1978

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THE UNIVERSITY OF ALABAMA IN BIRMINGHAM, PH.D., 1978

A SURVEY OF ALKYLDIOLS AS ANTIDOTES IN ETHYLENE GLYCOL TOXICITY IN MICE

by

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A DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in the
Department of Pharmacology in The Graduate School,
University of Alabama in Birmingham

BIRMINGHAM, ALABAMA

1978

"Don't keep forever on the public road, going only where others have gone. Leave the beaten track occasionally and dive into the woods. You will be certain to find something you have never seen before. Of course, it will be a little thing, but do not ignore it. Follow it up, explore all around it; one discovery will lead to another, and before you know it you will have something worth thinking about to occupy your mind. All really big discoveries are the results of thought."

Alexander Graham Bell

DEATH SPEAKS:

There was a merchant in Bagdad who sent his servant to market to buy provisions and in a little while the servant came back, white and trembling, and said, Master, just now when I was in the market-place I was jostled by a woman in the crowd and when I turned I saw it was Death that jostled me. She looked at me and made a threatening gesture; now, lend me your horse, and I will ride away from this city and avoid my fate. I will go to Samarra and there Death will not find me. The merchant lent him his horse, and the servant mounted it, and he dug his spurs in its flanks and as fast as the horse could gallop he went. Then the merchant went down to the market-place and he saw me standing in the crowd and he came to me and said, Why did you make a threatening gesture to my servant when you saw him this morning? That was not a threatening gesture, I said, it was only a start of surprise. I was astonished to see him in Bagdad, for I had an appointment with him tonight in Samarra.

W. Somerset Maugham

ACKNOWLEDGEMENTS

I am most grateful to my able advisor and chairman, Dr. Roy L. Mundy, for his patience, guidance, wisdom, and pithiness. I am indebted to Dr. Robert S. Teague for the opportunity to do this work and for his support and encouragement.

I wish to express my appreciation to my wife, Carolyn Rudd Holman, for her patience, support, and wisdom.

I wish to express my gratitude to my parents, Dr. and Mrs. Norman W. Holman, Sr., for their faith in my ability to perform and exceed.

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LIST OF ABBREVIATIONS

1,3-BD	1,3-Butanediol
CNS	Central Nervous System
ED_{50}	Dose of drug necessary to produce one-half maximal response
EG	Ethylene glycol
FID	Flame ionization detector
ip	Intraperitoneal
K_I	Inhibitor concentration necessary to reduce V_{max} by one half.
K_m	Substrate concentration necessary to produce one half V_{max}.
LD_{50}	Dose of drug necessary to produce 50% mortality
PG	Propylene glycol
po	Orally
PYR	Pyrazole
SEM	Standard error of the mean
sc	Subcutaneous
V_{max}	Maximum velocity
Wt.	Weight

INTRODUCTION

The ability of drugs to modulate or modify the expression or course of various toxicities has been a classical concern of pharmacology. If the lethality of a toxic agent is found to be reduced or abolished by administration of a drug, this drug may become useful as a therapeutic antidote to treat clinical cases with the particular intoxication. A case in point is the antidotal effects produced by ethanol and pyrazole in ethylene glycol toxicity (Peterson et al., 1963; Wacker et al., 1965; Mundy, Hall, and Teague, 1974; Van Stee et al., 1975). The fact that pyrazole and ethanol competitively inhibit liver alcohol dehydrogenase in vitro and are antidotes in vivo suggest that both compounds may limit ethylene glycol oxidation by inhibition of liver alcohol dehydrogenase; however, a study of the disappearance of ethylene glycol in plasma after pyrazole administration to elucidate the antidotal mechanism has not been reported in the literature.

Neither ethanol nor pyrazole is an ideal antidote from the practical viewpoint of clinical use because of the inherent toxicity of each compound (Wilson and Bottiglieri, 1962; Lelbach, 1969; Lieber et al., 1970; Petersen et al., 1963; Wacker et al., 1965; Borden and Bidwell, 1968). Therefore, it would be valuable to find new antidotes to replace either ethanol or pyrazole in treatment of ethylene glycol toxicity.

Liver alcohol dehydrogenase may metabolize a series of alkyldiols (Pietruszko, 1975b) and may be competitively inhibited at least by propylene glycol (Weiss and Coen, 1966; Brown et al., 1968). In addition, some of the alkyldiols appear to be less toxic than aliphatic monohydroxy alcohols of the same carbon chain length (Laug et al., 1939; Latven and Molitor, 1939; Smyth, Seaton, and Fischer, 1941; Williams, 1959; Stolman and Steward, 1960; Wallgren, 1960); however, a selected study of the individual pharmacology and toxicology of the alkyldiols with the specific intent of testing their possible application in ethylene glycol toxicity as antidotes has not been reported in the literature. The alkalinizing agent, tris (hydroxymethyl) aminomethane, an alkyldiol derivative, has apparently not been investigated as an antidote in ethylene glycol toxicity (Nahas, 1962; Nahas, 1963; Darby and Anderson, 1966; Strauss, 1968). The development of new alkyldiol-type antidotes was explored and compared with the known antidotes, ethanol and pyrazole, thereby expanding the scientific basis for effective treatment of ethylene glycol toxicity.

Background

The toxicity of ethylene glycol, its derivatives, or related compounds was unfortunately not recognized by the medical world until the 1930's when numerous uremic deaths occurred after the ingestion of Elixir of Sulfanilamide (Massengill), a therapeutic preparation containing 70% diethylene glycol (a hydrolysable ether

of ethylene glycol) as a solvent (Leech, 1937; Geiling and Cannon, 1938; Calvery and Klumpp, 1939). This tragedy spurred numerous investigators to expand the knowledge of the pathology and toxicology of some of these compounds (Wiley et al., 1938; Kesten, Mulinos, and Pomerantz, 1939; Latven and Molitor, 1939; Laug et al., 1939; Smyth, Seaton, and Fischer, 1941; Smith, 1951). Ethylene glycol was identified as the compound which produced a large number of clinical human intoxications due to the availability in the form of permanent automotive antifreeze and its appeal as a substitute alcoholic beverage (Wiley et al., 1938; Pons and Custer, 1946; Milles, 1946; Kahn and Brotcher, 1950; Ross, 1956; Harger and Forney, 1959). Most of these early authors observed a biphasic toxicity of ethylene glycol, consisting of an acute phase and a delayed phase. The acute human toxicity of ethylene glycol is evidenced by an alcohol-like inebriation and CNS depression, but without the alcohol odor on the breath (Kahn and Brotcher, 1950). The delayed toxicity of ethylene glycol was attributed to pathological changes in tissues such as the cardiovascular system, liver, central nervous system, and especially the kidney due to deposition of oxalate crystals. In fact, oxalate crystal deposition prior to death was first demonstrated by renal biopsy by Berman, Schreiner, and Feys (1957). Death during the acute phase of toxicity apparently resulted from respiratory failure, whereas death during the delayed phase of toxicity resulted from renal failure, climaxed by anuria and uremia (Pons and Custer, 1946; Milles, 1946; Kahn and Brotcher, 1950; Ross, 1956; Berman,

Schreiner, and Feys, 1957; Harger and Forney, 1959).

In 1961, Gessner, Parke, and Williams answered many questions concerning the appearance of oxalate in vivo by demonstrating in various animal species the catabolism of ethylene glycol to glycolaldehyde, glycolate, glyoxylate, oxalate, glycine, formate, and CO_2. If ethylene glycol was extensively metabolized by a species, formate and CO_2 were found to be major metabolites, whereas glycolaldehyde, glycolate, glyoxylate, glycine, and oxalate were found to be minor metabolites. In lower species, such as the rat, guinea pig, rabbit, the major excretory products were unchanged ethylene glycol eliminated in the urine and carbon dioxide in the expired air. In higher species, such as the cat, the major excretory product was still the unchanged ethylene glycol in the urine, but in an amount three to four times greater than observed in the rodents. In all species, oxalate was a minor metabolite, but the cat excreted in urine two to four times the amount of oxalate excreted by rodents. In spite of the fact that unchanged ethylene glycol is a major excretory product in all species, ethylene glycol seems to be most toxic to those species which excrete low amounts of carbon dioxide and high amounts of oxalate and unchanged ethylene glycol. Ethylene glycol is least toxic to those species which excrete a high amount of carbon dioxide and low amounts of oxalate and unchanged ethylene glycol. Gessner, Parke, and Williams (1960) had already found that no glucuronide derivatives could be found from either ethylene glycol or its metabolites.

In addition to oxalate formation, severe systemic acidosis of

an undetermined origin was recognized as a formidable consequence in delayed ethylene glycol toxicity (Hagemann and Chiffelle, 1948; Friedman et al., 1962; Flanagan and Libcke, 1964; Wacker et al., 1965). Since oxalate was only a minor metabolite of ethylene glycol (Gessner et al., 1961; McChesney et al., 1971; McChesney, Golberg, and Harris, 1972), it is apparent that endogenous oxalate production in vivo was insufficient to account for either the acidosis or the pathology and mortality observed in ethylene glycol toxicity. As early as 1938, Wiley et al. had reported suspicions that oxalate was not the sole toxic agent in ethylene glycol toxicity. Obviously, the importance of ethylene glycol catabolism in intermediary metabolism must be considered in greater detail.

A compilation of metabolic pathways for ethylene glycol metabolism described by Gessner, Parke, and Williams (1961), Hagler and Herman (1973), Parry and Wallach (1974), Williams and Smith (1968), Behrens (1941), Racker (1951), and Kermack and Matheson (1957) is shown in Figure 1. At first glance, the catabolism of ethylene glycol seems quite diffuse. Although the illustrated pathways have been demonstrated, all of the enzymes which may catalyze each reaction have not been identified and the enzyme kinetics of each reaction are only slightly understood in some cases or unknown in others. However, Richardson (1973) decreased the toxicity of ethylene glycol in male rats by partial hepatectomy and deduced that the liver is the central location of ethylene glycol catabolism.

The enzymes involved in the major pathway of ethylene glycol

Figure 1: Ethylene Glycol Metabolism. A compilation of metabolic pathways described by Gessner, Parke, and Williams (1961), Hagler and Herman (1973), Parry and Wallach (1974), Williams and Smith (1968), Behrens (1941), Racker (1951), and Kermack and Matheson (1957).

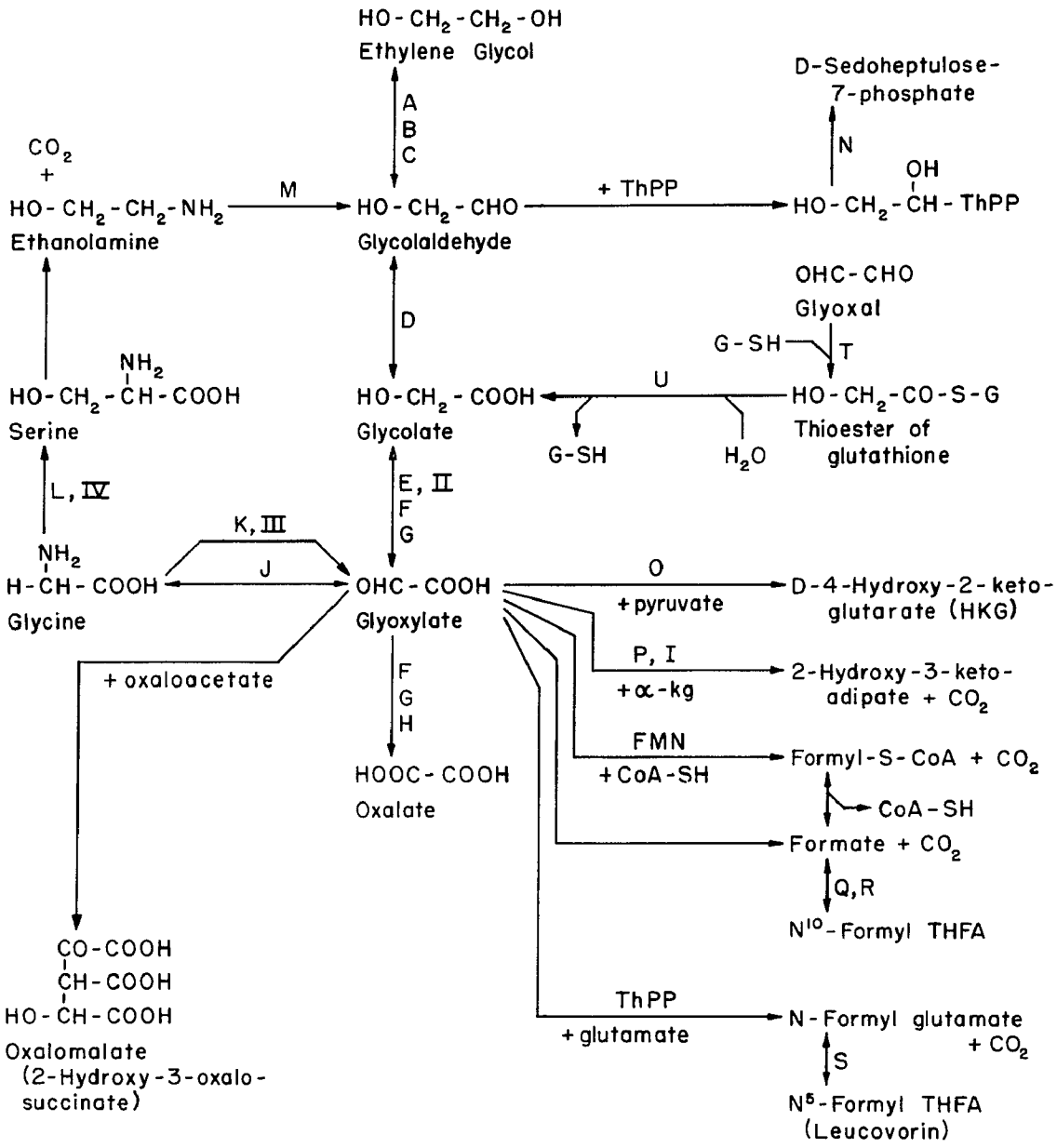
Symbol	Enzyme	Coenzymes(s)
A	Alcohol dehydrogenase	NAD^+
B	Catalase	--
C	Microsomal ethanol oxidizing system	--
D	Aldehyde dehydrogenase (or oxidase)	NAD^+
E	Glycerate dehydrogenase (Glyoxylate reductase)	NAD^+
F	Lactate dehydrogenase	NAD^+
G	Glycolic acid oxidase (2-Hydroxyacid dehydrogenase)	FAD
H	Xanthine oxidase	FAD
J	Transaminases	PyrP
K	Glycine oxidase (D-Aminoacid oxidase)	FAD
L	L-Serine hydroxymethyltransferase (Serine aldolase)	THFA, PyrP
M	Monoamine oxidase	PyrP
N	Transketolase	ThPP
O	2-Keto-4-hydroxyglutarate aldolase	PyrP
P	α-Ketoglutarate: glyoxylate carboligase	ThPP
Q	N^{10} - Formyl THFA deacylase	--
R	Formyl THFA synthetase	THFA
S	Formyl glutamate: formyl transferase	THFA
T	Glyoxalase I (G-S-thioester synthetase)	G-SH
U	Glyoxalase II (G-S-thioesterase)	--

Defects

I	type I hyperoxaluria, glycolic aciduria
II	type II hyperoxaluria, glyceric aciduria
III	hyperglycinemia, hypoxaluria
IV	hyperglycinemia

Coenzyme

α-kg	α-ketoglutarate
CoA-SH	coenzyme A
FAD	flavin-adenine dinucleotide
FMN	flavin mononucleotide
G-SH	glutathione
PyrP	pyridoxal phosphate (Vitamin B_6)
THFA	tetrahydrofolic acid
ThPP	thiamine pyrophosphate (Vitamin B_1)


HO - CH2 - CH2 - OH
Ethylene Glycol
A
B
C
D-Sedoheptulose-
7-phosphate
N
CO2
+
HO - CH2 - CH2 - NH2
Ethanolamine
M
HO - CH2 - CHO
Glycolaldehyde
+ ThPP
OH
HO - CH2 - CH - ThPP
D
OHC - CHO
Glyoxal
G - SH
T
NH2
HO - CH2 - CH - COOH
Serine
HO - CH2 - COOH
Glycolate
U
HO - CH2 - CO - S - G
Thioester of
glutathione
G - SH
H2O
L, IV
E, II
F
G
K, III
NH2
H - CH - COOH
Glycine
J
OHC - COOH
Glyoxylate
O
+ pyruvate
D-4-Hydroxy-2-keto-
glutarate (HKG)
P, I
+ α-kg
2-Hydroxy-3-keto-
adipate + CO2
+ oxaloacetate
F
G
H
FMN
+ CoA-SH
Formyl-S-CoA + CO2
CoA-SH
HOOC - COOH
Oxalate
Formate + CO2
Q,R
N10-Formyl THFA
CO-COOH
CH-COOH
HO-CH-COOH
Oxalomalate
(2-Hydroxy-3-oxalo-
succinate)
ThPP
+ glutamate
N-Formyl glutamate
+ CO2
S
N5-Formyl THFA
(Leucovorin)

catabolism have been identified and their involvement in ethylene glycol toxicity studied. Liver alcohol dehydrogenase is the major enzyme which oxidizes ethylene glycol to glycolaldehyde (Peterson et al., 1963; von Wartburg, Bethune, and Vallee, 1964; Blair and Vallee, 1966; Coen and Weiss, 1966; Weiss and Coen, 1966; Pietruszko, 1975b). Although other enzymes, such as catalase, may catalyze this first oxidation, their contribution to the oxidation is negligible (Coen and Weiss, 1966). The oxidation of glycolaldehyde to glycolate is catalyzed by either aldehyde dehydrogenase or aldehyde oxidase, and the relative importance of these two enzymes is not known (Hagler and Herman, 1973). The three enzymes, glycerate dehydrogenase (glyoxylate reductase), lactate dehydrogenase, and glycolic acid oxidase (2-hydroxyacid dehydrogenase), catalyze the oxidation of glycolate to glyoxylate, whereas the latter two of these three enzymes, plus xanthine oxidase, catalyze the oxidation of glyoxylate to oxalate (Hagler and Herman, 1973; Parry and Wallach, 1974; Williams and Smith, 1968). Of the four enzymes just mentioned, glycolic acid oxidase is the major enzyme responsible for the conversion of glycolate to oxalate (Liao and Richardson, 1973). In fact, hyperoxaluria and oxalosis in vivo are closely correlated to liver glycolic acid oxidase levels (Richardson, 1967). Since glycolic acid oxidase may be induced by testosterone in male rats at puberty, mature male rats produce more oxalate than mature female rats. Therefore, glycolic acid oxidase levels are responsible for sex differences in the tolerance of male and female rats to ethylene glycol toxicity (Richardson, 1964).

The enzymes involved in the minor pathways of ethylene glycol catabolism have not all been identified, but the pathways, nevertheless, do exist. Glyoxylate is visualized as the hub of numerous tangential minor pathways which, as a group, are relatively significant in overall ethylene glycol catabolism. Conversions of glyoxylate to glycine, α-hydroxy-β-keto-adipate, or glycolate are valuable as indicators of metabolic diseases caused by the genetic deficiency or absence of certain enzymes (see Roman numerals). Some findings in these diseases, such as excessive oxalate excretion, are identical to those of ethylene glycol poisoning, even though substrate saturation, rather than enzyme deficiency, is the cause in the latter case (Williams and Smith, 1968; Hagler and Herman, 1973). Gessner (1958) describes the formation of sedoheptulose as a possible detoxification pathway. A common thread links the sedoheptulose pathway and those pathways originating as glyoxylate. This thread is that certain enzymes depend on the availability of the B vitamin cofactors, thiamine pyrophosphate and pyridoxal phosphate, for enzymatic activity. Although B vitamin deficiency diseases like beriberi are rarely found in the Western World, Wernicke's syndrome is seen in chronic alcoholics. A Wernicke's case involved in ethylene glycol poisoning would suffer from inactivity of these pathways. Four studies involving experimental animals and man described the appearance of hyperoxaluria and oxalosis under pyridoxine deficiency (Williams and Smith, 1968). On the other hand, administration of pyridoxine to normal individuals decreases the normal oxalate excretion (Williams and

Smith, 1968). Pyridoxamine (vitamin B_6) deficiency not only eliminates the sex differences of ethylene glycol tolerance in rats, but is closely correlated to increased oxalosis because the pyridoxamine - dependent enzymes that metabolize glyoxylate are less active during pyridoxamine deficiency (Richardson, 1965; Richardson, 1967).

Ascorbic acid (vitamin C) has been recognized as a source of oxalate in vivo for a number of years and could possibly increase ethylene glycol toxicity. Although increased ascorbate intake would theoretically affect hyperoxaluria and oxalosis in an additive manner, several studies have confirmed that oxalate production is not influenced significantly by high ascorbate doses (Williams and Smith, 1968).

Gessner et al. (1961) and other researchers agree on the major toxic pathway of ethylene glycol catabolism. At least, glycolaldehyde is the product formed by alcohol dehydrogenase and is the substrate for aldehyde dehydrogenase. However, a suspicion exists that glyoxal may be an intermediate in the catabolism of ethylene glycol (Friedman et al., 1962; Gessner et al., 1961; Gessner, 1958). Doerr (1950) described pancreatic necrosis in cats after ethylene glycol and alloxan (mesoxalylurea) toxicity in which β cells are selectively destroyed to produce diabetes mellitus. Smith (1951) saw similar lesions of subacute focal pancreatitis in human necropsies. A clue to this toxicity is that alloxan is metabolized to glyoxal, but the pancreas lacks the glyoxalase system to continue the degradation (Doerr, 1949). From the work of

Behrens (1941), Racker (1951), and Kermack and Matheson (1957), the glyoxalase system (found in the liver and other tissues) is known to convert glyoxal to glycolate irreversibly in a two enzyme pathway (Figure 1); therefore, this system cannot be considered the source of glyoxal. If glyoxal is produced in vivo from ethylene glycol, the pathways have not been found. In isolation and identification procedures, Gessner et al. (1961) and others formed diphenylhydrazones (osazones) and quinoxalines from reactions of glycolaldehyde with a phenylhydrazine or O-phenylene diamine, respectively. Since glyoxal forms the same end products, there was no differentiation between chemically and possible enzymatically produced glyoxal. Recognizing the possible dual source of his osazone, Gessner (1961) expressed his results as the sum of glycolaldehyde and glyoxal.

