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16. Abstract (Limit: 200 words) The effect of changing concentrations of glutathione (GSH) in the male reproductive tract was investigated using sexually mature male Sprague-Dawley-rats sacrificed at 1, 2, 4, 8, or 16 hours following administration of various test compounds selected for their ability to perturb GSH levels. GSH content was determined in the liver, testes, and epididymides. GSH levels in all tested organs were significantly reduced following administration of isophorone (78591) (500mg/kg), phorone (504201) (250mg/kg), and diethylmaleate (500mg/kg). Isophorone treatment resulted in a significantly enhanced binding of tritium labeled ethyl-methanesulfonate (3H-EMS) to sperm heads. Hepatic and epididymal GSH was reduced following trimethyl-phosphate (512561) (600mg/kg), naphthalene (91203) (500mg/kg), and methyl-iodide (74884) (100mg/kg) administration, but no change was noted in testicular levels. Hepatic GSH was reduced following pentachlorophenol (87865) (25mg/kg) and acetaminophen (103902) (1500mg/kg) exposures, but no changes were apparent in reproductive tract GSH. In an additional study, rats exposed to phorone (250mg/kg) plus EMS showed a significant increase in the mortality of fetal implants during the third week, suggesting that depletion of GSH pools prior to EMS administration potentiated EMS induced dominant lethal clastogenesis. The authors suggest that chemically induced lowering of GSH in the male reproductive tract may be a mechanism for potentiation of chemically induced germ cell mutations. Subsequent tests indicated that treatment with acivicin plus N-acetyl-cysteine served to enhance the levels of GSH in reproductive tissue in-vivo. Studies are being conducted to determine whether this increase will offer protection against the mutagenic effects of ethyl-methanesulfonate.

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Small Grants for New Investigators

Epididymal GSH in Chemical-induced Germ Cell Mutations

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ABSTRACT

The importance of glutathione as a protective mechanism has been clearly established within the body, but little attention has been given to the reproductive tract where chronic toxicity or chemically-induced heritable changes could have serious consequences. The goal of this project was to investigate the effects of altering glutathione in the male reproductive tract. The study set out to identify chemicals capable of altering reproductive tract GSH and to establish a link between chemical-induced reduction of glutathione in the male reproductive tract and increased susceptibility of spermatozoa to chemical-induced mutations.

A number of chemicals were tested for their ability to perturb glutathione levels in the testes and epididymides as well as liver. Isophorone (500 mg/kg), phorone (250 mg/kg) and diethylmaleate (500 mg/kg) significantly reduced glutathione in the liver and in both reproductive organs examined. Trimethyl phosphate (600 mg/kg), naphthalene (500 mg/kg), and methyl iodide (100 mg/kg) affected only hepatic and epididymal glutathione, but had no effect on testicular levels. Pentachlorophenol (25 mg/kg) and acetaminophen (1500 mg/kg) significantly depressed hepatic glutathione but had no effects on reproductive tract glutathione. Also investigated was the ability of isophorone to enhance the covalent binding of tritiated ethyl methanesulfonate (^{3}H-EMS) to spermatocytes. Perturbation of reproductive tract glutathione by isophorone treatment significantly enhanced the extent of ^{3}H-EMS-induced binding to sperm heads. The temporal pattern of ethylations in sperm heads was consistent with the stage of sperm development known to be susceptable to ethylations by EMS. Phorone was tested for its ability to potentiate the dominant lethal effects of EMS. Male Sprague Dawley rats were exposed to either corn oil, phorone (250 mg/kg), EMS (50 mg/kg), or phorone (250 mg/kg) plus EMS (50 mg/kg) challenge for hours later. The combined phorne-EMS group caused 48% of the fetal implants to die in utero during week 3 compared to 13 %, 12%, and 16% of the controls (significance, $p<0.05$). These results indicate that depletion of GSH pools prior to EMS

administration potentiates EMS-induced dominant lethal clastogenesis. Therefore, chemical-induced lowering of glutathione in the male reproductive tract may be a mechanism for potentiation of chemical-induced germ cell mutations.

In order to determine the level of protection afforded by GSH, we evaluated various compounds for their ability to enhance reproductive tissue GSH levels *in vivo* . Compounds evaluated included N-Acetyl Cystiene (NAC) (250 mg/kg, every 2 hrs), L-2-oxothiozolidine-4- carboxylate (OCA) (1mM/kg), or AT-125 plus NAC (ANAC) (20 mg/kg AT-125 with 250 mg/kg NAC given every 2hrs) in male Sprague Dawley rats, ip, followed by measurement of hepatic and reproductive tract GSH. Of the compounds tested, ANAC was the most efficacious. Increases were liver-26.4%, caput-89.7%, cauda-35.6%, testis-10.4%.

INTRODUCTION

The importance of hepatic reduced glutathione in protecting against chemically induced injury is well established (Larsson et al., 1983; Orrenius & Moldeus, 1984; Kaplowitz et al., 1985). Significant depression of hepatic glutathione can markedly increase the chemical insult induced by compounds that employ GSH-conjugation as a major component in their detoxication. Glutathione conjugation is also recognized as a major detoxification mechanism in other organs such as kidney and lung (Kuo and Hook, 1982; Boyd, 1980)

The presence of all components of the GSH-mediated detoxication pathway has been demonstrated in male reproductive tissues. These components include GSH (Li, 1975), GSH-S-transferases (Kraus and Kloft, 1980; Hales et al., 1980; Dierickx and Asnong, 1982; Eidne and Kirsch, 1982 Guthenberg et al., 1983; Ketterer et al., 1983) GSH peroxidase (Brown et al., 1977), GSH reductase (Teaf et al., 1985), and gamma-glutamyl transpeptidase (DeLap et al., 1977). Therefore, it is likely that a protective role may also exist for GSH in reproductive tissues. Compromise of epididymal or testicular GSH-dependent protective mechanisms may lower the threshold for germ cell mutagenicity in a manner analogous to pretreatment regimens which deplete renal or hepatic GSH prior to administration of nephrotoxicants or hepatotoxicants (Mitchell et al., 1974).

Recently, Teaf et al. (1987) reported significant findings demonstrating a role for GSH in male reproductive tissues in protecting against chemically induced germ cell mutagenesis. Administration of buthionine sulfoximine (BSO) reduced testicular,

epididymal and vas deferen GSH levels in the Fisher 344 rat. Reduction of reproductive tract GSH was found to potentiate dominant lethal mutations induced by the well characterized germ cell mutagen, ethyl methanesulfonate (EMS). EMS is known to undergo GSH-dependent metabolism and excretion (Chasseaud, 1979; Roberts and Warwick, 1958). In addition, reduction of reproductive tract GSH by BSO also enhanced the incidence of EMS alkylation of spermatozoa, and this enhancement correlated in a dose-dependent and temporal manner with the enhanced EMS-induced dominant lethal mutations.

