

Pyrrolizidine Alkaloid Containing Plants

Bryan Stegelmeier
ADVS 586
February 12, 2008



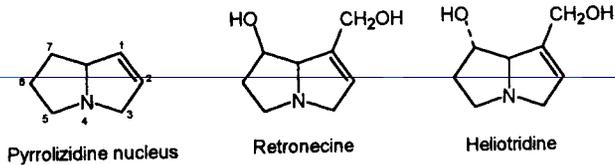
Pyrrolizidine alkaloid containing plants are the most widespread and expensive poisonous plant problem that affects plants, insects, animals and humans.

Outline

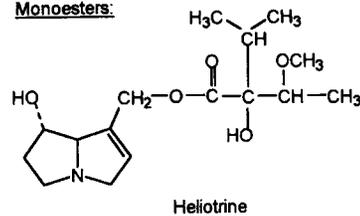


- Toxin
- Plants
- Poisoning
- Susceptibility
- Metabolism
- Clinical signs
- Lesions
- Diagnosis
- Current Research
- Future Objectives

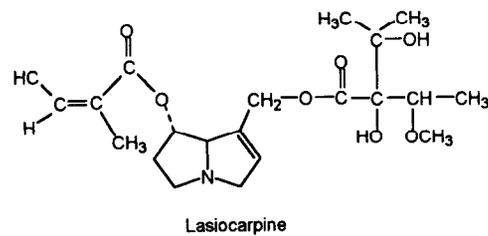
Pyrrolizidine alkaloids: Structure



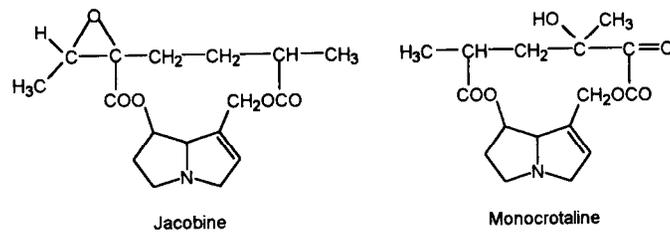
Monoesters:



Noncyclic diesters:



Cyclic diesters:



PA Global Problem

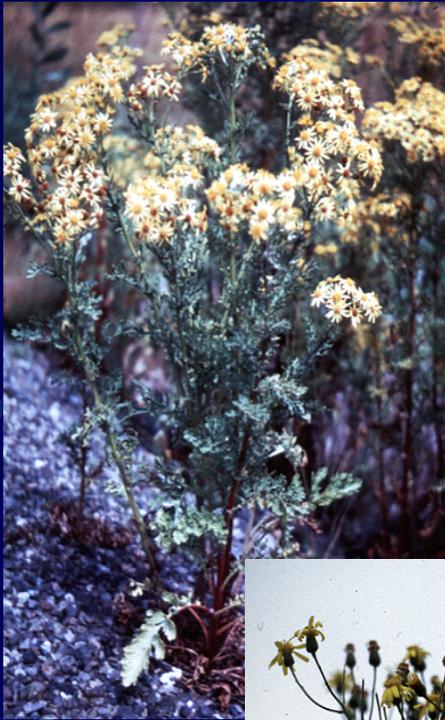


- >6000 plants contain PA's
- Most common poisonous plant affecting livestock, wildlife, and humans
- Invasive noxious weeds
- Contaminated feed, food and herbal preparations
- Wide range of susceptibility

Plants Containing Pyrrolizidine Alkaloids

- Compositae
 - Senecio (1200 species)
 - S. jacobaea (tansy ragwort)
 - S. vulgaris (common groundsel)
 - S. longilobus (threadleaf groundsel)
 - S. riddellii (Riddell groundsel)
- Fabaceae (Liguminosae)
 - Crotalaria (600 species)
 - C. sagittalis (rattlebox)
 - C. spectabilis (showy crotalaria)
 - C. retusa (wedge-leaf rattlebox)
 - C. pallida (smooth crotalaria)
 - C. juncea (sun hemp)
- Boraginaceae
 - Amsinckia intermedia (tarweed)
 - Borago officinalis (borage)
 - Cynoglossum officinale (hound's tongue)
 - Echium plantagineum (echium)
 - Echium vulgare (vipers bugloss)
 - Heliotropiu europaeum (heliotrope)
 - Symphytum officinale (comfrey)

Compositae



- Senecio (1200 species)
 - S. jacobaea (tansy ragwort)
 - S. vulgaris (common groundsel)
 - S. longilobus (threadleaf groundsel)
 - S. riddellii (Riddell groundsel)





Senecio jacobea

Senecio riddellii



*Senecio
longilobus*

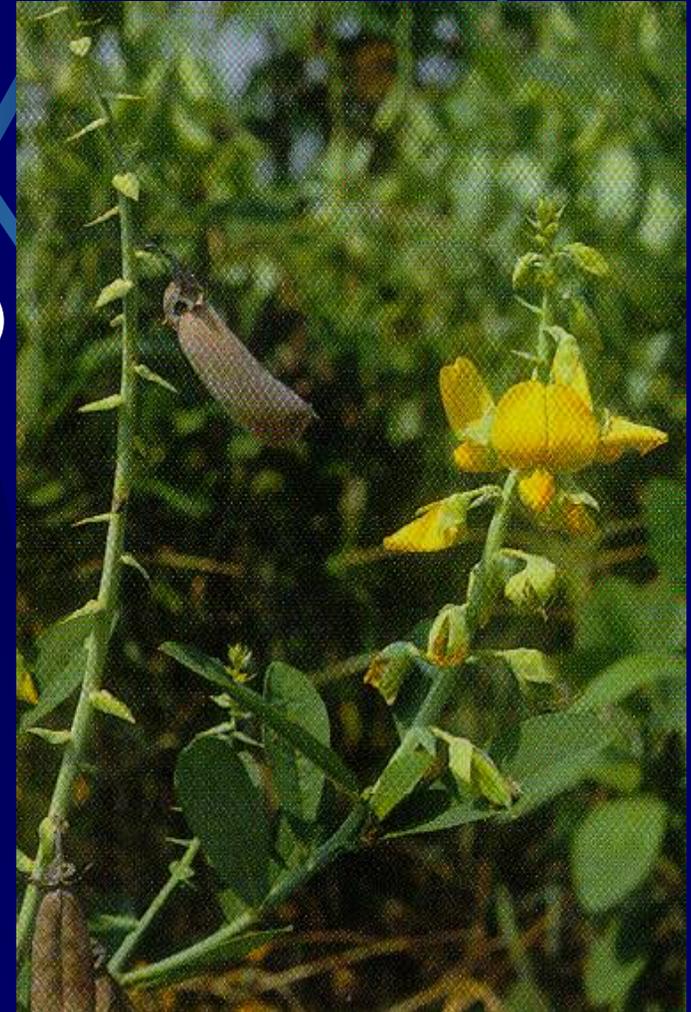






Fabaceae

- *Crotalaria* (600 species)
 - *C. sagittalis* (rattlebox)
 - *C. spectabilis* (showy crotalaria)
 - *C. retusa* (wedge-leaf rattlebox)
 - *C. pallida* (smooth crotalaria)
 - *C. juncea* (sun hemp)







● Boraginaceae

- *Amsinckia intermedia* (tarweed)
- *Borago officinalis* (borage)
- *Cynoglossum officinale* (hound's tongue)
- *Echium plantagineum* (echium)
- *Heliotropium europaeum* (heliotrope)
- *Symphytum officinale* (comfrey)

Cynoglossum officinale





PPRL 2008



PPRL 2008

Crop vs Weed

- *Echium plantagineum*
 - Patterson's Curse
 - Salvation Jane









PPRL 2008

Poisoning



- Accidental
- Palatability
- Feed Contamination
- Herbal Supplements

Feed and Food Contamination



- Native and introduced species invade ranges and fields.
- Though most are not palatable they are eaten in prepared feeds.
- Animal products?
- Human poisoning occurs.

9354
W. Sperl
H. Stuppner
I. Gassner
W. Judmaier
O. Dietze
W. Vogel

Reversible hepatic veno-occlusive disease in an infant after consumption of pyrrolizidine-containing herbal tea

Received: 27 April 1994
Accepted: 12 July 1994

W. Sperl (✉) · I. Gassner
Department of Paediatrics,
University of Innsbruck,
Anichstrasse 35,
A-6020 Innsbruck, Austria

H. Stuppner
Institute of Pharmacognosy,
University of Innsbruck,
Innsbruck, Austria

W. Judmaier
Department of Radiology
and Magnetic Resonance Imaging,
University of Innsbruck,
Innsbruck, Austria

O. Dietze
Institute of Pathology,
University of Innsbruck,
Innsbruck, Austria

W. Vogel
Department of Internal Medicine,
University of Innsbruck,
Innsbruck, Austria

Abstract Veno-occlusive disease was diagnosed in an 18-month-old boy who had regularly consumed a herbal tea mixture since the 3rd month of life. The boy developed portal hypertension with severe ascites. Histology of the liver showed centrilobular sinusoidal congestion with perivenular bleeding and parenchymal necrosis without cirrhosis. The tea contained peppermint and what the mother thought was coltsfoot (*Tussilago farfara*). The parents believed the tea aided the healthy development of their child. Pharmacological analysis of the tea compounds revealed high amounts of pyrrolizidine alkaloids. Seneciopylline and the corresponding N-oxide were identified as the major components by thin-layer chromatography, mass spectrometry and NMR spectroscopy. We calculated that the child had consumed at least 60 µg/kg body weight per day of the toxic pyrrolizidine alkaloid mixture over 15 months. Macroscopic and microscopic analysis of the leaf material

indicated that *Adenostyles alliariae* (Alpendost) had been erroneously gathered by the parents in place of coltsfoot. The two plants can easily be confused especially after the flowering period. The child was given conservative treatment only and recovered completely within 2 months.

Conclusion In all cases of veno-occlusive disease pyrrolizidine alkaloids ingestion should be excluded. The identity of collected plant material should be verified by pharmaceutically trained experts and information of composition, dosage and mode of administration should be included in guidelines for herbal preparations.

Key words Veno-occlusive disease
Pyrrolizidine alkaloids · Herbal tea

Abbreviations VOD-veno-occlusive disease · FAB/MS fast atom bombardment mass spectrometry · NMR nuclear magnetic resonance

Introduction

Veno-occlusive disease (VOD) of the liver is characterized by portal hypertension with severe ascites due to obliteration of centrilobular or sublobular hepatic veins. It is the most frequent cause of hepatic vein obstruction in children. Hepatic VOD in infants may be caused by hepatic irradiation, chemotherapeutic drugs and bone marrow transplan-

tation; in underdeveloped countries, the most common cause is ingestion of plants that contain hepatotoxic pyrrolizidine alkaloids. Epidemics of pyrrolizidine alkaloid intoxication have been reported from India, Afghanistan and Jamaica [1], whereas only sporadic cases are known from the United States of America, United Kingdom and Europe [4, 14, 18]. In the latter, comfrey products have led to an increased awareness of intoxication due to their widespread use in alternative medicine [4, 14, 15, 23].

Susceptibility

- Age
- Species
- Sex
- Nutritional Status

J. Vet. Med. A 40, 213-218 (1993)
© 1993 Paul Parey Scientific Publishers, Berlin and Hamburg
ISSN 0931-184X

This material may be protected
by copyright law (Title 17 U.S. Code)

*New South Wales Agriculture and Fisheries, Orange, and
The University of Queensland, Brisbane, Australia,
Toxicology Unit, Medical Research Council, Carshalton, England*

Pyrrolizidine Alkaloidosis in a Two Month Old Foal

A. C. SMALL¹, W. R. KELLY², A. A. SEAWRIGHT², A. R. MATTOCKS³ and R. JUKES³

Address of authors: ¹Diagnostic Veterinary Laboratories, Randwick, New South Wales, Australia

²Department of Veterinary Pathology, The University of Queensland, St. Lucia, 4072, Australia

³MRC Toxicology Unit, Carshalton, Surrey, SM54EF, England

With one figure and one table

(Received for publication June 30, 1992)

Summary

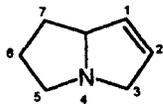
A foal, small and jaundiced from birth, succumbed after two months to chronic hepatic damage which was characterised by fibrosis, biliary ductular hyperplasia and the presence of pleomorphic hepatocytes containing either a single large nucleus or multiple nuclei. The fixed liver contained sulfur-bound pyrroles, which are derived from pyrrolizidine alkaloids. During pregnancy the pasture was heavily infested with the pyrrolizidine alkaloid-containing plant, *Senecio madagascariensis*. The hepatic disease affecting the foal appears to have been initiated by consumption of the alkaloids by the mare during gestation, and to represent a rare case of congenital pyrrolizidine alkaloidosis.

Species Susceptibility

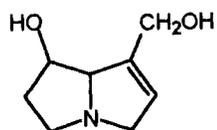
Species	Susceptibility to PA toxicosis	In vitro pyrrole production rate ^a	Lethal dose (as % of body weight) ^b	Reference
Cow	High	High	3.6	Cheeke <i>et al.</i> (1985)
Horse	High	High	7.3	Garrett <i>et al.</i> (1984)
Sheep	Low	Low	302	White <i>et al.</i> (1984)
Goat	Low	?	205	Goeger <i>et al.</i> (1982a)
Rat	High	High	21	Goeger <i>et al.</i> (1983)
Mouse	Intermediate	High	?	
Rabbit	Low	High	113	Pierson <i>et al.</i> (1977)
Guinea pig	Low	Low	119	Cheeke and Pierson-Goeger (1983)
Hamster	Low	High	338	Cheeke and Pierson-Goeger (1983)
Gerbil	Low	?	3640	Cheeke and Pierson-Goeger (1983)
Chicken	High	Low	39	Cheeke and Pierson-Goeger (1983)
Japanese quail	Low	Low	2450	Buckmaster <i>et al.</i> (1977)

^aAdapted from Shull *et al.* (1976).

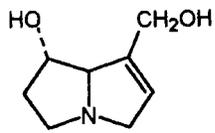
^bChronic lethal dose of *Senecio jacobaea*.



