Pyrrolizidine Alkaloid Containing Plants

Bryan Stegelmeier

ADVS 586

February 9, 2010

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

Outline

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

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)
- Boraginacea
  - 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

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Fabaceae

- Crotalaria (600 species)
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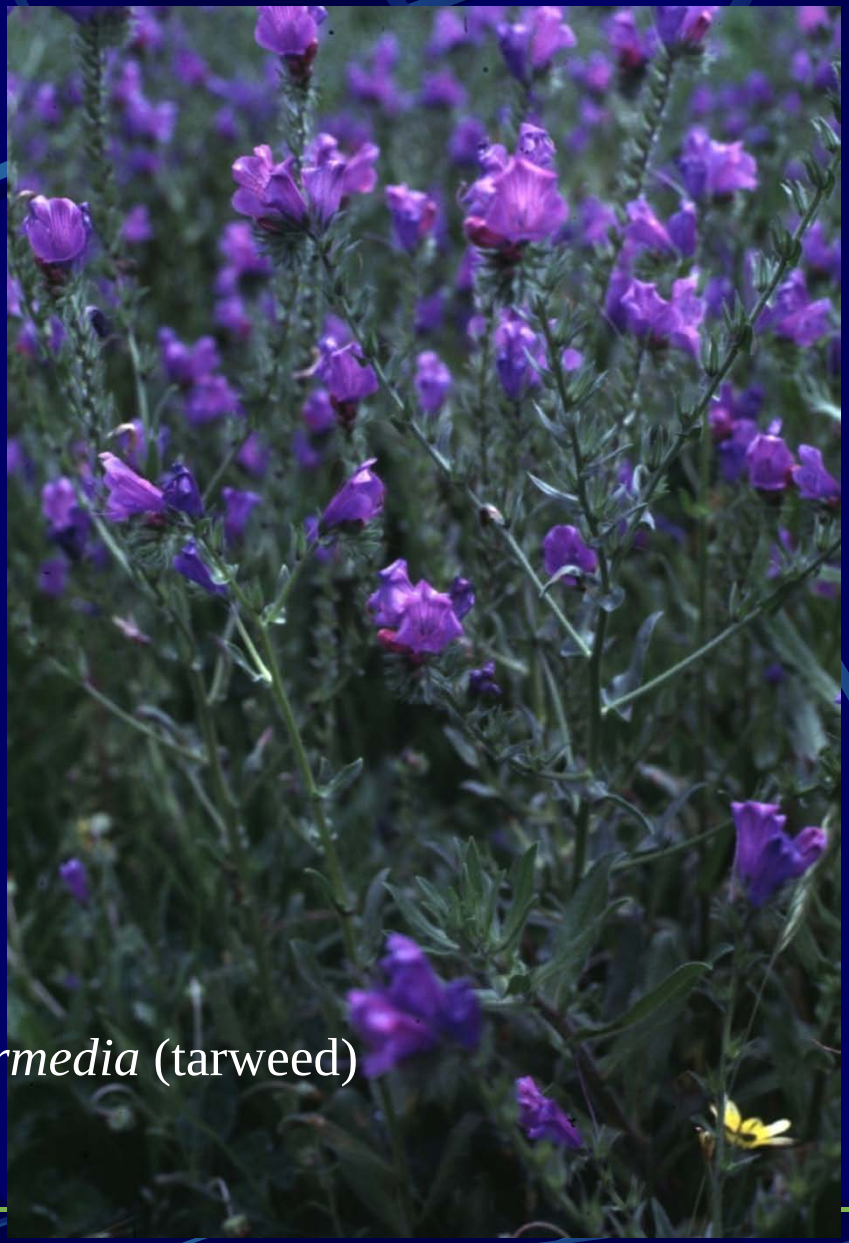
- Boraginacea
  - *Amsinckia intermedia* (tarweed)
  - *Borago officinalis* (borage)
  - *Cynoglossum officinale* (hound's tongue)
  - *Echium plantagineum* (Patterson's curse)
  - *Echium vulgare* (viper's bugloss)
  - *Heliotropium europaeum* (heliotrope)
  - *Symphytum officinale* (comfrey)

Cynoglossum officinale



Amsinckia intermedia (tarweed)

Crop vs Weed

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

Echium vulgare (viper's bugloss)


04/02/2004

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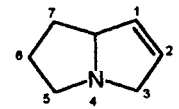
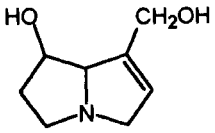
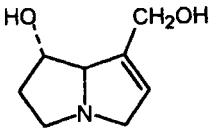


Pyrrolizidine nucleus

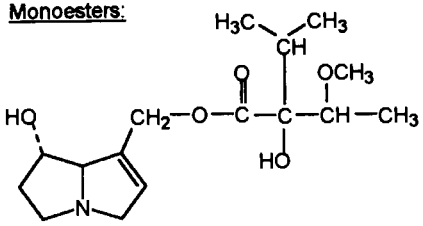


Retronecine



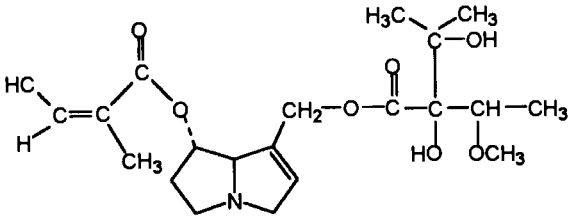
Heliotridine

Monoesters:



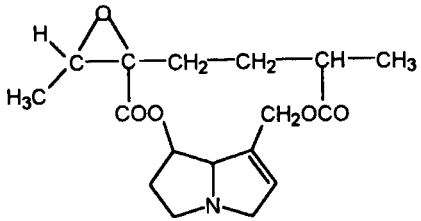
Heliotrine

Noncyclic diesters:

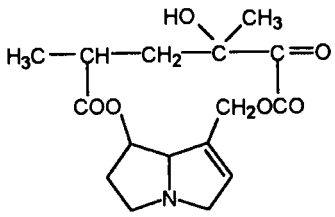


Lasiocarpine

Cyclic diesters:



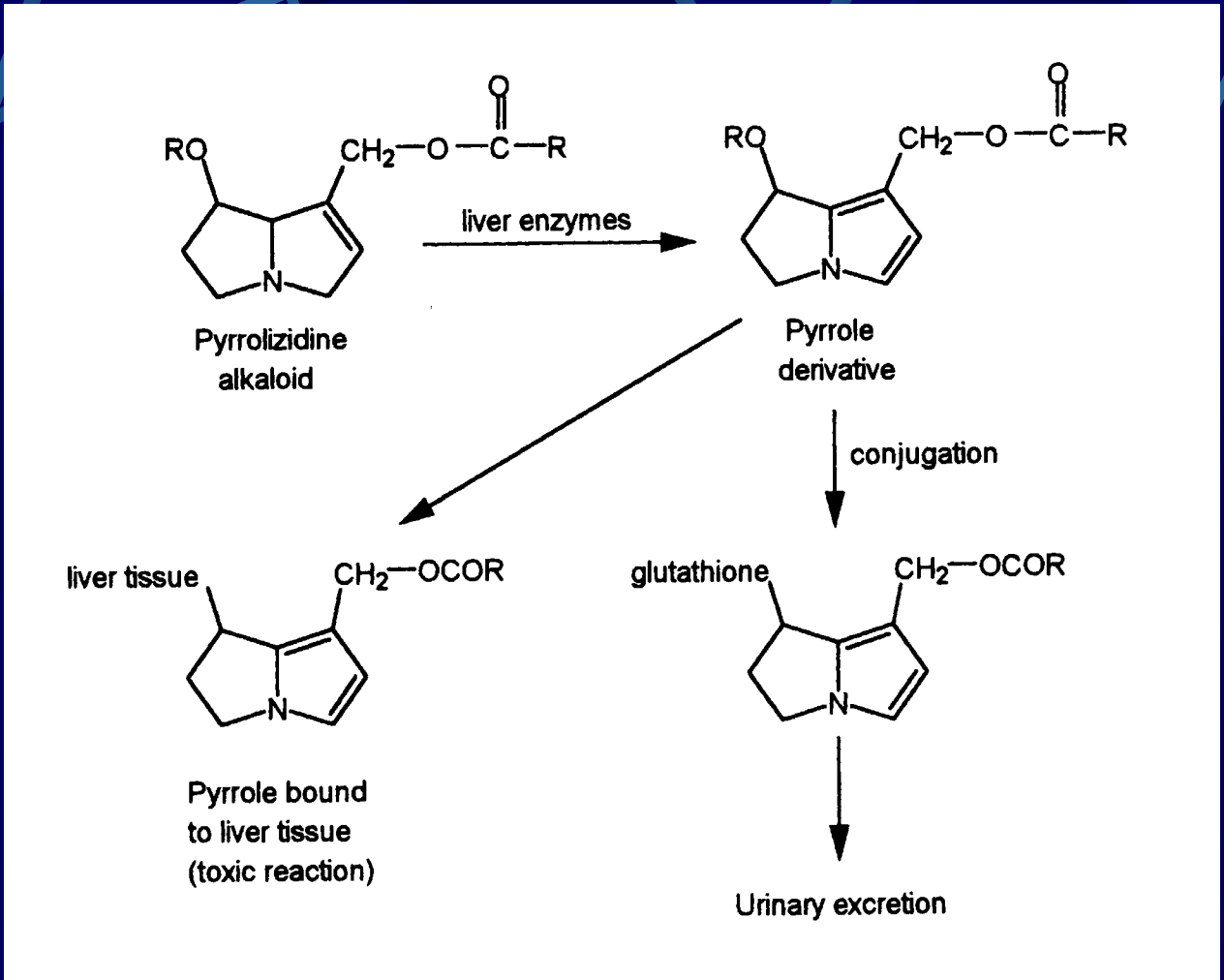
Jacobine

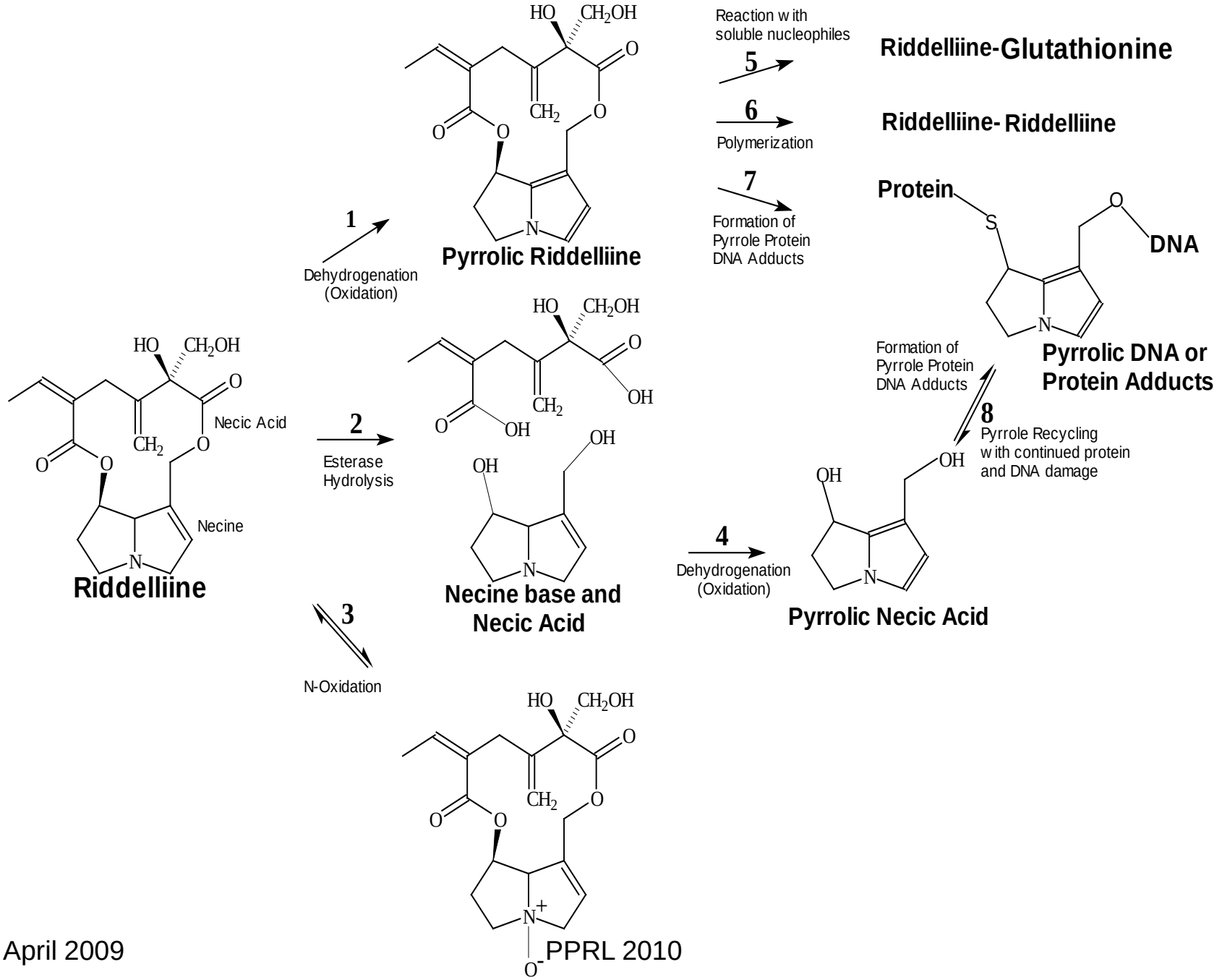


Monocrotaline

Pyrrolizidine alkaloids: Chemistry

Pyrrolizidine Alkaloids: Metabolism




Riddelliine
Necic Acid
Necine
1
Dehydrogenation
(Oxidation)
Pyrrolic Riddelliine
2
Esterase
Hydrolysis
Necine base and
Necic Acid
3
N-Oxidation
Riddelliine N-oxide
4
Dehydrogenation
(Oxidation)
Pyrrolic Necic Acid
Reaction with
soluble nucleophiles
5
Riddelliine-Glutathionine
6
Polymerization
Riddelliine- Riddelliine
7
Formation of
Pyrrole Protein
DNA Adducts
Protein
DNA
Pyrrolic DNA or
Protein Adducts
Formation of
Pyrrole Protein
DNA Adducts
8
Pyrrole Recycling
with continued protein
and DNA damage

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.

