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The synergistic effects of ethanol and acetaminophen-induced hepatotoxicity on hepatic glutathione and plasma glutamic oxaloacetic transaminase levels in mice

by

Romeo Smith

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

Department: Food and Nutrition
Major: Nutrition

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa

1985

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INTRODUCTION

Acetaminophen (p-hydroxy acetanilide) is a widely used analgesic and antipyretic drug. It is an ingredient in over 200 medicinal formulations. At therapeutic doses, it is relatively safe and has none of the adverse effects of aspirin (Ameer and Greenblatt, 1977; Hinson, 1980).

Overdoses of acetaminophen, however, may produce a fulminating hepatic necrosis in both humans and animals (Hinson et al., 1981; Jackson et al., 1984). This necrosis occurs mainly in the centrilobular region of the liver.

The hepatotoxicity produced after overdose is not due to acetaminophen itself, but is due to an electrophilic metabolite formed by the cytochrome P-450 mixed function oxidase system. This metabolite is currently thought to be an imidoquinone (Black, 1980; Grafstrom et al., 1979).

In low dose, acetaminophen is metabolized by the liver where it is conjugated with sulfate and UDP-glucuronate. In overdosage, due to the low stores of UDPGA and sulfate, the electrophilic metabolite of acetaminophen is conjugated to glutathione (Ameer and Greenblatt, 1977). When 70-80% of the glutathione is depleted, the toxic metabolite begins to covalently bind to tissue macromolecules and this depletion ultimately leads to cell death (Hinson et al., 1981; Jollow et al., 1973). Inhibitors of cytochrome P-450, e.g., cobaltous chloride and piperonyl butoxide, reduce the amount of toxic metabolite formed and thus limit the hepatotoxic effects (Potter et al., 1973; Jollow et al.,

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1973).

The hepatotoxic effects are also counteracted by N-acetylcysteine (Tredger et al., 1980), S-adenosyl-l-methionine (Stramentinoli et al., 1979), sodium sulfate (Slattery and Levy, 1977) and α-mercaptopropionylglycine (Labadarios et al., 1977).

The present study was undertaken in order to ascertain a possible synergistic effect of ethanol (an inducer of microsomal oxidation) and acetaminophen on the extent of liver necrosis as measured by total liver glutathione (GSH) levels and plasma glutamic oxaloacetic transaminase (PGOT) and to discover the relationship between PGOT activity and hepatic GSH levels after exposure of the liver to the toxic metabolite.

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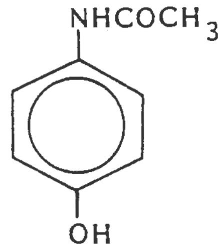
REVIEW OF LITERATURE

Acetaminophen

Acetaminophen (N-acetyl-p-aminophenol) (Figure 1) is a commonly used antipyretic and analgesic agent found in over 200 drug formulations. It does not interfere with blood coagulation or cause gastrointestinal erosion as aspirin does and is preferred over aspirin in many cases (Dietz et al., 1984; Black, 1980).



Acetaminophen

Figure 1 Chemical structure of acetaminophen (pHAA)

At low dose it is a safe and effective drug but overdoses of the drug have been linked to hepatotoxicity.

Acetaminophen was synthesized at Johns Hopkins University in 1877 and first used clinically by von Mehring in 1893. In 1949, Axelrod and Brodie confirmed its therapeutic effects and its use became extensive (Ameer and Greenblatt, 1977; Jackson et al., 1984).

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Pharmacologic Properties

Unlike aspirin, acetaminophen does not have anti-inflammatory or antirheumatic properties (Ameer and Greenblatt, 1977; Black, 1980). Acetaminophen's mode of action may be due to its inhibition of prostaglandin synthetase, an inhibition which may be greater in the central nervous system (CNS) than in the peripheral tissues (Ameer and Greenblatt, 1977).

Pharmacokinetics

The absorption rate of acetaminophen is influenced by the rate of gastric emptying. Cohen et al. (1974) using isolated perfused rat liver found that the systemic circulation concentration of acetaminophen was only 34% of that by intravenous injection. Cohen suggested that the rate of gastric emptying could also influence the "first pass" effect of acetaminophen metabolism. A high rate of gastric emptying could give high concentrations of acetaminophen in the portal vein resulting in a lower hepatic extraction ratio leading to higher systemic concentrations. A slow rate of gastric emptying would have a reverse effect.

Acetaminophen is a weak acid with a pKa of 9.5. In humans, it is rapidly absorbed from the gastrointestinal tract with peak plasma levels being reached in 30 to 60 minutes after oral ingestion. It is distributed uniformly throughout most body fluids (Volume of distribution Vd = 0.7 - 0.75 L/kg). It is also found bound to plasma proteins (25%) (Black, 1980). The absorption rate of acetaminophen is faster from an alcohol solution (Ameer and Greenblatt, 1977).

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Acetaminophen is metabolized by the liver and its metabolic products are excreted by the kidney. The plasma half-life of a normal dose of acetaminophen is 1-3 hours. The half life is prolonged in conditions such as overdosage or liver disease (Ameer and Greenblatt, 1977; Jackson et al., 1984). Renal dsyfunction, however, has no effect upon the half-life of the drug. The hepatic extraction ratio is estimated to be between .245 -.265 which indicates a 25% first pass metabolism effect (Ameer and Greenblatt, 1977). The total metabolic clearance is from 5.15-5.57 ml/min·kg. The percentages of the metabolites appearing in the urine are as follows: glucuronide conjugates (UDPGA) (55-63%), sulfate conjugates (30-34%), cysteine and mercapturic acid conjugates (3-4%) and free acetaminophen (1-4%) (Jackson et al., 1984).

Due to the limited hepatic stores of sulfate and UDPGA, more of the drug is oxidized to the cysteine and mercapturic acid conjugates with overdoses of the drug (Figures 2 and 3). These two conjugates are formed by the cytochrome P-450 mixed function oxidase system (Black, 1980; Jackson et al., 1984; Ameer and Greenblatt, 1977). This system is also thought to form an intermediate arylating compound, N-acetyl-p-benzoquinonemine. This compound is normally conjugated by glutathione (GSH), and the resulting mercapturic acids are excreted by the kidney (Jackson et al., 1984). In overdosage, the liver glutathione is depleted and the arylating metabolites attack liver cell macromolecules, resulting in cell death (Black, 1980).

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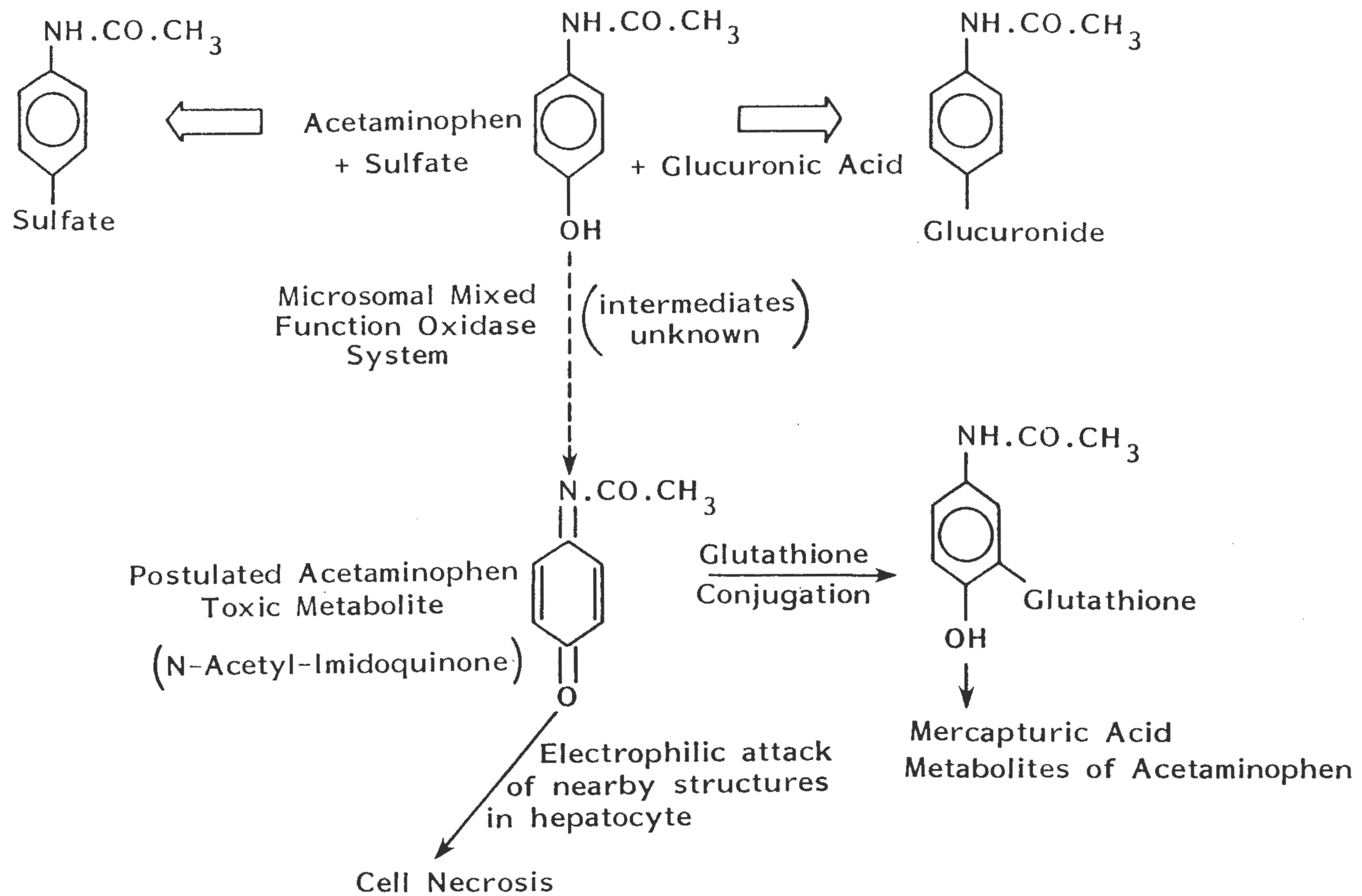