Pyrrolizidine nucleus

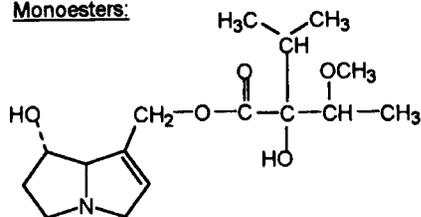


Retronecine



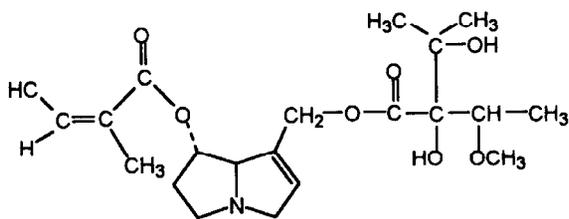
Heliotridine

Monoesters:



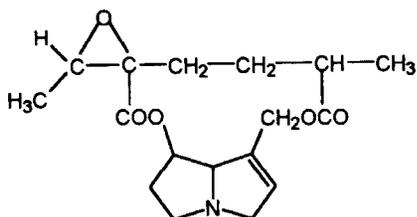
Heliotrine

Noncyclic diesters:

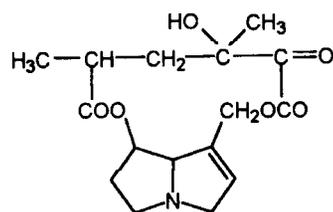


Lasiocarpine

Cyclic diesters:



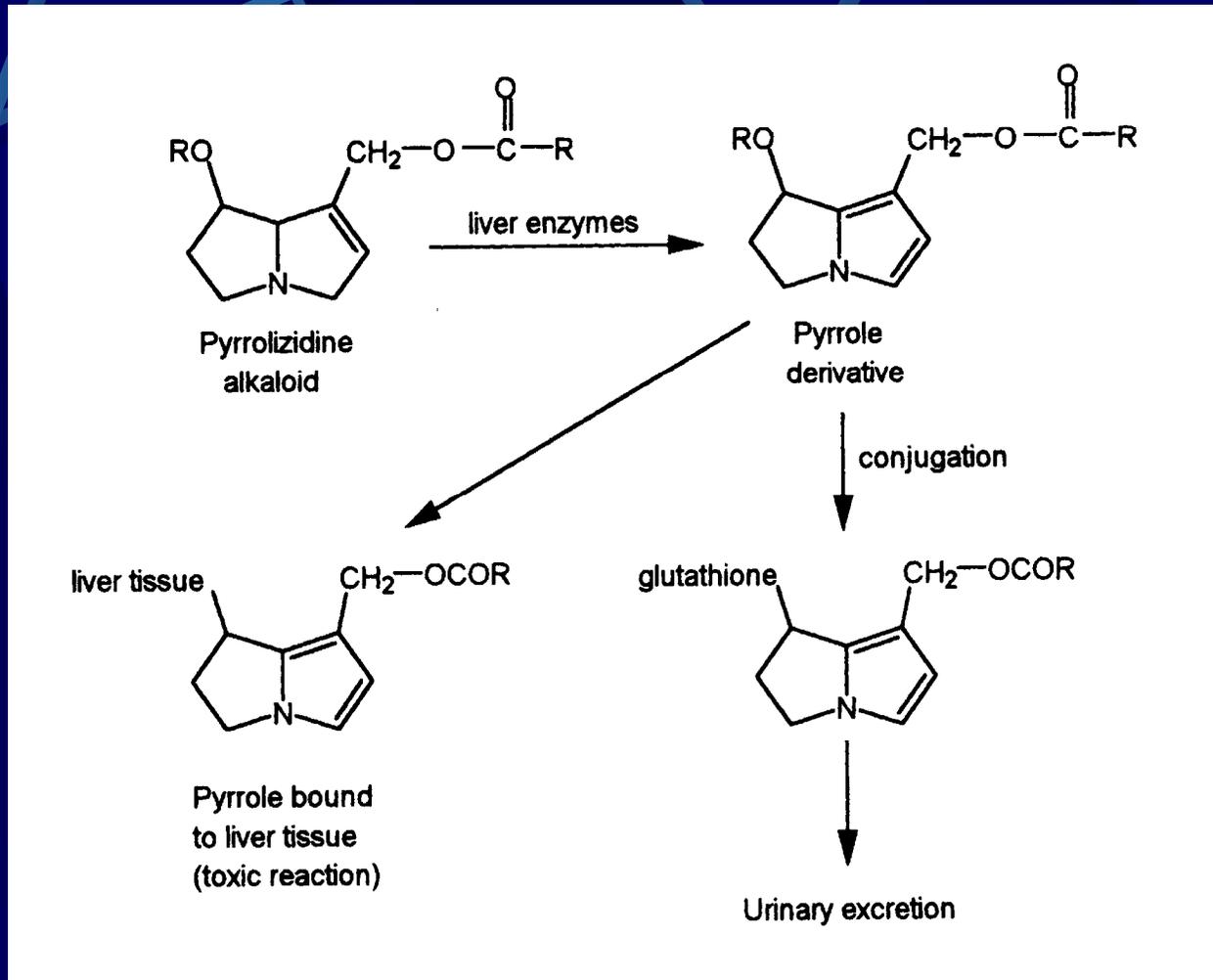
Jacobine

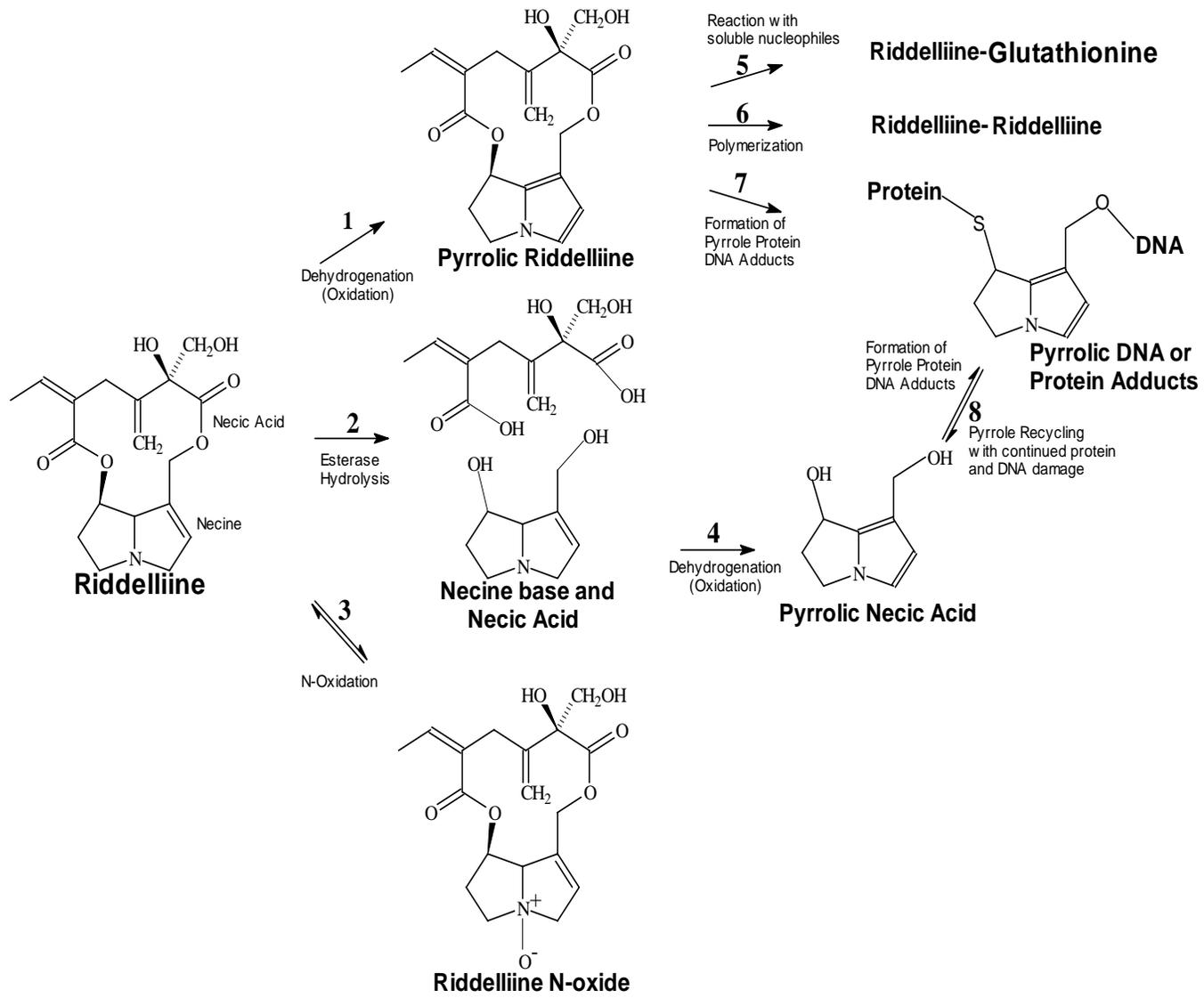


Monocrotaline

Pyrrolizidine alkaloids: Chemistry

Pyrrolizidine Alkaloids: Metabolism



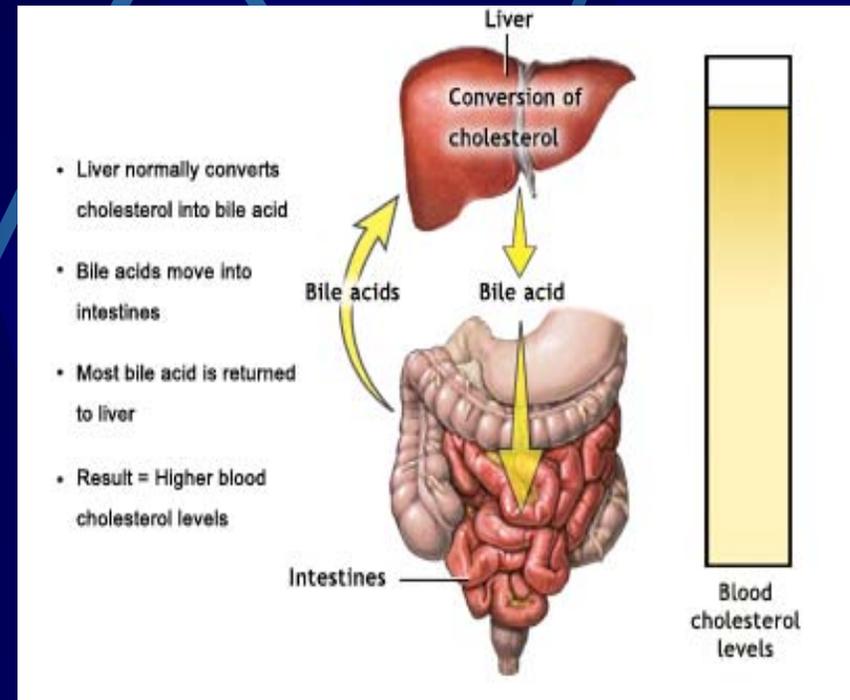
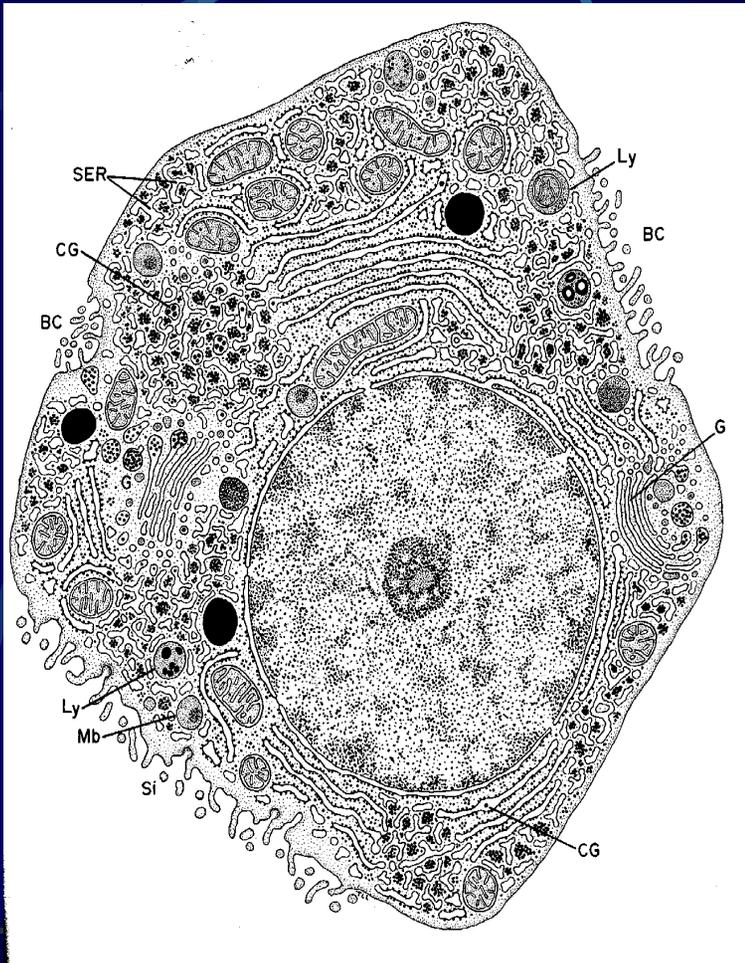


Clinical Lesions- Dose Dependent



- Hepatic Panel
- Bile Acids (BSP)
- Hepatic Biopsy
- Blood Pyrrole Analysis

Leakage enzymes (AST, ALT, SDH, LDH)
Biliary proliferation (ALP, GGT)
Cholestasis (Bilirubin, Bile Acids, Dye retention)



Clinical Signs

- Lethargy
- Anorexia
- Photosensitivity and solar dermatitis
- Diarrhea
- Weakness
- Wandering or blindness
- Belligerence
- Ascites







PPRL 2008



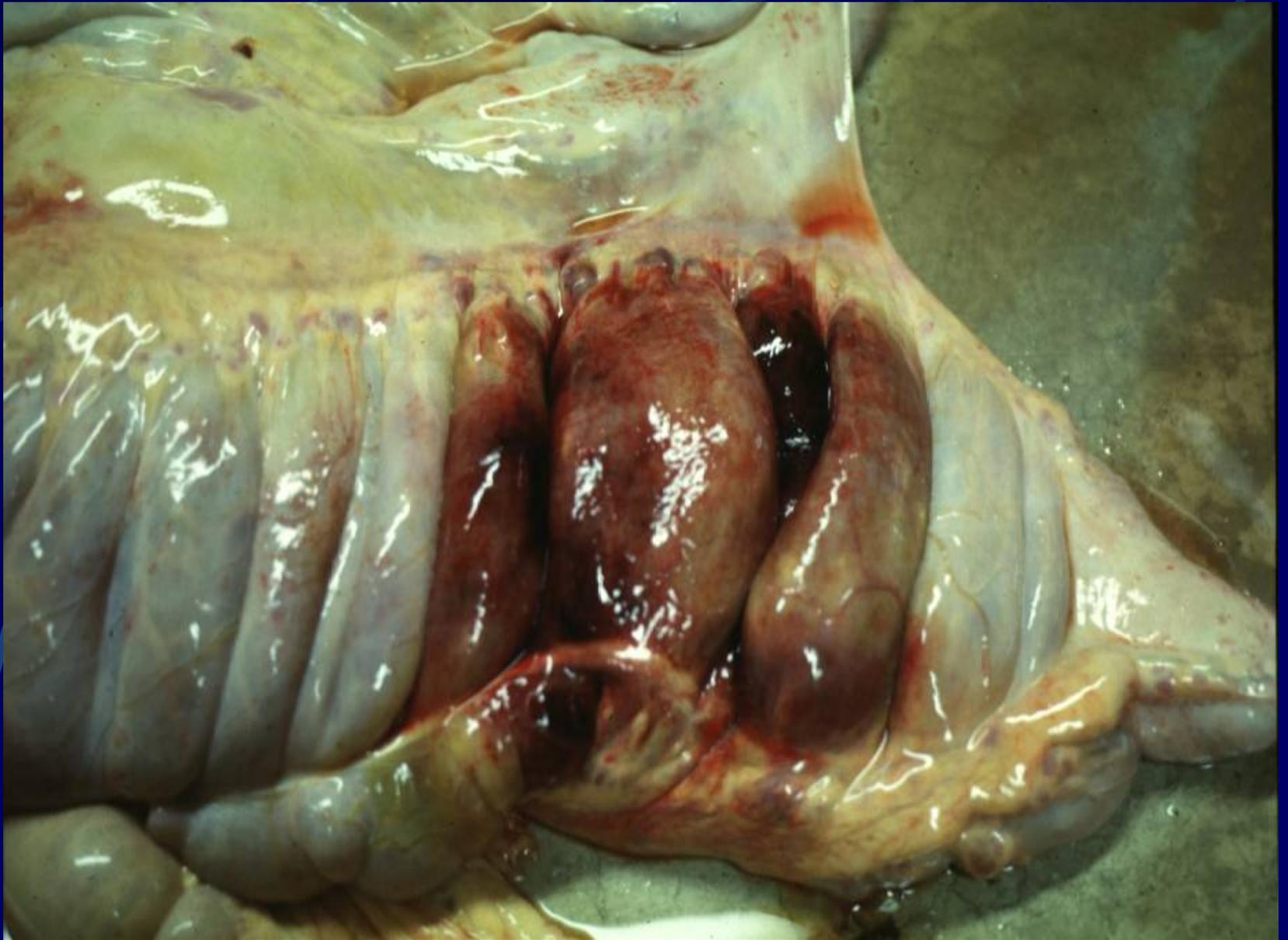
- Hepatic lesions
 - Yellow soft liver
 - Hepatocyte necrosis, fibrosis, biliary hyperplasia
- Generalized icterus
- Subcutaneous and visceral edema
- Species related extra-hepatic lesions