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ORIGINAL PAPER

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

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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. Senecyphylline 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 · *FABMS* fast atom bombardment mass spectrometry · *NMR* nuclear magnetic resonance

Introduction

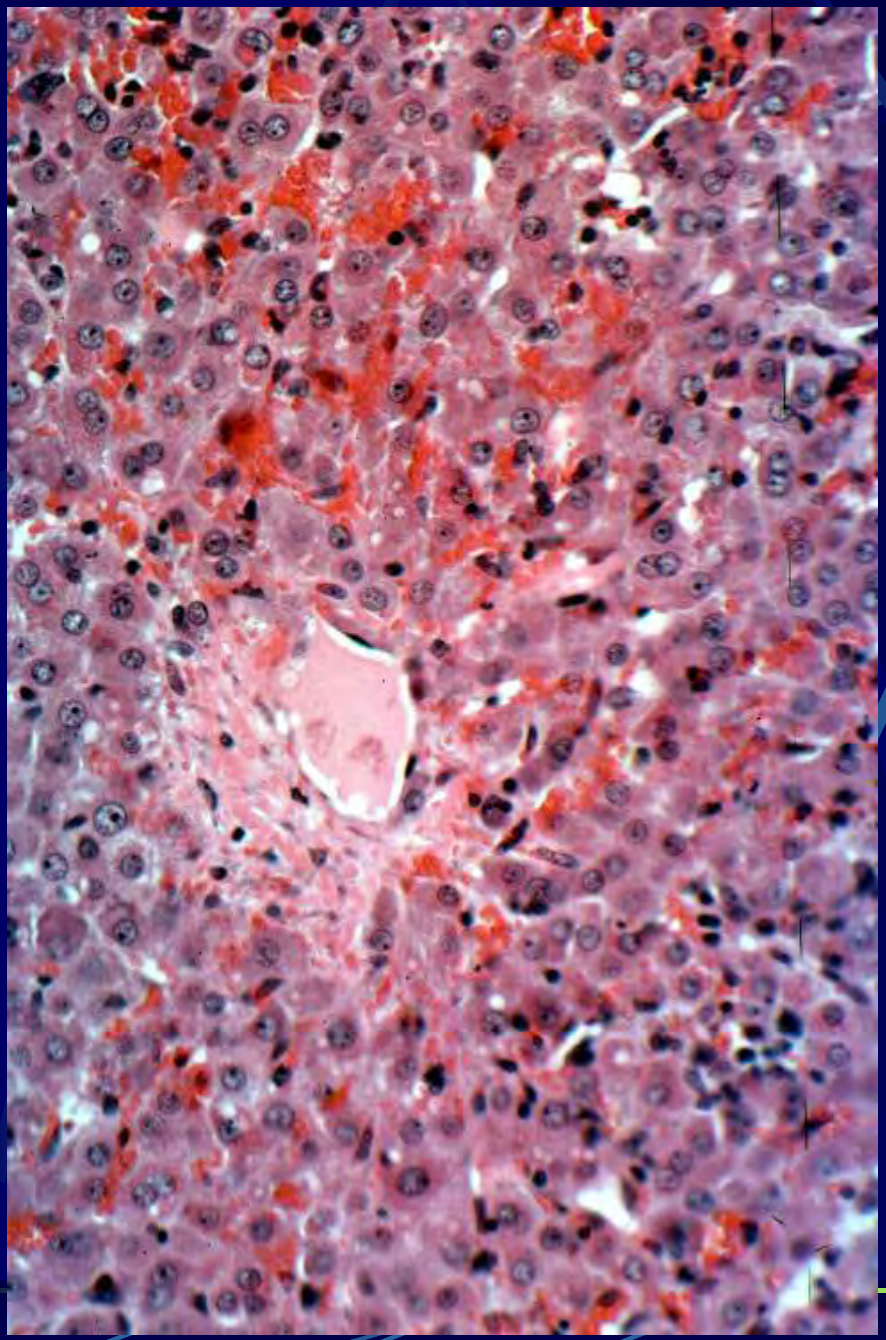
Veno-occlusive disease (VOD) of the liver is characterized by portal hypertension with severe ascites due to obliteration of centrilobular or subhepatic hepatic veins. It is the most frequent cause of hepatic vein obstruction in children. Hepatic VOD in infants may be caused by hepatic irradiation, chemotherapeutic drugs [19] bone marrow transplantation; 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

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



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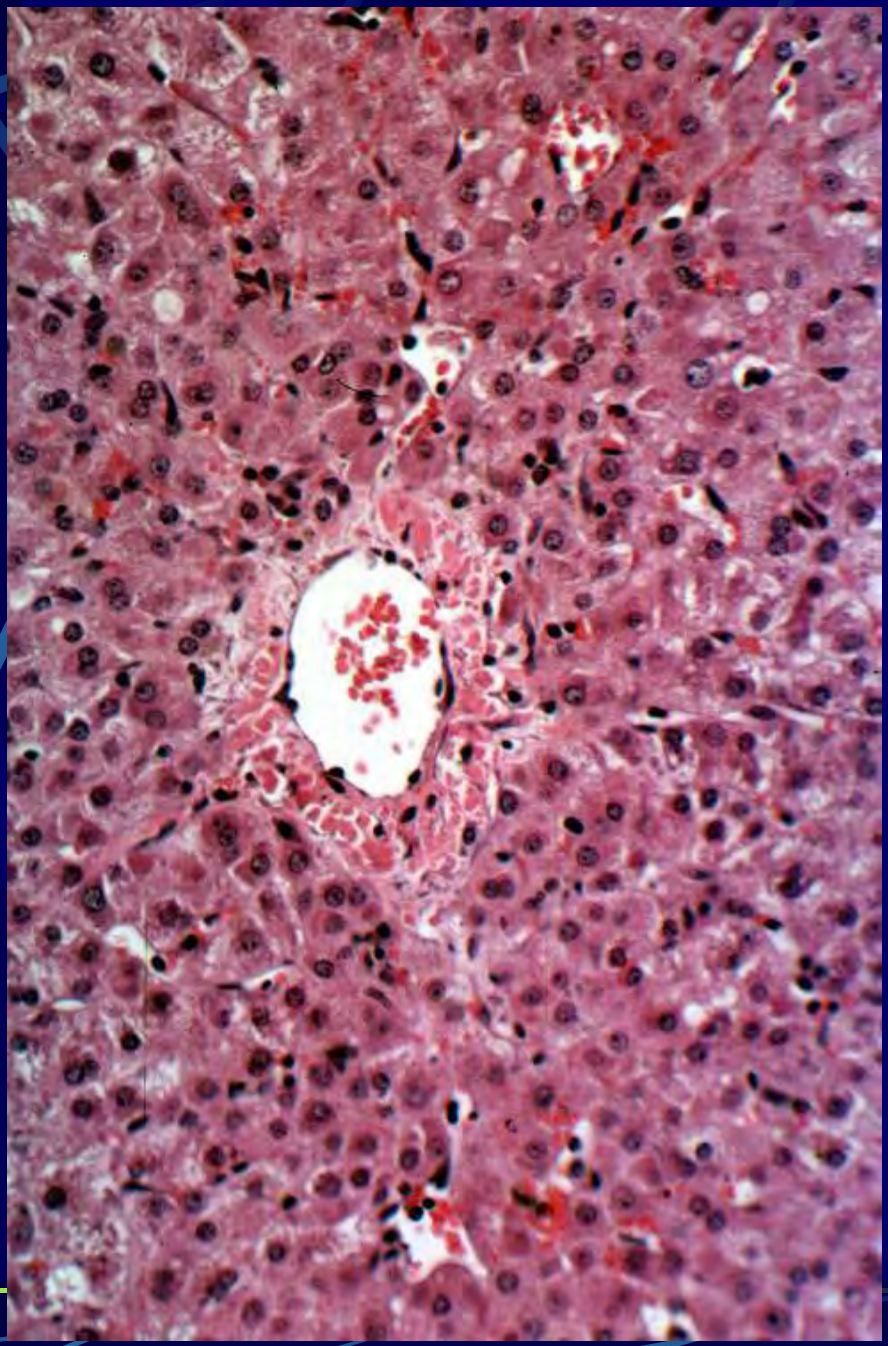

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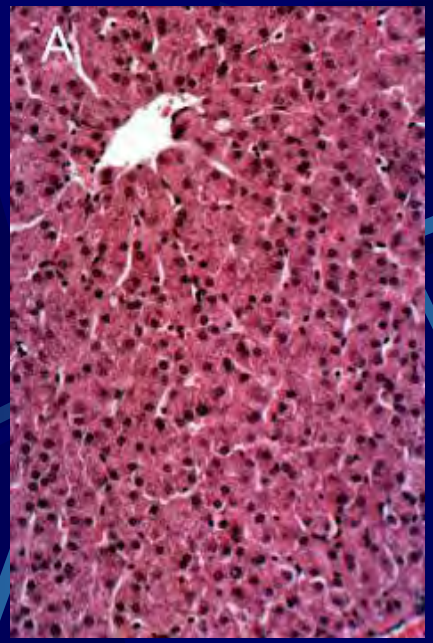

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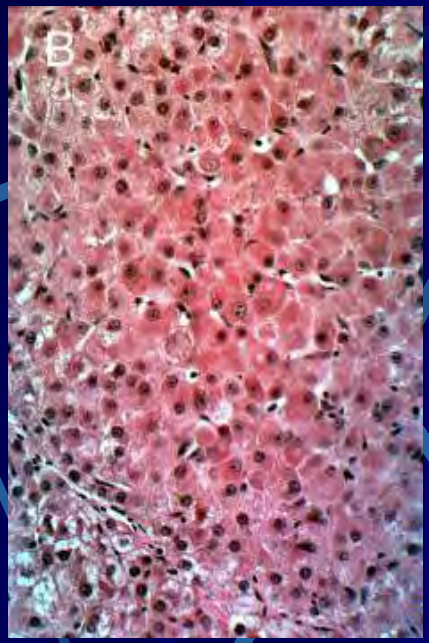

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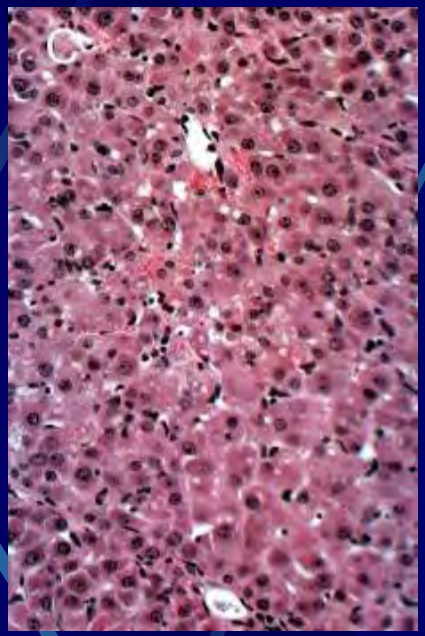

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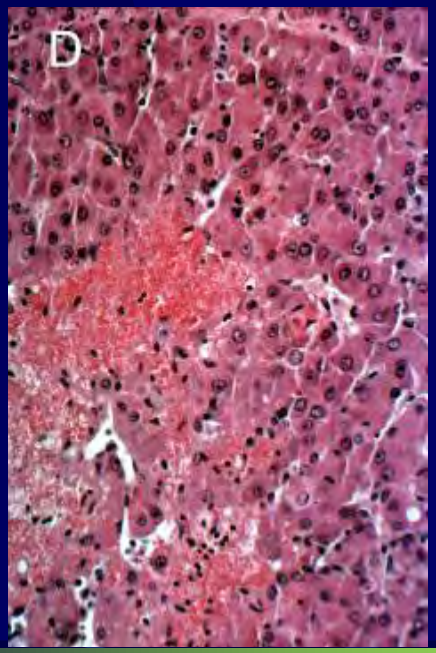

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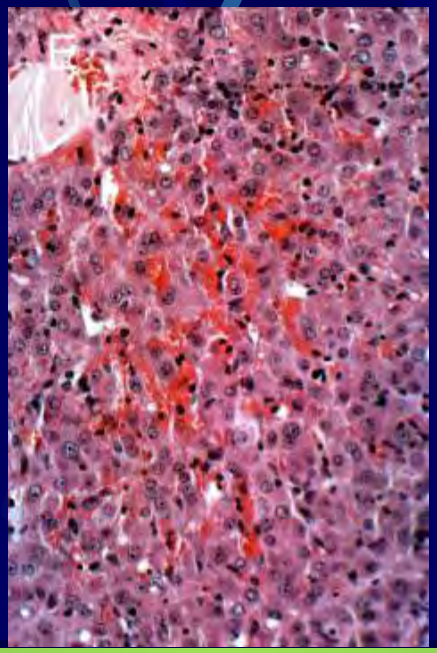

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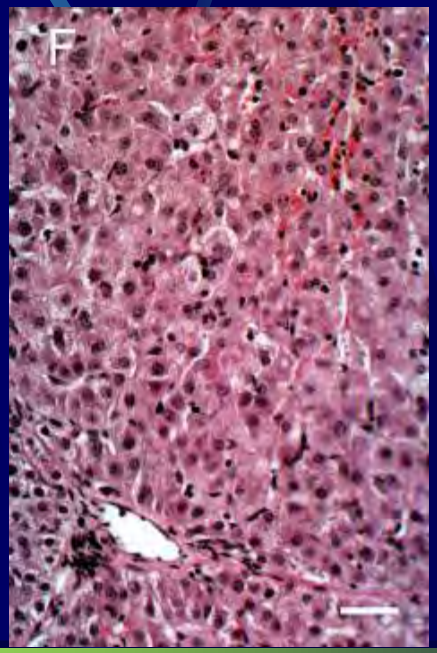

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Pyrrolizidine Alkaloidosis in a Two Month Old Foal

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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 *et al.* (1985)
Horse	High	High	7.3	Garrett *et al.* (1984)
Sheep	Low	Low	302	White *et al.* (1984)
Goat	Low	?	205	Goeger *et al.* (1982a)
Rat	High	High	21	Goeger *et al.* (1983)
Mouse	Intermediate	High	?	
Rabbit	Low	High	113	Pierson *et al.* (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 *et al.* (1977)

[a]Adapted from Shull *et al.* (1976).