In reviewing the catabolism of ethylene glycol, one may find several compounds other than ethylene glycol and oxalate which are probable mediators in ethylene glycol toxicity. In fact, this is the case and has been called the "metabolic intermediate hypothesis" by Chou and Richardson (1978). Bove (1966) found the metabolic products glycolaldehyde, glycolate, and glyoxylate were considerably more toxic than the parent compound ethylene glycol. All could produce renal tubular oxalosis, but the observed mortality could not always be attributed to oxalate. Bachmann and Golberg (1971) concentrated on the subcellular metabolic and cytotoxic effects of ethylene glycol, glycolate, glyoxylate, oxalate, and propylene glycol and found that glyoxylate significantly inhibited

numerous mitochondrial systems. This inhibition of mitochondrial function may be explained in part by the endogenous condensation of glyoxylate and oxaloacetate to form oxalomalate, a potent inhibitor of the citric acid cycle (Ruffo and Adinolfi, 1963; Ruffo et al., 1962a; Ruffo, Romano, and Adinolfi, 1959; Ruffo et al., 1962b; Ruffo et al., 1967). Glycolate, on the other hand, has no effect on mitochondrial function, but seems to accumulate and produce a metabolic acidosis in both acute and delayed phases of ethylene glycol toxicity (Chou and Richardson, 1978; Clay and Murphy, 1977; McChesney, Golberg, and Harris, 1972; McChesney et al., 1971).

Treatment of ethylene glycol toxicity has taken a variety of courses over the years, ranging from symptomatic support and hemodialysis to antidotal therapy with competitive enzymatic inhibitors. Prior to 1959, symptomatic clinical treatment was used with little success in cases of ethylene glycol poisonings. Schreiner et al. (1959) was the first group to successfully use hemodialysis to counteract delayed ethylene glycol toxicity in a human. Correction of the uremic syndrome, secondary to oxalate poisoning, and direct dialytic removal of the toxic agent were given as two reasons for the success of the treatment. Although the first claim could be substantiated, the second could not because of the lack of a method to measure ethylene glycol in biological materials or the dialysate. Subsequently, concomitant use of hemodialysis and supportive symptomatic clinical treatment have been used with varying success by Friedman et al. (1962), Flanagan and Libcke (1964), and Schreiner and Maher (1965) in cases of ethylene glycol poisoning.

Peterson et al. (1963) were not only the first to report the ability of human liver alcohol dehydrogenase to oxidize ethylene glycol, but were the first to double the LD_{50} of ethylene glycol in rats by treating them with ethanol. The urinary excretion of unchanged glycol in the first 24 hours in alcohol treated rats was twice that of control animals, whereas, in monkeys, glycol excretion was increased tenfold. Wacker et al. (1965) described the clinical usefulness of ethanol as an antidote in humans. Ethanol dosage for an average adult was approximately "one ounce of 100 proof ethanol every three to four hours". In all cases, vigorous bicarbonate therapy was necessary to control acidosis and, in some cases, oxygen and artificial respiration were used as supportive measures against CNS depression. This work has been confirmed by Borden and Bidwell (1968).

Mundy, Hall, and Teague (1974) first demonstrated that pyrazole treatment significantly increased the LD_{50} of ethylene glycol in mice. This experiment did not monitor or treat either acidosis or CNS depression. The theory was that pyrazole, like ethanol, could competitively inhibit liver alcohol dehydrogenase in vivo (Peterson et al., 1963; Theorell and Yonetani, 1963; Theorell, Yonetani, and Sjöberg, 1969; Li and Theorell, 1969; Blomstrand and Theorell, 1970; Goldstein and Pal, 1971). Using rats and dogs, Van Stee et al. (1975) confirmed Mundy's work, but complicated the treatment by using bicarbonate, fluid, vitamins, hydrocortisone, and glucose. Like Mundy et al., Van Stee et al. suggested that pyrazole might be an "acceptable risk" in cases of ethylene glycol

poisoning and claimed that pyrazole is more reliable than ethanol antidoting, especially in veterinary medicine, but should begin no later than 6 hours after initial ethylene glycol ingestion. They finally proposed that the study of liver aldehyde dehydrogenase inhibitors may prove useful in ethylene glycol poisoning.

Chou and Richardson (1978) reinvestigated the effect of pyrazole on ethylene glycol toxicity and metabolism in rats. They found the ability of pyrazole to act as an antidote depended on the time of administration as earlier observed by Van Stee et al. (1975). Tracing the fate of ethylene glycol, glycolaldehyde, glycolate, glyoxylate, and oxalate in blood and urine, Chou and Richardson discovered a direct correlation between glycolate levels in blood and urine and the percent mortality. Glycolic acid oxidase, as well as liver alcohol dehydrogenase, was found to be rate limiting in ethylene glycol oxidation. The observed accumulation of glycolate corresponded to the development of the acute toxicity and acidosis seen in ethylene glycol poisoning. With administration of pyrazole, no accumulation of glycolate was observed and as much as 88% of the administered ethylene glycol was recovered in the urine. These data were contrary to the implication of Gessner, Parke, and Williams (1961) that ethylene glycol was most toxic to those species that excreted large amounts of ethylene glycol in the urine. Instead, Chou and Richardson found that ethylene glycol toxicity decreased as a function of the increased renal elimination of ethylene glycol observed after the administration of the alcohol dehydrogenase inhibitor pyrazole.

Inhibition of enzymes other than liver alcohol dehydrogenase with the purpose of decreasing ethylene glycol toxicity in vivo has been unsuccessful. Mundy (personal communication, 1975) found that disulfiram (Antabuse^{R}) and diethyldithiocarbamate (DEDTC), both inhibitors of aldehyde dehydrogenase, failed to increase significantly the LD_{50} of ethylene glycol. Chou and Richardson (1978) inhibited aldehyde dehydrogenase and glycolic acid oxidase with butyraldoxime and d,l-phenyllactate, respectively, and found that these inhibitors increased ethylene glycol toxicity because of the accumulation of glycolaldehyde, glycolate, or glyoxylate. These studies not only confirm the metabolic intermediate hypothesis of ethylene glycol toxicity, but stress the need for inhibition of ethylene glycol catabolism at one, and only one, point, liver alcohol dehydrogenase.

Both ethanol and pyrazole are effective antidotes in the treatment of ethylene glycol delayed toxicity. Pyrazole is a good research tool, but has not been, and probably will not be, approved for clinical use in the United States of America because of the delayed hepatotoxic and nephrotoxic lesions it produces (Wilson and Bottiglieri, 1962; Lelbach, 1969; Lieber et al., 1970). Only ethanol is used clinically, but use of ethanol may be dangerous because it adds to the acidosis and CNS depression produced by ethylene glycol (Peterson et al., 1963; Wacker et al., 1965; Borden and Bidwell, 1968). The dilemma is to find an antidote of ethylene glycol toxicity without the side effects of either ethanol or pyrazole.

An immediate response to the problem of finding a suitable antidote of ethylene glycol toxicity is to look within the series of aliphatic alcohols of which ethanol is a member. Horse liver alcohol dehydrogenase has been known for many years to catalyze the oxidation of most primary aliphatic alcohols, except methanol, and some secondary aliphatic alcohols, except isopropanol, but no tertiary aliphatic alcohols (Lutwak-Mann, 1938; Bonnichsen and Theorell, 1951; Winer, 1958; Merritt and Tomkins, 1959; Sund and Theorell, 1963). Although isopropanol is not metabolized, it competitively inhibits the horse liver enzyme (Witter, 1960). However, human liver alcohol dehydrogenase can oxidize both methanol (Zatman, 1946; Stolman and Steward, 1960; von Wartburg, Bethune, and Vallee, 1964; Blair and Vallee, 1966) and isopropanol (Stolman and Steward, 1960). Methanol, the simplest member, would obviously not be a candidate because of its ocular toxicity in man and delayed systemic acidosis (Røe, 1943; Tephly, 1977). Working with human, horse, and rat liver alcohol dehydrogenases, Pietruszko et al. (1973) and Pietruszko (1975b) found that the Km values of saturated primary aliphatic alcohols decreased with increasing carbon chain length.

Williams (1959) and Stolman and Steward (1960) have indicated that the ease of oxidation of higher aliphatic alcohols and their isomers was decreased as a function of the molecular complexity from primary to secondary alcohols. The formation of water-soluble glucuronide derivatives increased as a function of the molecular complexity from primary to secondary to tertiary. In addition,

solubility of these higher aliphatic alcohols in water decreased with an increase in carbon chain length and oxidation of certain of the secondary alcohols can produce toxic ketones (Stolman and Steward, 1960).

Wallgren (1960) found that, in general, both toxicity and narcotic effects of aliphatic alcohols increased with an increase in carbon chain length, but decreased from primary to secondary to tertiary alcohols. Increasing carbon chain length decreased the Km values of the aliphatic alcohols, but, inversely, increased their toxicities. Molecular alterations of the aliphatic alcohols, such as isomerization, may decrease their toxicities, but coincidently decrease, or abolish, their metabolism by liver alcohol dehydrogenase. Therefore, an aliphatic alcohol, other than ethanol, that might produce an antidotal effect in ethylene glycol toxicity without the undesirable side effects of ethanol is not likely to be found. n-Propanol is the most likely of the higher aliphatic alcohols to have an antidotal effect similar to that of ethanol, but this, as of yet, is unproven.

The second choice in the search for the suitable antidote of ethylene glycol toxicity was to look within the series of alkyldiols of which ethylene glycol is a member. Various alkyldiols, such as ethylene glycol, propylene glycol, 1,3-propanediol, and 1,3-butanediol, have been found to be substrates for liver alcohol dehydrogenase in various species (Peterson et al., 1963; von Wartburg, Bethune, and Vallee, 1964; Blair and Vallee, 1966; Coen and Weiss, 1966; Weiss and Coen, 1966; Tate, Mehlman, and Tobin,

1971; Pietruszko, 1975b). Pietruszko (1975b) found that numerous alkyldiols were substrates for both human and horse liver alcohol dehydrogenase. The alkyldiols studied were ethylene glycol, propylene glycol, 1,3-propanediol, 1,3-butanediol, 1,4-butanediol, 1,5-pentanediol, and 1,6-hexanediol. In general the Km values of these alkyldiols decreased with an increase in carbon chain length or with an increase in the number of carbon atoms separating the two hydroxyls. Ethylene glycol had the highest Km value of the series. The Km values of the largest of these compounds were lower than, if not equal to, the Km value of ethanol. Tate, Mehlman, and Tobin (1971) found that rat liver alcohol dehydrogenase is the sole enzyme responsible for the initial oxidation of 1,3-butanediol and that this oxidation could be blocked by pyrazole.

Williams (1959) and Gessner, Parke, and Williams (1960) studied the metabolic fate of various alkyldiols and found that these compounds were oxidized to organic acids or conjugated with glucuronic acid. Ethylene glycol, 1,3-propanediol, 1,4-butanediol, 1,5-pentanediol, and 1,6-hexanediol were oxidized to the corresponding dicarboxylic acids: oxalate, malonate, succinate, glutarate, and adipate, respectively. Small amounts of 1,6-hexanediol were conjugated with glucuronic acid. Propylene glycol and 1,3-butanediol were oxidized to the corresponding monohydroxycarboxylic acids: lactate and β-hydroxybutyrate, respectively. In low doses, these alkyldiols were completely consumed by the body, whereas, in higher doses, the unchanged alkyldiols, or their organic acid

metabolites, were excreted in the urine. The metabolism of 1,2-butanediol has not been traced, but it is possible that an uncharacterized glucuronic acid derivative may be formed and excreted in the urine. 2,3-Butanediol was apparently not oxidized to any detectable extent, but was converted to a glucuronic derivative and excreted in the urine in large amounts.

The comparative toxicology of the alkyldiols has not been extensively studied. Kesten et al. (1939) found ethylene glycol was responsible for pathological lesions previously described, but propylene glycol was unable to produce such lesions. LD_{50} values are known in various species for ethylene glycol, propylene glycol, 1,3-propanediol, 1,3-butanediol, 1,4-butanediol, and 2,3-butanediol (Laug et al., 1939, Latven and Molitor, 1939; Smyth, Seaton, and Fischer, 1941; Williams, 1959). These authors used experimental conditions which enabled them to compare not only the relative potency of these alkyldiols to produce death within a species, but the sensitivity of various species to each of the alkyldiols. In general, propylene glycol and 1,3-butanediol were the least toxic, whereas 1,4-butanediol was the most toxic. In addition, Laug et al. (1939) found that dilution had no significant effect on observed mortality of ethylene glycol, but age related obesity increased mortality when LD_{50} values were compared on a body weight basis. Mehlman et al. (1971) chronically administered 1,3-butanediol to rats and observed the development and maintenance of a ketosis characterized by high blood levels of β-hydroxybutyrate and acetoacetate. No mortality resulted over a 7-week period in spite

of the fact that the amount of 1,3-butanediol in the rats' food was equal to 25% of the total caloric intake. Ethylene glycol is apparently the only compound among the alkyldiols that gives rise to metabolites more toxic than the parent compound.

With the exception of propylene glycol, the use of the alkyldiols as inhibitors of liver alcohol dehydrogenase has not been explored. Weiss and Coen (1966) were able to inhibit ethylene glycol oxidation *in vitro* with both ethanol and propylene glycol, but rejected the use of propylene glycol as a practical inhibitor solely on its higher K_I value and not on any other substantial scientific evidence. Brown *et al.* (1968) developed a gas chromatographic method for the detection of ethanol and propylene glycol in serum and used it to follow the decay kinetics of these compounds in mice. Ethanol disappearance followed zero order kinetics, whereas propylene glycol disappearance obeyed first order kinetics. Ethanol and propylene glycol did act as competitive inhibitors of each other *in vivo* and thus refuted the claim of Weiss and Coen as to the impracticality of propylene glycol as a competitive inhibitor *in vivo*. It was further suggested that propylene glycol may prove to be an inhibitor of choice because its inherent toxicity is less than that of ethanol.

The buffer, tris (hydroxymethyl) aminomethane (THAM, Tris) or 2-amino-2-hydroxymethyl-1,3-propanediol, is of interest to this investigation, not only because the compound is a derivative of the alkyldiol 1,3-propanediol, but because it is a good alkalinizing agent *in vivo* and might be used to treat the metabolic acidosis

observed in ethylene glycol toxicity. Unlike some other buffers or alkalinizing agents, there exists evidence to show that Tris is not at all an inert buffer and may in fact be able to influence the course of ethylene glycol toxicity by mechanisms other than prevention of metabolic acidosis. Tris buffer acts as an osmotic diuretic, like mannitol, and might possibly increase ethylene glycol excretion (Nahas, 1959; Manfredi et al., 1960; Nahas, 1962; Nahas, 1963; Darby and Anderson, 1966; Strauss, 1968). Tris buffer may form Schiff bases with aldehydes, such as glyoxylate, in an alkaline pH range in vitro and inhibit some enzymes by removal of the aldehyde substrate (Richardson and Tolbert, 1961; Thompson and Richardson, 1968; Richardson and Thompson, 1970). The ability of Tris to form Schiff bases in vivo with glyoxylate or other aldehydes, such as glycolaldehyde, and become, in effect, a carrier or scavenger of the toxic metabolites of ethylene glycol has not been studied. Finally, Tris may be a competitive inhibitor of liver alcohol dehydrogenase (Baker, 1960).

The work described herein is a survey of a series of alkyldiols and tris (hydroxymethyl) aminomethane with the specific hope of finding an antidote for ethylene glycol toxicity in mice better than the existing antidotes, ethanol and pyrazole. Ethanol and pyrazole were evaluated with the alkyldiols so that direct comparisons of pharmacological actions may be drawn. The study plan incorporated experiments designed to elucidate the mechanisms of action of each compound, such as enzymatic affinities, individual toxicities, and CNS effects.

MATERIALS AND METHODS

Animals and Housing

All of the animals used in this research were ICR strain, adult, female, Swiss albino mice obtained commercially from Southern Animal Farms, Prattville, Alabama. The weight range of these mice was 20 to 30 grams. With the exception of the water intake, urine output, and urinary excretion studies described later, the mice were housed six per cage in wire grid roofed plastic cages containing a hardwood chip floor. An overhead supply of tap water and rodent laboratory chow were available ad libitum. The animals were kept in a windowless, air-conditioned room whose temperature varied between 24 and 25° C and illumination was controlled for 12 hours of daylight and 12 hours of darkness.

Drugs and Chemicals

The drugs used in these experiments included: absolute ethanol from U. S. Industrial Chemicals Co.; ethylene glycol and propylene glycol from Fisher Scientific Co.; 1,3-propanediol and glycine from Eastman Kodak Co.; 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, 2,3-butanediol, 1,5-pentanediol, 1,6-hexanediol, pyrazole, and glyoxal from Aldrich Chemical Co.; glycolaldehyde,

tris (hydroxymethyl) aminomethane (Trizma^{R} Base), tris (hydroxymethyl) aminomethane hydrochloride (Trizma^{R} HCl), disodium pyrophosphate, semicarbazide HCl, and bovine albumin (Fraction V) from Sigma Chemical Co.; and sodium hydroxide from Mallinckrodt Chemical works. Drugs were dissolved in once-distilled, deionized water in all experiments. Placebo solutions consisted of 0.9% saline in once-distilled, deionized water.

General Gas Chromatographic Analyses

A Hewlett-Packard Model 402 High Efficiency Gas Chromatograph was equipped with a flame ionization detector (FID), a strip chart recorder, and a six foot, U-shaped glass column. The column contained the 60/80 mesh porous polymer packing Tenax-GC (Applied Science Laboratories, Inc.). Gas flows of 28 ml/min for helium, 37 ml/min for hydrogen, and 420 ml/min for air were maintained throughout the analyses. Other operating conditions varied according to the nature of the experiment, and will be described in pertinent sections.

Injection volumes of 2 µl were made into the gas chromatograph via 10 µl syringe pipets (Hamilton Co.). After each injection, the syringe pipets were disassembled and their barrels rinsed under vacuum with dilute nitric acid and then distilled water.

Ethanol, the alkyldiols, glycolaldehyde, glyoxal, and tris (hydroxymethyl) aminomethane (base form only) were qualitatively

analyzed in water solutions. Operating conditions included temperature programming from 80 to 250° C at a rate of 10° C per minute and constant temperatures of 300° C and 300° C for the flash heater and FID, respectively.

Gas Chromatographic Plasma Analysis

Ethylene glycol and glycolaldehyde were qualitatively and quantitatively analyzed in water and mouse plasma in vitro. Operating conditions of the gas chromatograph included constant temperatures of 150° C for the oven and 200° C for the flash heater and FID. Serial dilutions of ethylene glycol and glycolaldehyde in water and heparinized mouse plasma were compared with a fixed concentration of the internal standard, propylene glycol. Ethylene glycol and glycolaldehyde concentrations ranged from 5.36 mmoles/l to 398 mmoles/l and 5.00 mmoles/l to 400 mmoles/l, respectively. After 20 to 25 plasma samples were injected, the column was pulled for removal of the precipitated salt and protein plug that develops in the column mouth.

Ethylene glycol was qualitatively and quantitatively analyzed in untreated or pyrazole treated mice. Two groups of mice received 37.7 and 71.4 mmole/kg dose levels of ethylene glycol, ip, followed in 15 minutes by a saline placebo, ip. Three additional groups of mice were injected at the 71.4 mmole/kg dose level of ethylene glycol, ip. Two of these latter groups received a 4.41 mmole/kg dose level of pyrazole, either ip 15 minutes after or sc 60 minutes

prior to the administration of ethylene glycol. The third group received an 8.81 mmole/kg dose level of pyrazole, sc, 60 minutes prior to the administration of ethylene glycol. All solutions were made so that the mice received 0.01 ml of solution per gram body weight.

Mouse blood samples were obtained by puncturing the mucosa of the outer canthus of the eye with a Heparinized Capillary Tube (Durr Fillauer Medical, Inc.). The blood samples were collected from a mouse at timed intervals of 30 minutes for the first two hours and 60 minutes thereafter for the next six hours. The capillary tubes were immediately plugged with Critoseal (Sherwood Medical Industries) and centrifuged in an Adams Micro-Hematocrit Centrifuge (Clay-Adams, Inc.). Twenty µl aliquots of plasma were transferred by microliter pipets from the capillary tubes to a 6 x 50 mm borosilicate Disposable Glass Culture Tube (Kimble) and mixed with an equal volume of heparinized propylene glycol solution. Two µl samples were withdrawn from the culture tubes for gas chromatographic analysis.

Gas Chromatographic Urine Analyses

Ethylene glycol, propylene glycol, and 1,3-butanediol were qualitatively and quantitatively analyzed in water and mouse urine with 2,3-butanediol as the internal standard. In general, the methodology was the same as used in the plasma analyses; however, certain operating conditions varied with the particular analysis.

Ethylene glycol and propylene glycol were eluted with oven temperature of 150° C and flash heater and FID temperatures of 200° C, whereas ethylene glycol and 1,3-butanediol were eluted with an oven temperature of 180° C and flash heater and FID temperatures of 230° C.

