrease with increasing ethanol concentration in this series,

an effect opposite to that observed in the maternal infusion studies. This suggests that reflex responses in the maternal infusion studies may cause different cardiovascular effects in the fetus depending on the site of infusion. The only other change noted was a slight decrease in maternal blood pressure.

Newborn lambs were surgically prepared with indwelling catheters in the femoral artery and vein. The same ethanol solution was infused via the femoral vein at a rate of 0.6 g/kg over 1 hour. Arterial blood samples were collected every 30 minutes for 2 hours, and every 60 minutes thereafter until 8 hours after start of infusion. Peak plasma ethanol concentration, at the end of the infusion was 0.64 $\pm$ 0.03 g/L. Plasma ethanol clearance rates ranged from 22 - 56 mg/L/h, which is less than 25 percent of that observed in near term pregnant ewes. Plasma clearance in 3 or 5 day old lambs (46 - 56 mg/L/h) was greater than in 1 or 2 day old lambs (22 - 26 mg/L/h). Thus, ethanol disposition is impaired in the neonatal lamb. No significant cardiovascular changes were observed. These data are consistent with the interpretation that in the pregnant ewe, the fetus is primarily dependent on maternal metabolism for elimination of ethanol from its environment.

The activity of alcohol dehydrogenase (ADH) in maternal liver was determined to be 729 $\pm$ 150 mU/g tissue (n=4) with a pH optimum of 9.0. Fetal ADH activity was 72 $\pm$ 9 mU/g tissue (n=5) with a pH optimum of 9.6. This is similar to the activity of newborn lamb liver ADH, 54 $\pm$ 11 mU/g tissue (n=9), with a pH optimum of 9.6. Liver enzyme activity was found to increase with lamb age. The activity of placental ADH was much lower, 15 $\pm$ 3 mU/g tissue (n=4), with a pH optimum of 9.0. No correlation between plasma ethanol clearance and liver ADH at optimal pH

or at pH 7.4 and 25 ^{o}C or at pH 7.4 and 37 ^{o}C for the same animals could be shown for neonatal lambs.

ADH activity and the capacity for ethanol elimination in the neonate are diminished. This may present problems to the intoxicated neonate in his physiological adaptation to extrauterine environment after birth.

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INTRODUCTION

A. General Pharmacological Properties of Ethanol

Ethanol, commonly referred to as ethyl alcohol or alcohol, was once considered a remedy for practically all diseases. It is now recognized that its legitimate therapeutic use is more limited than its social use. Ethanol is the active ingredient of beer, wine, spirits, and other alcoholic beverages. It is widely used as a solvent for many drugs and is used as a vehicle for medicinal preparations. Ethanol concentrations of 40 percent or more may be found in some oral drug products (Parker and Baillie 1983). Ethanol is used topically as an astringent and skin disinfectant. It has also been used to improve appetite and digestion. Injection of dehydrated ethanol in close proximity to nerves or sympathetic ganglia results in relief of pain. It is sometimes used in trigeminal neuralgia and inoperable cancer (Ritchie 1975).

Excessive consumption of ethanol can create many psychological, medical and sociological problems. The costs to society in terms of lost production, crime, fire, welfare, motor vehicle accidents, health and medical care and alcohol programs and research are immense. Alcohol research cannot focus on any one aspect, but should encompass epidemiological, etiological, legal, and pharmacological studies. Certain diseases have been recognized as consequences of direct or indirect toxicity of ethanol. The acute and chronic physiological effects of ethanol have been studied in man and animals. However, the effects of ethanol on the developing fetus have only recently been realized and must be explored further.

a. Central Nervous System

The central nervous system (CNS) is affected by acute ethanol ingestion to a greater degree than any other body system. Most obvious

are the effects of acute ethanol intoxication. The effects of ethanol on the CNS are proportional to the blood ethanol concentration. Ethanol is a primary and continuous depressant of the CNS. At blood ethanol concentrations of 0.3 g/L, non-habituated drinkers experience mild euphoria. Depression of subcortical inhibitory control mechanisms results in unrestrained activity which has led to the misconception that ethanol is a stimulant. Motor performance, including speech and eye movement control, as well as complex motor skills and learning processes, becomes slower, less accurate and random as blood ethanol concentrations reach 1.0 g/L. As intoxication proceeds to approach blood ethanol concentrations of 4.0 g/L or greater, the depressant action spreads and general impairment of nervous function results. A condition of general anesthesia, respiratory depression and death may follow. The association of blood ethanol concentrations with CNS effects is not valid in chronic alcoholics who develop tolerance to the effects (Ritchie 1975).

Chronic excessive ethanol ingestion is directly associated with serious neurological and mental disorders including brain damage, memory loss, sleep disturbances and psychosis. Nutritional deficiencies in alcoholics may lead to neuropsychiatric syndromes. Wernicke's encephalopathy, Korsakoff's psychosis, alcoholic polyneuropathy, and cerebral degeneration result from chronic ethanol consumption (Nakada et al. 1984).

b. Cardiovascular System

Ethanol has significant and deleterious physiological and biochemical effects on the cardiovascular system. Generally, ethanol is a myocardial depressant after acute ingestion. Depressed contractility

of ventricular muscle due to ethanol has been demonstrated in vitro in rats (Hirota et al. 1976). Acute in vivo experiments in man have shown an increase in cardiac output associated with increases in heart rate or stroke volume (Riff et al. 1969). However, more recent studies using systolic time intervals have demonstrated that acute ethanol ingestion produces definite depression of left ventricular contractility in normal volunteers (Ahmed et al. 1973). Studies in non-alcoholic patients with heart disease have proven the myocardial depressant effects of ethanol (Gould et al. 1971). However, Greenberg et al. (1982) reported no evidence of acute deterioration of cardiac performance in patients with congestive heart failure. Segel et al. (1984) concluded that the net mechanical effects of acute ethanol ingestion in patients with cardiac disease will vary according to the relative influences of ethanol-induced peripheral vasodilation and direct myocardial depression as well as the underlying cardiac reserve.

Chronic ethanol ingestion is associated with alcoholic cardiomyopathy. The cellular mechanism of ethanol's direct depressant effect on cardiac muscle has not been established. The acute effects of ethanol may be related to increases in membrane fluidity, and the chronic effects may be due to alterations in membrane lipids (Katz 1982). Ethanol has been shown to inhibit mitochondrial and sarcoplasmic reticulum calcium ion transport activity (Retig et al. 1977). Increased triglyceride content, depressed mitochondrial respiration, depressed myofibrillar adenosine triphosphatase activity, and decreased protein synthesis have also been postulated as possible biochemical changes contributing to alcoholic heart disease (Bing 1982).

The acute effects of ethanol on the electrohysiological properties of the heart were studied in vivo by James and Bear (1967) who perfused sinus node arteries in dogs. No chronotropic changes were seen except with lethal doses of ethanol. However, a marked acceleration of heart rate was observed with acetaldehyde. Both atrial and ventricular arrhythmias have been observed after the onset of heart failure in alcoholics (Bashour et al. 1975), but no electrophysiological changes have been observed with acute ethanol ingestion by non-alcoholic subjects. The most consistent effect of ethanol on cardiac muscle in vitro is the shortening of action potential duration (Williams et al. 1980).

Moderate doses of ethanol cause vasodilation. Vasodilation of the coronary vasculature has led to the suggestion that low doses of ethanol may be beneficial to the myocardium. Vasodilation of the coronary vasculature has been consistently reported to be signifcantly greater in animals than in humans (Regan 1982). Acute doses of ethanol can produce peripheral vasodilation and lower blood pressure. These dilator actions may be related to calcium transport across vascular membranes (Altura and Altura 1982). Acute and chronic ethanol ingestion can result in cardiac and vascular alterations which may be due to ethanol itself or to its metabolite, acetaldehyde.

c. Skeletal Muscle

Ethanol ingestion is known to cause alcoholic myopathy. Acute alcoholic myopathy involves muscle swelling, myoglobinuria, and increased serum creatine phosphokinase. Chronic alcoholic myopathy, characterized by the gradual evolution of predominantly proximal weakness and muscle atrophy has been observed in chronic alcoholics. The

mechanisms underlying alcoholic myopathy are believed to be similar to those in alcoholic cardiomyopathy because of the similarities between cardiac and skeletal muscle (Rubin 1979).

d. Gastrointestinal Tract

Acute and chronic ethanol ingestion results in adverse effects on the gastrointestinal system. Increase in appetite after ethanol ingestion is attributed to stimulation of the end organs of taste and to a general sense of well being. Low concentrations of ethanol stimulate the gastric glands to produce acid, apparently by causing tissues to form or release histamine. Gastric secretions are inhibited by ethanol concentrations of about 20 percent. Concentrations of 40 percent and over are irritating to the mucosa and cause congestive hyperemia and a state of acute gastritis. There is no evidence to implicate ethanol as a cause of peptic ulcer disease. Acute and chronic ethanol ingestion interfere with the transport of fluid and nutrients in the small intestine. This may result in marked diarrhea and intestinal malabsorption. Acute and chronic ethanol ingestion alter pancreatic membrane structure and function (Burbige et al. 1984).

e. Metabolism and the Liver

Ethanol exerts numerous effects on intermediary metabolism in liver cells. Gluconeogenesis is inhibited, resulting in hypoglycemia. This may be associated with a reduction in the ratio of intracellular NAD:NADH caused by ethanol metabolism. Ethanol causes the accumulation of certain amino acids in hepatic cells. Decreased synthesis of transferrin, albumin, and complement result. Lipoprotein synthesis is increased and may cause hypertriglyceridemia. The decreased NAD:NADH ratio also causes an increase in lactate production which may lead to decreased urate

excretion and decreased activity of the tricarboxylic acid cycle. Increased generation of NADH may also be implicated in alcoholic ketoacidosis. Ethanol causes accumulation of fat in the liver, even with moderate intake. This is due to increased fat synthesis and mobilization of fat from peripheral tissues. The toxic effects of ethanol on the liver cause significant morbidity and mortality. Alcoholic hepatitis involving hepatocellular necrosis may present in chronic alcoholics. Cirrhosis is the irreversible end stage of alcoholic liver disease. It can follow alcoholic hepatitis but the relationship between cirrhosis and fatty liver is not clear (Isselbacker 1977).

Ethanol interferes with the metabolism of other drugs. Acute ethanol ingestion generally inhibits the metabolism of other drugs, most commonly by competition for a partially shared microsomal pathway in the liver. On the other hand, chronic ethanol ingestion generally enhances its own metabolism by increasing the activity of the microsomal ethanol oxidizing system (MEOS). This induction spills over to other drug metabolizing enzymes in hepatic micromsomes especially the cytochromes P-450 (Lieber 1977).

f. Kidney

Ethanol causes diuresis due to a transient supression of antidiuretic hormone release. It does not alter the sensitivity of the tubules to endogenous or exogenous antidiuretic hormone. The degree of diuresis seems to be related to the duration of the rise in ethanol concentration. It occurs only during the initial phase of ethanol administration and does not persist with prolonged drinking (Ritchie 1975).

The physiological effects of ethanol have been extensively studied in man and animals. However, until recently, the teratogenic and fetotoxic effects of ethanol have been overlooked. Whether the acute and chronic responses to ethanol in the fetus are similar to those in the adult or exaggerated has yet to be thoroughly investigated.

B. Ethanol Use During Pregnancy

a. Social Use

Although the deleterious effects of prenatal exposure to ethanol have long been recognized, not until the early 1970's has this association been seriously examined in scientific studies. Clinical alcohol intoxication is seen with alarming frequency during pregnancy. The estimated incidence of chronic alcoholism is between 0.42 and 1.2 per 1000 pregnancies (Hanson et al 1976). Maternal alcohol ingestion may result in fetal toxicity. A unique pattern of malformations in the offspring of chronic alcoholic mothers, identified as fetal alcohol syndrome (FAS) by Jones and colleagues (1973), has been well described. Over 450 cases have been reported in the literature (Sokol 1981). All cases have involved children of chronic alcoholic mothers (Sokol 1981). Features of the syndrome include; intrauterine and postnatal growth deficiency, as determined by weight, length, and head circumference, a distinctive pattern of physical malformations including microencephaly, shortened palpebral fissures, hypoplastic philtrum, thin upper lip, flat nasal bridge, joint, limb, eye and ear abnormalities, cardiac defects (primarily septal), and central nervous system dysfunction, including physiological depression, hypotonia, irritability, jitteriness, mental retardation, poor coordination, and hyperactivity during childhood (Newman & Correy 1980, Sokol 1981). The incidence of FAS is estimated to

be 1 to 2 live births per 1000 and partial expression of this syndrome occurs in about 3 to 5 live births per 1000. Depending on the population studied, the incidence of FAS in infants of chronic alcoholic mothers is between 30 and 40 percent (Streissguth et al. 1980). These figures are considered to underestimate the incidence of FAS as it often is misdiagnosed (Abel 1980). Alcohol related deficits may be missed in children at birth as the effects may not be immediately apparent or identifiable.

However, the amount of alcohol required to produce fetal damage is not known. There appears to be a continuum of effects of alcohol on the fetus with increased severity of outcome generally associated with higher alcohol intake by the mother (Little and Streissguth 1981). FAS is the most dramatic in the spectrum of adverse effects. Toxic rather than teratologic fetal effects are also observed. Stillbirths are found to occur twice as often in women having at least 3 drinks per day than in women having less than that amount. Spontaneous abortion in the second trimester has been shown to occur 3 times more frequently in women ingesting more than 3 drinks per day as in those having less than 1 drink per day. Disturbances in patterns of sleep and wakefulness in the newborn are attributed to maternal alcohol ingestion. There is no evidence that beverage content other than alcohol affects outcome. Outcome may be influenced by other variables such as smoking, hepatic dysfunction, other drugs, and poor nutrition (Rosett et al. 1983).

Certain differentials in alcohol related defects may depend on the stage of fetal development. The first trimester is critical for dysmorphology, the second trimester for fetal loss, and the third for impaired intrauterine growth. Rosett et al. (1983) reported that a

reduction in alcohol consumption in mid-pregnancy can benefit the newborn. Eighteen of 43 heavy drinkers (having not less than 45 drinks per month) markedly reduced alcohol consumption before the third trimester. Neonates born to women drinking heavily in early pregnancy, who reduced consumption before the third trimester were similar to offspring of rare and moderate drinkers in growth parameters, but exhibited more congenital abnormalities. More growth retardation was seen in the offspring of heavy drinkers (Rosett et al. 1983). The duration and timing of alcohol consumption require further exploration. The effect of binge drinking is not understood.

Despite its common occurrence, the acute effects of moderate alcohol consumption on the neonate are still not clearly defined. Inverse linear relationships between alcohol consumption and infant birth weight have been reported (Hanson et al. 1978). Other studies have found no relationship between moderate consumption and birth weight or frequency of physical anomalies (Tennes and Blackard 1980). Problems in study design make it difficult to obtain conclusive evidence. Self reports of drinking may not be reliable. The criteria for moderate drinking vary from 1 drink per month to 4 drinks per day and do not account for frequency and variability (Hanson et al. 1978). Further delineation of the kinds and degrees of fetal risk associated with light to moderate alcohol ingestion by the mother is required.

b. Medical Use

High morbidity and mortalilty have been associated with premature birth. Thus, different pharmacological methods, including the therapeutic use of alcohol, have been used to arrest labour and to postpone delivery when labour begins prior to term (Hemminki and

Starfield 1978). Fuchs et al. (1967) demonstrated that the administration of alcohol has definite inhibitory effects on uterine activity in early labour. Fuchs (1966) showed that alcohol inhibits the release of oxytocin by the posterior pituitary whereby it suppresses the milk ejection reflex and depresses uterine activity (Fuchs & Wagner 1963, Luukkainen et al. 1967). Alcohol does not act at the myometrial cellular level (Dilts 1970, Challis and Mitchell 1981). Fuchs et al. (1967) administered alcohol as a 9.5 percent v/v solution in 5 percent w/v dextrose in water intravenously to 68 patients in threatened premature labour. In the first 43 patients, a loading dose of 12.5 ml/kg body weight over one hour was followed by a maintenance dose of 1.25 ml/kg/hr. This dosage regimen, often associated with nausea, vomiting, and discomfort, was changed for the remaining 25 patients to a loading dose of 15 ml/kg over 2 hours, and a maintenance dose of 1.5 ml/kg/hr. This resulted in blood ethanol concentrations ranging from 0.9 to 1.6 g/L, as determined from the ethanol content of expired air. There were no real differences between the dose schedules with regard to final outcome. Alcohol was found to inhibit uterine contractions in all cases. In 43 cases, complete cessation of contractions was noted and in 25 cases, there was a distinct reduction in their intensity and frequency. The prevention of delivery was successful, meaning that labour was arrested and delivery was postponed for at least 3 days, in 35 of 42 patients with intact membranes, but in none of the 16 patients with ruptured membranes. It appeared that ethanol may be administered to patients in premature labour with some degree of clinical success (Fuchs et al. 1967). Fuchs & Fuchs (1981) reported a success rate of 64 percent in over 500 patients they have treated with ethanol since their first

publication. Some controversy exists over its effectiveness (Zlatnik et al. 1972, Graff 1971). Graff (1971) reported his attempts to arrest premature labour with alcohol a failure. However, inappropriate dosage schedules, early discontinuation of the infusion, and failure to repeat treatment may have contributed to failures (Fuchs & Fuchs 1981).

A prerequisite for any successful therapy is that it be safe for both mother and fetus. More knowledge concerning the effects of ethanol on the fetus is required. Many studies in the literature have investigated the fetal response to ethanol with conflicting results (Wagner et al. 1970, Idanpaan-Heikkila et al. 1972, Waltman et al. 1972). Neonatal toxicity and death associated with acute transplacental ethanol intoxication, have been reported when ethanol was used in attempts to prevent premature delivery (Cook et al. 1975, Jung et al. 1980, Peden et al. 1973). Wagner et al. (1970) investigated the effects of acute exposure to ethanol in a group of 6 premature infants and reported no significant changes in alertness, motor activity, circulation, respiration, or acid base status, even with blood ethanol concentrations as high as 0.9 g/L. Barrada et al. (1977) reported a significant decrease in the incidence of respiratory distress syndrome (RDS) and the mortality associated with it, in neonates of mothers treated with ethanol. They concluded that ethanol exerted a protective effect on RDS development in neonates, which is best realized after a full 6 hour course of treatment. Zervoudakis et al. (1980) reported a higher incidence of RDS in 165 ethanol-treated infants compared to control infants in a retrospective study. Blood ethanol concentrations for mother and fetus were not reported. They concluded that the intravenous administration of ethanol should be discontinued as soon as

it is evident that labour cannot be arrested in order to minimize risk to the immature fetus.

Idanpaan-Heikkila et al. (1972) studied placental transfer and elimination as well as the clinical and metabolic effects of ethanol in the mother, fetus, and newborn infant. Six healthy pregnant women received an infusion of 8 percent v/v ethanol in normal saline at a rate of 6 ml/minute beginning 1.5 to 3.5 hours prior to delivery and ending at delivery. This is equivalent to doses of either 0.39 to 0.52 g/kg over 1.5 hours or 0.90 to 1.20 g/kg over 3.5 hours. After 30 minutes of infusion, the average fetal ethanol concentration was 60 percent of maternal concentration. This concentration difference disappeared after 60 minutes of infusion and was not apparent at delivery. The mean ethanol elimination rate in the fetus, (77 mg/L/h) calculated over 4 hours, was almost half as fast as the mean maternal ethanol elimination rate (140 mg/L/h).

Seppala et al.(1971) reported a case where twins were born 4 hours after treatment of the mother with a total of 72 g ethanol. Blood ethanol concentrations at delivery were 0.58, 0.67, and 0.53 g/L in the mother, twin A, and twin B respectively. Elimination rates were determined to be 140, 80, and 70 mg/L/h respectively. Lower elimination rates in the fetus and neonates may be due to low alcohol dehydrogenase (ADH) levels (Pikkarainen and Raiha 1967).

C. Pathways of Ethanol Oxidation

A number of studies in different animal species have shown that the liver is the principal organ for ethanol elimination (Hawkins and Kalant 1972). Hepatic metabolism accounts for at least 75 percent of total ethanol elimination (Winkler et al. 1969). Ethanol metabolism occurs in

two steps. It is initiated by the oxidation of ethanol to acetaldehyde. The principal activity responsible for ethanol oxidation is the cytosol enzyme, ADH. Extrahepatic tissue contains little ADH. Acetaldehyde is further oxidized to acetate in the liver, by various aldehyde dehydrogenases. Most of the acetate and some of the acetaldehyde formed are released into the hepatic venous blood and metabolized in a number of tissues. Acetate is a readily utilized substrate for most tissues and may be oxidized to carbon dioxide and water via the Kreb's cycle. Only a small amount is oxidized further in the liver, or is converted to other intermediate metabolites such as fatty acids and ketone bodies. Venous catheterization studies in normal human subjects have shown that, on average, 75 percent of the ethanol taken up by the liver is released as acetate into the circulation (Lundquist et al. 1962).

The rate of ethanol oxidation by the liver is relatively constant. The maximal mean hepatic metabolic capacity for ethanol has been determined to be 1.6 mmol/min (Winkler et al. 1969). Extrahepatic metabolism in man has been estimated at 0.4 mmol/min (Larsen 1959). Alternate pathways to ADH may contribute to variation of ethanol oxidation rates (Lieber and DeCarli 1973).

The enzyme systems found to catalyze the oxidation of ethanol to acetaldehyde *in vitro* are ADH, catalase, and the microsomal ethanol oxidizing system (MEOS). The product of these reactions is acetaldehyde. Evidence for the participation of each system *in vivo* is indirect. Additionally, there exist some minor hepatic pathways and some extrahepatic pathways for metabolism of ethanol (Li 1977, Hawkins and Kalant 1972).

a. Alcohol Dehydrogenase

i. Enzymatic Properties

In experimental animals and in humans, liver ADH is primarily responsible for the metabolism of ethanol (Hawkins and Kalant 1972). ADH catalyzes the reversible interconversion of a large variety of alcohols and their corresponding aldehydes and ketones. Broad substrate specificity is a characteristic property of all mammalian liver ADH. Substrates include primary and secondary, but not tertiary, saturated aliphatic and aromatic alcohols and their corresponding aldehydes and ketones. Nicotinamide adenine dinucleotide (NAD) and reduced nicotinamide adenine dinucleotide (NADH) are coenzymes in the reaction. These enzymes utilize NAD(H) in preference to NADP(H). ADH catalyzes the reversible reaction:

$$NAD^+ + CH_3CH_2OH \rightleftharpoons NADH + CH_3CHO + H^+$$

The reaction proceeds from left to right provided acetaldehyde is removed, even though the position of the equilibrium is unfavourable for oxidation. Studies on ADH have been performed on horse, rat, guinea pig, monkey, and human livers. The reaction has a sequential mechanism:

$$ADH + NADH \rightleftharpoons ADH\text{-}NADH$$

$$H^+ + ADH\text{-}NADH + CH_3CHO \rightleftharpoons ADH\text{-}NAD^+ + CH_3CH_2OH$$

$$ADH\text{-}NAD^+ \rightleftharpoons ADH + NAD^+$$

The regulation of the kinetic mechanism has been described by Theorell and Chance (1951). The rate constants of binding of coenzyme are independent of whether the enzyme is free or occupied by substrate. The interconversion of the ternary complexes is rapid. The dissociation of the coenzymes from these complexes is much slower than the dissociation of the substrate, as the substrate is less tightly bound to the enzyme. The dissociation of the enzyme-coenzyme compound is facilitated by high pH values (Theorell and Winer 1959). The rate of the overall reaction of

ethanol oxidation is limited by the dissociation of the binary ADH-NADH complexes. This last step of the reaction is the slowest partial process in ethanol oxidation (Thieden 1975). Thus, the rate of NADH oxidation is rate limiting. Reoxidation occurs primarily in the mitochondria and is coupled to oxidative phosphorylation (Peters 1982). This mechanism may not be applicable to all mammalian liver ADH enzymes (Pietruszko 1975). The rate of ethanol oxidation depends on NAD supply, rather than on the amount of activity of ADH. However, recent evidence suggests that the initial burst of acetaldehyde formation may depend on ADH activity, not on the speed of NAD formation (Peters 1982).