Figure 2. Acetaminophen metabolism

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Figure 3. Mercapturic acid formation

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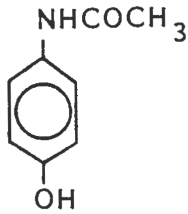
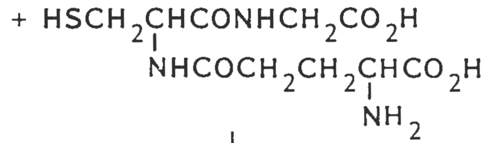
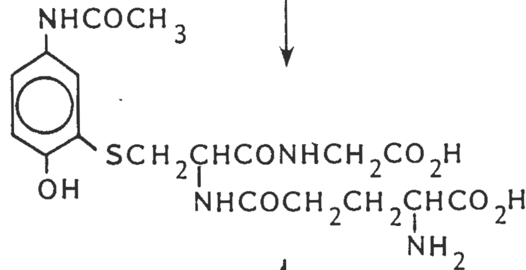
Acetaminophen



Glutathione

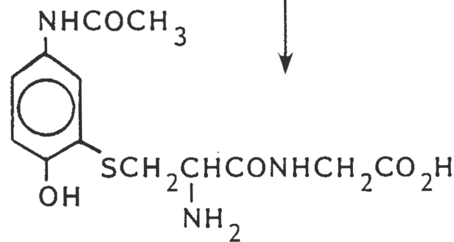


GSH-S-transferases



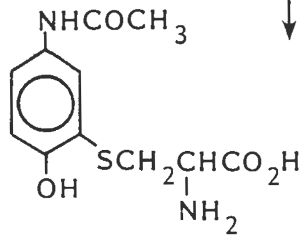
Glutamine

γ-Glutamyltranspeptidase



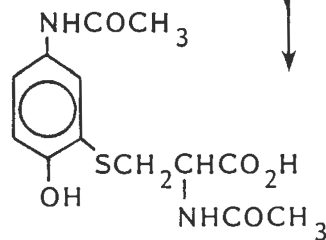
Glycine

Cysteinylglycinase



Acetyl-CoA

N-Acetylase



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Mechanism of Hepatotoxicity

An intermediate arylating compound, postulated to be N-acetyl-p-benzoquinoneimine is thought to be the causative agent of the fulminant centrilobular necrosis seen in cases of acetaminophen overdosage. This electrophilic metabolite is formed by the cytochrome P-450 mixed function oxidase system (Jackson et al., 1984).

The severity of the necrosis produced is influenced by many factors. Inducers of drug metabolism such as phenobarbital and 3-methylcholanthrene (3MC) which increase the cytochrome P-450 concentration have been found to increase both the incidence and severity of necrosis. Inhibitors of drug metabolism such as piperonyl butoxide, SKF-525A and cobaltous chloride prevent or limit liver necrosis (Mitchell et al., 1973b; Jollow et al., 1973; Potter et al., 1973). Piperonyl butoxide inhibits the oxidation of acetaminophen by combining with cytochrome P-450. Cobaltous chloride acts by blocking the synthesis of cytochrome P-450 at the ferrochelatase level (Mitchell et al., 1973a).

The toxic metabolite becomes covalently bound to tissue macromolecules leading to cell death. In mice, pretreatment with compounds which increase the severity of necrosis also increase the amount of covalent binding observed and those pretreatments which decrease the amount of covalent binding (Jollow et al., 1973). Administration of subtoxic doses of acetaminophen produces insignificant binding in the liver. Covalent binding is prominent in the centrilobular cells, localized in the endoplasmic reticulum and in the

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proteins of the cytoplasm (Jollow et al., 1973; Gillette et al., 1974).

Binding is regulated by cytochrome P-450 which is in high concentrations in the centrilobular region. The amount of binding is dose-dependent and peak binding occurs one to two hours before necrosis can be observed. NADPH and O_2 are required for binding to occur (Potter et al., 1973).

Although the amount of covalent binding has been highly correlated with the extent of necrosis, compounds exist which do not decrease the amount of binding yet decrease the severity the extent of necrosis. Two of these sulphydryl compounds are N-acetylcysteine and α-mercaptopropionglglycine (α-MPG) (Labadarios et al., 1977; Tredger et al., 1980; Gerber et al., 1977). α-MPG may act by either stabilizing the macromolecules against the effects of binding or by binding to the specific sties which are responsible for initiating cell necrosis (Labadarios et al., 1977).

Diet Effects

McLean and Day (1975) fed rats low protein diets and 25% yeast diets for at least one week before administration of acetaminophen alone or with inducers. The low protein diets were found to decrease the amount of cytochrome P-450/g liver, increase UDPGA transferase activity and to sharply decrease the liver glutathione levels. Phenobarbitone increased cytochrome P-450 levels but had no effect on glutathione levels. The rats fed the low protein diet were found to be highly sensitive to acetaminophen. This sensitivity was further increased by phenobarbitone elevation of cytochrome P-450. Cysteine and methionine

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when injected with acetaminophen were found to reduce the lethality of acetaminophen hepatotoxicity.

Stramentinoli et al. (1979) using mice injected both before and after acetaminophen administration with S-adenosyl-l-methionine observed a reduced acetaminophen-induced hepatotoxicity as evidenced by decreased necrosis and decreased GOT and GPT serum enzyme activities. Slattery and Levy (1977) found sodium sulfate injected intraperitoneally (i.p.) with acetaminophen reduced acetaminophen hepatotoxicity in mice.

Miller and Jollow (1984) using hamsters, observed that L-ascorbic acid partially inhibited covalent binding of acetaminophen metabolites in isolated hepatocytes but did not influence covalent binding of the reactive metabolite in vivo or decrease the extent of hepatic necrosis.

Pessayre et al. (1980) in studying effects of fasting and inducers alone and in combination on acetaminophen toxicity found that pretreatment with phenobarbital increased hepatic microsomal protein concentration, hepatic cytochrome P-450 levels, NADPH cytochrome c reductase activity, ethylmorphine N-demethylase activity and acetaminophen UDP-glucuronosyltransferase activity. Phenobarbital pretreatment had no effect on the amount of covalent binding in fed or fasted rats. Pretreatment with 3MC increased cytochrome P-448 and benzo[a]pyrene hydroxylase activity and the microsomal enzymes induced by phenobarbital except acetaminophen UDP-glucuronosyltransferase. 3MC increased the amount of covalent binding in both fed and fasted rats but the amount of binding was greater in the fasted group.

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Fasting the rats for a period of 42 hours in the absence of inducers tended to increase microsomal protein concentration and reduced hepatic GSH concentration but only slightly decreased mixed function oxidase activities.

Administration of a 500 mg/kg dose of acetaminophen further decreased the GSH concentration in the fasted rats as compared with the fed rats. Phenobarbital did not influence hepatic GSH concentration. Pretreatment with 3MC decreased hepatic GSH concentration in both the fed and fasted rats.

It was concluded that inducers which increase microsomal enzyme activity and fasting which decreases hepatic GSH may have an additive effect on acetaminophen hepatotoxicity.

Newman and Bargman (1979) in reporting a clinical case of acetaminophen in an anorexic adult male found no evidence of malnutrition potentiation of a hepatotoxic effect of acetaminophen. Although the prevention of liver damage may have been due solely to the administration of N-acetylcysteine, Newman and Bargman postulated that the dietary deficiencies present may have served to decrease the cytochrome P-450 activity relative to the GSH stores and thus prevented liver necrosis. It was concluded that if the postulate is in fact true, then malnourished patients/individuals may be more resistant to acetaminophen induced liver necrosis.

Effect of Age

Hart and Timbrell (1979) in studying the effects of age on acetaminophen hepatotoxicity found that neonatal mice were less

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susceptible than adult mice to the hepatotoxic effects of the reactive metabolite. They observed less GSH depletion, less covalent binding to tissue macromolecules, and less hepatic necrosis in the neonatal mice than in the adults. Neonate liver GSH reaches adult levels at about 10 days of age (Lambert and Thorgeirsson, 1976; Wirth and Thorgeirsson, 1978). Hart and Timbrell (1979) noted that hepatic cytochrome P-450 reached adult levels at 16 days of age, the age at which susceptibility to acetaminophen toxicity is about the same as in adult mice. They concluded that the reduced susceptibility observed in the neonate mouse might be due to the lower cytochrome P-450 levels coupled with low but sufficient glutathione levels to bind the reactive metabolite.

In human infants, the major urinary metabolite of acetaminophen is the sulfate conjugate, whereas in adults the major metabolite is the glucuronide conjugate. Thus, infants and children may have a reduced ability for glucuronide conjugation (Ameer and Greenblatt, 1977).

Toxic Effects

Hepatic

Walker et al. (1980) using light and electron microscopy to examine the morphological changes in the liver of white mice after a 500 mg/kg dose of acetaminophen discovered the following:

1. Numerous lipid droplets, particularly in the centrilobular region with only small amounts of glycogen visible in the midzonal and portal regions. The first visible histologic

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changes occurred at 3/4 to 1 1/2 hours after administration of the dose.