Ethylene glycol was qualitatively and quantitatively analyzed in urine from mice treated with saline, pyrazole, propylene glycol, or 1,3-butanediol. In addition, propylene glycol and 1,3-butanediol were qualitatively and quantitatively analyzed in urine from mice treated with either water or ethylene glycol. Urine samples for the above analyses of ethylene glycol, propylene glycol, and 1,3-butanediol were obtained every 12 hours from the water intake, urine output, and urinary excretion studies.

Twenty µl aliquots of urine were transferred by microliter pipets from the urine sample bottles to a 6 x 50 mm culture tube and mixed with an equal volume of 2,3-butanediol solution. Two µl samples were withdrawn from the culture tubes for gas chromatographic analysis. Results, expressed as the percentage of the original dose of a compound recovered and urinary concentration of that compound, were subjected to the Student's t test.

Preparation of Mouse Liver Alcohol Dehydrogenase

Mice were rendered unconscious by concussion and dissected. Whole livers from six mice were excised, blotted on clean filter paper to remove excess blood, crushed with a blunt glass rod, and

combined for the extraction procedure. Mouse liver alcohol dehydrogenase was partially purified by the method of Bonnichsen and Brink (1955). Homogenization was performed in a Teflon-pestled Thomas tissue grinder (A. H. Thomas Co.) driven by an electric motor, and centrifugation, at various steps, was performed on a model B-20A Centrifuge (International Equipment Co.) at 10,000 rpm and 5° C. Steps of the Bonnichsen method, involving hemoglobin extraction with ethanol-chloroform and refractionation with ethanol, respectively, were not performed. Apparently, trace amounts of ethanol sometimes remained in the enzyme preparation after dialysis and interfered with the reproducibility of kinetic data collected for some substrates of very low affinity for liver alcohol dehydrogenase. Protein content of the enzyme preparation was determined by the method of Lowry et al. (1951). Bovine albumin (Fraction V) was the protein used as a standard in the Lowry method.

Enzymatic Determination of Kinetic Constants

Mouse liver alcohol dehydrogenase was assayed at 30° C by the method of Bonnichsen (1965) modified to use a Gilford Model 220 Recording Spectrophotometer (Gilford Instrument Laboratories, Inc.). An initial pH of 8.9 was achieved by a buffer consisting of sodium pyrophosphate, semicarbazide HCl, glycine, and sodium hydroxide at concentrations of 59.8, 59.8, 17.8, and 53.4 mmoles/l, respectively. Nicotinamide-adenine dinucleotide was at an initial

concentration of 0.500 mmole/l. Serial dilutions of the substrates were made using a geometric factor of 0.316. The substrates and their starting concentration ranges in mmoles/l were ethanol (0.342 - 10.3), ethylene glycol (17.9 - 357), propylene glycol (4.08 - 136), 1,3-propanediol (1.38 - 41.5), 1,2-butanediol (1.12 - 33.5), 1,3-butanediol (1.12 - 33.5), 1,4-butanediol (0.226 - 6.77), 2,3 - butanediol (11.0 - 110), 1,5-pentanediol (0.191 - 5.73), 1,6-hexanediol (0.0167 - 0.508), and tris (hydroxymethyl) aminomethane - tris (hydroxymethyl) aminomethane HCl (0.826 - 247). Mouse liver extract containing alcohol dehydrogenase was at a concentration of 1.30 mg of protein/ml. Initial reaction velocities were measured by calculating the slope of the graph of absorption versus time within the first half minute, but reactions were not carried to completion as described in Bonnichsen's method. V_{max} and K_m were determined by the method of Lineweaver and Burk (1934). Maximum velocities, thus obtained, were normalized by the protein concentration determined by the Lowry method.

Toxicity Studies

The alkyldiols and tris (hydroxymethyl) aminomethane were surveyed as putative antidotes in mice to determine if any of these compounds produced in vivo antidotal effects comparable with ethanol or pyrazole in ethylene glycol toxicity. Because of the large

number of mice involved in the mortality studies, ethanol, pyrazole, and the putative antidotes were tested at three or more dose levels on a one-time injection basis only. Groups of six mice were orally administered geometrically increasing dose levels of ethylene glycol (geometric factor = 1.2) and then immediately injected ip with a placebo or putative antidote. The untreated groups received a saline placebo, whereas the treated groups received a solution containing one of the following: ethanol, pyrazole, tris (hydroxymethyl) aminomethane, or an alkyldiol. The tris (hydroxymethyl) aminomethane - tris (hydroxymethyl) aminomethane HCl was made at three concentrations at each of the pH levels, 7.8 and 10.8. In addition, a solution containing a mixture of tris (hydroxymethyl) aminomethane - tris (hydroxymethyl) aminomethane HCl and 1,3-butanediol was made at a single concentration for each compound at each of the pH levels 7.8 and 10.8. The dilutions of ethylene glycol were made so that the mice received 0.03 ml of solution per gram body weight, whereas the dilutions of ethanol, pyrazole, tris (hydroxymethyl) aminomethane, and the alkyldiols were made such that the mice received 0.01 ml of solution per gram body weight. The 0.03 ml of ethylene glycol solution per gram body weight was necessary to produce death in the mice.

Cumulative LD_{50} values and their 95% confidence limits were calculated at 144 hours by the method of Weil (1952).

The relative antidotal effect of a compound was calculated by dividing the LD_{50} of ethylene glycol for the treated groups by the LD_{50} of ethylene glycol for the untreated groups. The relative

antidotal effect will be used as an index of efficacy, since this value would increase as the antidotal effect increases.

Ethanol, pyrazole, the alkyldiols, and tris (hydroxymethyl) aminomethane (pH = 10.8) were tested in mice to determine the acute and delayed in vivo toxicities of each compound. Groups of six mice were given a single injection ip at geometrically increasing dose levels (geometric factor = 1.2) of each of the compounds. No placebo oral solution was given. The serial dilutions of ethanol, pyrazole, tris (hydroxymethyl) aminomethane, and the alkyldiols, except for propylene glycol and 1,3-butanediol, were made so that the animal received 0.01 ml of solution per gram body weight. The serial dilutions of propylene glycol and 1,3-butanediol were made such that the mice received 0.02 ml of solution per gram body weight. The 0.02 ml of propylene glycol or 1,3-butanediol solution per gram body weight was necessary to produce death in the mice.

Glycolaldehyde, the first metabolite of ethylene glycol, was tested in mice by two routes of administration to determine its acute and delayed in vivo toxicities. Groups of six mice were given a single injection either ip or sc (dorsal thoracic region) at geometrically increasing dose levels (geometric factor 1.2) of glycolaldehyde. No placebo oral solutions were given. The serial dilutions of glycolaldehyde were made such that the mice received 0.01 ml of solution per gram body weight.

Cumulative LD_{50} values and their 95% confidence limits were calculated at 24 and 144 hours by the method of Weil (1952). The exception to this is pyrazole, whose 48 hour LD_{50}, instead of 24

hour LD_{50}, was calculated. The intent of calculating these two LD_{50} values was to find not only the eventual LD_{50} for each compound, but also the time course of toxicity. If a compound had a marked delayed toxicity, the LD_{50} values for the two time periods should be statistically different.

The relative delayed toxicity of each compound was found by dividing the 144 hour LD_{50} by the 24 hour LD_{50}. The smaller the value of relative delayed toxicity, the greater the delayed toxicity of the compound.

For each of the compounds found to produce an antidotal effect, a therapeutic ratio was calculated by dividing the 144 hour LD_{50} by the dose which produced the maximum relative antidotal effect. The therapeutic ratio increased as the margin of safety increased. The therapeutic ratio is used in lieu of a therapeutic index since the ED_{50} for antidotal effect cannot be calculated from the data.

Hexobarbital Sleeping Time Studies

Ethanol, pyrazole, the alkyldiols, and tris (hydroxymethyl) aminomethane were tested in mice to determine what effect CNS depression had on either antidotal effect or toxicity. To insure that sleep was induced quickly, reliably, and reproducibly for each of the compounds tested, groups of six mice initially received 75 mg/kg doses of hexobarbital (Evipal^{R}), ip. Immediately thereafter, the untreated groups of mice received a saline placebo, whereas the

treated groups of mice received a solution of ethanol, pyrazole, tris (hydroxymethyl) aminomethane, or an alkyldiol, ip. Each mouse was placed in an individual cage on its back. Reacquisition of the righting reflex was the criterion of judging the recovery from depression. All drug solutions were made so that the mice received 0.01 ml of solution per gram of body weight.

Schiff Base Formaton

A 99.1 mmole/l solution of tris (hydroxymethyl) aminomethane - tris (hydroxymethyl) aminomethane HCl (pH @ 25° C = 8.92) was titrated in vitro with a 167 mmole/l solution of glycolaldehyde to test the possibility that the two compounds may form a Schiff base under alkaline conditions. Increments of 1.0 ml of glycolaldehyde solution were added to an initial 20 ml solution of tris buffer. In addition, the glycolaldehyde solution was titrated with a 0.1 N NaOH solution to check for acid content. An Expandomatic SS-2 pH meter (Beckman Instruments, Inc.) was used to measure pH.

Water Intake, Urine Output, and Urinary Excretion Studies

Pyrazole, propylene glycol, and 1,3-butanediol were tested in mice to determine what effect these compounds had on water intake, urine output, and urinary excretion of ethylene glycol. Groups of four mice were housed in 115 mm by 203 mm by 108 mm rodent metabolism cages made by Acme Research Products. The mice were allowed

access to rodent laboratory chow and tap water ad libitum. To minimize contamination in this experiment from foodstuffs, the chow pellets were ground into a fine powder to prevent transfer of whole pellets from the adjustable feeder to a position over the collection funnel. Once every twelve house, the collection funnels were cleaned and food was replenished. Urine was collected in small mouthed serum bottles attached to the collection funnels by vinyl tubing.

After an initial acclimation period of three days, measurement of water intake and urine output by weight were begun and recorded every twelve hours for the next three days. After water intake and urine output were measured at the termination of the second twelve hour period, the mice were given either water or a solution of ethylene glycol, orally, and saline or a solution of an antidote, ip. Ethylene glycol solutions and water were administered so that the mice received 0.03 ml of liquid per gram body weight, whereas antidote solutions or saline were administered such that the mice received 0.01 ml of liquid per gram body weight. The mice throughout these experiments received 108 mmoles/kg ethylene glycol, a dose about one half the oral LD_{50}. Those mice given pyrazole, propylene glycol, or 1,3-butanediol received 4.41, 27.2, and 22.3 mmoles/kg, respectively.

RESULTS

Purity

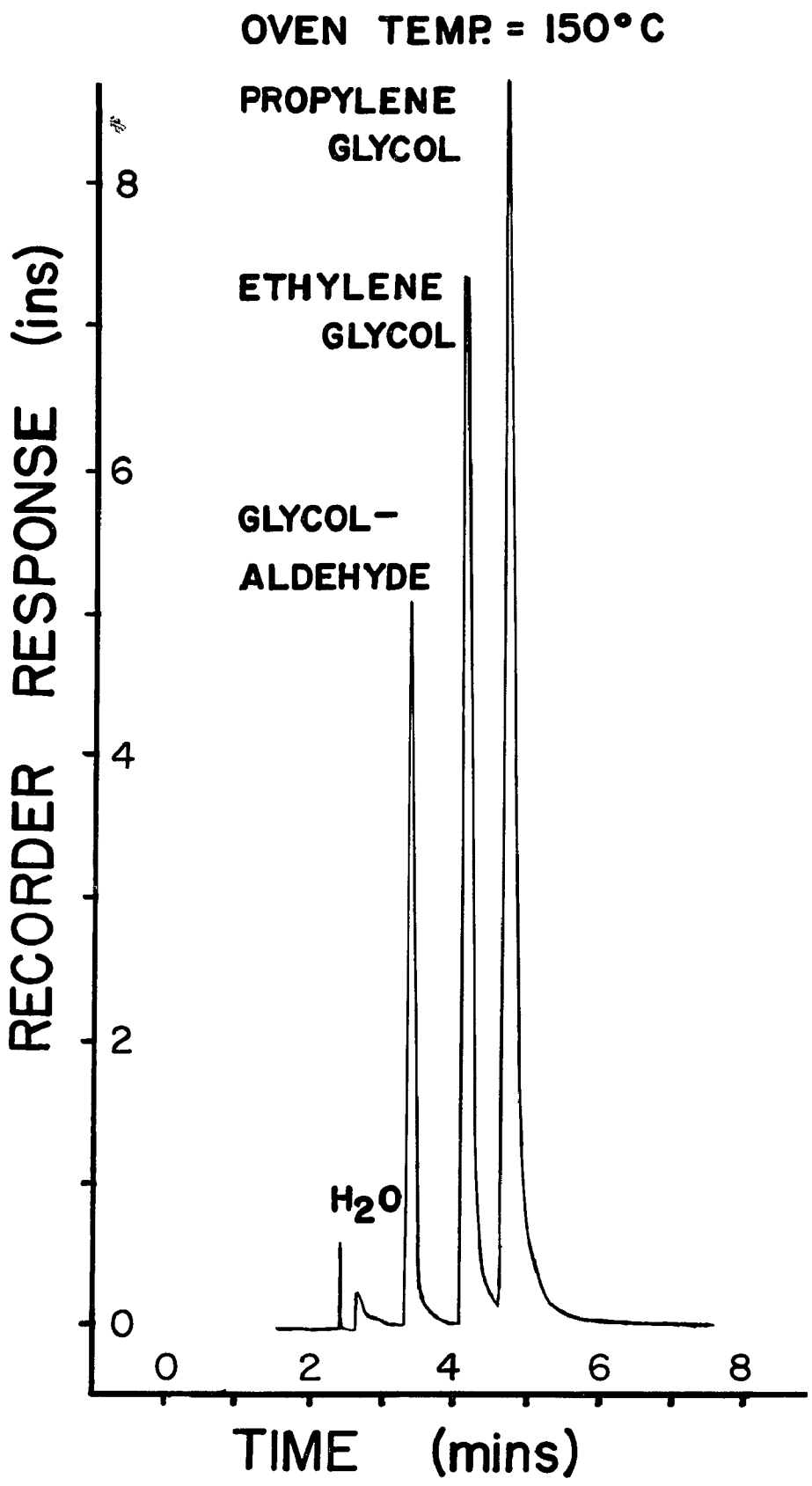
Ethanol, the alkyldiols, and glycolaldehyde were eluted after injections of their aqueous solutions into the gas chromatograph. Although elution time and peak height varied, one, and only one, well-defined peak was eluted after each injection. No secondary peaks with a height of at least 0.1% of the primary peak height were observed on the gas chromatograms at a sensitivity setting 100 times greater than used in the analyses. Glyoxal could not be eluted under any gas chromatographic conditions tried.

Plasma Analyses

Figure 2 shows the results of injecting a 2 μl aliquot of ethylene glycol, glycolaldehyde, and propylene glycol in aqueous solutions. Since the peaks are well separated and sharp, the criterion for quantitative determination of the compounds used was peak height.

A similar recording to that illustrated in Figure 2 was obtained when a mixture of ethylene glycol, glycolaldehyde, and propylene glycol in mouse plasma *in vitro* was injected. There were no normal constituents of mouse plasma that interfered with the

Figure 2: A facsimile of a gas chromatogram showing the *in vitro* separation of ethylene glycol and glycolaldehyde from water or mouse plasma with propylene glycol as the internal standard.


OVEN TEMP. = 150°C
PROPYLENE GLYCOL
ETHYLENE GLYCOL
GLYCOL-ALDEHYDE
H2O
RECORDER RESPONSE (ins)
0
2
4
6
8
TIME (mins)

qualitative and quantitative recording of these peaks.

Figure 3 shows the calibration curves obtained by injecting serial dilutions of ethylene glycol, glycolaldehyde, and propylene glycol in both water and plasma in vitro. The recovery of ethylene glycol from plasma was comparable with its recovery from water. As can be observed in Figure 3, the peak height ratios of ethylene glycol to propylene glycol in plasma were similar to those in water. The glycolaldehyde added to plasma in vitro disappears with time.

Figure 4 shows the decay of ethylene glycol at two dose levels in mouse plasma. The decay of ethylene glycol at these two dose levels appears to obey first order kinetics. The half-lives of the 35.7 and 71.4 mmoles/kg dose levels of ethylene glycol were 1.15 and 1.82 hours, respectively, whereas the volumes of distribution were 58.5 and 53.5 percent of total body weight.

Figure 5 shows the plasma decay of a 71.4 mmoles/kg dose level of ethylene glycol in mice treated with either saline or 4.41 mmoles/kg pyrazole, ip. Figure 6 shows the plasma decay of a 71.4 mmoles/kg dose level of ethylene glycol in mice treated with either saline or 8.81 mmoles/kg pyrazole, sc. In both Figures 5 and 6, the disappearance of ethylene glycol from saline treated mice represented by open circles, is previously shown in Figure 4. Pyrazole by two routes of administration was found to delay the disappearance of ethylene glycol from the mouse plasma (Figures 5 and 6). The disappearance of ethylene glycol after pyrazole administration is not compatible with a single compartment model of

Figure 3: Calibration curves *in vitro* for ethylene glycol and glycolaldehyde in water and mouse plasma with propylene glycol as the internal standard. Serial dilutions of ethylene glycol (5.36 to 398 mmoles/ l) and glycolaldehyde (5 to 400 mmoles/l) were diluted with an equal volume of propylene glycol internal standard (50 mmoles/l). Each point represents the mean of 6 replicate injections of a 2 microliter volume ± S.E.M. Lines of best fit were found by least squares.

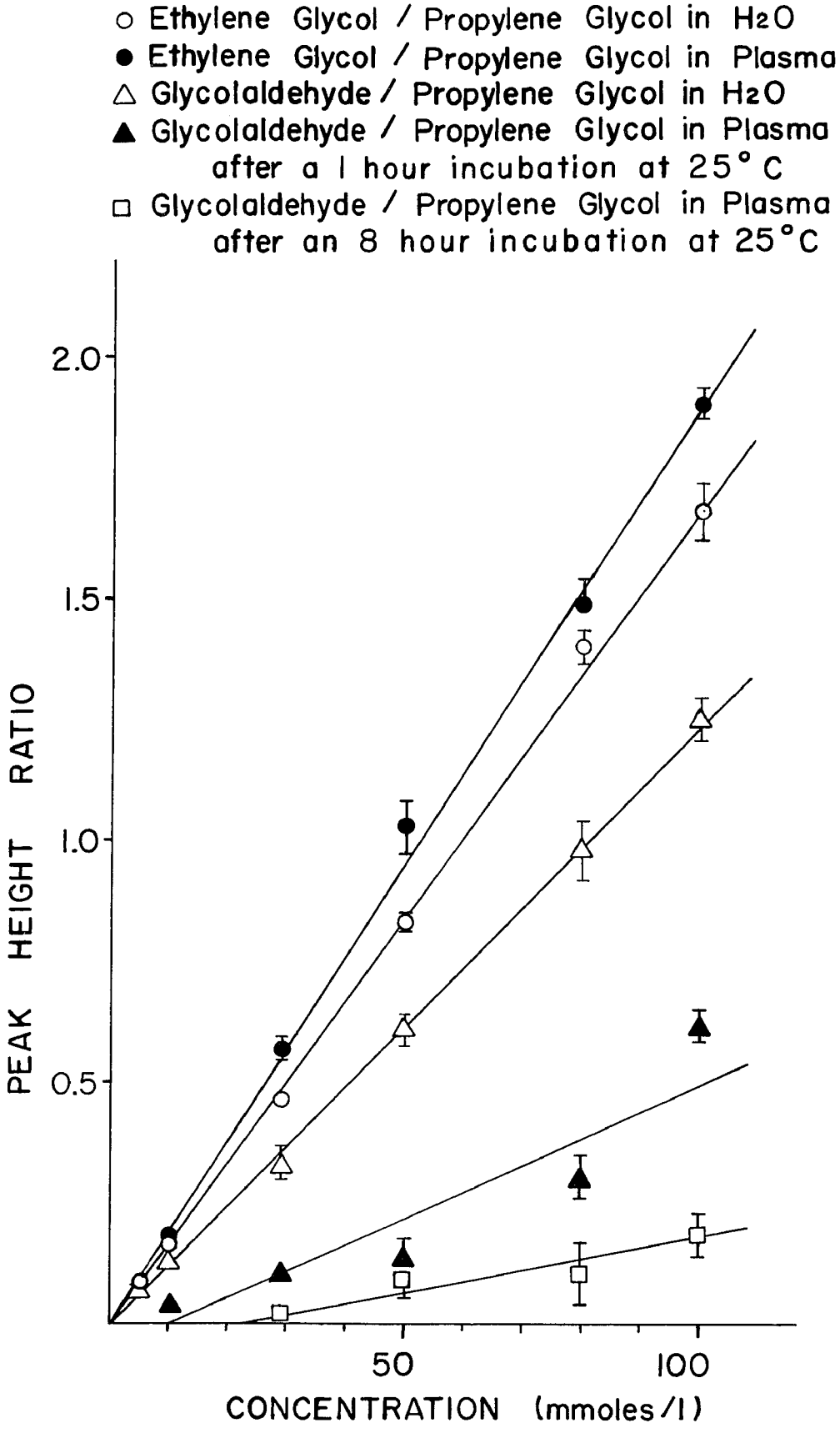

○ Ethylene Glycol / Propylene Glycol in H2O
● Ethylene Glycol / Propylene Glycol in Plasma
△ Glycolaldehyde / Propylene Glycol in H2O
▲ Glycolaldehyde / Propylene Glycol in Plasma after a 1 hour incubation at 25° C
□ Glycolaldehyde / Propylene Glycol in Plasma after an 8 hour incubation at 25°C
PEAK HEIGHT RATIO
2.0
1.5
1.0
0.5
50
100
CONCENTRATION (mmoles/l)

Figure 4: The plasma decay of ethylene glycol at two dose levels in saline treated mice. Ethylene glycol was injected ip at dose levels of 35.7 and 71.4 mmoles/kg. Saline was injected ip fifteen minutes later. Each point represents the antilog of the log mean of plasma concentrations in six mice. Bars represent the antilog of the log mean ± log S.E.M. The lines of best fit were found by least squares.