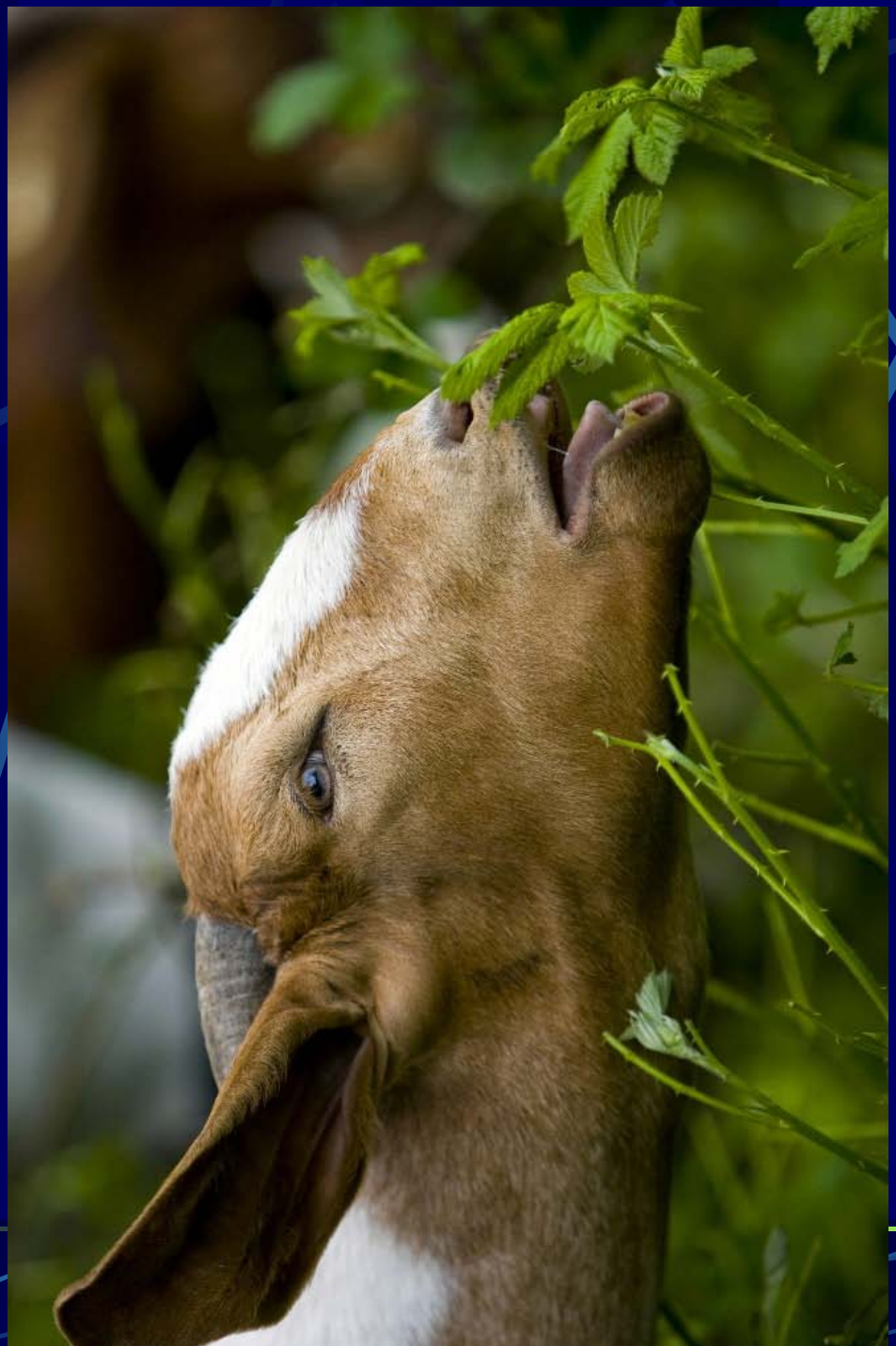
[b]Chronic lethal dose of *Senecio jacobaea*.

Veno-occlusive Disease

- **Afghanistan: "Charmak" disease still killing people and livestock**

16 December 2008 - Over 270 people have been diagnosed with a hepatic veno-occlusive disease (VOD), locally known as "camel belly" or "charmak" disease.

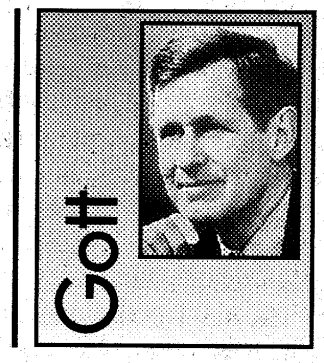
What about the pyrrole?



Is exposure changing?

Do herbal remedies cause cancer?

By Peter H. Gott, M.D.
Newspaper Enterprise Association



DEAR DR. GOTT: Do you have any recent evidence that certain herbal remedies cause health problems? My friend says that some cause cancer, which I find hard to believe.

DEAR READER: Hard to believe or not, your friend's statement is correct. As pointed out in an editorial in The New England Journal of Medicine, the Chinese herb Aristolochia fangchi has been shown to cause kidney and bladder cancer in humans.

Suspicions were first raised a decade ago when, in a Belgian clinic, women were given a Chinese weight-reduction herb that was contaminated by A. fangchi. Within three years, dozens of the patients developed progressive renal failure. In 1994, the first bladder cancers were reported.

Following publication of this information, the United Kingdom, Canada, Australia and Germany banned the use of herbal remedies that contain this toxin. Unfortunately, the product remains available in many American stores.

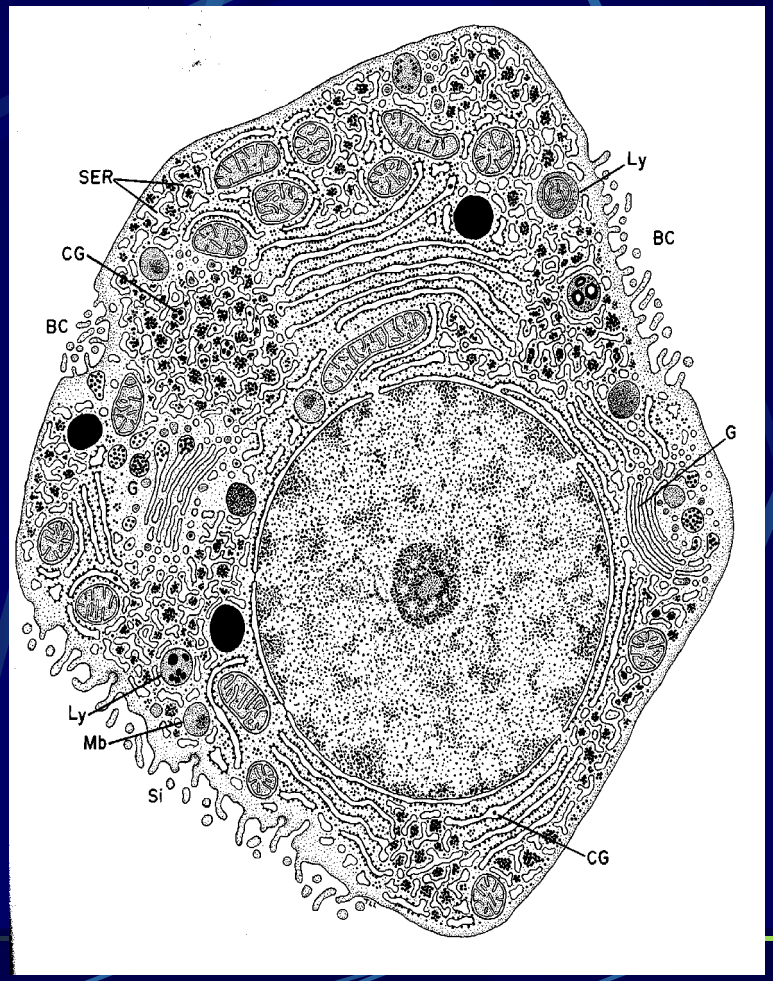
Because of the 1994 Dietary Supplement Health and Education Act, the Food and Drug Administration is no longer permitted to regulate the manufacture, purity and distribution of most herbal remedies, some of which — notably ephedra — are clearly associated with major health risks.

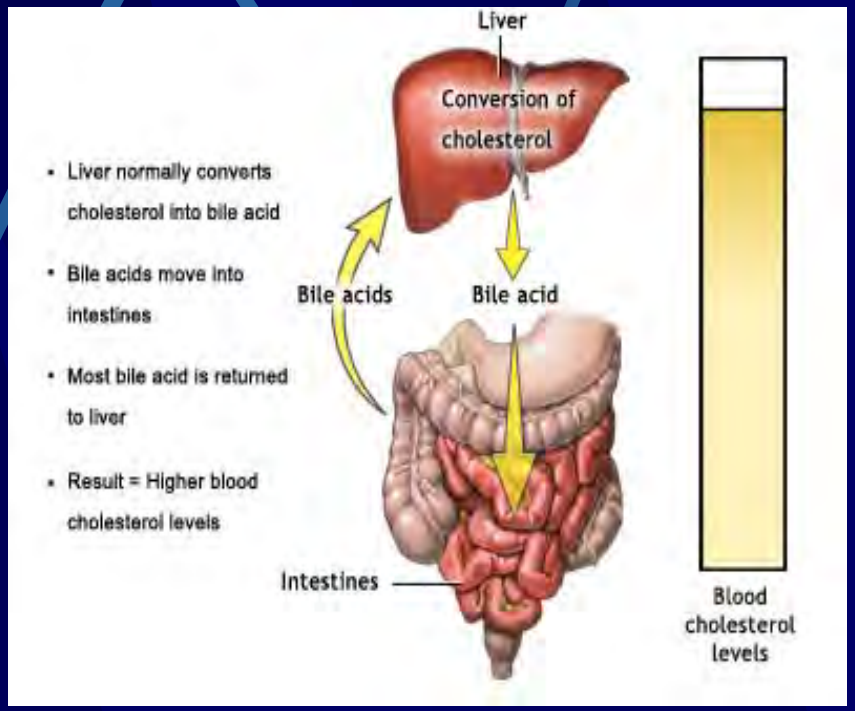
It is inconceivable to many scientists that Congress has shown so little interest in protecting the consumer from dangerous dietary supplements, fraudulent claims, products contaminated by lead and arsenic, and dietary supplements that are — in reality — medicines containing powerful prescription drugs (such as cortisone) that are not listed on the labels. Certainly, all of us deserve to be protected against pharmaceutical fraud and abuse, yet the public continues to believe that such herbal remedies rarely place people in real danger. Nothing could be further from the truth.

Until the Dietary Supplement Act can be revised and modified, our elected officials need to initiate an educational program that will inform consumers of the hazards of certain herbal remedies, especially those that are manufactured in parts of the world where quality control and public responsibility are, apparently, lacking. As with conventional medications, these unregulated supplements have the potential to cause harm. Take them at your own risk.

