

# *Astragalus Poisoning*

ADVS 486

Poisonous Plant Class- Spring 2008

**Bryan Stegelmeier**  
**March 25, 2008**

# *Astragalus* Poisoning

- Locoweeds (swainsonine)
- Nitrotoxins (misertoxins)
- Selenium



>500 species

# Common Toxic *Astragalus* Species

## Astragalus spp.

- Locoweeds: (A. lentiginosus, A. pubentissimus, A. wootoni, A. mollissimus).
- Nitro-containing plants: (A. emoryanus, A. convallarius, A. tetraapterus, and several A. miser varieties).
- Selenium accumulators: ( $\approx$  20 species).

## Oxytropis spp.

- Locoweeds: (O. sericea, O. lambertii).

# Selenium Accumulating *Astragalus*

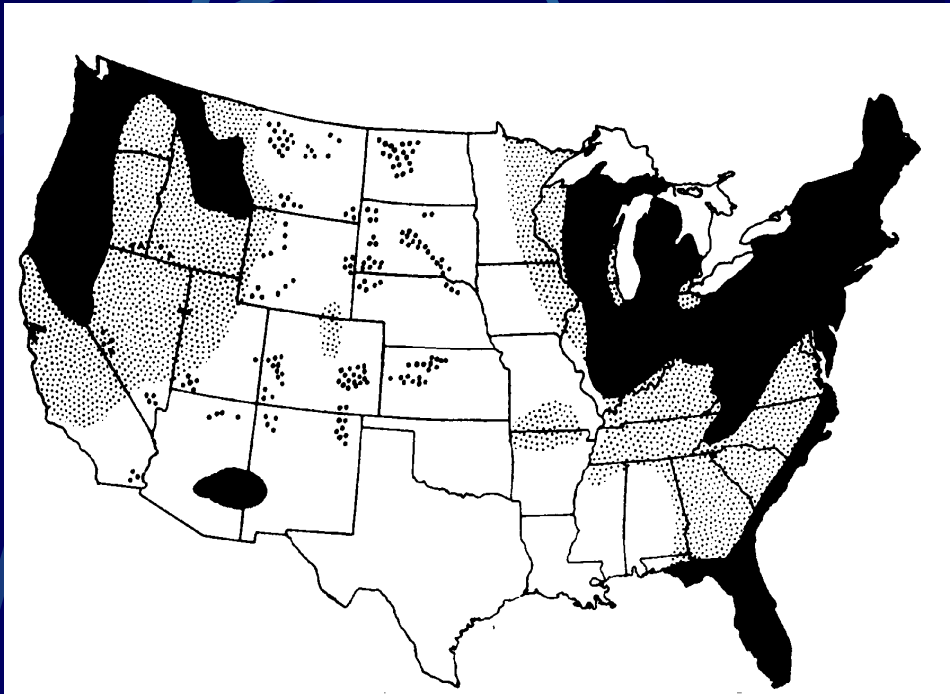


## *Astragalus bisulcatus*

- Unpalatable
- Indirect Toxicity
- Facultative Se Accumulators

“Indicator Plants” - *Astragalus* spp., woody aster, goldenweed, prince’s plum

# Selenium Distribution



- Black- low Se (<0.05 ppm)
- White- variable Se (0.1 ppm)
- Black dots- Se Accumulators (>50 ppm)

# Selenium Toxicity

Mechanism unknown- thought to cause glutathione depletion and lipid peroxidation

Poisoning Syndromes:

- Acute Selenium Toxicity
- Chronic Selenium Toxicity
- Blind Staggers

# Acute Selenium Toxicity



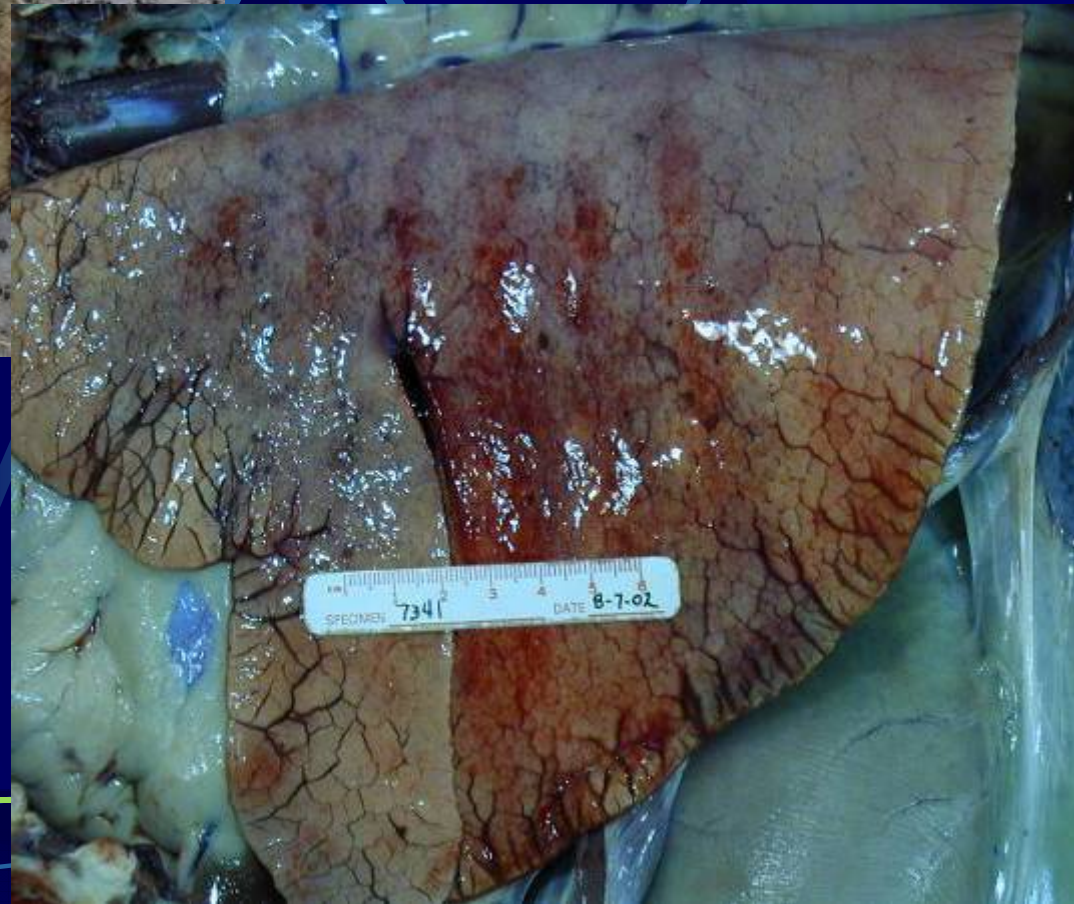
- High Se forage (>100 ppm) or iatrogenic
- Within a week develop staggering, incoordination, lethargy, dyspnea, cyanosis, nasal discharge, teeth grinding, anorexia, prostration, mydriasis, elevated temperature, pulmonary edema, hydrothorax, myocardial necrosis
- Pigs develop poliomyelomalacia