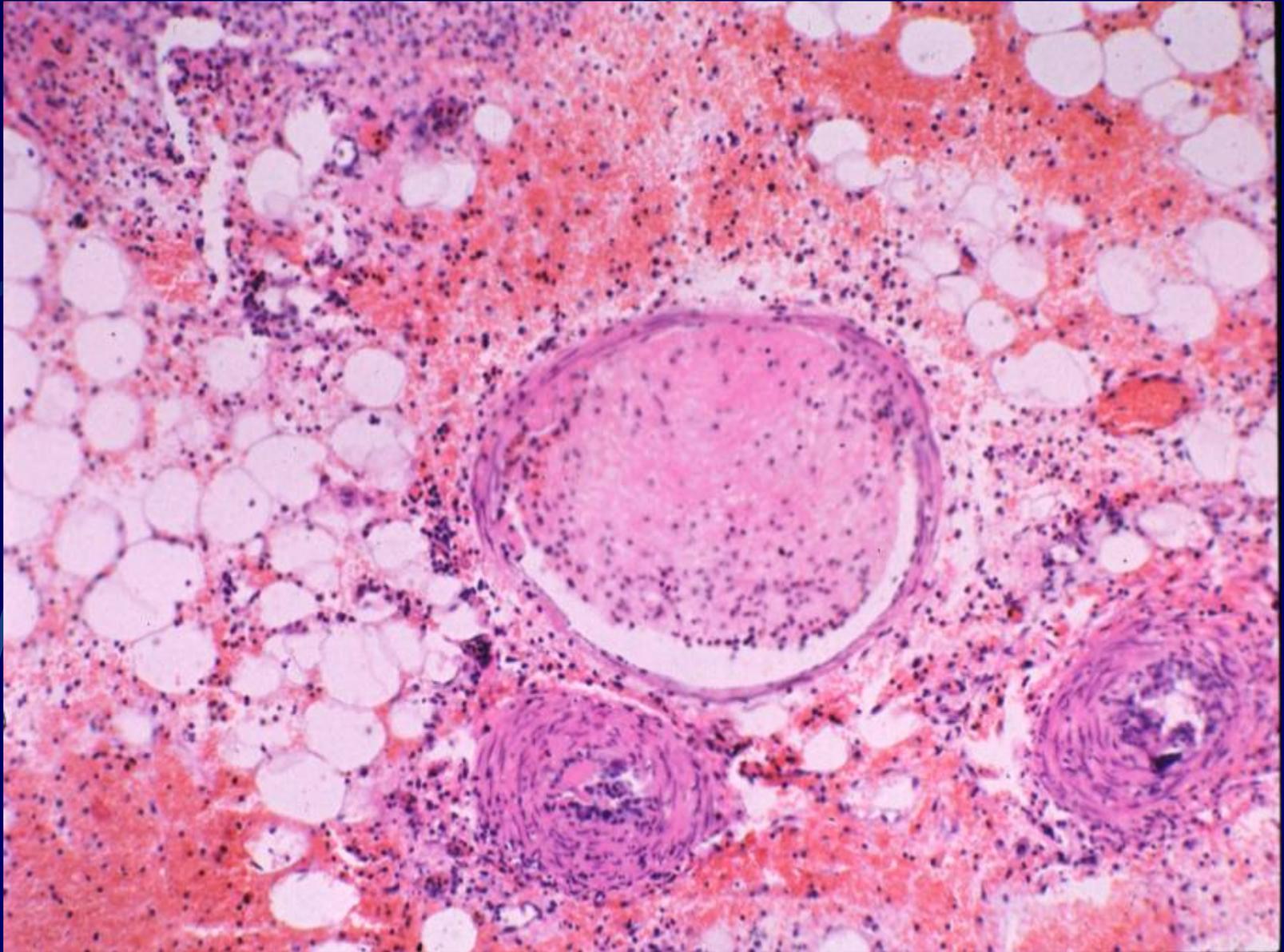
Secondary Lesions

- Wasting cow
- Hepatic encephalopathy
- Icteric and hyperbilirubinemia
- Edema and dilated lymphatics
- Gross liver necrosis
- Edema (colonic and abomasal)
- Vascular thrombosis and intestinal infarction
- Photosensitivity and dermal necrosis

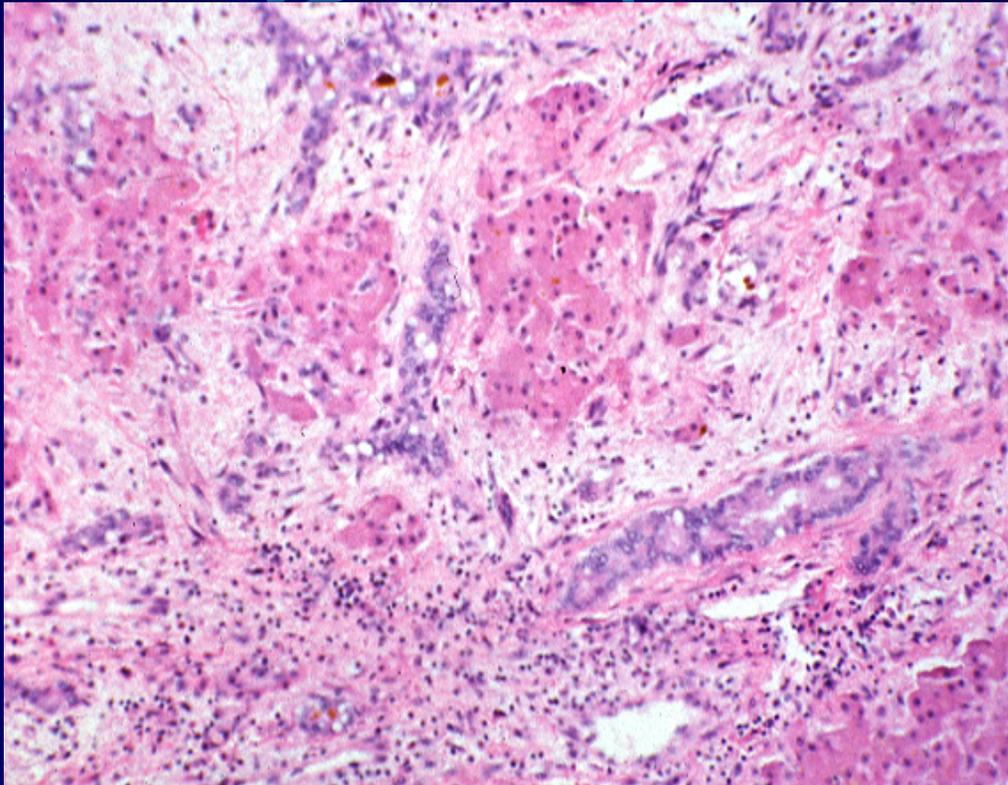




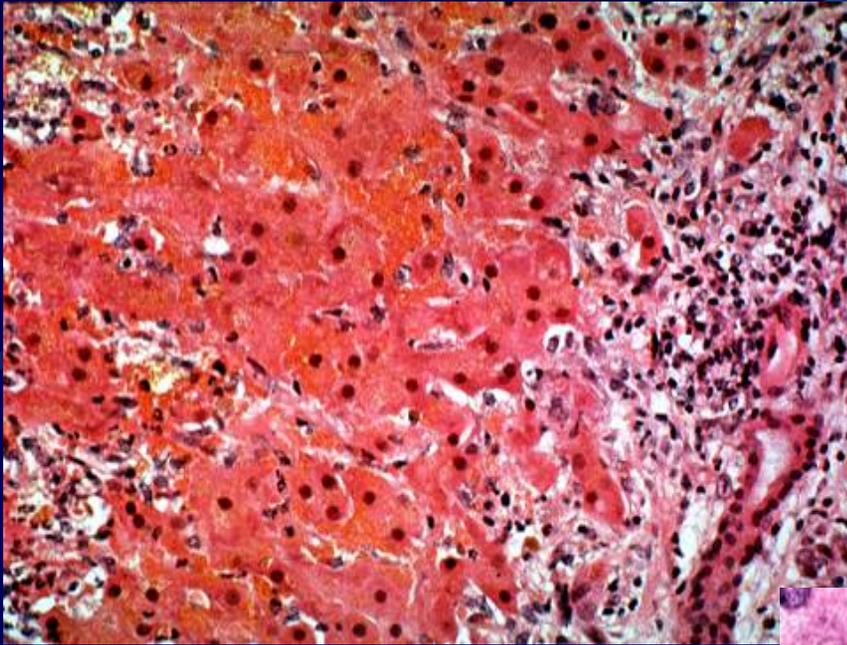




Dose Dependent- Histologic Lesions

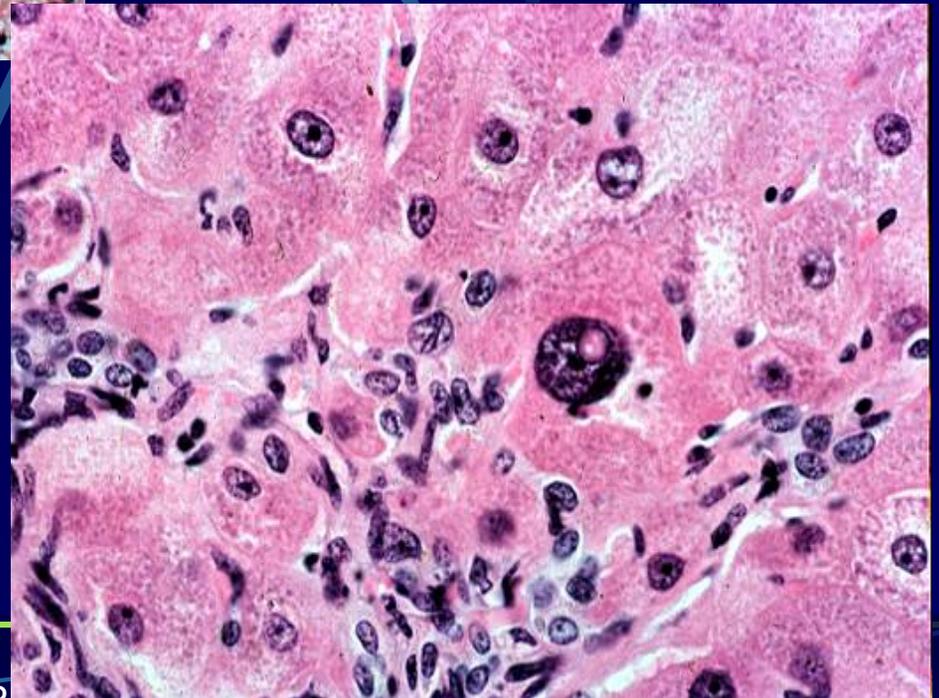


- Portal circulation
- Hepatic metabolism
- Hepatocyte response
- Classical response
 - Necrosis
 - Fibrosis
 - Biliary hyperplasia