The pH optimum for ethanol oxidation in man is about 10.8. At pH 7.0, activity is 40 percent maximal and decreases to zero at pH values less than 6.0 (von Wartburg et al. 1964). The K_m values for ethanol oxidation for human, monkey, and rat are summarized in table 1. K_m values for human ADH in vitro vary depending on the amount of isoenzymes present in the preparations (Blair et al. 1966, von Wartburg et al. 1964, Pikkarainen and Raiha 1967, Li 1977) and are in agreement with those obtained in vivo (Lundquist and Wolthers 1958, Li 1977). K_m values of ethanol oxidation for rat and monkey in vitro (Reynier 1969, Theorell et al. 1955) and in vivo (Makar and Mannering 1970) are similar and within the range of human values (table 1). The K_m for catalase and MEOS is about 10×10^{-3} M (Lieber and DeCarli 1970a, Thurman et al. 1972).

ii. Molecular Properties

Human, horse, and monkey ADH are similar in their molecular weights, amino acid composition, and zinc content (Li 1977). The molecular weight has been calculated to be 80,000 for all enzymes by summing the molecular weights of component amino acids. All enzymes

Table 1. Comparison of reported K_m values for ethanol oxidation *in vitro* and *in vivo* in man, monkey and rat.

Species	$K_m \times 10^{-3}$ M	
	in vitro	*in vivo*
Man	0.4 to 18	2 to 3
Monkey	1.8, 2.1	4.6
Rat	0.5, 1.9	2.7

appear to be dimeric molecules, containing two subunits of equal molecular weight. Horse and human liver ADH contain one NADH molecule per subunit (Pietruszko 1975).

Human, horse, rat, and monkey liver ADH all contain four atoms of firmly bound zinc per mol enzyme or two atoms per subunit (Drum et al. 1967, Arslanian et al. 1971, Dafeldecker et al. 1981a,b). Drum et al. (1967) demonstrated that loss of two of the four zinc atoms resulted in complete loss of catalytic activity, while the tertiary structure was maintained. Thus, it was postulated that ADH contains two types of zinc molecules. The first type is concerned with enzymatic activity (catalytic zinc) and the second type contributes to maintenance of structure (structural zinc). Each subunit contains one of each type of zinc molecule. Zinc is not required for coenzyme or substrate binding to the ADH active site. Its role in the catalytic mechanism of ADH is uncertain (Pietruszko 1975).

iii. Multiple Molecular Forms

ADH exists in multiple molecular forms with different catalytic and kinetic properties. Horse, monkey, and human liver ADH display multiple molecular forms, whereas only one molecular form for rat liver ADH has been identified. All enzymes produce multiple bands on gel electrophoresis (Pietruszko 1975). The molecular basis of the 3 major isoenzyme forms in horse liver, due to the formation of dimers from dissimilar polypeptide chains, has been elucidated (Pietruszko 1969). The amino acid sequence for rat liver ADH appears homogeneous with no structurally distinct subunits. Separation on electrophoresis appears to be an artifact of the purification procedure (Pietruszko 1975). Although the molecular weights for mammalian liver ADH are identical, the amino

acid sequence can vary, indicating that polypeptide chains from which dimeric molecules of ADH are assembled may be coded by different genes. Differences in the amino acid sequence in the polypeptide chains of human liver ADH have been observed (Smith et al. 1971).

Horse, human, and rat liver ADH have similar but not identical amino acid composition, and a similar number of amino acid residues per subunit (Pietruszko 1975). Eighty percent amino acid homology is observed among horse, human, and rat liver ADH, and homology between horse and human liver ADH is greater than 90 percent (Pietruszko 1975). Similar data have not been reported for human and monkey liver ADH, although a high percentage of homology is anticipated (Dafeldecker et al. 1981a,b). No data are available for sheep liver ADH.

Heterogeneity in the molecular forms of human liver ADH develops perinatally, and is present in adult livers to variable and different extents (von Wartburg et al. 1964). In some livers, as many as 7 to 9 major bands, identified by electrophoresis, are observed. The specific activity of ADH varies widely between livers. The complex multiplicity of differently charged molecular forms of ADH has been recognized. Problems in purification of human ADH enzyme, including low yield, failure to separate molecular forms from each other and from contaminating protein, and variable results have been minimized with the introduction of a new method of purification. This method, known as double ternary complex affinity chromatography, described by Lange and Vallee (1976), is specific for ADH.

Smith et al. (1971,1972) suggested a genetic basis for molecular forms of human ADH as isoenzymes. There exist 3 separate gene loci; ADH_1, ADH_2, and ADH_3, that code for 3 structurally distinct polypeptide

chains; alpha, beta, and gamma respectively. Genetic polymorphism at the ADH_3 locus is commonly present. Here, 2 different alleles coding for a distinct form of the gamma chain (1 and 2) exist. Since ADH is a dimer, molecular forms may be homodimeric, consisting of two identical chains, or heterodimeric, consisting of different chains.

Subsequently, pi-ADH, and chi-ADH have been identified as molecular forms of human liver ADH (Bosron et al. 1979). The genetic model proposed by Smith et al. (1971) cannot account for these newly discovered molecular forms. The kinetic properties of pi-ADH differ from those of other isoenzymes. Their K_m value for ethanol oxidation exceeds that of other forms by as much as 100 times, and it is insensitive to the known potent inhibitors of mammalian ADH, pyrazole and 4-methylpyrazole. The recent isolation of pyrazole-insensitive and pyrazole sensitive ADH isoenzymes from the squirrel monkey provides evidence for similar molecular forms in another species (Dafeldecker et al. 1981b). Pi-ADH, as well as simian pyrazole-insensitive isoenzymes of Saimiri scuireus and Macaca mulatta, share similar electrophoretic and kinetic properties. They also display higher Km values for ethanol and are less susceptible to 4-methylpyrazole inhibition than other cathodic forms. The Km of pyrazole-insensitive ADH in Macaca mulatta for NAD^+ at pH 10.0 was determined to be 0.06 mM (Dafeldecker et al. 1981b). Maximal activity for ethanol oxidation is seen at pH 11.0 and 10.6 for the simian pyrazole-insensitive and pyrazole-sensitive forms respectively (Dafeldecker et al. 1981a). The anodic pyrazole-insensitive enzyme of Macaca mulatta presents features identical with those of human chi-ADH (Dafeldecker et al. 1981b). The presence of these isoenzymes in primates implicates them as a most suitable model for the study of human alcohol

oxidation. Other species, such as sheep, must be evaluated for their suitability.

iv. Variations in Specific Activity

Variation in ethanol tolerance is known to exist between different races and individuals, and at different stages during development. During human development, ADH activity in the fetus is low and reaches adult levels at about 5 years of age (Pikkarainen and Raiha 1967). Variants in liver ADH during human development exist. Pikkarainen and Raiha (1969) demonstrated 4 electrophoretically distinct bands in the adult human liver and one band in the fetal human liver, corresponding to band 3 in the adult liver. The enzymatic activity of the fetal liver is only 3 to 4 percent of adult activity. Pikkarainen (1971) demonstrated that the 10 to 16 week old fetus cannot metabolize ethanol. During human development, liver ADH shows a distinct progression in its isoenzyme pattern from only one form during fetal life to 4 forms in the adult (Pikkarainen and Raiha 1967). More recently, as many as 7 to 9 activity bands have been identified in adults (Li 1977).

According to Smith et al. (1973), only the alpha,alpha polypeptide chain form is present in the early fetus. The more mature fetus shows both alpha,alpha and alpha,beta forms and the alpha,alpha and alpha,beta and beta,beta forms are observed in the neonate. Molecular forms including the gamma chains do not appear until after birth (Smith et al. 1973).

Pikkarainen and Raiha (1967) studied the development of ADH in fetal and postnatal human liver. ADH activity in human fetal liver ranged from 111 to 797 mU/g liver wet weight. Activity was present in 2 month old fetal livers, but only at about 3 to 4 percent of adult

activity. Activity increased linearly and adult activity was attained at 5 years of age. ADH activity for adults ranged from 945 to 6530 mU/g liver wet weight (Pikkarainen and Raiha 1967).

Smith et al. (1971) determined average ADH activity in liver samples obtained from 56 fetuses, 37 infants, and 129 adults. Activity of ADH in infants less than 1 year old was about 40 percent of adult activity. Fetal activity was about 31 percent of adult activity, and appeared to increase with gestational age (Smith et al. 1971).

Raiha et al. (1967) reported liver ADH activity in the rat to be 25 percent of adult activity at birth. Adult levels were attained at approximately 18 days after birth. Administration of ethanol to pregnant rats during the latter half of gestation had no effect on ADH activity in the liver of the newborn (Raiha et al. 1967). ADH activity in the newborn guinea pig was reported to be 20 percent of adult activity (Raiha et al. 1967). Similar studies in mice demonstrated lower ADH activity in newborn mouse liver than in the adult liver (Krasner et al. 1974).

Table 2 summarizes the K_m values obtained for adult, newborn, and fetal liver ADH from human and animal studies. The K_m values are higher in the newborn human and mouse livers than in the adult. This indicates a lower affinity for ethanol substrate in the newborn. The significance of this in relation to activity is not understood (Krasner et al. 1974). The higher K_m for the newborn mouse was attributed to the fact that one isoenzyme dominates in the newborn, whereas other more developed isoenzymes are present in the adult (Krasner et al. 1974).

Krasner et al. (1976) reported the presence of ADH amongst the numerous enzymes in human placental tissue. Four electrophoretic bands

Table 2. Summary of reported K_m values for ethanol oxidation *in vitro* in adult (A), newborn (N), and fetal (F) livers.

Study	Species	K_m x 10^{-3} M			pH		
		A	N	F	A	N	F
Pikkarainen and Raiha (1967)	Man	1.1	-	3.4	10.8	-	10.0
Raiha et al. (1967)	Rat	0.33	-	0.24	8.7	-	8.7
Krasner et al. (1974)	Mouse	0.25	0.45	-	9.6	9.6	-

were present at pH 9.6. The Km value was determined to be 5×10^{-3} M with an optimal pH of 10.0. The authors suggested that the presence of ADH in placental tissue might contribute to the control of the distribution of energy regulating processes in the placenta.

Racial differences in the handling of ethanol have been attributed to different elimination rates. An atypical form of ADH, with an optimum pH of 8.5, rather than 10.0 or greater, has been identified in certain populations. According to Smith et al. (1973), the atypical behavior is a result of genetic polymorphism at the ADH_2 locus. Two alleles produce an enzyme of either high or low activity. In former years, the assessment of ADH type was based on activity ratios at different pH values, (von Wartburg and Schurch 1968) but they can now be identified electrophoretically (Harada et al. 1978). ADH^1 is the commonly observed allele at the ADH_2 locus. This corresponds to the beta-1 polypeptide chain. The atypical allele is ADH^2 at the ADH_2 locus (beta-2 polypeptide chain) (Smith et al. 1973). The low activity type is considered "normal", whereas the high activity ADH type is called "atypical". The frequency of occurrence of the variant "atypical" variant enzyme has been estimated to be 20% of the Swiss population, 5-10% of the British and American white population, and as high as 85% of the Japanese population (Smith et al. 1973). A small number of individuals with the atypical ADH_2 phenotype have exhibited increased ethanol oxidation *in vivo* (von Wartburg and Schurch 1968) but larger studies must confirm this. The low activity enzyme predominates in western Europeans and their descendents.

v. ADH activity and ethanol elimination *in vivo*

Westerfield et al. (1943) demonstrated that acetaldehyde disappeared from the blood much faster than ethanol did *in vivo* in the dog. This led to the conclusion that ADH activity is rate limiting. It is believed to be the major enzyme responsible for ethanol elimination *in vivo*. The kinetics of ADH *in vitro* are similar to the kinetics of ethanol disappearance *in vivo* (table 1).

A lack of correlation between the rate of removal of ethanol *in vivo* and ADH activity may be dependent on experimental conditions. Correcting for pH and temperature, the amount of ADH activity in man is almost sufficient to account for the metabolic rate (Mourad et al. 1967).

Direct comparison of ADH activity and the rate of ethanol metabolism may be subject to numerous errors. Autolysis may inactivate the enzyme. Extraction of the soluble enzyme may be incomplete unless detergent is used (Raiha and Koskinen 1964). The lack of an agent, such as semicarbazide, to prevent acetaldehyde oxidation by NAD^+ and endogenous NAD reductases and NADH oxidases in liver homogenates may affect determination of activity (Plapp 1975). The theoretical problem of whether maximum velocities observed *in vitro* are attained with the concentrations of substrates found *in vivo*, which may be as low as the Michaelis constants of the enzymes, must be considered. However, Zahlten et al. (1980) attributed underestimation of ADH activity to two major causes. Firstly, highly dependent pH backgrounds, as a result of ethanol contamination of commercially available NAD, could falsely lower the calculated overall ADH activities when subtracted from rates after substrate addition. Another problem may depend on the concentration of substrate used. Strong substrate inhibition has been observed at 600 mM

ethanol, particularly as the assay system approaches physiological pH. Yeast ADH reached V_m rates at 600 mM ethanol whereas rat liver ADH was 60 percent inhibited at this concentration (Zahlten et al. 1980). Proper measurement of ADH activity yields values that exceed the in vivo disappearance rates of ethanol (Zahlten et al. 1980).

Humans who have "atypical" enzyme metabolize ethanol at the normal rate, even though the atypical enzyme is three to five times more active in vitro at pH 8.8. This suggests ADH may not be rate limiting.

vi. ADH Inhibition and Induction

The rate of oxidation of NADH is rate limiting in the metabolism of ethanol and acetaldehyde. Reoxidation occurs primarily in the mitochondria and is coupled to oxidative phosphorylation. When NADH oxidation is increased by fructose, pyruvate, or glyceraldehyde, an increase in ethanol oxidation rate is observed (Li 1977).

The presence of inhibitors, including a wide variety of primary and secondary alcohols, aldehydes and ketones, could lower ADH activity in vivo. Fatty acids, adenosine monophosphate, and aminoazo dyes also inhibit ADH (Plapp 1975).

Pyrazole, and its derivatives, are potent inhibitors of ADH in vitro and also markedly decrease ethanol metabolism in vivo (Theorell and Yonetani 1963). In rats, acute pyrazole administration results in competitive inhibition of ethanol elimination of greater than 85 percent (Goldberg and Rydberg 1969). It has only a slight or no inhibitory effect on catalase and MEOS activity under these conditions. In man, methylpyrazole inhibits the conversion of ethanol-1-^{14}C to $^{14}CO_2$ in a dose dependent manner (Blomstrand and Theorell 1970). The formation of $^{14}CO_2$ was inhibited by 50 percent 1.5 hours after administration of 10

mg/kg methylpyrazole (Bloomstrand and Theorell 1970). The inhibition constant, (K_i), was calculated to be 0.2mM. K_i for 4-methylpyrazole with human liver ADH in vitro is 0.21 uM at pH 7.4 and 23 ^{o}C (Li and Theorell 1969). The wide discrepancy may appear as $^{14}CO_2$ release measures acetate oxidation in extrahepatic tissues, or because 4-methyl pyrazole is metabolized in man. Eighty percent inhibition of ADH is observed in the guinea pig after intraperitoneal injection of 200 mg/kg 4-methyl pyrazole (Zahlten et al. 1980). The effects observed in vitro seem to correlate well with predicted ADH inhibitory activity in vivo.

Oxidation of ethanol by NAD^+ produces NADH, which reduces various metabolites. Hepatic vein catheterization studies in man have shown increases in the ratios of lactate/pyruvate and beta-hydroxybutyrate/acetoactetate after ethanol administration (Tystrup et al. 1965, Peters 1982). These changes are equivalent to a reduction in the NAD^+/NADH ratio. A role for ADH in vivo is demonstrated since only the ADH catalyzed reaction for ethanol oxidation causes a decrease in the NAD^+/NADH ratio.

The rate of ethanol disappearance from the blood is linear with time until a concentration of 0.2 g/L or 4×10^{-3} M. Linearity suggests the ethanol metabolizing system is saturated. As the concentration decreases below 3 mM, the rate of disappearance decreases and the apparent K_m can be calculated. Comparison of kinetic constants in vitro and in vivo for ADH shows reasonably good agreement, consistent with a rate limiting role for ADH. The apparent K_m values in vivo may be representative of more than one enzymatic process, and error in K_m measurements may result (Lundquist and Wolthers 1958). Presently, a predominant rate limiting role for ADH in ethanol metabolism cannot be assumed until further studies are done.

b. Catalase

A role for catalase in the oxidation of ethanol was first reported by Keilen and Hartree (1945). Ethanol oxidation by catalase (Cat), in the presence of excess hydrogen peroxide (H_2O_2) can be represented by the following equations:

$$Cat + H_2O_2 \longrightarrow Cat\text{-}H_2O_2$$

$$Cat\text{-}H_2O_2 + CH_3CH_2OH \longrightarrow Cat + 2H_2O + CH_3CHO$$

where $Cat\text{-}H_2O_2$ is a catalase-hydrogen peroxide intermediate (Thieden 1975). The degree of participation of this enzyme in ethanol oxidation is determined by the rate of H_2O_2 production in relation to catalase and ethanol concentrations. Hydrogen peroxide production is the rate limiting factor in ethanol peroxidation (Oshino et al. 1975a). In experiments with isolated perfused rat livers, ethanol produced a decline in the steady-state concentration of the catalase-H_2O_2 complex (Theorell et al. 1972). This is strong evidence for catalase mediated ethanol oxidation as an alternate pathway in the rat liver. Zahlten et al. (1981) found that guinea pig liver contained 3.4 times more catalase than rat livers. Seventy-six percent of the catalase in the guinea pig liver is in the cytosolic fraction, in contrast to only 50 percent in the rat. It has not yet been studied in man. The contribution of hepatic catalase to ethanol oxidation in the rat is reported to be less than 10 percent (Oshino et al. 1975b).

Studies with the catalase inhibitor 3-amino-1,2,4-triazole, and 4-methyl pyrazole in isolated rat livers have demonstrated little contribution of catalase to ethanol oxidation at ethanol concentrations less than 20 mM. The administration of 3-amino-1,2,4-triazole to the guinea pig produced 98 percent inhibition of catalase, but subsequent

ethanol injection (1.7 g/kg) did not show a significant difference in ethanol elimination in catalase inhibited animals compared to untreated controls (Zahlten et al. 1981). The role of catalase is suggested to increase with increasing ethanol concentration, but this is by no means certain (Thurman et al. 1975).

c. Microsomal Ethanol Oxidizing System (MEOS)

Orme-Johnson and Ziegler (1965) first reported the ability of the microsomal fraction of rat, rabbit, and pig livers to convert ethanol into acetaldehyde. They assumed the reaction was catalyzed by mixed-function oxidases according to the equation:

$$CH_3CH_2OH + NADPH + H^+ + O_2 \longrightarrow CH_3CHO + NADP + 2H_2O$$

Lieber and DeCarli (1968) described a similar microsomal system which they defined as MEOS. They found that ethanol oxidation is inhibited by carbon monoxide, which is known to inhibit microsomal drug oxidizing mixed-function oxidases (Orrenius et al. 1964). However, MEOS is not inhibited by 1mM SKF525A, which is a known inhibitor of many microsomal drug metabolizing enzymes (Lieber and DeCarli 1970a). Acute ethanol ingestion decreases the activity of a number of drug metabolizing enzymes in rat liver microsomes (Rubin et al. 1970).

Conflicting opinions exist regarding the identity of the enzymes responsible for ethanol oxidation. Some investigators attribute ethanol oxidation to enzymes of the mixed function oxidase system, utilizing NADPH and oxygen, but not involving ADH or catalase (Lieber and DeCarli 1970a). Liver microsomes contain NADPH oxidase, which is capable of generating H_2O_2 in the presence of NADPH and oxygen (Thurman et al. 1972). A number of investigators state that the activity of MEOS is mainly due to catalase, the oxidative enzyme which contaminates the

microsomal fraction, and that the mixed function oxidase merely serves to furnish H_2O_2 for the reaction (Thurman et al. 1972, Roach et al. 1969). In microsomal preparations of rat liver, the requirement of NADPH can be abolished when an H_2O_2 generating system is added (Thurman et al. 1972), suggesting catalase may be a factor in microsomal ethanol oxidation. Arguments stating that catalase is not involved are derived from experiments using inhibitors to differentiate between MEOS, catalase, and ADH (Lieber and DeCarli 1968). MEOS was found to be 17 percent inhibited by 0.01 mM cyanide (Lieber and DeCarli 1968) whereas the same concentration almost completely inhibited catalase (Lundquist et al. 1963). The intraperitoneal injection of the catalase inhibitor, 3-amino-1,2,4-triazole to rats produced a much smaller change in MEOS activity than in catalase activity (Lieber and DeCarli 1970a). Hassinen and Ylikahri (1972) found no role for cytochrome P-450 in ethanol oxidation.

In catalase-free microsomes from acatalitic mouse liver, no ethanol metabolic activity, but metabolic activity for other drugs was reported (Vatsis and Schulman 1973). Lieber and DeCarli (1972) determined that the apparent K_m of MEOS *in vitro* correlated well with the apparent K_m *in vivo* of blood ethanol disappearance after pyrazole adminstration. In a later study, Lieber and DeCarli (1973) attributed 25 percent of ethanol oxidation to MEOS. Subsequently, Teschke et al. (1974) separated hepatic MEOS from ADH and catalase by column chromatography.

d. Minor Hepatic Pathways

Several minor hepatic pathways of ethanol metabolism have been described. These include conjugation reactions to form ethyl sulfate, and ethyl glucuronide, the formation of fatty acid esters, and

condensation of acetaldehyde with pyruvate and alpha-ketoglutarate. Quantitatively, however, these reactions are considered insignificant (Hawkins and Kalant 1972).

e. Extrahepatic Elimination

About 20 percent of total ethanol elimination in man occurs outside the liver. The kidney contains ADH and is responsible for most of the extrahepatic elimination (Thieden 1975). The mucosa of the alimentary tract is involved in extrahepatic ethanol elimination. Muscle tissue may also contribute, although its capacity for ethanol oxidation is considered insignificant (Thieden 1975).

f.Factors Which May Increase Ethanol Metabolism

ADH is generally considered to be the normal rate limiting step in ethanol oxidation. In order for the reaction with ADH to proceed, acetaldehyde must first be removed, and NADH must quickly be reoxidized in the mitochondria via the electron-transport chain. The re-oxidation of NADH is the principal rate limiting step under normal conditions. The rate of NADH oxidation regulates ethanol oxidation because the steady state concentration of free NADH in the cytosol affects the rate of dissociation of the ADH-NADH complex, which is the actual rate limiting step for the ADH reaction *in vivo* and *in vitro*. Ethanol oxidation is increased by agents that increase NADH oxidation. These include fructose, pyruvate, and glyceraldehyde. An increase of 25 to 75 percent in the ethanol metabolic rate has been reported with fructose administration (Lundquist and Wolthers 1958, Tygstrup et al. 1965). An increase in the steady state concentration of free NADH is indicated by an increase in the ratio of lactate to pyruvate, which serves as an index of free NADH to NAD^+ (Thieden 1975). Observations that these

oxidizable substrates can increase ethanol metabolism provide further proof for the rate limiting role of NADH oxidation in ethanol metabolism in man.