Clinical Lesions- Dose Dependent

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





Clinical Signs

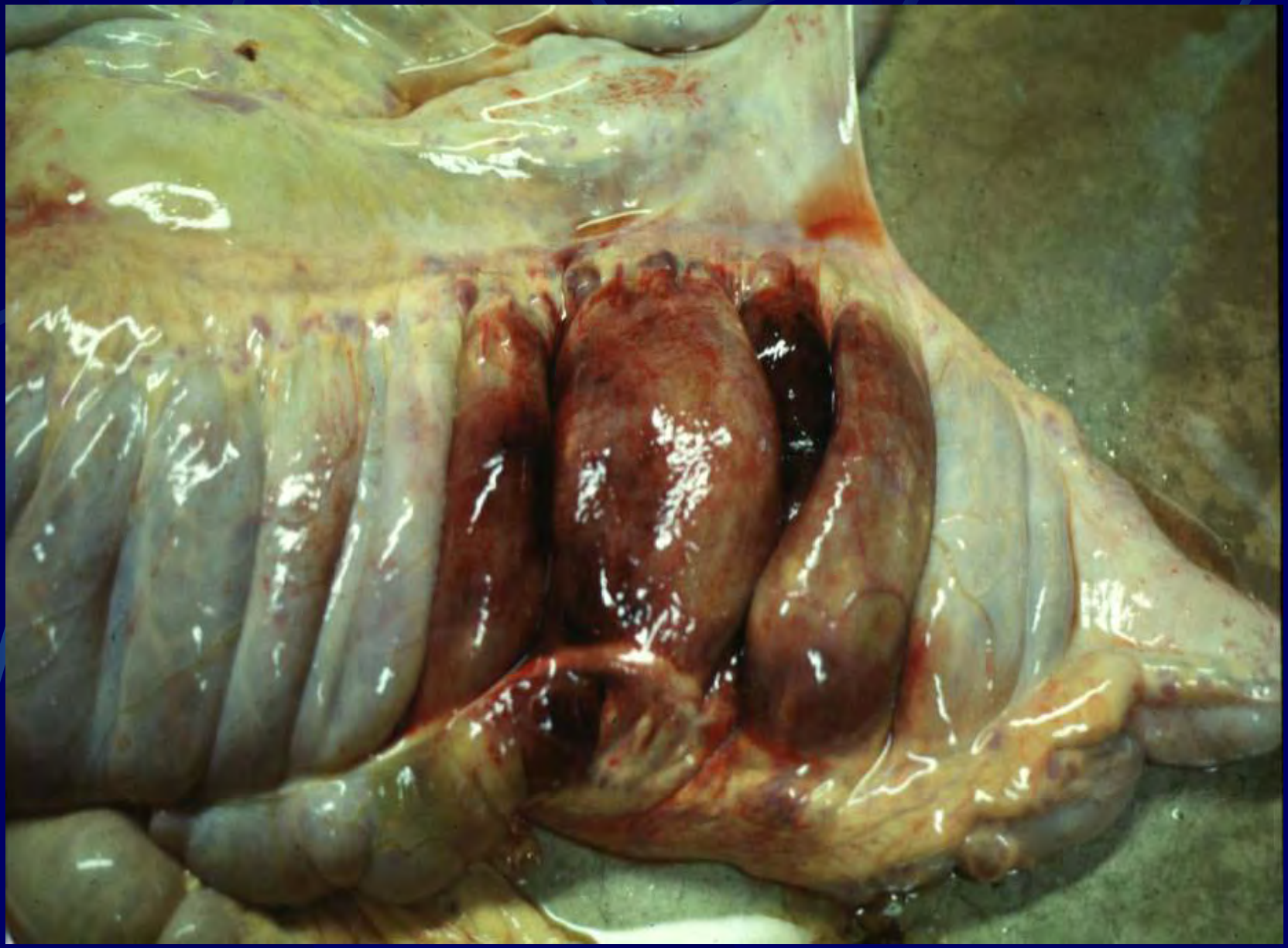
- Lethargy
- Anorexia
- Photosensitivity and solar dermatitis
- Diarrhea
- Weakness
- Wandering or blindness
- Belligerence
- Ascites

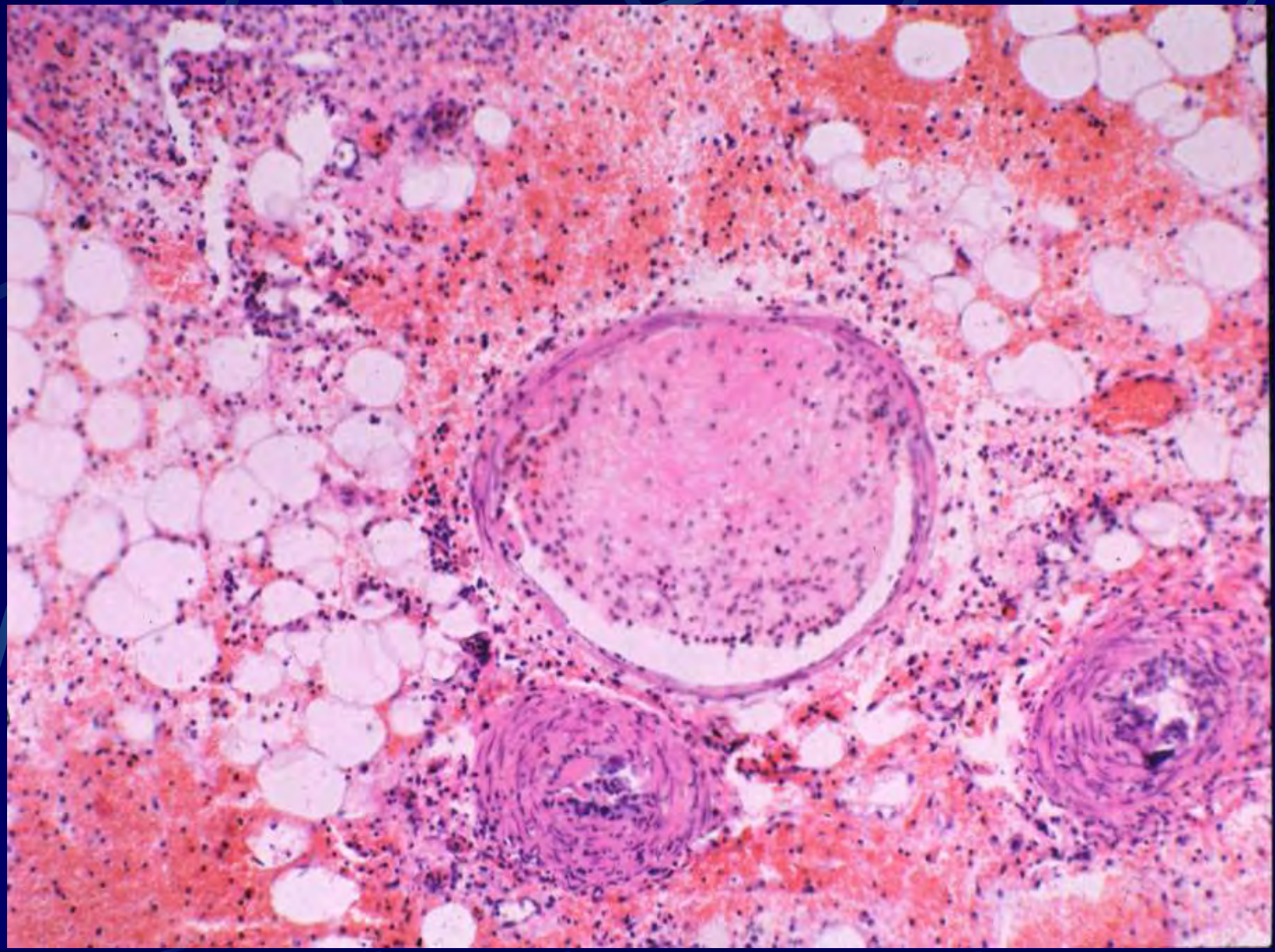
- Hepatic lesions
  - Yellow soft liver
  - Hepatocyte necrosis, fibrosis, biliary hyperplasia
- Generalized icterus
- Subcutaneous and visceral edema
- Species related extra-hepatic lesions