2. Vacuolization along sinusoidal margins of centrilobular cells occasionally communicating with Disse's space. The cytoplasm appeared foamy due to dilation of both rough and smooth endoplasmic reticulum.

3. Vesiculation of the rough endoplasm reticulum resulting in random dispersal of ribosomes, disaggregation of polyribosomes and possibly a decrease in protein synthesis were seen. The mitochondria appear swollen with detached cristae fragments and have bizarre forms.

4. Three hours after dosage there was centrilobular congestion due to red blood cells (RBC) packed in the sinusoids, beneath sinusoidal lining cells and in some large cell vacuoles. There was also an increase seen in centrilobular fat.

5. After three hours' loss of microvilli, gross disorganization of organelles, increase in platelet aggregates and obstruction of blood flow by narrowing of sinusoids are observed.

Necrosis was maximum between 24 to 48 hours and regeneration evident at 48 to 72 hours. Inducers of drug metabolism were found to potentiate the extent of necrosis but not the onset of the histologic changes.

Serum enzyme glutamic-oxaloacetic transaminase (SGOT), glutamic-pyrovic transaminase (SGPT), sorbitol dehydrogenase (SSDH), and hepatic triglyceride are elevated after administration of an acute dose of .5

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g/kg to 1.0 g/kg of acetaminophen in mice (Dixon et al., 1975; Buttar et al., 1976). Maximal hepatotoxic effects were observed 12-18 hours after administration of the dose. A close positive correlation was found between SGPT and SGOT levels and the extent of hepatic necrosis. Thus, the levels of these enzymes may be an indication of the severity of necrosis. In humans, the symptoms of acetaminophen overdosage progress gradually from nausea, malaise and vomiting within 12-24 hours after the overdosage to observable histologic changes occurring 4 to 6 days later. There is a sharp rise in SGOT and SGPT serum enzyme levels which returns to normal levels in 7 to 14 days. Mild acidosis, jaundice, abdominal pain, possible hepatic coma and death may also occur (Ameer and Greenblatt, 1977; Jackson et al., 1984).

In diseased states

Acetaminophen intake has been implicated in a number of patient case histories as either a cause of acute and chronic hepatic inflammation and fibrosis or as an aggravator of underlying liver disease (Johnson and Tolman, 1977; Ware et al., 1978; Craig, 1980; Neuberger et al., 1980; Olsson, 1978; Bronkowsky et al., 1978). In many of these cases there were other underlying causes or confounding variables which may have been responsible for the actual disease. However, challenge tests with subtoxic doses of acetaminophen were found to elevate serum enzyme levels.

Hypersensitivity reactions to acetaminophen have also been reported (Olsson, 1978).

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Strubelt et al. (1979) reported finding an acetaminophen-induced protection against acetaminophen toxicity in rats. They concluded that this protection was due to a decrease in microsomal mixed function oxidase activity brought about by three days administration of a 1000 mg/kg dose of acetaminophen. The hepatic GSH level was found to continually decrease over the three day period to 46% of the control level after which GSH concentration exceeded the control level by 85%.

Renal

Renal tubular necrosis has been reported after intake of large doses of acetaminophen. Tubular damage occurs primarily in the proximal tubules but the distal tubules are also affected. Renal damage is usually secondary to hepatic necrosis, although cases of renal damage in the absence of hepatic necrosis have been reported (Hinson, 1980; Jackson et al., 1984).

Effect of Ethanol

Acute and chronic ingestion of ethanol has been found to influence the following hepatic parameters: to decrease hepatic reduced glutathione levels (Fernandez and Videla, 1981), increase lipoperoxidation (Videla and Valenzuela, 1982; Videla et al., 1980), potentiate the toxicity of a variety of hepatotoxic agents (Strubelt et al., 1978), alter the appearance of liver mitochondria (Kiessling and Pilstrom, 1966a, 1966b), and to induce drug metabolizing enzymes (Liu et al., 1975; Coon et al., 1984; Peterson et al., 1980). The depletion of GSH by ethanol is thought to take place via acetaldehyde (Viña et al., 1980b).

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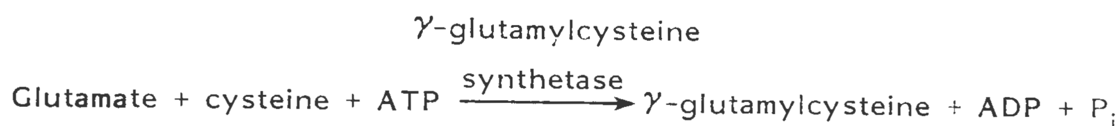
Ethanol induction of drug metabolism takes place via induction of the ethanol inducible isoenzyme 3a of cytochrome P-450 (Coon et al., 1984). This induction has been demonstrated both in vivo and in vitro by a number of researchers in rats (Liu et al., 1975; Teschke et al., 1979; Moldeus et al., 1980), in mice (Strubelt, 1984; Walker et al., 1983; Banda and Quart, 1984) and in humans (Licht et al., 1980; McClain et al., 1980; Dietz et al., 1984). This research finding specifically relates to the potentiation of acetaminophen toxicity by ethanol. This potentiation is due to both its depletive effects on hepatic GSH (via acetaldehyde) and its induction of microsomal enzymes. Isoenzyme 3a is also active toward amiline but not paranitroanisole.

Glutathione

Glutathione, γ-glutamylcysteinylglycine, is found in almost all mammalian cells. Its functions are related to its thiol group and its γ-glutamyl moiety (Figure 4) (Binkley and Nakamura, 1948; Meister, 1982; Stryer, 1981).



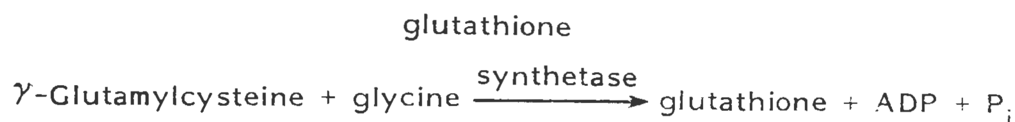


Figure 4. Glutathione biosynthesis

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Glutathione performs a variety of functions. It functions as an intracellular reductant, in the destruction of free radicals and peroxides, in the maintenance of red cell structure, keeping hemoglobin in the ferrous state, in amino acid transport and in the detoxification of xenobiotic compounds (Meister, 1982; Stryer, 1981; Meister, 1984; Sies et al., 1982; Levine, 1982; Mitchell et al., 1973b; Davis et al., 1974; Prescott, 1982; Cagen and Klassen, 1980; Griffith and Meister, 1979; Sekura and Meister, 1974). Glutathione S-transferases play an important role in detoxification (Grover, 1982; Ketterer et al., 1982; Jakoby, 1978; Uotila, 1973; Keen and Jakoby, 1978; Keen et al., 1976).

Cellular glutathione levels are affected by diet (Tateishi et al., 1982; Maruyama et al., 1968), fasting (Lauterberg and Mitchell, 1982; Jaeger et al., 1974) and diurnal cycles (Boor, 1979; Jaeger et al., 1973; Beck et al., 1958).

Depletion of glutathione to less than 30% of normal can result in increased toxicity of electrophilic metabolites (Plummer et al., 1981). Depletion of GSH can result in increased toxicity of 1,1-dichloroethylene (Jaeger et al., 1974), ethionine (Glaser and Mager, 1974), bromobenzene (Thor et al., 1979; Jollow et al., 1974) carbonyl compounds (Boyland and Chasseaud, 1970), acetaminophen (Davis et al., 1975; Benedetti et al., 1975; Viña et al., 1980a; Mitchell et al., 1973a) and a variety of other chemical compounds.

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MATERIALS AND METHODS

Experiment 1

Adult male Swiss Webster mice (Laboratory Supply Inc., Indianapolis, IN.) were acclimated to the laboratory environment for a period of one week in plastic cages under controlled light (12 hour, 7a.m.-7p.m. light/dark cycle) and temperature (22°C) conditions. The mice were fed distilled water and laboratory chow (Teklad, Winfield, IA) ad lib. After week one, the mice were randomly assigned to three treatment groups: H_2O + pHAA, 10% ethanol (EtOH), and 10% ethanol+pHAA which received either distilled water or a solution of 10% (V/V) ethanol. Food, fluid intake, and animal weights were measured weekly. After five weeks of treatment, the mice were fasted for a period of 24 hours after which they were presented with either distilled water or acetaminophen (pHAA) in the drinking water 1000 mg/kg b.w. in each 3 ml of drinking water) ad lib. for a period of 2 hours. The mice were then killed by decapitation at various times after introduction of the acetaminophen. The liver was removed and frozen quickly in liquid nitrogen and stored at -80°C for analysis of total glutathione concentration. Blood was collected in heparinized tubes, centrifuged and the plasma immediately analyzed for plasma oxaloacetic transaminase, (PGOT) activity or was stored at -80°C until the next day.

Experiment 2

Adult male Swiss Webster mice were exposed to the same conditions as in Experiment 1 excepting the one week acclimation period. All mice

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received 10% (V/V) ethanol in the drinking water. After five weeks, the mice were fasted for a period of 24 hours after which they were given either 10% ethanol or pHAA (1000 mg/kg b.w. in each 3 ml of drinking water) ad lib. The animals were sacrificed and tissues collected as noted in experiment one.

Experiment 3

Adult male Swiss Webster mice were exposed to the same conditions as in Experiment 1 excepting the one week acclimation period. The mice were divided into three groups receiving either distilled water or a 10% (V/V) ethanol solution.