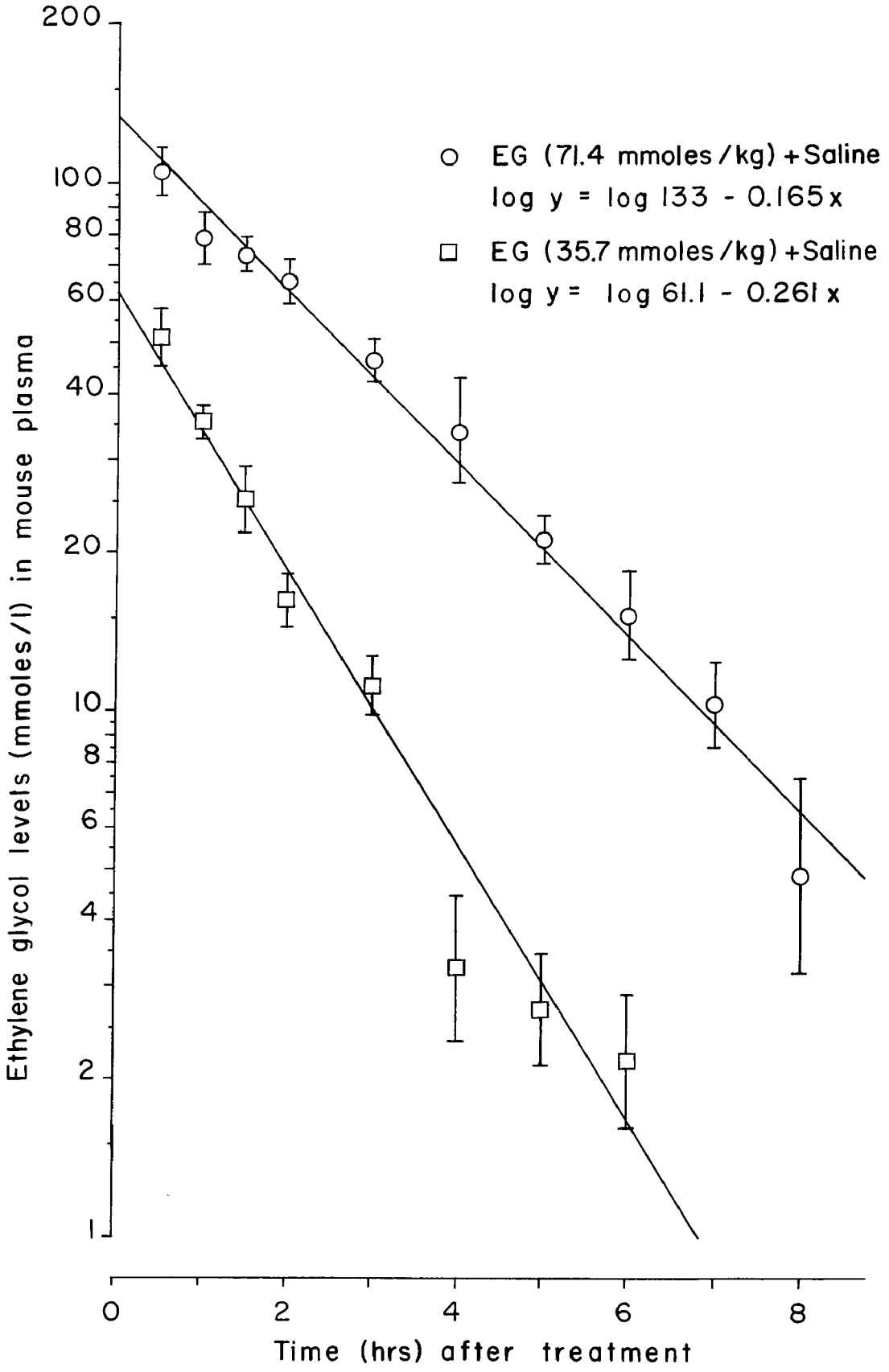

200
100
80
60
40
20
10
8
6
4
2
1
Ethylene glycol levels (mmoles/l) in mouse plasma
EG (71.4 mmoles/kg) + Saline
log y = log 133 - 0.165x
EG (35.7 mmoles/kg) + Saline
log y = log 61.1 - 0.261x
0
2
4
6
8
Time (hrs) after treatment

Figure 5: The plasma decay of a 71.4 mmoles/kg dose level of ethylene glycol in mice treated with either saline or 4.41 mmoles/kg pyrazole, ip. Saline and pyrazole were injected 15 minutes after the injection of ethylene glycol. Each point represents the antilog of the log mean of plasma concentrations in six mice. Bars represent the antilog of the log mean ± log S.E.M. The lines of best fit were found by least squares.

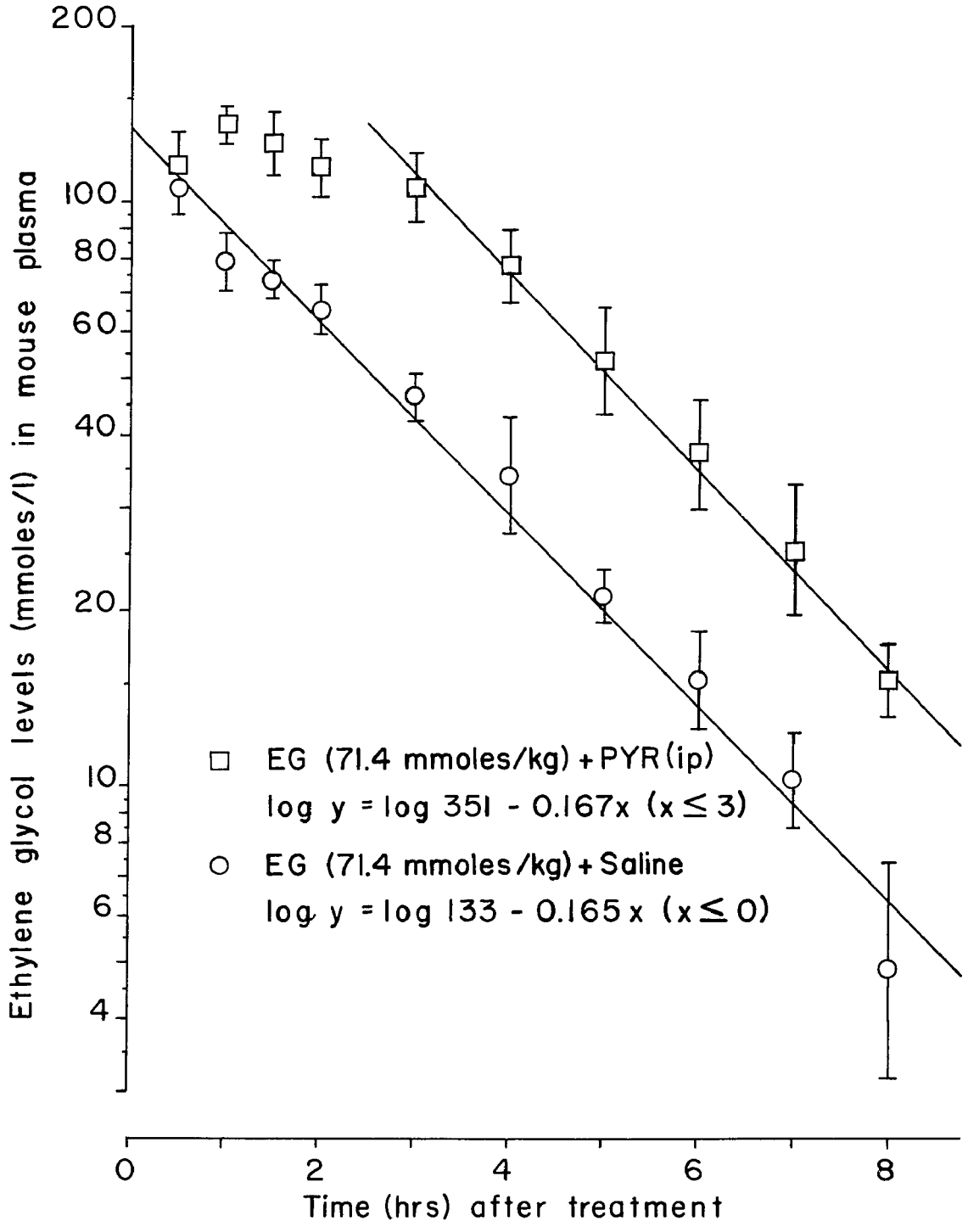
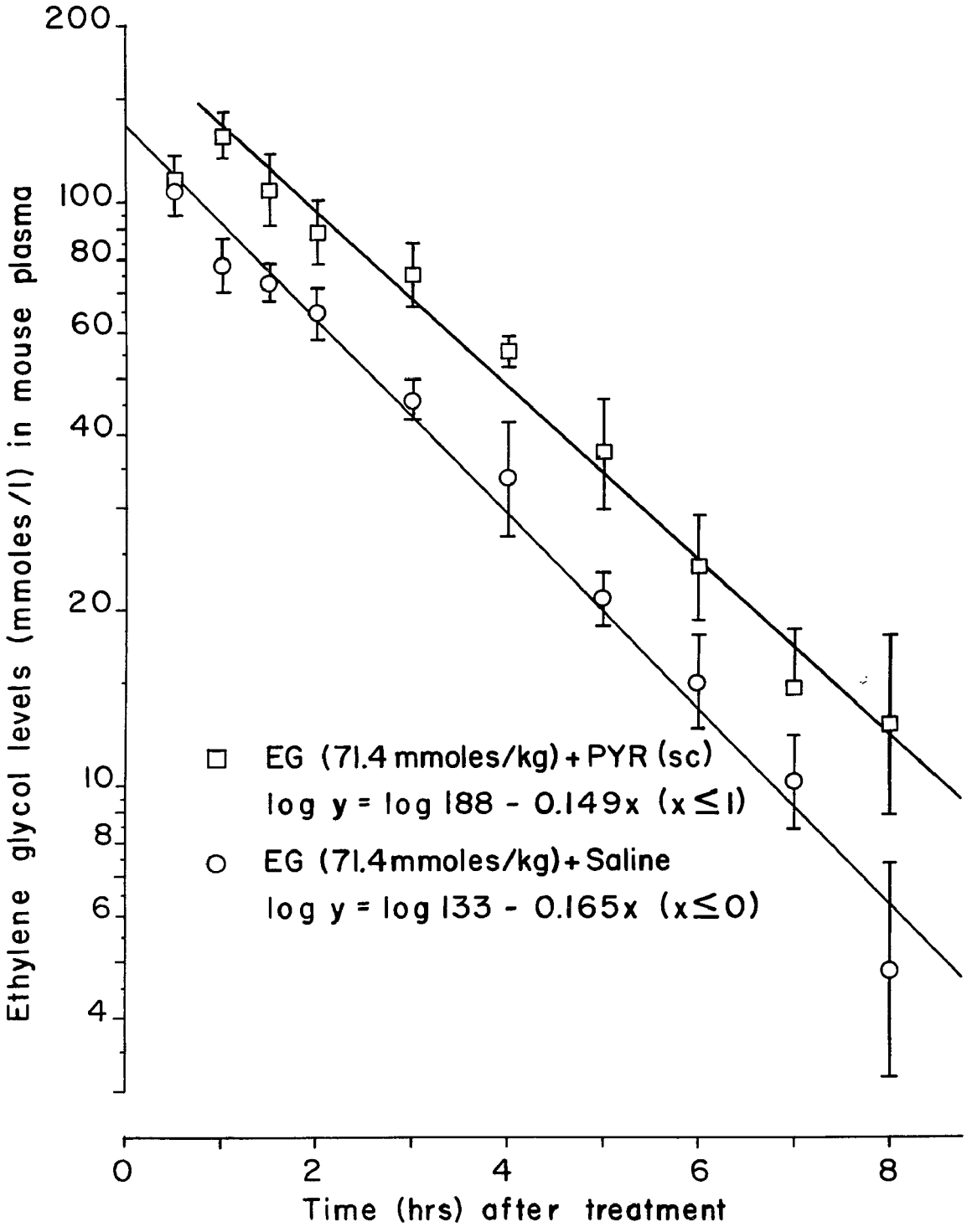

200
100
80
60
40
20
10
8
6
4
Ethylene glycol levels (mmoles/l) in mouse plasma
□ EG (71.4 mmoles/kg) + PYR (ip)
log y = log 351 - 0.167x (x ≤ 3)
○ EG (71.4 mmoles/kg) + Saline
log y = log 133 - 0.165 x (x ≤ 0)
0
2
4
6
8
Time (hrs) after treatment

Figure 6: The plasma decay of a 71.4 mmoles/kg dose level of ethylene glycol in mice treated with either saline or 8.81 mmoles/kg pyrazole, sc. Saline was injected fifteen minutes later, whereas pyrazole was injected subcutaneously one hour prior to the ethylene glycol injection. Each point represents the antilog of the log mean of plasma concentrations in six mice. Bars represent the antilog of the log mean ± log S.E.M. The lines of best fit were found by least squares.


200
100
80
60
40
20
10
8
6
4
Ethylene glycol levels (mmoles/l) in mouse plasma
□ EG (71.4 mmoles/kg) + PYR (sc)
log y = log 188 - 0.149x (x ≤ 1)
○ EG (71.4mmoles/kg) + Saline
log y = log 133 - 0.165x (x ≤ 0)
0
2
4
6
8
Time (hrs) after treatment

analysis.

This same type of experiment was done with a 4.41 mmoles/kg dose of pyrazole sc one hour before injection of ethylene glycol. Although this decay curve is not shown, this lower dose of pyrazole failed to affect the decay of ethylene glycol.

At no time was glycolaldehyde detected in mouse plasma in vivo after the administration of ethylene glycol.

Substrate Specificity of Mouse Liver Alcohol Dehydrogenase

Mouse liver alcohol dehydrogenase was found to catalyze the oxidation of ethanol and the alkyldiols, including ethylene glycol, propylene glycol, 1,3-propanediol, 1,2-, 1,3-, 1,4- and 2,3-butanediols, 1,5-pentanediol and 1,6-hexanediol. Tris (hydroxymethyl) aminomethane in a high concentration (25 mmoles/l) and at a pH equal to that of Bonnichsen's buffer (pH = 8.9) was neither a substrate for mouse liver alcohol dehydrogenase nor an inhibitor of ethanol oxidation.

Kinetic Constants of Mouse Liver Alcohol Dehydrogenase With Ethanol and the Alkyldiols

K_m and V_{max}, determined by the method of Lineweaver and Burk (1934), were found with ethanol and each of the alkyldiols tested (Table 1). The Km values of the alkyldiols, in general, decreased with an increase in carbon chain length or with an increase in the

TABLE I: SUBSTRATE SPECIFICITY AND KINETIC CONSTANTS OF MOUSE LIVER ALCOHOL DEHYDROGENASE WITH ETHANOL, THE ALKYLDIOLS, AND TRIS(HYDROXYMETHYL)AMINOMETHANE

Substrate	Km (mmoles/l)	Vmax (μmoles/min/mg protein)
Ethanol	0.4	6.01
Ethylene Glycol	53	3.25
Propylene Glycol	14	5.70
1,3-Propanediol	5.4	5.83
1,2-Butanediol	3.8	6.31
1,3-Butanediol	1.5	4.45
1,4-Butanediol	0.5	3.96
2,3-Butanediol	56	2.41
1,5-Pentanediol	0.23	6.11
1,6-Hexanediol	0.031	5.78
Tris(hydroxymethyl)-aminomethane	—	0
Ethanol + Tris-(hydroxymethyl)-aminomethane (24.8 mmoles/l)	0.4	6.00

number of carbon atoms separating the two hydroxyls. The comparative ranking of the relative affinities of ethanol and the alkyldiols for mouse liver alcohol dehydrogenase in order of increasing K_m values is: 1,6-hexanediol > 1,5-pentanediol > ethanol > 1,4-butanediol > 1,3-butanediol > 1,2-butanediol > 1,3-propanediol > propylene glycol > ethylene glycol > 2,3-butanediol. With the exception of 2,3-butanediol, the alkyldiols in this series and ethanol have Km values lower than that of ethylene glycol. The V_{max} obtained with the alkyldiols is similar to that obtained with ethanol, with the exception of poor substrates such as ethylene glycol and 2,3-butanediol.

Effects of Known Antidotes on Ethylene Glycol Toxicity

Ethanol and pyrazole were tested on a one-time injection basis to determine their antidotal effects in ethylene glycol toxicity in mice (Table 2). Both compounds were found to produce significant antidotal effects, but the range of these effects was different. Ethanol, tested at dose levels of 17.1, 34.3, and 68.5 mmoles/kg, only produced a significant antidotal effect at the 34.3 mmoles/kg dose level. Pyrazole, tested at dose levels of 1.10, 2.20, and 4.41 mmoles /kg produced significant antidotal effects at all three dose levels tested. The maximum relative antidotal effects produced by ethanol was 1.49, whereas that produced by pyrazole was 2.65. When tested on a one-time injection basis, pyrazole is both more potent and more efficacious an antidote than ethanol. More

TABLE 2: ANTIDOTAL EFFECTS OF KNOWN ANTIDOTES FOR ETHYLENE GLYCOL TOXICITY IN MICE TESTED ON A ONE TIME INJECTION SCHEDULE

Drug	Dose (mmoles/kg)	144 hr. LD50 of Ethylene Glycol (95% Confidence Limits)		Relative Antidotal Effect [c]
		Saline Treated (mmoles/kg)	Antidote Treated (mmoles/kg)	
Ethanol	17.1	160 (139-184) [a]	214 (178-258)	1.34
	34.3	160 (139-184) [a]	239 (207-276)	1.49 *
	68.5	160 (139-184) [a]	168 (128-221)	1.05
Pyrazole	1.10	178 (149-213) [b]	414 (370-463)	2.32 *
	2.20	178 (149-213) [b]	405 (363-455)	2.28 *
	4.41	178 (149-213) [b]	473 (445-502)	2.65 *

* $P<0.05$ (Significance of antidote treated as compared with saline treated)

a and b Superscripts indicate those LD50 values from the same saline treated mice.

[c] LD50 of antidote treated / LD50 of saline treated

importantly, an experimental design has been established by which any antidotal effects produced by the alkyldiols and tris buffer may be detected and compared with the two known antidotes, ethanol and pyrazole.

Effects of Putative Antidotes on Ethylene Glycol Toxicity

The alkyldiols were tested at three or more dose levels under the same experimental conditions as ethanol and pyrazole. The three-carbon chain alkyldiols tested were propylene glycol and 1,3-propanediol (Table 3). Propylene glycol was tested at 6.81, 13.6, 27.2, and 54.5 mmoles/kg dose levels and produced significant antidotal effects at the three highest dose levels. 1,3-Propanediol was ineffective at all dose levels tested. The maximum relative antidotal effect produced by propylene glycol was 1.61. Propylene glycol appears to be equivalent, but not superior, to ethanol in both potency and efficacy.

The four-carbon chain alkyldiols tested were 1,2-butanediol, 1,3-butanediol, 1,4-butanediol, and 2,3-butanediol, each at three dose levels (Table 3). 1,2-Butanediol and 1,3-butanediol were effective as antidotes at all dose levels tested, whereas neither 1,4-butanediol nor 2,3-butanediol were effective at any dose level tested. 1,2-Butanediol and 1,3-butanediol produced maximum relative antidotal effects of 1.84 and 1.87, respectively. The efficacy of these two butanediols is about 1.2 times that of either ethanol or propylene glycol, but only about 0.70 times that of

TABLE 3: ANTIDOTAL EFFECTS OF THE ALKYLDIOLS ON ETHYLENE GLYCOL TOXICITY IN MICE TESTED ON A ONE TIME INJECTION SCHEDULE

Drug	Dose (mmoles/kg)	144 hr. LD50 of Ethylene Glycol (95% Confidence Limits)		Relative Antidotal Effect [g]
		Saline Treated (mmoles/kg)	Alkyldiol Treated (mmoles/kg)	
Propylene Glycol	6.8	160 (139-184) [a]	214 (167-275)	1.34
	13.6	160 (139-184) [a]	234 (209-317)	1.47 *
	27.2	160 (139-184) [a]	257 (233-284)	1.61 *
	54.5	160 (139-184) [a]	246 (204-296)	1.54 *
1,3 - Propanediol	6.9	178 (149-213) [b]	224 (185-272)	1.26
	13.8	154 (133-179) [c]	192 (166-222)	1.24
	27.6	178 (149-213) [b]	190 (153-236)	1.06
1,2 - Butanediol	5.6	178 (164-194) [d]	235 (201-275)	1.32 *
	11.2	160 (139-184) [e]	295 (245-354)	1.84 *
	22.3	178 (164-194) [d]	257 (237-279)	1.44 *
1,3 - Butanediol	5.6	178 (164-194) [d]	242 (219-267)	1.36 *
	11.2	160 (139-184) [e]	282 (253-314)	1.76 *
	22.3	160 (139-184) [e]	299 (277-325)	1.87 *
1,4 - Butanediol	5.6	178 (149-213) [b]	< 178	< 1
	11.3	154 (133-179) [c]	< 154	< 1
	22.6	178 (149-213) [b]	< 178	< 1
2,3 - Butanediol	5.5	178 (157-203) [f]	163 (136-196)	0.91
	11.0	178 (157-203) [f]	163 (130-204)	0.91
	22.0	178 (157-203) [f]	149 (124-178)	0.83
1,5 - Pentanediol	4.8	178 (149-213) [b]	207 (178-239)	1.16
	9.5	154 (133-179) [c]	163 (130-204)	1.06
	19.1	178 (149-213) [b]	< 178	< 1
1,6 - Hexanediol	4.2	178 (157-203) [f]	208 (188-230)	1.16
	8.5	178 (157-203) [f]	168 (128-221)	0.94
	16.9	178 (157-203) [f]	< 178	< 1

* $P<0.05$ (Significance of alkyldiol treated as compared with saline treated)

[a,b,c,d,e, and f] Superscripts indicate those LD50 values from the same saline treated mice.