Studies by Teaf et al. (1985; 1987) indicate that BSO is capable of perturbing reproductive tract GSH, and that this has important functional significance in the protection against germ cell mutagens. Because of this demonstrated protective role of GSH in the male reproductive tract, we have initiated an investigation to see if other chemicals are capable of perturbing this protective mechanism. Therefore, one of the objectives of this study was to further identify additional chemical classes that are capable of altering testicular and/or epididymal glutathione and to characterize the structural requirements for reproductive tract GSH depletion.

Once individual chemicals were identified as capable of perturbing reproductive glutathione, one of these compounds was tested to assess the potential functional consequences of this when animals are exposed to a known mutagen. Phorone was administered in a dominant lethal assay to test if the perturbation of reproductive tract glutathione induced by this compound would affect the extent of dominant lethal mutations induced by the mutagen, ethyl methanesulfonate.

In addition, various chemicals known to enhance hepatic glutathione were tested in different treatment regimens in an effort to increase reproductive tract glutathione levels.

MATERIALS AND METHODS:

Animals:

Sexually mature male Sprague-Dawley rats (400-600g) were used for all experiments. Animals were obtained from the vivarium of the National Center for Toxicological Research, maintained on a 12/12 hour light/dark cycle, and were provided food and water *ad libitum.*

Chemicals and Treatments:

Acetaminophen, methyl iodide, phorone, and isophorone were purchased from Sigma Chemical Co. (St. Louis), and diethylmaleate, pentachlorophenol, naphthalene, and trimethyl phosphate were purchased from Aldrich Chemical Co. (Milwaukee). Treatment compounds were mixed in either saline (0.9%) or corn oil and administered, intraperitoneally, to the male rats between the hours of 8 and 10 a.m. Control animals were administered the appropriate vehicle (2 ml/kg) at the same time. Concurrent control groups were maintained for all time points examined to account for the circadian fluctuations of GSH throughout the day. Each treatment and control group contained at least 4 animals per group.

All enzyme reagents and co-factors used in the assay of GSH were of the highest purity commercially available. Tritiated ethyl methanesulfonate (^{3}H-EMS) was obtained from New England Nuclear (specific activity, 4.8 Ci/mmol).

Sample Preparation and GSH Assay Procedure:

Animals were sacrificed at 1, 2, 4, 8, or 16 hours after

administration of the test compounds. Liver, testes, and epididymides were excised for determination of total GSH content. Tissue samples were immediately placed in ice cold saline (0.9%). Further handling of the tissues was done on ice. The tissues were rinsed, and all fat and connective tissue were carefully removed. The tissues were then blotted, weighed, and placed in 5 volumes (w:v) of ice cold 1% sulfosalicylic acid. The tissues were then homogenized with a Brinkman Polytron at a setting of 7 for 20 seconds, and centrifuged for 20 minutes at 10,000 x g and 4°C.

The spectrophotometric assay used for measuring GSH levels was a modification of the methods of Tietze (1969) and Griffith and Meister (1980). These procedures were adapted for use on an Instrumentation Laboratories Multistat III centrifugal spectrophotometric autoanalyzer. The continuous formation of 2-nitro-5-thiobenzoic acid at 30°C was measured at 405 nm. All activities were measured using substrate concentrations sufficiently greater than K_m to ensure a linear reaction.

The measurements were analyzed by regressing the absorbance data from twelve time points from each sample against the time that each absorbance value was measured. Tissue GSH concentrations were determined by regressing the absorbance data against time, determining the slope of the regressed curve, and comparing the slope to a standard curve generated concurrently with each measurement in the autoanalyzer.

Radiolabeled binding of ^{3}H-EMS to sperm heads:

The extent of EMS-induced alkylations of spermatozoa was determined by treating rats, in vivo, with ^{3}H-EMS and measuring the radioactivity bound to sperm heads (Teaf et al., 1987). Male rats were given a pretreatment of isophorone (500 mg/kg), followed by a

four-hour post-treatment challenge dose of ^{3}H-EMS (50 mg/kg; 1.34 mCi/kg). Control animals were treated with saline (2 ml/kg) followed by the challenge dose of ^{3}H-EMS. At various time points after treatment, animals were sacrificed and caudal epididymal sperm cells were isolated by mincing the cauda for 2 minutes in 3 ml of 0.9% NaCl/10 mM EDTA. Purified sperm heads were then isolated by differential centrifugation by the method of Sega et al. (1974) as modified by Teaf et al., (1987). A 100-µl aliquot of the isolated sperm heads was solubilized in 1.0 ml of NCS tissue solubilizer for 24 hours and neutralized with 30 µl glacial acetic acid. Fifteen ml of scintillation fluid was added, and the samples were counted for bound radioactivity in a Packard Tricarb 300 liquid scintillation counter (Teaf et al., 1987).

Dominant lethal mutation assay:

Phorone was used as a pretreatment to alter glutathione levels prior to the administration of the mutagen, ethyl methanesulfonate. Three groups of 10 male rats were exposed to ethyl methanesulfonate at 50 mg/kg four hours after exposure to either 25, 100, or 250 mg/kg of phorone, i.p. Three control groups of 10 male rats each were exposed to either corn oil (vehicle), 250 mg/kg phorone or 50 mg/kg EMS. On the eighth day after exposure, a female was placed with each male for the duration of one estrous cycle (5 days). Two days later a second female was placed with each male for one estrous cycle. The described method of mating is a balance which maximizes the number of successful matings while limiting the range of post-meiotic age of spermatozoa represented by the data. This limitation is important because the dominant lethal test as conducted is sampling a differential susceptibility of stages of spermatozoa to chemical mutagens and must present discrete

intervals as data points. Because EMS is known to induce its dominant lethal effects only in mating weeks 2 and 3 post treatment, only these mating periods were tested. Eighteen days after being placed with a male, each female was sacrificed and the corpora leutea and implant status was determined.

GSH Enhancement:

Enhancement of male reproductive tract GSH was attempted through chemical inducement of the production of GSH by the introduction of large quantities of the precursors of GSH. This was attempted by one of the following methods:

1. Increasing sulfhydryl donors through pretreatment with cysteine or methionine and as a second method with N-acetyl cysteine. Male rats received three doses of N-acetyl cysteine (250 mg/kg) at 0, 2 and 4 hours and were sacrificed at 6 hours (Hill et al., 1985).

2. Increasing the activity of GSH synthetic reactions by providing 2-oxo-thiazolidine-4-carboxylate (OCA) as a substrate for 5-oxyprolinase. Male rats were treated with 250 mg/kg OCA, i.p., and animals were sacrificed after 4 hours (Williamson et al., 1982).

