

“Metabolism and Mechanisms of Renal Cellular Injury Induced by Trichloroethylene”

Lawrence H. Lash, Ph.D.

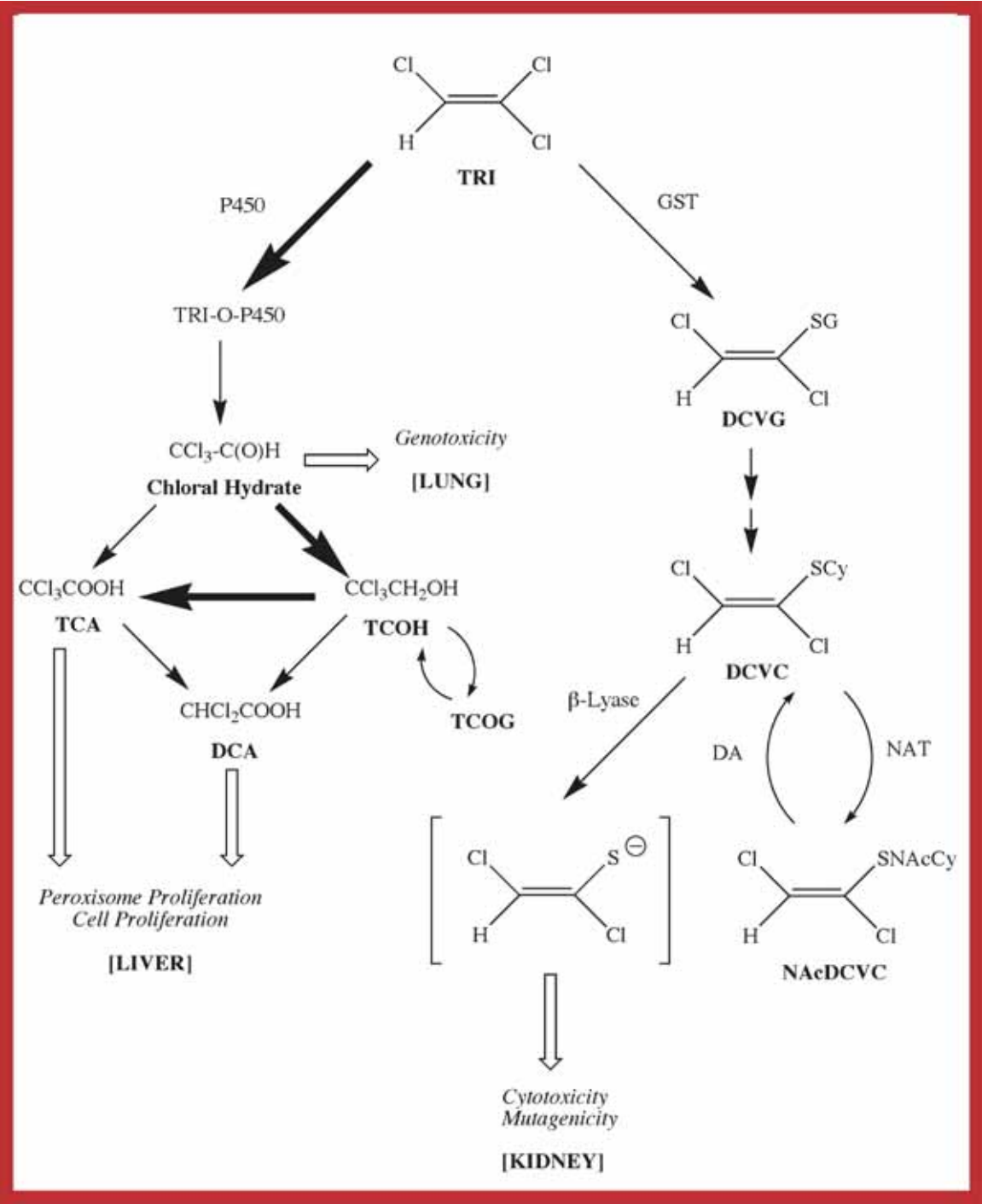
Department of Pharmacology

Wayne State University School of Medicine

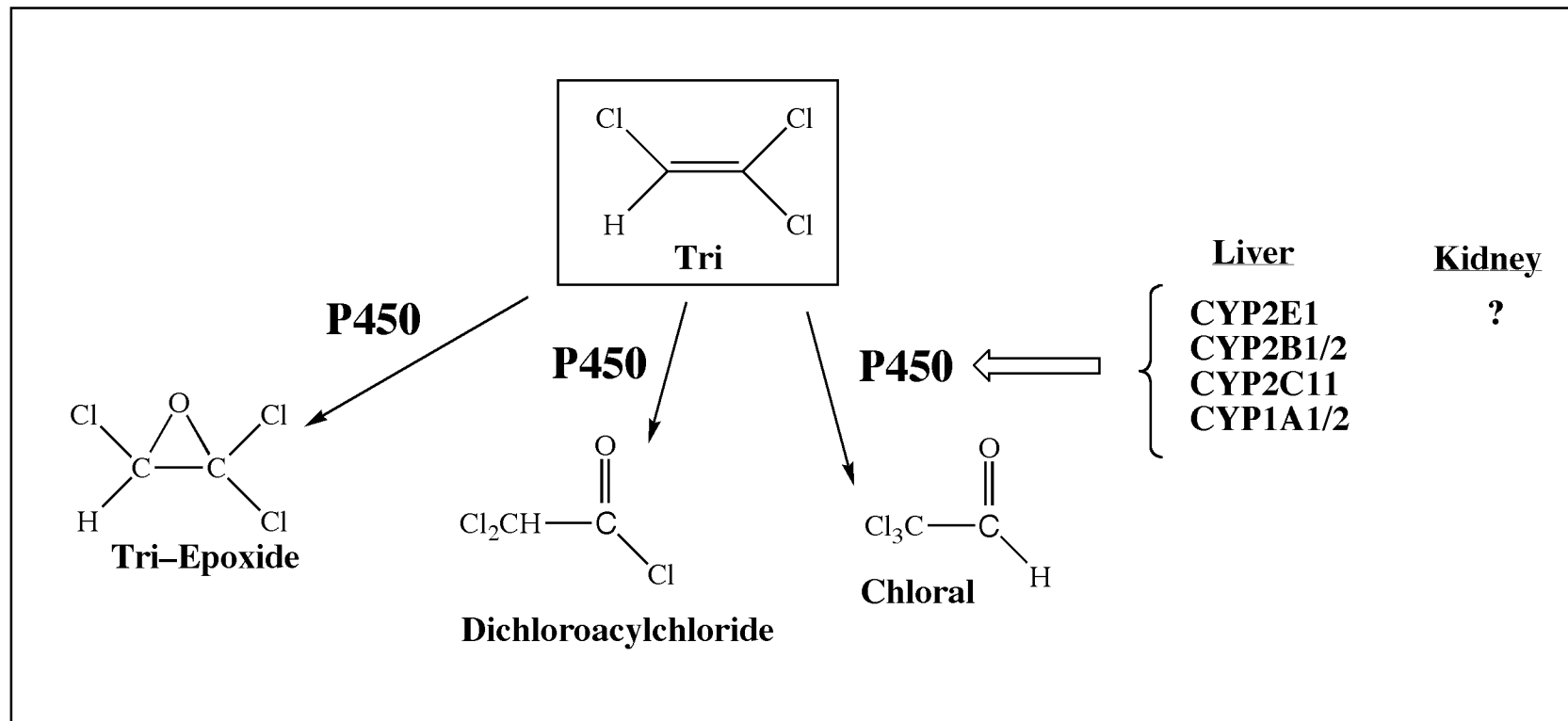
Detroit, Michigan

Outline of Talk

- Metabolism
 - P450 vs. GST: Species Differences
 - Kidney: Beta-Lyase vs. S-Oxidase
 - Male reproductive system: Implications for toxicity
- DCVC-Induced Renal Toxicity
 - Sex- and species-related differences in acute toxicity: Rat, mouse, human
 - Role of specific GST isoforms in TCE bioactivation
 - Sublethal injury and repair: Rat PT cells
 - Apoptosis, necrosis, and cell proliferation: Human PT cells
 - Role of FMO in DCVC bioactivation: Rats, humans
- In Vivo Evidence for GST Pathway



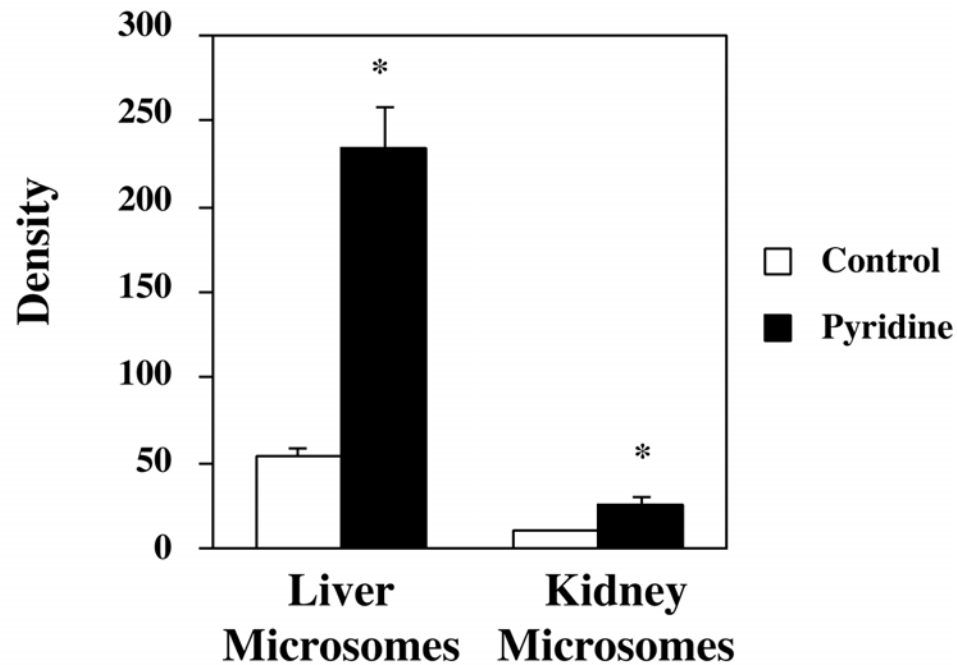
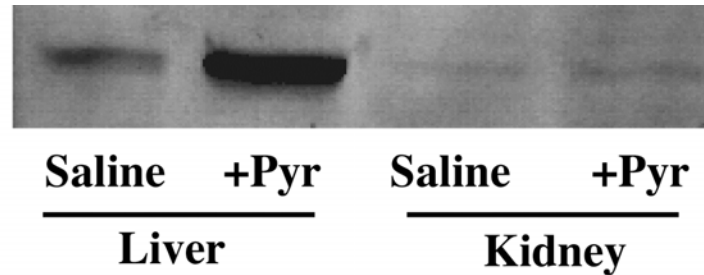
Role of Individual P450 Enzymes in TCE Bioactivation in Liver and Kidney.



Renal CYP2E1: Rats vs. Humans.

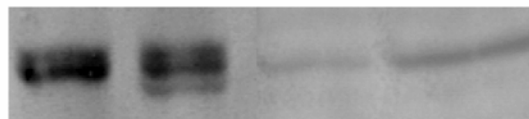
- Rats:
 - Readily detectable
 - Major P450 in PT cells; < 10% of hepatic content
- Humans:
 - No CYP2E1 by pNP hydroxylase activity or Western blot
 - Virtually no detectable P450-dependent metabolism of TCE

Effect of Pyridine on CYP2E1 Expression in Rat Liver and Kidney Microsomes:

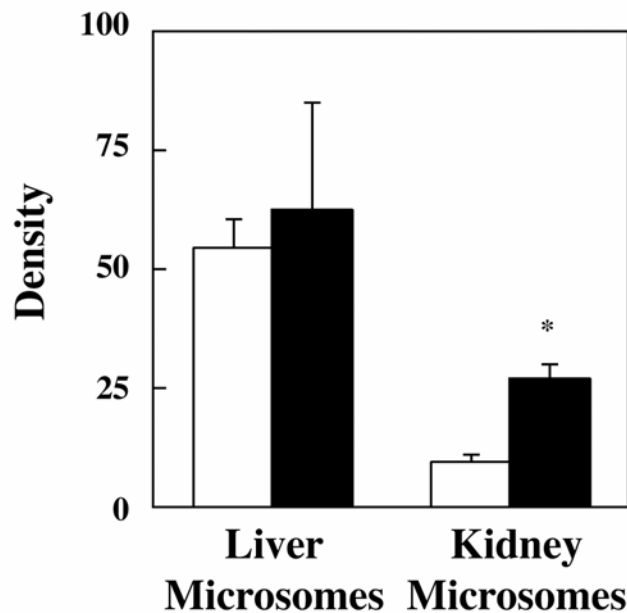


Effect of Clofibrate on Expression of CYP2E1 and CYP2C11 in Rat Liver and Kidney Microsomes:

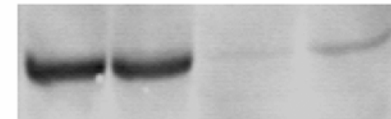
CYP2E1



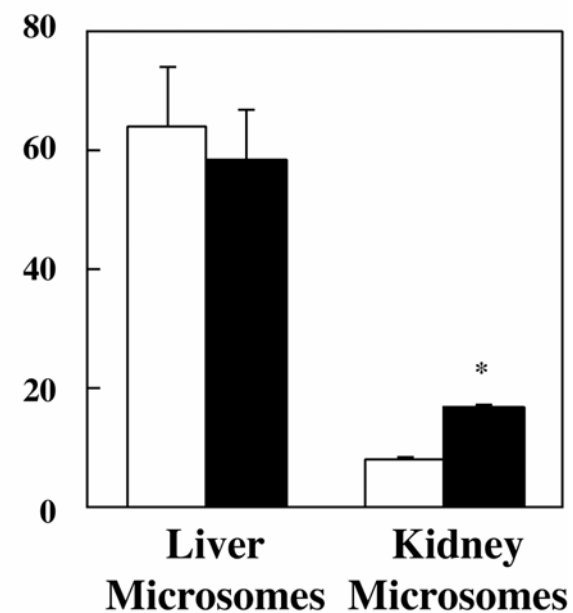
1 2 3 4
Saline Clof Saline Clof
Liver Kidney