Chronic ethanol administration may result in increased ethanol metabolism by 50 to 70 percent in man and animals (Mezey and Tobon 1971). The mechanism of this increased rate has not yet been elucidated although changes in the activities of the responsible enzyme systems are being investigated. Some investigators report an increase in ADH activity with chronic ethanol ingestion (Hawkins et al. 1966) whereas others report no change or a decrease in activity (Lieber and DeCarli 1973, Tobon and Mezey 1971). Possibly, an increase in activity of ADH is offset by deficiencies in protein and zinc as a result of chronic alcoholism (Rothschild et al. 1971, Huber et al. 1975). This would decrease ADH activity (Li 1977). Liver ADH activity generally decreases in chronic alcoholics and patients with cirrhosis or liver disease (Dow et al. 1975). Catalase activity does not appear to be affected by chronic alcoholism (Ishii et al. 1973). An increase in MEOS activity or catalase and an increase in H_2O_2 generation via NADPH oxidase may account for about 10 to 20 percent of the increased ethanol metabolism observed in chronic alcoholic rats (Videla et al. 1973). Chronic alcoholism leads to hyperplasia of the smooth endoplasmic reticulum of rat liver. This is associated with a 70 to 80 percent increase in ethanol oxidation ability, and in NADPH oxidase activity in rat liver (Lieber and DeCarli 1970b). Proliferation of the smooth endoplasmic reticulum and increased MEOS and mixed function oxidase activity have also been shown in man (Mezey and Tobon 1971). Increased NADPH-dependent ethanol oxidizing capacity has been demonstrated in both man and

animals. However, the NADPH-oxidase remains elevated for prolonged periods of times after ethanol withdrawal, whereas the rate of ethanol elimination rapidly decreases to normal (Mezey 1972). After withdrawal of ethanol in man, its elimination returns to normal within 7 days, whereas increased MEOS activity persists for 21 days (Mezey and Tobon 1971).

Phenobarbital has been found to increase ethanol elimination and to increase ADH activity, but no effect on MEOS could be demonstrated (Mezey and Robles 1974). Increased MEOS or increased H_2O_2 generation by NADPH oxidase does not appear to be sufficient to explain the increased rate of ethanol metabolism during chronic ethanol ingestion.

Chronic alcoholism has been shown to increase the activity of Na^+-K^+-ATPase and NADH oxidation by mitochondria (Israel et al. 1970). In rat liver, chronic alcohol ingestion results in increased oxygen consumption, increased ATP utilization, and increased NADH oxidation. These changes require further clarification in all species.

g. Aldehyde Dehydrogenase

i. Enzymatic Properties

The product of alcohol dehydrogenase reaction is acetaldehyde. Acetaldehyde dehydrogenase (AlDH) is responsible for the formation of acetate. It is nonspecific for aldehyde substrates and catalyzes the reaction:

$$\text{Aldehyde} + NAD^+ \longrightarrow \text{carboxylic acid} + NADH + H^+$$

The K_m of mitochondrial AlDH is between 0.1 and 0.5 x 10^{-3} M. The K_m values for the cytosolic enzymes are higher and vary between species. The K_m of horse liver cytosolic enzyme is 70 x 10^{-3} M, whereas the values for rat and man are in the millimolar range (Koivula and Koivusalo 1975).

AlDH is specific for NAD coenzymes. NAD^+ binds preferentially to the free enzyme to form a binary complex of NAD^+ and enzyme, followed by binding of aldehyde to form a ternary complex (Eckfeldt and Yonetani 1976).

The site of acetaldehyde oxidation in rat liver has been shown to vary as a function of acetaldehyde concentration. During ethanol oxidation in vivo, liver acetaldehyde concentration is 50 to 150 uM. At these concentrations, mitochondria account for 75 to 80 percent, microsomes for 14 to 17 percent, and the cytosolic fraction for 5 to 7 percent of the total AlDH activity of the liver (Marchner and Tottmar 1976). At low concentrations, the low K_m mitochondrial enzyme is primarily responsible for oxidation, and at higher concentrations of acetaldehyde, the high K_m cytosolic and mitochondrial enzymes are more important (Li 1977).

The study of subcellular distribution of liver AlDH in several other animal species indicates that there is considerable interspecies variation. The cytosolic fraction of horse liver contains most of the activity (66 percent) whereas only 26 percent of the activity is present in mitochondria. The cytosolic fraction of sheep liver contains 35 to 40 percent, the microsomal fraction contains 10 percent, and the mitochondrial fraction contains about 35 percent of total activity (Crow et al. 1974).

The cytoplasmic fraction of human liver contains at least 2 chromatographically distinct AlDH's, one having a high K_m and the other with a low K_m value, which account for about 30 percent total activity. More than 50 percent of the activity is present in the mitochondrial fraction and 10 percent activity is present in the microsomal fraction (Koivula 1975).

ii. Molecular Properties

The soluble high and low Km enzymes have been partially purified from sheep, rat and rabbit liver (Koivula and Koivusalo 1975, Crow et al. 1974). Horse and human enzymes have been purified to homogeneity (Feldman and Weiner 1972, Sidhu and Blair 1975). The molecular weight of the horse liver enzyme is between 200,000 and 300,000, with subunits of molecular weights between 52,000 and 57,000. There are 2 isozymic bands of AlDH (Harada et al. 1980). The high affinity enzyme is generally more positively charged than the enzyme with low affinity for acetaldehyde (Feldman and Weiner 1972).

iii. Variations in Activity

In humans, the high affinity band is absent in 50 percent of Oriental subjects, but is not absent in Caucasians (Teng 1981). Facial flushing and other unpleasant side effects after ethanol ingestion are experienced by many Orientals (Wolff 1972). Ninety percent of Orientals have ADH with high activity, causing an initial burst of acetaldehyde formation. Combined with the low capacity to remove acetaldehyde due to lack of efficient AlDH, accumulation of acetaldehyde results (Kalow 1982). A similar accumulation of acetaldehyde has been suggested for alcohol intolerance in American Indians (Kalow 1982).

iv. Inhibition of AlDH

Disulfiram, which is used in the treatment of alcoholism as a form of aversion therapy, causes the accumulation of acetaldehyde. Its mode of action has been examined in mitochondrial and cytosolic AlDH from sheep liver (Kitson 1975). Two phases of inhibition occur in vitro. The initial rapid inhibition is followed by a gradual irreversible loss of activity. Disulfiram, or its metabolite, diethyldithiocarbamate, may be

responsible for its mode of action in vivo. The cytoplasmic enzymes are more sensitive to inhibition than the mitochondrial enzymes in purified horse and sheep liver (Kitson 1975, Eckfeldt et al. 1976). The K_m for disulfiram of horse cytosolic enzyme is about 1×10^{-3} M, whereas that for the mitochondrial enzyme is 30×10^{-3} M. This suggests that the cytosolic enzyme is functionally more important than the mitochondrial enzyme, but this needs to be confirmed (Eckfeldt et al. 1976).

D. Considerations for Study of the Maternal-Fetal Unit

It is clear that the capacity of the human fetus to metabolize ethanol is low. Therefore, the distribution of ethanol across the placenta and maternal hepatic and possibly placental metabolism are important for fetal ethanol elimination. It is now evident that the placenta cannot be regarded as a barrier to protect the fetus from compounds ingested by the mother during pregnancy. For years it was assumed that placental transfer involved simple diffusion. Although this assumption holds for some substances such as electrolytes, increasing evidence shows that active processes do exist (Mirkin 1973). Generally, lipid-soluble, non-ionized compounds with molecular weights of less than 600 readily traverse the placenta. Under steady state conditions, placental transfer is a function of placental permeability, rates of blood flow on either side of the placenta, and the arterial concentration differences between maternal and fetal blood (Bonds et al. 1980).

Ethanol, a relatively polar molecule, has a molecular weight of 46. Virtually any molecule can and will cross the placenta if present in sufficient quantities in the maternal circulation (Mirkin 1973). Many factors appear to influence the transfer of pharmacological agents. The

important concerns are how much drug reaches the fetus, how rapidly it enters fetal circulation, and how long it remains in the fetus.

a. Placental Blood Flow

i. Regulation of Placental Blood Flow

Difficulty arises when assessing the regulation of placental blood flow. This is due to the complexity of the uterine vasculature, the fetal arterial system, the transient nature of the placenta, and rapidly occurring changes in blood flow throughout pregnancy. Blood flow to the non-pregnant ovine uterus is about 100 ml/min, whereas blood flow to the near-term uterus is approximately 800 ml/min (Rankin and McLaughlin 1979). Neural, mechanical, and chemical factors affect placental blood flow. The degree of autonomic innervation to the uterine vasculature during pregnancy varies widely among species. Cholinergic supply to the parametrial artery is present in humans but not in sheep (Bell 1972). Autonomic fibres have not been identified in the human placenta and umbilical cord. Fibres in the ductus venosus are not believed to influence umbilical blood flow (Bell 1972). The mechanical regulation of maternal and fetal blood flow is complex. Resistance of the maternal and fetal placental circulations do not appear to be influenced by changes in fetal arterial and venous pressures respectively. The uterine and umbilical blood flows may be influenced by circulating angiotensin II, catecholamines, steroids, and prostaglandins (Rankin and McLaughlin 1972).

ii. Ethanol and Blood Flow

Uterine blood flow in the human at term is about 10 percent of cardiac output (Mirkin 1973). The rate of placental transfer may be influenced by changes in the amount of drug delivered to the site of

transfer. Placental transfer of highly diffusable molecules is a function of both uterine and umbilical blood flow. Wilkening et al. (1982) examined the effect of variations of uterine blood flow on placental transfer in 6 chronic sheep preparations by measuring the placental clearances of ethanol and antipyrine. Uterine blood flow was reduced by norepinephrine, hemmorhage, and occlusion of the terminal aorta. The decrease in uterine blood flow had no demonstrable effect on umbilical blood flow. Maximum placental clearance of ethanol occurred when the uterine/umbilical blood flow ratio was 1 (Wilkening et al. 1982).

Bonds et al. (1980) compared the placental clearances of ethanol and antipyrine in 6 chronic sheep preparations. Experiments were performed under steady state conditions in which uterine and umbilical blood flows and transplacental diffusion rate were constant. The results indicate that placental transfer is primarily flow limited, as these molecules possess different placental permeability coefficients.

The effects of drugs on placental blood flow could cause secondary effects on placental transfer. Jones et al. (1981) reported a significant decrease in blood flow to the placenta in rats fed alcohol before and during gestation. Fetuses of alcohol-treated rats were lighter and the placentae were heavier as compared to controls. They concluded that chronic alcohol consumption leads to redistribution of blood, with less supplying the placenta. They suggested that this may contribute to growth retardation in FAS. This reduction in blood flow may be unique to the rat. The effects of acute ethanol administration on fetal blood flow have not been determined.

iii. Placental Characteristics

Placental metabolism of drugs may affect transfer, although its significance has not been determined (Juchau 1972). Maturation of the placenta leads to altered permeability due to a decrease in thickness. Studies in hamsters indicate that the concentrations of ethanol in the fetus in late pregnancy were higher than or equal to concentrations of ethanol in the early stages (Idanpaan-Heikkula et al. 1971). However, histopathology of the placenta may not be the sole factor in determining the placental transfer of drugs (Boulos et al. 1972).

b. Fetal Considerations

i. Fetal Circulation

The distribution of fetal circulation is probably of great importance in the determination of the amount of drug presented to the fetus. After a drug crosses the placenta to enter the umbilical vein, 60 to 85 percent of the flow in the umbilical vein enters the liver via the portal vein, and 15 to 40 percent passes directly via the ductus venosus into the inferior vena cava. Initially a large proportion of drug will be delivered to the liver. Thus the metabolic activity of the liver will influence the amount of drug delivered to more distal parts of the circulation. The concentration of drug in the inferior vena cava is subject to dilution by venous drainage (Mirkin 1973). Whether these factors influence drug effects on the fetus has not been determined. The effects of drugs on the fetal circulation are not known. Drug-induced changes in fetal circulation may influence the responses of the fetus to drug administration (Heymann 1972).

ii. Fetal Characteristics

Certain characteristics of the fetus may influence the disposition of drugs transferred across the placenta. The permeability of

specialized membranes such as the blood brain barrier, and the selective uptake of lipophilic drugs by organs with a relatively high lipid content may influence distribution (Mirkin 1973).

Several attempts have been made to obtain direct evidence for fetal drug elimination. It appears that human and nonhuman primate fetuses are exceptional in that they possess well developed oxidative, hydrolytic, and conjugative enzyme systems rather early in gestation, which are usually less active than those of the adult (Pelkonen 1980). Some studies have indicated that drugs, including alcohol, administered to mothers for long periods and in large doses, may induce drug metabolic activity in the human fetal liver during the first half of pregnancy (Pelkonen et al. 1973). The emergence and disappearance of different forms of enzymes, and even their inducibility, may be regulated independently, leading to typical and definite patterns of drug metabolism at different ages and in different species (Pelkonen et al. 1973). No data are available for alcohol in the last half of pregnancy. The sheep model has not been investigated with respect to this characteristic.

c. Amniotic Fluid Considerations

It is assumed that rapid equilibrium occurs between the maternal and fetal circulations and that blood concentrations of drug and metabolites are comparable in both circulations. However, more than simple transfer is involved (Horning et al. 1973). The drug and its metabolites may accumulate in fetal tissues and redistribution to other compartments such as the amniotic fluid may occur.

Boulos et al. (1971) examined the placental transfer of constant levels of a lipid soluble drug, sulfanilamide. Changes in sulfanilamide

concentration in maternal and fetal blood and in amniotic fluid were compared. Maternal concentrations were constant during the 240 minute infusion, but fetal concentrations did not attain steady state until 120 minutes after start of the infusion. The fetal/maternal concentration ratio during steady state was 0.62. The amniotic fluid/fetal concentration ratio at the same time was 0.65. They attributed the non-equilibrium of maternal and fetal blood sulfanilamide concentrations to the presence of appreciable amounts of sulfanilamide in amniotic fluid.

i. Amniotic Fluid Dynamics

Alterations of the amniotic fluid compartment may parallel changes in the fetus, indicating that exchanges between the fetus and amniotic fluid are significant and result in a relatively short time lag before amniotic fluid evidence of fetal status appears (Seeds 1980). Significant exchanges of water and solutes take place between the amniotic fluid and fetus near term by several pathways, including fetal swallowing (Gresham et al. 1972). The human fetus swallows between 200 and 450 ml of amniotic fluid per day. Total volume is 800 ml. The fetus produces about 600 to 800 ml hypotonic urine per day at term. Fetal swallowing can account for removal of only about half of the daily urine production. Thus, a large quantity of urine must be resorbed by other pathways, since human amniotic fluid gains no more than 10 ml of fluid per day in the last trimester (Seeds 1980). Fetal plasma is significantly hypertonic to amniotic fluid. Net transfer of fluid to the fetal compartment could occur wherever fetal capillary beds and amniotic fluid are in close proximity. Within the fetal respiratory tract, exchange may occur where the alveolar capillary bed is perfused by

amniotic fluid transported in and out by respiratory movements (Seeds 1980).

The transfer of highly lipid soluble drugs with low molecular weights may occur across the fetal skin. This is more likely in early pregnancy before keratinization of this tissue makes it relatively impermeable. This occurs at 24 to 26 weeks gestation in the human (Seeds 1980). However, changes in amniotic fluid P_{CO_2} closely paralleled changes in fetal P_{CO_2} in the rhesus monkey, suggesting rapid equilibration of small highly lipid soluble compounds between the fetus and amniotic fluid (Seeds et al. 1967).

ii. Maternal Drug Administration

The amniotic fluid is completely the product of significant steady state exchanges with the fetus by numerous pathways in the latter part of pregnancy. Small lipid soluble drugs diffuse rapidly into the fetus and appear rapidly and in similar concentrations in the amniotic fluid. Diffusion probably occurs across fetal skin or on the fetal surface of the placenta. Larger water soluble compounds, such as ampicillin (molecular weight 349), cross more slowly. Bray et al. (1966) compared the concentration of ampicillin in the amniotic fluid of a living fetus and following intrauterine death after a maternal intravenous bolus dose. Peak concentrations in the living fetus appeared within 30 to 60 minutes and a significant quantity was found in the amniotic fluid at 120 minutes, reaching peak concentrations at 6 to 12 hours. Very little ampicillin reached the amniotic fluid when the fetus was dead, suggesting that maternal-amniotic fluid exchange in late pregnancy primarily occurs indirectly through the fetus.

The rapid transfer of larger lipid soluble compounds such as meperidine from mother to fetus and amniotic fluid suggests diffusion may also occur across fetal skin or the fetal side of the placenta from fetal capillary beds. Rapid transfer to the fetus is followed by slower entry into the amniotic fluid. In a study by Szeto et al. (1978), steady state was achieved in the mother and fetus within 60 minutes during maternal infusion of meperidine (0.06 mg/kg/min over 2 h). At steady state, the fetal-maternal meperidine concentration ratio was 0.3. The concentration of meperidine in the amniotic fluid increased to exceed maternal concentration (1.69 and 1.0 ug/ml respectively) at the end of infusion. This amniotic fluid level reflects the addition of a drug in the fetal urine and the inability to back diffuse to the fetal compartment.

Infusion of meperidine (150 mg) into the amniotic fluid resulted in a maternal peak concentration at 15 minutes. Fetal peak concentration, at 75 minutes, was only 9 percent of maternal peak concentration. The slow appearance of meperidine in the fetal plasma could result either from drug absorption from the amniotic fluid via fetal swallowing or via transfer from the mother. These results suggest that the amniotic fluid serves as a source of drug for the mother, rather than for the fetus (Szeto et al. 1978).

Ho et al. (1972) demonstrated that a single dose of ethanol, when measured radiographically, can be found uniformly distributed throughout the fetus. Idanpaan-Heikkula et al. (1971) found high concentrations of ethanol in monkey amniotic fluid during late pregnancy and noted a slight tendency for ethanol and degradation products to stay longer in the fetus than in the mother.

Himwich et al. (1977) administered 1.2 to 2.0 g ethanol, depending on body weight and number of fetuses, to near-term pregnant cats, via an arterial catheter. Ethanol concentration in the amniotic fluid, maternal blood and fetal blood, using 1 fetus from a litter each time, was determined for up to 3 hours after the dose. The amniotic fluid and fetal blood ethanol concentrations peaked after maternal concentrations in both experiments reported. One experiment demonstrated that ethanol concentration in the amniotic fluid continued to rise, whereas maternal concentration declined over a 70 minute period following the dose. The authors reported that the rate of elimination from the amniotic fluid was slower than elimination from maternal blood, although given data were insufficient to calculate clearance and the statistical significance was not stated.

After a drug has been transferred across the placenta to the fetal compartment, it can be metabolized, excreted into the amniotic fluid or transferred back to the mother. Thus, pharmacokinetic considerations indicate the need to fully characterize the kinetics of drug distribution and elimination in the mother and fetus.

d. Pharmacokinetic Models in the Maternal-Fetal Unit

It is necessary to focus on the time course of a drug in the fetus. The ratio of maternal and fetal areas under the concentration-time curves may serve as an index of relative fetal exposure to a drug. The primary concern is the adverse effects these drugs may produce on the developing fetus. Pharmacokinetic study provides a meaningful basis for the determination of potential effects of maternally ingested drugs on the developing fetus.

Studies of placental transfer of drugs are limited. Human studies are limited to single point determinations of maternal and fetal concentration ratios at the time of delivery. Single point determinations are no indication of the extent of the transfer between mother and fetus (Waddell and Marlowe 1981).

Most of the currently available information on maternal-fetal pharmacokinetics has been obtained using pregnant sheep. Serial blood samples can be obtained from the mother and fetus using the chronic preparation with indwelling catheters (Szeto 1982). Pharmacokinetic models aid in designing protocols to maximize information obtained from placental transfer studies, and are useful in the interpretation of maternal-fetal concentration ratios (Szeto 1982).

A suitable pharmacokinetic model, based on the knowledge of the anatomical and physiological processes of the real system, must first be developed. Mathematical expressions describing the system should be derived. Then experimental data must be collected and goodness of fit must be determined (Szeto 1982). Any pharmacokinetic model is a simplification of the real biological system. Generally the simplest model is first proposed, although the development of more complex models provides a closer approximation to reality. The complexity of a model is limited by the experimental data that can be obtained (Szeto 1982).

The simplest model of the maternal-fetal unit is a two-compartment model. The mother and fetus are each represented as single compartments, with bidirectional transfer between them. There are 3 possible two compartment models, according to Szeto (1982). In the first model, drug elimination occurs only from the maternal compartment. Drug elimination occurs only from the fetus in the second hypothetical model, although

this is improbable. Thirdly, drug elimination may occur from both the maternal and fetal compartments. The first model is used by most investigators, although it is now recognized that the third model may be appropriate where fetal metabolic activity is present (Szeto 1982).

Waddell and Marlowe (1981) described 3 general types of pharmacokinetic curves to represent drug concentrations in the mother and fetus. The type 1 curve applies to substances which freely cross the placenta and rapidly distribute in a single fetal compartment. The rapid equilibrium between maternal and fetal drug concentrations persists throughout the time course of drug disposition. The fetal concentration is slightly higher as the only route of elimination is by tranfer back to the mother. The concentration ratio between mother and fetus is negligible because of rapid transfer from the relatively small fetal compartment to the mother.

In the type 2 pharmacokinetic curve, a large fetal compartment causes delayed equilibrium. This is followed by a crossover after which the fetal concentration remains greater than the maternal concentration. Only a small fraction of the large fetal compartment is cleared by transfer back to the mother. A pH gradient or extensive fetal plasma protein binding could create this situation. The delayed equilibrium illustrates the importance of adequate definition of the kinetic curve through serial measurements (Waddell and Marlowe 1981). If the interval to crossover is longer than the sampling times, the data may be erroneously interpreted to indicate limited transfer to the fetus, binding to maternal plasma, a pH gradient causing retention in maternal plasma, or fetal metabolism (Waddell and Marlowe 1981).

The type 1 and 2 pharmacokinetic curves describe the possibilities for the first model described by Szeto (1982). Waddell and Marlowe (1981) have described a type 3 kinetic curve which is in keeping with the third two-compartment model of Szeto (1982). In this model, the drug may be eliminated by both mother and fetus. The concentration of drug is always lower in fetal than in maternal blood. This relationship may be attributed to many different factors including maternal plasma protein binding, or a pH gradient favoring higher maternal concentrations. Also, metabolism by the fetus, excretion by the fetus directly into the uterine lumen, or disposition by the fetus into a large compartment such as the amniotic fluid are possible explanations. The rates of these processes are greater than the rate of drug transfer from the mother, resulting in lower fetal concentrations (Waddell and Marlowe 1981).

E. Ethanol Pharmacokinetics

a. Ethanol Disposition

The intensity and duration of a drug effect on the fetus are influenced by its pharmacokinetic disposition in the maternal-fetal unit. The duration and extent of toxic and therapeutic effects of ethanol are dependent on the rate of its metabolic degradation. Thus, the study of ethanol phamacokinetics in the maternal-fetal unit is warranted to further understand the effects of ethanol on the fetus.

All drug biotransformations and many biological processes involve enzyme or carrier systems. These systems are relatively specific with respect to substrate and are saturable. The Michaelis-Menten equation often best describes these types of reactions (Gibaldi and Perrier 1975). For a given substrate (drug):

$$-dC/dt = V_m C/(K_m + C)$$

where -dC/dt is the rate of change of drug concentration vs. time, C is the drug concentration, t is time, V_m is the theoretical maximum rate of the process, and K_m is the Michaelis constant, indicating affinity between substrate and enzyme. When $V=V_m/2$, $K_m=C$. When C is greater than 0.1 K_m, and less than 10 K_m, Michaelis-Menten kinetics apply. Two limiting cases of Michaelis-Menten kinetics occur. When C is small ($\ll 0.1K_m$), $-dC/dt=kC$, since V_m and K_m are constants. This is termed apparent first order kinetics, with respect to concentration. When C is large ($\gg 10K_m$), the elimination process is essentially saturated and a constant amount of drug is removed per unit time, independent of concentration. This is called apparent zero order kinetics. The most appropriate model for the description of a time course for drug disposition is determined by multiple blood sampling after a dose to obtain the plot of concentration versus time (Gibaldi and Perrier 1975).