3. Inhibiting the gamma-glutamyl transpidase cycles with AT-125 (i.e. Acivicin) to prevent chemical depletion of GSH. Acivicin was used in conjuction with N-acetyl cysteine (NAC) using the treatment regimen of Hill et al. (1985). Six male rats received a 25 mg/kg dose, i.p. of Acivicin followed by a 250 mg/kg dose, i.p., of NAC every 2 hours starting at time 0. Six control animals received an equal volume of saline every 2 hours. Tissues were removed, processed and measured for glutathione content as previously described above.

After the optimum compound for GSH enhancement was

determined, dose-response experiments were conducted to determine the optimum dosing regimen for GSH enhancement and protection in the male reproductive tract.

Data of GSH measurements were analyzed by analysis of variance followed by Duncan's multiple range test using the SAS® system, while radiolabeled binding data were analyzed by Student's-t test (Steel and Torrie, 1960). A p value less than 0.05 was considered significant.

RESULTS:

The chemicals tested by single i.p. injection for their ability to deplete reproductive tract GSH were trimethyl phosphate, naphthalene, pentachlorophenol, isophorone, phorone, acetaminophen, methyl iodide, and diethylmaleate. Initial dosages for each compound were chosen based on literature reports and personal experience. Isophorone (500 mg/kg), phorone (250 mg/kg), and diethylmaleate (500 mg/kg) significantly ($p \leq 0.05$) reduced GSH in the testes and epididymis, as well as the liver. Isophorone maximally reduced hepatic and testicular glutathione by 4 hours after administration, while maximal reduction of epididymal GSH was detected at 8 hours (Figure 1a). The extent of GSH reduction in liver, testes and epididymis at these time points of maximum effect was 40%, 82%, and 72% of concurrent control values, respectively. Phorone maximally reduced hepatic, epididymal and testicular glutathione at 2 and 4 hours (Figure 1b). The difference between the 2 and 4 hour time points for the three tissues was not statistically significant. The maximally depressed values of liver, epididymis and testis GSH following phorone administration

were 6%, 63%, 24% of control values, respectively. The effects of diethylmaleate (500 mg/kg) on hepatic and reproductive tract GSH were followed through 4 hours. At this time, GSH levels were 1%, 7%, and 51% of concurrent control values, respectively (Figure 1c).

Trimethyl phosphate (600 mg/kg), naphthalene (500 mg/kg), and methyl iodide (100 mg/kg) reduced only hepatic and epididymal GSH. Trimethyl phosphate induced maximal effects on hepatic GSH at 8 hours (53% of control), while maximum reduction of epididymal GSH was measured at 16 hours (62% of control) (Figure 2a). Naphthalene administration produced a reduction of epididymal GSH of up to 53% of control at 4 hours, while hepatic GSH was depressed to 11% of control at 16 hour (Figure 2b). Methyl iodide at 100 mg/kg lowered hepatic GSH to levels that were not detectable at 1-hour after administration, and hepatic levels remained significantly depressed through 8 hours. However, only the 1-hour measurement of epididymal GSH showed significant depression (Figure 2c).

Pentachlorophenol (25 mg/kg) and acetaminophen (1500 mg/kg) both significantly depressed hepatic GSH levels, but neither compound significantly perturbed GSH levels in either the epididymis or testes (Figure 3a & b). Lower doses of acetaminophen (500 and 1000 mg/kg) also had no effect on reproductive tract glutathione levels, although significant depression of hepatic glutathione was measured after 16 hours (data not shown).

Radiolabeled binding of ^{3}H-EMS to sperm heads:

The effects of isophorone pretreatment on EMS-induced alkylations in sperm heads were investigated at 2 hr, 1, 8, and 15 days following a challenge administration of ^{3}H-EMS. Treatment with isophorone (500 mg/kg) 4 hours prior to administration of ^{3}H-EMS significantly increased the binding of labeled material to

isolated caudal sperm heads when measured 8 days post-treatment (Figure 4). At the other time points, the extent of ^{3}H-EMS binding was no different in sperm heads from animals pretreated with either saline or isophorone.

Dominant Lethal Assay:

The effects of chemical-induced reduction of reproductive tract glutathione on EMS-induced dominant lethal mutations was investigated at mating weeks 2 and 3. Experiments described above revealed that phorone was efficacious in perturbing both hepatic and reproductive glutathione levels; therefore, this compound was chosen as the pretreatment chemical for glutathione reduction. Corn oil, and phorone (250 mg/kg) alone produced from 7 to 15 percent loss of viable fetuses at both mating week 2 and mating week 3 (Table 1). Likewise, EMS treatment (50 mg/kg) alone did not result in a significantly greater percent loss than the corn oil control groups, indicating that this is a subthreshold dosage for EMS-induced dominant lethal mutations in the Sprague-Dawley rat. However, when phorone was administered as a pretreatment and followed by EMS challenge, significantly greater loss of viable fetuses were measured at the week 3 mating period. This was measured regardless of the dose of phorone pretreatment (25, 100 or 250 mg/kg).

GSH Enhancement:

Treatment with NAC or OCA alone did not result in an increase in the glutathione levels in the reproductive tract or in hepatic tissue (data not shown). However, the regimen for Acivicin plus NAC (ANAC) showed significant increases as shown in Figure 5 & 6. A dose response experiment was performed to determine the minimum dose that would result in maximum glutathione enhancement. The NAC

dose level remained constant at 250 mg/kg every two hours while the Acivicin dose was varied among different treatment groups (10, 20, and 30 mg/kg). The 20 mg/kg dose of Acivicin produced the highest response in the caput and cauda, which are the primary tissues of concern (Figure 7).

Using the 20 mg/kg dose of Acivicin, a time course study was completed to examine the temporal pattern of GSH enhancement in the reproductive tissues. Each animal was treated, i.p., with Acivicin (20 mg/kg) plus 250 mg/kg NAC every 2 hours until sacrifice at 3, 9 12 or 15 hours. The 9-hour time point provided the maximum increase in glutathione concentration in the caput (Figure 8), which is where sperm cells reside that are in a stage of development susceptible to EMS (Sega and Owens, 1978). It was noted at the 9-hour sacrifice time that the animals were not absorbing the i.p. injections well.

DISCUSSION

Many cell types in the testes, especially the spermatogenic epithelium, are protected by the blood-testis-barrier (BTB). The BTB is anatomically and functionally similar to the blood-brain-barrier and acts to restrict the transfer of compounds to the spermatogenic epithelium. However, major portions of the male reproductive tract, such as the rete testis and epididymis, are not protected by a BTB. Therefore, because of higher accessibility in these regions of the reproductive tract, germ cells in these areas may be more severely affected by xenobiotics, thereby accentuating the potential importance of testicular and epididymal metabolic protective mechanisms. It has been suggested

that reproductive tract glutathione plays a central role in protecting germ cells from chemical insult. However, currently there is a paucity of data concerning the effect of chemicals on the epididymis and the ultimate consequences to spermatozoa.