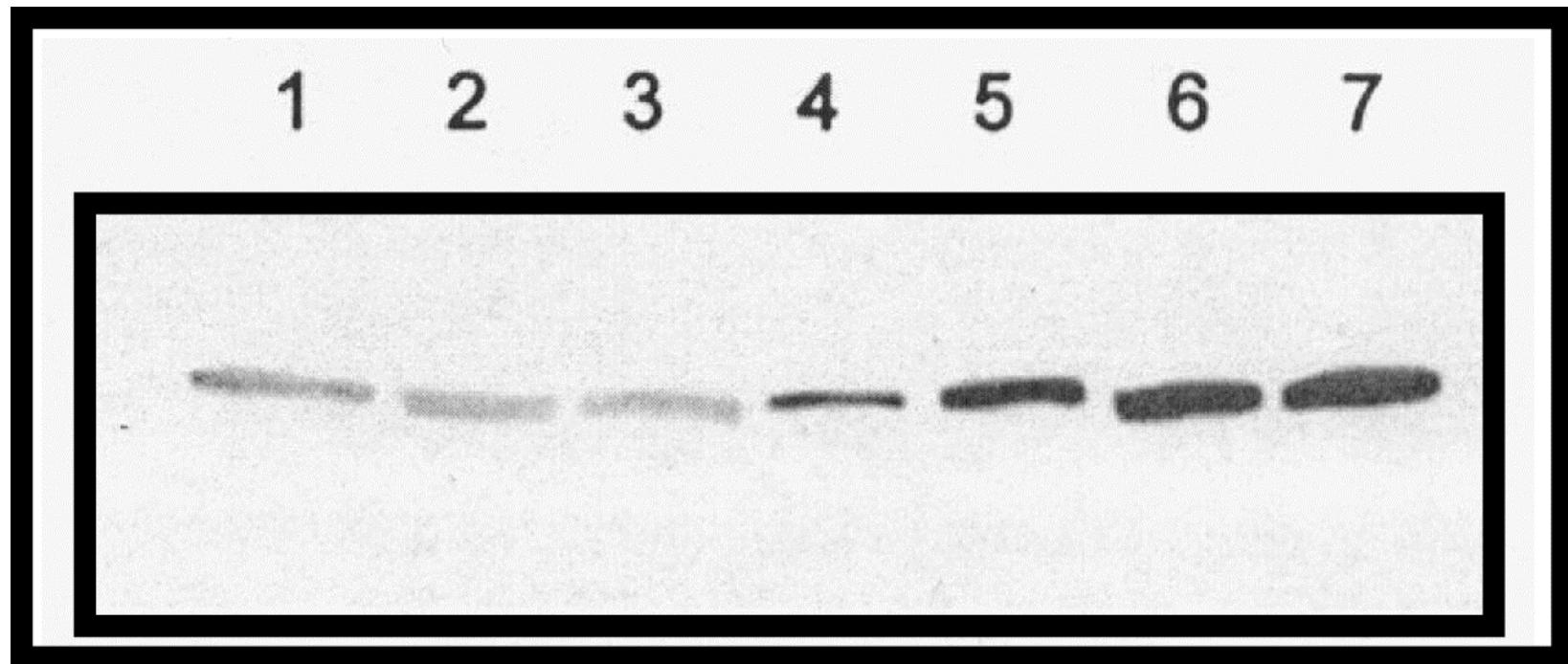
CYP2C11



1 2 3 4
Saline Clof Saline Clof
Liver Kidney



CYP2E1 Expression in Mouse Testis and Epididymis:

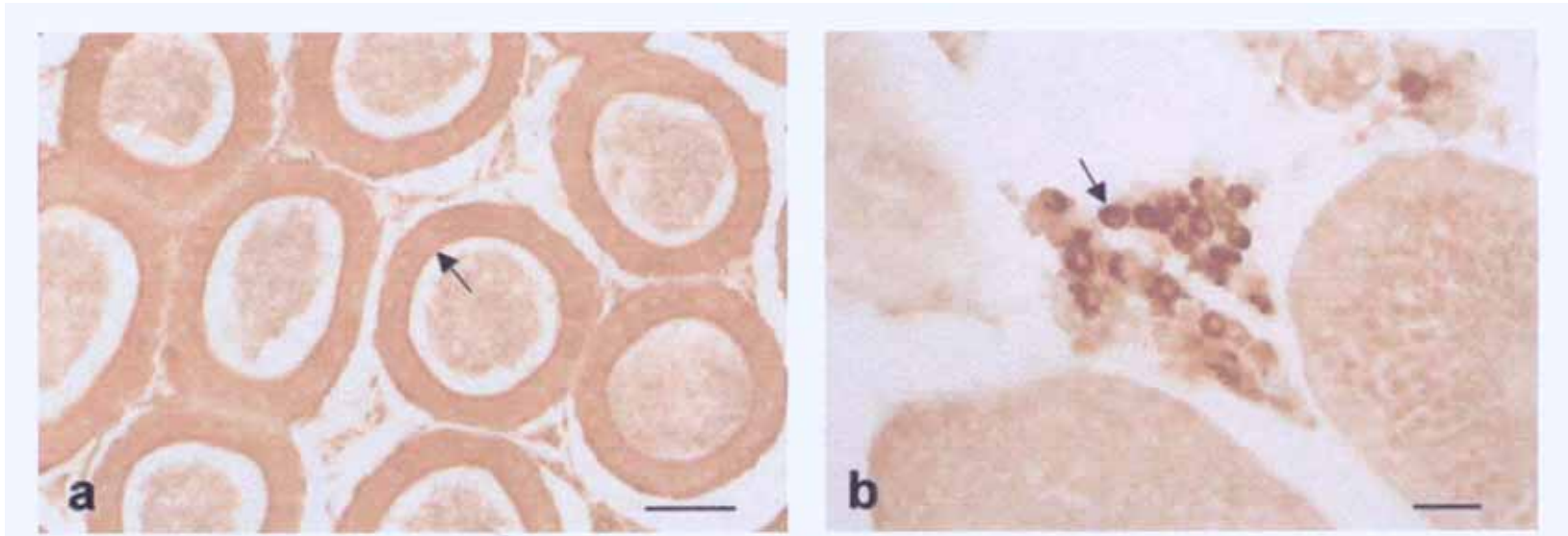


Lanes 1-3: Testis (10, 25, 50 µg protein);

Lanes 4-6: Epididymis (5, 10, 25 µg protein);

Lane 7: Liver (2 µg protein)

Localization and Distribution of CYP2E1 in Mouse Epididymis and Testis:

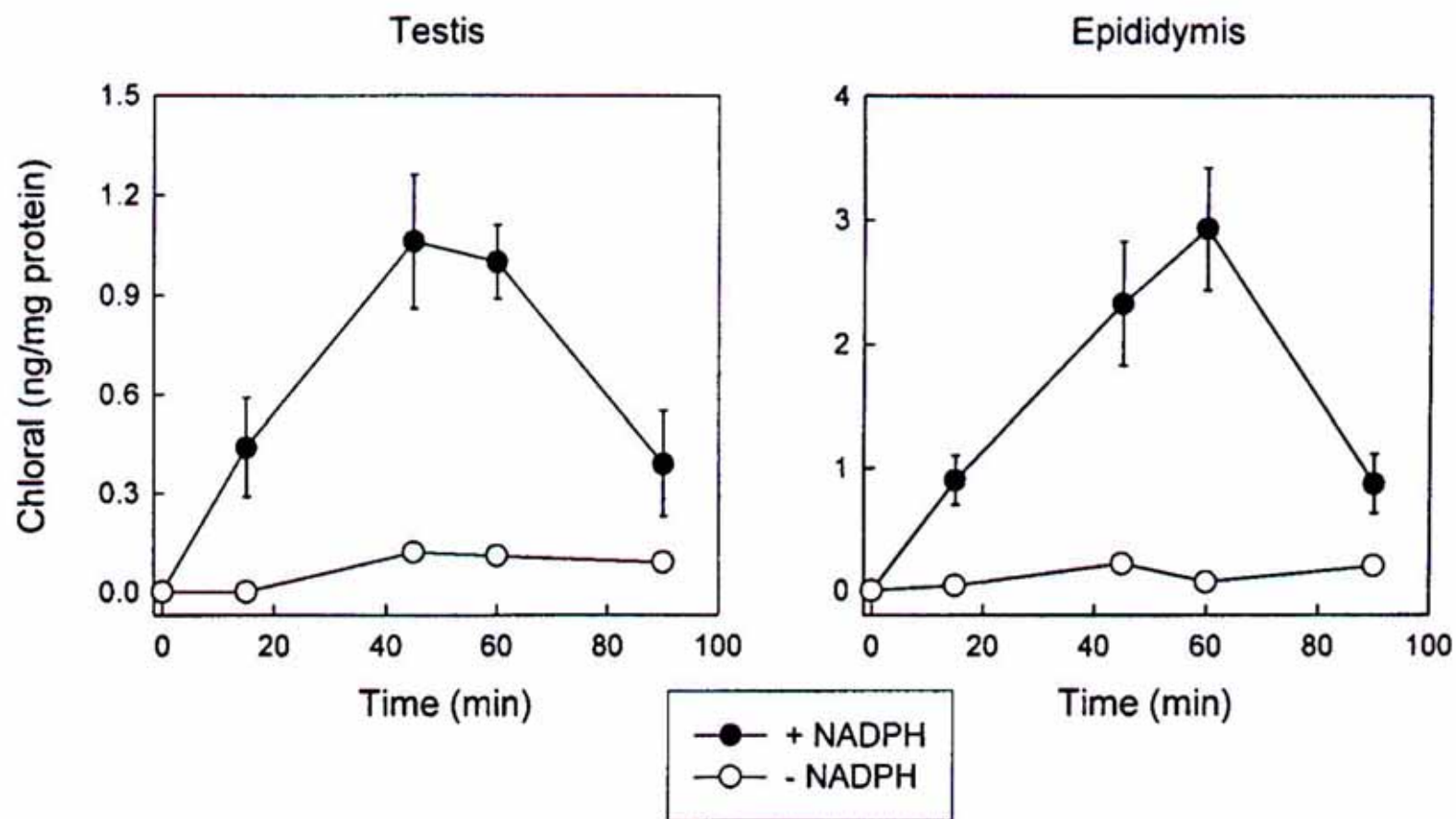


Bar = 50 μ m

Bar = 25 μ m

- a. Epididymis: Epithelial cells (arrow)
- b. Testis: Leydig cells (arrow)

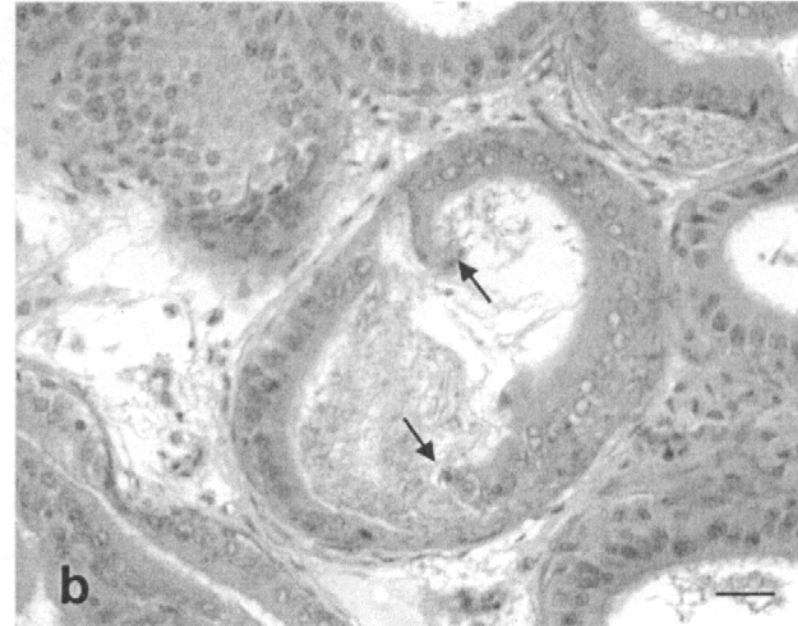
Time-Dependent Formation of Chloral from TCE in Incubations of Microsomes from Mouse Testis and Epididymis:



pNP Hydroxylase Activity (pmol/min per mg protein):

Testis = 3.01 ± 0.78 ; Epididymis = 7.17 ± 1.01 .

**Microscopic Evidence of Damage to Mouse Epididymis
from TCE: 1000 ppm TCE by Inhalation
(6 hr/day x 5 days/week x 4 weeks).**

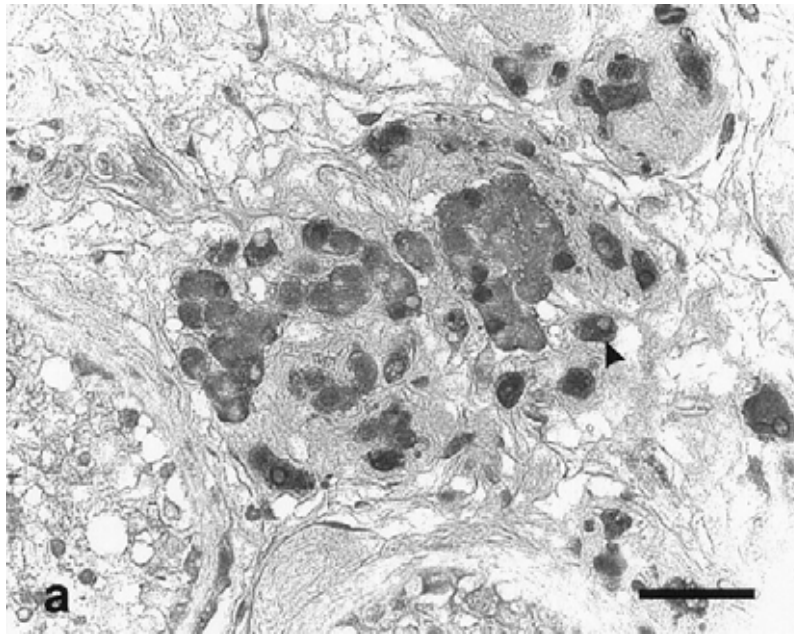


Bar = 25 μ m

TCE and Metabolites in Human Seminal Fluid:

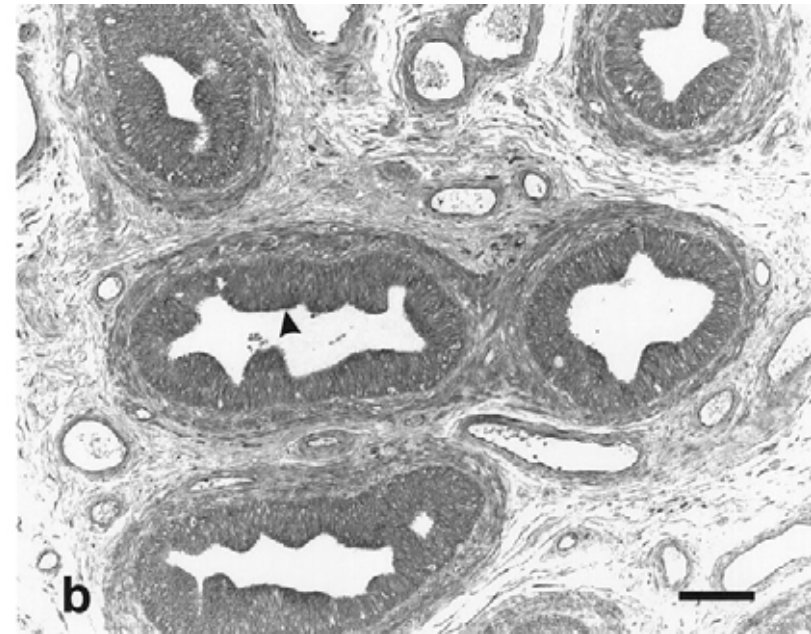
Subject	TCE	TCE Metabolites			
		CH	TCOH	TCA	DCA
		pg/extract			
1	98.8	62.7	16.2	< 100	< 100
2	1122	510	9.4	< 100	< 100
3	641	1739	10.8	< 100	< 100
4	5419	69.1	25.5	< 100	13342
5	20.4	108	14.7	< 100	< 100
6	194	119	3.5	< 100	< 100
7	1618	116	2.7	5504	9439
8	673	61.2	3.2	< 100	< 100

Localization of CYP2E1 in Human Testis and Epididymis.