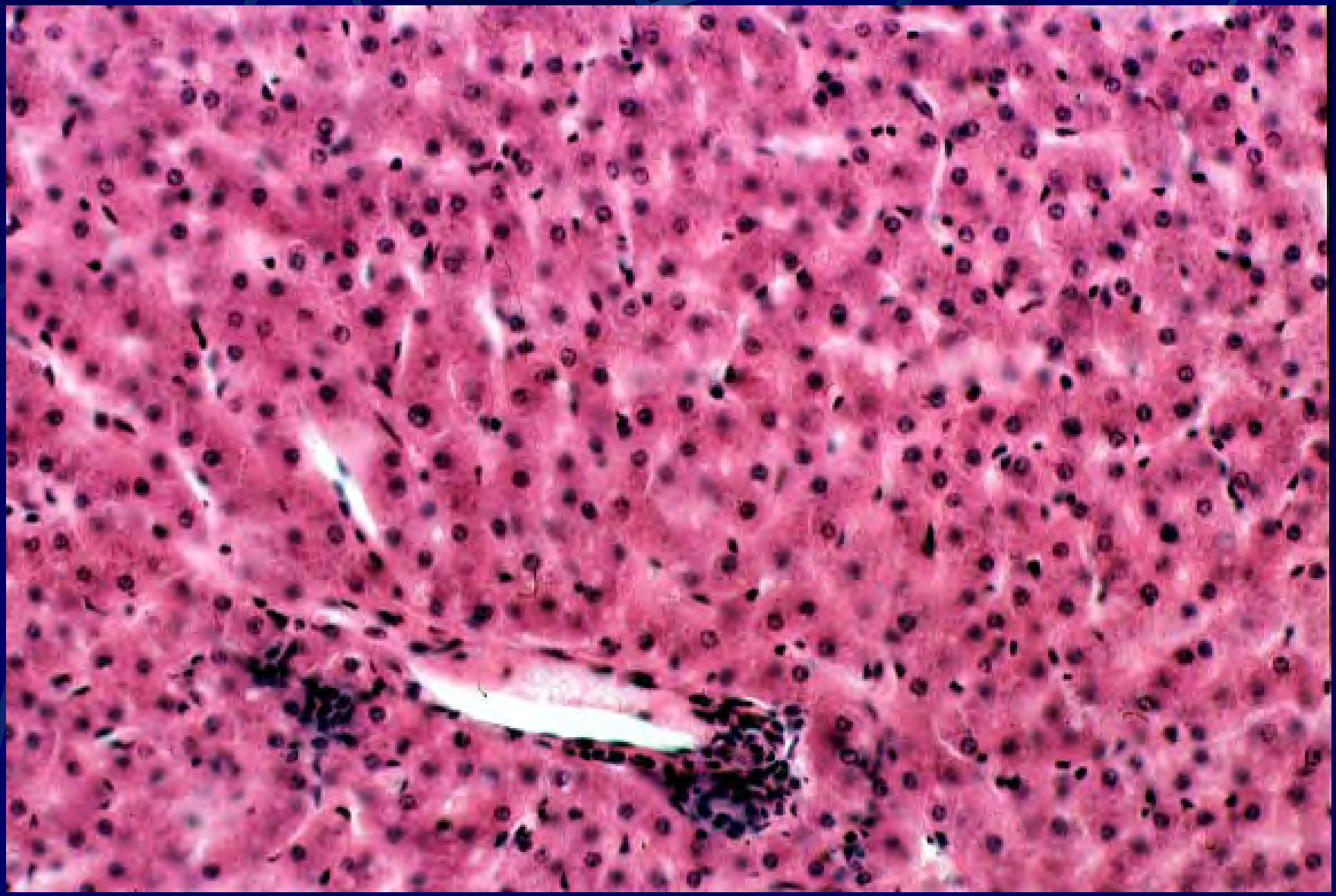
Secondary Lesions

- 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

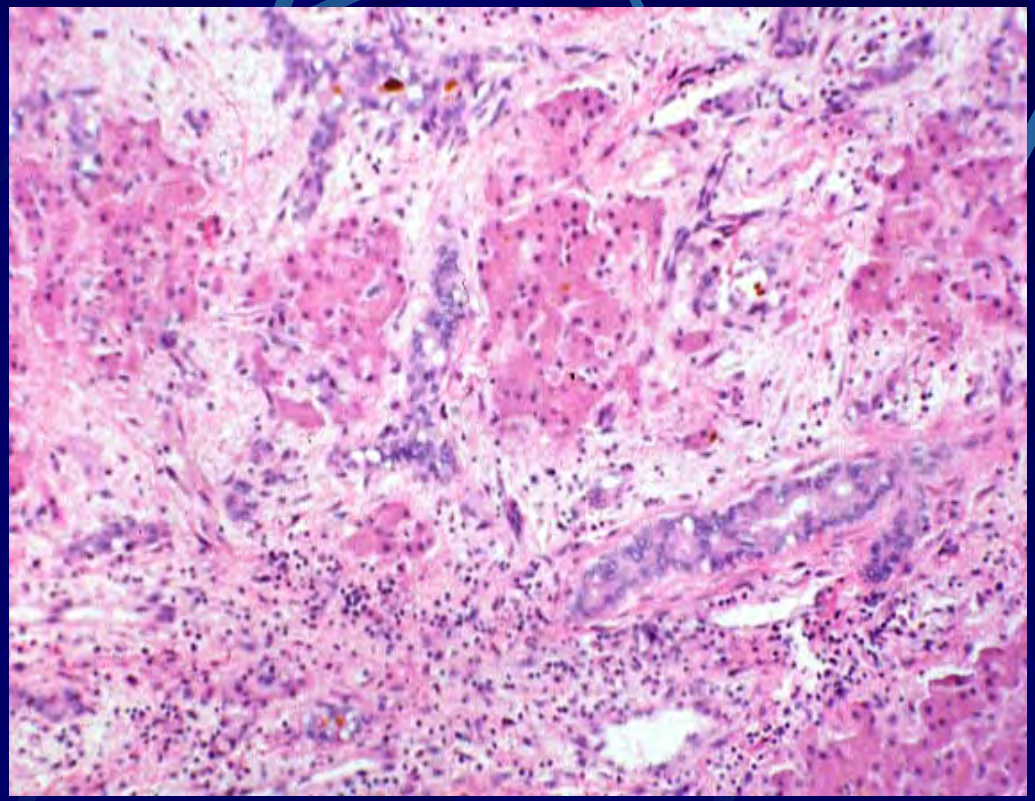

Bay Horse
11/23/92



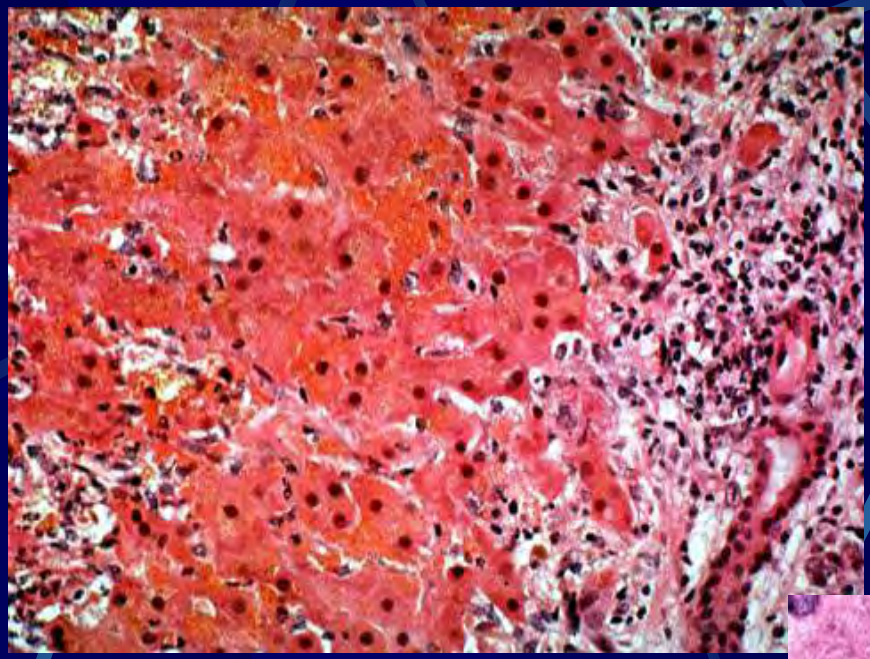




Dose Dependent- Histologic Lesions

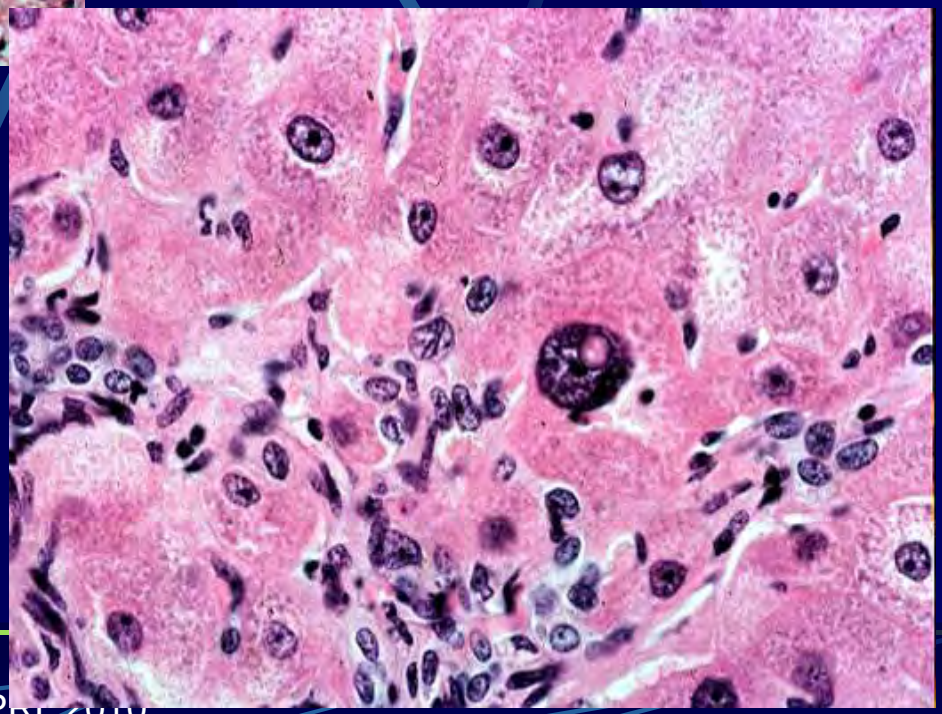


- Portal circulation
- Hepatic metabolism
- Hepatocyte response
- Classical response
  - Necrosis
  - Fibrosis
  - Biliary hyperplasia