Acute Toxicity

Chronic Toxicity

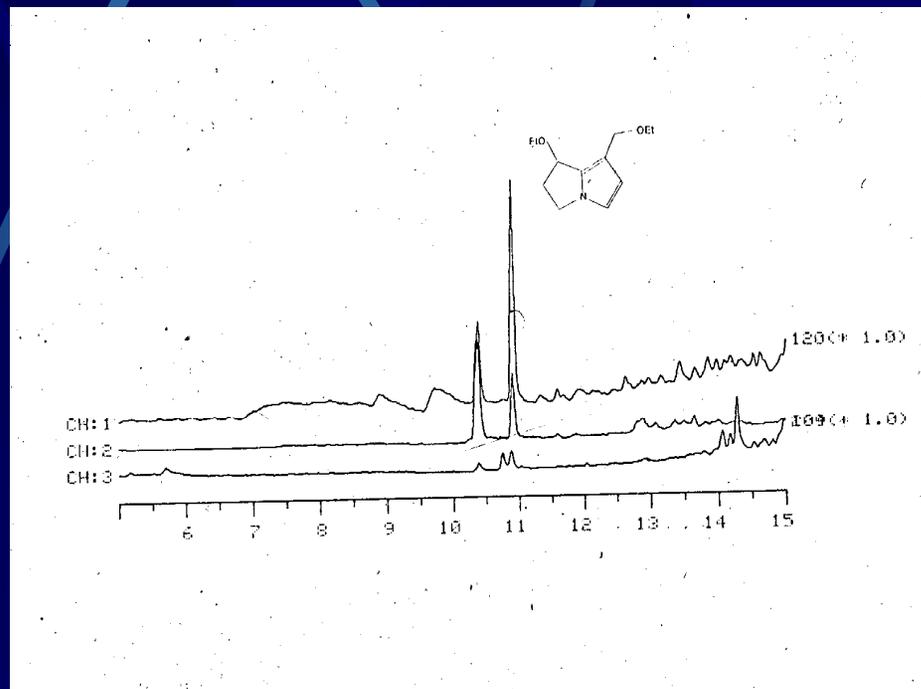


Diagnostic Techniques



- Hepatic Panel
- Bile Acids (BSP)
- Hepatic Biopsy
- Blood Pyrrole Analysis

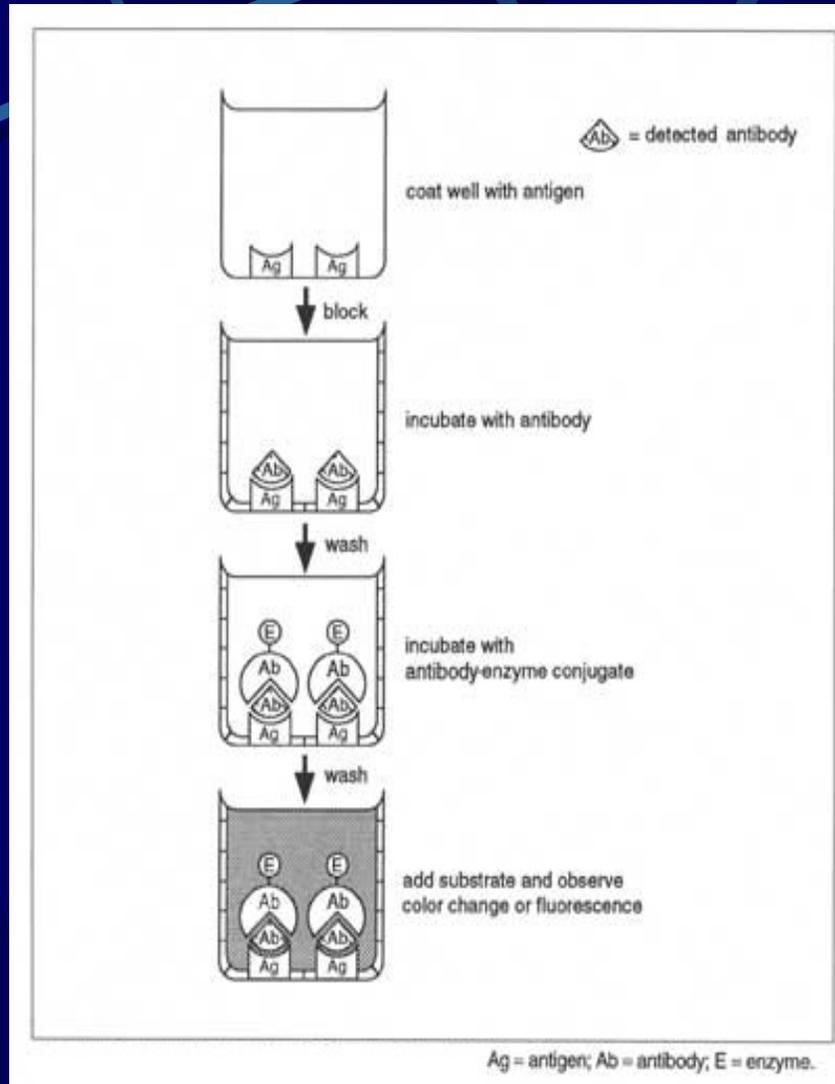
Pyrrole Detection



Research Goals

- ELISA techniques to monitor contamination of feeds and food.
- Better diagnostic techniques to identify poisoned animals and form a prognosis.
- Better characterization of risk and susceptible species- especially neonatal toxicity.
- Evaluate potential therapies, antidotes and vaccines.
- Determine molecular pathogenesis of PA toxicity- fate of PA adducts and delayed toxicities.
- Integrate results and models to predict risk of PA toxicosis in humans and animals.

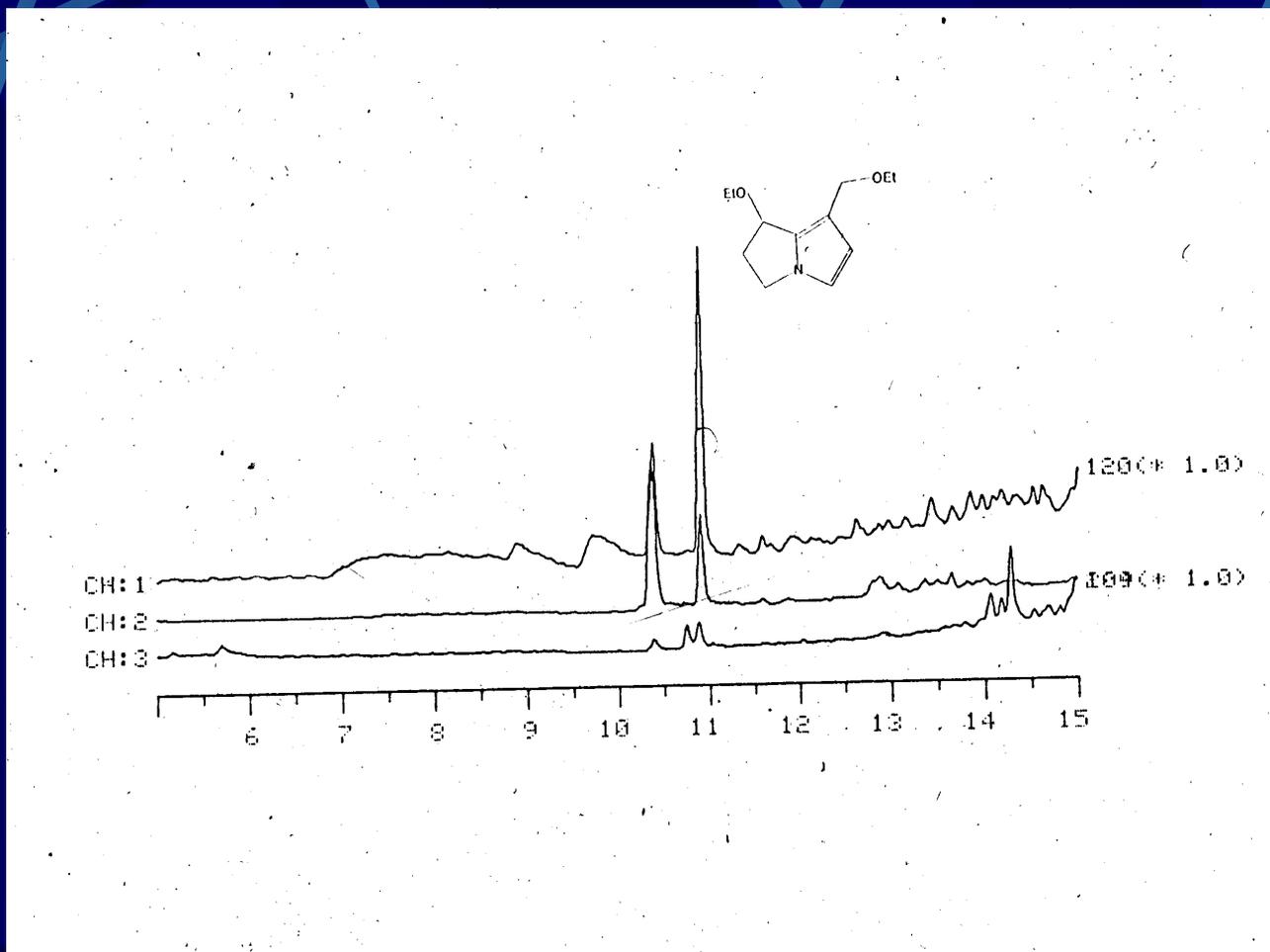
ELISA Studies

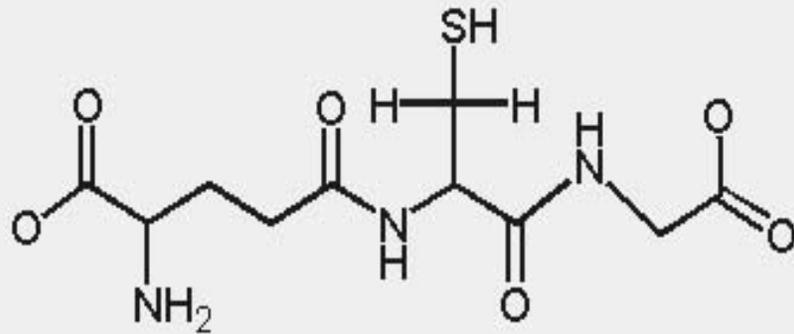
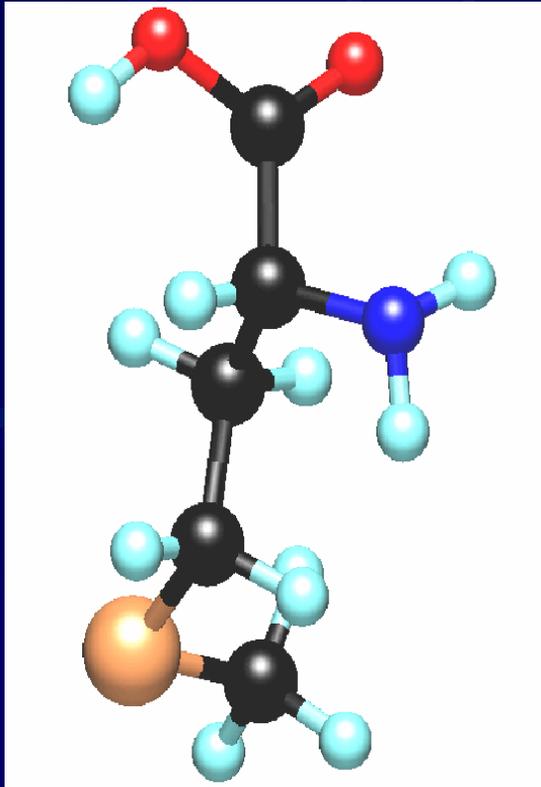


Class and alkaloid specific ELISA's



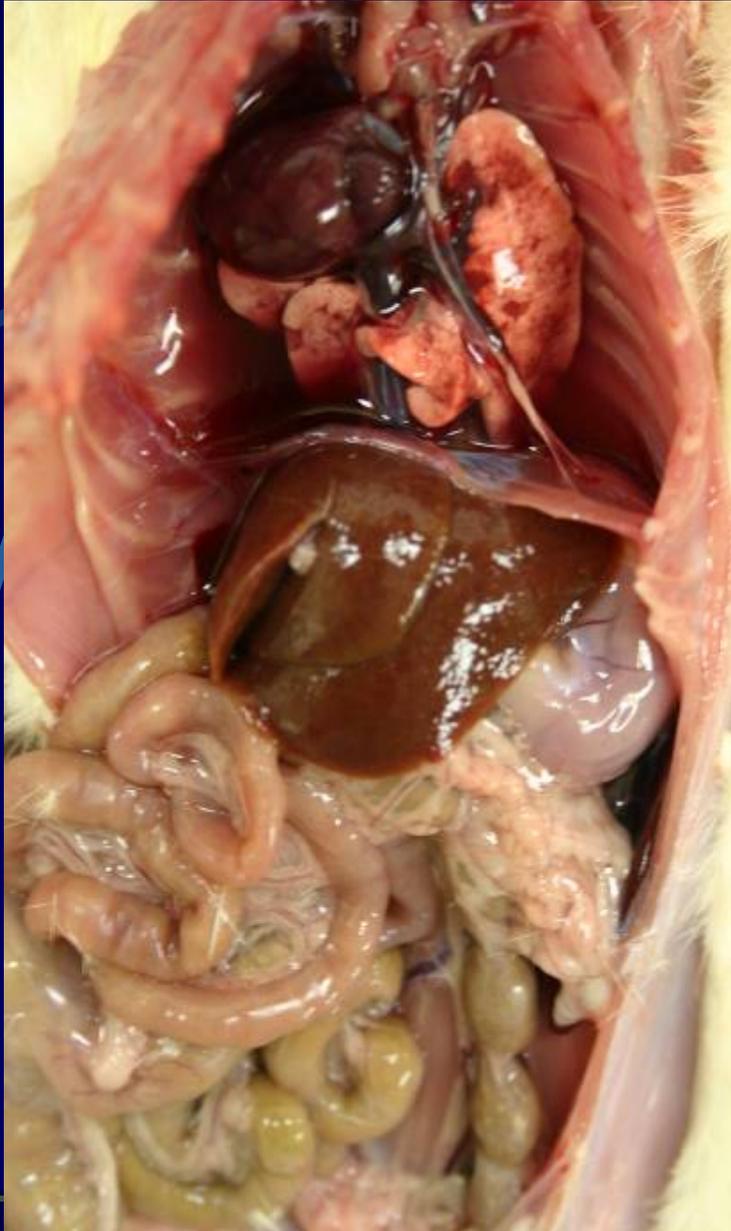
Pyrrole Detection





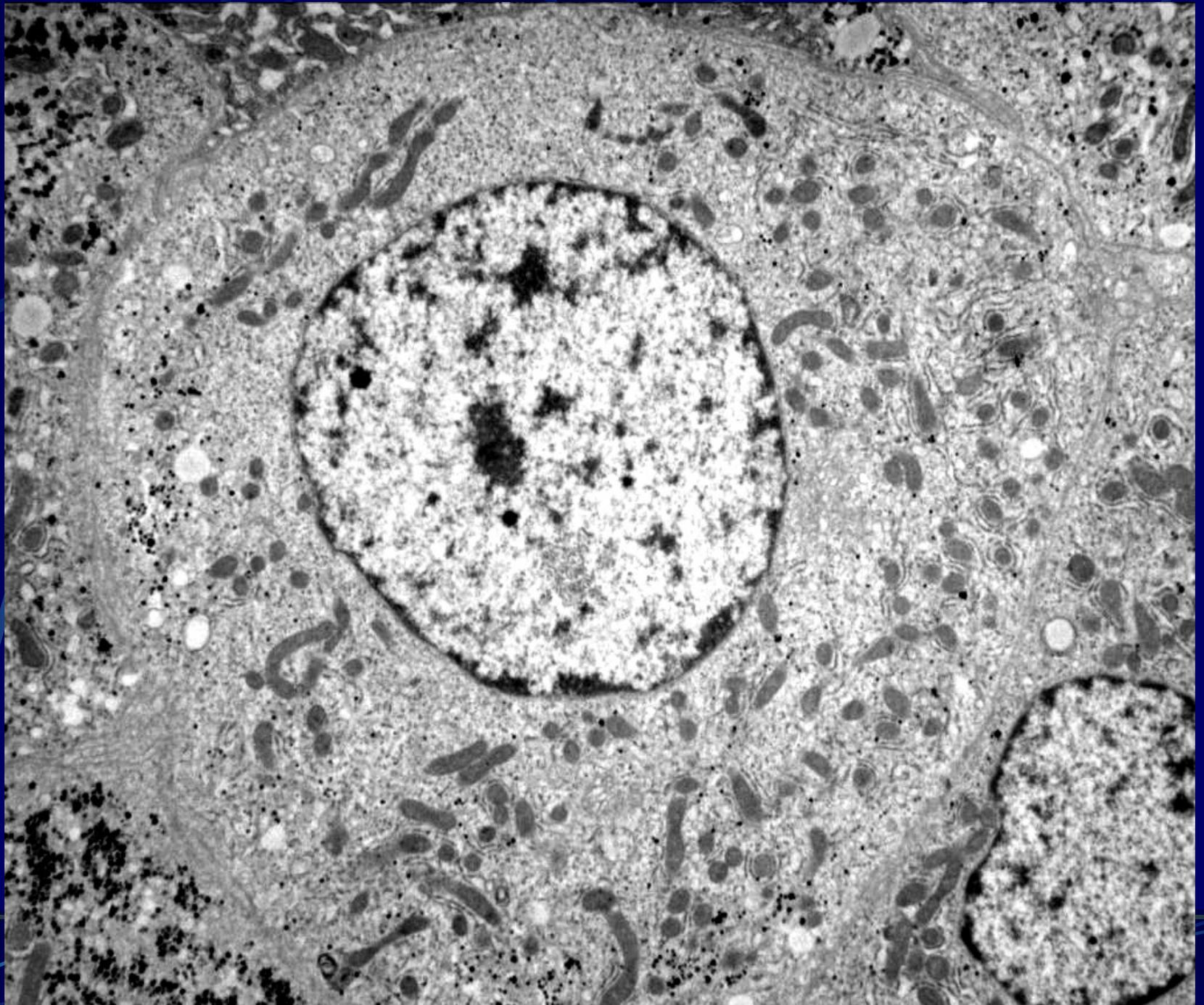
GLUTATHIONE (GSH)

gamma-glutamyl-cysteinyl-glycine





Diagnosis



Sample Studies

- Pig Study
 - Age susceptibility
 - N-oxide toxicity
 - Pyrrole toxicity
- Riddelliine Vaccination Study