Bar = 25 μm .

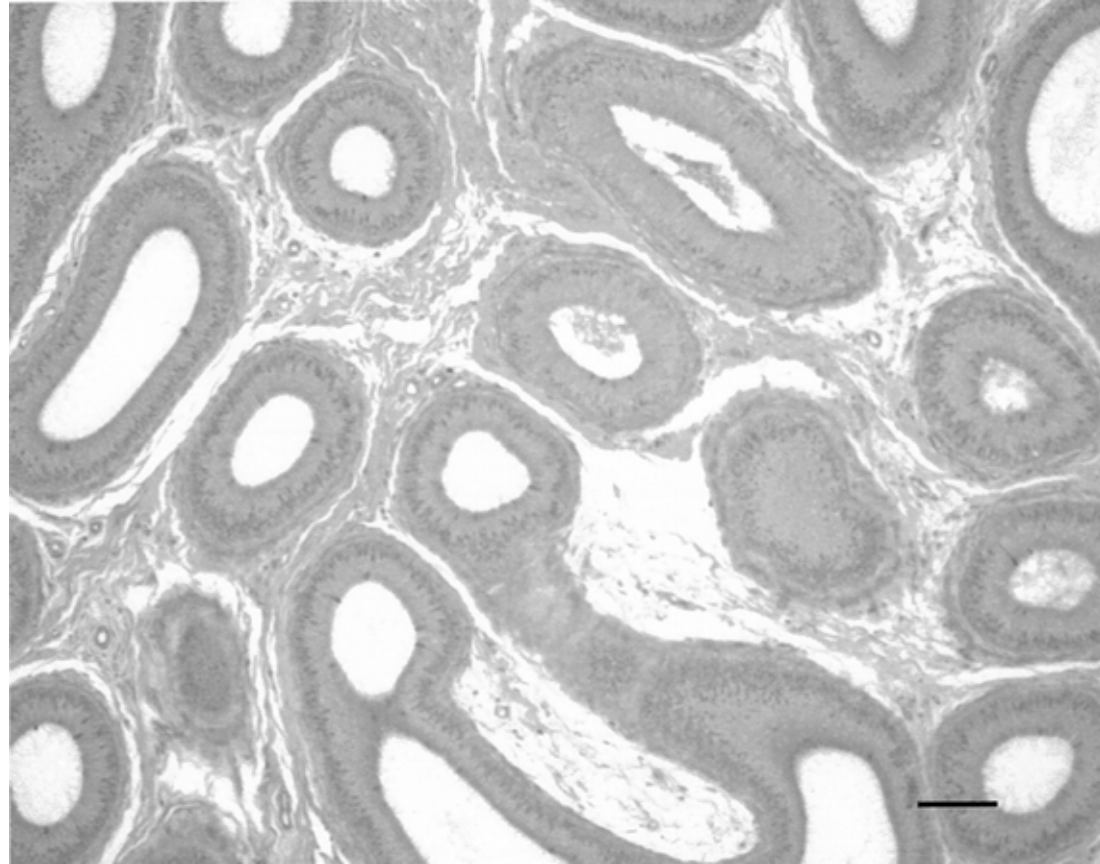
Testis: Arrow = Leydig cells.



Bar = 50 μm .

Epididymis: Arrow = Epithelium.

Localization of CYP2E1 in Monkey Epididymis.



Bar = 200 μm .

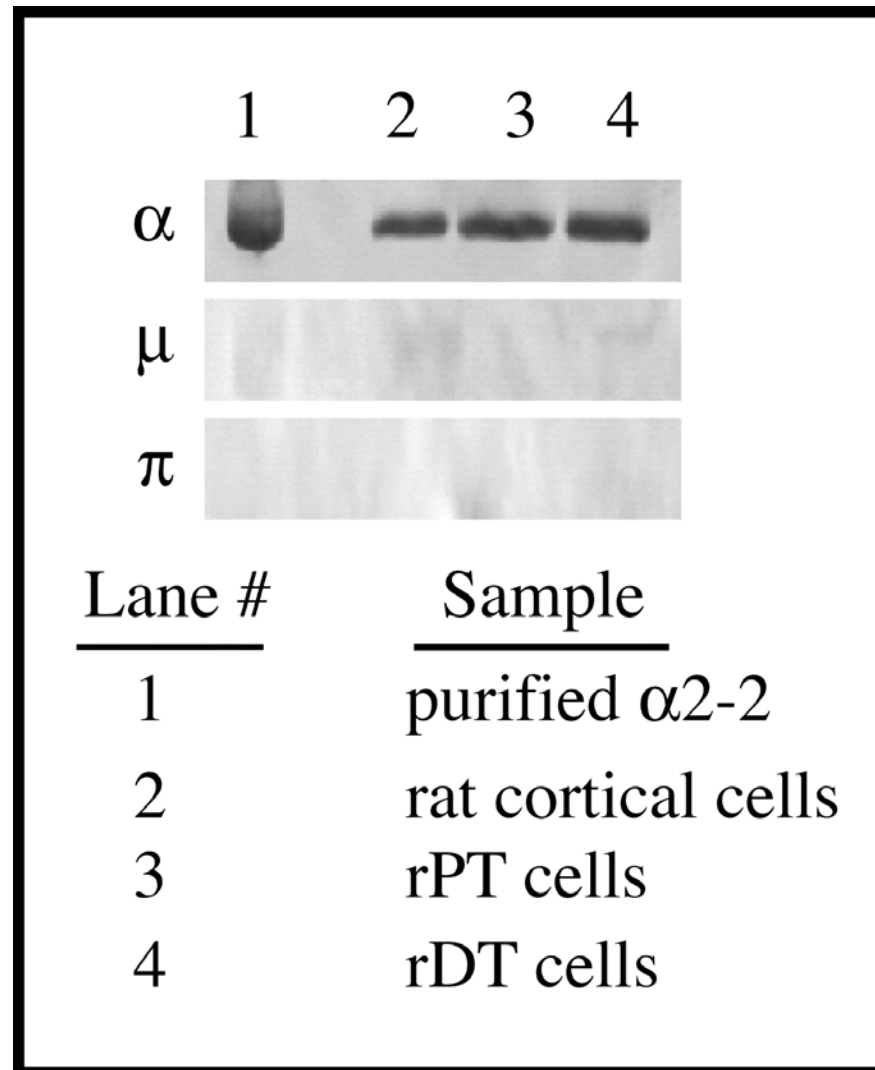
Testicular Metabolism and Toxicity of TCE: Conclusions.

- CYP2E1, the major P450 enzyme that metabolizes TCE, is present in testis of mouse, a non-human primate, and humans.
- Activity and expression of CYP2E1 are highest in the epididymis.
- Histopathology observed in epididymis of mice exposed to 1000 ppm inhalation x 6 h/d x 5 d/wk x 4 w.
- Humans exposed occupationally to high levels of TCE exhibit both TCE and its metabolites in seminal fluid.
- Data consistent with role for CYP2E1 in animals and humans in bioactivation of TCE leading to testicular toxicity; likely a fairly high dose needed.

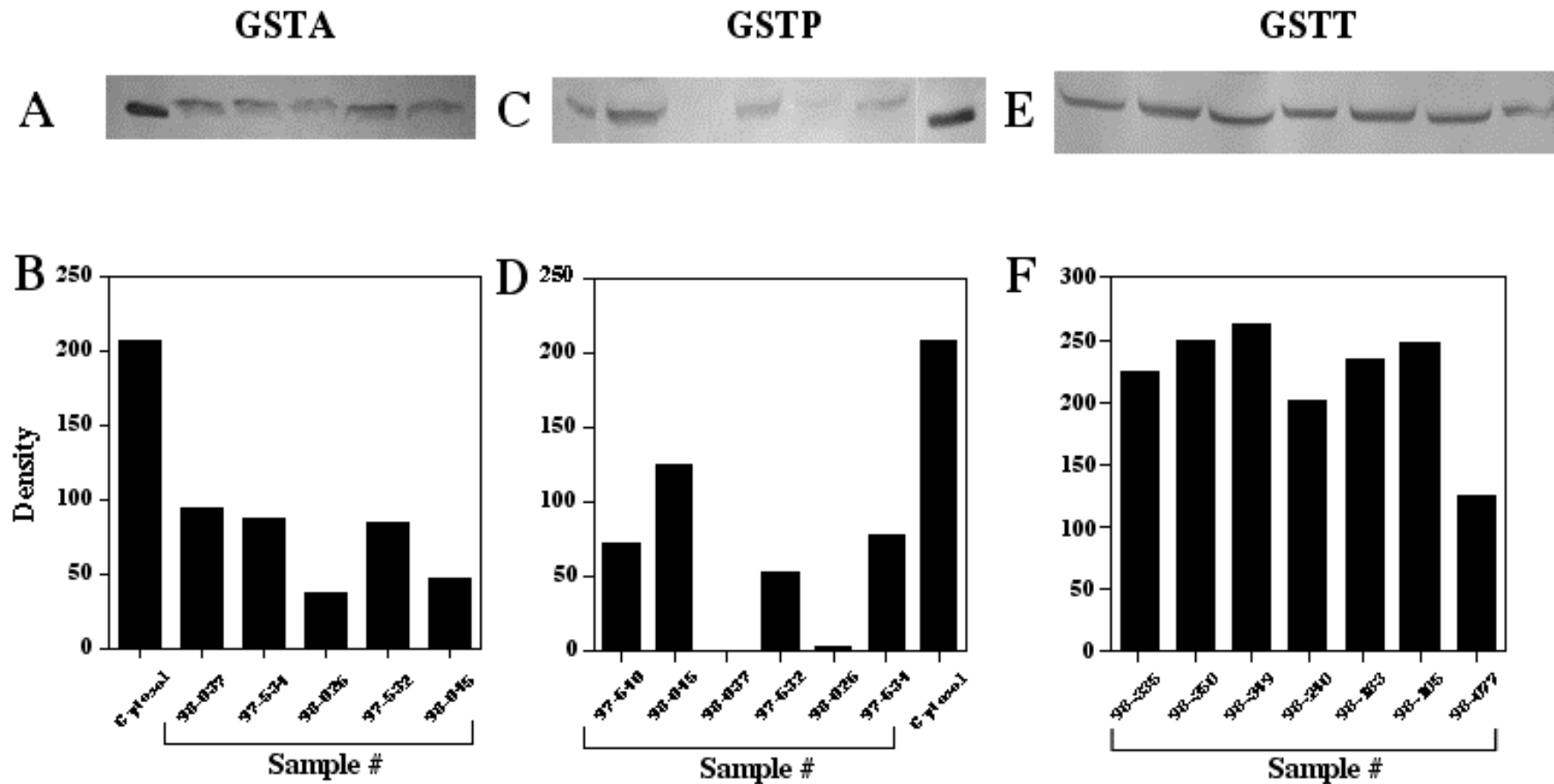
Relative Rates of TRI Metabolism in Rats and Humans:

Enzyme	Rat	Human
P450	50	30
GST	20	30
GGT	200	60
Beta-Lyase	10	1

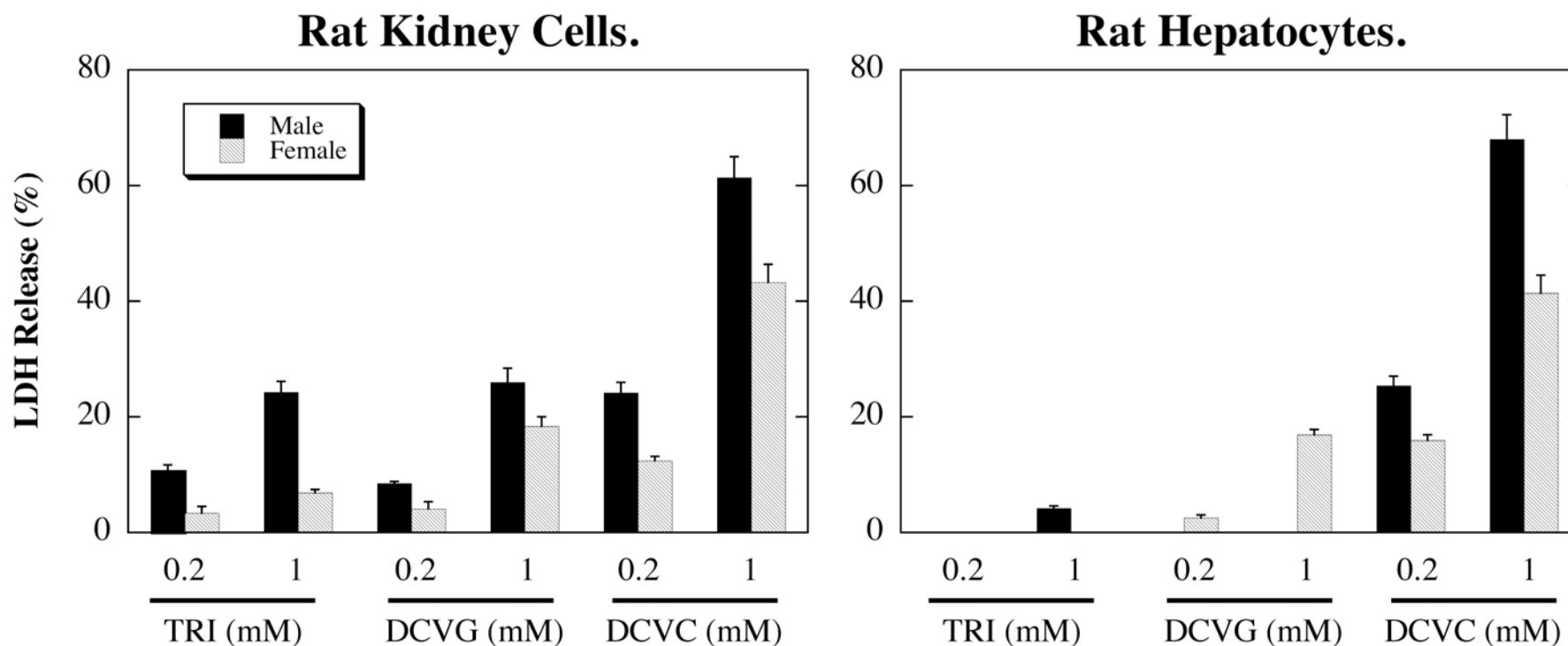
GST Expression in Rat Kidney:



GST Expression in hPT Cells:



Comparison of Acute Nephrotoxicity and Hepatotoxicity of TRI, DCVG, and DCVC in Male and Female F344 Rats.