After four weeks, the mice were fasted for 24 hours and injected intraperitoneally (i.p.) with either acetaminophen (750 mg/kg) dissolved in propylene glycol (60 mg/ml) or an equivalent volume of propylene glycol and sacrificed at various times after injection as in experiment one.

Assays

Total glutathione was determined according to Jollow et al. (1973) by the method of Ellman (1959). PGOT was determined by using a reagent kit (Sigma Chemical Company, St. Louis, Mo.). Acetaminophen used was obtained from Sigma Chemical Company.

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RESULTS AND DISCUSSION

Effects on Glutathione Levels

In Experiment 1, highly significant time, treatment and treatment x time interactions were observed (Tables 1 and 2; Figure 5). All treatments were significantly different from one another, however, only the 18 hr results were significantly different from those at the 4, 6, and 10 hour times.

There was a trend observed for a negative correlation for GSH and PGOT levels and treatment, but it was not significant. This same trend was observed for the PGOT and GSH levels at the various times, but again, it was not significant (Tables 3 and 4).

In Experiment 2, significant treatment differences were found, but there were no significant time or treatment x time effects (Tables 5 and 6; Figure 5). No significant correlations between GSH and PGOT levels were observed (Tables 7 and 8).

In Experiment 3, highly significant time and treatment effects were observed (Table 9; Figure 7). Only the 10% ethanol + 0 group was responsible for the difference. The 10% ethanol + pHAA and H_2O + pHAA groups were not significantly different (Table 11).

A negative correlation between GSH and PGOT levels was observed for all treatments, however, only the H_2O + pHAA and 10% ethanol + 0 groups had a significant negative correlation between GSH and PGOT levels. All times were negatively correlated for GSH and PGOT levels, but only the 0.5 hr and 1.0 hr times were significantly correlated (Tables 11 and 12).

In Experiment 1, the significance at 18 hr was probably due to the

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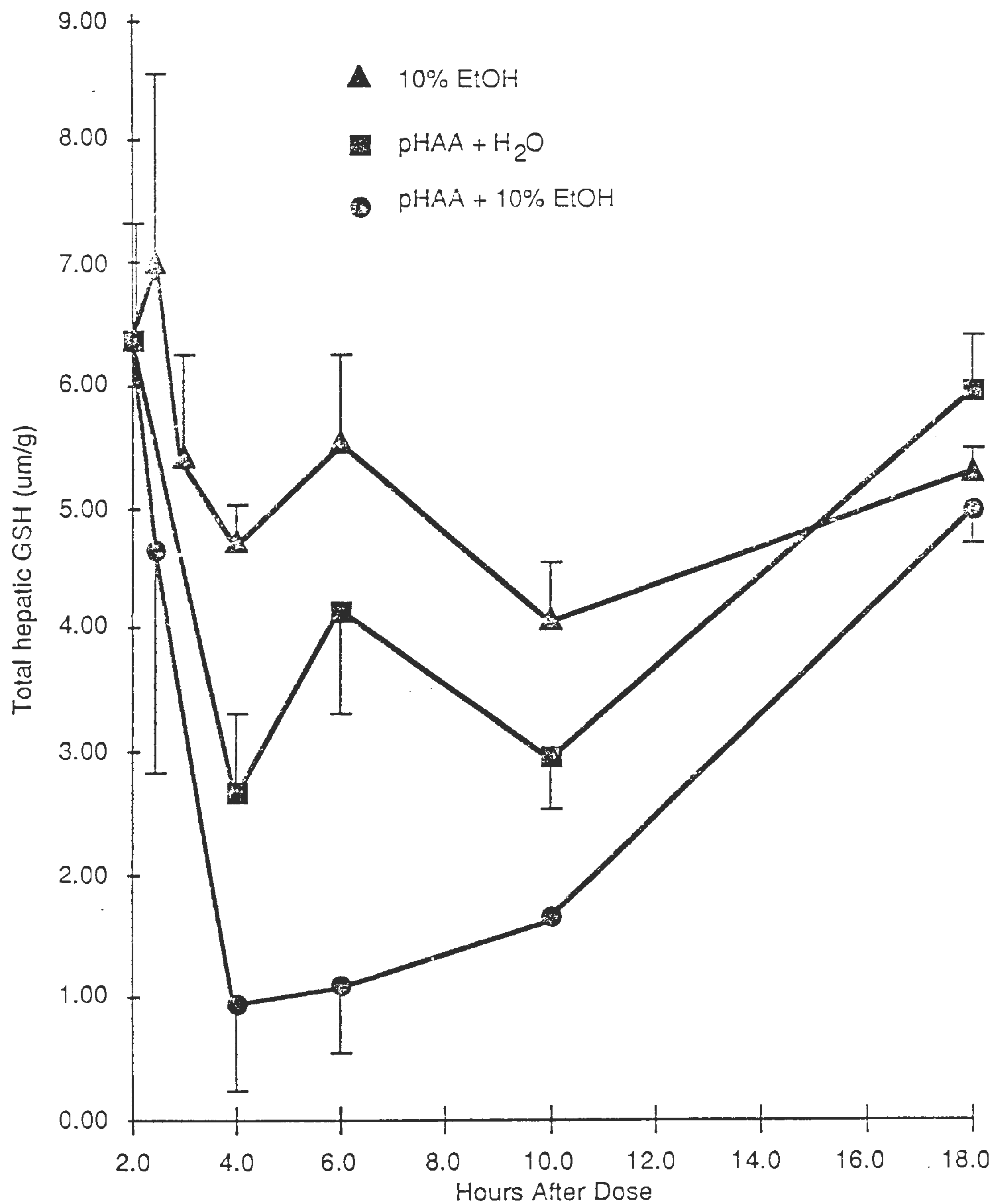

Figure 5. Time-dependent depletion of hepatic glutathione after administration of an ad lib. dosage (1000 mg kg^{-1} 3 ml^{-1}) of acetaminophen

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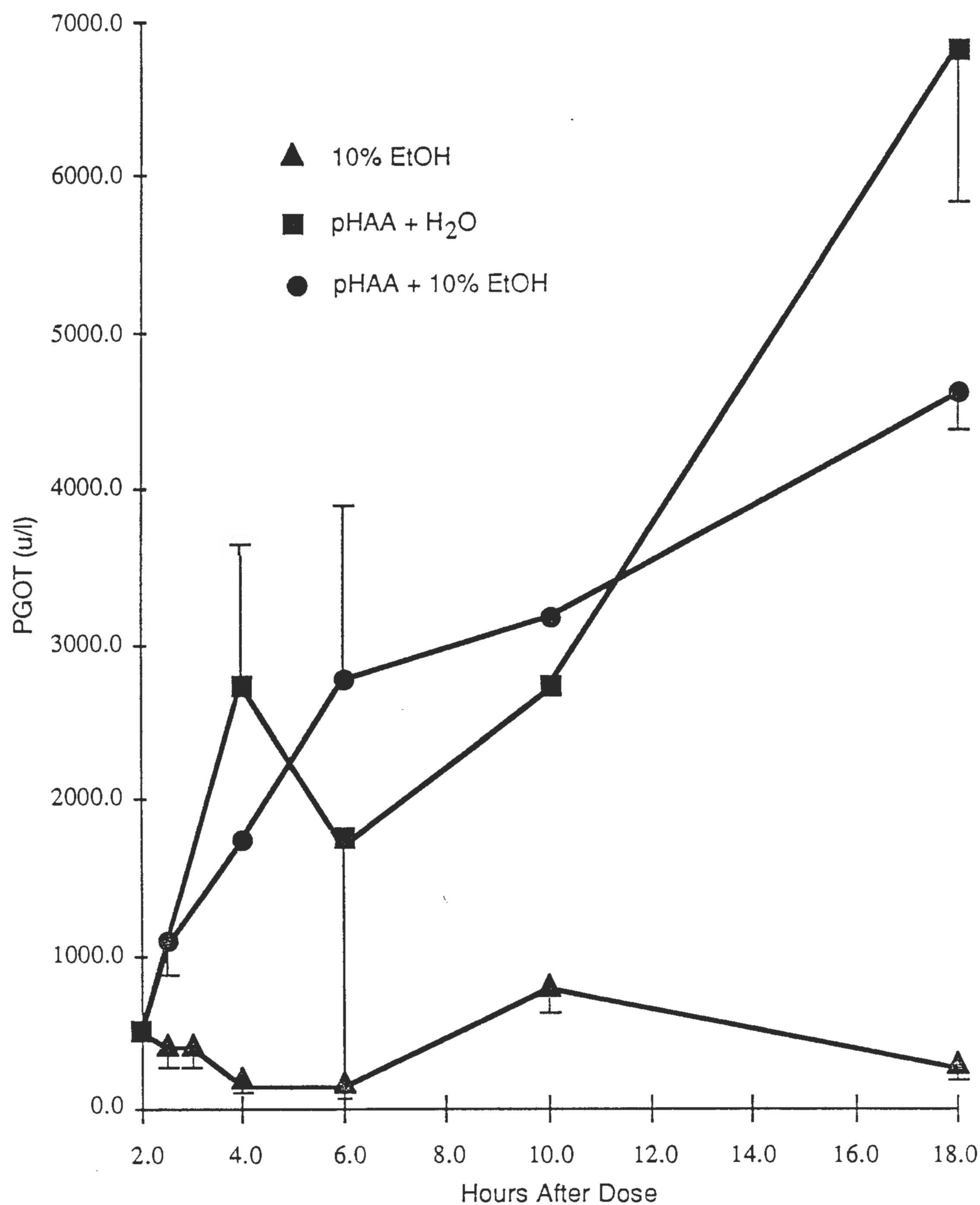