Pig Riddelliine Studies

- Age susceptibility study
 - Determine toxic dose in different aged growing pigs
- Pyrrole and N-oxide toxicity study
 - Determine the comparative toxicity of n-oxide riddelliine and protein bound riddelliine metabolites (pyrroles)
- Neonatal effects of maternal toxicity
 - Determine the neonatal effects of maternal ingestion of riddelliine

Neonatal riddelliine toxicity of pigs



- 5 different age groups of 12 pigs
- neonates, 3 week old, 6 weeks old, 12 weeks old, 24 weeks old, and year old crossbred pigs
- dosed with riddelliine at 0.0, 5.0, 10.0 and 20.0 mg/kg for 14 days

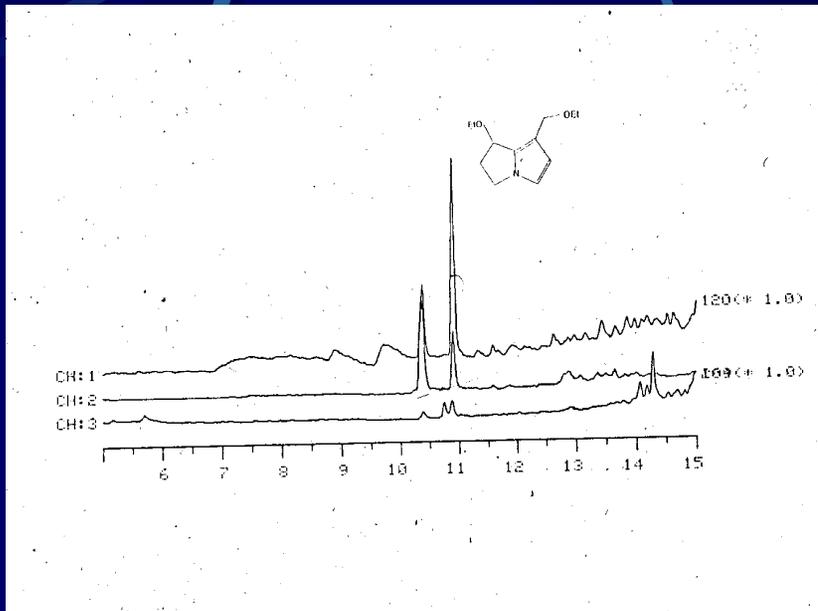
Indicators of Toxicity

- Weight gains and clinical signs
- Serum and hematological changes
- Gross and microscopic lesions
- Chemical and immunologic detection and monitoring of PA metabolites

Serum Biochemistries

- 20 mg/kg
 - Decreased Albumin, Total Protein and CO₂
 - Increased AST, GGT, and CK
 - Increased Bilirubin (Total and Direct) and Bile Acid
 - Sporadic changes in BUN, CREA, GLU, Na, and Cl
- 10 mg/kg
 - Sporadic Reduced Albumin and Total Protein, Mild AST, GGT and CK Elevations, and Mild Elevations in Bilirubin
- 5 mg/kg
 - Minimally Decreased Albumin and Total Protein

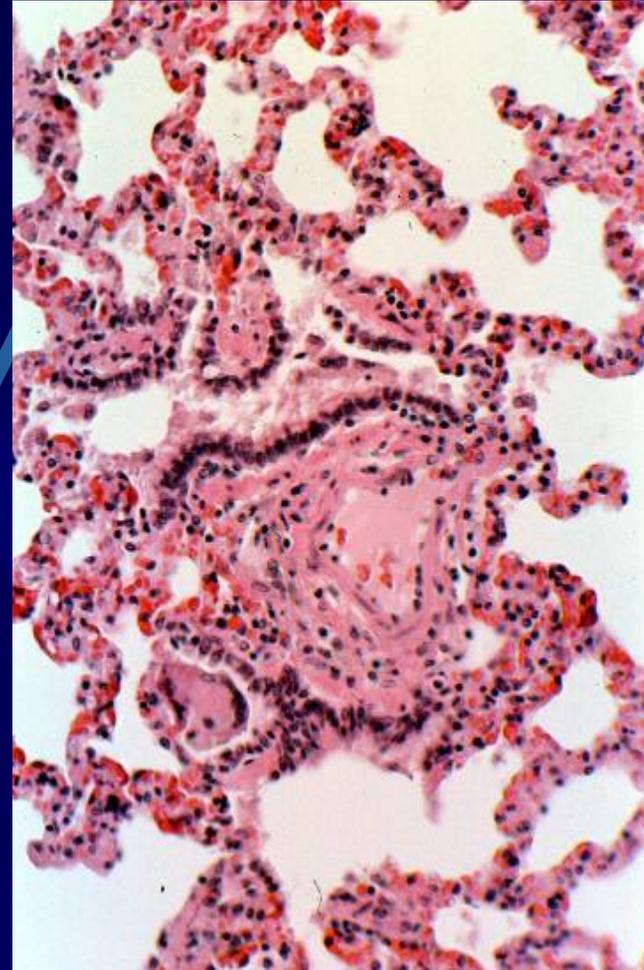
Pyrrole Detection

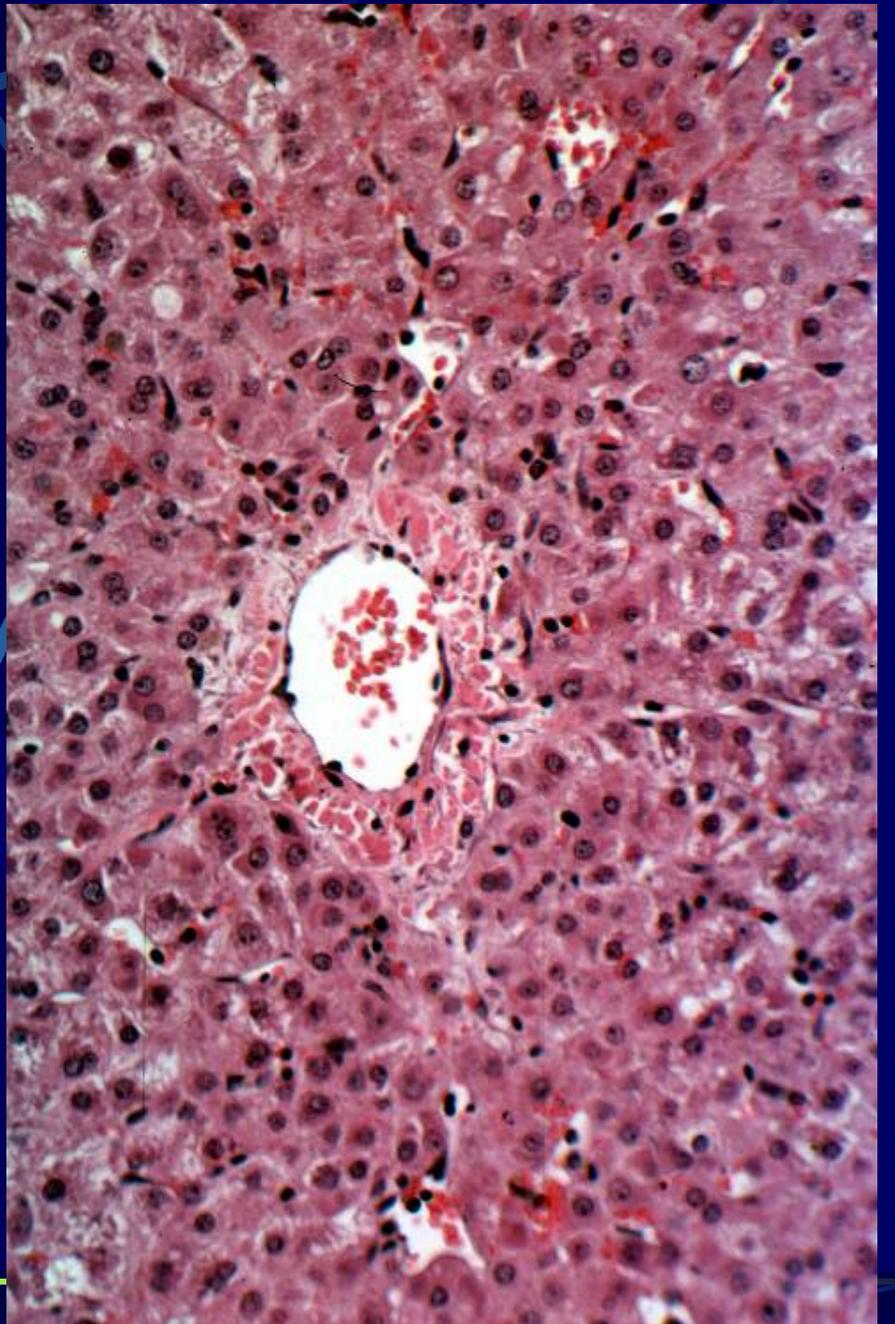
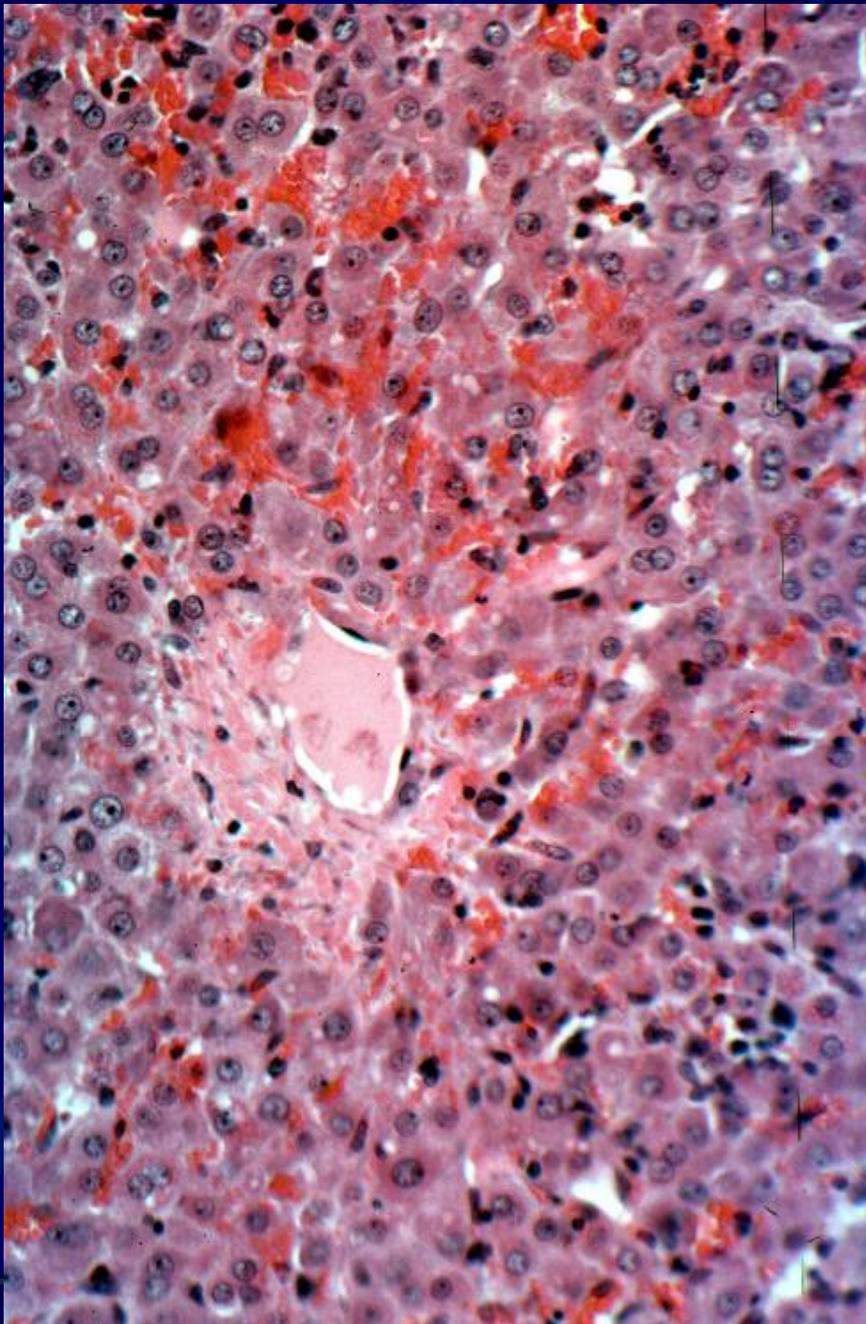


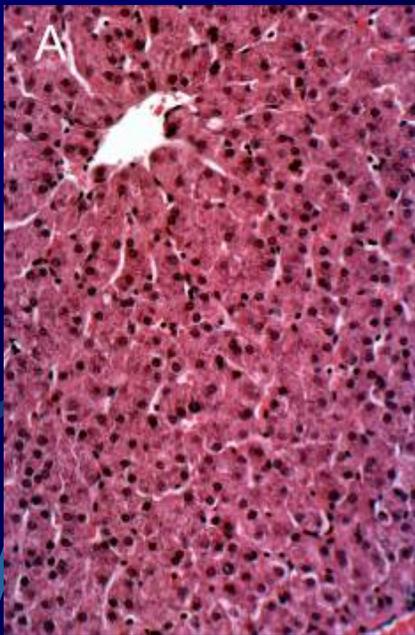
- Pilot Study: Pyrroles were detected in sow liver and serum, piglets results were variable.
- All exposed pigs in age related study were positive. Assay not yet quantitative.