Ethanol is soluble in water in all proportions which facilitates its rapid and uniform distribution throughout total body water by simple diffusion. It requires no energy facilitated transport mechanism and does not appear to be bound or stored. Elimination is primarily through metabolic conversion, although significant amounts may also be excreted unchanged in the urine and breath (Li 1977). Non-metabolic routes of elimination are more important at higher blood ethanol concentrations. Original observations by Widmark (1933) indicated that alcohol disappearance from the blood is linear and that the rate is constant above blood alcohol concentrations of 0.30 g/L. This traditional concept of elimination independent of blood concentration, termed zero order kinetics, has been challenged by several investigators. (Wilkinson et

al. 1976, Wagner et al. 1976, Lundquist and Wolthers 1958, Rangno et al. 1981).

Some investigators assume zero order kinetics because part of the downslope of the blood alcohol concentration-time curve appears linear (Widmark 1933, Raskin 1975, Goldstein 1970, Hawkins and Kalant 1972). This is a consequence of Michaelis-Menten kinetics (Wagner 1973), and as Lundquist and Wolthers (1958) suggested, the curve gradually approaches baseline. Other investigators (Widmark 1933, Raskin 1975, Goldstein 1970, Hawkins and Kalant 1972) believe that liver alcohol dehydrogenase is saturated at low concentrations of alcohol. The percent saturation of an enzyme system in Michaelis-Menten kinetics is given by:

$$\text{percent saturation} = 100(-dC/dt)/V_m = 100C/(K_m+C).$$

Using various values for C, Wagner et al. (1976) showed that the enzyme system is never saturated, even at high levels of alcohol.

Metabolic rates determined by the formula proposed by Widmark (1933) have been determined to be about 100 mg/kg/h (2 mmole/kg/h) (Hawkins and Kalant 1972) whereas calculations based on Michaelis-Menten kinetics yield metabolic rates of 120 to 150 mg/kg/h (Wagner et al. 1976). Thus the maximum capacity for alcohol elimination is underestimated by zero order kinetics. Also, alcohol elimination rates have been demonstrated to increase with alcohol concentration (Newman et al 1937, Grunnet & Thieden 1972, Rangno et al. 1981).

A summary of K_m and V_m values is presented in table 3. Human K_m values are similar with the exception of Rangno et al. (1981) who reported wide variation between subjects, routes of adminstration and doses with no detectable trend. The V_m values reported by Wagner et al. (1976), Wilkinson et al. (1976) and Lunquist and Wolthers (1958) are

Table 3. Summary of reported K_m and V_m values for ethanol in humans.

Study	$K_m \times 10^{-3}$ M	V_m (g/L/h)
Wagner et al. (1976)	3.0 (8)[a]	0.29
Wilkinson et al. (1976)	1.8 (6)	0.23
Lundquist and Wolthers (1958)	2.0 (10)	0.22
Korsten et al. (1975)	2.3 (6)	-
Rangno et al. (1981)	0.65 (8)	0.12 g/kg/h[b]

[a] number of subjects

[b] corrected for body weight

similar but are given in units customarily used for slope. Using data from Wagner et al. (1976) and Wilkinson et al. (1976), Rangno et al. (1981) recalculated the maximum ethanol elimination rate, the product of "V_m" and V_d, to be 0.12 g/kg/h. This resembled the V_m from the data of Rangno et al. (1981) (table 3). They determined the Widmark B60, a classical parameter used to describe ethanol elimination to be similar (0.11 g/kg/h) to the V_m obtained from Michaelis-Menten analysis (table 3), suggesting that ethanol elimination can be determined by both techniques.

Wagner et al. (1976) reasoned that if alcohol elimination could be described by zero order kinetics, then the absolute value of the slope of linear decline of the blood alcohol concentration would be independent of slope or initial concentration. Four different oral doses of alcohol were administered to 8 normal males using a crossover design The slope increased with increasing alcohol dosage (Wagner et al. 1976). In the study by Rangno et al. (1981), 8 normal male volunteers each received 3 intravenous doses of ethanol and 4 of the subjects received 4 oral doses. The slope of decline of ethanol concentration tended to increase with increased dose, but no significant difference was detected. The disproportionate increase in the area under the concentration time curve (AUC) with increased dose, as determined by the ratio of AUC to dose, is consistent with Michaelis-Menten kinetics (Rangno et al. 1981).

Ethanol is distributed in total body water, which, in the average man, represents about 60 percent (0.6 L/kg) of lean body weight. Wilkinson et al. (1976) estimated the volume of distribution (V_d) to be 0.54 L/kg, which is compatible with values given for total body water,

allowing for variation due to age and body weight. Wilkinson et al. (1976) concluded that the one compartment open model with zero order input and Michaelis-Menten kinetics is an operationally useful model to describe ethanol pharmacokinetics. Dedrick and Forrester (1973) suggested a two compartment model with allowance for hepatic blood flow. Using a two compartment open model with Michaelis-Menten elimination, Rangno et al. (1981) determined the V_d (0.47 L/kg) to be smaller than in previous studies (Wilkinson et al. 1976) and attributed this to the different model used and also to the fact that plasma rather than blood ethanol concentrations were used for the kinetic estimations.

b. Animal Studies

i. Sheep Studies

Further study and clarification of human data have been facilitated by the introduction of animal models. Many investigators have used the chronic sheep preparation in which near-term pregnant sheep have been surgically prepared with implanted catheters allowing the repeated sampling of maternal and fetal blood and the monitoring of maternal and fetal blood pressure, heart rate, and arterial blood gases (Van Petten et al. 1978). The major advantage is that the relatively large size of the fetus facilitates implantation of catheters. The use of the chronic preparation allows the animals several days of recovery from surgery and anesthesia before any studies are performed (Szeto 1982).

Reported studies using pregnant sheep were performed by the intravenous infusion of 10 percent v/v ethanol in 5 percent w/v dextrose in water at a rate of 15 ml/kg over 2 hours (1.2 g/kg over 2 hours). This is in accordance with Fuchs et al. (1967). Blood ethanol concentrations in mothers and in fetuses were not significantly

different at anytime after 30 minutes of infusion (Kirkpatrick et al. 1976, Ayromlooi et al. 1979, Cook et al. 1981, Mann et al. 1975), with one exception. Rose et al. (1981) reported a significantly lower fetal peak concentration than the maternal peak concentration. Alcohol is detectable in fetal blood within 2 minutes after start of maternal infusion (Ng et al. 1982, Cottle et al. 1980) The close correlation between maternal and fetal values indicate rapid placental transfer to the fetus, consistent with the molecular size, lipid solubility, and charge characteristics of ethanol.

Peak ethanol concentrations in studies where 1.2 g/kg of ethanol were administered range from 1.22 to 2.37 g/L in the mother and 1.21 to 2.22 g/L in the fetus (table 4). These concentrations occurred at 2 hours after start of infusion in all but the study by Mann et al. (1975) in which maternal ethanol concentrations were higher and peaked at 1.5 hours after start of infusion. However, this may be due, in part, to the acute anesthetized preparation used and may be used as evidence that alcohol disposition in pregnancy is altered during stress. Ng et al. (1982) administered 0.5 g/kg ethanol over 0.5 hours intravenously to 3 pregnant sheep. Maternal and fetal blood ethanol concentrations were similar (table 4). The peak amniotic fluid ethanol concentration was about half of the maternal concentration and occurred much later, at 2 hours after start of infusion. Cottle et al. (1980) similarily reported an amniotic fluid peak ethanol concentration of 70 to 78 percent of fetal peak concentration, occurring at 1.8 to 3.3 hours after start of infusion. These data require confirmation with studies using a larger sample size.

Table 4. Summary of reported ethanol pharmacokinetics in pregnant sheep

Study	Dose of Ethanol (g/kg)	Peak Plasma Ethanol Concentration (g/L)			Plasma Ethanol Clearance (mg/L/h)		
		M	F	A"	M	F	A"
A n=9	1.2	1.22	1.21	X	184[a]	206[a]	X
B n=13	1.2	1.49	1.44	X	204	205	X
C n=X	X	X	X	X	140-230	130-190	140-190
D n=20	1.2	1.63	1.22	X	X	X	X
E n=6	1.2	X	X	X	X	X	X
F n=10	1.2	2.37	2.22	X	X	X	X
G n=3	0.5	0.66-.12	0.61-0.82	0.38-0.52	145	143	107
H n=7	2.0	2.40	1.90	X	298[a]	214[a]	X

M - mother, F - Fetus, A"- amniotic fluid, [a]calculated from limited mean data, A - Ayromlooi et al. (1979), B - Cook et al. (1981), C - Cottle et al. (1980), D - Dilts (1970), E - Kirkpatrick et al. (1976), F - Mann et al. (1975), G - Ng et al. (1982), H - Rose et al. (1981) , X - not reported.

Mean clearance data were reported only in the study by Cook et al. (1981)(table 4). Clearance data reported by Rose et al. (1981), 400 mg/L/h and 100 mg/L/h for mother and fetus respectively do not correlate well with previous findings. However, recalculation from limited mean data yielded apparent clearance rates which are similar to other studies. In the report by Ng et al. (1982), the amniotic fluid clearance rate was significantly lower than maternal and fetal values in the 3 sheep studied. However, amniotic fluid clearance did not differ from maternal and fetal clearance in the study by Cottle et al. (1980). Amniotic fluid clearances merit further study.

Using the sheep model, different investigators have reported conflicting hemodynamic and blood gas alterations after acute ethanol adminstration in pregnancy. Variable effects on maternal and fetal heart rate, blood pressure, and arterial blood gases have been demonstrated, as shown in table 5. No change in maternal heart rate was noted in 3 studies (Ayromlooi et al. 1979, Mann et al. 1975, Ng et al. 1982), whereas Rose et al. (1981) reported an increase in maternal heart rate during infusion and Cook et al. (1981) demonstrated an increase in maternal heart rate during infusion and until 1 hour post-infusion. Cook et al. (1981) found a similar increase in fetal heart rate. These finding are in keeping with those of Ayromlooi et al. (1979) who also showed an increase in fetal heart rate until 1 hour post-infusion. No significant changes in fetal heart rate were reported in other studies (Kirkpatrick et al. 1976, Mann et al. 1975, Rose et al. 1981, Ng et al. 1982)(table 5).

Rose et al. (1981) reported an increase in maternal blood pressure during infusion, but no changes in blood pressure were observed in the

Table 5. Summary of reported pharmacodynamics in pregnant sheep

Study	Heart Rate		Blood Pressure		Arterial Blood Gases	
	M	F	M	F	M	F
A	0	$+^{b}$	0	$+^{a}$	$-$ pH^{a}	$+pO_2{}^{a,b}$
B	$+^{b}$	$+^{b}$	0	0	0	0
C	X	X	X	X	X	X
D	X	X	X	X	0	0
E	X	0	X	0	0	0
F	0	0	0	$-^{a}$	0	$-pH^{a}$,$+pO_2{}^{a}$
G	0	0	0	0	X	X
H	$+^{a}$	0	$+^{a}$	0	X	X

X - not reported, 0 - no change, + - increase, - - decrease in pharmacodynamic parameter, ^{a} during infusion, ^{b} after infusion, M - mother, F - fetus, A - Ayromlooi et al..(1979), B - Cook et al. (1981), C - Cottle et al. (1980), D - Dilts (1970), E - Kirkpatrick et al. (1976), F - Mann et al. (1975) G - Ng et al. (1982), H - Rose et al. (1981).

other studies (table 5). Ayromlooi et al. (1979) reported an increase in fetal blood pressure, whereas Mann et al. (1975) observed a decrease in fetal blood pressure during infusion and until 30 minutes post-infusion. This difference may again be due to the chronic preparation used in the former study and the acute preparation used in the latter study.

Changes in maternal blood gas parameters were found only by Ayromlooi et al. (1979). A decrease in blood pH was observed during infusion. They also reported an increase in fetal P_{O_2} during infusion which remained elevated. On the other hand, Mann et al. (1975) reported no change in fetal P_{O_2} but observed a decrease in arterial blood pH and an increase in P_{CO_2} during infusion and until 30 minutes post-infusion. No changes in fetal blood gases were reported in other studies (Cook et al. 1981, Dilts 1970, Kirkpatrick et al. 1976). Using sonocardiometry, Kirkpatrick et al. (1976) found that ethanol reduced fetal left ventricular output, and the extent and velocity of left ventricular fibre shortening without changing left ventricular end diastolic diameter or systemic arterial pressure.

Some of the observed differences may be attributed to surgical trauma or anesthesia in the acute preparation. However, different results reported using chronic sheep preparations may be related to ethanol dosage. Further studies are required to clarify existing data.

ii. Other Species

In a study by Horiguchi et al. (1971), the intravenous infusion of 2 to 4 g/kg of ethanol over one hour to 13 pregnant monkeys in the third trimester resulted in a peak maternal ethanol concentration of 2.37 $\pm$ 0.35 g/L at the end of the infusion, and a peak fetal concentration of 1.65 $\pm$ 0.30 g/L at 1.5 hours after start of infusion. Fetal tachycardia,

hypotension, and acidosis were observed during the infusion with only minimal maternal acid base changes. Operative stress in this acute preparation may have contributed to the failure of ethanol to inhibit uterine contractions and to the fetal acidosis (Fuchs & Fuchs 1981). Using limited mean data from this study, it was possible to calculate the apparent clearance of ethanol as 140 mg/L/h in the mother and 85 mg/L/h in the fetus. Hill et al. (1981) reported nearly identical elimination rates for mother and fetus in 9 pregnant monkeys at 154 days gestation (term 167 days), but after delivery of the fetus, the maternal clearance of 149 $\pm$ 16 mg/L/h was four times that of the neonate (36 $\pm$ 2 mg/L/h). They concluded that fetal exposure to ethanol is determined primarily by maternal elimination capacity. In one experiment, Horiguchi et al. (1971) infused ethanol directly into the fetus. A dose of 2 g/kg fetal body weight (based on assumed fetal body weight of 0.5 kg) was infused directly into the fetal jugular vein over 90 minutes. Blood ethanol concentrations were not stated. Fetal pH and blood pressure decreased while fetal heart rate, P_{CO_2} and base deficit increased during ethanol infusion. This may indicate a higher susceptibility to ethanol by the fetus.

Mukherjee and Hodgen (1982) reported ethanol-induced impairment of umbilical circulation causing hypoxia and acidosis. A 35 percent v/v ethanol in 5 percent w/v dextrose in normal saline (3.0 g/kg body weight) was administered as a bolus (1 to 2 minutes) into the maternal femoral vein of 5 pregnant monkeys (120 to 147 days gestation). This dose is much higher than those used previously. Collapse of umbilical vessels began within 10 to 15 minutes after ethanol administration and gradual recovery was seen after about 30 minutes. Fetal blood pH

declined from 7.30 ± 0.08 at 15 minutes to 6.81 ± 0.05 at 30 minutes. Fetal P_{O_2} dropped from 38 ± 2 at time zero to 11 ± 2 mm Hg at 30 minutes. Maternal peak ethanol concentrations, at 15 minutes, were reported to be 2.50 ± 0.82 g/L. Fetal concentrations peaked at 30 minutes, at which time they were reported to be one-third of maternal concentrations. The lag in fetal ethanol uptake may suggest impaired placental transfer of ethanol. From limited data, fetal peak concentrations were estimated at 0.75 g/L. Fetal clearance could not be calculated from the data given. Calculated maternal ethanol clearance from mean data was 113 mg/L/h. Graphical representation showed fetal clearance to be much slower than maternal clearance. The authors attributed this difference to differences in metabolism or continued distribution of ethanol pools after recovery of the umbilical circulation. These data show that even brief exposure to ethanol may have deleterious effects, but data relating dosage to the degree of fetal hypoxia are required, as the high dose administered over a short time period may be responsible.

The short term effects of ethanol on the fetus when it is used to prevent premature labour are largely unknown. Despite attempts by several investigators to clarify existing data, conflicting results appear in the literature (Mann et al. 1975, Ayromlooi et al. 1979, Kirkpatrick et al. 1976, Cook et al. 1981). It is assumed that the risks of ethanol use are less significant than the risk of prematurity and its complications. However, the importance of fetal risk associated with light to moderate ethanol ingestion for medical or non-medical reasons, and how it relates to long term growth, development and behaviour of the child must be assessed. Further studies must elaborate

on the physiological effects of acute exposure to ethanol on the fetus and on the neonate.

F. Dissertation Objectives

1. to determine the kinetic disposition of ethanol in the pregnant ewe, fetal lamb, and newborn lamb

2. to assess the acute effects of ethanol on cardiovascular function of the pregnant ewe, fetal lamb, and newborn lamb

3. to determine ADH activity of the livers of the adult ewe, fetal lamb, and newborn lamb, and placental tissue

4. to determine correlation between ethanol disposition *in vivo* and ADH activity *in vitro*

5. to relate these data to those in other species in order to determine the usefulness of this model for the studies of ethanol disposition and effect in pregnancy

METHODS

A. Surgical preparation

a. Near-term Pregnant Sheep

Studies were performed on 8 near-term pregnant sheep, weighing 60 to 80 kg, and their fetuses, gestational age 124 to 142 days (term 150 days). At least 2 days prior to study, each ewe was anesthetized with 1.5 percent halothane in oxygen, intubated, and ventilated to maintain normal arterial P_{CO_2}. Maternal femoral arterial and venous catheters were inserted. Through a midline incision, the uterus was exposed and a small hysterotomy incision was made. A fetal hind limb was withdrawn and polyvinyl catheters were placed in the fetal femoral artery and vein, and in the fetal axillary artery. In some studies, a catheter was placed in the amniotic fluid sac and tied to a fetal hind limb. All incisions were closed and catheters were tunnelled subcutaneously to the ewe's flank and stored in a protective pouch. Immediately following the surgical procedure, catheters were filled with a 4 IU/ml heparin in saline solution. Penicillin G (2,000,000 IU) and gentamicin (120 mg) were administered intravenously to the ewe. At the same time, the fetus received penicillin G (1,000,000 IU) and gentamicin (40 mg) intravenously. The procedure lasted about 1.5 hours.

b. Newborn Lambs

For surgical preparation, lambs (1 to 5 days old) received 70 percent nitrous oxide and 30 percent oxygen inhalational analgesia by mask. All incisions were infiltrated with 2 percent lidocaine. Catheters were inserted in the femoral artery and vein. The incisions were closed and catheters were tied to the tail. Catheters were filled with a 4 IU/ml heparin in saline solution. The procedure lasted about 30 minutes. Lambs were allowed at least 1 hour recovery prior to study.

B. Experimental Protocols

a. Maternal Infusion Study

On the day of investigation, ewes were transported in a cart to a quiet study room with a companion sheep and left for at least 1 hour prior to the experiment. They had free access to food and water during the procedure. In the maternal infusion study, a 9.5 percent v/v solution of ethanol in 5 percent w/v dextrose in water was administered via the maternal femoral vein by infusion pump at a rate of 15 ml/kg total body weight over 2 hours.

Simultaneous blood samples were obtained from the maternal (1.0 ml) and fetal (0.75 ml) femoral arteries at 30 minute intervals, starting just prior to the infusion and continuing for 5 to 7 hours after the end of ethanol infusion. Amniotic fluid samples (1.0 ml) were similarly obtained from the same animals in some studies. At each sampling time, the heparinized saline was removed from the catheters. A volume of blood approximately equal to twice the catheter dead space was removed, and re-infused after sampling. The samples were placed in 10 ml Vacutainer^{R} tubes containing 20 mg potassium oxalate and 25 mg sodium fluoride, and stored on ice.

Additional blood samples were drawn into heparinized syringes at 1 hour intervals for immediate blood gas analysis, using a Corning pH/blood gas instrument model 165/2 (Canlab, Winnipeg, Manitoba). Hematocrit was determined every 3 hours.

Maternal and fetal heart rate and blood pressure were monitored with Statham transducers connected to indwelling catheters, and recorded on a Hewlett Packard model 8824C Dynograph strip chart multichannel recorder (Hewlett Packard, Mississauga, Ontario). At the end of the experiment, ewes were anesthetized with intravenous sodium pentothal 2.5

percent solution (1 g) and sacrificed with intravenous saturated potassium chloride solution (50 ml) to allow for verification of catheter placement and for liver removal for ADH determinations.

b. Fetal Infusion Study

To assess the transfer of ethanol from the fetus to the mother, ethanol was administered directly to the fetus as described below. In one fetal infusion study, the ethanol solution, as described above, was administered via the fetal femoral vein at a rate of 7.5 ml/kg total body weight over 1 hour.Two successive studies used infusion rates of 6.1 ml/kg over 4 hours, and 4.0 ml/kg over 3.5 hours. Blood samples were collected as in the maternal infusion studies, starting just before and continuing for 2 to 3 hours after ending ethanol infusion. Blood gas analysis, heart rate, blood pressure, and hematocrit were determined as in the maternal infusion studies.

c. Amniotic Fluid Infusion Study

The same ethanol solution was administered via the amniotic fluid catheter at a rate of 3.0 ml/kg total body weight over 1 hour. Samples from the maternal and fetal femoral arteries and from the amniotic sac were obtained as in the maternal infusion studies. Sampling continued for 5 hours after the end of the infusion.

d. Lamb Infusion Study

Studies were performed on newborn lambs, ages 1 to 5 days, weighing 2.5 to 5.8 kg. The ethanol solution, as previously described, was infused via the femoral vein at a rate of 7.5 ml/kg over 1 hour with a Harvard infusion pump, either model 940 (double barrel) or model 901 (single barrel) (Harvard Apparatus Co. Inc., Milles, Mass.). Blood glucose was maintained between 4.4 and 6.7 mmol/L by intravenous

infusion of 5 percent w/v dextrose solution. Blood samples were obtained from the femoral artery (0.75 ml) at 30 minute intervals until 2 hours after start of the infusion, and at 60 minute intervals thereafter, until 7 to 9 hours after end of infusion. The samples were taken and processed as in the maternal infusion studies.

Arterial blood gas analysis and hematocrit were determined at 1 hour intervals for the first 2 hours, and at 2 hour intervals thereafter. Heart rate and arterial blood pressure were monitored with Statham transducers connected to indwelling catheters, and recorded on a Grass model 5 polygraph recorder (Grass Instrument Co., Quincy, Mass.). At the end of the experiments, lambs were anesthetized with an intravenous injection of 10 ml sodium pentothal 2.5 percent w/v solution (250 mg) and sacrificed with 20 ml of intravenous saturated potassium chloride solution to verify catheter placement and for liver removal for ADH determinations.

C. Sample Collection

a. Plasma and Amniotic Fluid Samples for Ethanol Determination

Within 2 hours of collection, blood samples were centrifuged for 2 minutes using a Fisher microcentrifuge (model 235A). The plasma was separated and stored immediately at -20 ^{o}C. The amniotic fluid samples were also stored at -20 ^{o}C. Ethanol analysis by gas-liquid chromatography was performed within 1 month after the experiment.

b. Tissue for ADH Determination

Tissue samples from maternal, fetal, and lamb livers and from placenta were obtained after sacrifice and immediately put on ice. The samples were weighed and 10 to 50 percent homogenates were prepared with a Polytron Homogenizer (setting 5, Brinkmann Instruments Ltd., Toronto,

Ontario) in ice-cold 0.25 M sucrose containing 1 percent v/v Triton X-100, to give maximal ADH activity (Raiha and Koskinen 1964). After centrifugation of the homogenate for 20 minutes at 9000 x g, the supernatant samples were immediately frozen until analyzed. The samples were analyzed for ADH activity and protein content. Analyses were completed within 2 months of tissue preparation.

D. Sample Analyses

a. Ethanol Determination

Plasma and amniotic fluid ethanol concentrations were determined by gas-liquid chromatography with a Hewlett Packard model 402 instrument, using a flame ionization detector and a Poropaks Q/R (1/1) column (80-100 mesh)(Solon et al. 1972). Operating parameters included a column and injector port temperature of 150 ^{o}C and a detector temperature of 250 ^{o}C. Nitrogen was used as the carrier gas.