Previous work from our group has demonstrated the presence and differential localization of GSH in testis, epididymis, and vas deferens of the male rat and confirmed the longitudinal variation in specific activity of the GSH-S-transferases in epididymis (Teaf et al., 1984; 1985). Additionally, it has been demonstrated that GSH may be perturbed in these tissues both by direct chemical depletion and by inhibition of synthesis (Teaf, et al., 1987; Gandy et al., 1987). Therefore, in the current study we have investigated a number of compounds for their ability to perturb reproductive tract glutathione levels. The compounds tested were chosen because they 1) are known to have a great affinity for sulfhydryl groups in biological systems, or 2) the parent compound or one of its intermediate metabolites are reactive electrophiles that will react with GSH and the sulfhydryl groups on the enzymes of the GSH system.

Three of the compounds tested resulted in significant depression of both testicular and epididymal GSH levels. These compounds, isophorone, phorone, and diethylmaleate, generally induced a depression of GSH in the target tissues shortly after administration, usually within 2 to 4 hours. This is consistent with the fact that these compounds distribute quickly and are reactive electrophiles that directly conjugate with glutathione without requiring metabolic activation.

Three of the compounds produced a differential effect on GSH levels in the reproductive tract. Trimethyl phosphate,

naphthalene, and methyl iodide significantly depressed epididymal GSH, but had no significant effect on testicular GSH levels. The reasons for this differential lowering of reproductive tract GSH are not known, but it could likely be due to distributional properties of these compounds, which may be influenced by the BTB. However, our current study does not provide insight into this.

Pentachlorophenol and acetaminophen are two compounds that are known to undergo glutathione conjugation in the liver and to significantly reduce hepatic glutathione levels following administration. It is well established that the hepatotoxicity of acetaminophen is directly correlated with glutathione status. Therefore, these compounds were also tested for their effect on reproductive tract glutathione. While both compounds significantly depressed hepatic glutathione levels, neither perturbed glutathione in either the testes or epididymis. Reduction of GSH and the ultimate toxicity produced by these two compounds is dependent on the production of a reactive intermediate (Testa and Jenner, 1976; Hinson 1980). Therefore, it is likely that the reactive intermediate is produced and conjugated in the liver and is not formed in significant amounts in the reproductive tract, nor distributed to the reproductive tract from the site of activation in the liver.

In previous studies (Teaf et al., 1987), the increased incidence of EMS-induced dominant lethal mutations resulting after depletion of reproductive tract glutathione was also correlated with an increase in the extent of EMS-induced alkylations of sperm heads. The temporal pattern of dominant lethal mutation induction by EMS has been has been well correlated with the ethylation of sperm head (Sega and Owens, 1978; 1983). Therefore, one of the

compounds, isophorone, that was effective in reducing both testicular and epididymal GSH, was investigated to see if this compound was also capable of increasing EMS-induced ethylations to sperm heads. The effectiveness of EMS as a germ cell mutagen is greatest at the late spermatid to early spermatozoal stages of germ cell development (Cattanach et al., 1968; Rohrborn, 1970; Sega, 1974). These stages of germ cell development coincide with transit from the rete testes through the epididymides. Spermatozoa in these tissue regions at the time of isophorone/EMS treatment would be available for ejaculation in 2 to 3 weeks (LeBlond and Clermont, 1952). This is consistent with the measurement at day 15 following treatment of higher ethylations in caudal sperm heads from animals receiving isophorone pretreatment and supports the hypothesis that reproductive tract GSH is important in protecting against the clastogenic action of EMS.

Chemical-induced depression of glutathione prior to EMS administration potentiated EMS-induced germ cell mutations. The effectiveness of EMS as a germ cell mutagen is greatest at the late spermatid - early spermatozoal stages of rodent germ cell development. No elevation of mutation rate above control values typically is observed in premeiotic stages (spermatogonia, spermatocytes), except at very high dosages. Modification of the EMS baseline dominant lethal effect by phorone was observed in mating week 3 (day 15-19 post-treatment), indicating effects on those germ cells which were in late testicular stages or were caput epididymal spermatozoa at the time of EMS treatment. This is in agreement with the increased binding of ^{3}H-EMS measured when animals were pretreated with isophorone.

Initial screening for compounds that might enhance glutathione

levels in the reproductive tract focused on two compounds which the literature indicated gave considerable increases in hepatic and plasma glutathione levels. NAC and OCA treatment has been reported to increase hepatic glutathione 1.5 and 2.0 fold, respectively (Hill et al., 1985; Williamson et al., 1982). However, a significant increase was not obtained in this study. Results for all tissues, liver, caput epididymides, cauda epididymides, and testis, were inconclusive.

Acivicin plus N-acetyl cysteine treatment regimen did significantly increase reproductive tract glutathione levels. Experiments are currently underway to determine if this increase offers protection against the mutagenicity induced by ethyl methanesulfonate.