[g] LD50 of alkyldiol treated / LD50 of saline treated

pyrazole.

1,5-Pentanediol and 1,6-hexanediol, having carbon chain lengths greater than four, were tested at three dose levels (Table 3). Neither 1,5-pentanediol nor 1,6-hexanediol was effective at any dose level tested.

Tris (hydroxymethyl) aminomethane - tris (hydroxymethyl) aminomethane HCl was tested on a one-time injection basis at two pH levels, both alone and with 1,3-butanediol (Table 4). Tris buffer was ineffective at all dose levels tested at both pH = 7.8 and pH = 10.8. When 1,3-butanediol was administered concomitantly with tris buffer, significant antidotal effects were produced at both pH = 7.8 and pH = 10.8, but the relative antidotal effects of 1.42 and 1.46, respectively, at these pH levels were markedly less than the maximum relative antidotal effect of 1.87 produced by 1,3-butanediol alone (Table 3).

Acute and Delayed Toxicities

The toxicities of ethanol, pyrazole, the alkyldiols, and tris (hydroxymethyl) aminomethane were determined at the 24 and 144 hour time periods (Table 5). In general, the toxicities of the alkyldiols having two end-chain hydroxyls increased with an increase in carbon chain length and with the number of atoms separating the hydroxyls. The toxicities of the alkyldiols having one end-chain and one mid-chain hydroxyl increased with an increase in carbon chain length, but not with the number of carbon atoms separating

TABLE 4: ANTIDOTAL EFFECTS OF TRIS(HYDROXYMETHYL)AMINOMETHANE ALONE OR CONCOMITANTLY WITH 1,3-BUTANEDIOL ON ETHYLENE GLYCOL TOXICITY IN MICE TESTED ON A ONE TIME INJECTION SCHEDULE

Drug(s)	pH	Dose (mmoles/kg)	144 hr. LD50 of Ethylene Glycol (95% Confidence Limits)		Relative Antidotal Effect [c]
			Saline Treated (mmoles/kg)	Drug Treated (mmoles/kg)	
Tris(hydroxymethyl)-aminomethane	10.8	4.13	160 (139-184)[a]	212 (175-253)	1.33
		8.26	160 (139-184)[a]	196 (163-235)	1.23
		16.5	160 (139-184)[a]	192 (167-220)	1.20
	7.8	4.13	172 (151-196)[b]	202 (183-222)	1.17
		8.26	172 (151-196)[b]	207 (181-236)	1.20
		16.5	172 (151-196)[b]	156 (123-196)	0.91
Tris(hydroxymethyl)-aminomethane + 1,3-Butanediol	10.8 —	4.13 22.3	168 (131-214)	239 (221-259)	1.42 *
Tris(hydroxymethyl)-aminomethane + 1,3-Butanediol	7.8 —	4.13 22.3	168 (131-214)	246 (223-277)	1.46 *

* $P < 0.05$ (Significance of drug treated as compared with saline treated)

a and b Superscripts indicate those LD50 values from the same saline treated mice.

[c] LD50 of drug treated / LD50 of saline treated

TABLE 5: ACUTE AND DELAYED TOXICITIES OF ETHANOL, PYRAZOLE, THE ALKYLDIOLS AND TRIS(HYDROXYMETHYL)AMINOMETHANE IN MICE

Drug	LD50 (95% Confidence Limits)		Relative Delayed Toxicity [a]
	24 hr. - Acute (mmoles/kg)	144 hr. - Delayed (mmoles/kg)	
Ethanol	123 (114-132)	109 (96-132)	0.885
Pyrazole	17.1 (14.9-19.9) [b]	11.3 (10.1-12.6)	0.662 *
Propylene Glycol	178 (161-196)	168 (158-178)	0.941
1,3-Propanediol	68.8 (63.7-74.3)	62.8 (57.0-69.3)	0.913
1,2-Butanediol	66.5 (60.2-73.7)	46.5 (42.4-51.1)	0.700 *
1,3-Butanediol	122 (109-136)	114 (104-126)	0.941
1,4-Butanediol	18.3 (16.0-20.9)	18.3 (16.0-20.9)	1.00
2,3-Butanediol	87.2 (62.1-106)	67.4 (63.3-71.5)	0.773
1,5-Pentanediol	21.6 (20.3-22.9)	21.6 (20.3-22.9)	1.00
1,6-Hexanediol	14.9 (12.8-17.3)	14.7 (13.0-16.7)	0.989
Tris(hydroxymethyl)-aminomethane (pH=10.8)	30.5 (28.8-32.4)	30.5 (28.8-32.4)	1.00

* $P<0.05$ (Significance of delayed toxicity as compared with acute toxicity)

[a] Delayed LD50 / Acute LD50

[b] 48 hr. LD50 in lieu of the 24 hr. LD50

the two hydroxyls. Alkyldiols having two end-chain hydroxyls are in general more toxic than those having one end-chain and one mid-chain or two mid-chain hydroxyls. Pyrazole and 1,2-butanediol are the only compounds which show a significant delayed toxicity.

The comparative ranking of ethanol, pyrazole, tris (hydroxymethyl) aminomethane, and the alkyldiols by their acute toxicities in order of decreasing 24 hour LD_{50} values is: propylene glycol > ethanol ≅ 1,3-butanediol > 2,3-butanediol > 1,3-propanediol > 1,2-butanediol > THAM > 1,5-pentanediol > 1,4-butanediol > pyrazole > 1,6-hexanediol. The comparative ranking of these compounds by their delayed toxicities in order of decreasing 144 hour LD_{50} values is: propylene glycol > 1,3-butanediol ≅ ethanol > 2,3-butanediol > 1,3-propanediol > 1,2-butanediol > THAM > 1,5-pentanediol > 1,4-butanediol > 1,6-hexanediol > pyrazole. Pyrazole produced a delayed toxicity sufficient to shift its place with 1,6-hexanediol between the 24 and 144 hour periods. In spite of the delayed toxicity produced by 1,2-butanediol, its ranking remained the same.

The toxicity of glycolaldehyde, the first metabolite of ethylene glycol, was found at the 24 and 144 hour time periods and by two routes of administration (Table 6). Glycolaldehyde was twice as toxic to mice after ip administration as compared with sc administration and about 30 times more toxic than orally administered ethylene glycol. Glycolaldehyde produced a marked relative delayed toxicity only by ip administration.

Mice injected with glycolaldehyde ip displayed symptoms not

TABLE 6: ACUTE AND DELAYED TOXICITIES OF GLYCOLALDEHYDE, THE FIRST METABOLITE OF ETHYLENE GLYCOL, IN MICE

Drug	Route of Administration	LD50 (95% Confidence Limits)		Relative Delayed Toxicity [a]
		24 hr. - Acute (mmoles / kg)	144 hr. - Delayed (mmoles / kg)	
Glycolaldehyde	Intraperitoneal	6.78 (6.04-7.58)	5.00 (4.46-5.43)	0.737 *
	Subcutaneous	11.6 (10.1-13.2)	10.7 (9.3-12.4)	0.929

* $P < 0.05$ (Significance of delayed toxicity as compared with acute toxicity)
[a] Delayed LD50 / Acute LD50

seen after injections of other drugs used in this study. Within minutes of the injections, the mice assumed a tenesmus-like position with arched backs, distended abdomens, and withdrawn flanks. Feet were drawn close together as if the mice were trying to balance on a small area. Walking was ataxic, but no writhing was apparent. The nose, eyelids, and other mucous membranes became red, the eyes became bloodshot, and the tenesmus-like position was interrupted by rapid nose-scratching with the forepaws. Mice that died early developed a marked antemortem depression. Many of the mice surviving the first 24 hours had ruffled fur and an ungroomed appearance. Several mice had rectal prolapse whereas others developed unilateral or bilateral eyelid closure and could not immediately locate the source of water in the cage. Many mice that survived the first 24 hours became progressively sick and lethargic and deaths occurred during each 24 hour period through the 144 hour period. Autopsies revealed no remarkable changes in the gross appearance of the internal organs.

Mice injected subcutaneously with glycolaldehyde did not display the symptoms seen after ip injection of glycolaldehyde, but developed a brown spot on the fur and skin at the point of injection. Depression was the major acute symptom. Eighty-seven percent of the mice surviving the first 24 hour period sloughed fur and skin around the site of injection to reveal necrotic subcutaneous tissues. Mice that did not die during the period of ulcer development improved markedly after scab formation over the ulcer. Only 2 of the 39 surviving mice died after scab formation and

during the following recuperative period.

Hexobarbital Sleeping Time Studies

In order to determine what effect CNS depression had on either antidotal effect or toxicity, hexobarbital sleeping time experiments were performed (Table 7). The alkyldiols having two end-chain hydroxyls, with the exception of 1,3-propanediol, or two mid-chain hydroxyls produced a marked extension in hexobarbital sleeping time which increased with an increase in carbon chain length and with the number of carbon atoms separating the two hydroxyls. Neither the alkyldiols having one end-chain and one mid-chain hydroxyl, with the exception of propylene glycol, nor ethanol and tris (hydroxymethyl) aminomethane had any effect on hexobarbital sleeping time at the doses tested. Four of the eight mice treated with pyrazole and hexobarbital died during the experiment. In the remaining four mice, the hexobarbital sleeping time was twice that of the sleeping time produced by 1,6-hexanediol.

Therapeutic Ratios

The therapeutic ratios for ethanol, pyrazole, propylene glycol, 1,2-butanediol, and 1,3-butanediol were calculated as a means to judge the possible margin of safety inherent in using each compound (Table 8). Ethanol and pyrazole have low therapeutic ratios when compared with propylene glycol, 1,2-butanediol, and

TABLE 7: EFFECTS OF ETHANOL, PYRAZOLE, THE ALKYLDIOLS, AND TRIS(HYDROXYMETHYL)AMINO - METHANE ON HEXOBARBITAL SLEEPING TIME IN MICE

Drug	Dose (mmoles / kg)	Hexobarbital Sleeping Time (mean ± standard error of the mean) [f]		Extension of Sleeping Time [d] (mins)	Relative Extension of Sleeping Time [e]
		Saline Treated (mins)	Drug Treated (mins)		
Etnanol	17.1	19.3 ± 4.1 [a]	19.0 ± 3.3	- 0.3	0.98
Pyrazole	4.41	29.2 ± 5.5 [b]	263.0 ± 39.6 [g]	233.8	8.99 *
Propylene Glycol	13.6	19.3 ± 4.1 [a]	31.6 ± 7.8	12.4	1.64
1,3- Propanediol	13.8	19.3 ± 4.1 [a]	26.7 ± 3.8	7.5	1.39
1,2 - Butanediol	11.2	22.7 ± 7.2 [c]	22.0 ± 4.7	- 0.7	0.97
1,3 - Butanediol	11.2	22.7 ± 7.2 [c]	18.5 ± 5.6	- 4.2	0.82
1,4 - Butanediol	11.3	22.7 ± 7.2 [c]	100.8 ± 4.5	78.1	4.44 *
2,3 - Butanediol	11.0	29.2 ± 5.5 [b]	61.7 ± 9.9	32.5	2.11 *
1,5 - Pentanediol	9.5	29.2 ± 5.5 [b]	129.9 ± 15.8	100.7	4.44 *
1,6 - Hexanediol	8.5	29.2 ± 5.5 [b]	130.6 ± 15.8	101.4	4.47 *
Tris(hydroxymethyl) - aminomethane (pH = 10.8)	8.3	29.2 ± 5.5 [b]	30.2 ± 6.6	1.0	1.03

* $P < 0.05$ (Significance of drug treated as compared with saline treated)

[a,b, and c] Superscripts indicate those sleeping time values from the same saline treated mice.

[d] Drug treated sleeping time – Saline treated sleeping time

[e] Drug treated sleeping time / Saline treated sleeping time

[f] Dose of hexobarbital (Evipal) = 75 mg / kg

[g] 4 out of 8 mice died. Mean is the average sleeping time of the 4 remaining mice.

TABLE 8: THERAPEUTIC RATIOS AND RELATIVE SAFETY OF THE KNOWN AND NEW ALKYLDIOL ANTIDOTES AT THE DOSE WHICH PRODUCED THE MAXIMUM RELATIVE ANTIDOTAL EFFECT IN MICE

Antidote	Dose (mmoles/kg)	Maximum Relative Antidotal Effect	144 hr. LD50 (mmoles/kg) [d]	Incidence of Significant Delayed Toxicity [d]	Therapeutic Ratio [a]
Ethanol	34.3 [b]	1.49 [b]	109	0	3.17
Pyrazole	4.41 [b]	2.65 [b]	11.3	+	2.56
Propylene Glycol	27.2 [c]	1.61 [c]	168	0	6.16
1,2 - Butanediol	11.2 [c]	1.84 [c]	46.5	+	4.15
1,3 - Butanediol	22.3 [c]	1.87 [c]	114	0	5.11

[a] 144 hr. LD50 / Dose of antidote which produced the maximum relative antidotal effect

[b] Data from Table 2

[c] Data from Table 3

[d] Data from Table 5

1,3-butanediol. The comparative ranking of these antidotes in order of decreasing therapeutic ratio is: propylene glycol > 1,3-butanediol > 1,2-butanediol > ethanol > pyrazole.

Schiff Base Formation

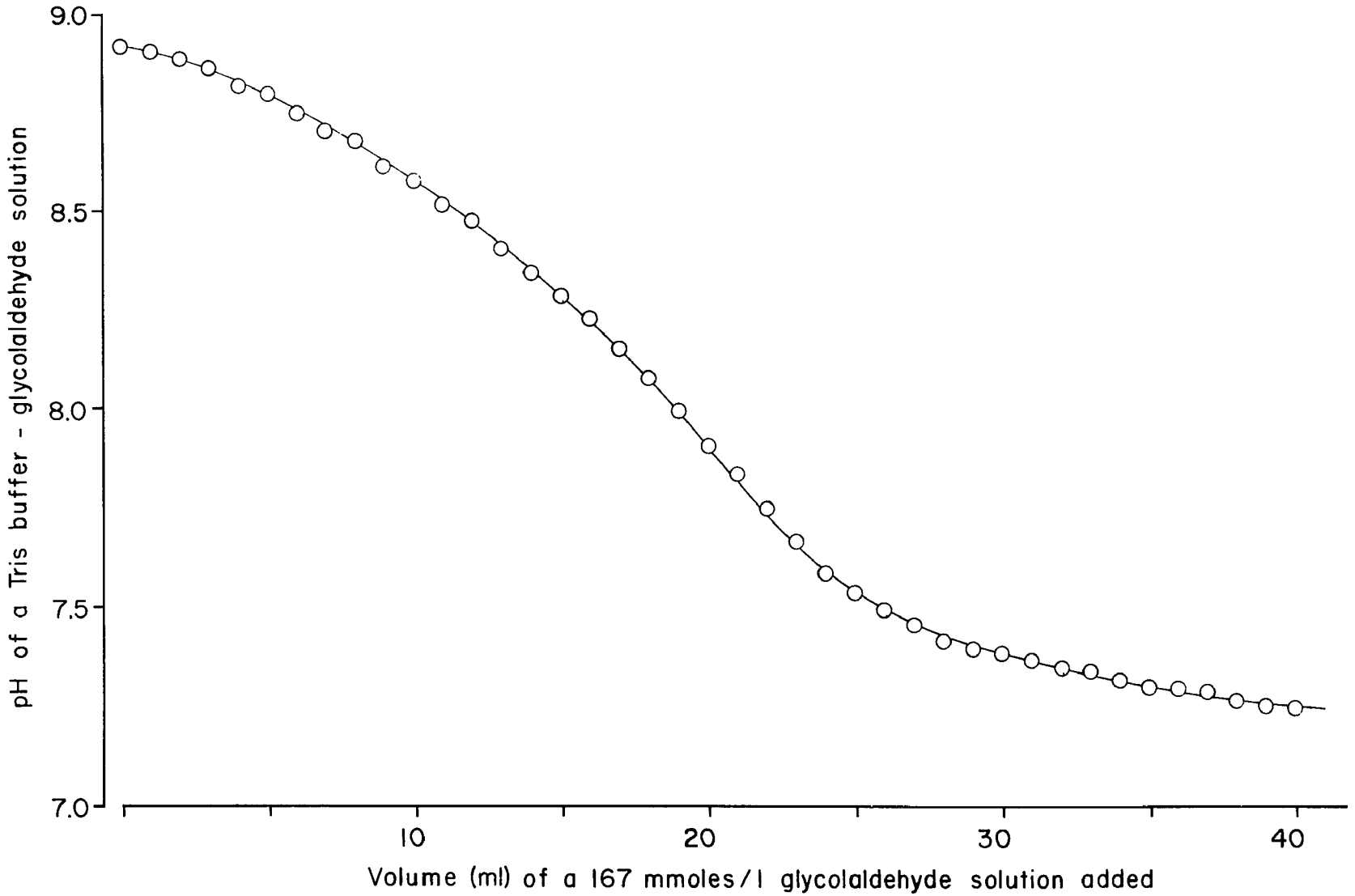
The pH of 5 ml of the glycolaldehyde solution used in the tris titration was measured to be 4.35. However, one drop (approximately 20 µl) of 0.1 N NaOH raised the pH to 9.91. Therefore, the glycolaldehyde solution was deemed to be free of any titratable acid.

The results of titrating a 20 ml solution of 99.1 mmoles/ml of tris (hydroxymethyl) aminomethane - tris (hydroxymethyl) aminomethane HCl with a 167 mmoles/ml solution of glycolaldehyde is shown in Figure 7. The addition of glycolaldehyde to the tris produced a lowering of the measured pH value. A plot of the data resulted in a sigmoid-shaped curve.

Calculations were performed to determine the steady-state equilibrium constant for the reaction using the Henderson-Hasselbach equation and the law of mass action. A mean of the overall equilibrium constant, plus or minus the standard error of the mean, was found to be $4.93 \pm 0.33\ M^{-1}$.

At a pH of 7.60, 95% of the original amount of tris base had been consumed. At pH levels less than 7.60, the ability of glycolaldehyde to lower the pH of the reaction mixture decreased markedly.

Figure 7: Titration of tris (hydroxymethyl) aminomethane with glycolaldehyde. A 99.1 mmole/l solution of tris buffer (pH at 25° C = 8.92) was titrated with a 167 mmole/l solution of glycolaldehyde. Increments of 1.0 ml of glycolaldehyde were added to an initial 20 ml volume of tris buffer solution.


9.0
8.5
8.0
7.5
7.0
pH of a Tris buffer - glycolaldehyde solution
10
20
30
40
Volume (ml) of a 167 mmoles/l glycolaldehyde solution added

Water Intake and Urine Output Studies

The results of the water intake and urine output studies on mice treated with ethylene glycol and/or an antidote are illustrated in Figures 8, 9, and 10. Three sets of six metabolism cages were used for each of the antidotes pyrazole, propylene glycol, and 1,3-butanediol. The data collected from each of the three sets were plotted on a separate graph. The top graph in each figure represents the data collected from those sets of mice which received ethylene glycol and saline, whereas the middle and lower graphs in each figure represent the data collected from those sets of mice which received ethylene glycol and an antidote or water and an antidote, respectively. On the average, four untreated mice consume 8 to 10 ml of water each 12 hours and excrete 3 to 4 ml of water during the same period.

Figure 8 shows the effects of ethylene glycol and pyrazole on water intake and urine output in mice. Both urine output and water consumption markedly increased in those mice that received ethylene glycol and saline, but only during the first 12 hour period post-treatment. Urine output, but not water intake, markedly increased during the first 12 hour period posttreatment in those mice that received ethylene glycol and pyrazole. Neither water consumption nor urine output increased in those mice that received water and pyrazole. Water consumption and urine output by all sets of mice during the three subsequent 12 hour collection periods were not different from the pretreatment levels.

Figure 8: Water intake and urine output in mice after administration of ethylene glycol and pyrazole. Groups of 4 mice were housed in rodent metabolism cages. Water intake and urine output by weight were measured once every 12 hours. At the start of the third 12 hour period, mice were given either water or 108 mmoles/kg ethylene glycol, orally, and either saline or 4.41 mmoles/kg pyrazole, intraperitoneally. Each bar represents the mean of intake or output for 6 cages of 4 mice each ± S.E.M.