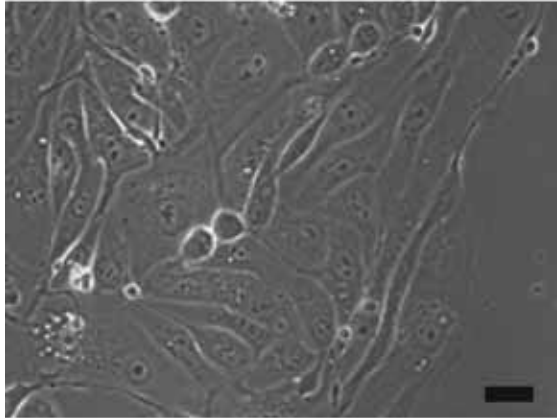


Medium for Primary Cultures of rPT and hPT Cells:

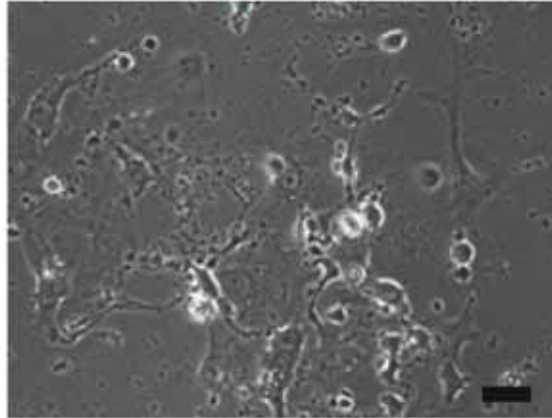
- DMEM:Ham's F12 (1:1)
- Basic Supplements:
 - NaHCO₃ (20 mM)
 - Hepes (15 mM)
 - Antibiotics (penicillin, streptomycin, amphotericin B; day 0-3 only)
- Growth Factors and Hormones:
 - Insulin
 - Hydrocortisone
 - Transferrin
 - Sodium selenite
 - EGF
 - T₃

Photomicrographs of hPT Cells Treated with Sts and DCVC: 24 hr.

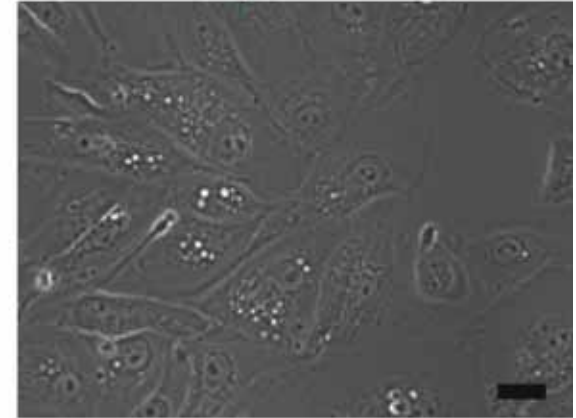
Control



1 μ M Sts

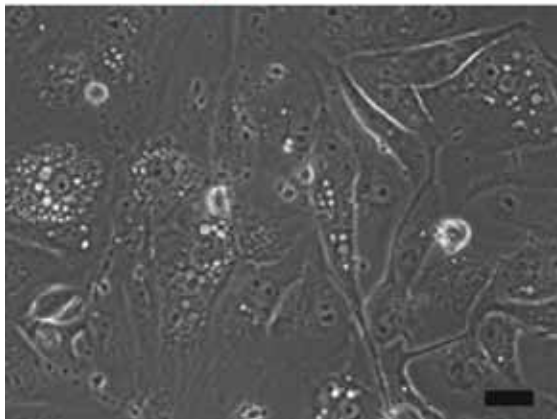


100 μ M DCVC

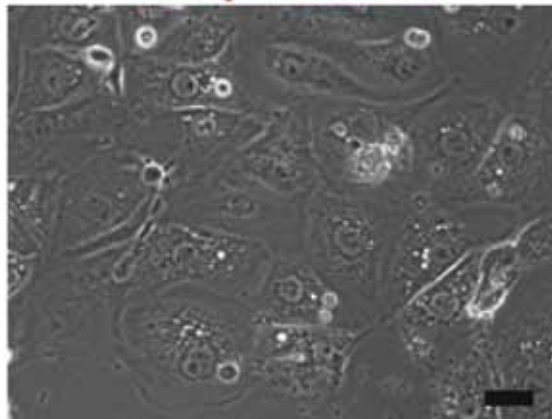


Bar = 5 μ m

200 μ M DCVC

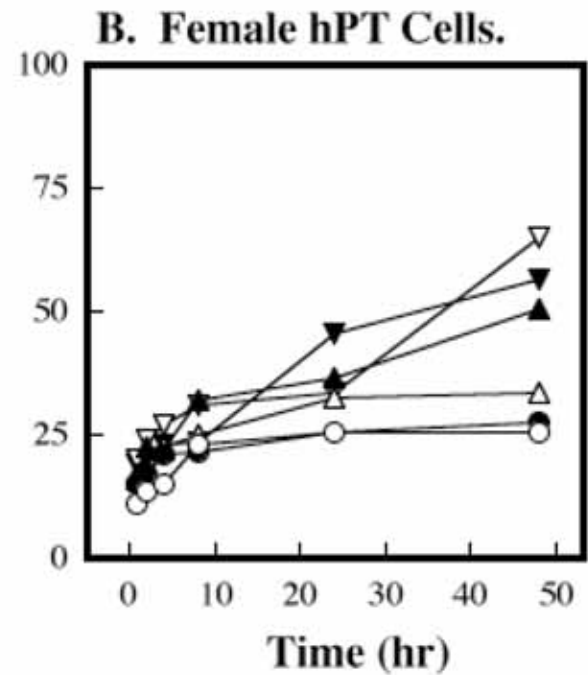
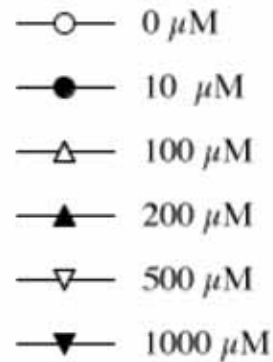
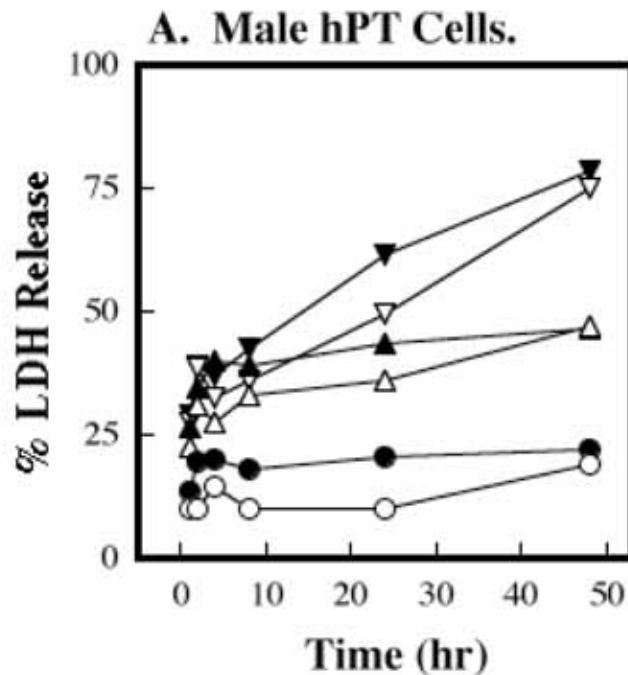


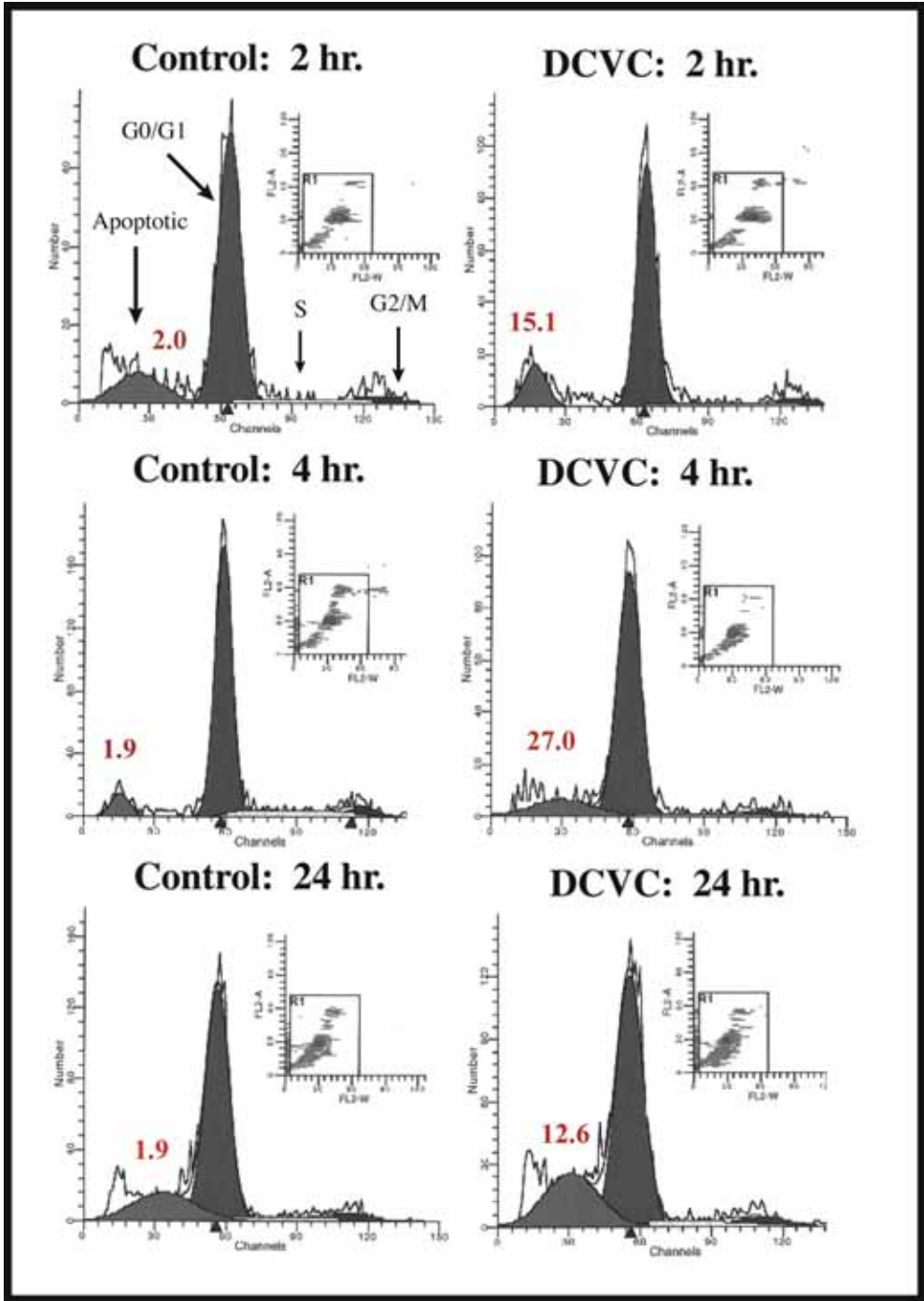
500 μ M DCVC



Control cells at 24 hr exhibit a generally normal epithelial appearance, although there are some elongated cells and intracellular vesicles. As at 8 and 16 hr, cells treated for 24 hr with 1 μ M Sts exhibit extensive cellular debris and little or no recognizable intact cellular structure. Cells treated for 24 hr with DCVC exhibit extensive intracellular vesicularization, elongated morphology, and apoptotic bodies.