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Figure 1

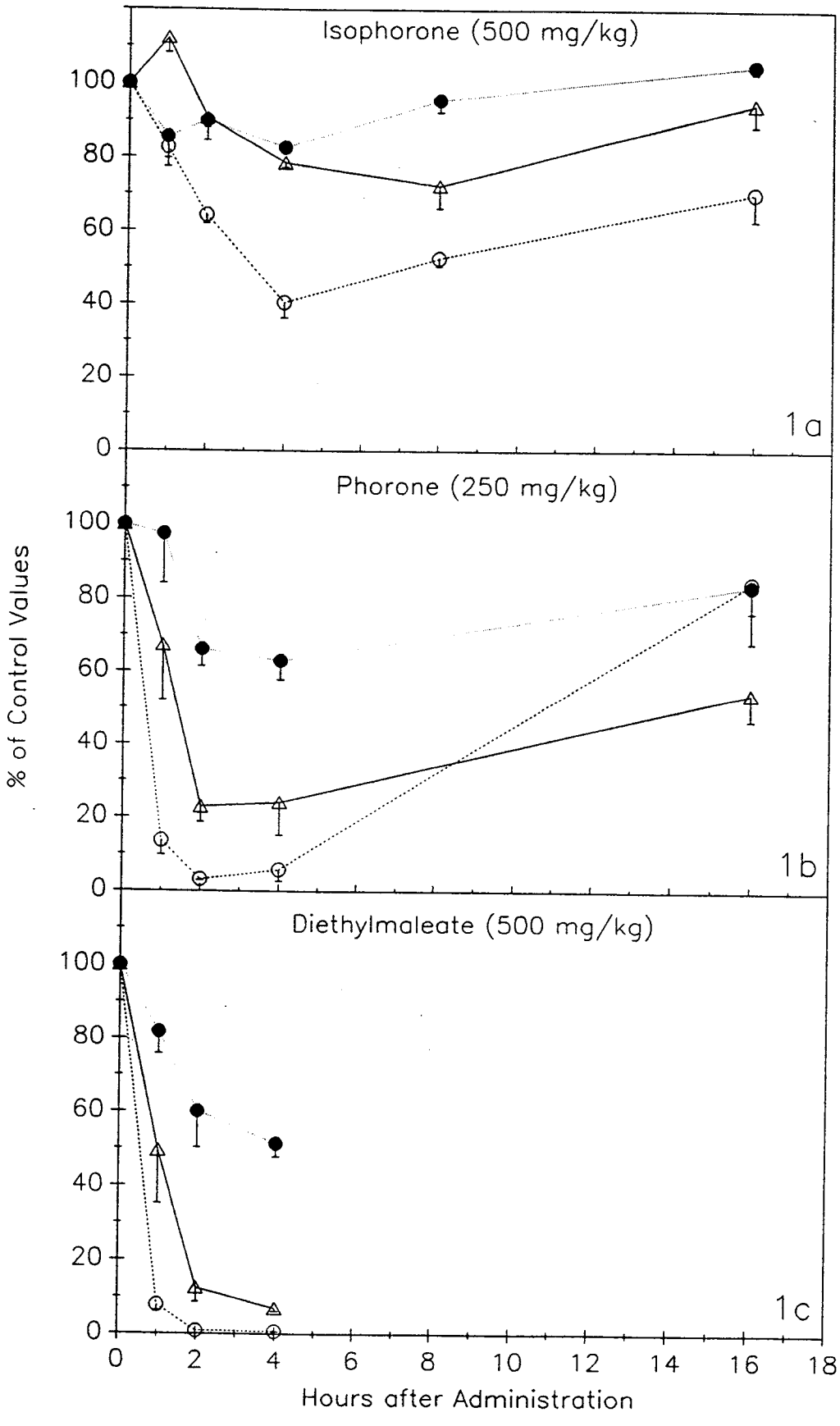

Isophorone (500 mg/kg)
1a
Phorone (250 mg/kg)
1b
Diethylmaleate (500 mg/kg)
1c
% of Control Values
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
18
Hours after Administration
O Liver
Testis
Epididymis

Figure 2

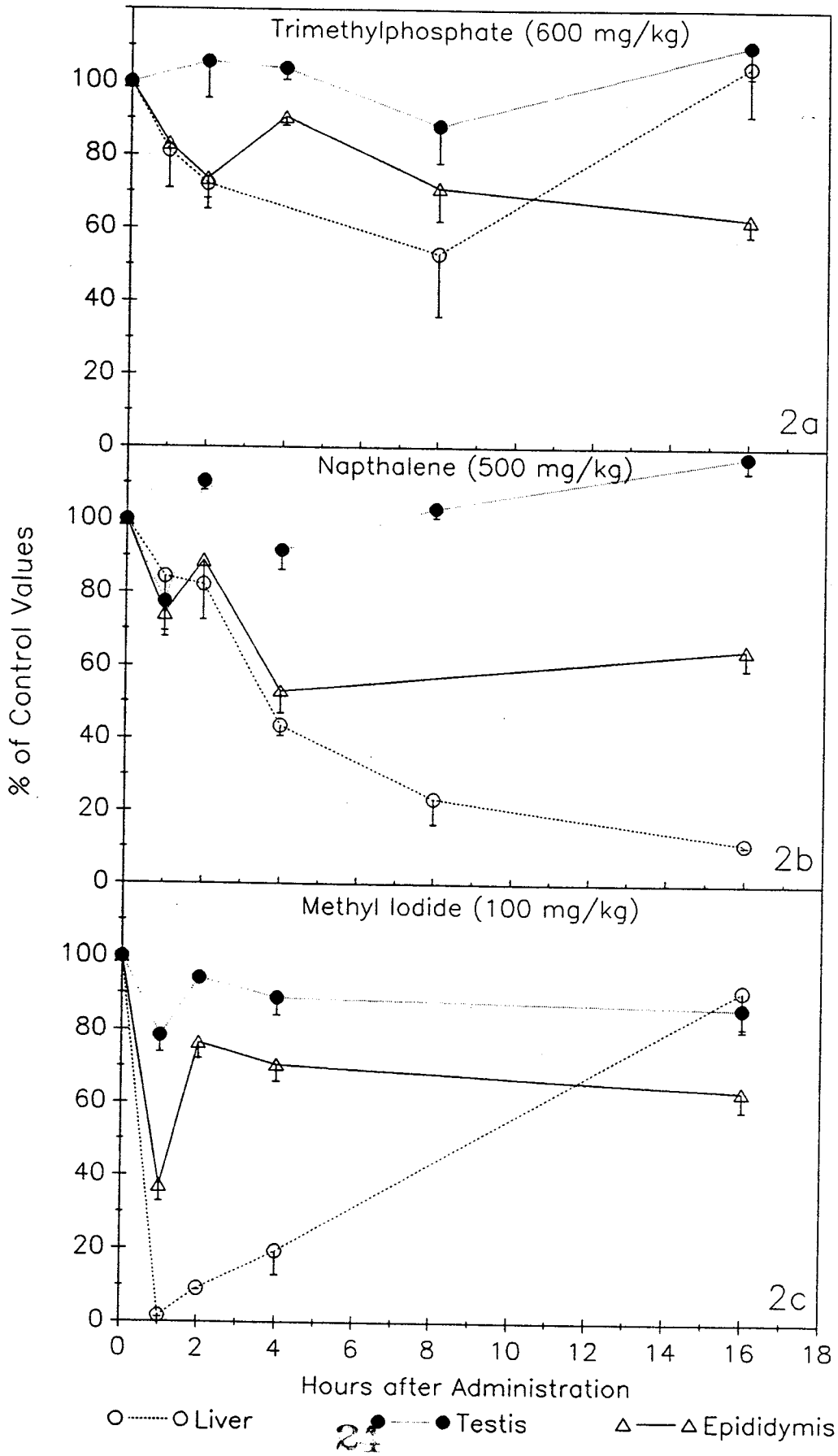

Trimethylphosphate (600 mg/kg)
2a
Napthalene (500 mg/kg)
2b
Methyl Iodide (100 mg/kg)
2c
% of Control Values
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16
18
Hours after Administration
Liver
Testis
Epididymis