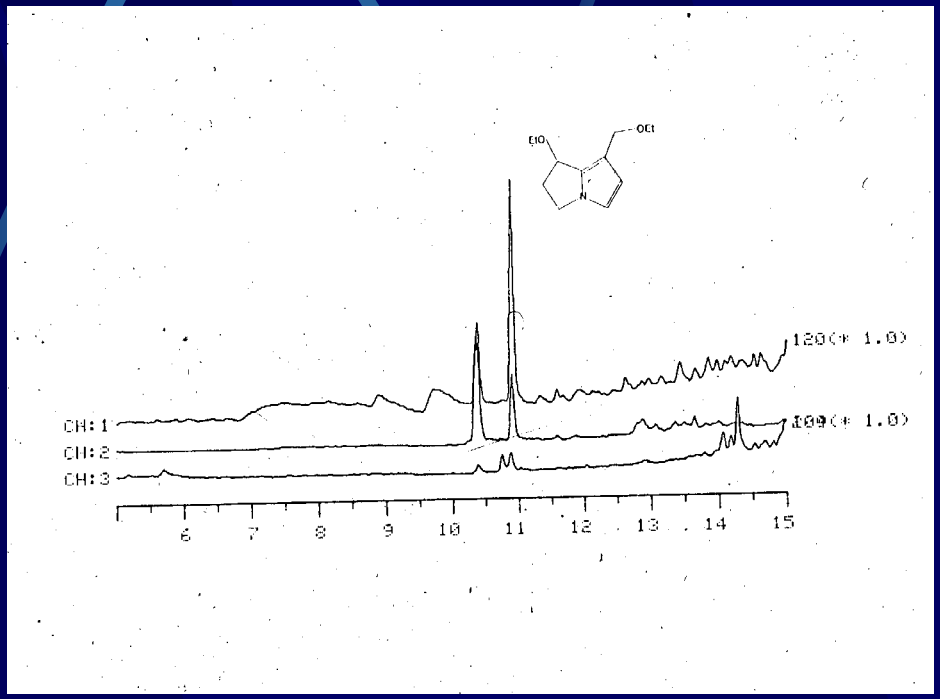


Acute Toxicity

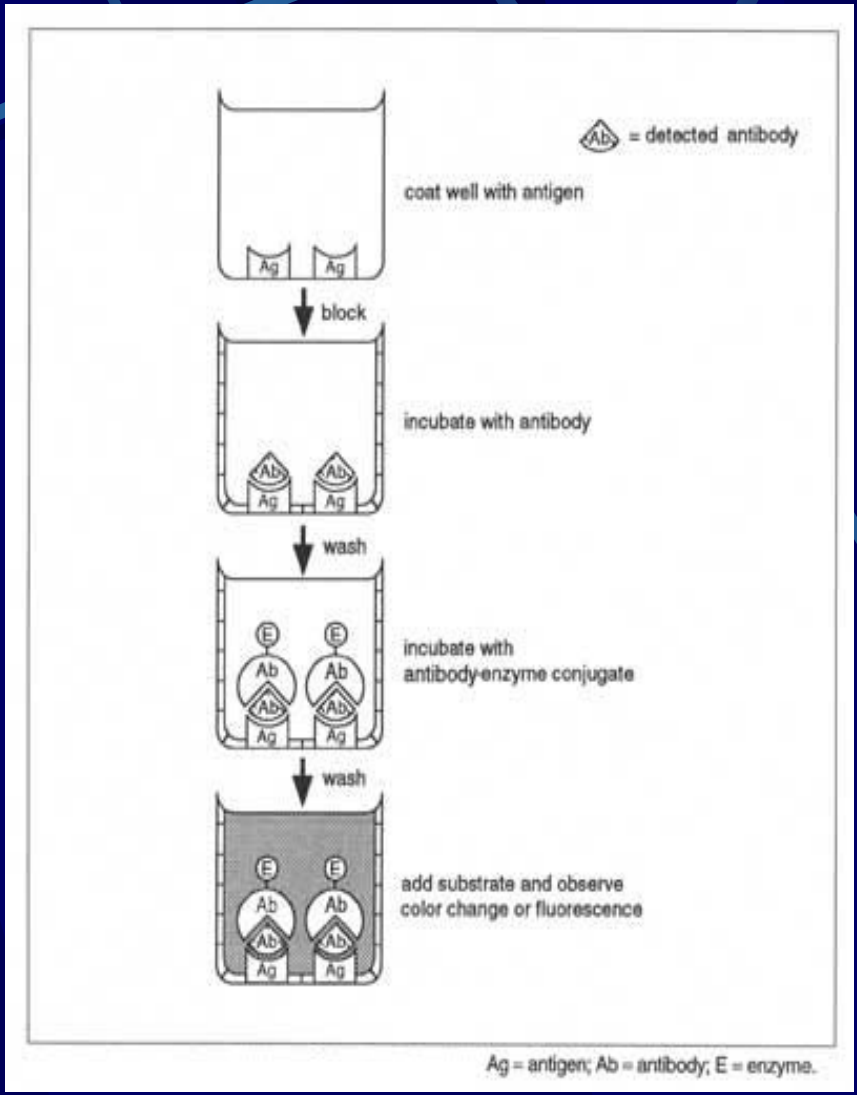
Chronic Toxicity

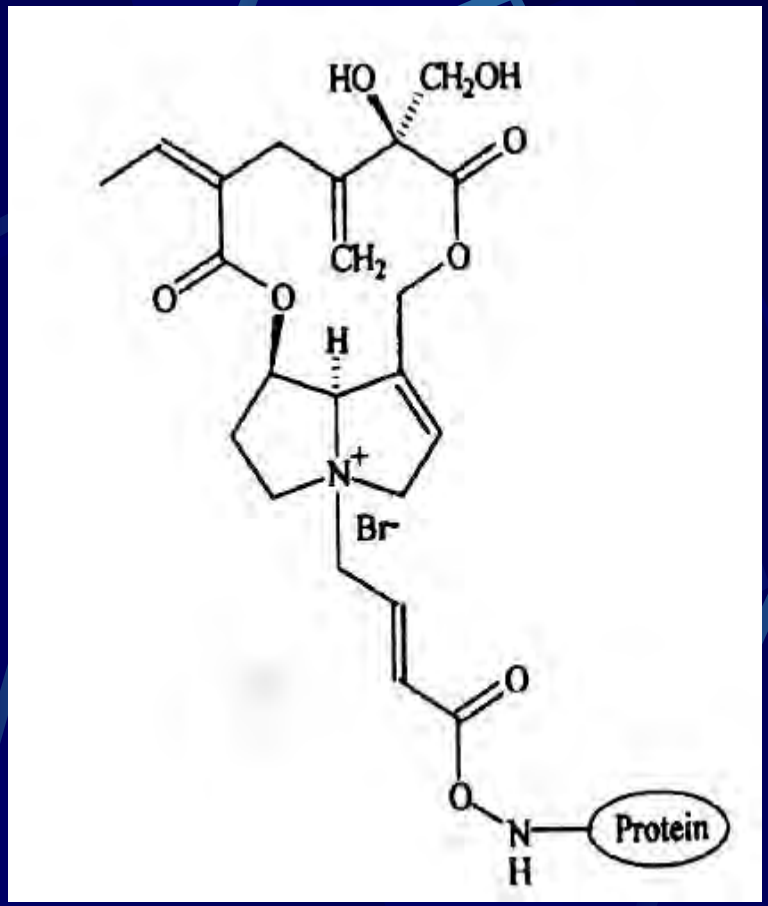


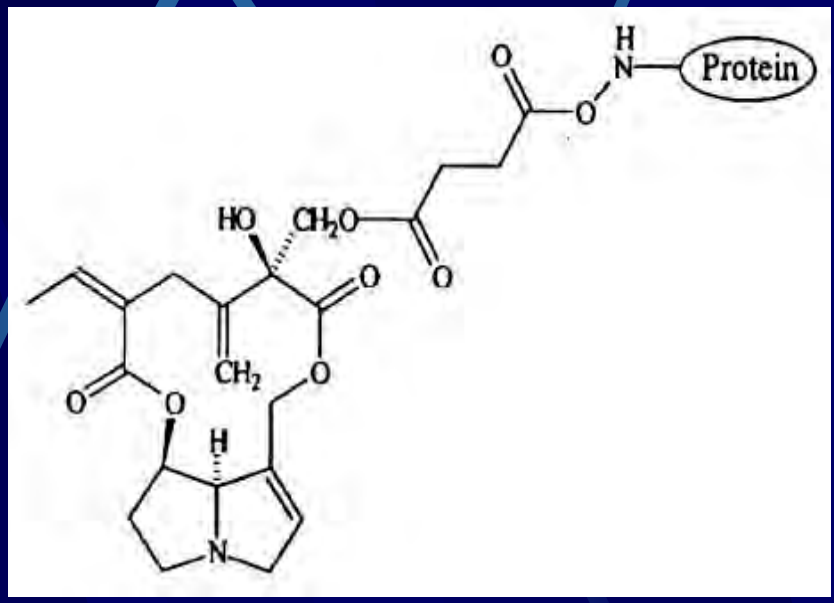
Pyrrole Detection



ELISA Studies



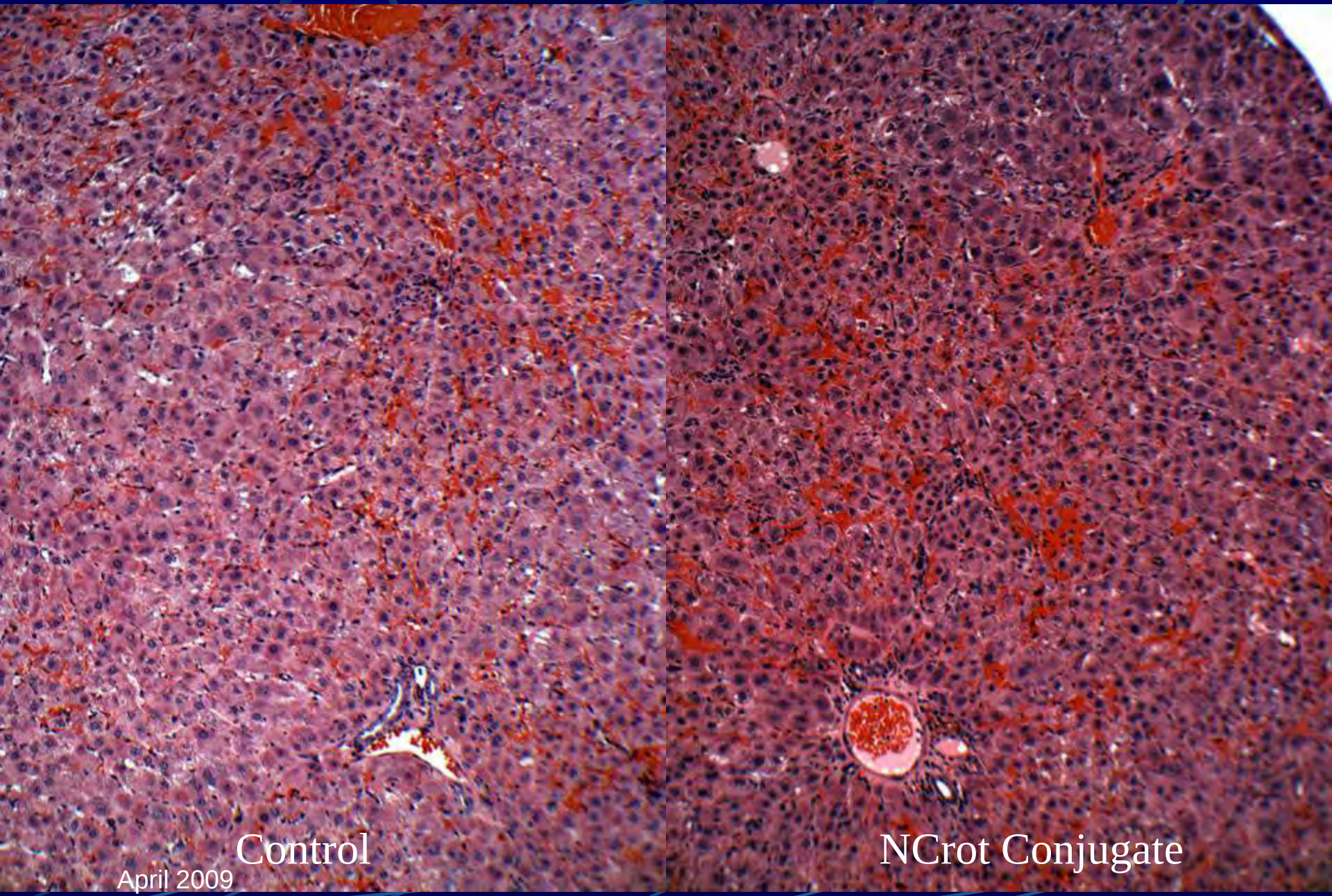

HO
CH2OH
O
CH2
O
O
O
H
N+
Br
O
O
N
H
Protein


O
H
N
Protein
O
HO
CH2O
O
O
CH2
O
O
O
H
N

Class and alkaloid specific ELISA's

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..



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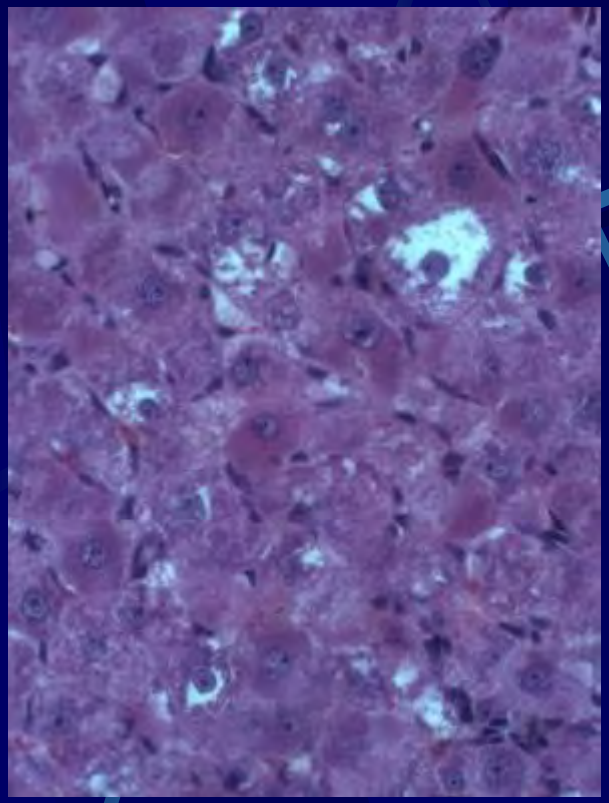
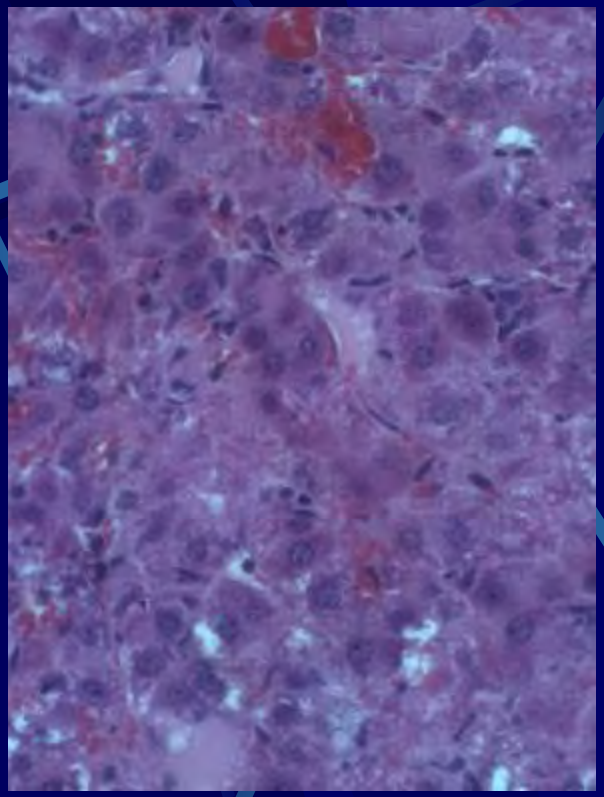
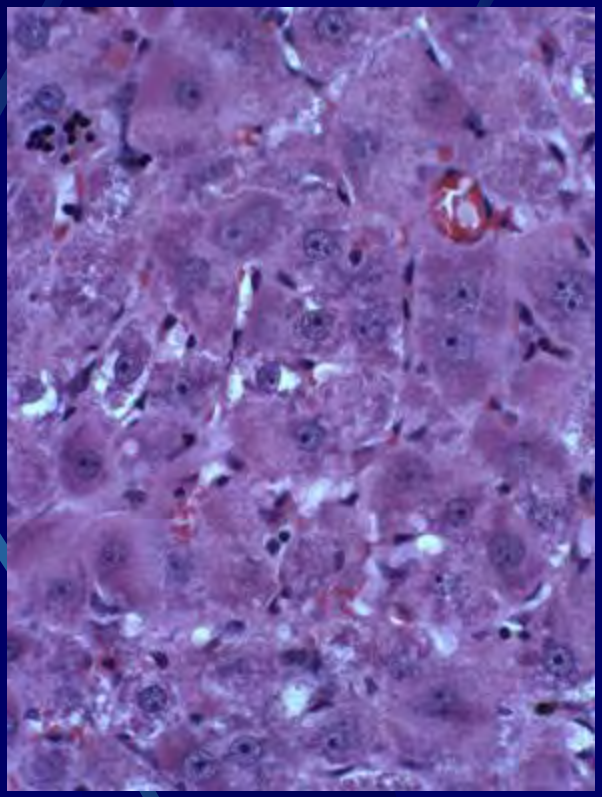
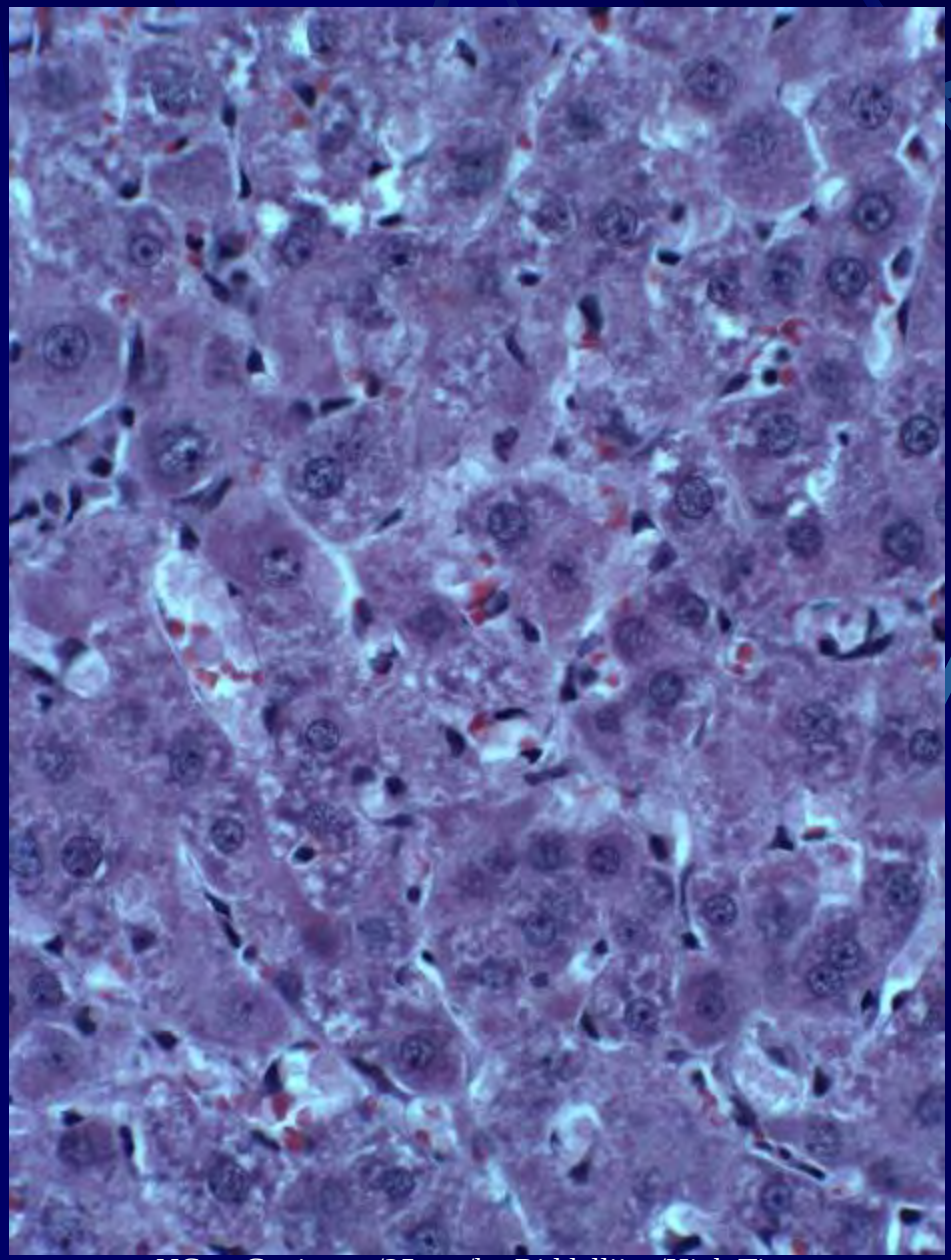
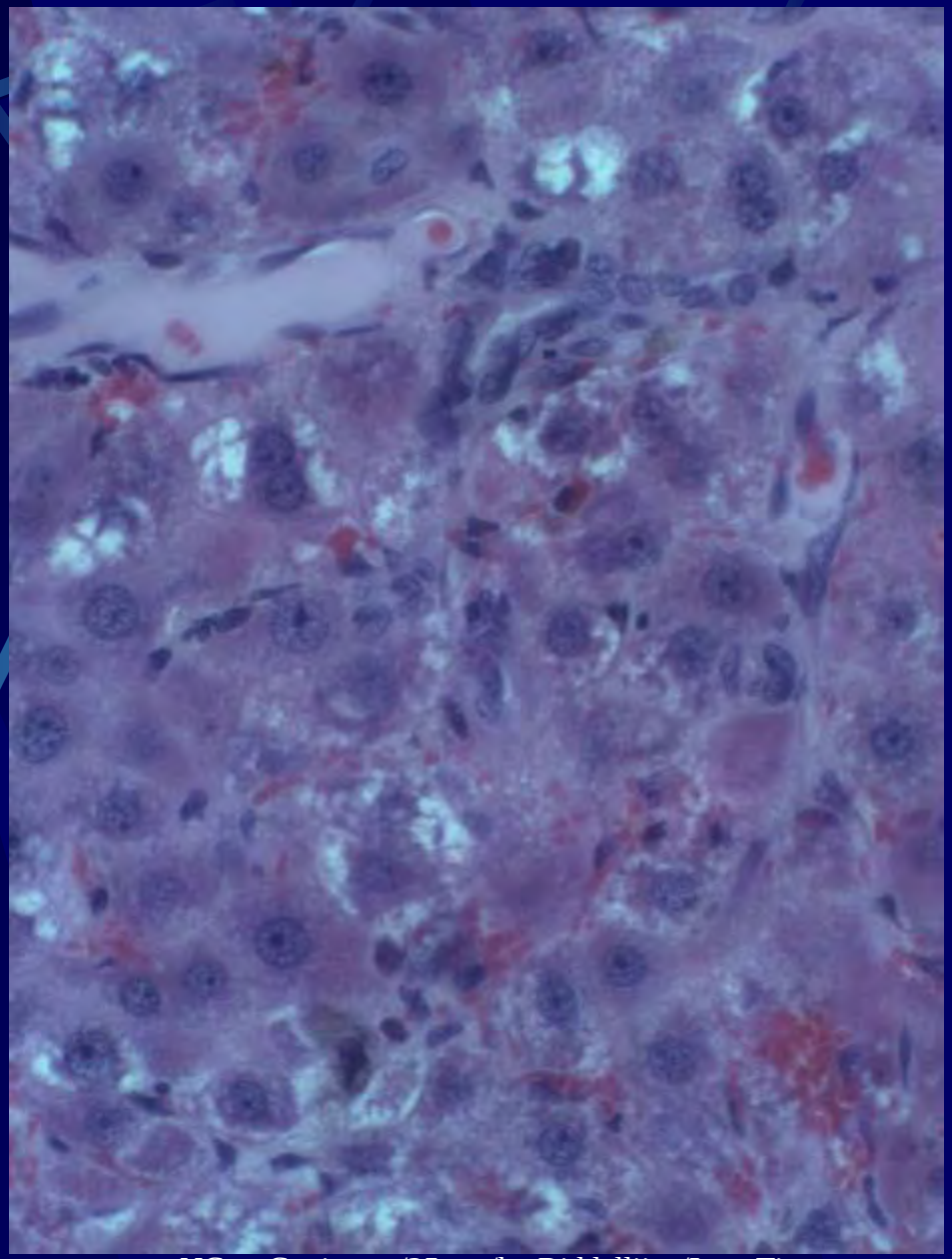