Figure 6. Time-dependent elevation of glutamic oxalogoeteic transaminase after administration of an ad lib. dosage (1000 mg kg^{-1} 3 ml) of acetaminophen

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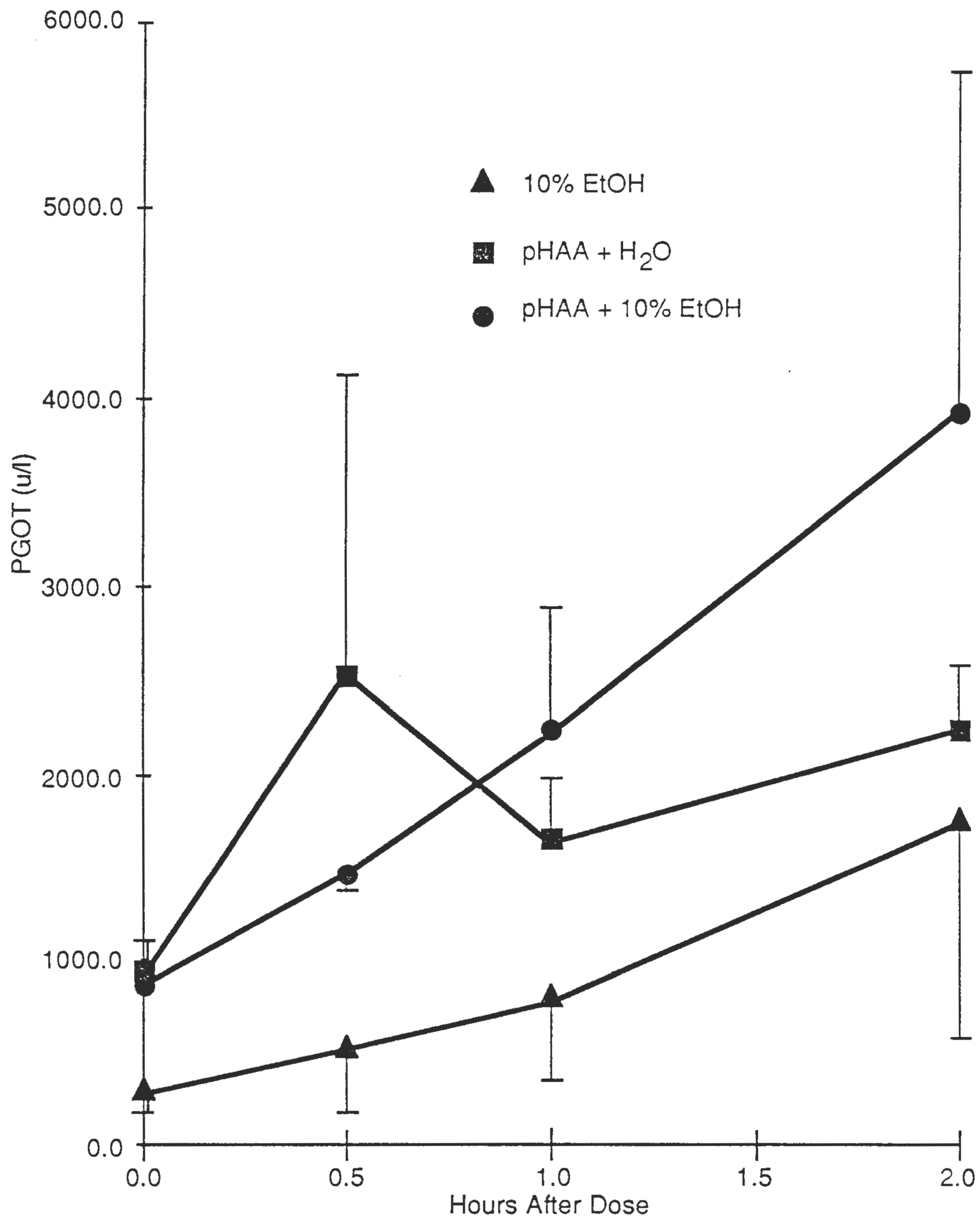

Figure 7. Time-dependent elevation of plasma glutamic oxaloacetic transaminase after administration of a 750 mg kg^{-1} i.p. dose of acetaminophen

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Table 1. Exp. 1. Analysis of variance of glutathione and plasma oxalo-acetic transaminase

	GSH		PGOT	
Source	F Value	PR > F	F Value	PR > F
TRT	46.97	.0001	19.25	.0006
TIME	30.83	.0001	5.38	.0214
TRT*TIME	6.36	.0043	2.18	.1409

Table 2. Exp. 1. Average glutathione and plasma oxaloacetic transaminase values

GSH (umoles/g liver)		PGOT (U/L)	
TRT[1]	TIME (hr)	TRT[1]	TIME (hr)
1. 3.92[a2]	4. 2.74[a2]	1. 3600.[a2]	4. 1508.8[a2]
2. 4.87[b]	6. 3.58[b]	2. 331.2[b]	6. 1543.0[a]
3. 2.22[c]	10. 3.13[a,b,c]	3. 3285.7[a]	10. 1851.9[a]
	18. 5.38[d]		18. 3892.4[b]

[1]Treatment one = H_2O + pHAA, treatment two = 10% EtOH only, treatment three = 10% EtOH + pHAA.

[2]Means with same letter are not significantly different at $P \leq .05$.

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Table 3. Exp. 1. Glutathione: Plasma glutamic oxaloacetic transaminase correlation coefficients (r)

TRT	TIME (hr)	
1. -.634 (.250)[a]	4. -.634 (.251)	10. -.919 (.081)
2. -.603 (.114)	6. -.553 (.255)	18. .365 (.477)
3. .6314 (.179)		

[a]PR > F appears under correlation coefficient.

effects of both diurnal variation (Jaeger et al., 1973) and the increase in glutathione synthesis observed after depletion. In this rebound period, GSH levels can increase up to twice that of control values (Plummer et al., 1981).

Fasting also lowers hepatic GSH levels by about 30% (Lauterberg and Mitchell, 1982. This decrease in GSH levels occurs during the first 12 hours, after which the levels remain constant. The mice used in this series of experiments were fasted in order to increase the absorption of acetaminophen. The mice were sacrificed between the hours of 10 AM and 2 PM, the time that the level of hepatic GSH is at its zenith in the diurnal cycle (Jaeger et al., 1973). At least partly due to its glutathione lowering effect, fasting also affects the toxicity of various xenobiotics (Pessayre et al., 1980; Lauterberg and Mitchell,

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1982; Plummer et al., 1981). In Experiments 2 and 3, the significant treatment effects were due to the effect of the acetaminophen alone. No induction of microsomal oxidation by ethanol seems to have occurred although such induction has been reported widely in the literature (Banda and Quart, 1984; Moldeus et al., 1980; McClain et al., 1980; Coon et al., 1984; Teschke et al., 1979; Peterson et al., 1980; Licht et al., 1980; Walker et al., 1983; Charles River Breeding Laboratories, 1976; Strubelt et al., 1978). Depletion of GSH due to the effect of ethanol itself has also been reported (Fernandez and Videla, 1981). This is thought to be due to acetaldehyde, a metabolite of ethanol, because inhibition of alcohol dehydrogenase eliminates the observed depletion.

Table 4. Exp. 2. Analysis of variance of glutathione and plasma oxalo-acetic transaminase

	GSH		PGOT	
Source	F Value	PR > F	F Value	PR > F
TRT	7.55	.0125	91.77	.0001
TIME	1.48	.2525	.85	.4441
TRT*TIME				

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Table 5. Exp. 2. Average glutathione and plasma oxaloacetic transaminase values

GSH (umoles/g liver)				PGOT (U/L)			
TRT[1]		TIME (hr)		TRT[1]		TIME (hr)	
1.	6.52[a2]	2.0	6.39[a2]	1.	437.6[a2]	2.0	495.6[a2]
2.	4.61[b]	2.5	6.04[a]	2.	1052.9[b]	2.5	663.0[b]
		3.0	5.32[a]			3.0	424.9[a]

[1]Treatment one = 10% EtOH only, treatment two = 10% EtOH + pHAA.

[2]Means with same letter are not significantly different at $P \leq .05$.

Table 6. Exp. 2. Glutathione: Plasma oxaloacetic transaminase correlation coefficients (r)

TRT		TIME (hr)			
1.	.049	2.0	.635	3.0	.884
	(.846)[a]		(.175)		(.310)
2.	-.482	2.5	-.489		
	(.333)		(.064)		

[a]PR > F appears under correlation coefficient (r).

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Effect on PGOT

In Experiment 1, highly significant treatment effects and significant time effects were observed (Table 1). The treatment significance was due to the 10% ethanol + 0 group, the pHAA + H_2O and pHAA + 10% ethanol groups were not significantly different (Table 2; Figure 6). Only the 18 hr time was significantly different from all other times. This was due to the elevation of PGOT after administration of pHAA, which elevation is maximal at 24 hr (Dixon et al., 1975).

In Experiment 2, there was a highly significant treatment effect but no significant time effect (Table 5; Figure 8). Again, as in Experiment 1, the treatment significance was due to the presence of acetaminophen since both groups were treated with 10% ethanol in this experiment (Table 6).

In Experiment 3, highly significant time and treatment effects were observed (Table 9; Figure 7). The time effect observed in this experiment but not in Experiment 2 was probably due to the method of acetaminophen administration; ad libidium in drinking water in Experiment 2 as opposed to intraperitoneal (i.p.) injection in Experiment 3. The i.p. injection allowed smaller time interval measurements after administration and also a constant dosage per animal. The treatment effect observed, as in the two previous experiments was due to the 10% ethanol + 0 group (Table 10).