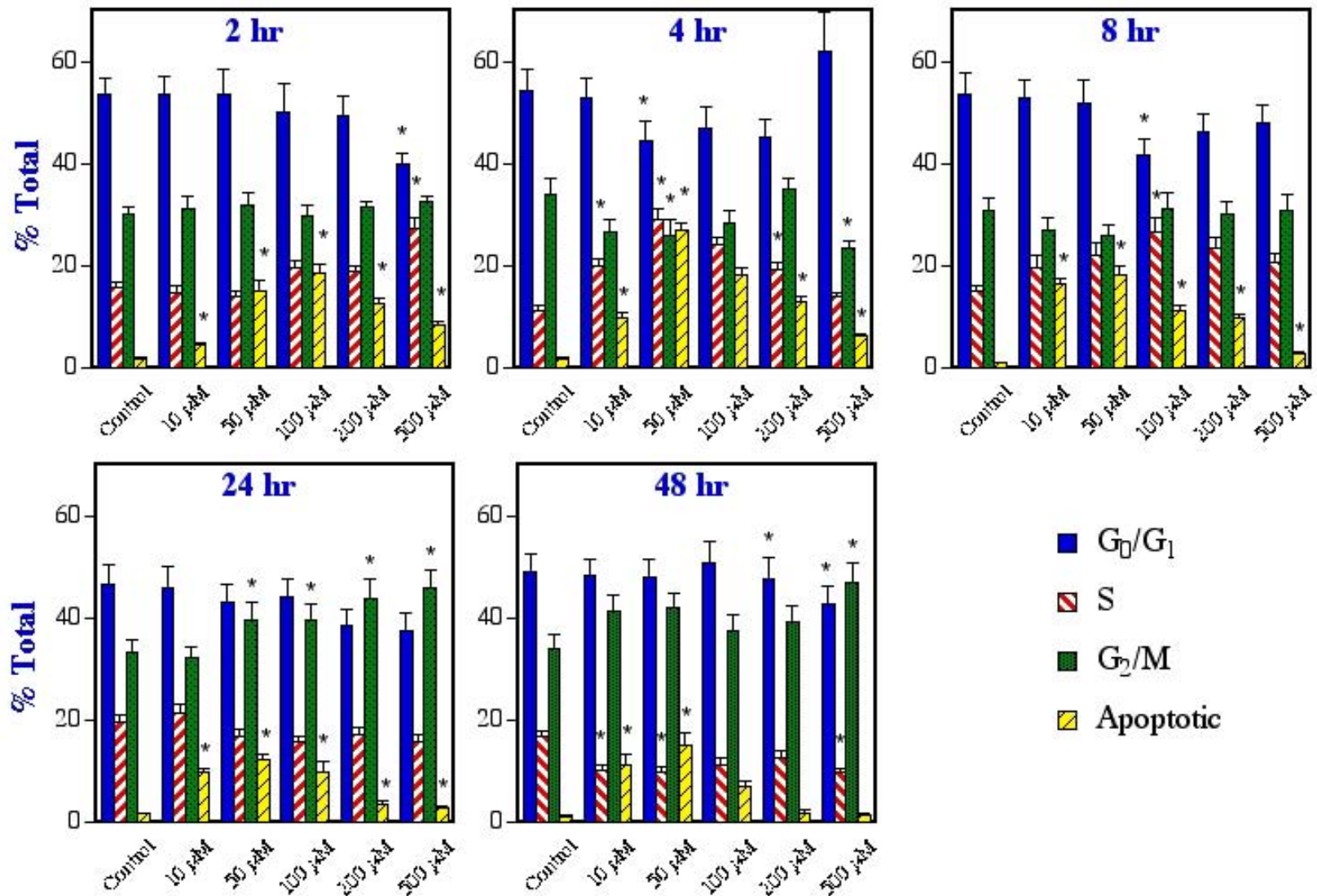
Time and Concentration of LDH Release in hPT Cells Exposed to DCVC:



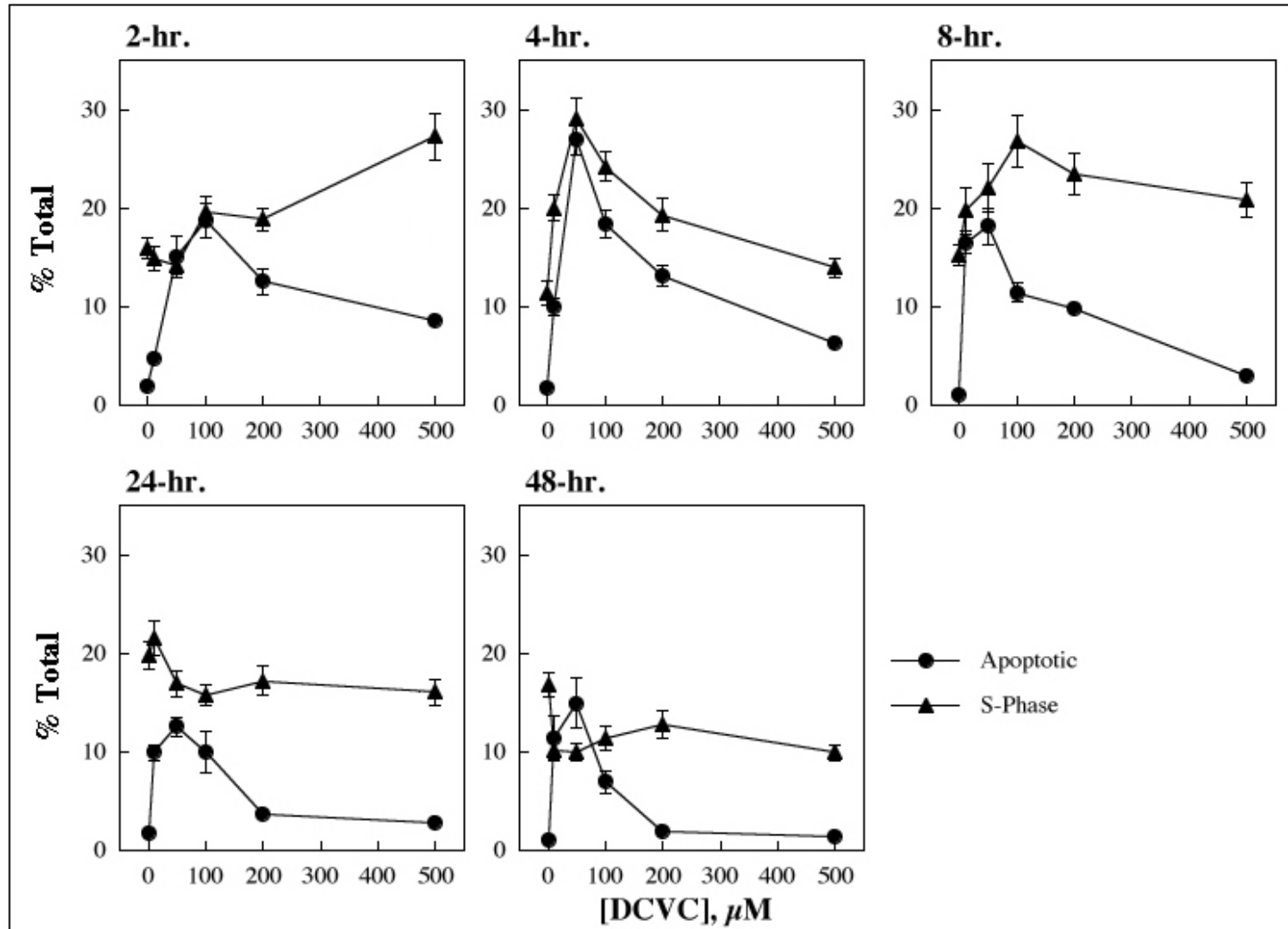


DCVC-Induced Apoptosis in hPT Cells.

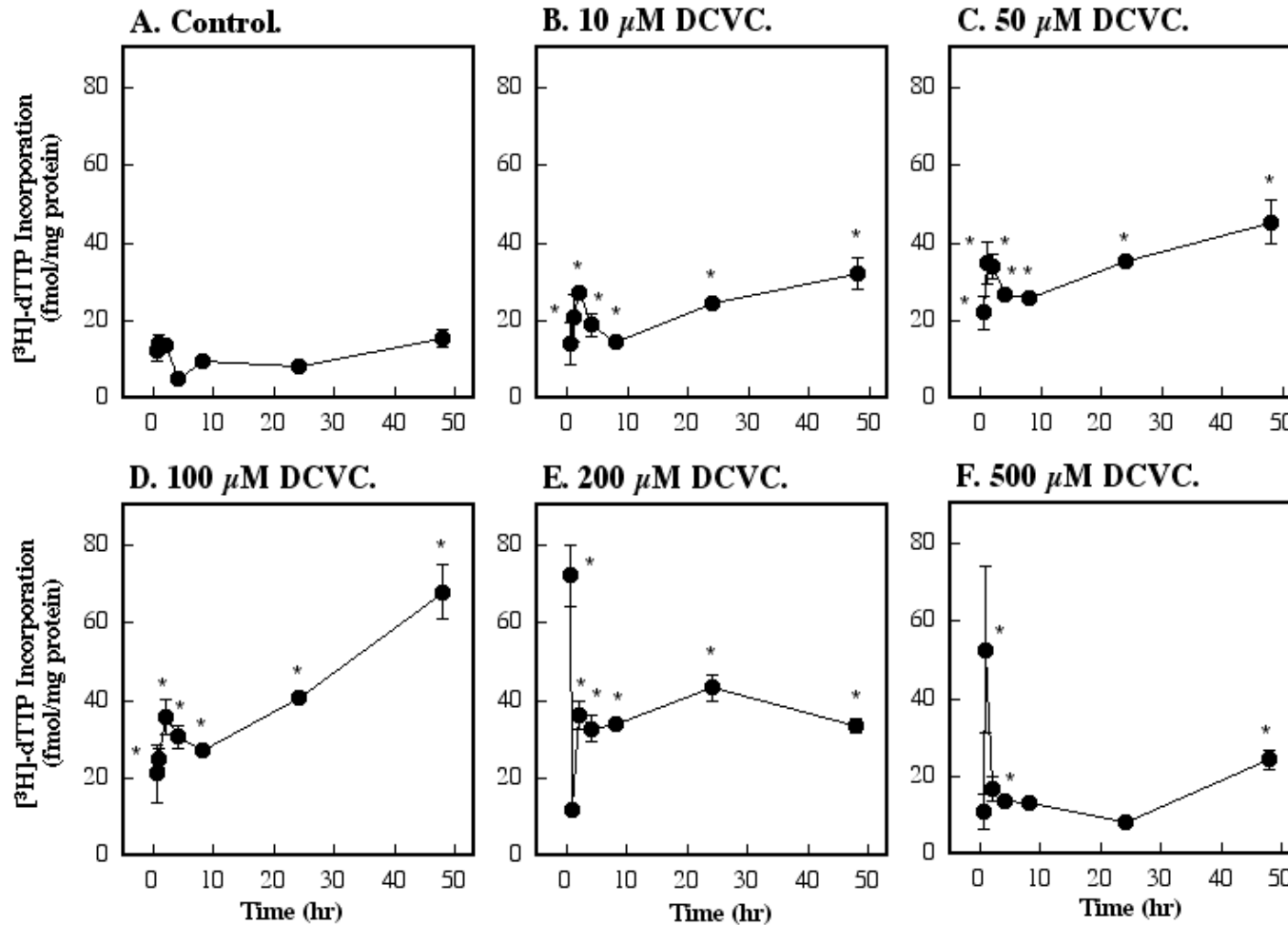
Time and Concentration Dependence of DCVC-Induced Changes in hPT Cell Cycle.



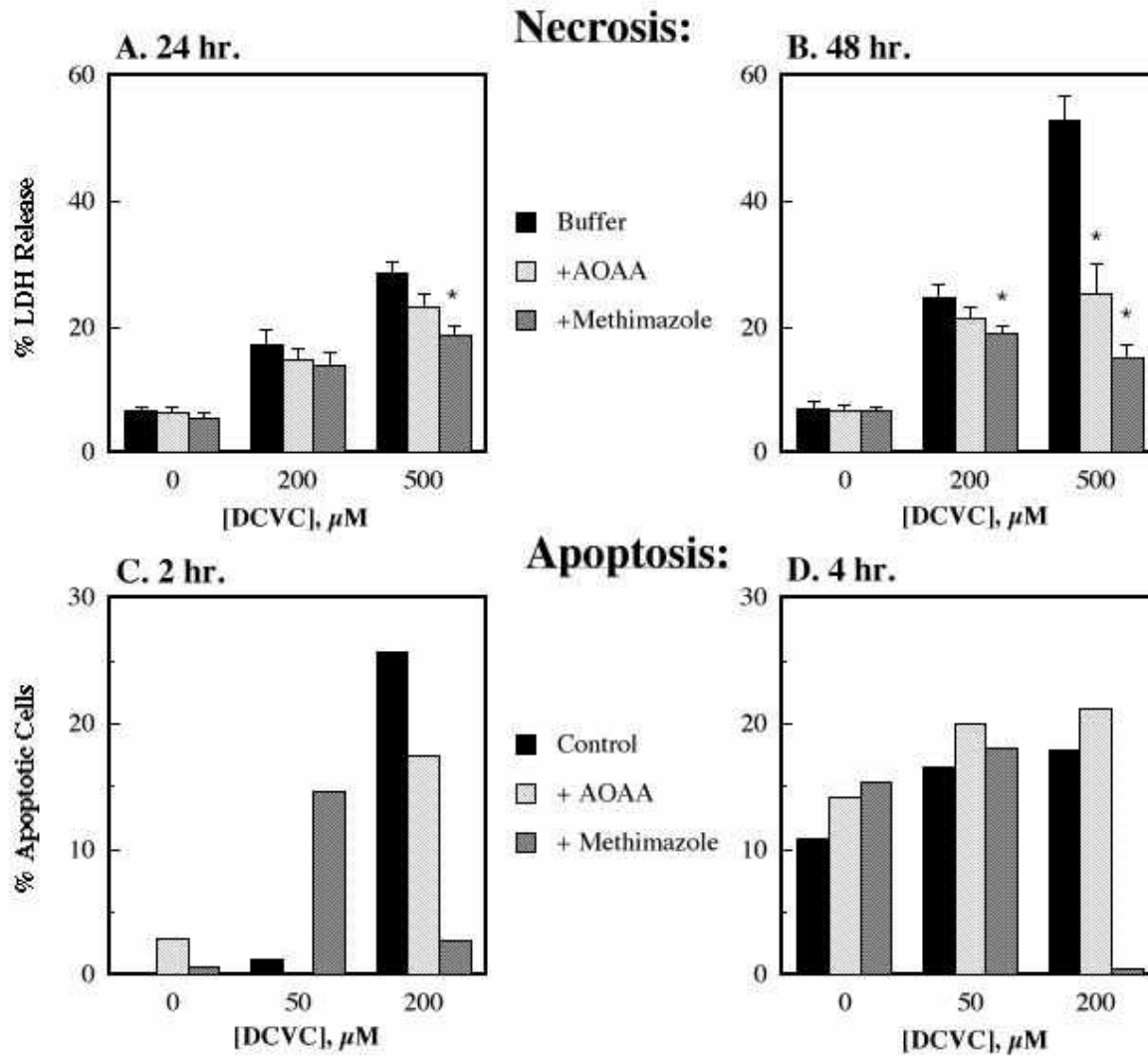
Time and Concentration Dependence of DCVC-Induced Changes in Apoptosis and S-Phase hPT Cells.