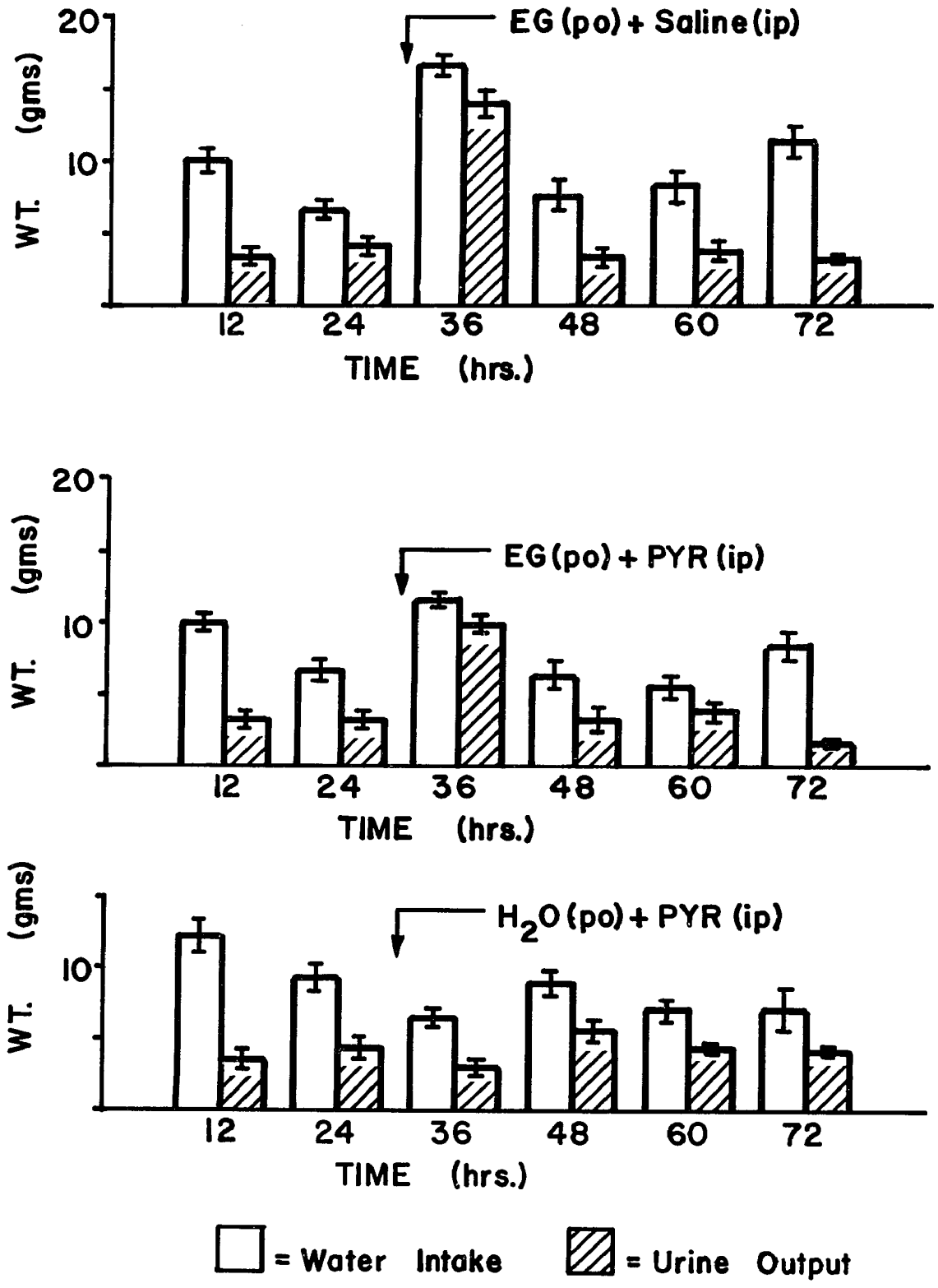

EG (po) + Saline (ip)
EG (po) + PYR (ip)
H2O (po) + PYR (ip)
WT. (gms)
20
10
12
24
36
48
60
72
TIME (hrs.)
= Water Intake
= Urine Output

Figure 9: Water intake and urine output in mice after administration of ethylene glycol and propylene glycol. Groups of 4 mice were housed in rodent metabolism cages. Water intake and urine output by weight were measured once every 12 hours. At the start of the third 12 hour period, mice were given either water or 108 mmoles/kg ethylene glycol, orally, and either saline or 27.2 mmoles/kg propylene glycol, intraperitoneally. Each bar represents the mean of intake or output for 6 cages of 4 mice each ± S.E.M.

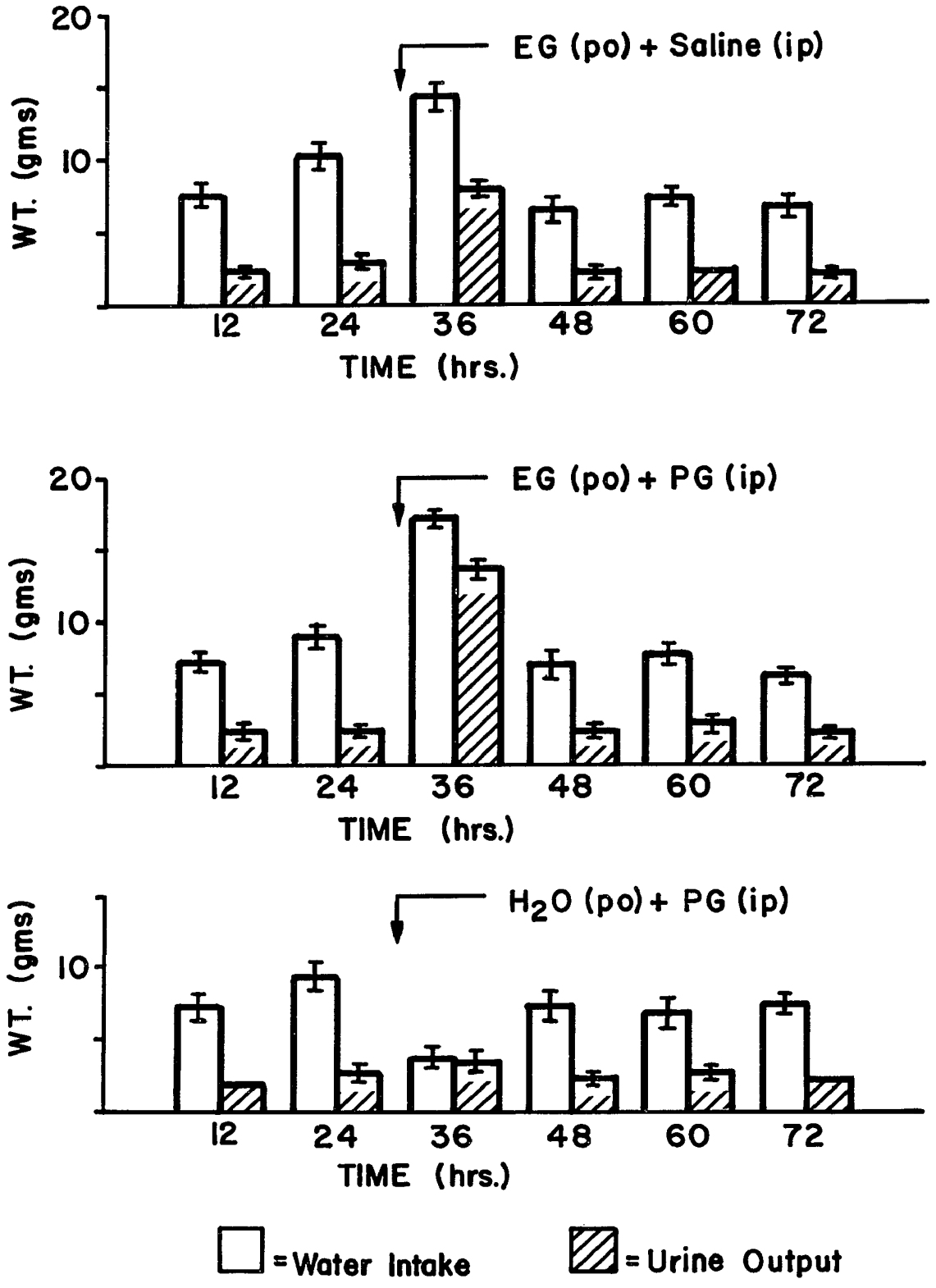

20
10
WT. (gms)
EG (po) + Saline (ip)
12
24
36
48
60
72
TIME (hrs.)
20
10
WT. (gms)
EG (po) + PG (ip)
12
24
36
48
60
72
TIME (hrs.)
10
WT. (gms)
H2O (po) + PG (ip)
12
24
36
48
60
72
TIME (hrs.)
= Water Intake
= Urine Output

Figure 10: Water intake and urine output in mice after administration of ethylene glycol and 1,3-butanediol. Groups of 4 mice were housed in rodent metabolism cages. Water intake and uring output by weight were measured once every 12 hours. At the start of the third 12 hour period, mice were given either water or 108 mmoles/kg ethylene glycol, orally, and either saline or 22.3 mmoles/kg 1,3-butanediol, intraperitoneally. Each bar represents the mean of intake or output for 6 cages of 4 mice each ± S.E.M.

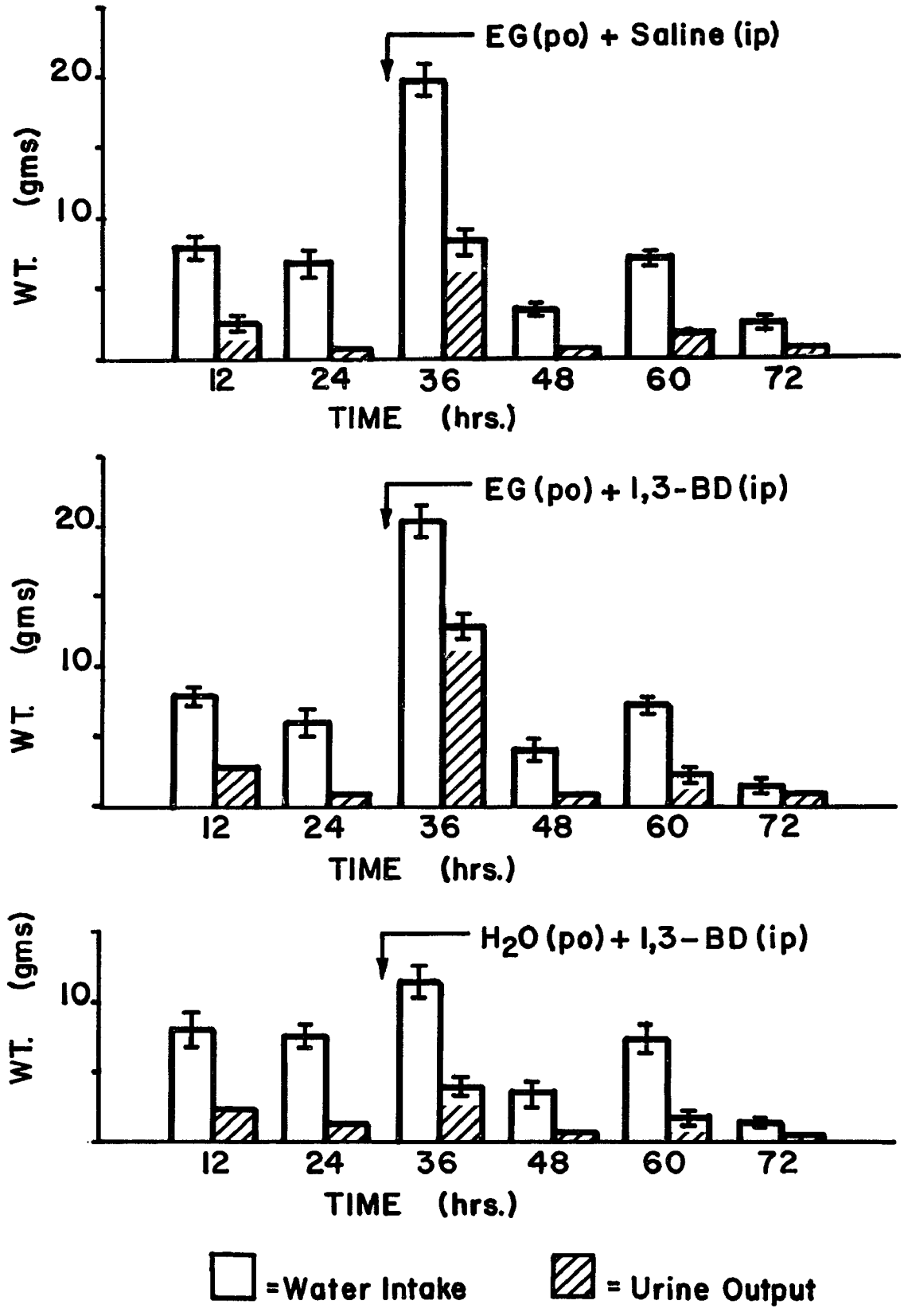

EG(po) + Saline (ip)
20
10
WT. (gms)
12
24
36
48
60
72
TIME (hrs.)
EG (po) + 1,3-BD (ip)
20
10
WT. (gms)
12
24
36
48
60
72
TIME (hrs.)
H2O (po) + 1,3-BD (ip)
10
WT. (gms)
12
24
36
48
60
72
TIME (hrs.)
= Water Intake
= Urine Output

Figure 9 shows the effects of ethylene glycol and propylene glycol on water intake and urine output in mice. Both urine output and water consumption markedly increased in those mice that received ethylene glycol, whether or not propylene glycol was given concomitantly, but only during the first 12 hour period posttreatment. Urine output during the first 12 hour period posttreatment remained the same in those mice that received water and propylene glycol, but water intake during the same period decreased. Water consumption and urine output by all sets of mice during the three subsequent 12 hour collection periods were not different from the pretreatment levels.

Figure 10 shows the effects of ethylene glycol and 1,3-butanediol on water intake and urine output in mice. Both urine output and water consumption markedly increased in those mice that received ethylene glycol, whether or not 1,3-butanediol was given concomitantly, but only during the first 12 hour period posttreatment. Those mice that received water and 1,3-butanediol increased water consumption, but not urine output during the first 12 period posttreatment. Water consumption and urine output by all sets of mice during the three subsequent 12 hour collection periods returned to pretreatment levels. Unlike propylene glycol, mice treated with water and 1,3-butanediol increased water consumption and urine output during the first 12 hour period posttreatment.

Urinary Excretion Studies

The results of urinary excretion studies on mice treated with ethylene glycol and/or an antidote are illustrated in Figures 11, 12, 13, and 14. Figure 11 shows the results of injecting a 2 µl aliquot of ethylene glycol, propylene glycol, and 2,3-butanediol or ethylene glycol, 2,3-butanediol, and 1,3-butanediol. The criterion chosen for quantitative determination was peak height since the peaks were well separated and sharp.

A similar recording to that illustrated in Figure 11 was obtained when a mixture of the compounds in mouse urine was injected. There were no normal constituents of mouse urine that interfered with the qualitative and quantitative recording of these peaks.

Calibration curves were obtained by injecting serial dilutions of the alkyldiols in water and urine. Although these curves are not shown, recovery of the glycols from urine was not significantly different from results in water.

Figure 12 shows the percentage of the original dose of ethylene glycol and the concentration of ethylene glycol found in the urine of saline and pyrazole treated mice. Ethylene glycol was found only in those urine samples collected during the 36 hour period. The amount of ethylene glycol excreted by the pyrazole treated mice was significantly greater than that excreted by the saline treated mice. The urinary concentration of ethylene glycol was almost twice that in the pyrazole treated mice when compared

Figure 11: Facsimiles of gas chromatograms showing the *in vitro* separation of ethylene glycol and propylene glycol from water or plasma or ethylene glycol and 1,3-butanediol from water or plasma with 2,3-butanediol as the internal standard in both cases.

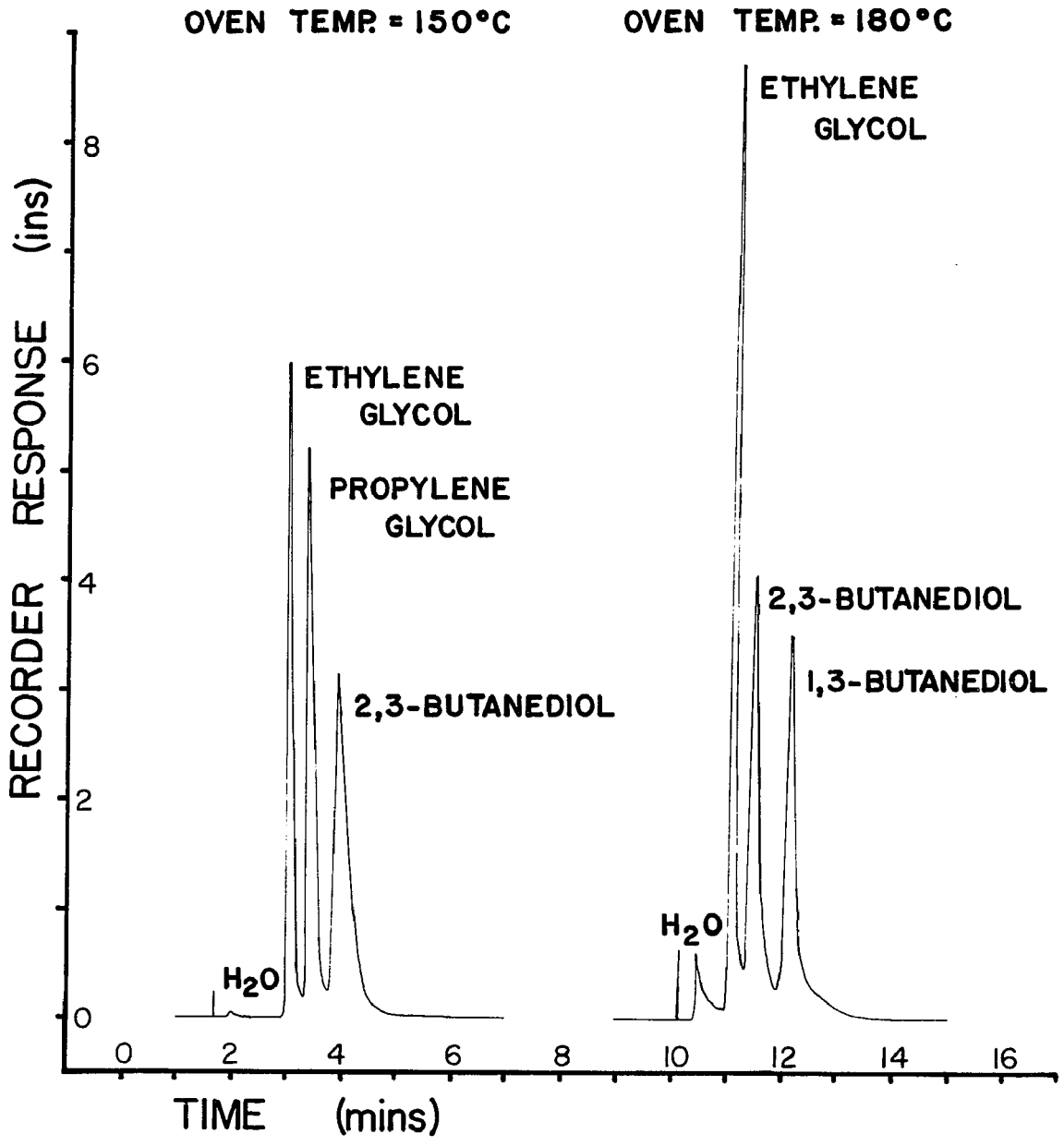

OVEN TEMP. = 150°C
OVEN TEMP. = 180°C
RECORDER RESPONSE (ins)
0
2
4
6
8
ETHYLENE GLYCOL
PROPYLENE GLYCOL
2,3-BUTANEDIOL
H2O
ETHYLENE GLYCOL
2,3-BUTANEDIOL
1,3-BUTANEDIOL
H2O
0
2
4
6
8
10
12
14
16
TIME (mins)

Figure 12: Urinary excretion and urine concentrations of ethylene glycol after saline or pyrazole treatments in mice. Twelve hour samples of urine from the water intake and urine output studies were analyzed for ethylene glycol. The data illustrated are from the first 12 hour period after ethylene glycol administration and are expressed as both percent of the original dose of ethylene glycol recovered in the urine and the concentrations of ethylene glycol in the samples. Mice were given 108 mmoles/kg ethylene glycol, po, and either saline or 4.41 mmoles/kg pyrazole, ip. Each bar represents the mean recovery or concentrations for 6 cages of 4 mice each ± S.E.M.