The (0.5 hr, 0 hr) and (0.5 hr, 1.0 hr) times were not significantly different. The 2 hr time was significantly different from all others (Table 10).

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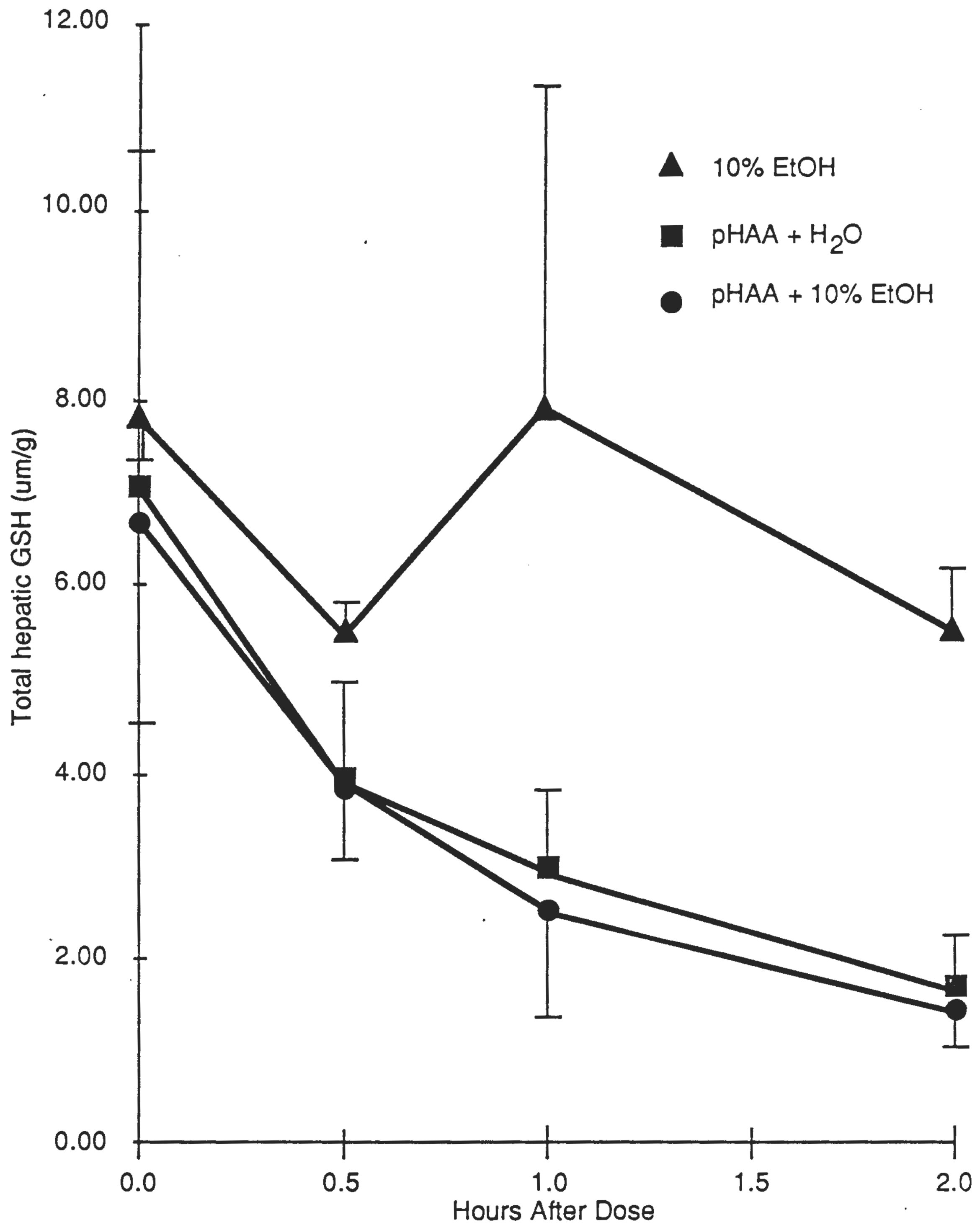

Figure 8. Time-dependent depletion of hepatic glutathione after administration of a 750 mg Kg^{-1} i.p. dose of acetaminophen

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Table 7. Exp. 3. Analysis of variance of glutathione and plasma oxaloacetic transaminase

	GSH			PGOT		
Source	df	F Value	PR > F	df	F Value	PR > F
TRT	2	17.60	.0001	2	11.03	.0002
TIME	3	13.53	.0001	3	11.23	.0001
TRT*TIME	6	1.97	.0981	6	1.90	.1131

Table 8. Exp. 3. Average glutathione and plasma glutamic oxaloacetic transaminase values

GSH (umoles/g liver)				PGOT (U/L)			
TRT[1]		TIME (hr)		TRT[1]		TIME (hr)	
1.	3.67[a2]	0	7.26[a]	1.	1838.8[a]	0	652.3[a]
2.	6.74[b]	0.5	4.43[b]	2.	830.1[a]	0.5	1392.8[a,b]
3.	3.64[a]	1.0	2.86[c]	3.	2206.4[a]	1.0	1603.3[b,c]
		2.0	4.33[b,d]			2.0	2644.8[d]

[1]Treatment one = H_2O + pHAA, treatment two = 10% EtOH only, treatment three = 10% EtOH + pHAA.

[2]Means with same letter are not significantly different at $P \leq .05$.

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Table 9. Exp. 3. Glutathione: Plasma glutamic oxaloacetic transaminase correlation coefficients (r)

TRT		TIME (hr)			
1.	-.662	0	-.014	1.0	-.651
	(.009)[a]		(.968)		(.030)
2.	-.174	0.5	-.957	2.0	-.427
	(.552)		(.0002)		(.166)
3.	-.626				
	(.017)				

[a]PR > F appears under correlation coefficient (r).

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A negative correlation between GSH and PGOT levels was expected in the above experiments. Serum transaminase levels are thought to give a reliable index of the extent of cellular damage (Dixon et al., 1975; Buttar et al., 1976). Because the electrophilic metabolite of acetaminophen would tend to decrease glutathione levels acutely and thus increase the extent of hepatic necrosis over time, serum enzyme activity was postulated to increase over time and thus be negatively correlated to the level of hepatic glutathione.

Negative correlations were observed for both treatment and time in Experiments 1 and 3, but only for one time period in Experiment 2. Although negative correlations were observed in Experiment 1, they were not significant. Only in Experiment 2 were significant correlations observed.

Effect of Diet and Fasting

The ethanol treated mice ingested significantly less fluid than their water treated counterparts (Figures 9-12). This may be due to an aversion to ethanol or to its caloric content. In Experiment 1, food consumption was approximately the same for both groups, but in Experiment 3 food consumption was significantly different (Figures 11 and 12). This may have been due to the one-week acclimatization period used in Experiment 1. Fasted animals weighed an average of 4.02 g less than non-fasted ones, a statistically significant difference (Table 13). Liver weight for the ethanol treated group was significantly higher than the water treated group in Experiment 3.

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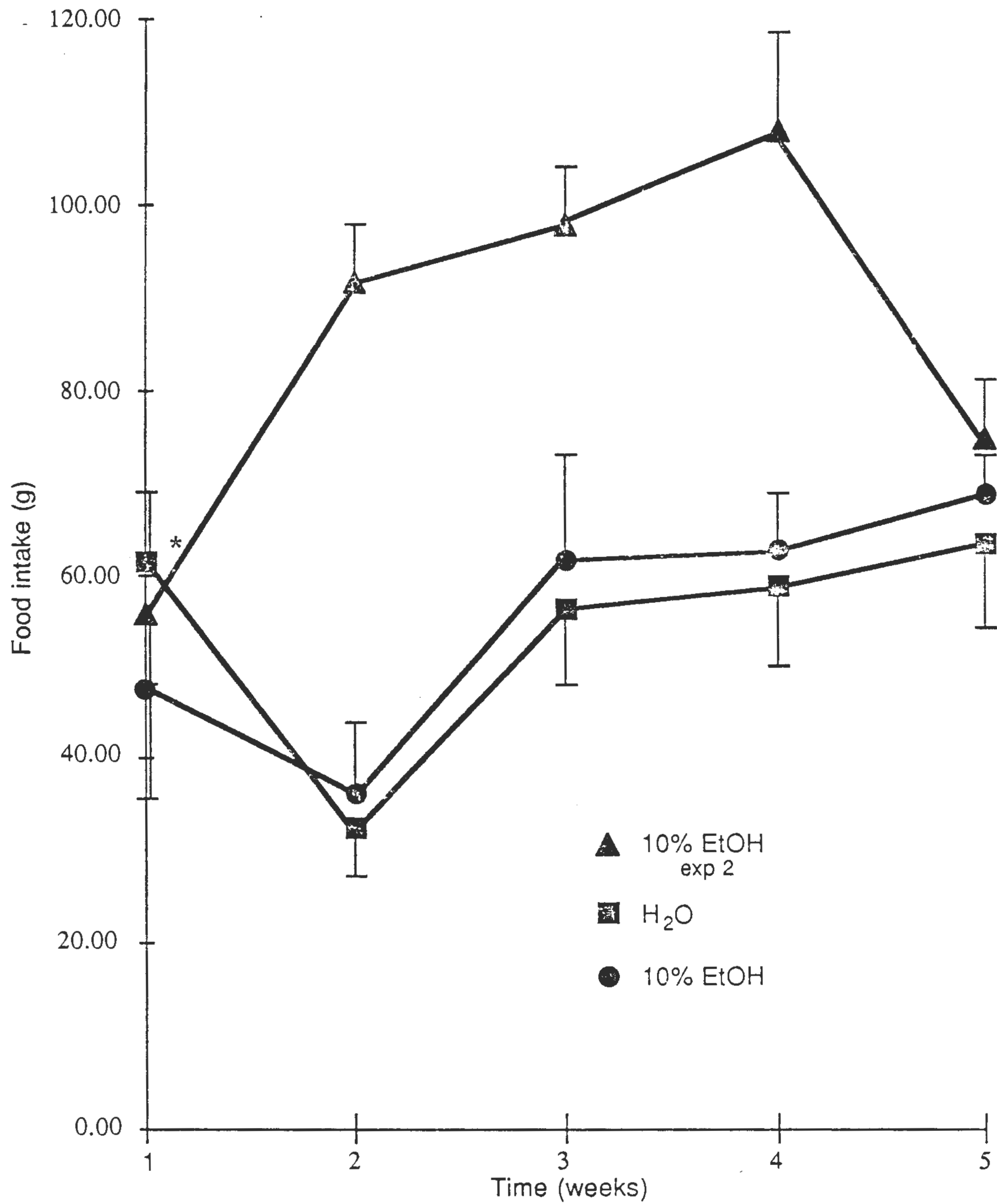