Propanol-1 (0.2% v/v) was used as internal standard. Plasma or amniotic fluid (50 μl) and internal standard (50 μl) were stirred for 30 seconds with a vortex-type mixer. Using a Hamilton microlitre syringe, 2 μl of this solution were injected into the injection port. The syringe was rinsed repeatedly with a sodium bicarbonate (10 percent w/v) solution followed by distilled water. A calibration curve was determined by serial dilution of a solution of ethanol (1.52 g/L) in plasma. The method used to determine the concentration of ethanol was the peak height ratio between ethanol and propanol-1, monitored on a Fisher Recordall Series 5000 recorder. The assay was sensitive to 0.01 g/L plasma ethanol. The analysis for each sample required 5 minutes. Samples were re-checked at various intervals to assure that there was no difference in measurements over the 4 month assay period.

i. Principles of Gas Chromatography

The term, chromatography, is applied when components of a mixture are separated between a mobile and a stationary phase by making use of differences in their partition coefficients. In gas-liquid chromatography, a gaseous moving phase and a liquid stationary phase, usually adsorbed on an inert solid, are used. The gaseous moving phase comes into contact with the stationary phase by passing through a glass, metal or plastic column. In our experiments, nitrogen gas was the gaseous moving phase, and Porapaks Q/R were the solid stationary phase in a glass column. Porapaks are actually solids with lipophilic characteristics.

Small quantities of sample vapour dissolve in both phases, to an extent dependent on the partition coefficients of the components. Carrier gas and sample vapour equilibrate with the stationary phase as they move throughout the length of the column. The amount of vapour eluted from the column reaches a maximum and then falls to zero as all vapour passes through it.

A detector produces an electrical signal, the magnitude of which is proportional to the quantity of each component present per unit time, as they are eluted. Using a flame ionization detector, combustion of the solute components in the carrier gas in a hydrogen flame causes ionization. The conducting gases cause current to flow between 2 electrodes which are held at a constant potential. The current change is used to provide the signal which is recorded as the chromatogram (Pattison 1973).

An internal standard is often included to account for errors inherent in the experiment. The relative peak heights of the sample to the standard are proportional to the amount of sample present.

b. Alcohol Dehydrogenase Activity

Maternal, fetal, and lamb liver and placental ADH activity was determined at 25 ^{o}C and at 37 ^{o}C by recording the generation of NADH at 340 nm in a Beckman DU-8 spectrophotometer. The buffers used were 0.2 M sodium phosphate (pH 6.5 to 8.0) or 0.2 M glycine-sodium hydroxide (pH 8.6 to 10.4) (Dawson et al. 1969). The reaction mixture contained 95 percent v/v ethanol solution (0.01 ml), NAD 10 mg/ml (0.1 ml), and liver or placenta supernatant (0.05-0.2 ml) in 3.0 ml of buffer. The mixture was incubated in a water bath at 25 ^{o}C prior to recording of the absorbance. Ethanol was added to start the reaction. A blank determination was also performed without the addition of ethanol. This value was subtracted from the increase in absorbance found in the presence of substrate. The change in absorbance after 3.2 minutes was noted. Determinations were performed in triplicate. One unit (U) of enzyme is defined as that amount which will catalyze the transformation of one micromole of substrate (ethanol) per minute under defined temperature and pH conditions (Bonnichsen and Brink 1955). Activity was expressed as mU/g tissue.

c. Protein Determination

The protein content of samples was determined by the method of Lowry et al. (1951). Bovine serum albumin (50 ug/ml) was used as the standard. Liver and placenta homogenates (0.33 ml) were diluted to 100 ml with distilled water. Triplicate aliqouts (1.0 ml) of these solutions were further diluted to 5.0 ml with distilled water, and 1.0 ml of this dilution was taken and placed into 15 ml test tube. A blank solution containing 0.25 M sucrose-Triton X-100 buffer was similarily prepared.

Reagents:

a) 2% Na_2CO_3 in 0.1N NaOH

b) 1% $CuSO_4 \cdot 5H_2O$

c) 2% NaK tartrate

d) equal parts of (b) and (c)

e) 98% reagent (a) and 2% reagent (d)

f) commercial phenol reagent diluted 1:1 with distilled water

Reagent e (5.0 ml) was added to each test tube, mixed, and allowed to stand at room temperature for 10 minutes. Reagent f (0.5 ml) was added and mixed immediately. After standing for 30 minutes, the samples were read in a Beckman model DU-8 spectrophotometer at 750 nm. Determinations were performed in duplicate.

i. Principles of Spectrophotometry

Spectrophotometry involves the measurement of the ability of a dissolved substance to absorb electromagnetic radiation of defined and narrow wavelength ranges. Most measurements are made in the ultraviolet (200-400 nm) and visible (400-750 nm) ranges of the spectrum. Absorptions are measured at wavelengths characteristic of the chemical composition of the absorbing substance. Blank determinations are made on the solvents and reagents used to obtain a correction for their inherent absorbances.

The essential parts of a spectrophotometer include a source of radiant energy, a dispersing device with a slit for wavelength band selection, a cell for the sample, and a photometer to indicate the intensity of the transmitted radiation.

An unknown concentration of a known compound, if it conforms to Beer's law, can be determined by the equation:

$$\log I_0/I = \epsilon . c . l$$

where I_0 is the intensity of the incident light, I is the intensity of the transmitted light, ϵ is the molar absorptivity, c is the concentration, and l is the pathlength. The term log I_0/I or log (1/T) is referred to as absorbance A, or optical density (OD) (Osol et al. 1975).

The determination of ADH activity is based on the spectrophotometric measurement of the reduction of the coenzyme NAD in the presence of ethanol. NAD^+ has an absorbance peak at 260 nm. The reduced form, NADH, has an additional peak at 340 nm and can be detected without interference from the oxidized form.

E. Data Analysis

Data for the calibration curve for ethanol quantitation and the elimination rates for ethanol from maternal, fetal, and lamb circulation and amniotic fluid were analyzed by least squares linear regression analysis followed by analysis of variance to assess goodness of fit (Goldstein 1964). The apparent volume of distribution (V_d) was determined by dividing the total dose by the initial concentration (C_o). The initial concentration was determined by extrapolating the peak concentration to zero time and correcting for elimination of ethanol during the time interval for the animal under investigation. Area under the concentration versus time curve (AUC) was determined by the trapezoidal rule from the beginning of drug administration to the end of the experiment, and extrapolated to zero ethanol concentration using the elimination rate experimentally determined in the same preparation. All data are presented as mean $\pm$ standard error. Comparisons of data for significant differences were done using analysis of variance, correlation analysis or paired t-test. The minimum level for a significant difference is $p \leq 0.05$.

RESULTS

A. Ethanol Assay

Sample chromatograms are shown in figure 1. The standard curve of the peak height ratios for ethanol/propranol-1 in plasma was linear in the concentration range of 0.10 to 1.5 g/L. Correlation coefficient values for definition of the linearity of these standard curves were always greater than 0.99.

B. Kinetic Disposition of Ethanol

a. Maternal Infusion Study

Representative plasma ethanol concentration-time curves for a maternal ethanol infusion experiment are shown in figure 2. A total dose of 1.2 g/kg ethanol over 2 hours resulted in peak maternal and fetal ethanol concentrations as shown in table 6 (studies 1-5). Peak maternal and fetal concentrations occurred at the end of infusion, with one exception. In study 4, the fetal peak occurred at 2.5 hours. During infusion, fetal concentrations were slightly lower than maternal ethanol concentrations, but were higher at most times after the end of ethanol administration (figure 2). However, these differences were not significant. The calculated maternal and fetal areas under the concentration-time curve (AUC) were similar, and the apparent volume of distribution (V_d) did not vary widely. Maternal and _in utero_ fetal plasma ethanol clearance rates were similar (table 6).

In experiments 6-9 (table 6), involving loading and maintenance doses, maternal and fetal peak ethanol concentrations were similar. In studies 6,7, and 9, amniotic fluid peak concentrations were determined to be about 2/3 of maternal and fetal peak plasma concentrations. Maternal and fetal peak concentrations occurred at the end of the bolus infusion or during the maintenance infusion (1-2h), with the exception

Figure 1. Chromatograms (A-plasma standard, B-experimental plasma sample and C-plasma blank) from the gas chromatographic analysis of plasma for ethanol with propranol-1 as the internal standard. Peak 1 represents ethanol and peak 2 represents propanol-1. Inj designates the time of sample injection.

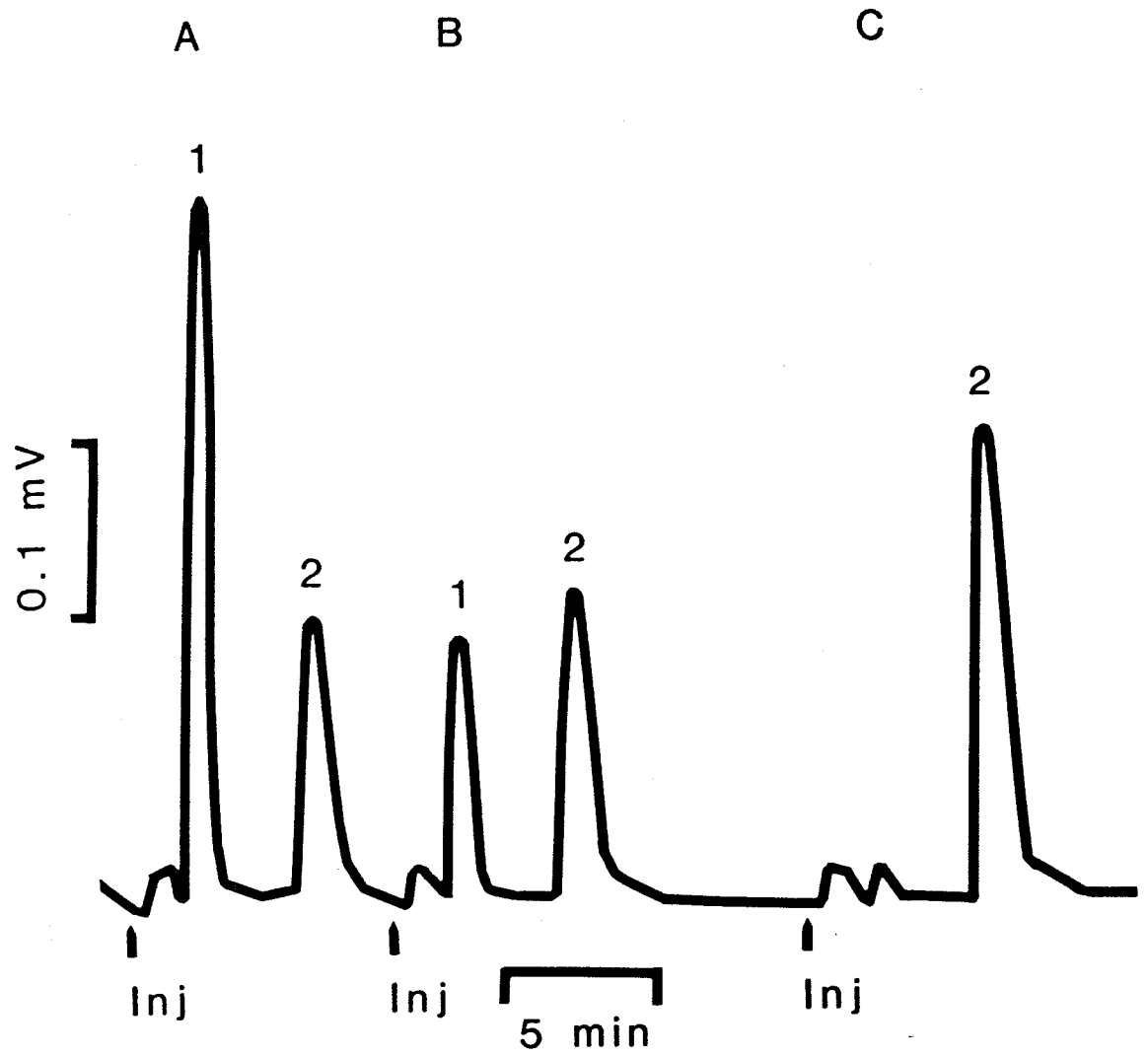

A
B
C
1
2
1
2
2
0.1 mV
Inj
Inj
Inj
5 min

Figure 2. Representative plasma ethanol concentration versus time curve from maternal (●) and fetal (Δ) blood samples after maternal intravenous infusion of 9.5% v/v ethanol in 5% w/v dextrose solution at 1.2 g/kg over 2 hours (experiment 1, table 6).

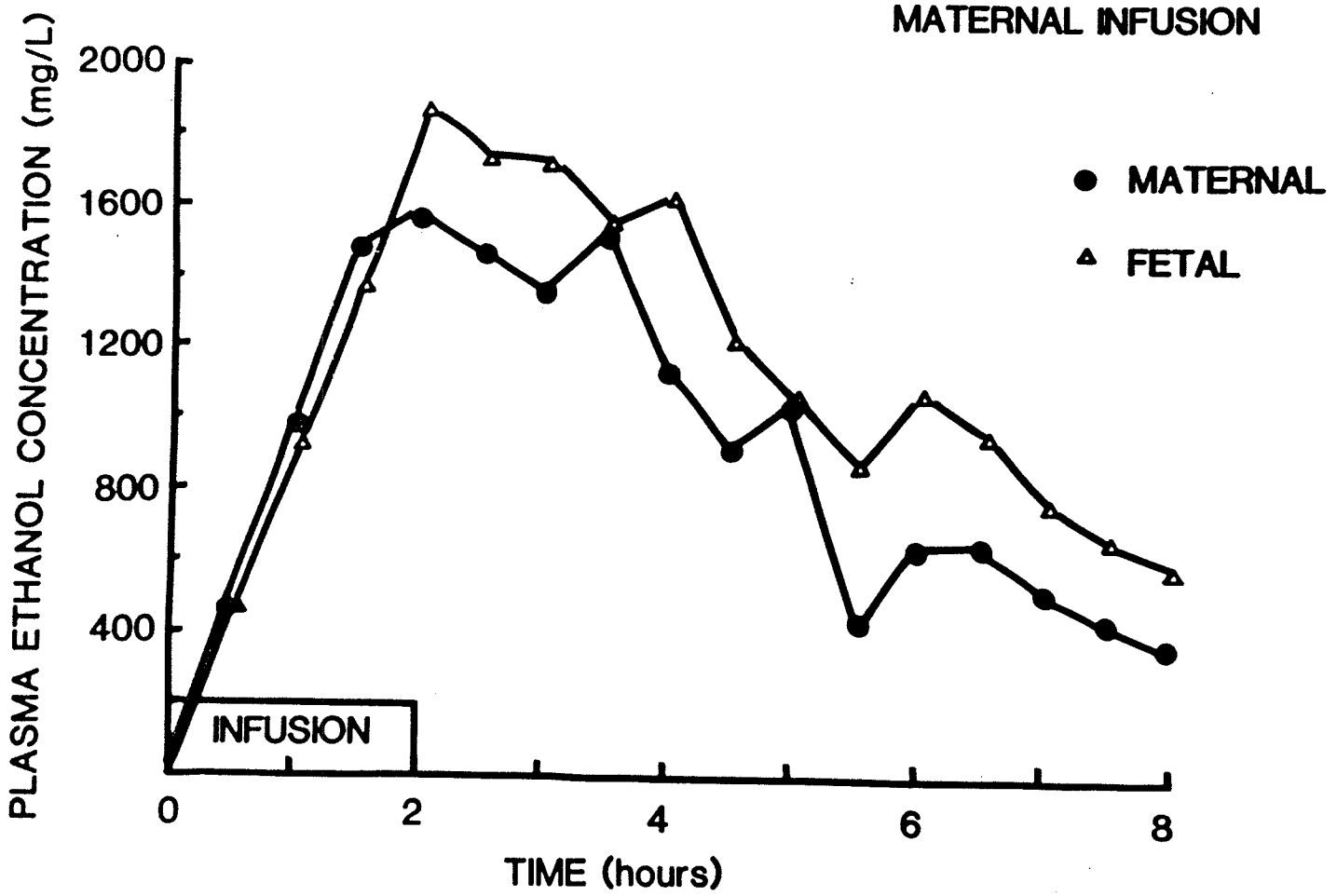

MATERNAL INFUSION
MATERNAL
FETAL
PLASMA ETHANOL CONCENTRATION (mg/L)
2000
1600
1200
800
400
INFUSION
0
2
4
6
8
TIME (hours)

Table 6. Pharmacokinetic disposition after maternal intravenous infusion of ethanol to pregnant sheep.

	Dose (g/kg)	Peak Concentration (g/L)			Clearance (mg/L/h)			AUC (g.h/L)			V_d (L/kg)
		M	F	A	M	F	A	M	F	A	
1.	1.2	1.5 (2)[a]	1.8 (2)	-	216	218	-	7.2	8.9	-	0.68
2.	1.2	2.1 (2)	1.6 (2)	-	236	198	-	7.2	6.5	-	0.51
3.	1.2	1.6 (2)	2.1 (2)	-	168	198	-	7.6	9.9	-	0.69
4.	1.2	2.1 (2)	1.8 (2.5)	-	215	200	-	9.4	8.4	-	0.53
5.	1.2	1.7 (2)	2.0 (2)	-	179	218	-	7.6	7.5	-	0.65
mean		1.8	1.9	-	203	206	-	7.8	8.2	-	0.61
S.E.		0.1	0.1	-	13	5	-	0.4	0.6	-	0.04
6.	b-0.6 m-.12	0.9 (1)	1.0 (1)	0.7 (2.5)	-	-	-	-	-	-	-
7.	b-0.8 m-.12	0.8 (3)	1.1 (1.75)	0.7 (6)	171	210	-	3.6	3.4	3.6	0.83
8.	b-0.8 m-.12	1.5 (1)	1.4 (1.5)	-	-	-	-	-	-	-	-
9.	b-0.8 m-.12	1.3 (1.25)	1.2 (1.5)	0.7 (5)	191	177	151	5.3	5.7	4.4	0.71

M-mother
F-fetus
A-amniotic fluid
b-bolus dose
m-maintenance dose
[a] time at which peak concentration occurred (h)

of the maternal peak concentration in experiment 7 which was lower and occurred later. In all cases, the amniotic fluid peak concentration occurred after the end of ethanol infusion. Maternal and fetal plasma ethanol clearance rates in experiments 6-9 were similar to those determined in experiments 1-5. In one experiment (9), amniotic fluid ethanol clearance was determined and was lower than maternal or fetal clearance. Calculated AUC was lower than those in experiments 1 to 5, and V_d in this small sample was slightly higher.

b. Fetal Infusion Study

The results of the fetal infusion experiments are summarized in table 7. Fetal infusion of 0.6 g/kg over 1 hour (experiment 1) resulted in fetal death 5 minutes after end of infusion. Representative plasma ethanol concentration-time curves from the fetal infusion studies are shown in figure 3. Fetal infusion of 6.1 ml/kg total body weight over 4 hours (0.5 g/kg) resulted in peak maternal and fetal ethanol concentrations of 0.3 g/L and 1.2 g/L respectively at the end of the infusion (experiment 2, table 7). A four-fold difference in maternal and fetal peak concentrations was observed in both fetal infusion studies described (numbers 1 and 2). This difference was minimized in a third study using a lower infusion rate (number 3). The apparent maternal plasma ethanol clearance rates were similar to those in the maternal infusion experiments. Apparent fetal clearance was determined in only 1 experiment because of fetal death in experiment 1 and insufficient data points in experiment 2. The AUC calculated in experiment 2 was 4 times greater in the fetus than in the mother and about 50% greater in experiment 3. The calculated V_d varied more than in the maternal infusion studies (table 7).

c. Amniotic Fluid Infusion Study

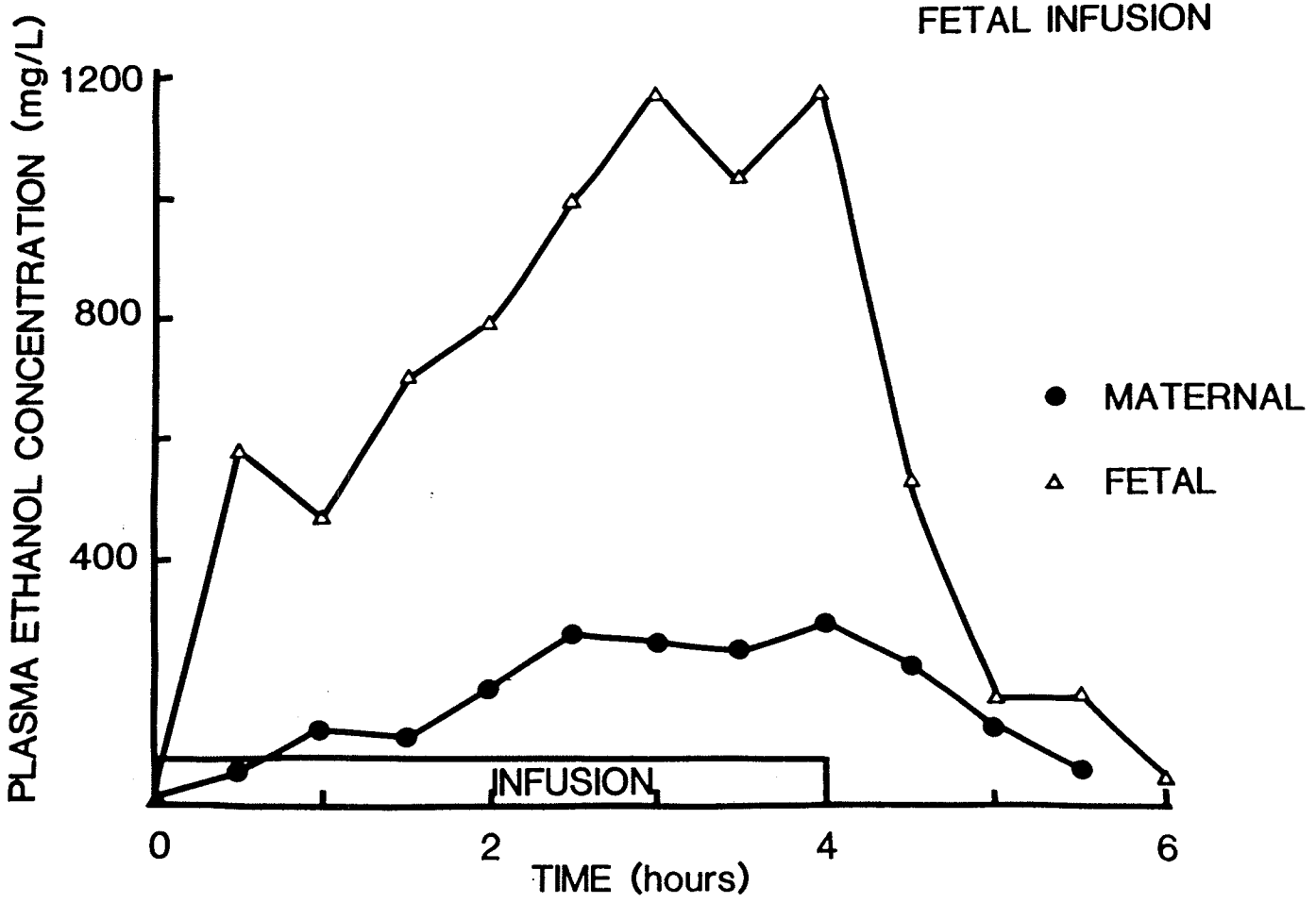
Table 7. Ethanol pharmacokinetic disposition after fetal intravenous infusion to sheep.

	Dose (g/kg)	Peak Concen -tration (g/L)		Clearance (mg/L/h)		AUC (g.h/L)		V_d (L/kg)
		M	F	M	F	M	F	
1.	0.6	1.2 (1)[a]	4.7 (1)	202	-	-	-	0.41
2.	0.5	0.3 (4)	1.2 (4)	164	-	0.9	3.9	1.03
3.	0.3	0.4 (3.5)	0.5 (2.5)	175	197	1.2	1.7	0.50
mean				180				0.65
S.E				11				0.19

M - mother
F - fetus

[a] time at which peak concentration occurred (h)

Figure 3. Representative plasma ethanol concentration versus time curve from maternal (●) and fetal (△) blood samples after fetal intravenous infusion of 9.5% v/v ethanol in 5% w/v dextrose solution at 0.5 g/kg over 4 hours (experiment 2, table 7).