Which rat was vaccinated? Real blind study.



NCrot Conjugate/25 mg/kg Riddelliine/High Titer



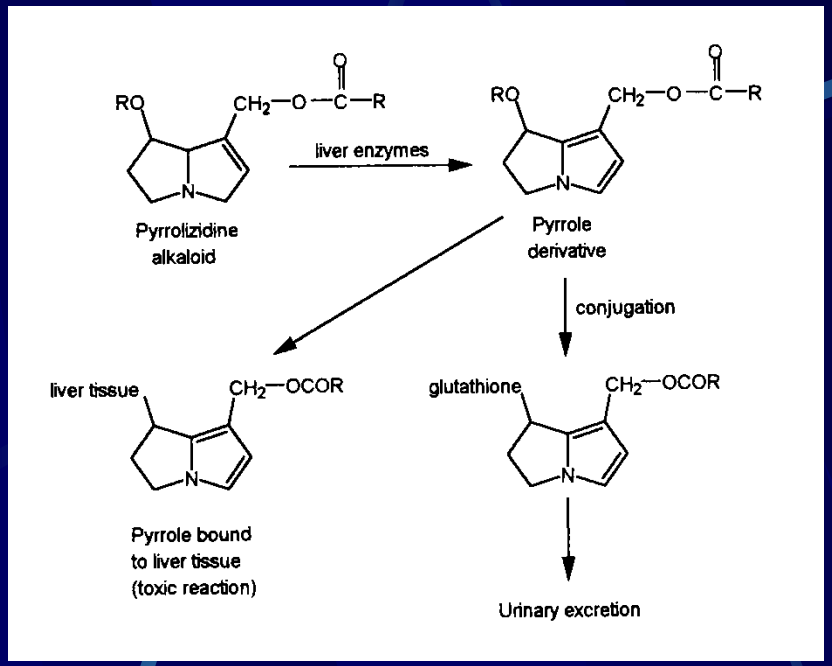
NCrot Conjugate/25 mg/kg Riddelliine/Low Titer

Diagnosis

Current Research Plan Objectives

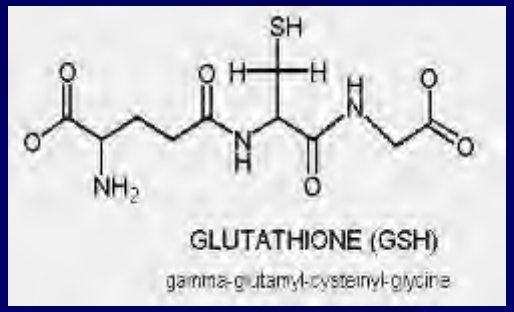
- **Objective 1:** ***Develop diagnostic techniques*** *and biomarkers to better identify animals poisoned by pyrrolizidine alkaloids (PA's) and their subsequent metabolites, and develop techniques to monitor foods and feeds for PA-contamination.*
- **Objective 2:** ***Determine pyrrole toxicity and 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.

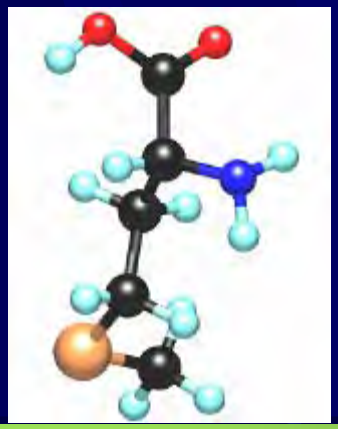
Immunologic Diagnostics



DNA Conjugates
Thymidine Conjugates

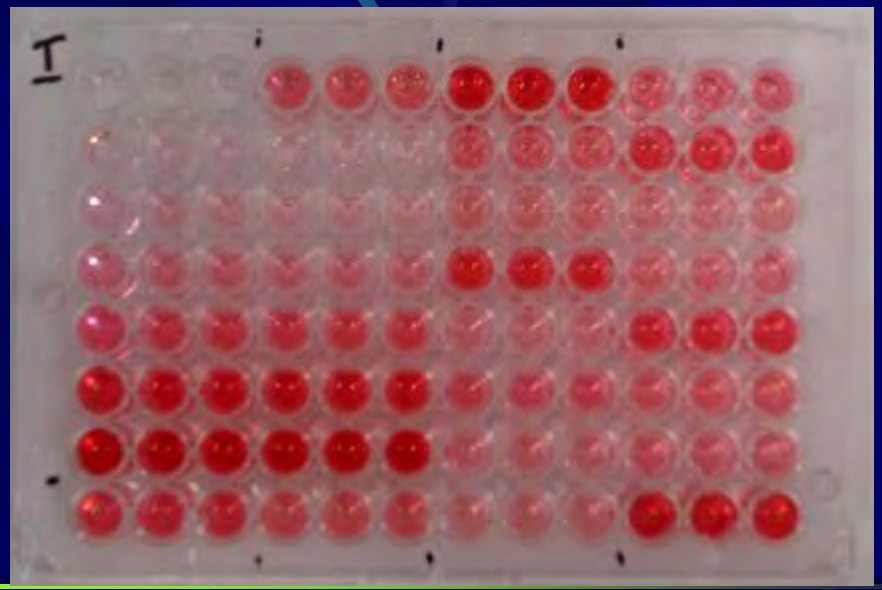
Glutathione Conjugates



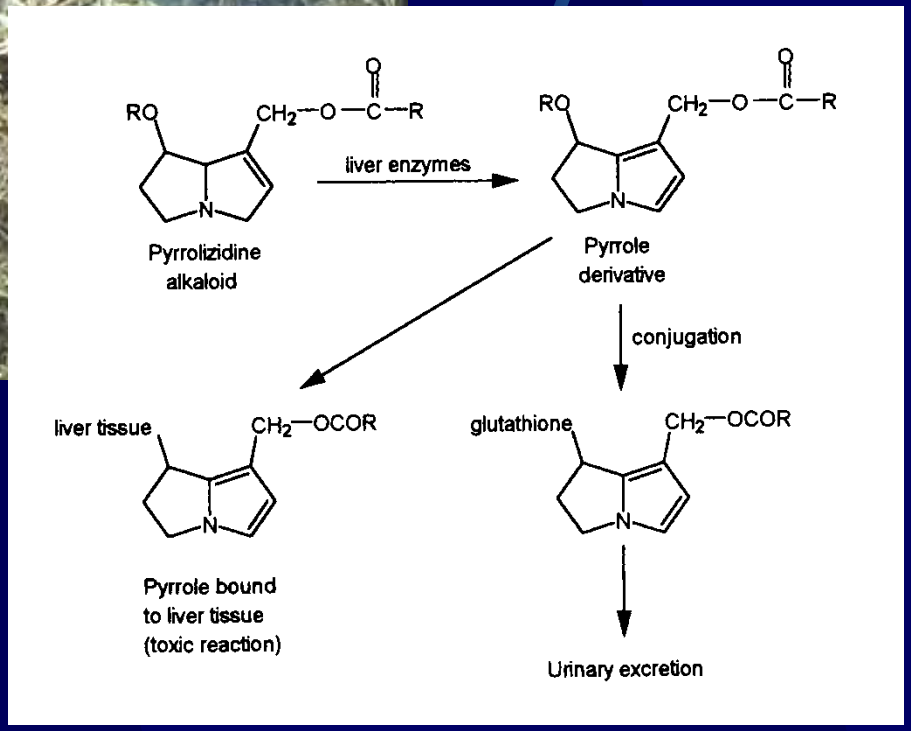


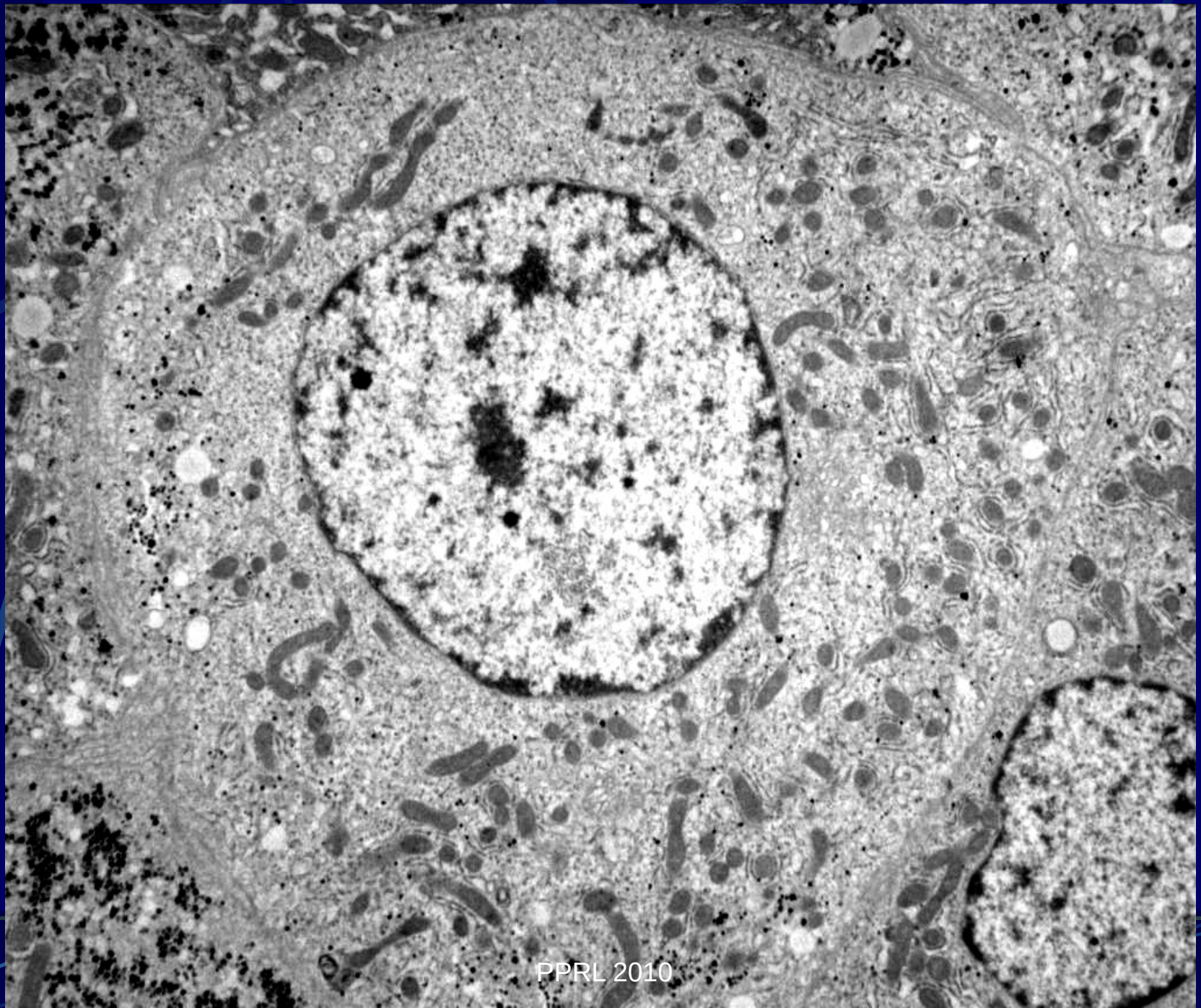
Protein Conjugates

Diagnostic Immunochemistry (ELISA)