Figure 9. Food intake comparisons of water and ethanol treated animals (Exp. 1 and 2) (*=Significant difference from H_2O group $P \leq .05$)

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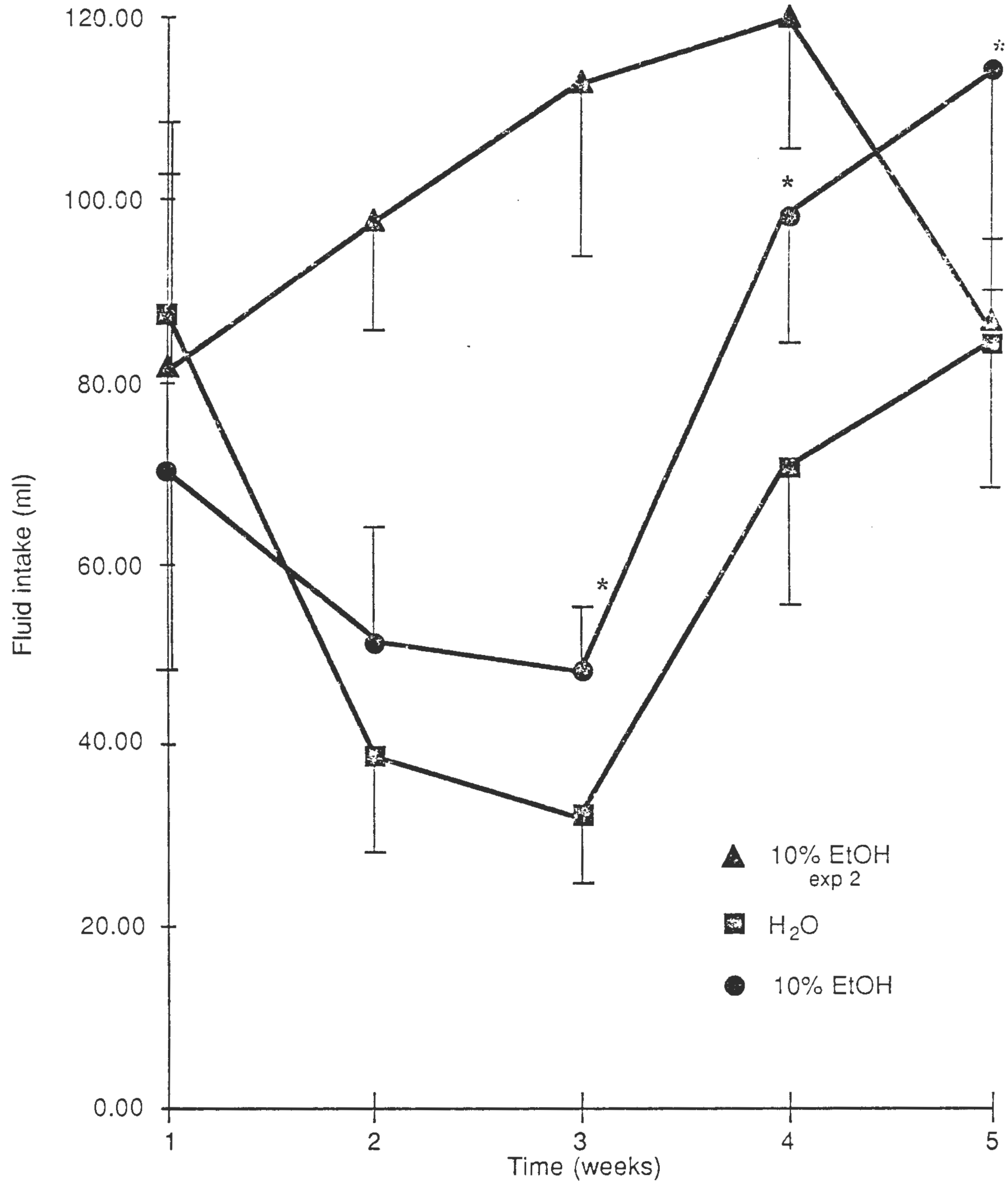

Figure 10. Fluid intake comparisons of water and ethanol treated animals (Exp. 1 and 2) (*=Significant difference from H_2 group $P \leq .05$)

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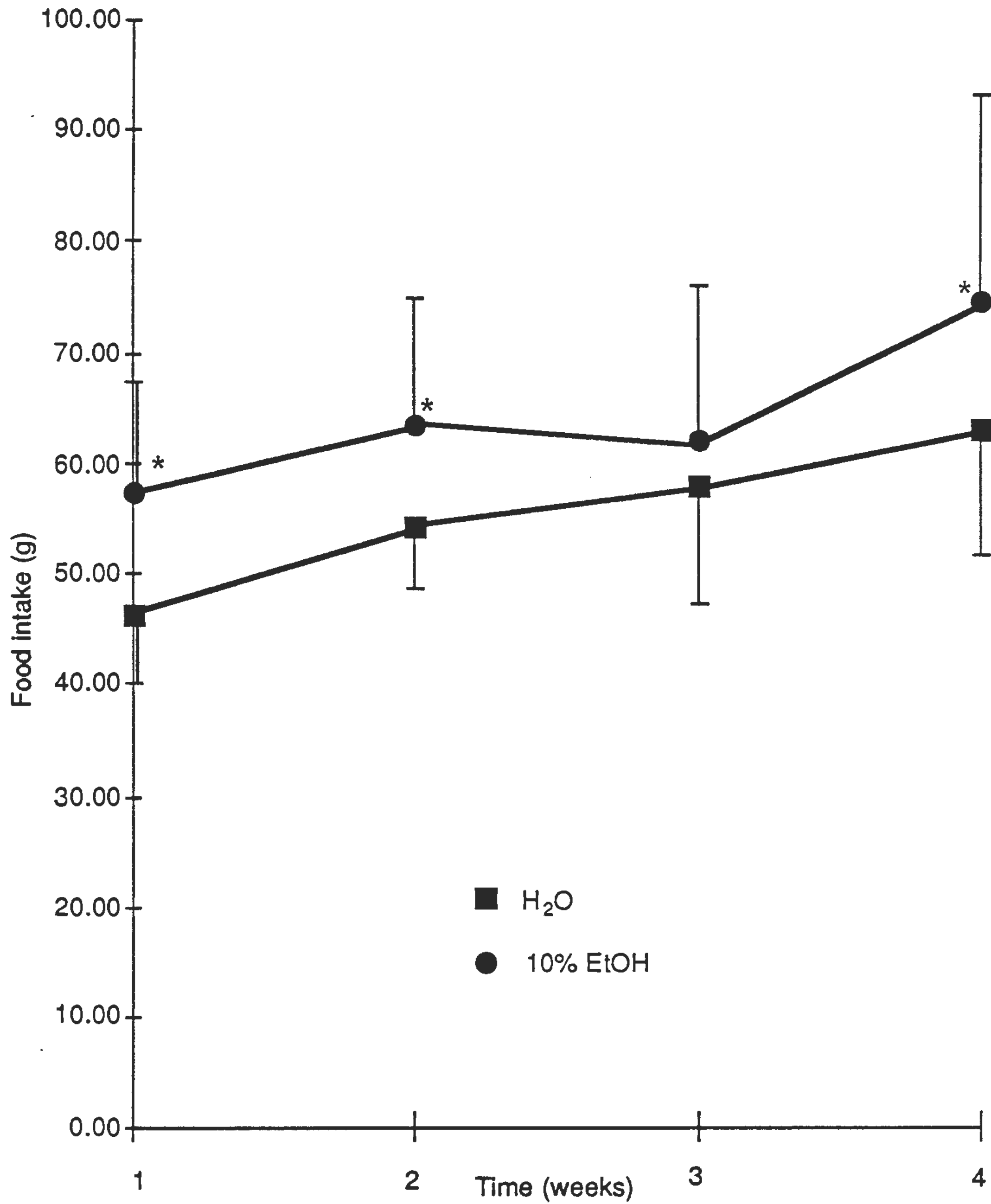

Figure 11. Food intake comparisons of water and ethanol treated animals (Exp. 3) (*=Significant difference from H_2O group $P \leq .05$)