FETAL INFUSION
PLASMA ETHANOL CONCENTRATION (mg/L)
1200
800
400
MATERNAL
FETAL
INFUSION
0
2
4
6
TIME (hours)

The results of an experiment in which ethanol was infused directly into the amniotic fluid are summarized in table 8. The peak amniotic fluid ethanol concentration could not be determined as only one catheter was available for sampling and infusion. The peak fetal plasma ethanol concentration was higher than the maternal peak and occurred earlier. However, both maternal and fetal peak concentrations were reached after the infusion was ended. The calculated AUC for the fetus was twice the maternal value. Maternal and fetal plasma ethanol clearance rates could not be determined due to concentrations lower than our assay sensitivity, but amniotic fluid clearance was determined.

The plasma ethanol clearance rates from the maternal, in utero fetal and amniotic fluid infusion studies are summarized in table 9. Maternal and in utero fetal clearance rates were similar regardless of whether the infusion was to the mother or fetus. Amniotic fluid clearance was less than maternal and fetal rates in one maternal infusion and in the amniotic fluid infusion experiment.

d. Lamb Infusion Study

A representative lamb plasma ethanol concentration-time curve is shown in figure 4. A total of 0.6 g/kg ethanol was infused over 1 hour. Table 10 summarizes the results of this study. Peak ethanol concentrations occurred at the end of infusion in all experiments. There was little variation in the AUC and V_d values. The plasma ethanol clearance rates varied from 22 to 57 mg/L/h. Figure 5 illustrates the relationship between plasma ethanol clearance and lamb age. Plasma ethanol clearance increased with age ($F_{1,7}=22.99; p<0.005; r=0.88$).

C. Relationship between Pharmacokinetics and Pharmacodynamics

a. Maternal Infusion Study

Table 8. Pharmacokinetic disposition after infusion of ethanol into amniotic fluid of pregnant sheep.

Dose (g/kg)	Peak Concentration (g/L)			Clearance (mg/L/h)			AUC (g.h/L)		
	M	F	A	M	F	A	M	F	A
0.23	0.2 (2.5)[a]	0.3 (1.5)	- (1)	-	-	116	0.5	1.0	-

M - mother
F - fetus
A - amniotic fluid

[a] time at which peak concentration occurred

Table 9. Apparent plasma and amniotic fluid clearance of ethanol. Data are presented as mean ± S.E.

Infusion Site	Apparent Ethanol Clearance (mg/L/h)		
	M	F	A
Maternal	197 ± 10 (7)[n]	203 ± 5 (7)	151 (1)
Fetal	180 ± 11 (3)	197 (1)	-
Amniotic Fluid	-	-	116 (1)

M - maternal

F - fetal

A - amniotic fluid

[n] number of experiments

Figure 4. Representative plasma ethanol concentration versus time curve from lamb blood samples after intravenous infusion of 9.5% v/v ethanol in 5% w/v dextrose solution at 0.6 g/kg over 1 hour (experiment 8, table 10).

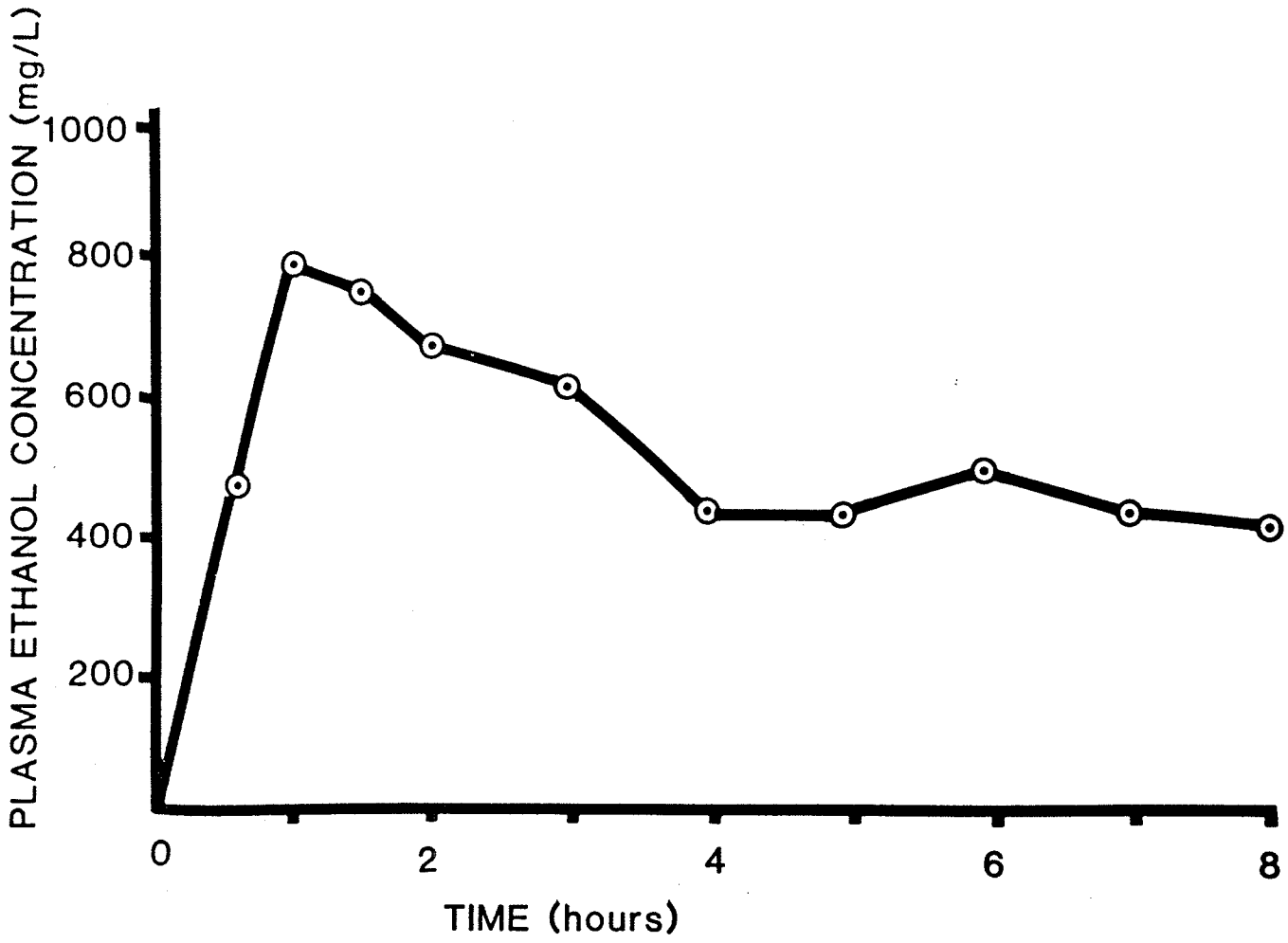
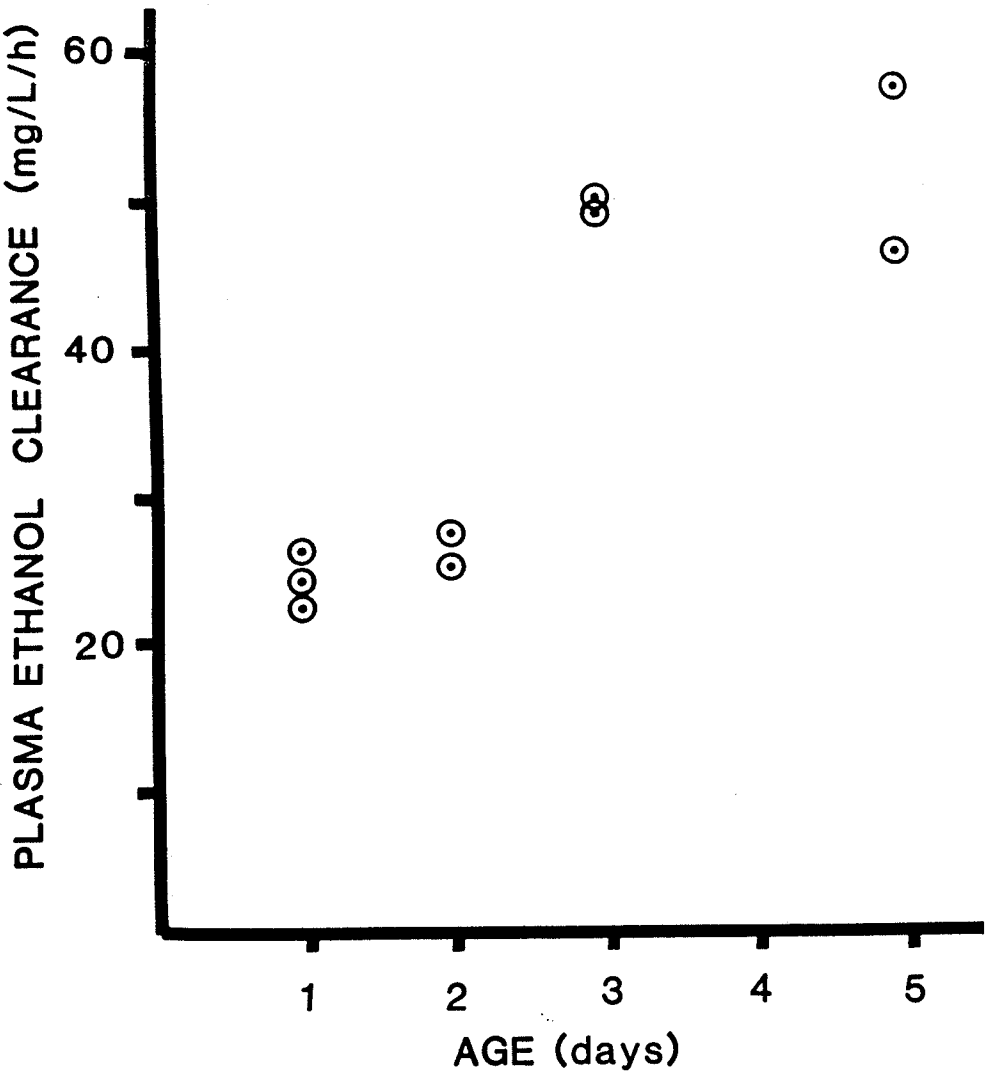

PLASMA ETHANOL CONCENTRATION (mg/L)
1000
800
600
400
200
0
2
4
6
8
TIME (hours)

Table 10. Pharmacokinetic disposition following intravenous infusion of ethanol to newborn lambs.

	Dose (g/kg)	Peak Concentration (g/L)	Clearance (mg/L/h)	AUC (g.h/L)	V_d (L/kg)
1.	0.6	0.7	22	3.6	0.85
2.	0.6	0.6	26	3.6	0.82
3.	0.6	0.5	24	3.2	1.04
4	0.6	0.7	25	3.4	0.82
5.	0.6	0.5	27	3.3	1.01
6.	0.6	0.7	50	3.7	0.69
7.	0.6	0.8	49	4.0	0.68
8.	0.6	0.6	46	3.1	0.84
9.	0.6	0.7	57	3.2	0.72
mean		0.64	36	3.5	0.83
S.E.		0.03	5	0.1	0.04

Figure 5. Relationship between apparent plasma ethanol clearance rate and lamb age ($F_{1,7}=22.99;p<0.005;r=0.88$).


PLASMA ETHANOL CLEARANCE (mg/L/h)
60
40
20
1
2
3
4
5
AGE (days)

Concurrent pharmacodynamic assessment of cardiovascular parameters during the maternal infusion studies showed the following weak but statistically significant associations. The relationship between maternal heart rate and maternal plasma ethanol concentration is presented in figure 6. Maternal heart rate varied between 96 and 156 beats per minute and increased with ethanol concentration ($F_{1,54}$=8.72;$p<0.01$;r=0.37). Fetal heart rate varied between 120 and 228 beats per minute and also increased with fetal plasma ethanol concentration ($F_{1,54}$=5.89;$p<0.05$;r=0.31) (figure 7). No consistent changes were observed in maternal (70 to 116 mm Hg) or fetal (36 to 70 mm Hg) mean arterial blood pressure with time or treatment. Similarly, maternal pH (7.32 to 7.59), P_{CO_2} (19 to 40 mm Hg), or P_{O_2} (67 to 112 mm Hg) and fetal pH (7.23 to 7.51), P_{CO_2} (30 to 56 mm Hg), or P_{O_2} (10 to 28 mm Hg) did not change with time or treatment.

b. Fetal Infusion Study

The following weak but statistically significant associations were observed in the fetal infusion studies. Maternal blood pressure increased with maternal plasma ethanol concentration ($F_{1,31}$ =4.91;$p<0.05$;r=0.37)(figure 8). In contrast to the maternal infusion study, fetal heart rate was inversely related to fetal ethanol concentration ($F_{1,26}$=15.81;$p<0.001$;r=-0.61) (figure 9). No changes were found in fetal blood pressure (40 to 86 mm Hg), maternal heart rate (100 to 150 beats per minute), maternal pH (7.38 to 7.59), P_{CO_2} (25 to 37 mm Hg) or P_{O_2} (74 to 101 mm Hg) and fetal pH (7.27 to 7.46), P_{O_2} (40 to 50 mm Hg) or P_{O_2} (9 to 22 mm Hg) with time or treatment.

c. Lamb Infusion Study

Figure 6. Relationship between maternal heart rate and maternal plasma ethanol concentration during and following maternal infusion of 9.5% v/v ethanol in 5% w/v dextrose solution at 1.2 g/kg over 2 hours ($F_{1,54}=8.72; p<0.01; r=0.37$).

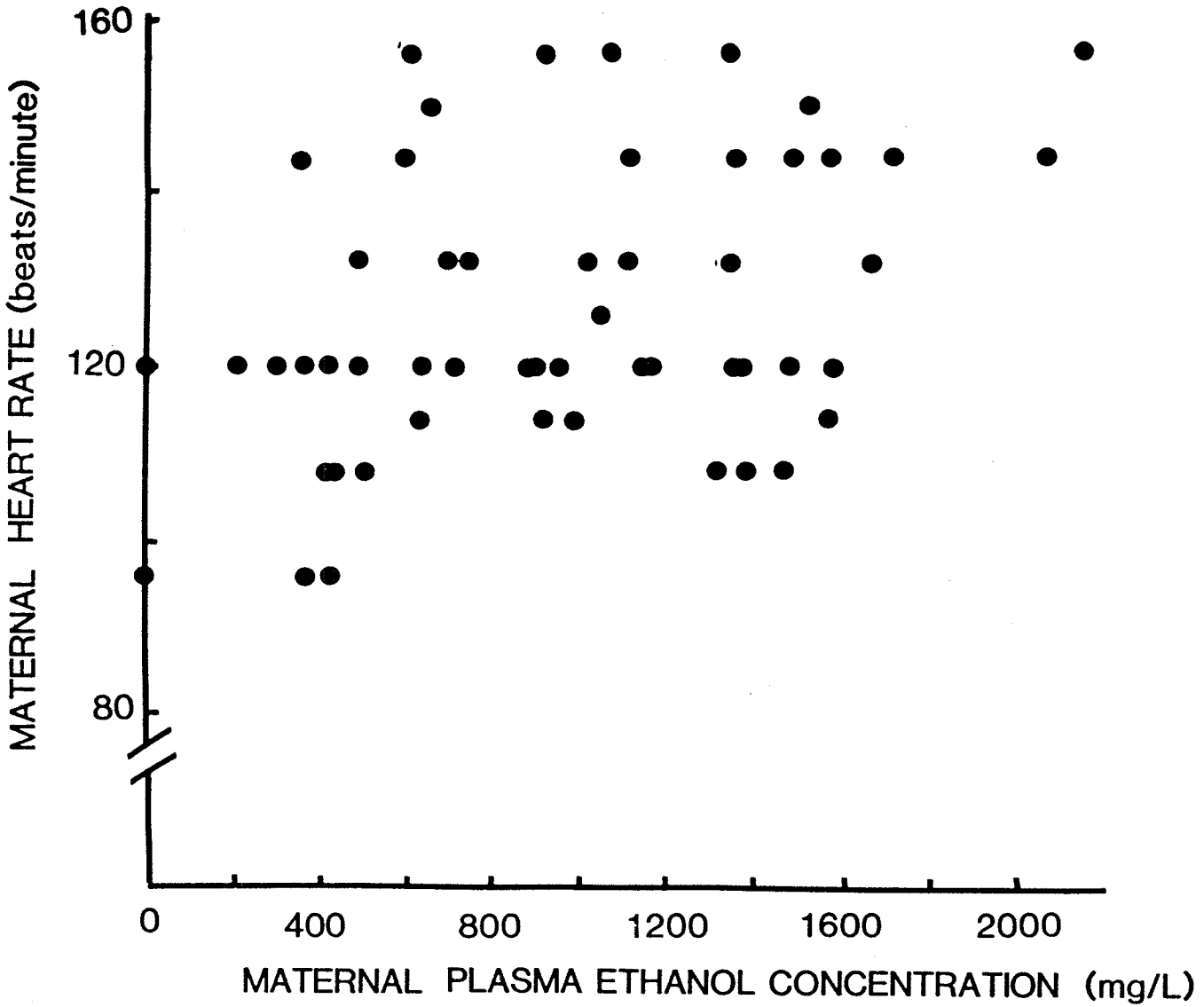

160
120
80
MATERNAL HEART RATE (beats/minute)
0
400
800
1200
1600
2000
MATERNAL PLASMA ETHANOL CONCENTRATION (mg/L)

Figure 7. Relationship between fetal heart rate and fetal plasma ethanol concentration during and following maternal infusion of 9.5% v/v ethanol in 5% w/v dextrose solution at 1.2 g/kg over 2 hours ($F_{1,54}=5.89; p<0.05; r=0.31$).

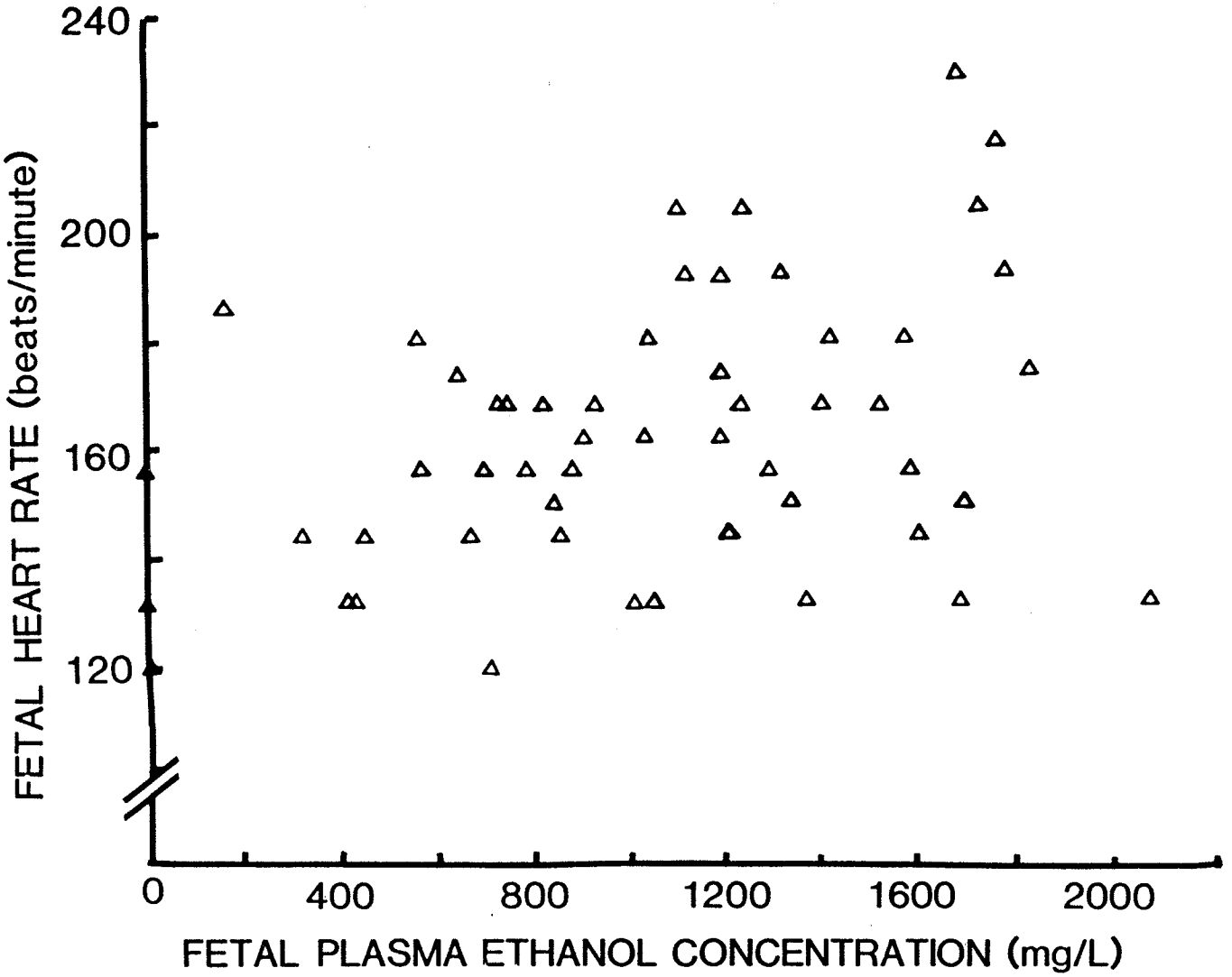

240
200
160
120
FETAL HEART RATE (beats/minute)
0
400
800
1200
1600
2000
FETAL PLASMA ETHANOL CONCENTRATION (mg/L)

Figure 8. Relationship between maternal blood pressure and maternal ethanol concentration during and following fetal infusion of 9.5% v/v ethanol in 5% w/v dextrose solution ($F_{1,31}=4.91;p<0.05;r=0.37$).