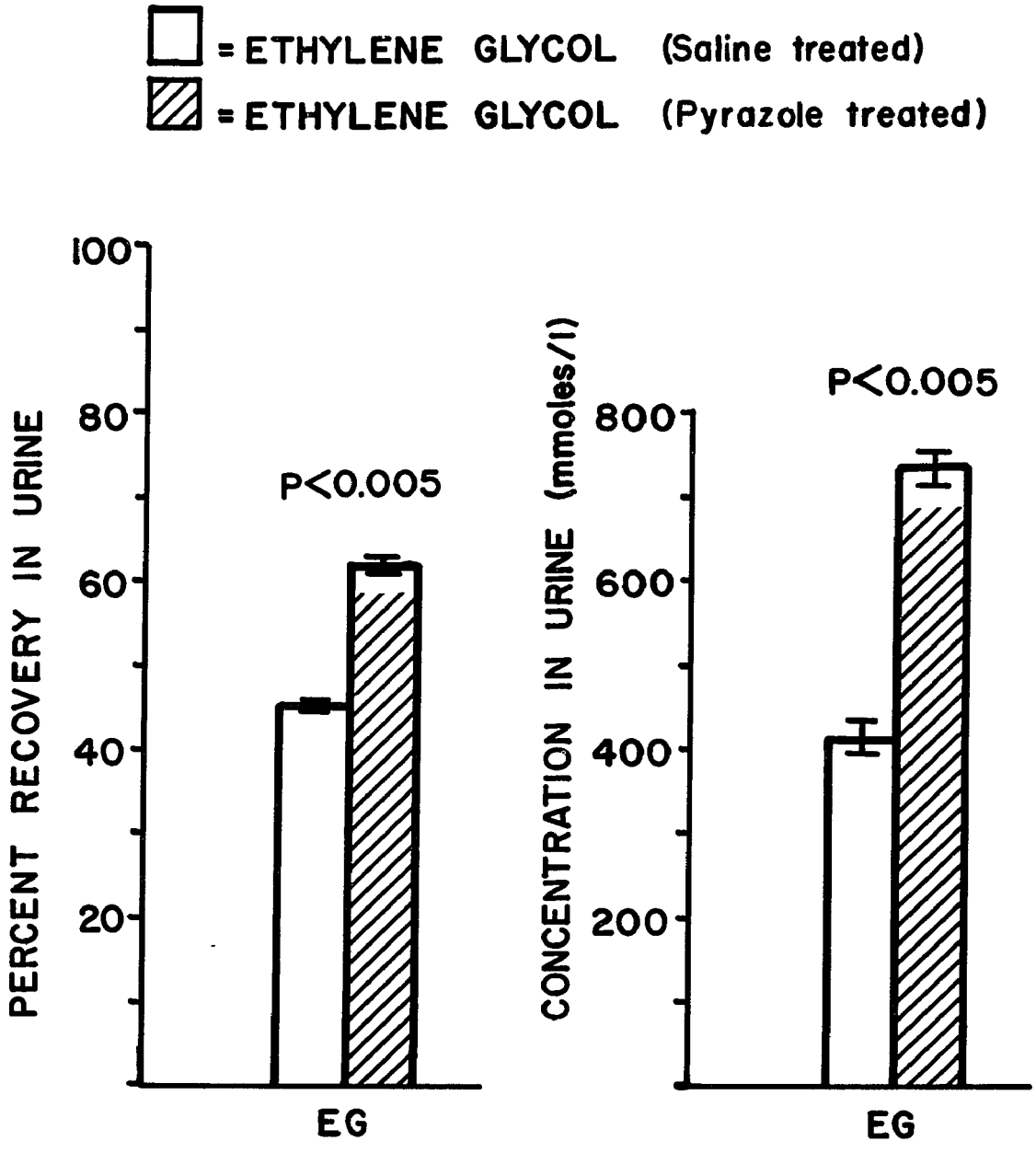

= ETHYLENE GLYCOL (Saline treated)
= ETHYLENE GLYCOL (Pyrazole treated)
PERCENT RECOVERY IN URINE
100
80
60
40
20
P<0.005
EG
CONCENTRATION IN URINE (mmoles/l)
800
600
400
200
P<0.005
EG

Figure 13: Urinary excretion and urine concentrations of ethylene glycol after saline or propylene glycol treatments and of propylene glycol after water or ethylene glycol treatments in mice. Twelve hour urine samples from the water intake or urine output studies were analyzed for ethylene glycol or propylene glycol. The data illustrated are from the first 12 hour period after alkyldiol administration and are expressed as both percent of the original dose of alkyldiol recovered in the urine and the concentrations of alkyldiol in the urine. Mice were given either water or 108 mmoles/kg ethylene glycol, po, and either saline or 27.2 mmoles/kg propylene glycol, ip. Each bar represents the mean recovery or concentrations for 6 cages of 4 mice each ± S.E.M.

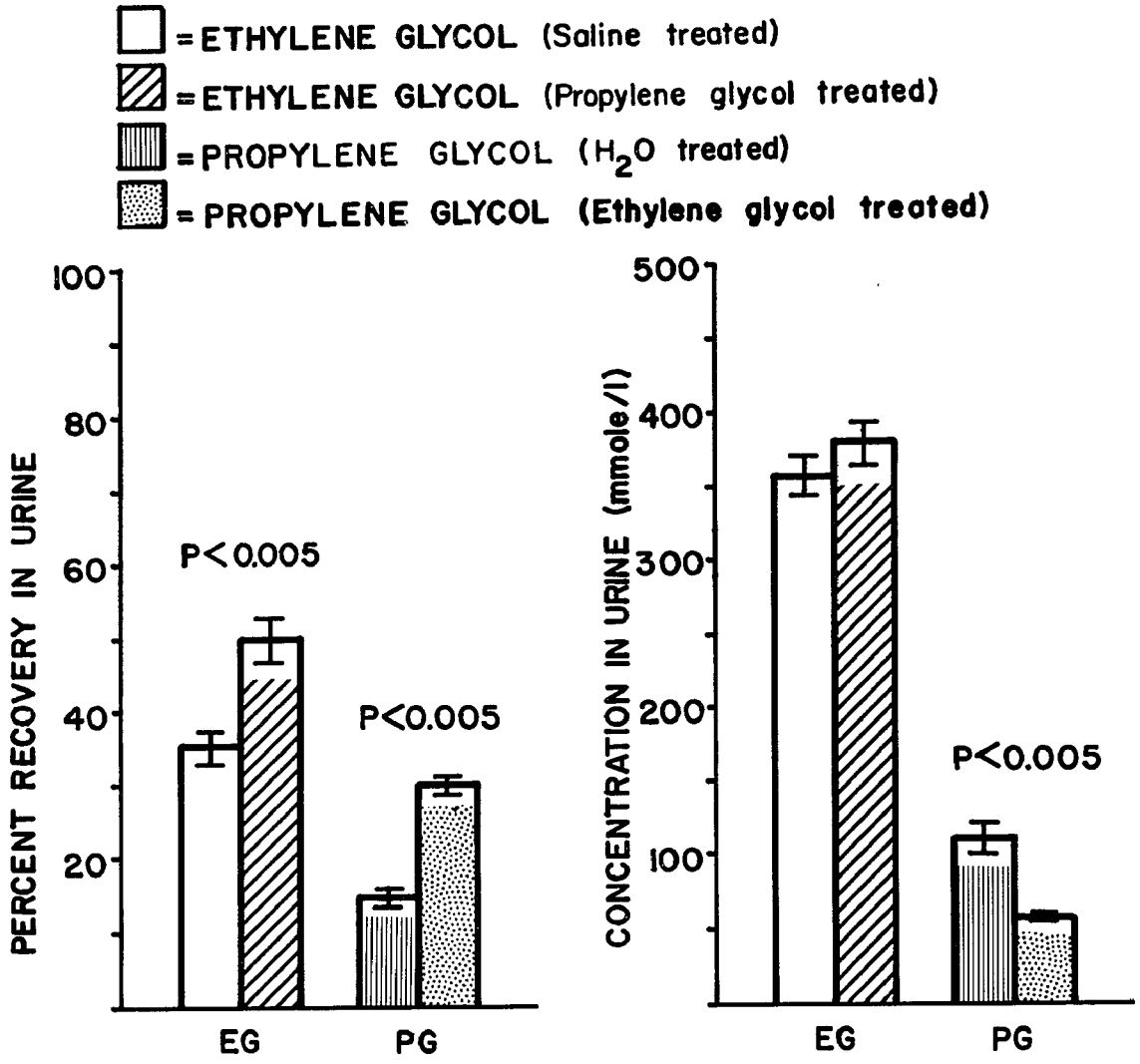
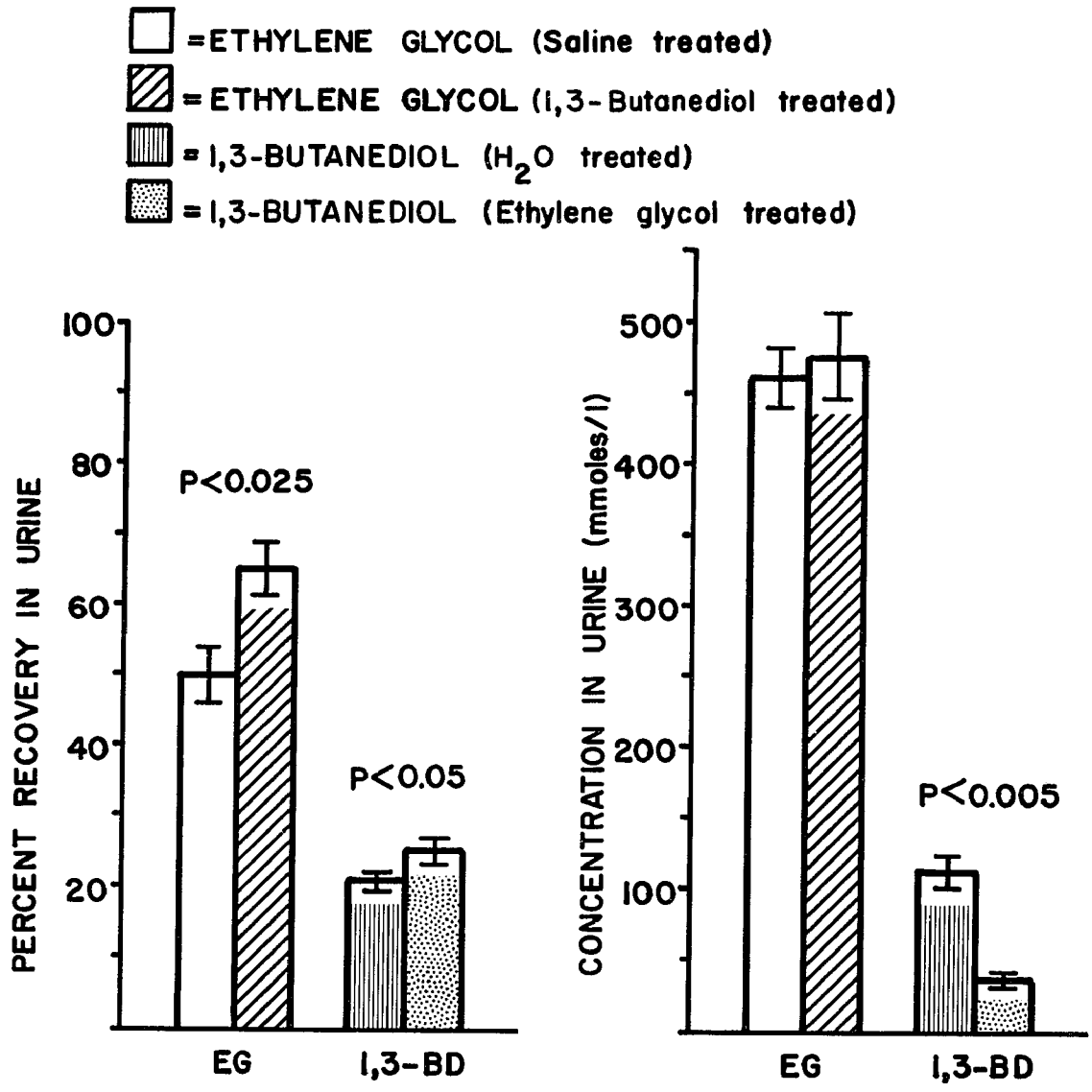

= ETHYLENE GLYCOL (Saline treated)
= ETHYLENE GLYCOL (Propylene glycol treated)
= PROPYLENE GLYCOL (H_2O treated)
= PROPYLENE GLYCOL (Ethylene glycol treated)
PERCENT RECOVERY IN URINE
100
80
60
40
20
P<0.005
P<0.005
EG
PG
CONCENTRATION IN URINE (mmole/l)
500
400
300
200
100
P<0.005
EG
PG

Figure 14: Urinary excretion and urine concentrations of ethylene glycol after saline or 1,3-butanediol treatments and of 1,3-butanediol after water or ethylene glycol treatments in mice. Twelve hour urine samples from the water intake or urine output studies were analyzed for ethylene glycol or 1,3-butanediol. The data illustrated are from the first 12 hour period after alkyldiol treatment and are expressed as both percent of the original dose of alkyldiol recovered in the urine and the concentrations of alkyldiol in the urine. Mice were given either water or 108 mmoles/kg ethylene glycol, po, and either saline or 22.3 mmoles/kg 1,3-butanediol, ip. Each bar represents the mean recovery or concentrations for 6 cages of 4 mice each ± S.E.M.


=ETHYLENE GLYCOL (Saline treated)
=ETHYLENE GLYCOL (1,3-Butanediol treated)
= 1,3-BUTANEDIOL (H_2O treated)
= 1,3-BUTANEDIOL (Ethylene glycol treated)
PERCENT RECOVERY IN URINE
100
80
60
40
20
P<0.025
P<0.05
EG
1,3-BD
CONCENTRATION IN URINE (mmoles/l)
500
400
300
200
100
P<0.005
EG
1,3-BD

with the untreated mice.

Figure 13 shows the percentages of the original doses of ethylene glycol and propylene glycol found in the urine of various sets of treated mice. Ethylene glycol and propylene glycol were found only in those urine samples collected during the first twelve hours after treatment. Ethylene glycol excretion was significantly increased by administration of propylene glycol, but, unlike pyrazole, the concentrations of the ethylene glycol in urine of saline and propylene glycol treated mice were not significantly different. Conversely, propylene glycol excretion was doubled by administration of ethylene glycol. The urine concentration of propylene glycol was significantly less in the group treated with ethylene glycol when compared with the water treated group, in spite of the fact that the total amount of propylene glycol in this latter sample was greater.

Figure 14 shows the percentages of the original dose of ethylene glycol and 1,3-butanediol found in the urine of various sets of treated mice. Ethylene glycol and 1,3-butanediol was found only in those urine samples collected during the first twelve hours after treatment. 1,3-Butanediol significantly increased the excretion of ethylene glycol, and, as with propylene glycol, the concentrations of ethylene glycol in the urine of saline and 1,3-butanediol treated mice were not significantly different. Ethylene glycol significantly increased the excretion of 1,3-butanediol. As with propylene glycol, the urine concentration of 1,3-butanediol was significantly less in the set of mice treated with ethylene glycol when

compared with the water treated group, in spite of the fact that the total amount of 1,3-butanediol in this latter sample was greater.

DISCUSSION

Harger and Forney (1959), Russell, McChesney, and Golberg (1969), and Peterson and Rodgerson (1974) have reported methods for the qualitative and quantitative analysis of ethylene glycol in biological materials. However, these methods were not used for the following reasons. The colorimetric method of Harger and Forney (1959) has been shown to be nonspecific since both normal body constituents and various metabolites of ethylene glycol interfere with an accurate determination of ethylene glycol (Russell, McChesney, and Golberg, 1969). The colorimetric method of Russell, McChesney, and Golberg (1969) and the gas chromatographic method of Peterson and Rodgerson (1974) are highly specific for ethylene glycol and more sensitive than the method of Harger and Forney. These two latter methods both involve complicated, time-consuming extraction procedures that, as admitted by the authors, increase the variability of and, correspondingly, decrease the precision of the results. In addition, the extraction procedures are not easily adaptable for a large number of samples. Therefore, the method described herein was developed and is most similar in technique to a gas chromatographic method developed by Brown et al. (1968) for the detection of ethanol and propylene glycol in whole mouse homogenate.

The proposed method has several distinct advantages over the

previous methods. It was found to be highly specific for not only ethylene glycol, but numerous other alkyldiols and glycolaldehyde, the first metabolite of ethylene glycol. Neither detectable interferences from other compounds, including normal body constituents nor false positive blanks from untreated biological materials were observed. The linearity of detector response and simplicity of analytical procedures gave highly reproducible results. With the exception of glycolaldehyde, samples were stable at room temperature for same-day analysis or, if frozen, for up to six weeks. The time required for a single analysis is usually less than 30 minutes.

The major disadvantage of this new method is its lack of sensitivity. Unlike the method of Peterson and Rodgerson (1974), an amplification of the detector signal is not achieved by formation of a derivatized ethylene glycol of larger molecular weight. Yet, the sensitivity was sufficient for the purposes of this research and equal to that of the method of Brown et al. (1968).

An attempt was made during the development of the method to settle the old question of whether or not glyoxal, as well as glycolaldehyde, is a metabolite of ethylene glycol. Although glycolaldehyde could be eluted from the gas chromatograph, glyoxal could not be detected in either water or plasma dilutions under any gas chromatographic conditions tried.

Mundy, Hall, and Teague (1974) found pyrazole produced an antidotal effect in ethylene glycol toxicity comparable to the antidotal effect produced by ethanol as reported by Peterson et al.

(1963). If pyrazole and ethanol inhibit the metabolism of ethylene glycol by the liver, then pyrazole would be expected to modify the plasma decay kinetics of ethylene glycol *in vivo* just as ethanol and propylene glycol acted as competitive inhibitors of each other in mice as found by Brown *et al.* (1968). In order to test this hypothesis, the plasma analyses of ethylene glycol were performed.

The plasma pharmacokinetics of ethylene glycol were studied in mice treated with either saline or pyrazole. The present findings indicate that the disappearance of ethylene glycol in the plasma of saline-treated mice was an exponential decay. However, since the slopes of the decays at two dose levels (Figures 5 and 6) are not similar, the single compartment model of analysis is not applicable. These results in mice are similar though to the findings of McChesney *et al.* (1971) in monkeys that the disappearance of ethylene glycol from plasma obeys first order kinetics. Mice apparently eliminate ethylene glycol at a rate faster than monkeys, since the halflives of a 71.4 mmole/kg dose in mice and 17.9 mmole/kg dose in monkeys (McChesney *et al.*, 1971) are 1.8 and 2.7 hours, respectively. The plasma levels of ethylene glycol in both cases were practically undetectable at 24 hours after treatment. These data suggest that if the treatment of ethylene glycol poisoning with a liver alcohol dehydrogenase inhibitor is to be successful, the inhibitor must be administered within the first several hours after the ethylene glycol was consumed. This seems to be the case in that Van Stee *et al.* (1975) and Chou and Richardson (1978) found that pyrazole was effective in ethylene glycol toxicity only during

a short time span of no more than about 6 hours. Lastly, the volume of distribution of ethylene glycol in mice was about 55% of total body weight. Extrapolation of the data of McChesney et al. (1971) and appropriate calculations yielded volumes of distribution of ethylene glycol in monkeys that were between 50 and 60 percent of total body weight.

The present findings indicate that the treatment of mice with pyrazole prolongs the existence of ethylene glycol in the plasma. Although this effect of pyrazole was demonstrated by two routes of administration, the resulting disappearance of ethylene glycol could not totally be described by an exponential function, as seen in the saline-treated mice. Even though this anomaly of altered decay could not be explained by the data collected, the fact that pyrazole prolongs the existence of ethylene glycol in the plasma lends indirect evidence to the theory that pyrazole does inhibit alcohol dehydrogenase in vivo and the antidotal effect of pyrazole is due to the decreased formation of more toxic metabolites. In the final analysis, the increased plasma levels of ethylene glycol after pyrazole administration do not account for the antidotal effect of pyrazole if CNS depression is considered the major cause of acute mortality. This indicates that the acute, as well as the delayed, mortality of ethylene glycol poisoning is due to the early formation of metabolic intermediates as suggested by Chou and Richardson (1978).

Since the metabolic intermediate hypothesis seems best to

explain the acute and delayed toxicity of ethylene glycol poisoning, the need for an alcohol dehydrogenase inhibitor to block formation of the toxic metabolites of ethylene glycol is reaffirmed. Yet, the CNS depression and acidosis produced by ethanol (Peterson et al., 1963; Wacker et al., 1965; Borden and Bidwell, 1968) or the delayed hepatotoxic and nephrotoxic lesions produced by pyrazole (Wilson and Bottiglieri, 1962; Lelbach, 1969; Lieber et al., 1970) seriously limit the use of either ethanol or pyrazole as ideal antidotes. Since previous researchers had reported that some members of the series of alkyldiols of which ethylene glycol is a member had relatively high affinities for liver alcohol dehydrogenase and relatively low toxicities, the possibility of finding an antidote of ethylene glycol toxicity within the alkyldiol series more suitable than either ethanol or pyrazole seemed likely.

The substrate specificity and kinetic properties of mouse liver alcohol dehydrogenase were investigated to establish in the mouse model observations previously reported for other species. The present findings that mouse liver alcohol dehydrogenase will metabolize alkyldiols confirmed the findings of Peterson et al. (1963), von Wartburg, Bethune, and Vallee (1964), Blair and Vallee (1966), Coen and Weiss (1966), Weiss and Coen (1966), Tate, Mehlman, and Tobin (1971), and Pietruszko (1975b) in various species. The present study included the alkyldiols 1,2-butanediol and 2,3-butanediol. These two alkyldiols have not been studied as substrates for liver alcohol dehydrogenase. The structure-activity

relationships of the alkyldiols with mouse liver alcohol dehydrogenase confirmed the relationships found by Pietruszko (1975b) with horse and human liver alcohol dehydrogenase.

The Km values obtained in the enzyme kinetic studies were used to assess the relative affinity of the alkyldiols for mouse liver alcohol dehydrogenase. Since the alkyldiols, with the exception of 2,3-butanediol, were found to have Km values lower than that of ethylene glycol, one might predict that these alkyldiols would have an antidotal effect like that of ethanol in ethylene glycol toxicity. In addition, should an antidotal effect manifest itself, one would want to know whether or not the alkyldiol that produced the antidotal effect had any advantages over ethanol or pyrazole. Therefore, in addition to performing experiments to test the hypothesis that alkyldiol antidotes for ethylene glycol toxicity might be found, ethanol and pyrazole were tested again to establish these known antidotes as references.