Histologic Changes

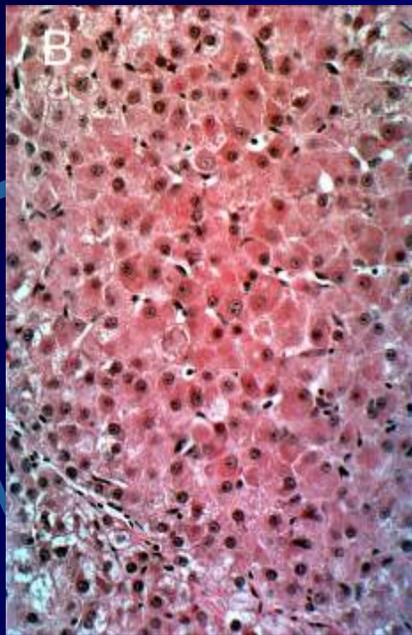
- Extrahepatic lesions rare.
- Hepatocellular swelling, necrosis, hemorrhage and inflammation.
- Hepatic vascular lesions.



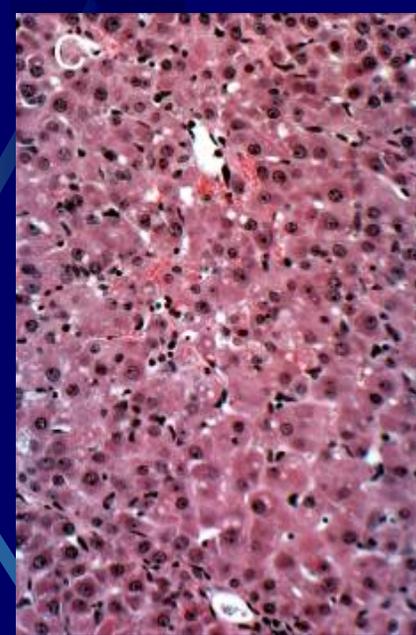




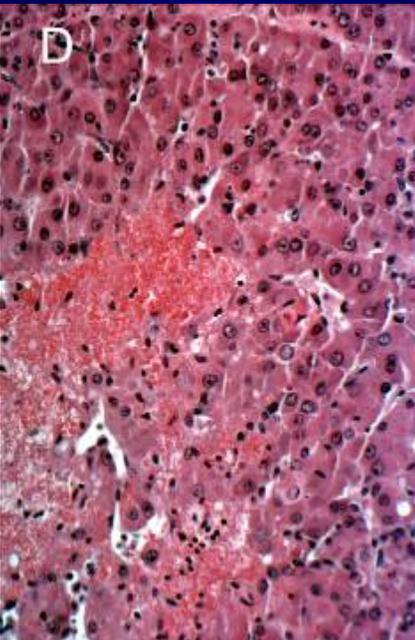
0



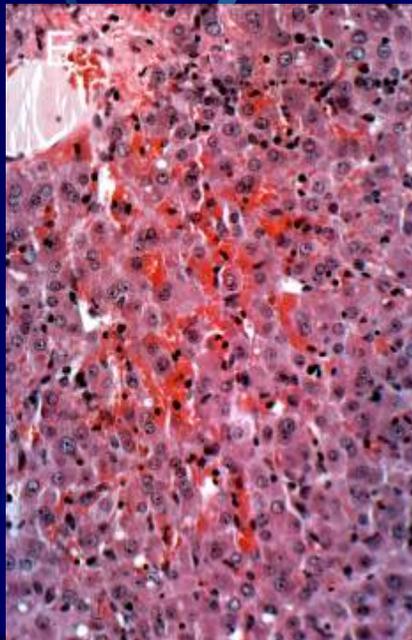
33



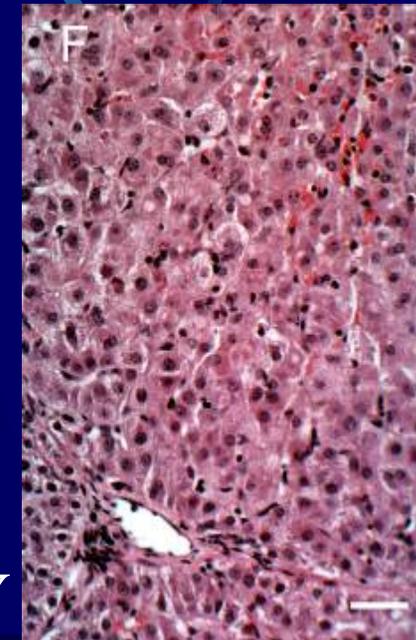
6



12



24



Y

Summary

- Clinically 3 and 6 week old pigs were most severely affected.
- 0, 3 and 6 week old pigs were hypoalbuminemia and hypoproteinemia.
- 12 and 64 week old pigs had increased GGT, bilirubin and bile acids.
- Histologically 3 and 6 week old pigs had most severe necrosis and hemorrhage.

N-oxide toxicity



- Toxicity 90% that of riddelliine free base
- Similar lesion distribution and severity

Conclusions



- Weanling pigs are most susceptible to PA toxicosis (3 and 6 weeks old)
- Older pigs have toxicity similar to adults
- N-oxide riddelliine is about 90% the toxicity of free base riddelliine in pigs

What about the pyrrole?





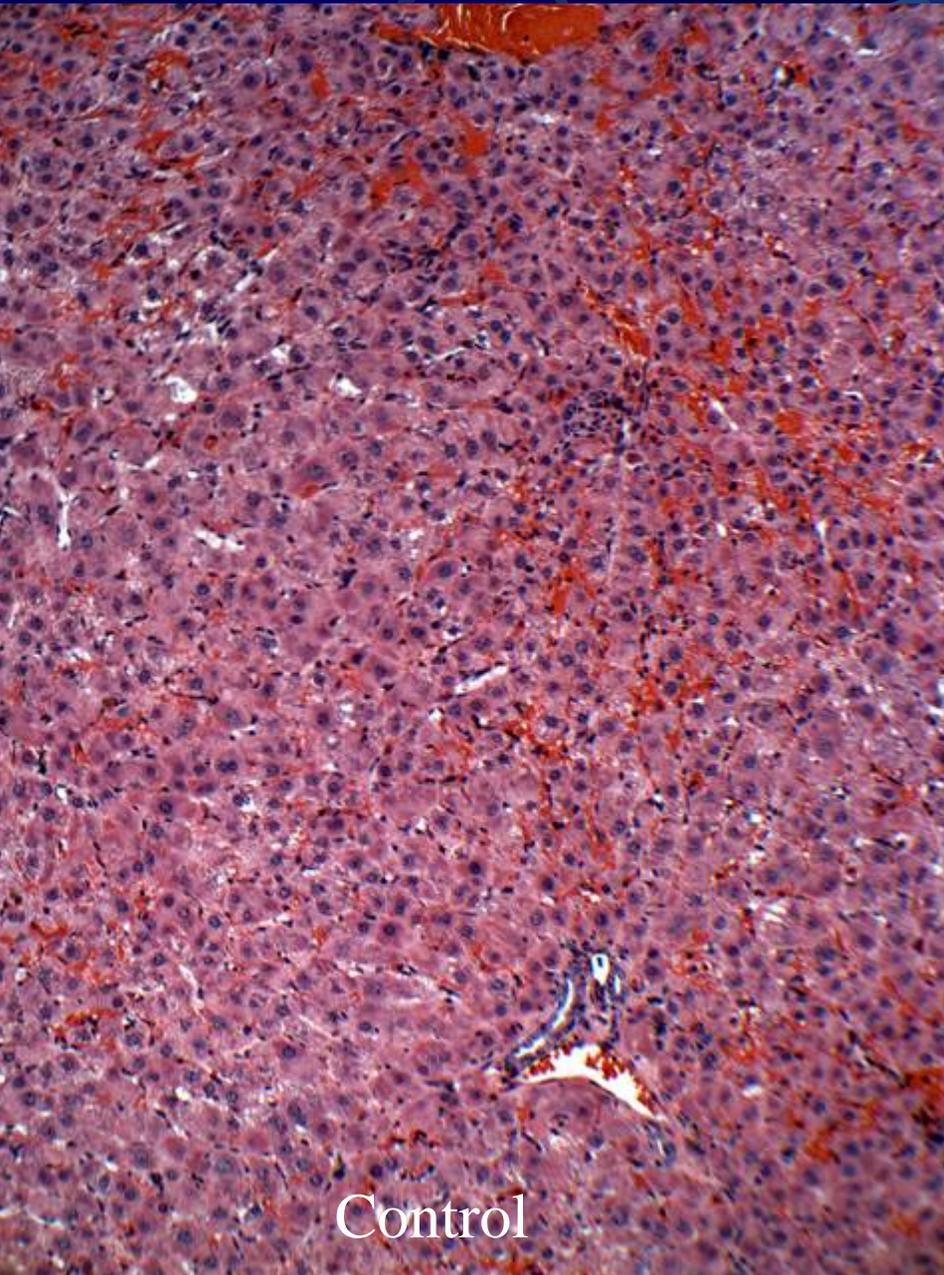
Is exposure changing?

Riddelliine conjugate vaccine trial

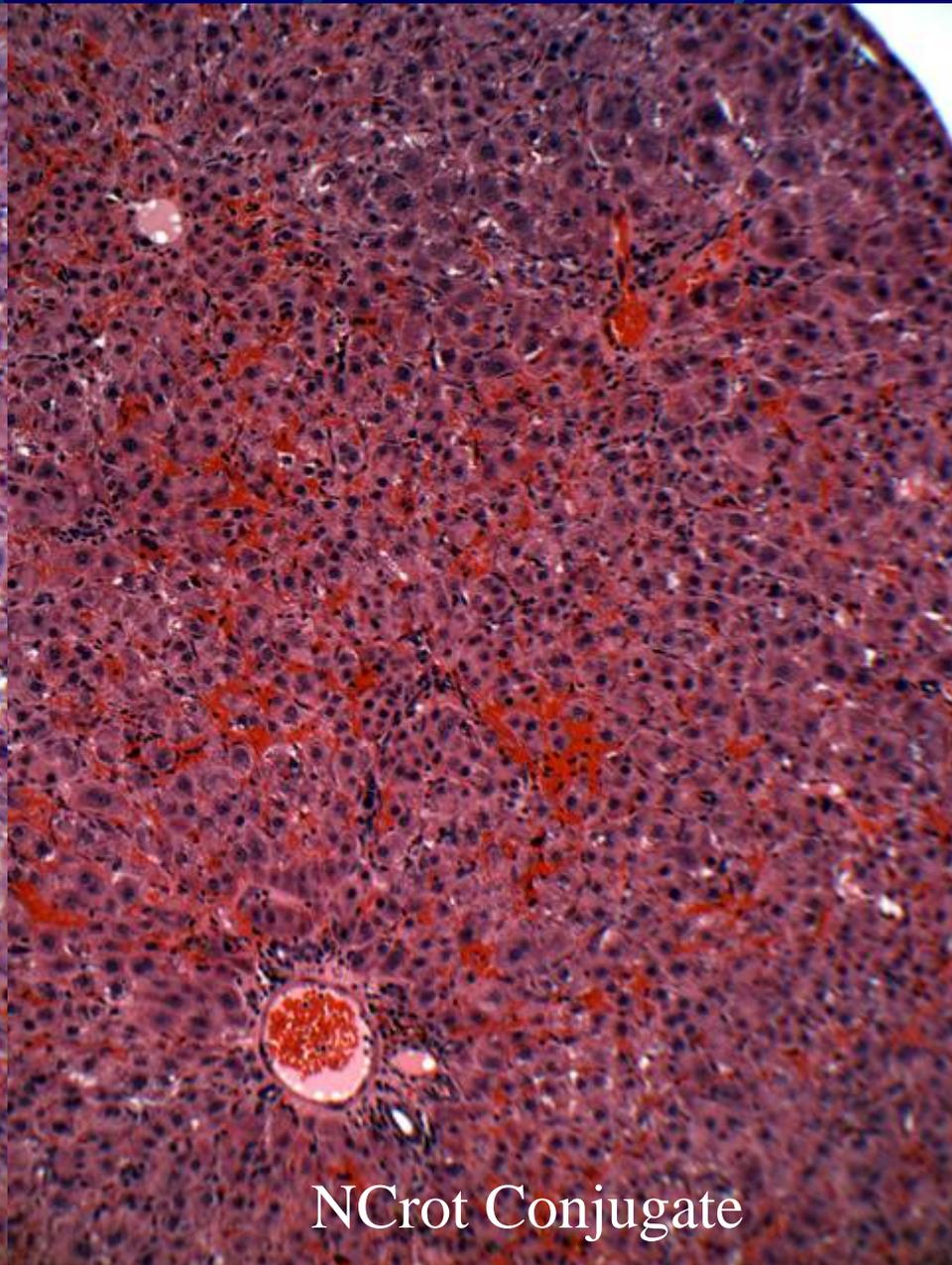
- Three groups of 30 rats were immunized with two riddelliine conjugates and a sham ovalbumin conjugate.
- three groups of 10 dosed with riddelliine of 5 mg/kg (25% LD50), 15 mg/kg (75% LD50) and 30 mg/kg (150% LD50) for 10 days.
- Serum was collected and tissues were collected for evaluation..