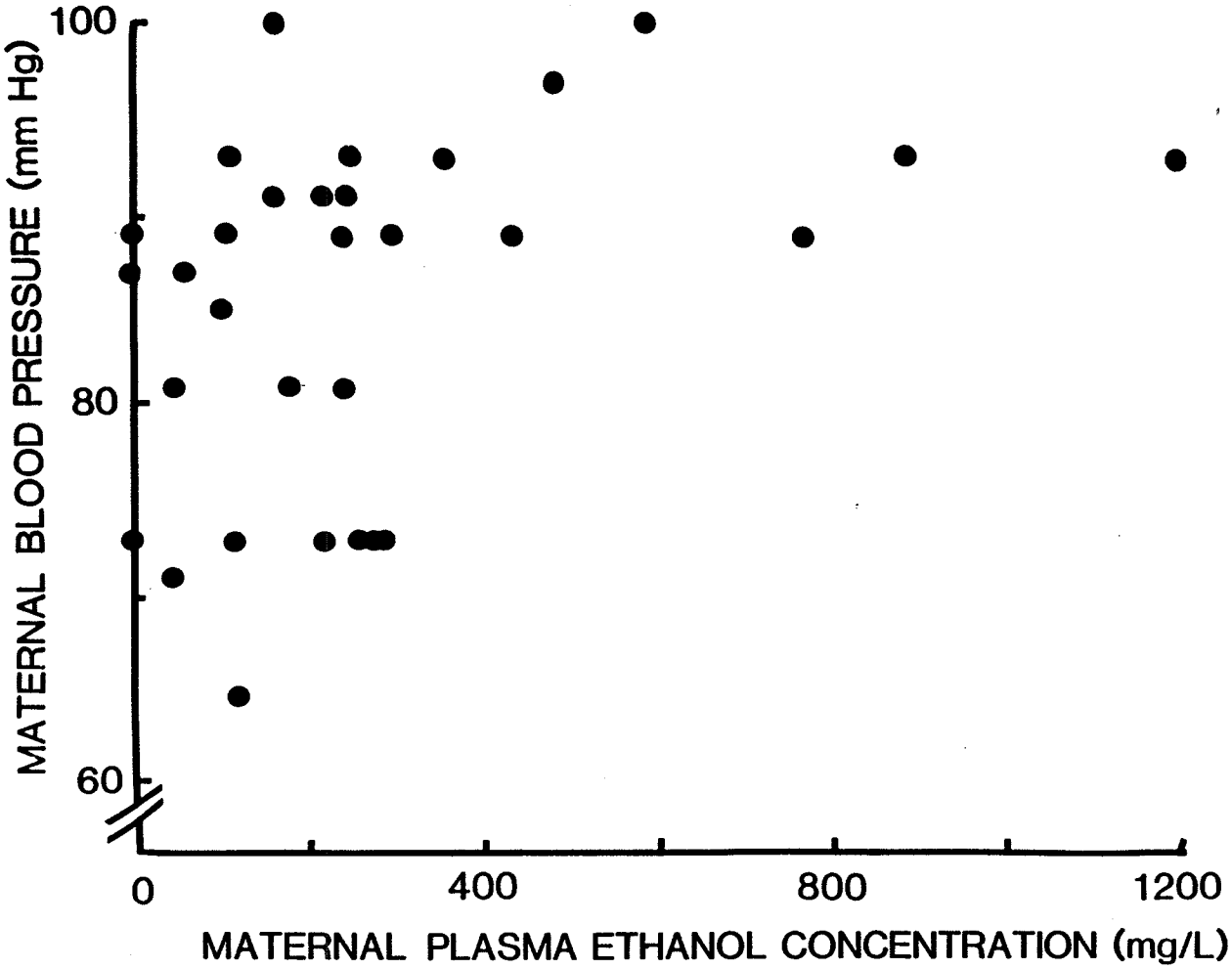
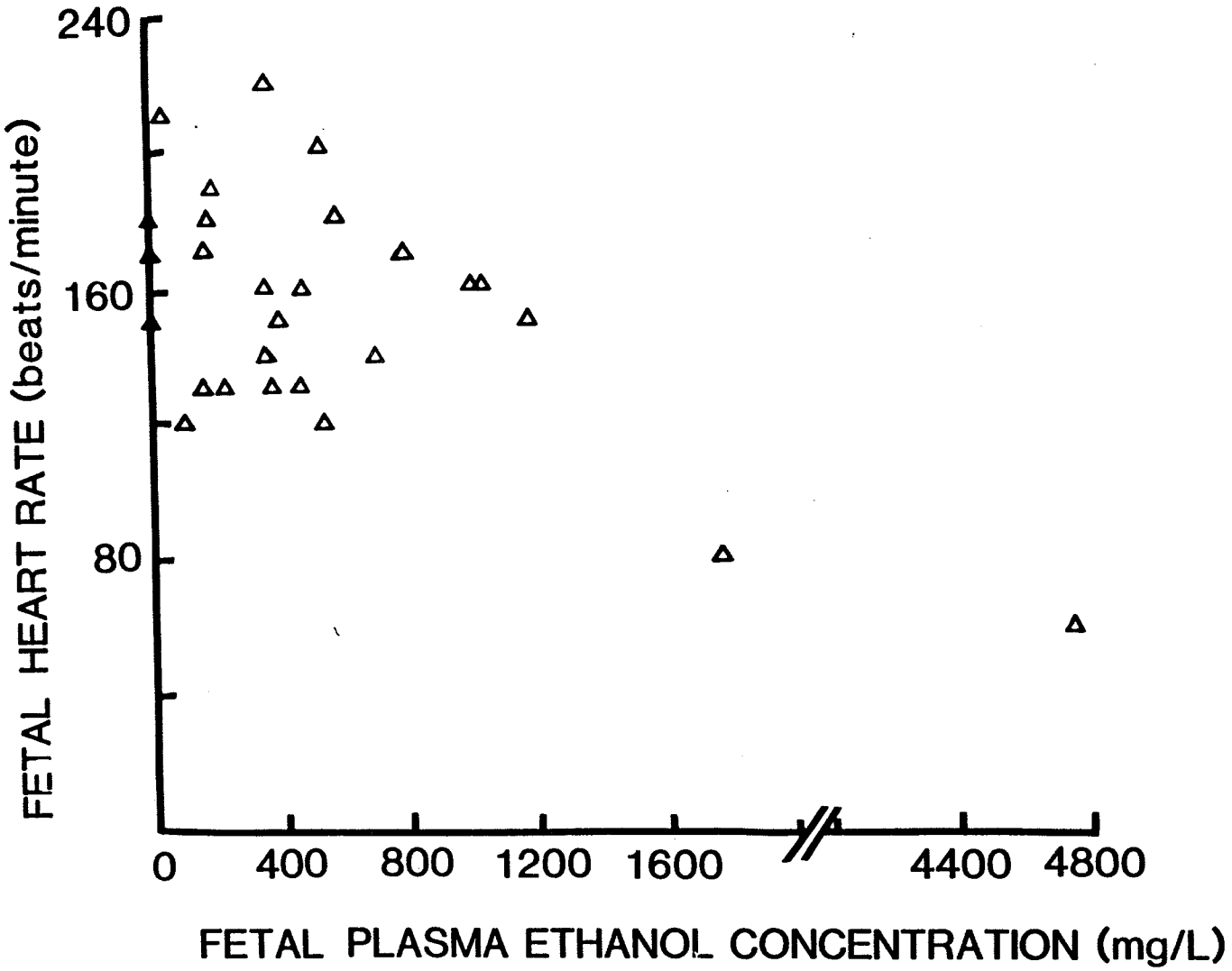

100
80
60
MATERNAL BLOOD PRESSURE (mm Hg)
0
400
800
1200
MATERNAL PLASMA ETHANOL CONCENTRATION (mg/L)

Figure 9. Relationship between fetal heart rate and fetal ethanol concentration during and following fetal infusion of 9.5% v/v ethanol in 5% w/v dextrose solution ($F_{1,26}=15.81;p<0.001;r=-0.61$).


240
160
80
FETAL HEART RATE (beats/minute)
0
400
800
1200
1600
4400
4800
FETAL PLASMA ETHANOL CONCENTRATION (mg/L)

Heart rate in neonatal lambs ranged between 156 and 276 beats per minute. This was associated with mean arterial blood pressures between 40 and 90 mm Hg. Blood pH ranged from 7.39 to 7.56. This was associated with P_{O_2} values between 50 and 103 mm Hg and P_{CO_2} values between 26 and 44 mm Hg. None of these measurements correlated with time or treatment.

D. Alcohol Dehydrogenase

a. Optimal pH for Activity

The relationship between ADH activity and pH at 25 ^{o}C is presented in figures 10 and 11. The optimal pH for adult liver, fetal liver, and placental tissue was determined to be 9.0, 9.6, and 9.0 respectively (figure 10). The pH optimum for 1 and 2 day old lamb liver ADH was determined to be 8.0, and for 3 and 5 day old lamb livers, 9.6 (figure 11).

b. Maximum Activity

Table 11 summarizes the results of the determination of ADH activity in maternal, fetal and lamb liver, and in placenta. The relationship between activity of ADH at optimal pH and 25 ^{o}C and liver age is shown in figure 12. Adult enzyme activity was at least 10 times greater than fetal or lamb ADH activity. Fetal and lamb ADH activities were similar. Placental enzyme activity was much lower, about 1/4 of that in fetal and neonatal lamb liver or 1/40 of maternal liver ADH.

Figure 13 illustrates the relationship between lamb liver ADH activity at optimal pH and 25 ^{o}C and lamb age. Enzyme activity was found to increase with age ($F_{1,7}=5.73; p<0.05; r=0.67$). ADH activity increased from 37 $\pm$ 11 mU/g tissue in the 1 and 2 day old lambs (n=5) to 75 $\pm$ 16 mU/g tissue in the 3 and 5 day old lambs (n=4).

c. Activity at Physiological pH as a Function of Temperature

Figure 10. Relationship between ADH activity at 25°C and pH in adult and fetal livers, and placenta.

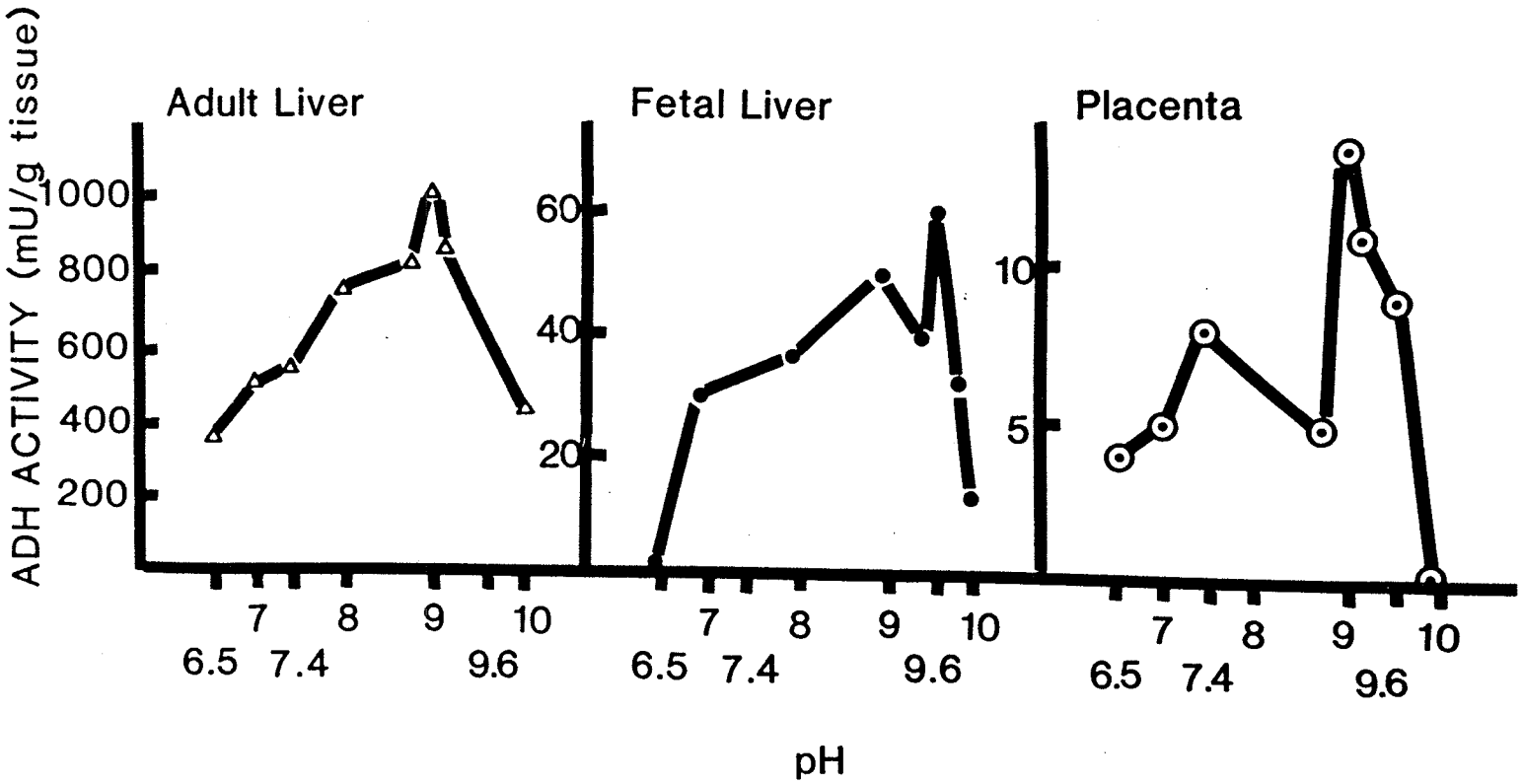

ADH ACTIVITY (mU/g tissue)
Adult Liver
Fetal Liver
Placenta
1000
800
600
400
200
60
40
20
10
5
7
8
9
10
6.5
7.4
9.6
pH

Figure 11. Relationship between ADH activity at 25^{o}C and pH in 1,2,3 and 5 day old lamb liver.

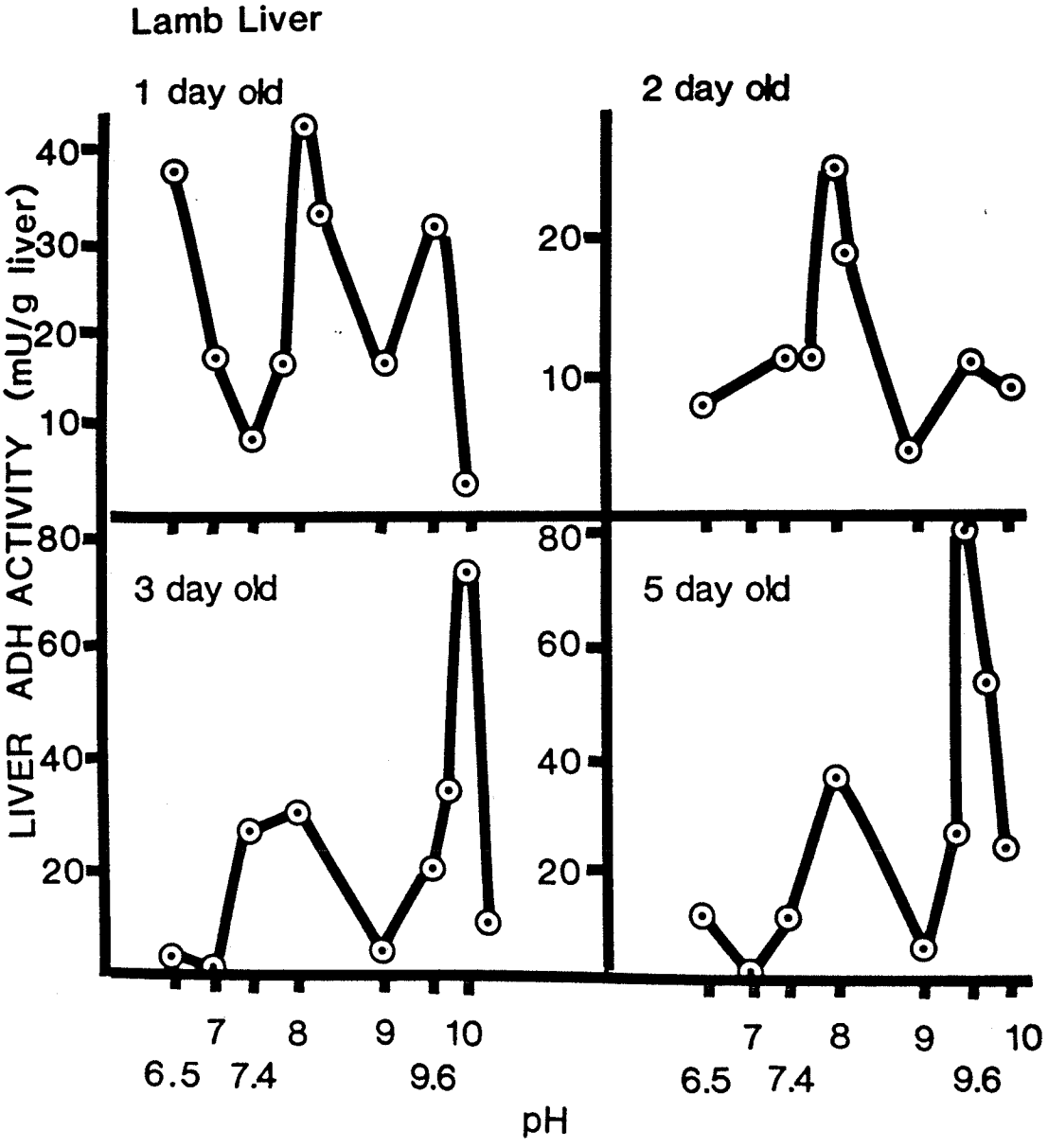

Lamb Liver
1 day old
2 day old
3 day old
5 day old
LIVER ADH ACTIVITY (mU/g liver)
40
30
20
10
80
60
40
20
7
8
9
10
6.5
7.4
9.6
pH

Table 11. Alcohol dehydrogenase activity at optimal pH and 25°C in maternal, fetal and lamb livers and placenta.

Sample	Activity at pH optimum (mU/g tissue)	pH optimum
Maternal Liver		
1	1005	9.0
2	559	9.0
3	958	9.6
4	395	9.6
mean	729	
S.E.	150	
Fetal Liver		
1	62	9.0
2	59	9.6
3	95	9.6
4	51	9.6
5	91	9.6
mean	72	
S.E.	9	

Sample	Activity at pH optimum (mU/g tissue)	pH optimum
Lamb Liver		
1 (1)[a]	42	8.0
2 (1)	77	8.0
3 (1)	22	9.6
4 (2)	21	9.6
5 (2)	25	8.0
6 (3)	73	10.0
7 (3)	33	10.0
8 (5)	112	9.6
9 (5)	81	9.6
mean	54	
S.E.	11	
Placenta		
1	14	9.0
2	10	9.0
3	24	9.0
4	11	9.0
mean	15	
S.E	3	

[a] age in days

Figure 12 .Relationship between liver ADH activity at optimal pH and 25 ^{o}C and age in adult (Δ) and fetal (●) sheep and in newborn lambs (⊙).

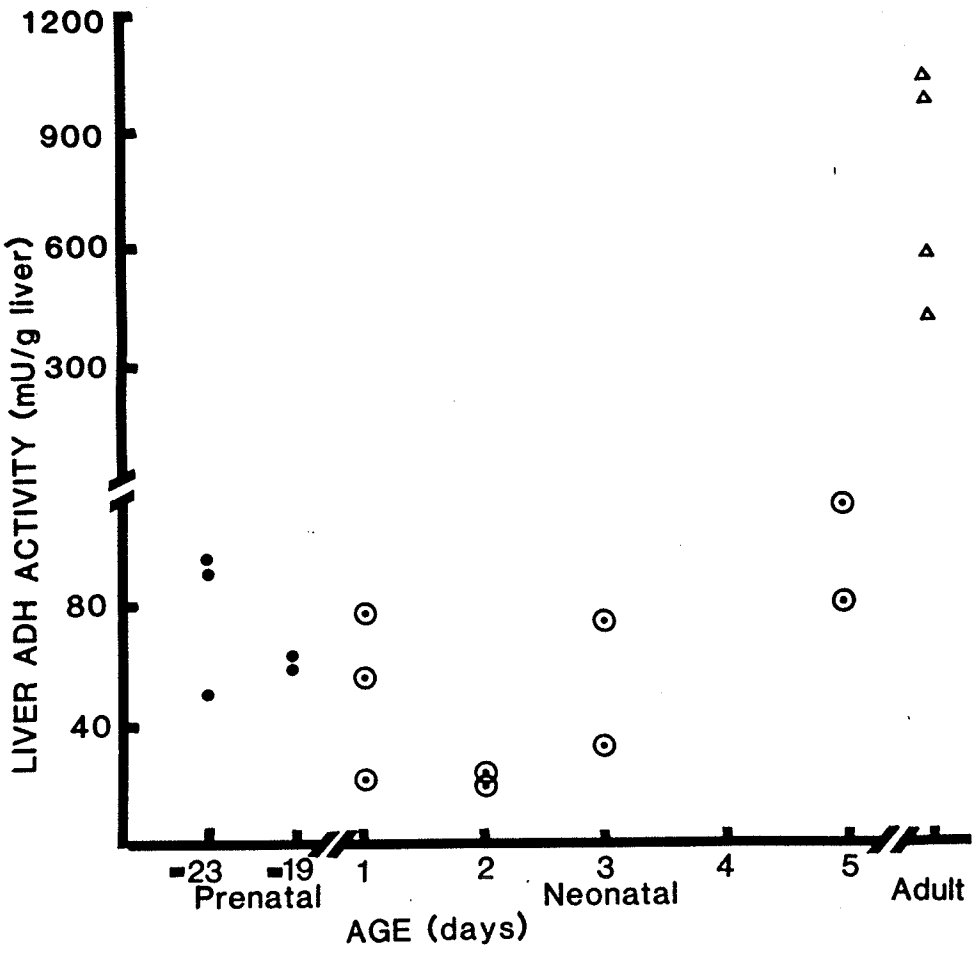

1200
900
600
300
80
40
LIVER ADH ACTIVITY (mU/g liver)
-23
-19
1
2
3
4
5
Prenatal
Neonatal
Adult
AGE (days)

Figure 13. Relationship between lamb liver ADH activity at optimal pH and 25 ^{o}C and age ($F_{1,7}$=5.73;p<0.05;r=0.67).

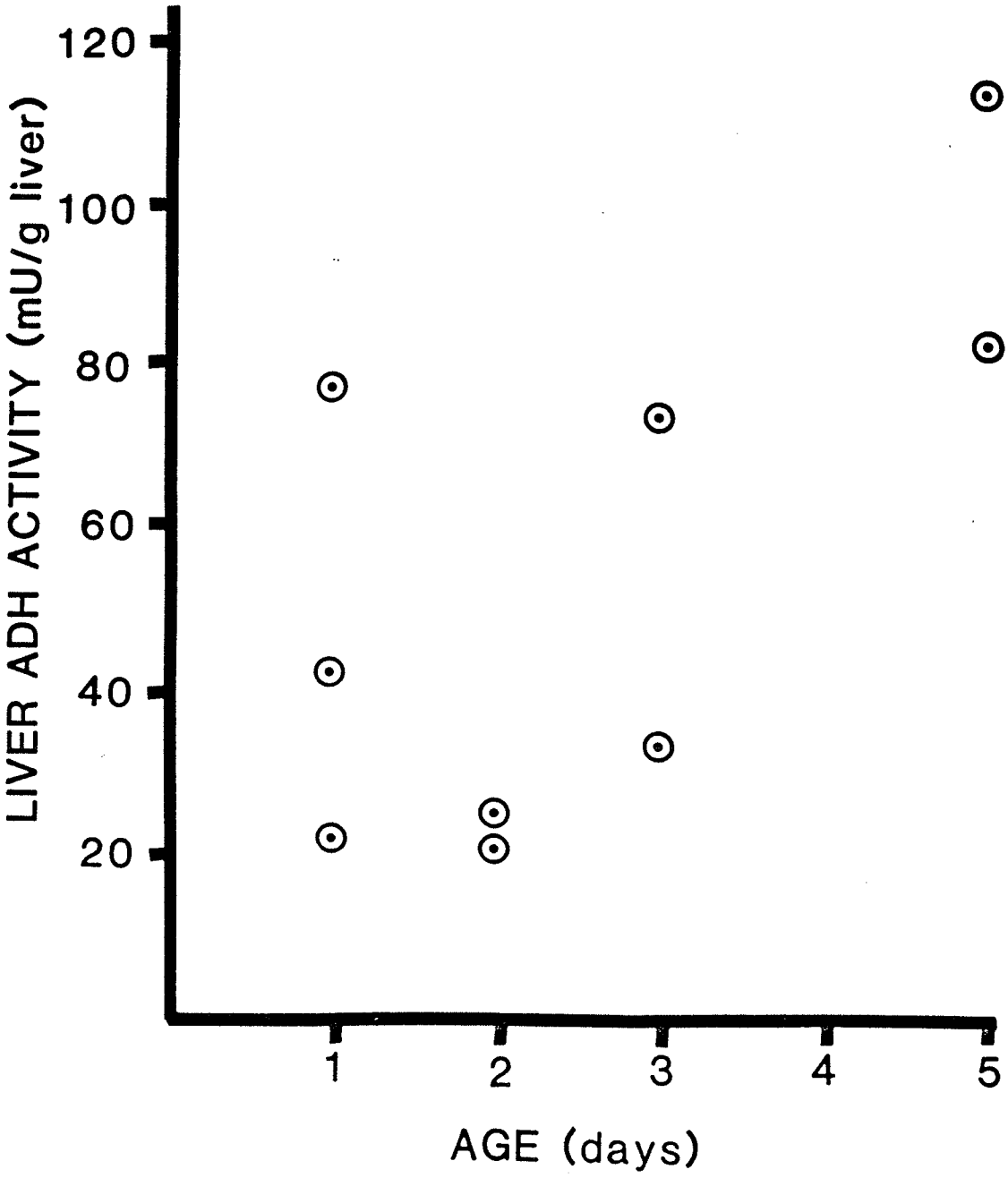

LIVER ADH ACTIVITY (mU/g liver)
120
100
80
60
40
20
1
2
3
4
5
AGE (days)

Table 12 compares the activity of ADH at 25 °C and at 37 °C in maternal, fetal and lamb liver, and placenta at pH 7.4. Activity is greater at 37 °C in all tissues, but the ratio of activity at 37 °C to 25 °C varies.