Peterson et al. (1963) and Mundy, Hall, and Teague (1974) doubled the LD_{50} of ethylene glycol in different species with ethanol and pyrazole, respectively. The major difference in these two reports, other than the drugs and species used, was the schedule of drug administration. Mundy, Hall, and Teague (1974) gave pyrazole on a one-time injection basis, whereas Peterson et al. (1963) gave ethanol on a multiple injection schedule that included an initial loading dose and smaller maintenance doses at times intervals, thereafter. The present findings indicate that both ethanol and pyrazole produce antidotal effects in ethylene glycol

toxicity when both drugs are given on the same one-time injection schedule. However, when this schedule is used, the present findings confirm only the findings of Mundy, Hall, and Teague (1974) with pyrazole. The failure of ethanol to double the LD_{50} of ethylene glycol in mice is probably a contribution of several pharmacokinetic factors. Apparently, the 17.1 mmoles/kg dose of ethanol did not protect the mice because this level was probably insufficient to inhibit ethylene glycol metabolism, whereas the 68.5 mmoles/kg dose of ethanol may have inhibited ethylene glycol metabolism, but did not protect the mice because this level was close to the LD_{50} of ethanol and produced death by CNS depression, or some other mechanism, in an additive manner with ethylene glycol. However, the intermediate 34.3 mmoles/kg dose of ethanol protected the mice because this dose was probably sufficient to inhibit the enzyme without producing death by its own toxicity.

Propylene glycol, 1,2-butanediol, and 1,3-butanediol were the only alkyldiols found to have an antidotal effect in ethylene glycol poisoning. Based on structural formulas, all alkyldiol antidotes have one end-chain and one mid-chain hydroxyl, a fact which precisely defines a structure-activity relationship. No antidotal effect was expected from 2,3-butanediol because its Km value was greater than that of ethylene glycol, but it was surprising to find no antidotal effects produced by 1,4-butanediol, 1,5-pentanediol, and 1,6-hexanediol, substrates of mouse liver alcohol dehydrogenase whose Km values were closest to or less than the Km value of ethanol. The Km values of the three alkyldiols

found to be antidotes were surprisingly greater than that of ethanol and, if ranked in order of decreasing value, directly correlated with the quantitative ability of the alkyldiols to produce an antidotal effect. Obviously, the Km value of an alkyldiol was important, but was not the only determining factor in whether or not an antidotal effect was manifested.

Additional experiments were then performed to elucidate the mechanisms of action of the alkyldiols, other than enzymatic inhibition, that might explain the antidotal effect. The LD_{50} values determined in the toxicity studies were within the limits of LD_{50} values reported for ethanol by Williams (1959), for pyrazole by Lelbach (1969), and Goldstein and Pal (1971), for some of the alkyldiols by Laug et al. (1939), Latven and Molitor (1939), Smyth, Seaton, and Fischer (1941), and Williams (1959), for tris (hydroxymethyl) aminomethane by Darby and Anderson (1966) and for glycolaldehyde by Laborit et al. (1971). In addition, the toxicity experiments confirmed the findings of Lelbach (1969) that pyrazole produces a marked delayed toxicity. On the other hand, the hypnotic effects of alkyldiols have not been studied. The acute and delayed toxicity and hexobarbital sleeping time experiments indicated that the alkyldiols that protected the mice were, in general, the least toxic and least hypnotic of the alkyldiols. Those alkyldiols having relatively low Km values, but producing no antidotal effects, were, in general, most toxic and hypnotic of the alkyldiols. With the exception of 1,2-butanediol, the hypnotic action of the alkyldiols correlated directly with their toxicity and inversely

with their ability to produce an antidotal effect. 1,2-Butanediol manifested an unexplicable delayed toxicity not observed with any other alkyldiol.

The inclusion of glycolaldehyde in the acute and delayed toxicity experiments provided a compound with delayed toxicity to check the validity of the experimental system for detecting a marked delayed toxicity. Laborit et al. (1971) had noted that glycolaldehyde produced a delayed toxic effect. The present findings not only increase the validity of the method described herein for qualitatively detecting a delayed toxic effect and confirm the observation of Laborit et al. (1971), but quantitatively define such a delayed toxicity. However, the present findings indicate that the route of administration determines the manifestation of and symptomatic expression of a delayed toxic effect. In addition, it was found that glycolaldehyde had a necrotic effect which has not been reported in the previous literature (Bove, 1966; Laborit et al., 1971; Lamothe, Thuret, and Laborit, 1971).

Although not directly related to the discussion at hand, it is nevertheless important to digress momentarily and note the serendipitous finding in the hexobarbital sleeping time experiments that pyrazole potentiates the hypnotic effects of hexobarbital. Lieber et al. (1970) studied the acute and chronic effects of pyrazole on activity of hepatic microsomal phenobarbital hydroxylase and found that acute administration of pyrazole had no effect on enzyme activity, whereas chronic administration of pyrazole decreased the enzyme activity over 50 percent. The present findings that the

acute administration of pyrazole does potentiate hexobarbital sleeping time suggest that pyrazole may have an acute as well as chronic effect on barbiturate metabolism.

The organic buffer and alkyldiol derivative tris (hydroxymethyl) aminomethane was considered as a possible antidote since it is a good alkalinizing agent in vivo and might be used to treat ethylene glycol-induced metabolic acidosis (Nahas, 1959; Manfredi et al., 1960; Nahas, 1962; Nahas, 1963, Darby and Anderson, 1966, Strauss, 1968). This idea was suggested by the fact that Borden and Bidwell (1968) observed an antidotal effect of sodium bicarbonate in ethylene glycol poisoning. In addition, possible mechanisms of action, other than as an alkalinizing agent, might be as an osmotic diuretic, as a carrier or scavenger via Schiff base formation, and as an enzymatic inhibitor (Strauss, 1968; Richardson and Tolbert, 1961; Thompson and Richardson, 1968; Richardson and Thompson, 1970; Baker, 1961).

The present findings indicate that tris buffer may form a Schiff base with glycolaldehyde as it has been found with glyoxylate (Richardson and Tolbert, 1961; Thompson and Richardson, 1968; Richardson and Thompson, 1970). However, the ability of tris buffer to act as a carrier in vivo by Schiff base formation is dubious. The pH range within which Schiff base formation occurs readily is above the normal body pH limits. Within the normal body pH range, less than 20 percent of tris buffer is in the base form and available for Schiff base formation. Calculated on a mole for

mole basis, the estimated amount of tris buffer required to scavenge that fraction of ethylene glycol metabolized to toxic products would exceed the LD_{50} of tris buffer. The present findings failed to confirm the *in vitro* observation of Baker (1960) that tris buffer inhibited ethanol oxidation by liver alcohol dehydrogenase. In spite of the expectations for tris buffer as an antidote, it was not found to produce a significant antidotal effect and, therefore, investigations to assess diuretic and alkalinizing actions were discontinued. However, concomitant use of tris buffer and 1,3-butanediol, an efficacious alkyldiol antidote, was tried to determine if the two compounds produced additive antidotal effects in ethylene glycol toxicity. Ethanol and sodium bicarbonate were found by Borden and Bidwell (1968) to produce additive antidotal effects in rats. In opposition to expectations, tris buffer was found to decrease the antidotal effect produced by 1,3-butanediol. Until this attenuation of the antidotal effect of 1,3-butanediol by tris buffer can be explained by future research, the combination of tris buffer and alkyldiol antidotes may be contraindicated in ethylene glycol poisoning.

The liver and the kidney are the two sites of removal of ethylene glycol *in vivo*. If one route is blocked or inhibited, the removal by the other route is correspondingly increased. This seems to be the case in that Chou and Richardson (1978) found that pyrazole, an alcohol dehydrogenase inhibitor, increases the urinary excretion of ethylene glycol and decreases the production of toxic metabolites of ethylene glycol. If the alkyldiol antidotes inhibit

liver alcohol dehydrogenase like pyrazole, then the alkyldiol antidotes should increase the urinary excretion of ethylene glycol as does pyrazole. Therefore, propylene glycol and 1,3-butanediol were compared with pyrazole in this study. However, two major differences distinguish the present experimental procedures from those procedures of Chou and Richardson (1978) and affect the interpretation of the present results. First, the mice were given a dose of ethylene glycol equal to about half the LD_{50} of ethylene glycol to minimize the likelyhood of deaths in the animals receiving ethylene glycol alone. Secondly, the mice were allowed access to food as well as water during the entire course of the experiment to prevent variation in water intake due to lack of food intake.

The present findings indicate that both propylene glycol and 1,3-butanediol are efficacious, like pyrazole, in increasing the urinary excretion of ethylene glycol. The pyrazole data confirms at sublethal ethylene glycol levels the results of Chou and Richardson (1978) that pyrazole increases the urinary excretion of potentially lethal levels of ethylene glycol. The increase in excretion of ethylene glycol following the administration of alcohol dehydrogenase inhibitors other than pyrazole has not been studied. The fact that ethylene glycol was detected in only those urine samples collected during the first twelve hours after administration of ethylene glycol, whether or not an alcohol dehydrogenase inhibitor was given concomitantly with the ethylene glycol, is in agreement with the plasma disappearance of ethylene glycol

described herein. In the case of mice, these data indicate that a 48 hour urine collection period, as used by Chou and Richardson (1978), may not be necessary. Also, it seemed unusual that only about 40 percent of the water consumed by mice in the first 24 hour period appeared as urine; however, the apparently large amount of insensible water loss (60%) is in total agreement with the data of Altman and Dittmer (1961).

During the collection of these data, several findings became apparent and seem worthy of mention. First, ethylene glycol doubled the urine output in mice, whether or not an alcohol dehydrogenase inhibitor, with the exception of pyrazole, was given concomitantly. These data contradict the findings of Borden and Bidwell (1968) that diuresis was not produced by the administration of ethylene glycol. It was not determined if the ethylene glycol-induced diuresis in mice is a dose-dependent response whose manifestation is abolished by potentially lethal ethylene glycol levels, such as used by Borden and Bidwell (1968) or Chou and Richardson (1978). Also, the possible involvement of ethylene glycol with the secretion of antidiuretic hormone was not investigated. Secondly, the marked polyuria was accompanied by a marked polydipsia in all groups of mice that received ethylene glycol, except in that group which received pyrazole in addition to ethylene glycol. These data do not distinguish which effect, polyuria or polydipsia, comes first and produces, in turn, the other, but, if polydipsia is a response to compensate for fluid lost by diuresis, then it appears that pyrazole may inhibit or block in some way

this compensation mechanism. The practical effect of this phenomenon was that the concentration of ethylene glycol in urine of animals given pyrazole was higher than the concentration of ethylene glycol in the urine of animals given either saline or an alkyldiol. Thirdly, propylene glycol and 1,3-butanediol were excreted in the urine of mice given either compound, irrespective of whether or not ethylene glycol was also administered. This is in disagreement with Gessner, Parke, and Williams (1960) who did not detect either of these alkyldiols in the urine of animals after treatment. However, this difference may be due to the comparatively high alkyldiol doses used in this study. Fourthly, it became apparent that ethylene glycol increased the excretion of either alkyldiol, just as either alkyldiol increased the excretion of ethylene glycol. Such an interaction of the excretion of alkyldiols has not been previously reported. The interaction between ethylene glycol and the alkyldiol antidotes in urinary excretion suggests common sites of action of the interacting drugs. This interaction, coupled with the findings described in the preceding paragraph that the alkyldiols, like pyrazole, increase the urinary excretion of ethylene glycol, lends further indirect evidence to the idea that, indeed, the mechanism of action of the alkyldiols is inhibition of liver alcohol dehydrogenase. This is consonant with the findings of Majchrowicz, Hunt, and Piantadosi (1976) that 1,3-butanediol suppresses the ethanol withdrawal syndrome in rats.

The idea that inhibition of enzymes other than liver alcohol dehydrogenase may produce an antidotal effect in ethylene glycol

toxicity is not a new one. Chou and Richardson (1978) found that butyraldoxime and d,l-phenyllactate, inhibitors of aldehyde dehydrogenase and glycolic acid oxidase, respectively, actually increased the toxicity of the metabolites of ethylene glycol. These findings suggest that these two inhibitors facilitate an increase in the toxicity of ethylene glycol by allowing the production and accumulaton of glycolaldehyde, glycolate or glyoxylate. An increased mortality is manifested since glycolaldehyde, glycolate, or glyoxylate have been found to be more toxic than the parent compound (Bove, 1966; Laborit et al., 1971). However, Chou and Richardson (1978), in finding that glycolate seemed to be the sole culprit in acute ethylene glycol poisoning, suggested that inhibition of any enzymatic step prior to glycolate formation might have an antidotal effect. Aldehyde dehydrogenase is the only major enzyme, other than liver alcohol dehydrogenase, which catalyzes a metabolic step prior to glycolate formation. In spite of the fact that butyraldoxine did not produce an antidotal effect, the possibility existed that another aldehyde dehydrogenase inhibitor might be used successfully. However, Mundy (personal communication, 1975) found no antidotal effects produced by either disulfiram (Antabuse^{R}) or diethyldithiocarbamate (DEDTC), both aldehyde dehydrogenase inhibitors. These data suggest that inhibition of enzymes, other than liver alcohol dehydrogenase, will not result in successful antidotal therapy of ethylene glycol poisoning and increase the validity of the metabolic intermediate hypothesis and the necessity of having suitable alcohol dehydrogenase inhibitors

to use as antidotes. In this respect, increased emphasis may be placed on the present findings that some alkyldiols are efficacious antidotes in ethylene glycol poisoning.

The relative study and advantages of using the alkyldiol antidotes in ethylene glycol toxicity, instead of ethanol or pyrazole, is an important consideration. The therapeutic ratios found for the alkyldiol antidotes suggest that these glycols may be safer to use than either ethanol or pyrazole. In spite of the fact that pyrazole was the most potent and efficacious antidote of any tested, it may be eliminated as a practical clinical antidote because of its marked delayed toxicity. Likewise, the marked delayed toxicity of 1,2-butanediol may preclude the use of this alkyldiol in spite of its efficacy. Because the maximum relative antidotal effect of propylene glycol was no greater than that produced by ethanol, propylene glycol does not seem to offer any advantages over ethanol other than in safety alone. However, 1,3-butanediol was found to be both safer and more efficacious than ethanol and have no delayed toxicity. The safety of using 1,3-butanediol is also emphasized by the fact that it has been extensively studied in humans (Kies et al., 1973; Tobin et al., 1975) and animals (Tate, Mehlman, and Tobin, 1971; Mehlman et al., 1971; Dymsza, 1975; Young, 1975; Mehlman, Tobin, and Mackerer, 1975; Romsos, Belo, and Leveille, 1975; Mackerer et al., 1975; Romsos and Leveille, 1977) for potential uses as an energy source in the form of a food additive, a food preservative, an antilipogenic agent, and a replacement for propylene glycol, sorbitol, or glycerol in some foods.

1,3-Butanediol has already received FDA approval as a food flavor and cosmetic solvent and as an indirect food additive from packaging materials (Dymsza, 1975). Studies of the teratogenic and mutagenic effects of 1,3-butanediol have never given positive results (Dymsza, 1975). Therefore, if the advantages of the antidotal use of 1,3-butanediol in ethylene glycol poisoning are confirmed in other species, 1,3-butanediol may well become the drug of choice in ethylene glycol toxicity.

The fact that the alkyldiols were limited to a one time-injection basis may preclude a quantitative manifestation of an antidotal effect as efficacious as that of pyrazole. Just as repeated administration of ethanol has been shown to double the LD_{50} of ethylene glycol (Peterson et al., 1963), repeated administration of an alkyldiol antidote may double the LD_{50} of ethylene glycol. In addition, alkyldiols, other than those found to be antidotes in this study, may manifest an antidotal effect after repeated administration.

The larger alkyldiols, having two end-chain hydroxyls, produced marked hypnotic effects, but had relatively low Km values. If the low Km values are found to be correlated with a high rate of metabolism and a correspondingly short biological half-life, then the observations suggest that some alkyldiols having two end-chain hydroxyls may be found to have applications as hypnotics or short-acting anesthetics.

The present study suggests the possibility that 1,3-butanediol may have an application in another and probably more prevalent form

of toxicity. For many decades, methanol has been known to produce blindness in primates and a delayed systemic acidosis in most species (Røe, 1943; Tephly, 1977). Røe (1943) first found that ethanol could be used as an antidote in methanol toxicity and Zatman (1946) first showed that the mechanism of action of ethanol was competitive inhibition of liver alcohol dehydrogenase. Although pyrazole has not been found to be an antidote of methanol toxicity, pyrazole has been shown to inhibit oxidation of methanol by human liver alcohol dehydrogenase (Pietruszko, 1975a). Due to the relatively low affinity of methanol for liver alcohol dehydrogenase compared with that of ethanol, 1,3-butanediol may be found in the future to competitively inhibit methanol oxidation and the resulting manifestation of methanol toxicity in man or other species (Pietruszko, 1975a; Blair and Vallee, 1966).

SUMMARY

Ethanol and pyrazole are known to be antidotes of ethylene glycol toxicity. Neither ethanol nor pyrazole is an ideal clinical antidote. Therefore, a survey of a series of alkyldiols was conducted with the specific objective of finding a superior antidote for ethylene glycol toxicity.

The findings that pyrazole and ethanol competitively inhibit liver alcohol dehydrogenase *in vitro* and are antidotes *in vivo* suggest that both compounds limit ethylene glycol oxidation by inhibition of liver alcohol dehydrogenase. Based on previous findings that liver alcohol dehydrogenase catalyzes the oxidation of some alkyldiols, mouse liver alcohol dehydrogenase was assayed with ethanol, a series of alkyldiols, and tris (hydroxymethyl) aminomethane. The K_m values in mmoles/l determined from the assays were 0.4 with ethanol, 53 with ethylene glycol, 14 with propylene glycol, 5.4 with 1,3-propanediol, 3.8 with 1,2-butanediol, 1.5 with 1,3-butanediol, 0.5 with 1,4-butanediol, 56 with 2,3-butanediol, 0.23 with 1,5-pentanediol, 0.031 with 1,6-hexanediol, and undeterminable with tris (hydroxymethyl) aminomethane. These data indicated that ethanol and the alkyldiols, with the exception of 2,3-butanediol and tris (hydroxymethyl) aminomethane, had higher affinities for mouse liver alcohol dehydrogenase than ethylene glycol.

Toxicity studies were performed to test the hypothesis that

alkyldiols of higher affinity than ethylene glycol for mouse liver alcohol dehydrogenase were antidotes in ethylene glycol toxicity. Propylene glycol (27.2 mmoles/kg), 1,2-butanediol (11.2 mmoles/kg), and 1,3-butanediol (22.3 mmoles/kg) were the only alkyldiols found to protect the mice, like ethanol and pyrazole, against ethylene glycol poisoning. Acute and delayed toxicity studies and hexobarbital sleeping time studies indicated that the alkyldiols that protected the mice were, in general, the least toxic and least hypnotic of the alkyldiols.

Therapeutic ratios found by dividing the eventual LD_{50} of an antidote by the dose which produced the maximum antidotal effect were 3.17 for ethanol, 2.56 for pyrazole, 6.16 for propylene glycol, 4.15 for 1,2-butanediol, and 5.11 for 1,3-butanediol. These data suggest that the alkyldiol antidotes may be safer to use than either ethanol or pyrazole.

A gas chromatographic method was used to detect ethylene glycol in mouse plasma and alkyldiols in mouse urine. Dose levels of pyrazole at 4.41 mmoles/kg, ip, or 8.81 mmoles/kg, sc, were found to delay the disappearance of a 71.4 mmole/kg dose level of ethylene glycol in mouse plasma. These data strengthen the theory that pyrazole inhibits liver alcohol dehydrogenase in vivo and that the antidotal effect of pyrazole is due to the lack of formation of toxic metabolites. The increased plasma levels of ethylene glycol after pyrazole administration do not account for the antidotal effect of pyrazole if CNS depression is considered the major cause of acute mortality in ethylene glycol poisoning.

The liver and the kidney are the two sites of removal of ethylene glycol in vivo. If one route is blocked or inhibited, the removal of ethylene glycol by the other route is correspondingly increased. This has been found previously to be the case in the disposition of ethylene glycol after pyrazole administration in vivo. In the present study, pyrazole (4.41 mmoles/kg), propylene glycol (27.2 mmoles/kg), and 1,3-butanediol (22.3 mmoles/kg) increased significantly the percentage of the original 108 mmoles/kg dose level of ethylene glycol excreted in mouse urine. Propylene glycol and 1,3-butanediol were excreted in mouse urine as ethylene glycol was excreted. Ethylene glycol increased significantly the percentage of the original dose levels of either propylene glycol or 1,3-butanediol excreted in mouse urine. These data suggest that the mechanism of action of the alkyldiols, like pyrazole, is the inhibition of liver alcohol dehydrogenase.

Urine output was tripled and water intake was doubled in mice during the first 12 hour period after treatments with ethylene glycol (108 mmoles/kg), po, and one of the following, ip: saline, propylene glycol (27.2 mmoles/kg), or 1,3-butanediol (22.3 mmoles/kg). Urine output was doubled, but water intake remained the same in mice during the first 12 hour period after treatments with ethylene glycol (108 mmoles/kg), po, and pyrazole (4.41 mmoles/kg), ip. The increased urine output could not be attributed to the volume of drug solutions administered. Therefore, the observation that ethylene glycol induced an early diuresis was unexpected in view of the findings that death during the delayed

phase of ethylene glycol toxicity has been attributed to oliguria and anuria. These data do not distinguish which effect, polyuria or polydipsia, comes first and produces, in turn, the other. If polydipsia is a response to compensate for fluid lost by diuresis, then it appears that pyrazole may inhibit or block in some way this compensation mechanism. On the other hand, if ethylene glycol-induced polyuria is depressed by pyrazole, then the necessity of evoking the response of polydipsia is diminished.

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GRADUATE SCHOOL
UNIVERSITY OF ALABAMA IN BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Norman Willard Holman, Jr.

Major Subject Pharmacology

Title of Dissertation A Survey of Alkyldiols as Antidotes in Ethylene Glycol Toxicity in Mice

Dissertation Committee:

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Date 25 August 1978