Figure 3

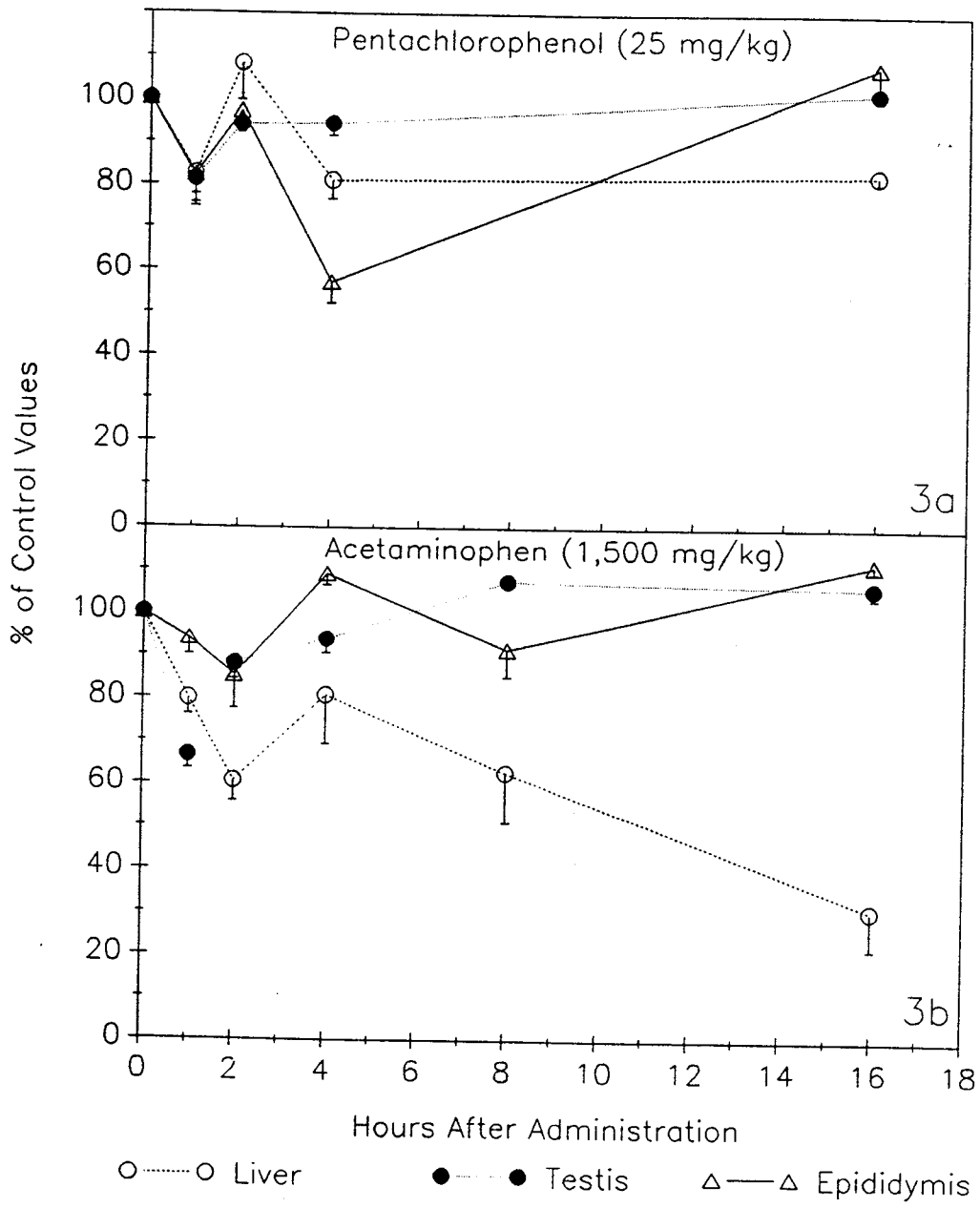

Pentachlorophenol (25 mg/kg)
3a
Acetaminophen (1,500 mg/kg)
3b
% of Control Values
0
20
40
60
80
100
0
2
4
6
8
10
12
14
16
18
Hours After Administration
Liver
Testis
Epididymis

Figure 4

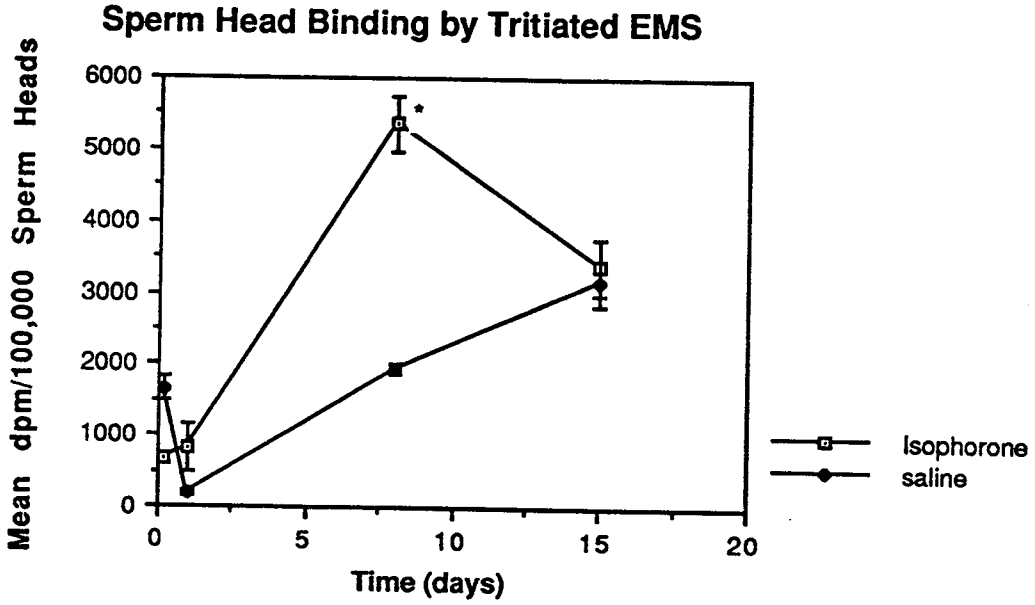
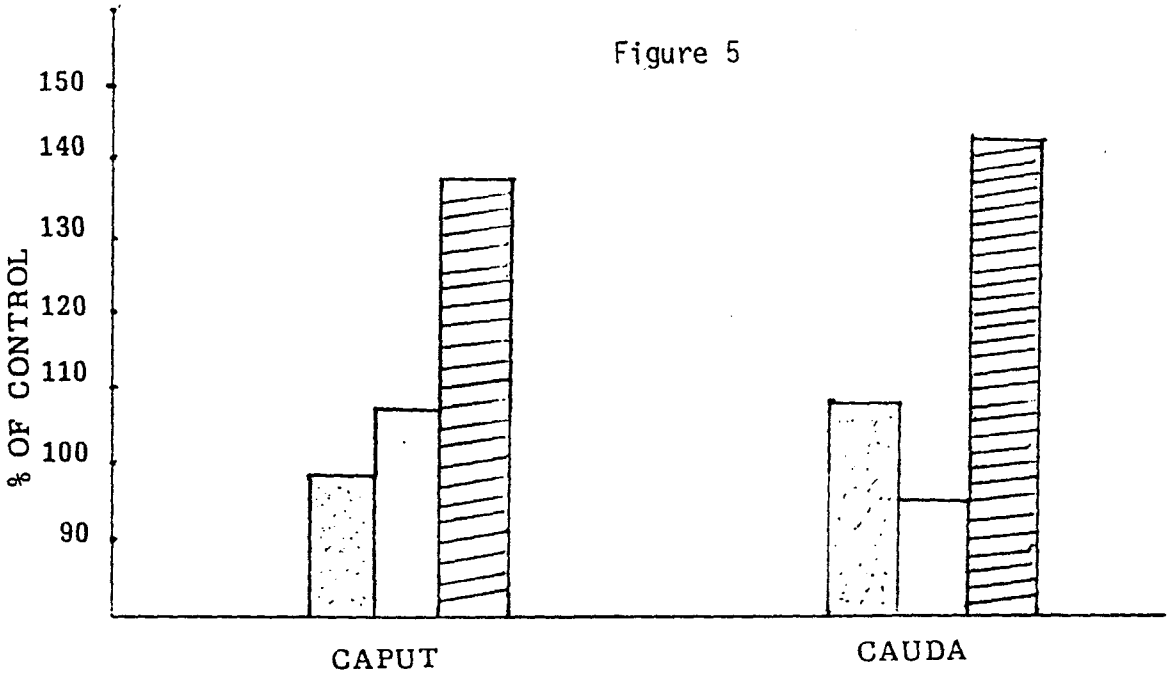