Results

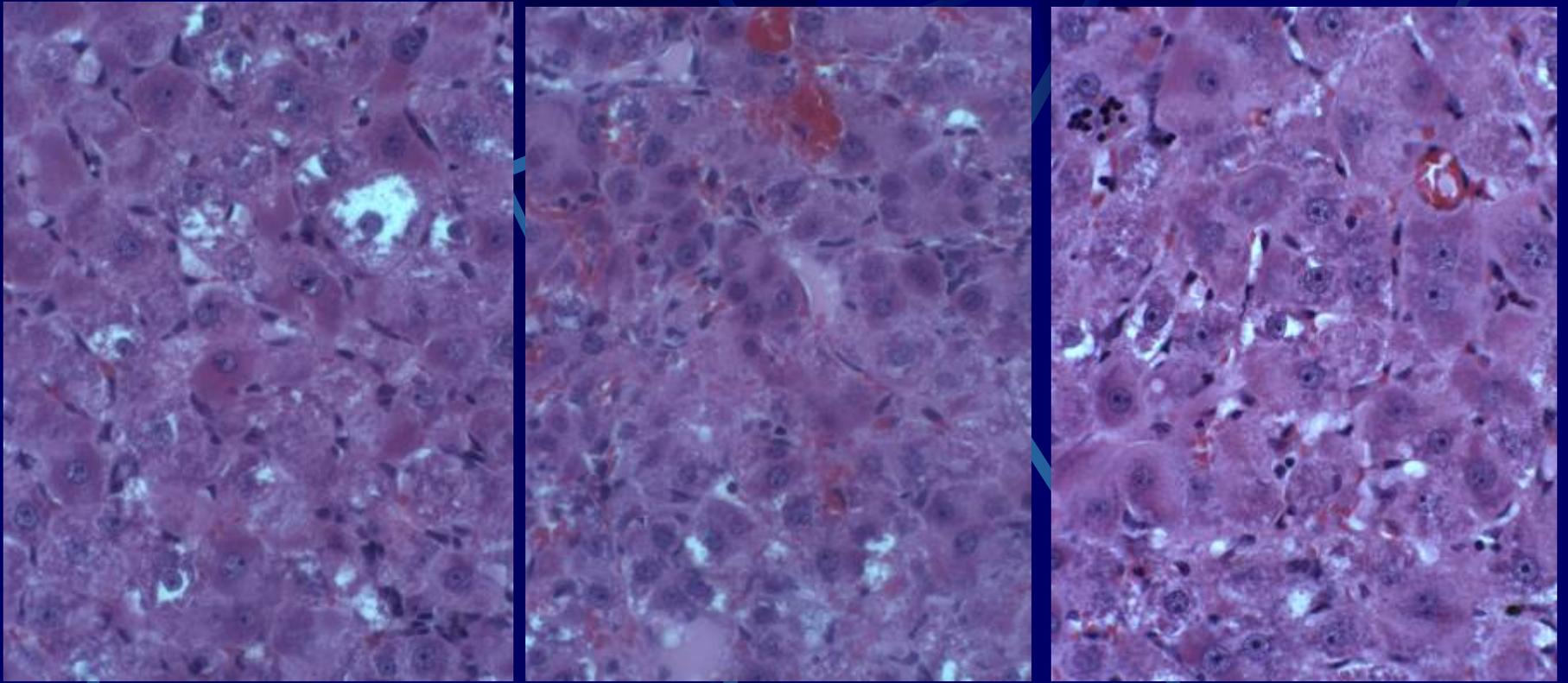
- Mild decrease in GGT, ALP and AST
- Additional evaluations with titer are pending
- Decreased hepatocellular swelling and individualization



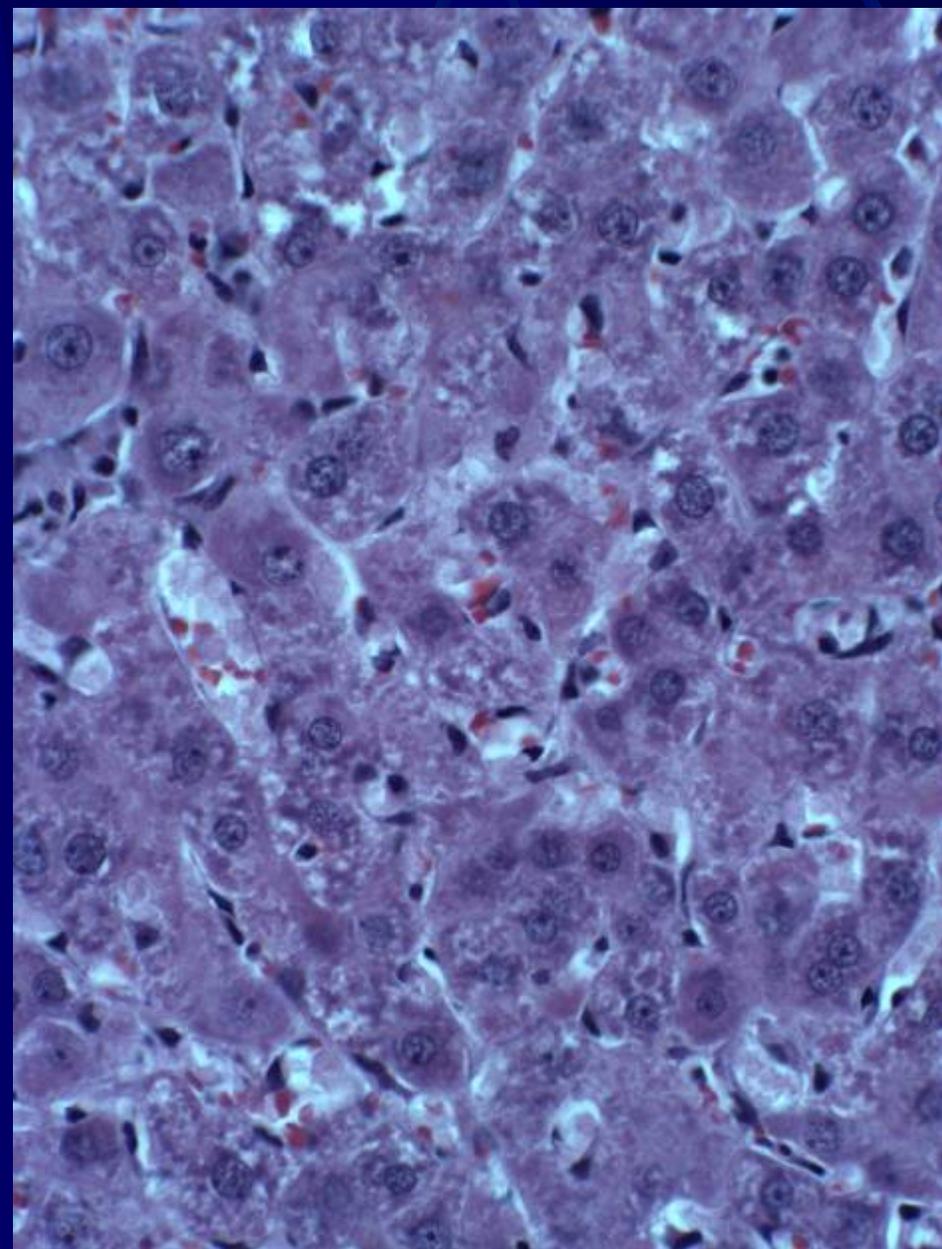
Control



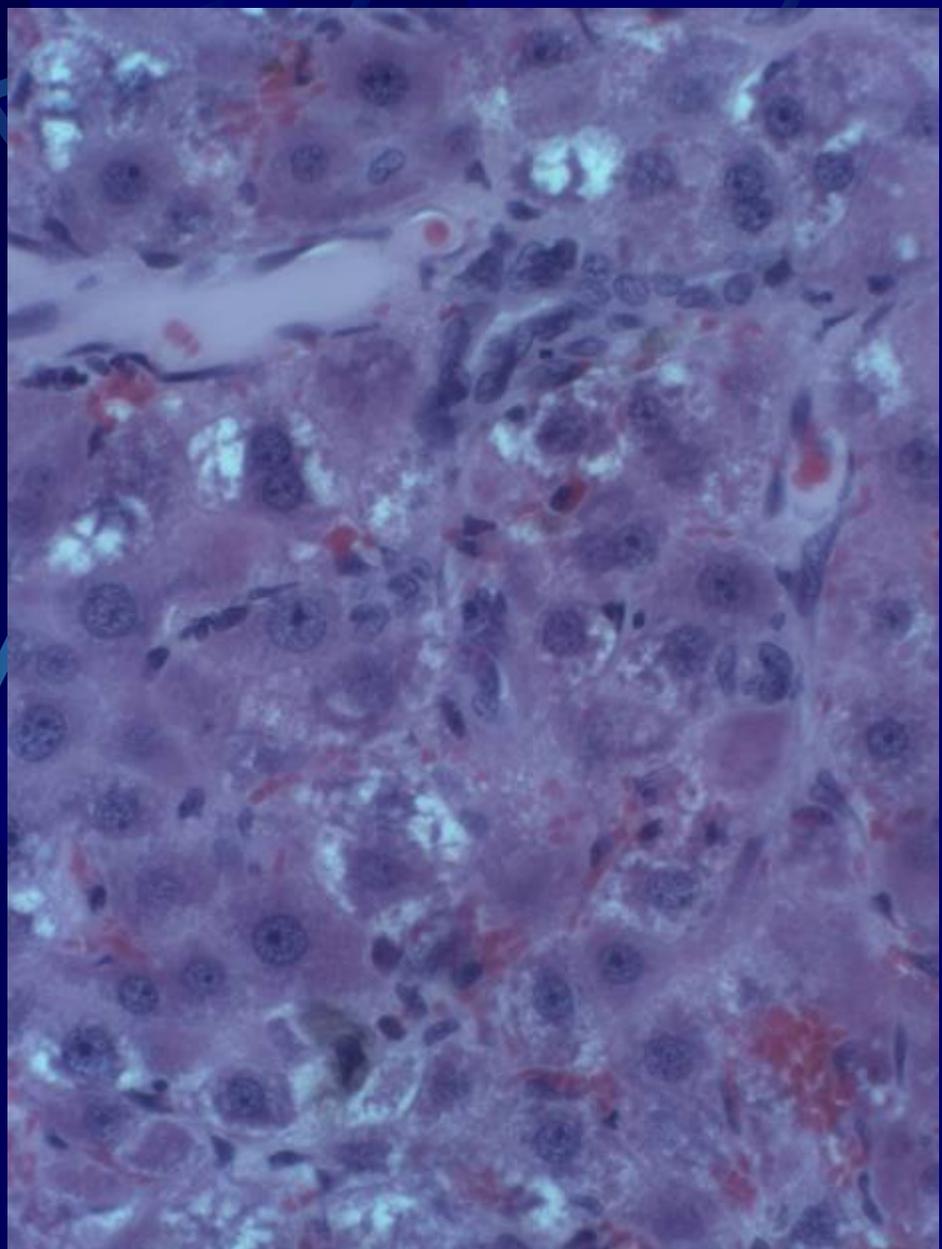
NCrot Conjugate



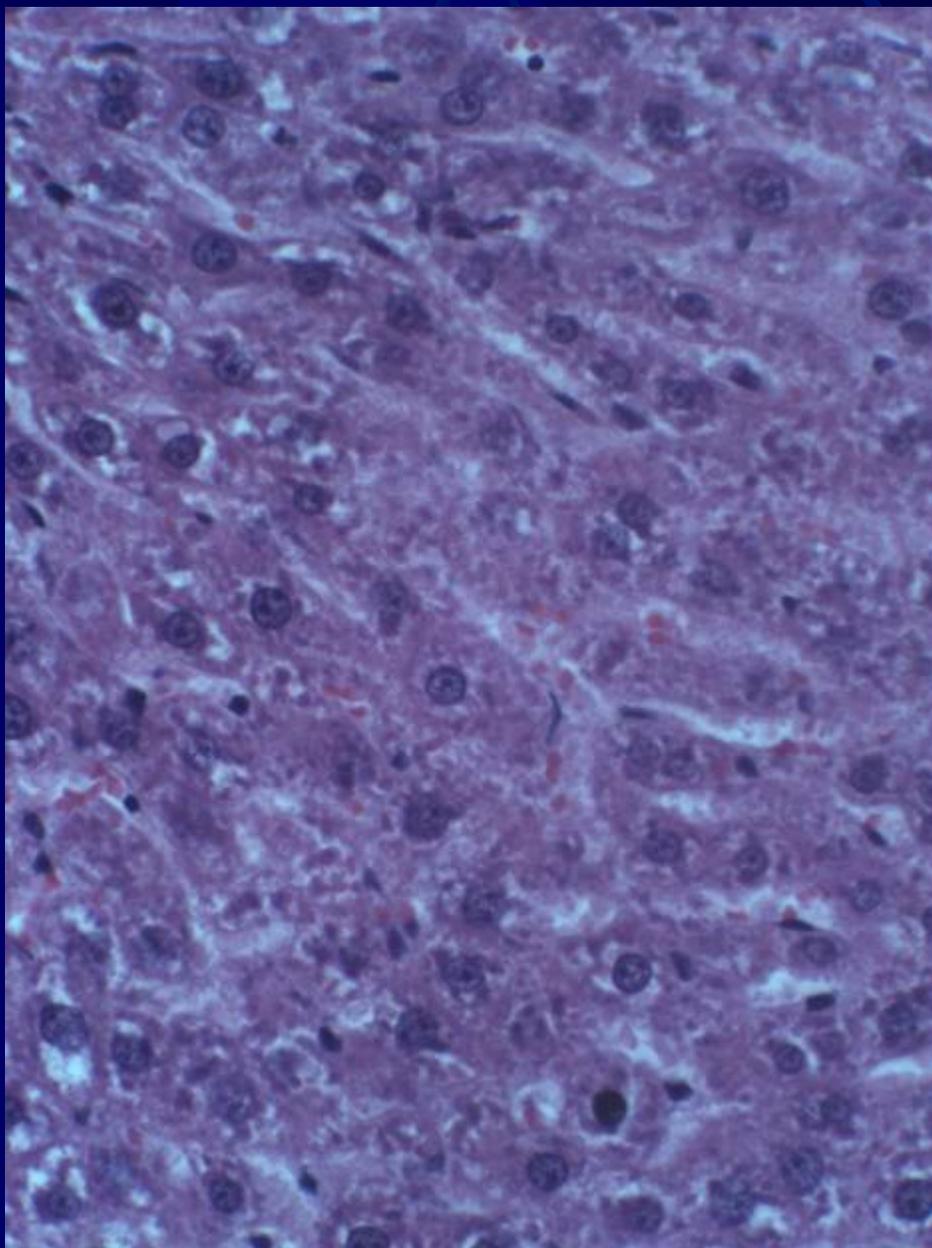
Which rat was vaccinated? Real blind study.



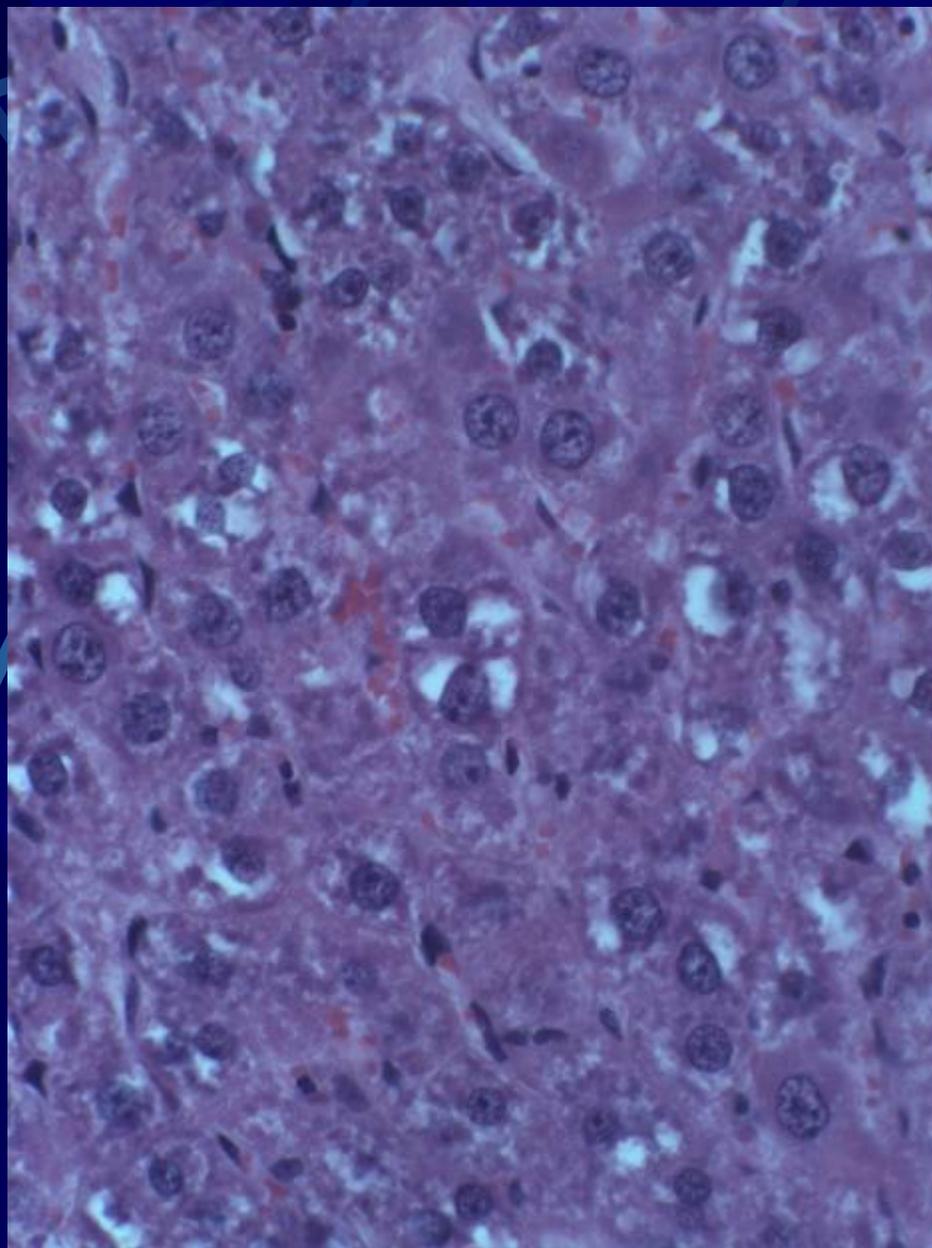
NCrot Conjugate/25 mg/kg Riddelliine/High Titer