E. Relationship between ADH Activity and Ethanol Pharmacokinetics

The relationship between *in vitro* ADH activity at pH optimum and 25°C and *in vivo* plasma ethanol clearance rates is presented in table 13. Maternal and fetal *in utero* plasma ethanol clearance rates are similar. However, the mean fetal liver ADH activity is only about 10 percent of maternal liver ADH activity. Both liver ADH activity and plasma ethanol clearance are lower in the lambs than in the maternal sheep. However, no correlation between plasma ethanol clearance and liver ADH activity at optimal pH and 25°C (figure 14), or at physiological pH and either 25 °C or 37 °C for the same animals was demonstrated for neonatal lambs.

Table 12. Alcohol dehydrogenase activity at pH 7.4. Comparison between activity at 25 °C and 37°C in maternal, fetal and lamb liver and placenta.

Sample	ADH Activity (mU/g tissue)		Activity Ratio 37°/25°C
	25°C	37°C	
Maternal Liver	556	1008	1.81
Fetal Liver	31	65	2.10
Placenta	8	10	1.25
Lamb Liver (1 day)	7	11	1.57
Lamb Liver (2 day)	11	16	1.45
Lamb Liver (3 day)	26	35	1.35
Lamb Liver (5 day)	11	20	1.82

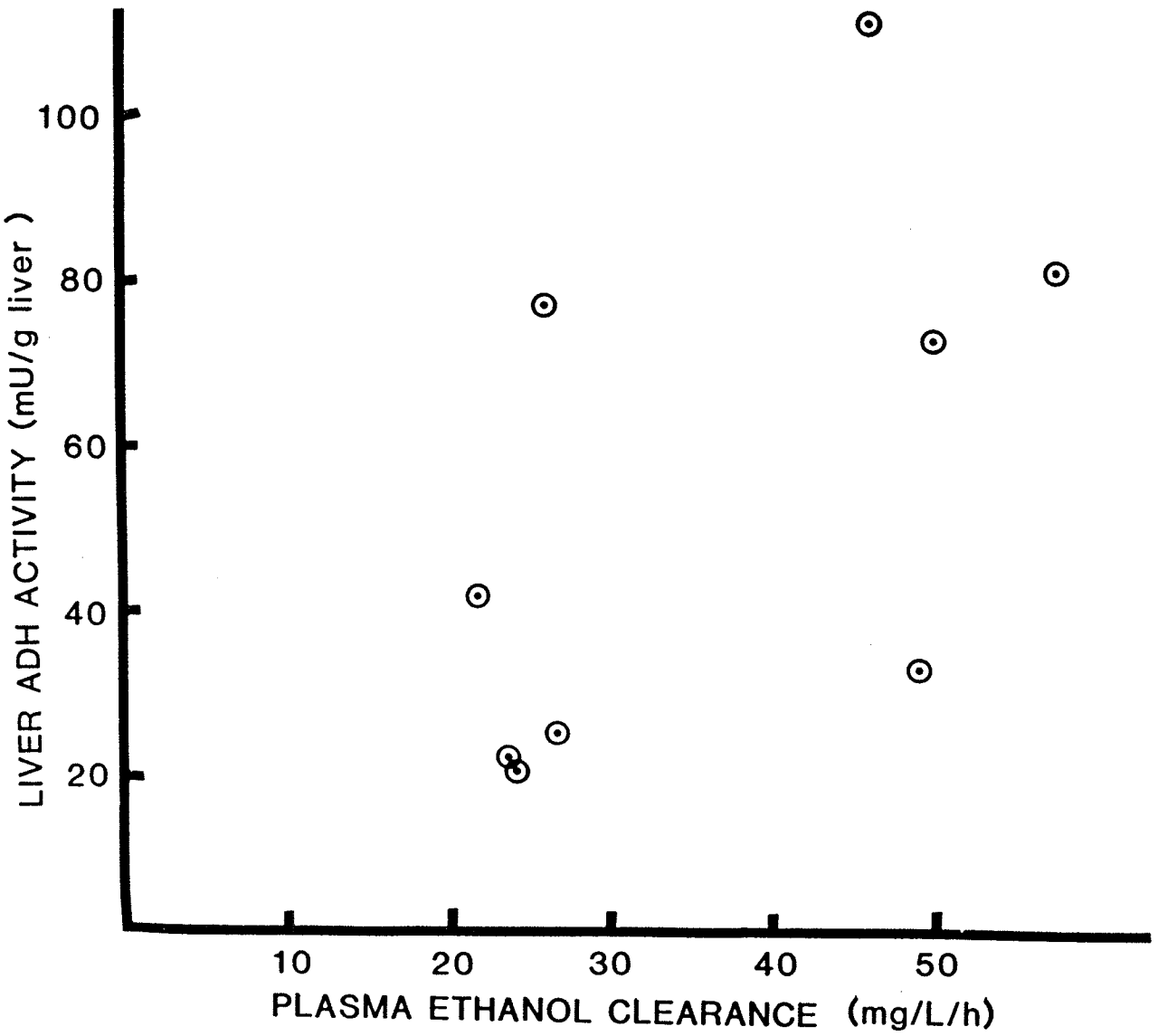
Table 13. Correlation between plasma ethanol clearance and ADH activity in maternal and fetal sheep and newborn lambs. Data are expressed as mean ± S.E..

Sample	ADH Activity (mU/g tissue)	Apparent Plasma Clearance (mg/L/h)
Fetal	72 ± 9 (5)[a]	206 ± 5[b] (5)
Lamb	54 ± 11 (9)	36 ± 5 (9)
Adult	729 ± 150 (4)	203 ± 13 (5)

[a] number of experiments

[b] determined in utero

Figure 14. Relationship between lamb liver ADH activity at 25°C and optimal pH and plasma ethanol clearance in the same animals after infusion of 9.5% v/v ethanol in 5% w/v dextrose solution at 0.6 g/kg over 1 hour.


LIVER ADH ACTIVITY (mU/g liver)
100
80
60
40
20
10
20
30
40
50
PLASMA ETHANOL CLEARANCE (mg/L/h)

DISCUSSION

A. Kinetic Disposition of Ethanol

a. Maternal Infusion Study

Peak maternal ethanol concentrations in experiments 1-5 (table 6) were similar to those reported by Fuchs et al. (1967) for the prevention of premature labour in humans who received the same infusion rate. In our study, no differences between maternal and fetal peak concentrations which occured at the end of infusion were noted. Dilts (1970) and Rose et al. (1981) reported peak maternal concentrations to be higher than fetal concentrations. This might be associated with the use of a higher ethanol dose (Rose et al. 1981) or the use of an acute preparation (Dilts 1970). This implies that conditions of stress, including surgical procedures and possibly ethanol-induced impairment of umbilical circulation may alter ethanol pharmacokinetics in pregnancy.

During infusion, fetal ethanol concentrations increased at a slower rate than maternal concentrations. This delay has been attributed to general hemodynamic mechanisms of placental circulation (Shapiro et al. 1967). However, the difference is small, indicating that rapid placental transfer of ethanol from mother to fetus must occur. Similar findings have been reported by others (Dilts 1970, Rose et al. 1981, Ng et al. 1982), but Cook et al. (1981) and Ayromlooi et al. (1979) found no difference.

The observation that fetal ethanol concentrations exceed maternal concentrations at the end of infusion is supported by previous studies (Mann et al. 1975, Rose et al. 1981). However, fetal ethanol concentrations closely paralleled maternal concentrations. These findings are consistent with observations made in human fetuses and newborns where ethanol elimination is much slower than in adults

(Idanpaan-Heikkula et al. 1972, Wagner et al. 1970, Seppala et al. 1971). Whether this difference represents differences in fetal metabolism or limitations in placental hemodynamics is yet to be established.

In experiments 6-9 (table 6), a bolus infusion was followed by the same maintenance dose as used by Fuchs et al. (1967). It appeared that steady state conditions were attained, although a longer maintenance period would have confirmed this. Maternal and fetal peak concentrations were similar and amniotic fluid peak concentrations were lower and occurred after the end of infusion, suggesting delayed transfer to the amniotic fluid. These observations confirm previous reports (Cottle et al. 1980, Ng et al. 1982).

Maternal and fetal plasma ethanol clearance rates from all studies were similar. This correlates well with findings of Cook et al. (1981) and with clearance rates calculated from limited mean data of Ayromlooi et al. (1979). This is not in agreement with Rose et al. (1982) who reported fetal clearance to be 1/4 of maternal clearance. Ng et al. (1982) calculated maternal and fetal clearance rates to be similar, but lower than in our studies. Amniotic fluid clearance in one study was lower than maternal and fetal clearance rates and is in agreement with Ng et al. (1982).

b. Fetal Infusion Study

Infusion of ethanol into the fetus has not previously been reported. Since ethanol is a relatively small molecule, rapid transfer across the placenta is expected. Different characteristics between maternal-fetal and fetal-maternal exchange are not anticipated. However, infusion of ethanol into the fetus at the same rate given in the

maternal infusion studies (0.6g/kg over 1 hour) resulted in a four-fold difference in maternal and fetal peak ethanol concentrations (table 7, study 1). Infusion of a total dose of 0.5g/kg over 1 hour (table 7, study 2) also produced a four-fold difference. This difference may represent limitations of placental blood flow resulting in impaired transfer to the maternal circulation. Whether this impaired transfer results from ethanol itself remains to be established. A lower dose produced a smaller difference in maternal and fetal peak concentrations. Maternal and fetal clearance rates in the fetal infusion studies were similar to those in the maternal infusion studies in spite of the difference in placental transfer rate.

c. Amniotic Fluid Infusion Study

Infusion of ethanol into the amniotic fluid has not previously been reported. Direct ethanol infusion enabled us to examine the transfer of ethanol from the amniotic fluid compartment. The delay in peak concentrations in the maternal and fetal circulations suggests that transfer of ethanol from the amniotic fluid is a slow process. This observation is supported by our maternal infusion study and maternal infusion studies by Ng et al. (1982) and Cottle et al. (1980). The relative times to peak ethanol concentrations suggest that the fetus is first exposed to ethanol. Drugs administered to the amniotic fluid are rapidly absorbed by the fetus (Van Petten et al. 1978). Ethanol reaches the maternal circulation from the fetus and the amniotic fluid. The reason for the difference in maternal and fetal calculated AUC is not clear but could be due to impaired transfer to the mother or deficient ADH in the fetus. Maternal and fetal clearance rates could not be determined, but similar rates would suggest metabolism from the maternal

compartment only. The amniotic fluid clearance rate was determined to be lower than that after maternal infusion. Amniotic fluid ethanol concentrations used to determine clearance were higher in the amniotic fluid infusion than in the maternal infusion experiment. These observations do not support evidence that ethanol elimination rates may increase with ethanol concentration (Newman et al. 1937, Grunnet and Thieden 1972, Rangno et al. 1981), although our concentration range was not great enough to determine this. Thus, the effect of the site of administration on ethanol clearance rates needs to be examined further. This study indicates that the amniotic fluid may act as a reservoir for ethanol, resulting in prolonged fetal exposure. The extent of this exposure is not represented by maternal ethanol concentrations. Data from the maternal infusion and amniotic fluid infusion studies suggest impaired bidirectional transfer of ethanol in the amniotic fluid. Further studies are necessary to define the mechanisms contributing to our observations.

d. Lamb Infusion Study

Ethanol disposition in the newborn lamb has not previously been reported. These experiments were performed to assess the ability of the neonate to eliminate ethanol in comparison to the fetus in utero. Ethanol elimination rates in the human neonate have been determined to be about half the maternal rate (Sepalla et al. 1971, Idanpaan-Heikkula et al. 1972). The newborn monkey eliminates ethanol at about 1/4 the rate of the mother (Hill et al. 1983). In our lamb experiments, ethanol elimination in the newborn lamb was also lower, at about 1/6 the maternal and fetal in utero rates. Plasma ethanol clearance increased with lamb age, to about 1/4 maternal and in utero clearance rates at 5

days. This confirms the reliance of the fetus on the mother for ethanol elimnation and identifies the risk to the neonate. The neonate of an intoxicated mother will be exposed to significant ethanol concentrations for a considerably longer period of time than in utero when maternal metabolism would limit fetal exposure.

B. Relationship between Pharmacodynamics and Pharmacokinetics of Ethanol Disposition

a. Maternal Infusion Study

The increase in maternal heart rate with ethanol infusion has been previously observed (Cook et al. 1981, Rose et al. 1981), but others (Ayromlooi et al. 1979, Mann et al. 1975, Ng et al. 1982) reported no change. A cardiostimulatory action of ethanol may be due to several mechanisms. Acetaldehyde stimulation of the sinus node (James and Bear 1967), decreased total peripheral vascular resistance (Stein et al. 1963), direct myocardial effects (Kirkpatrick et al. 1976) and indirect catecholamine release (Kirkpatrick et al. 1976) have been postulated. Fetal tachycardia has also been reported (Mann et al. 1975, Ayromlooi et al. 1979, Cook et al. 1981) and is in agreement with our findings.

Our studies confirm earlier observations that maternal ethanol infusion does not affect maternal (Ng et al. 1982, Ayromlooi et al. 1979, Cook et al. 1981, Mann et al. 1975) or fetal (Ng et al. 1982, Rose et al. 1982, Cook et al. 1981, Kirkpatrick et al. 1976) blood pressure. Ayromlooi et al. (1979) reported a slight increase in fetal blood pressure during infusion, and Mann et al. (1975) noted fetal hypotension. The use of an acute preparation by Mann et al. (1975) in which the fetus is exteriorized may explain their observation.

We observed no changes in maternal or fetal blood gas parameters in our studies, which is in keeping with most other studies (Dilts 1970, Kirkpatrick et al. 1976, Cook et al. 1981). Ayromlooi et al. (1979) reported an increase in fetal P_{O_2}, and suggested an ethanol-induced increase in uterine blood flow and improvement in fetal oxygenation. Fetal acidosis reported by Mann et al. (1975) may be attributed to the acute preparation, as well as higher peak maternal and fetal ethanol concentrations. Arterial blood gas parameters may not accurately reflect major alterations in fetal cardiovascular status (Kirkpatrick et al. 1976, Cook et al. 1981). Kirkpatrick et al. (1976) observed no changes in fetal blood gas parameters with maternal ethanol infusion but demonstrated a significant reduction in fetal cardiac contractility. The consequences of acute ethanol exposure on the fetal heart need to be examined further.

b. Fetal Infusion Study

Fetal infusion studies were performed to determine if the direct infusion of ethanol to the fetus resulted in different cardiovascular effects. Fetal ethanol infusion produced fetal bradycardia. This has not been reported in maternal infusion studies. Kirkpatrick et al. (1976) reported a significant decrease in fetal cardiac contractility associated with maternal ethanol intake. During maternal ingestion, the increases in maternal and fetal heart rate may be due to catecholamine release by the mother (Kirkpatrick et al. 1976). In our fetal infusion studies, the decrease in heart rate was not accompanied by changes in maternal heart rate. This may be because of the low ethanol concentration attained in the maternal circulation. The mechanism for the decrease in fetal heart rate is not readily apparent. However, it is

believed to be independent of maternal influences. The negative inotropic action of ethanol on fetal heart rate may represent underlying cardiac biochemical changes which could adversely affect post-natal cardiovascular adaptation. Further pharmacokinetic and pharmacodynamic studies of ethanol are needed to assess potential adverse effects related to prolonged exposure to ethanol when the fetus is separated from its intrauterine protective environment at birth.

c. Lamb Infusion Study

We found no significant changes in heart rate, blood pressure, or acid-base status in lambs during or after ethanol infusion. These findings are consistent with those of Wagner et al. (1970) who reported no significant changes in alertness, motor activity, circulation, respiration, or acid-base status in 6 premature infants with blood ethanol concentrations of up to 0.9 g/L. In our studies, the peak ethanol concentrations were lower (table 10). Changes may occur at higher concentrations. In all nine lambs, infusion of 5 percent w/v dextrose solution was required to maintain normal blood glucose concentrations. Wagner et al. (1970) reported 2 cases of ethanol-induced hypoglycemia. Ethanol impairs hepatic glucose output by inhibiting gluconeogenesis. The consequences include tachycardia, sweating, tremulousness, irritability, headache and mild hypothermia (Williams 1984). In our studies, dextrose infusion may have prevented the observation of any adverse cardiovascular effects. As previously stated, these parameters may not be appropriate indicies of more fundamental changes. Neonatal toxicity and death associated with acute transplacental ethanol intoxication have been reported (Cook et al. 1975, Jung et al. 1980, Peden et al. 1973). The primary features of

toxicity were respiratory and CNS depression (Cook et al. 1975). Neonates delivered during or after unsuccessful attempts to inhibit premature labour with ethanol or to intoxicated mothers could be expected to have a higher incidence of neonatal depression. Further studies are needed to delineate the effects of ethanol on the neonate.

C. Alcohol Dehydrogenase

a. Effect of pH

The optimal pH for human fetal and adult liver ADH are 10.0 and 10.4 respectively (Pikkarainen and Raiha 1967). Differences in optimal pH for adult, fetal and lamb liver ADH were observed in our studies, and may reflect the presence of electrophoretically different bands of ADH (Von Wartburg et al. 1964). During human development, liver ADH shows a distinct progression in isoenzyme pattern from only one form during fetal life to as many as 7 to 9 major forms in the adult (Pikkarainen and Raiha 1967, Li 1977). Isoenzyme studies were not performed in our experiments, but they may reveal changes in isoenzyme patterns associated to enzyme activity. Difference in activity at pH 7.4 to optimal pH indirectly supports a similar situation in the sheep model.

b. Maximal Activity

The enzymatic and molecular properties of sheep liver ADH have not previously been characterized. Wide variation in ADH activity was observed in our experiments. It is expected that sheep liver ADH, like horse, monkey, and human ADH displays multiple molecular forms. Heterogeneity in molecular forms of human liver ADH develops perinatally, and is present in adult livers to variable and different extents (von Wartburg 1964). Human fetal ADH activity is 10 to 30 percent of adult activity (Pikkarainen and Raiha 1967, Smith et al.

1971). Fetal activity in humans appears to increase with gestational age (Smith et al. 1971). Pikkarainen (1971) demonstrated that the 10 to 16 week human fetus cannot eliminate ethanol at all. We found the activity of near-term fetal liver ADH to be about 10 percent of adult activity which is consistent with differences reported for human (Pikkarainen and Raiha 1967), rat, and guinea pig (Raiha et al. 1967) liver.

During human development, ADH activity in the fetus is low and reaches adult levels at about 5 years of age (Pikkarainen and Raiha 1967). Newborn lamb liver ADH activity was similar to fetal activity, suggesting increases in enzyme activity occur only after birth, and begin within the first 5 days of life. A distinct increase in activity was noted at 3 days. This may be related to progression in isoenzyme pattern (Pikkarainen and Raiha 1967) and in observed changes in pH optimum. The low placental ADH activity indicates a minor role for it in ethanol metabolism.

c. Activity at Physiological pH as a Function of Temperature

ADH activity was determined at pH 7.4 to simulate physiological conditions. Activity was less at pH 7.4 than at optimal pH and is consistent with the activity of human ADH (von Wartburg et al. 1964). The increase in activity with temperature has previously been observed. Plapp (1975) reported the activity of horse liver ADH at 25 ^{o}C to be about 60 percent of the activity at 37 ^{o}C. The calculated activity ratio 37 ^{o}C:25 ^{o}C of about 1.67 is within the range of our data (table 12). The ratio of activity 37^{o}C:25^{o}C in our studies were similar but dependent on the tissue. This suggests the presence of various forms of ADH in the tissue samples.

D. Relationship between Pharmacokinetics and Alcohol Dehydrogenase Activity

The mean fetal liver ADH activity is 1/10 maternal ADH activity, whereas maternal and *in utero* fetal ethanol clearance rates are similar. This implies that the fetus relies on maternal metabolism for the elimination of ethanol, and that elimination is dependent primarily on the mother. This interpretation is supported by data which demonstrates that fetal and lamb liver ADH activity are similar, but lamb ethanol clearance is about 1/6 of maternal and *in utero* fetal rates. The lack of correlation between plasma clearance rates and ADH activity in newborn lambs may be explained by experimental conditions. ADH activity was determined *in vitro* at optimal pH and 25°C, which does not mimic physiological conditions. Also, the detergent may unmask enzyme activity which is not expressed physiologically. It could also be due to the narrow range of age and small sample size in our study. No correlation was found at physiological pH and 25 or 37°C. It is clear that ADH activity and the capacity for ethanol elimination in the neonate are diminished, and that this may present problems in the initial expression of physiological function in the intoxicated neonate. Whether this has long term consequences is not presently understood.

Although ethanol has been widely replaced by beta agonists in the treatment of premature labour, the effects of fetal exposure to ethanol must still be considered in view of its widespread social use. There is no doubt that increased fetal risk is associated with heavy maternal ethanol intake. However, the fetal risks associated with light to moderate ethanol intake require further consideration. A major question that arises is the influence of timing, exposure and pattern of drinking

on the outcome. The possibililty of acquired tolerance to repeated exposure could be explored. Our studies explored the pharmacokinetics of ethanol to provide information about the transfer of ethanol across biological membranes. Further investigation of the significance of the amniotic fluid as a reservoir is warranted. Assessment of the dose-related effect of ethanol on umbilical circulation and its effect on bidirectional placental transfer would be of value. Whether intravenous and oral administration of ethanol produce equivalent adverse effects on the human fetus must be examined.

We observed different pharmacodynamic changes with different infusion sites. More precise measurements of cardiac performance would be an asset. Identification of the biochemical changes associated with ethanol exposure would contribute to an understanding of the effects of ethanol. Direct infusion of acetaldehyde into the fetus would help identify if the observed effects are directly due to ethanol or its metabolite.

Kinetic data from our lamb studies confirmed that the fetus has little capacity for ethanol metabolism. The determination of ADH activity in sheep tissues has provided preliminary data for identification of molecular properties of sheep ADH. Isolation of isoenzyme bands may lead to understanding of liver ADH development in sheep. Although animal studies are not necessarily reflections of human responses, our studies of the kinetics of ethanol in pregnant sheep and in newborn lambs have contributed to a most useful animal model for study of the maternal-fetal unit. An understanding of the effects of fetal exposure of ethanol is crucial to the prevention of abnormal outcomes of preganancy associated with ethanol ingestion.

CONCLUSIONS

Ethanol is rapidly transferred from the maternal to fetal circulation when it is administered to the mother during pregnancy.

On the other hand, the ability of the fetus to excrete or metabolize a large dose of ethanol administered directly to it is impaired.

The amniotic sac may serve as a reservoir for ethanol resulting in prolonged exposure of the fetus to ethanol.

Ethanol elimination rates in newborn lambs are lower than in the mother or the fetus *in utero*.

Direct infusion of ethanol into the fetus produces different cardiovascular effects than when infused into the mother.

Lamb and fetal liver ADH activity are similar and are less than adult liver activity.

The fetus relies on the maternal circulation for the elimination of ethanol.

Neonates born to intoxicated mothers will be exposed to ethanol for prolonged periods because of decreased capacity for metabolism and elimination of ethanol.

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