Sperm Head Binding by Tritiated EMS
Mean dpm/100,000 Sperm Heads
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
Time (days)
*
Isophorone
saline

Table 1
Potentiation of EMS Dominant Lethality by Phorone

	WEEK 2			WEEK 3		
DOSE GROUP	TOTAL RESORBED	TOTAL IMPLANTS	% LOSS	TOTAL RESORBED	TOTAL IMPLANTS	% LOSS
Oil Control	2.1	14.1	15	1.6	12.0	13
Phorone Control (250 mg/kg)	1.0	14.6	7	1.5	12.1	12
EMS Control (50 mg/kg)	1.9	13.4	14	2.4	14.9	16
Phorone 25 mg/kg & EMS 50 mg/kg	1.4	14.3	10	4.8	13.8	35
Phorone 100 mg/kg & EMS 50 mg/kg	2.6	12.9	20	3.2	13.3	24
Phorone 250 mg/kg & EMS 50 mg/kg	1.5	11.8	13	5.4	11.3	48

Figure 5



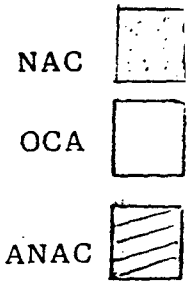


Figure 6

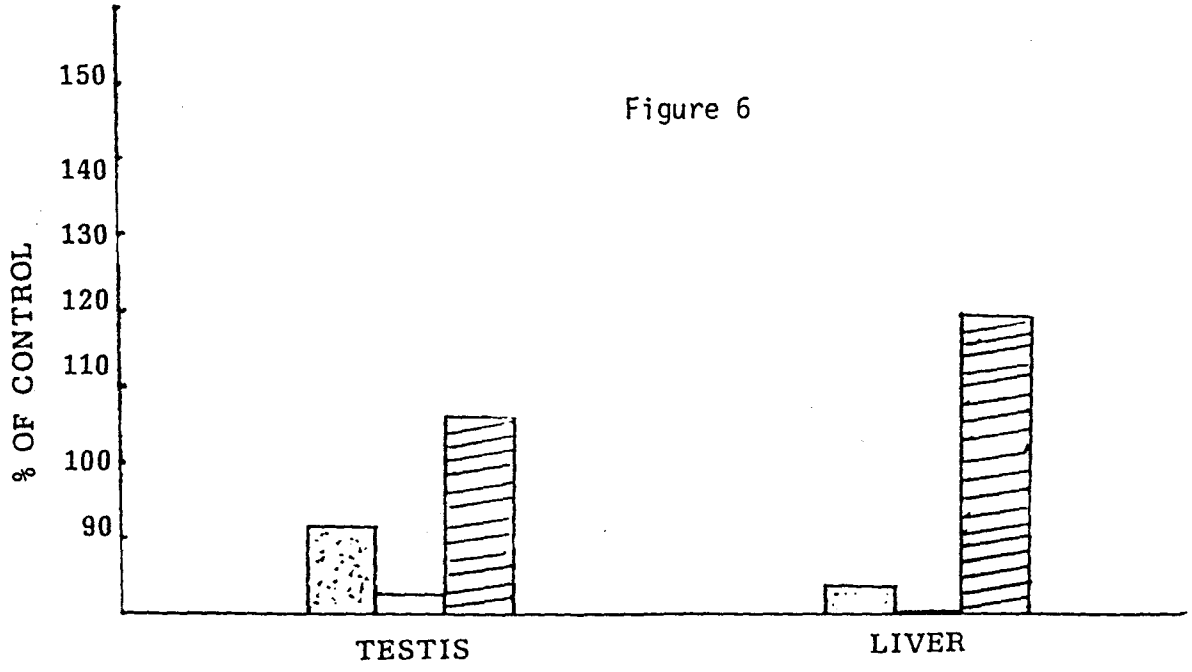


Figure 7

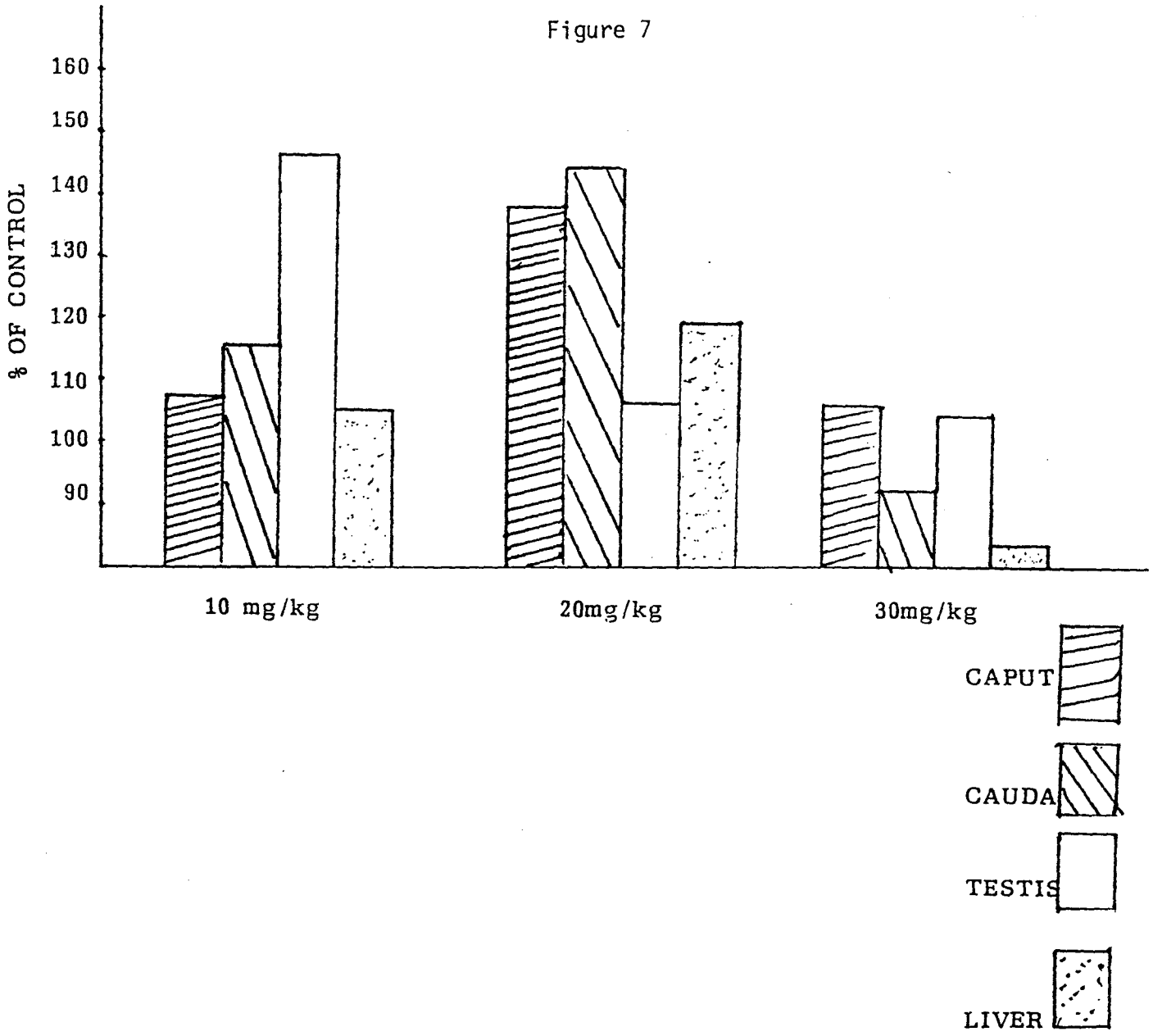
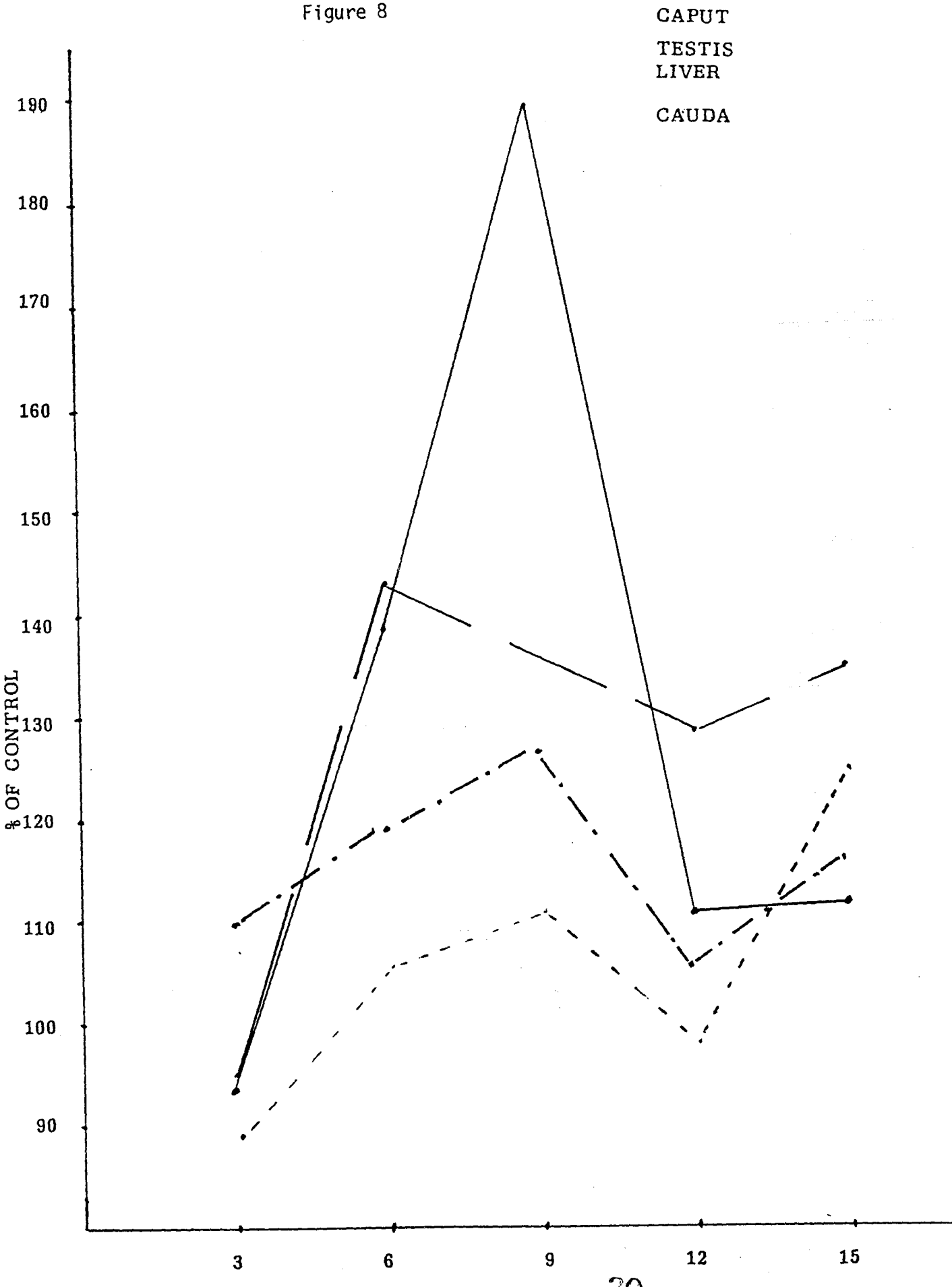

160
150
140
130
120
110
100
90
% OF CONTROL
10 mg/kg
20mg/kg
30mg/kg
CAPUT
CAUDA
TESTIS
LIVER

Figure 8


CAPUT
TESTIS
LIVER
CAUDA
190
180
170
160
150
140
130
120
110
100
90
% OF CONTROL
3
6
9
12
15
TIME, hrs