Pyrrole kinetics

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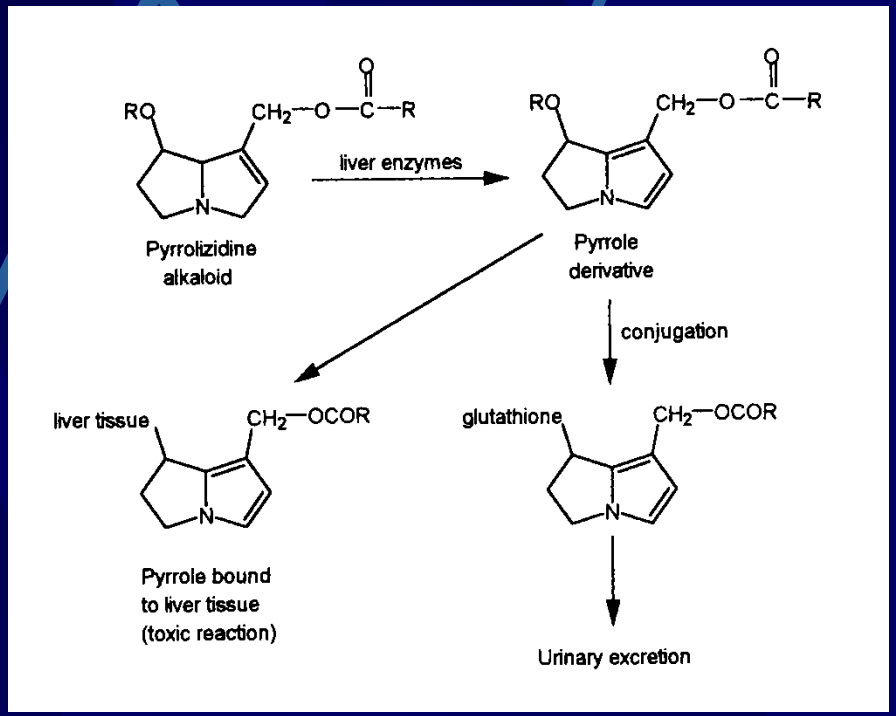
- **Objective 2:** *Determine pyrrole toxicity and carcinogenicity and compare pyrrole toxicity with that of PA and PA-n-oxides. Characterize the risk to fetuses and neonates that are exposed by maternal PA-ingestion.*
- **Sub-Objective 2.1:** Determine pyrrole toxicity and carcinogenicity.
- **Sub-Objective 2.2:** Characterize transplacental and transmammary toxicity of various PA's.

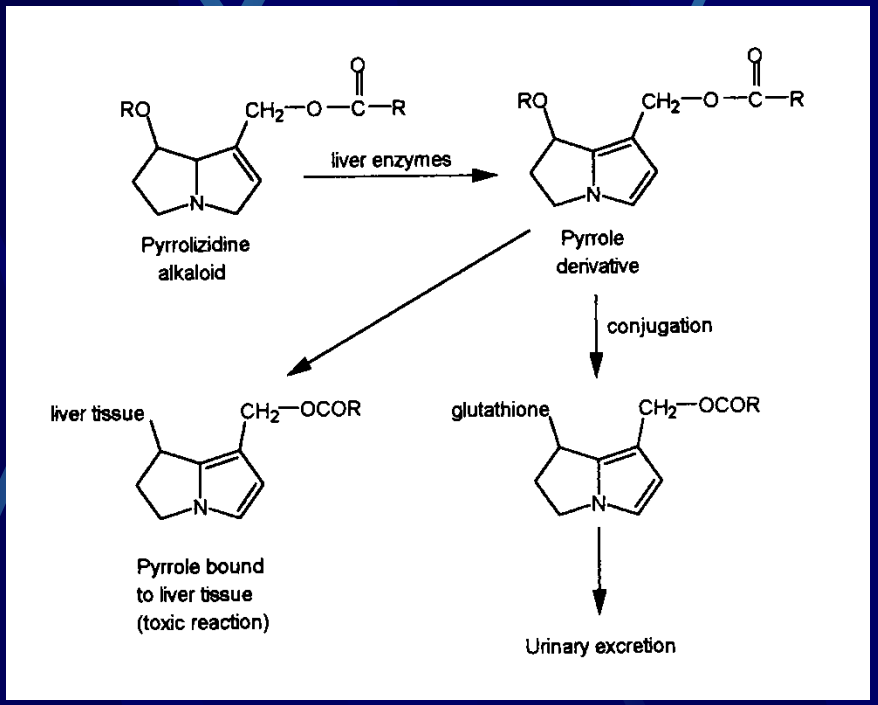
Upregulated or sensitized mice model

- P450 upregulation
  - Phenobarbital
  - Spironolactone
- Glutathione depletion (BSO)
- Pilot Study: Groups of 3 sensitized mice dosed with riddelliine for 14 days

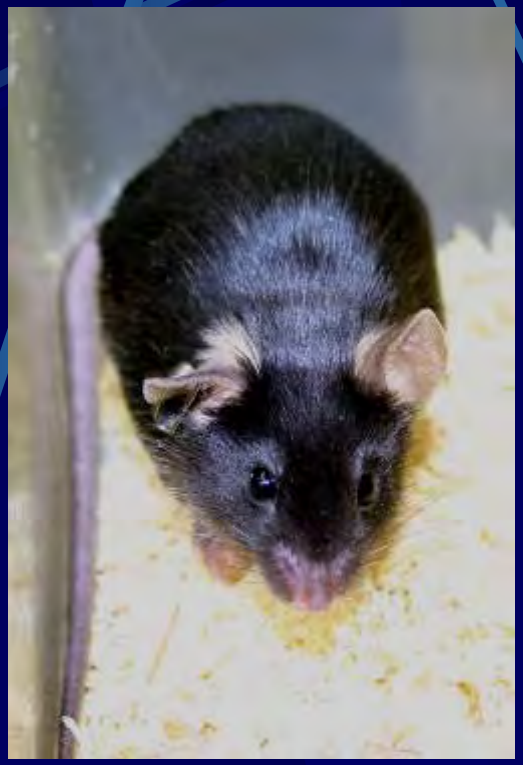


Pilot Study

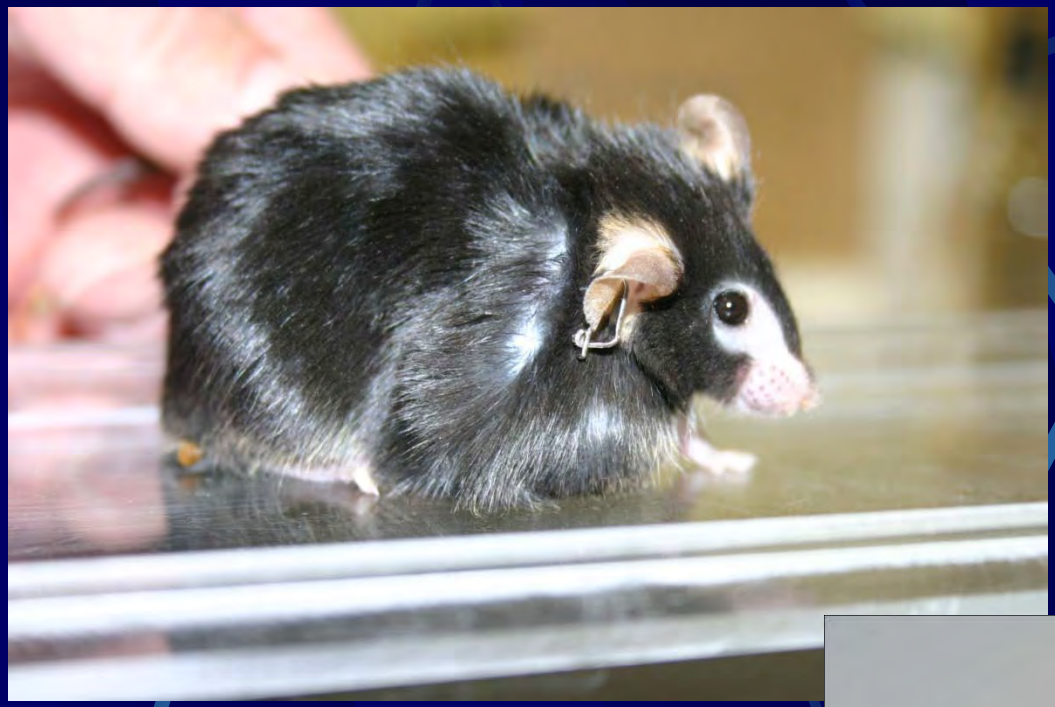
- Phenobarbital and spironolactone increased susceptibility
- BSO (L-buthionine (S,R)-sulfoximine) alone or in combination was poorly tolerated
- Histology studies are underway

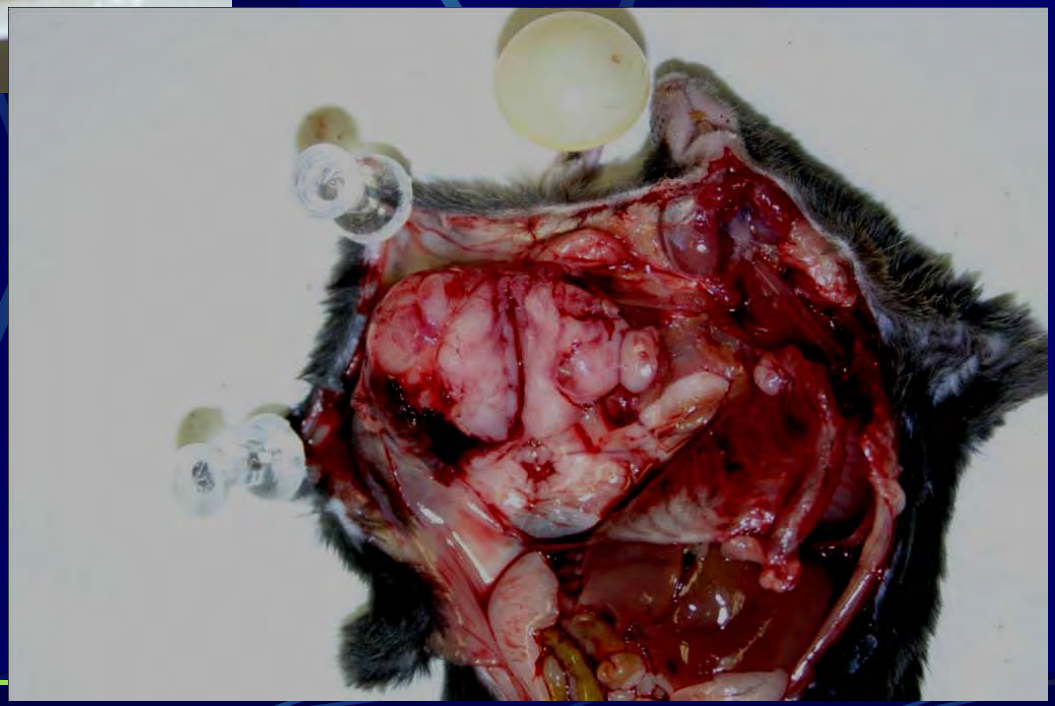

RO
CH2—O—C—R
liver enzymes
Pyrrolizidine alkaloid
Pyrrole derivative
conjugation
liver tissue
CH2—OCOR
glutathione
Pyrrole bound to liver tissue (toxic reaction)
Urinary excretion

Heterozygote TRP53 mutated mice model



- Carcinogenesis endpoint
- Riddelliine challenge at 0, 5, 15, and 45 mg/kg/day for 14 days.
- Monitor neoplastic transformation





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Current Direction

- Continue synthesizing pyrrole conjugates
- Further define and characterize small animal model (sensitized mouse)
- Compare free base, n-oxide and pyrrole toxicity *in vitro* and sensitized mouse models
- Complete characterization of P53 knockout carcinogenicity model

Reading Assignment:

Cheeke: Natural Toxicants in Feeds, Forages and Poisonous Plants

Pyrrolizidine Alkaloids 338-352