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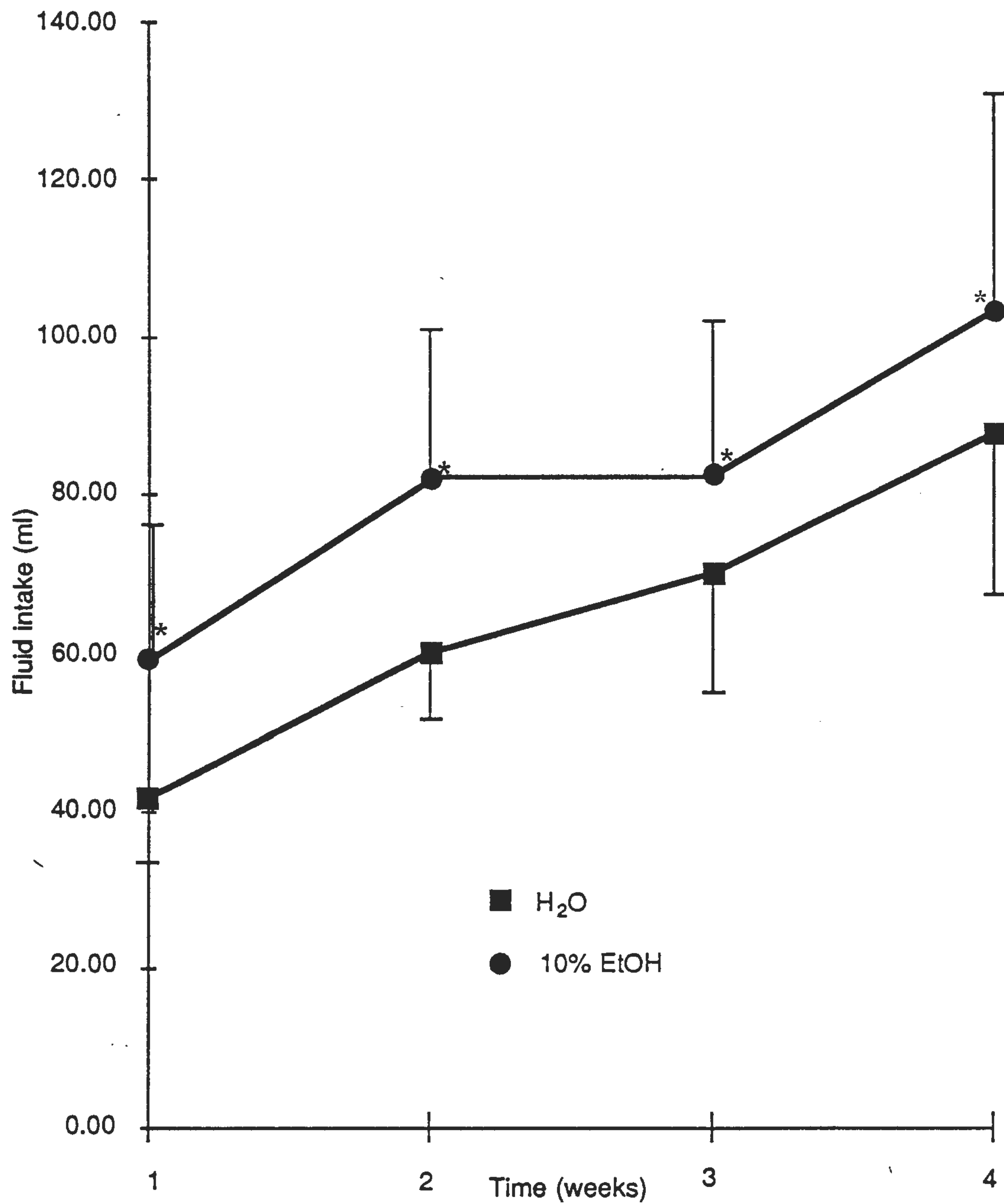

Figure 12. Fluid intake comparisons of water and ethanol treated animals (Exp. 3) (*=Significant difference from H_2 group $P \leq .05$)

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Table 10. Exp. 1. Total hepatic glutathione content and plasma glutamic-oxaloacetic transaminase activity after ad lib. acetaminophen dose 1000 mg/kg/3 ml[a]

Treatment condition	Time (hr) after administration	GSH umoles/g liver[b] wet weight	PGOT (U/L)[b]
1. H_2O + pHAA	4	2.63 ± .69 (2)	275.3 ± 927.9 (2)
	6	4.16 ± .77 (2)	1700.5 ± 2234.3 (2)
	10	2.98 ± .38 (2)	2709 (1)
	18	5.92 ± .54 (2)	6793.7 ± 987.7 (2)
2. 10% EtOH	4	4.67 ± .24 (2)	147.6 ± 29.9 (2)
	6	5.50 ± .71 (2)	166.7 ± 47.1 (2)
	10	4.04 ± .57 (2)	761.9 ± 119.7 (2)
	18	5.27 ± .18 (2)	248.7 ± 37.4 (2)
3. 10% EtOH+pHAA	4	.92 ± .65 (2)	1746 (1)
	6	1.07 ± .47 (2)	2761.9 ± 2110.1 (2)
	10	1.64 ± (1)	3174.6 (1)
	18	4.95 ± .25 (2)	4634.9 ± 269.4 (2)

[a]Avg. dose for treatment one = 1.57 ± .29 ml (521.7 mg/kg), avg. dose for treatment two = 3.00 ml 10% EtOH, avg. dose for treatment three = 2.32 ± ml (771.7 mg/kg).

[b]Each value is the mean ± the standard error of the mean of n observations. Numbers in parentheses are the number of observations.

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Table 11. Exp. 2. Total hepatic glutathione content and plasma glutamic oxaloacetic transaminase activity after ad lib. acetaminophen dose 1000 mg/kg/3 ml[a]

Treatment condition	Time (hr) after administration	GSH umoles/g liver[b] wet weight	PGOT (U/L)[b]
1. 10% EtOH	2.0	6.39 ± .88 (6)	495.6 ± 89.0 (6)
	2.5	7.00 ± 1.65 (9)	403.1 ± 113.9 (9)
	3.0	5.33 ± 1.02 (3)	424.9 ± 149.1 (3)
2. 10% EtOH+pHAA	2.5	4.61 ± 1.77 (6)	1052.9 ± 191.5 (6)

[a]Avg. dose for treatment one = 1/58 ± .97 ml 10% ethanol, avg. dose for treatment two = 1.25 ± .35 ml (416.7 mg/kg).

[b]Each value is the mean ± the standard error of the mean of n observations. Numbers in parentheses are the number of observations.

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Table 12. Exp. 3. Total hepatic glutathione content and plasma glutamic-oxaloacetic transaminase activity after ad lib. acetaminophen dose of 750 mg/kg body weight

Treatment condition	Time (hr) after administration	GSH umoles/g liver[a] wet weight	PGOT (U/L)[a]
1. H_2O + pHAA	0	7.07 ± .32 (3)	892.5 ± 199.2 (3)
	0.5	3.86 ± 1.13 (4)	2520.5 ± 1568.8 (3)
	1.0	2.93 ± .84 (4)	1627.4 ± 307.7 (4)
	2.0	1.67 ± .55 (4)	2248.7 ± 347. (4)
2. 10% EtOH	0	7.79 ± 2.90 (4)	265.8 ± 76.7 (4)
	0.5	5.43 ± .37 (3)	515.9 ± 138.1 (4)
	1.0	7.89 ± 3.49 (4)	768.1 ± 41.5 (3)
	2.0	5.52 ± .67 (4)	1755.1 ± 1151.9 (4)
3. 10% EtOH+pHAA	0	6.68 ± 2.13 (4)	858.6 ± 202.0 (4)
	0.5	3.86 ± .76 (3)	1455.0 ± 37.4 (2)
	1.0	2.48 ± 1.10 (4)	2205.6 ± 651.5 (4)
	2.0	1.39 ± .39 (4)	3930.6 ± 1803.0 (4)

[a]Each value is the mean ± standard deviation of the mean of n observations. Numbers in parentheses are the number of observations.

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Table 13. Effect of diet and fasting

Treatment	Avg. wt. gain	Avg. wt at termination	Liver wt.
Exp. 1			
EtOH	3.69 ± 3.48	33.25 ± 5.27	1.60 ± .38
H_2O	8.13 ± 2.64	29.88 ± 1.89	1.40 ± .27
Exp. 2			
EtOH	23.52 ± 2.96	34.51 ± 3.28	1.45 ± .26
Exp. 3			
EtOH	10.55 ± 2.54	32.42 ± 2.60	1.40 ± .13*
H_2O	10.00 ± 2.41	31.67 ± 3.67	1.28 ± .15
Nonfasted		32.27 ± 2.79	
Fasted		28.07 ± 2.75**	

*Means significantly different at $P \leq .01$.

**Means significantly different at $P \leq .001$.

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SUMMARY

The results from this study confirm an inverse relationship between liver GSH levels and PGOT activity after administration of an acute high dose of acetaminophen.

No evidence for increased susceptibility to acetaminophen-induced hepatic necrosis by ethanol was observed. Ethanol treated animals tended to ingest less fluid and consume less solid food than water treated animals. However, body weights at termination of the experiments and average weight gain were not significantly different and thus are not thought to have influenced data.

Onset of changes in the levels of GSH and PGOT as influenced by acetaminophen dosage was noted to occur between 0 minutes and 30 minutes of dosing.

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ACKNOWLEDGEMENTS

Appreciation is given to Dr. J. N. Hathcock for his advice, guidance, and for stimulating my interest in toxicology.

I am grateful to Cynthia Shriver for her technical assistance in the laboratory.

Special thanks are given to the members of the Ames United Pentecostal Church; my brothers and sisters in Christ (Eccl. 4:9,10).

Deepest appreciation is expressed to the members of my family: my parents Tom and Mary Pope, Lucious and Ruby Chieves; and my brothers Michael, Richie, William, and Ron Chieves.

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