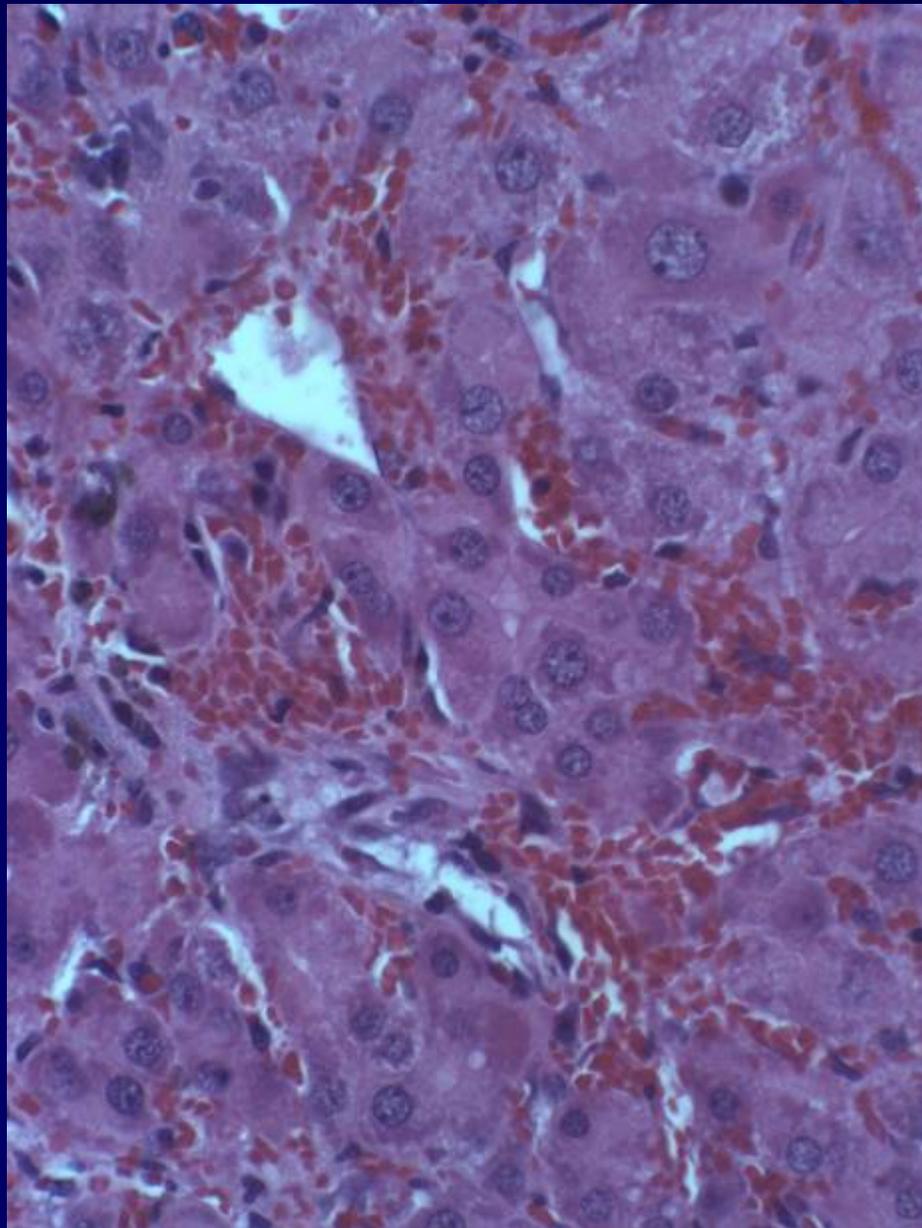
NCrot Conjugate/25 mg/kg Riddelliine/Low Titer



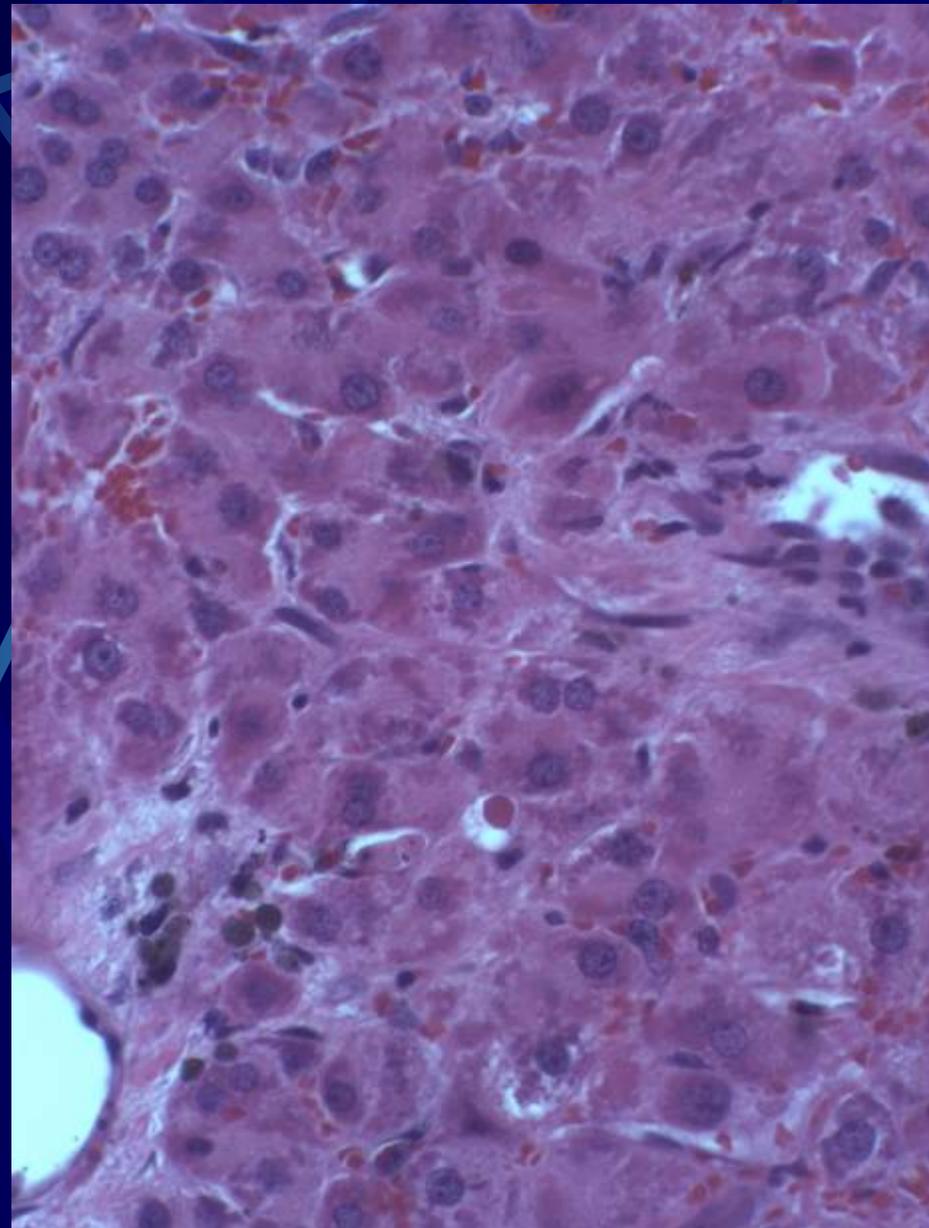
NCrot Conjugate/5 mg/kg Riddelliine/High Titer



NCrot Conjugate/5 mg/kg Riddelliine/Low Titer



SAFet Conjugate/25 mg/kg Riddelliine/High Titer



SAFet Conjugate/25 mg/kg Riddelliine/Low Titer

Conclusion

- Rats had variable and inadequate response to vaccination
- Animals with high titers may have less hepatocellular swelling

Implications?



**New CRIS Project:
The Toxicity of Pyrrolizidine Alkaloid-
Containing Plants and Other
Hepatotoxic and Neurotoxic Plants**

**Old Project Number
5428-32000-013-00D**

Scientific Staff Years 1.85

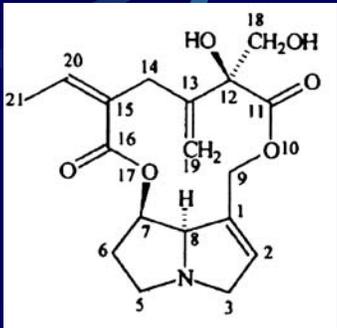
Objectives

- Objective 1: Develop **diagnostic techniques and biomarkers** to better identify animals poisoned by pyrrolizidine alkaloids (PA's) and their subsequent metabolites, and develop techniques to monitor foods and feeds for PA-contamination.
- Objective 2: Determine pyrrole toxicity and carcinogenicity and compare pyrrole toxicity with that of PA and PA-n-oxides. Characterize the risk to fetuses and neonates that are exposed by maternal PA-ingestion.
 - Sub-Objective 2.1: Determine **pyrrole toxicity and carcinogenicity**.
 - Sub-Objective 2.2: Characterize **transplacental and transmammary toxicity** of various PA's.

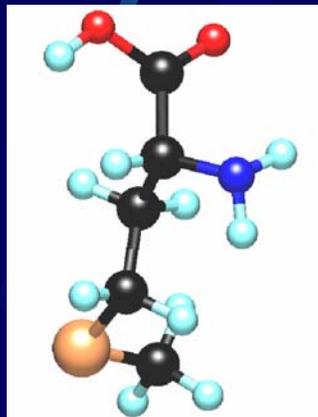


- Heterozygote TRP53 mutated mice model
- P450 upregulation
 - Phenobarbital
 - Spironolactone
- Glutathione depletion

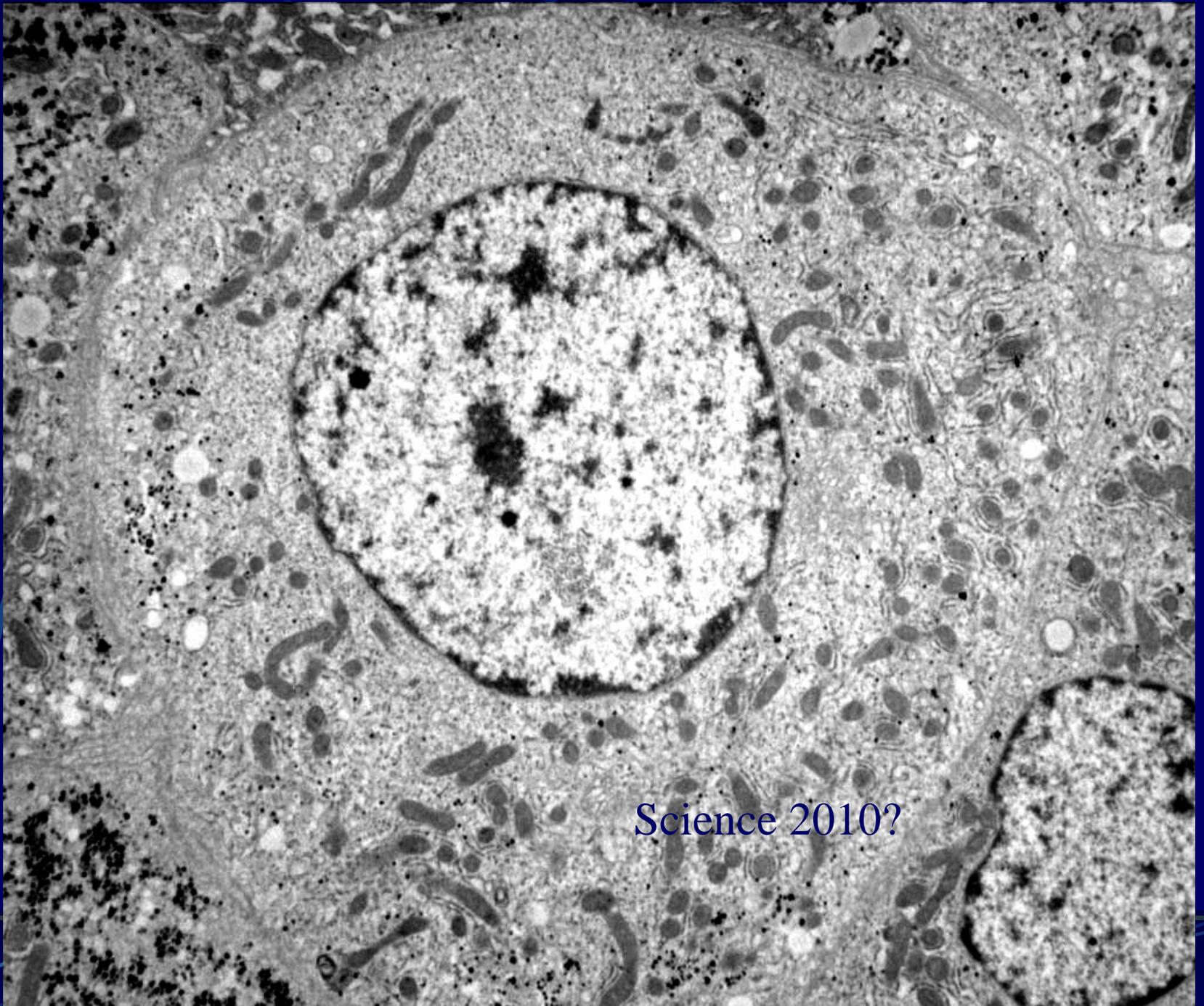
Immunologic Diagnostics



DNA Conjugates
Thymidine Conjugates



Protein Conjugates
Methionine Conjugates



Science 2010?