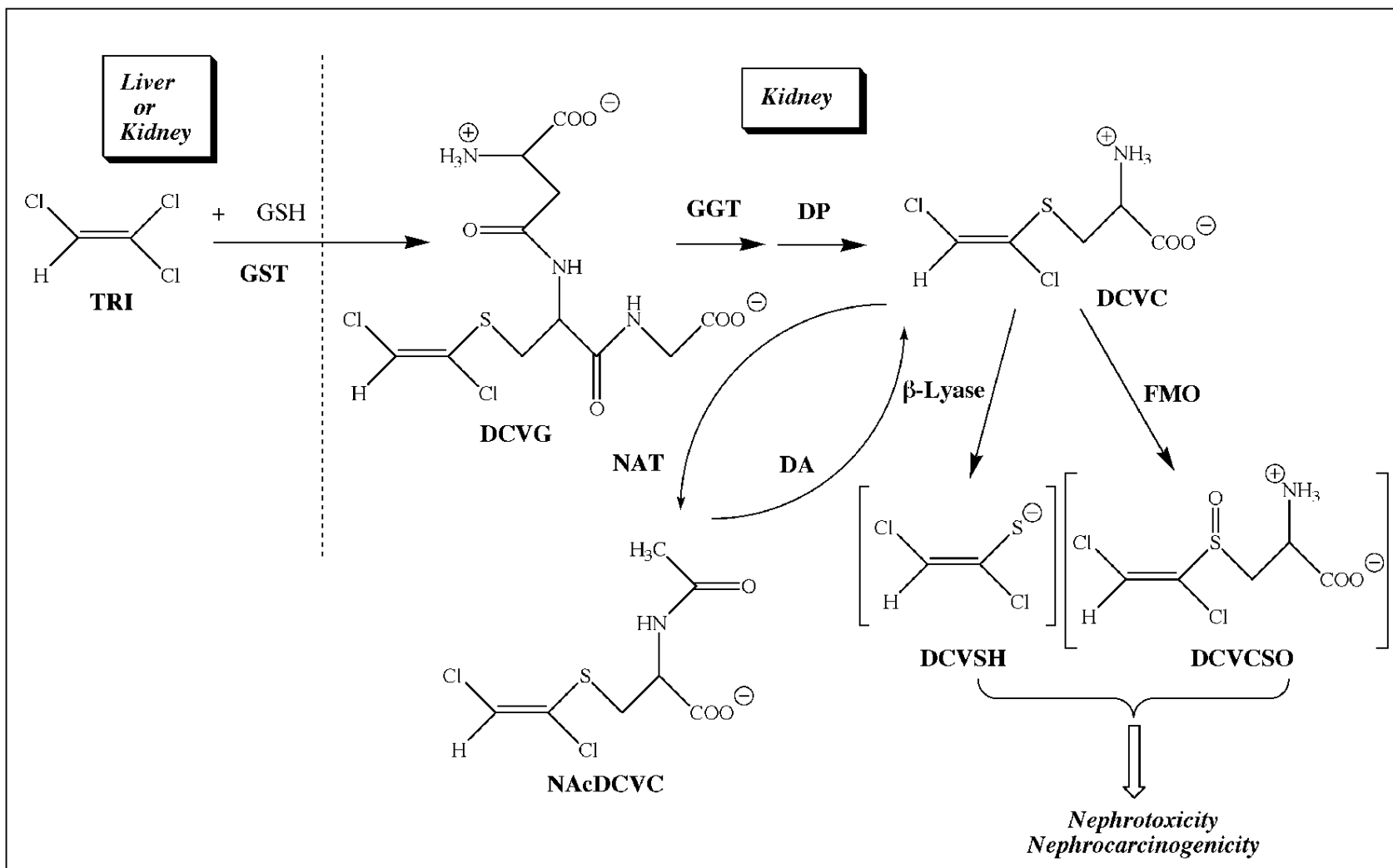
Effects of DCVC on DNA Synthesis in hPT Cells.



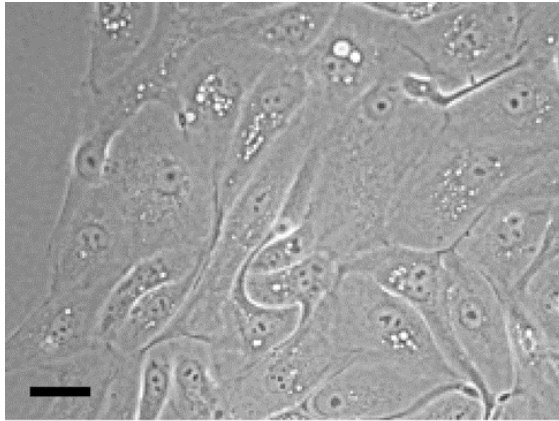
Effect of Inhibitors of Beta-Lyase and S-Oxidase on DCVC-Induced Necrosis and Apoptosis in hPT Cells.



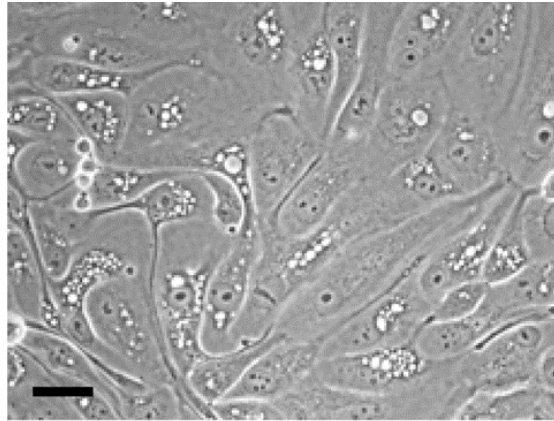
Bioactivation of DCVC: Beta-Lyase vs. FMO.



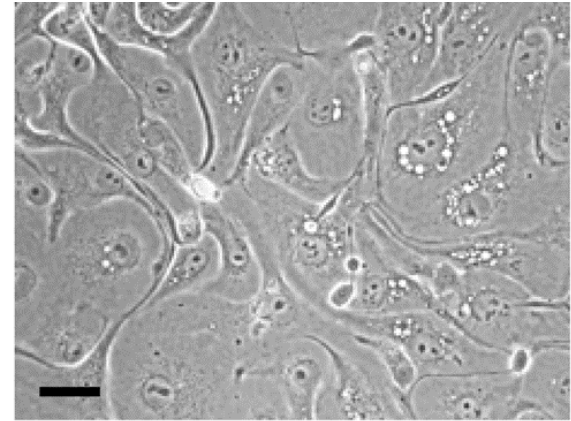
Morphology of hPT Cells Treated for 24 hr with DCVCSO:



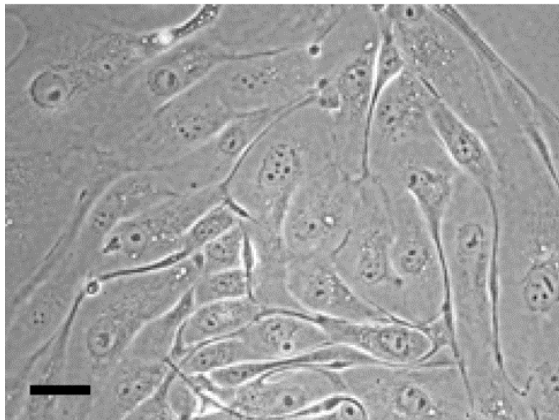
Control



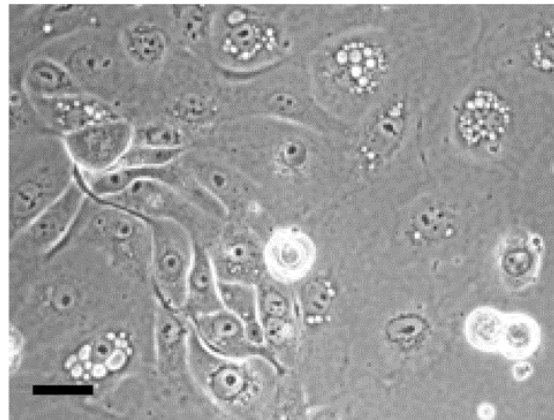
10 μM DCVCSO



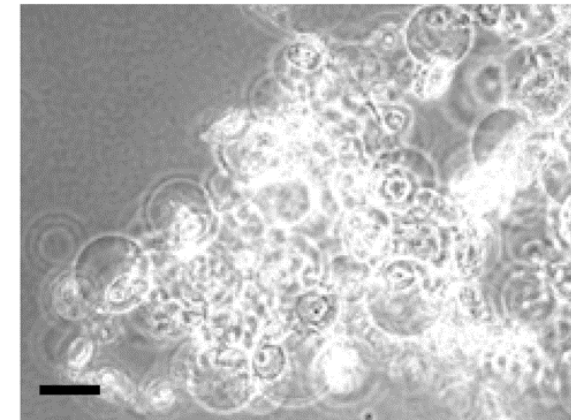
50 μM DCVCSO



100 μM DCVCSO

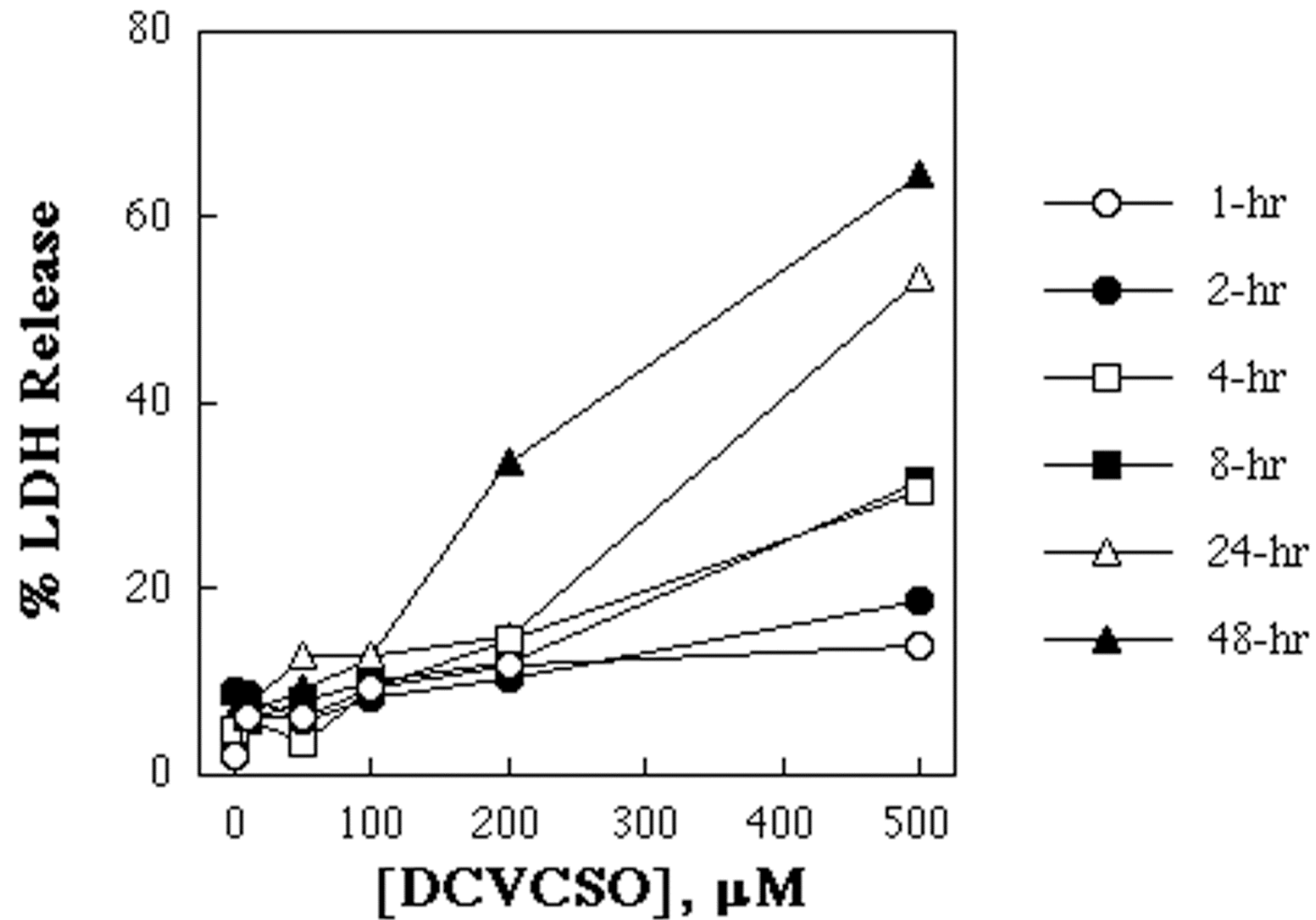


200 μM DCVCSO

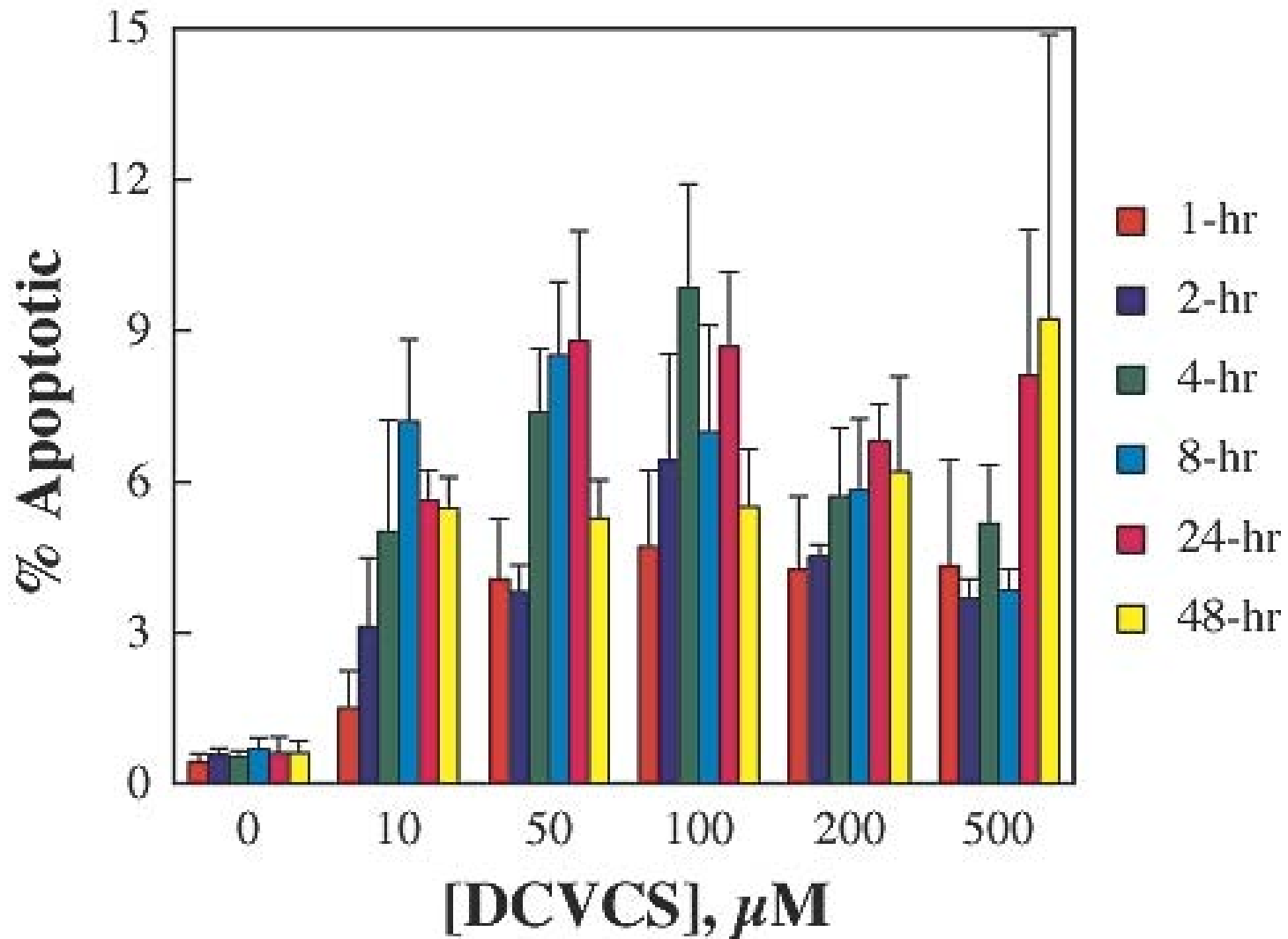


500 μM DCVCSO

DCVCS-Induced Necrosis in hPT Cells:



DCVCS-Induced Apoptosis in hPT Cells:



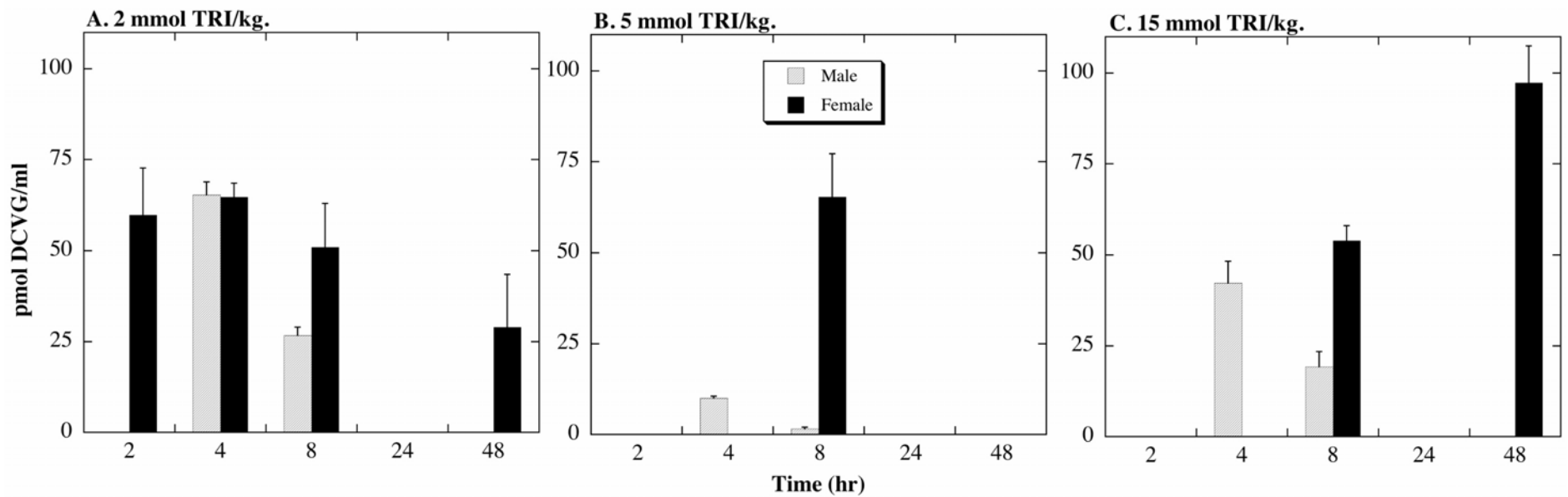
Role of Beta-Lyase vs. S-Oxidase:

- Beta-Lyase more important in rat kidney.
- S-Oxidase more important in human kidney.
- Apoptosis in hPT cells:
 - $DCVC > DCVCS$
- Necrosis in hPT cells:
 - $DCVCS > DCVC$

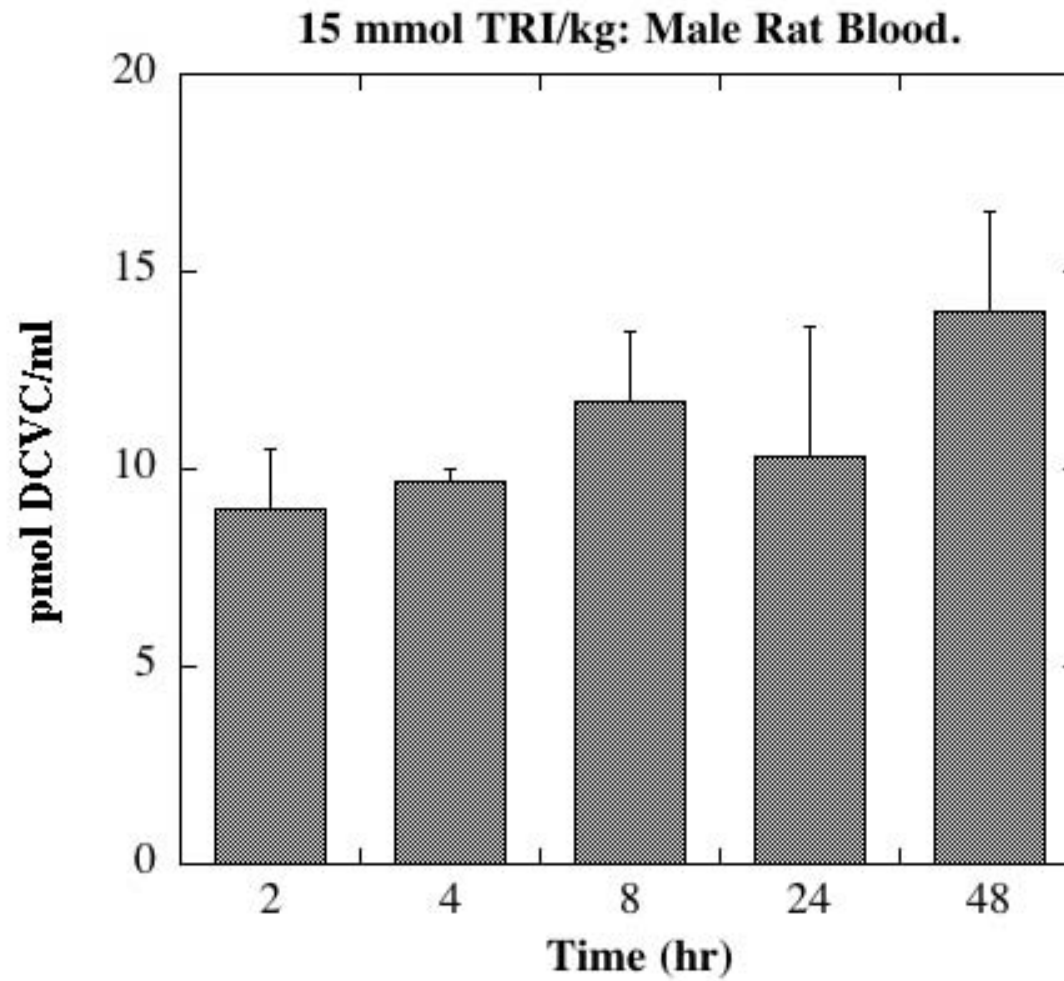
In Vivo Disposition of TRI Administered to Male and Female Rats by Oral Gavage:

- Male and female F344 rats administered either 2, 5, or 15 mmol/kg TRI in corn oil by oral gavage
- Measured P450- and GST-derived metabolites in blood and urine (24, 48 hr) and in liver and kidney (2, 4, 8, 24 , 48 hr)

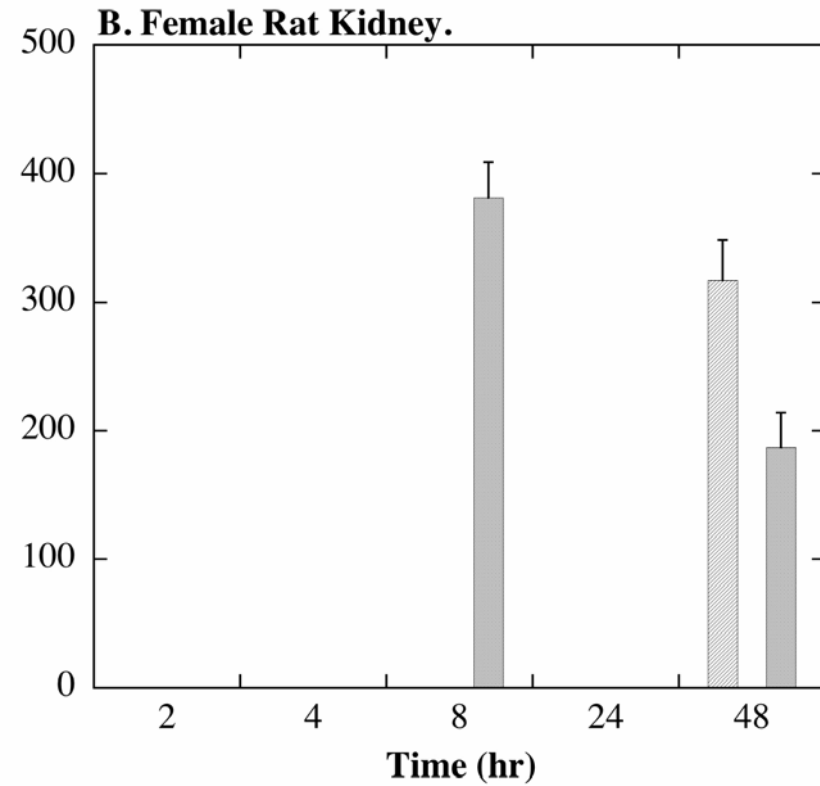
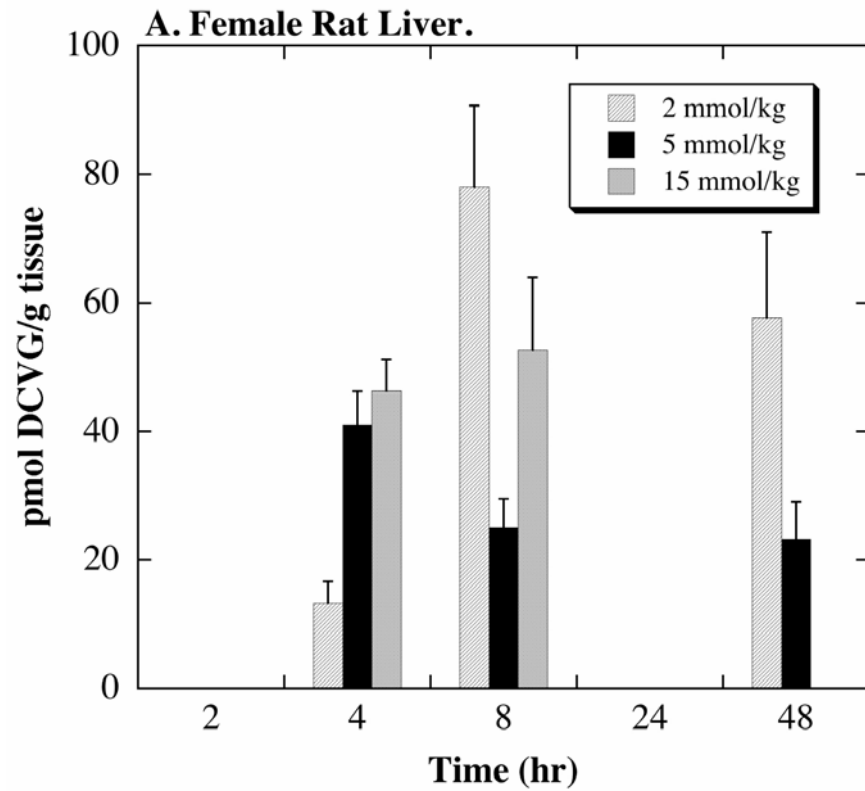
DCVG in Rat Blood:



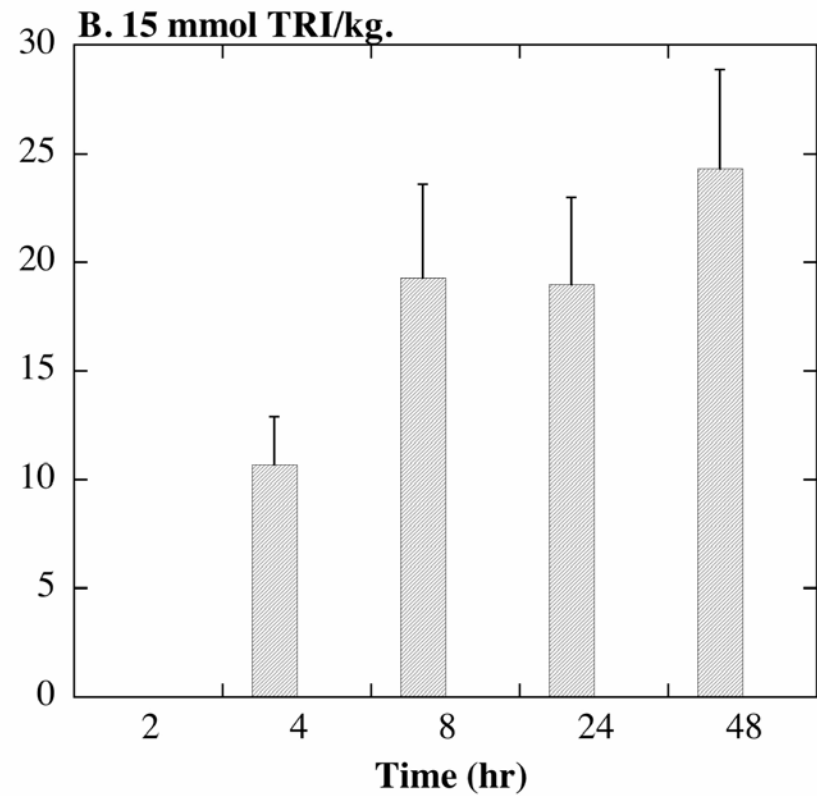
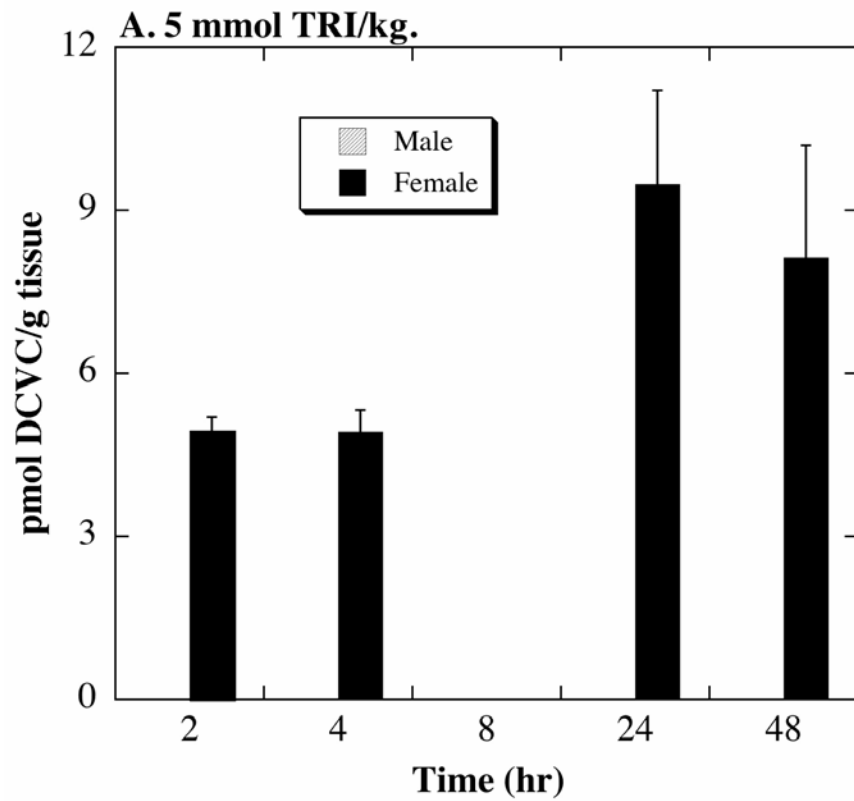
DCVC in Rat Blood:



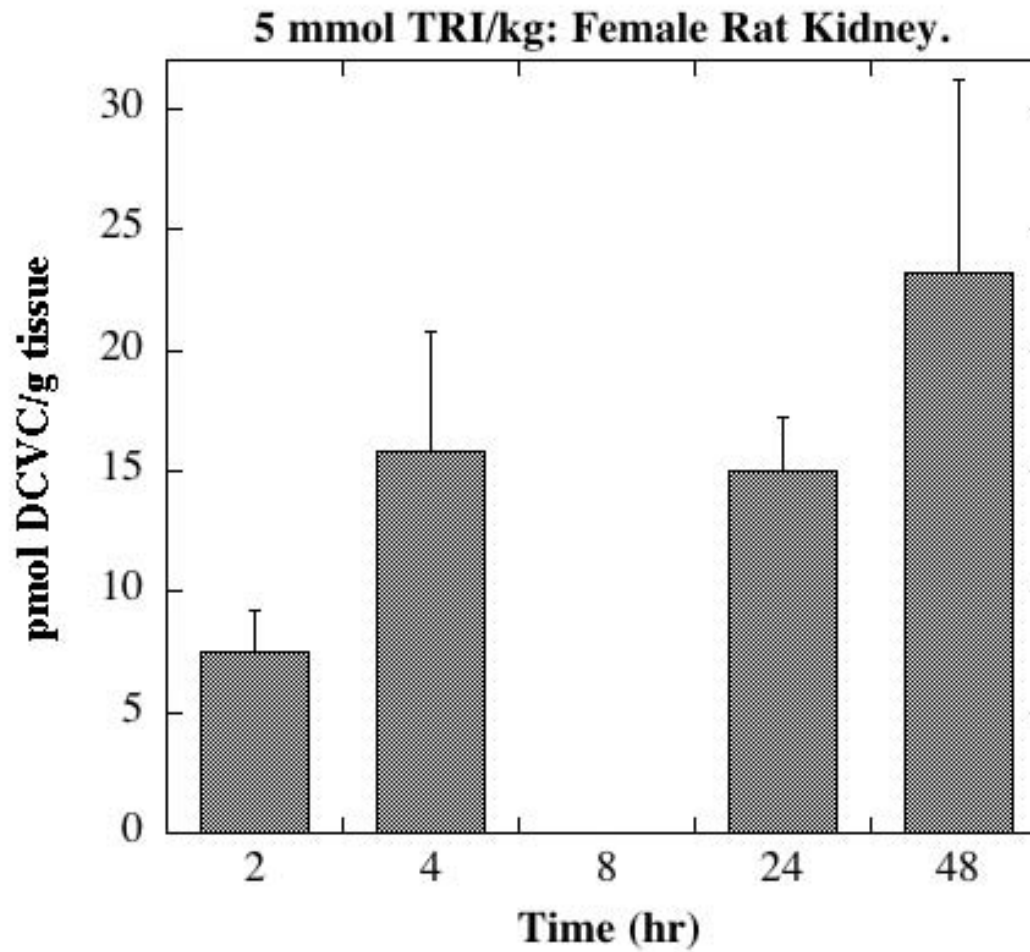
DCVG in Rat Liver and Kidney:



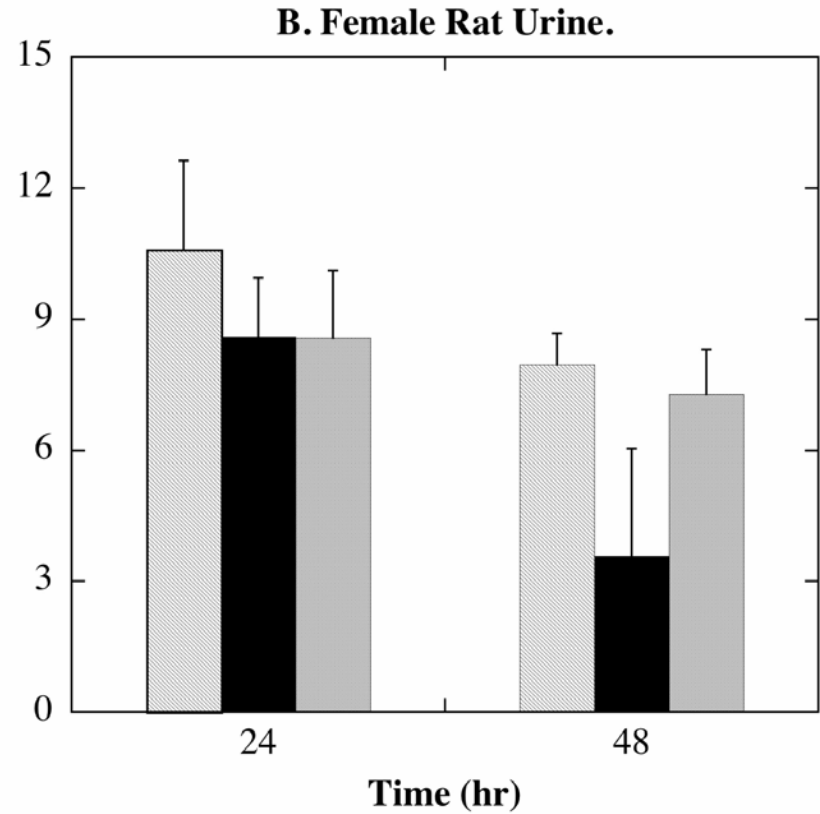
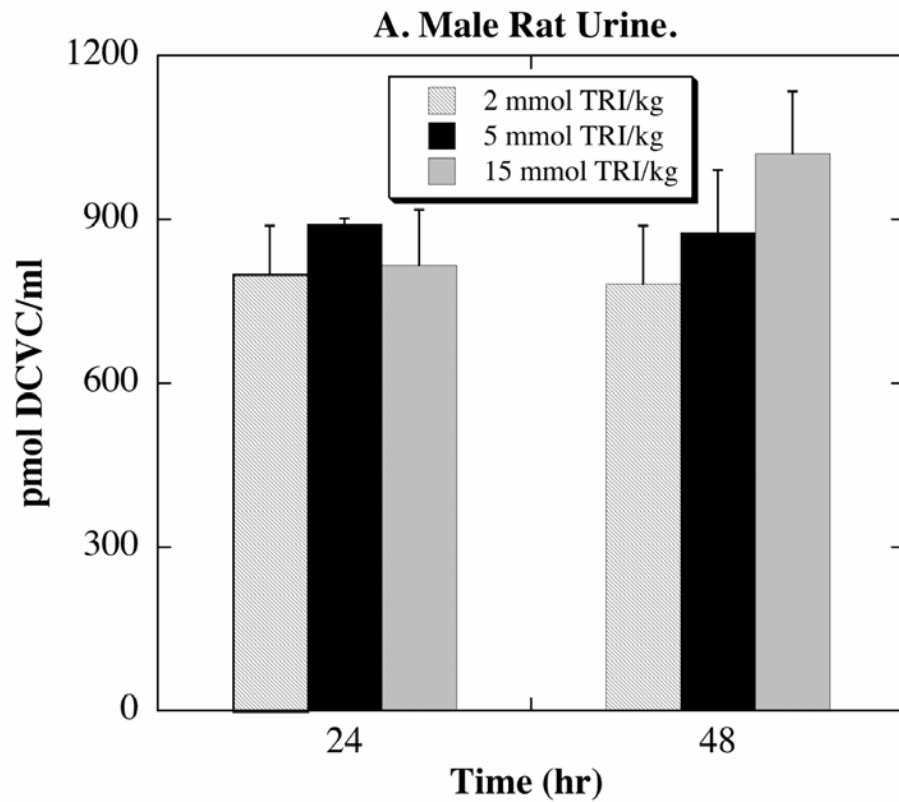
DCVC in Rat Liver:

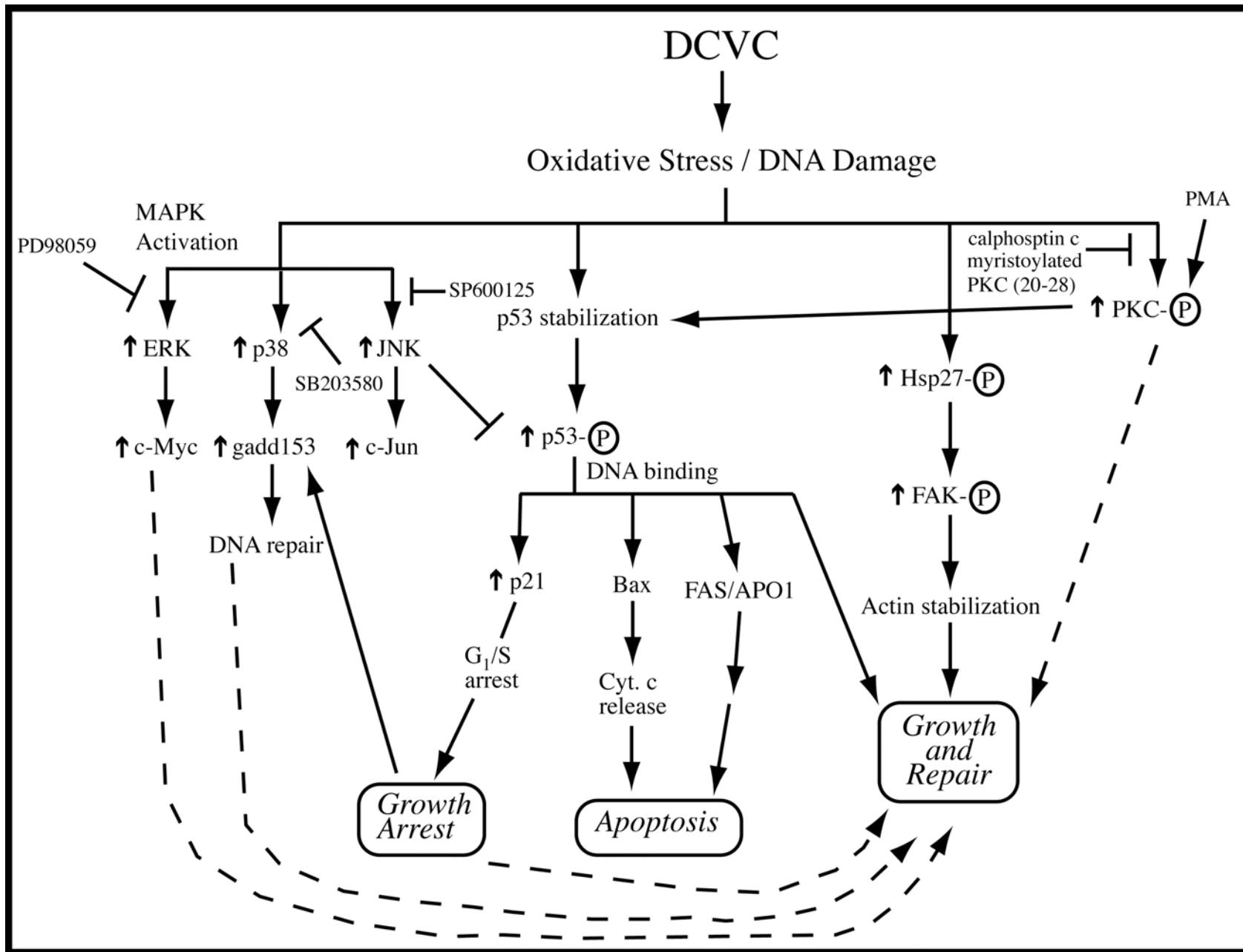


DCVC in Rat Kidney:



DCVC in Rat Urine:





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