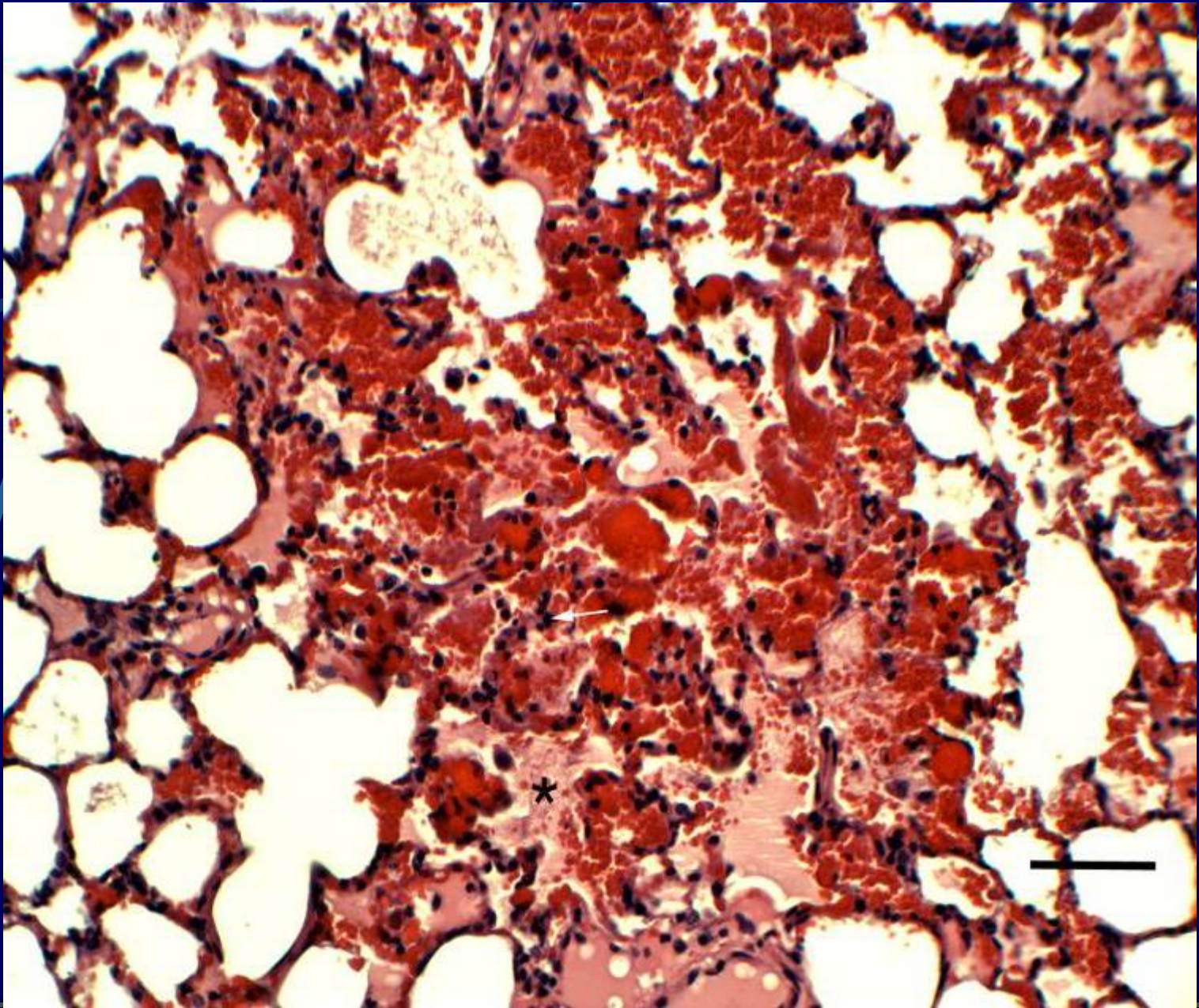


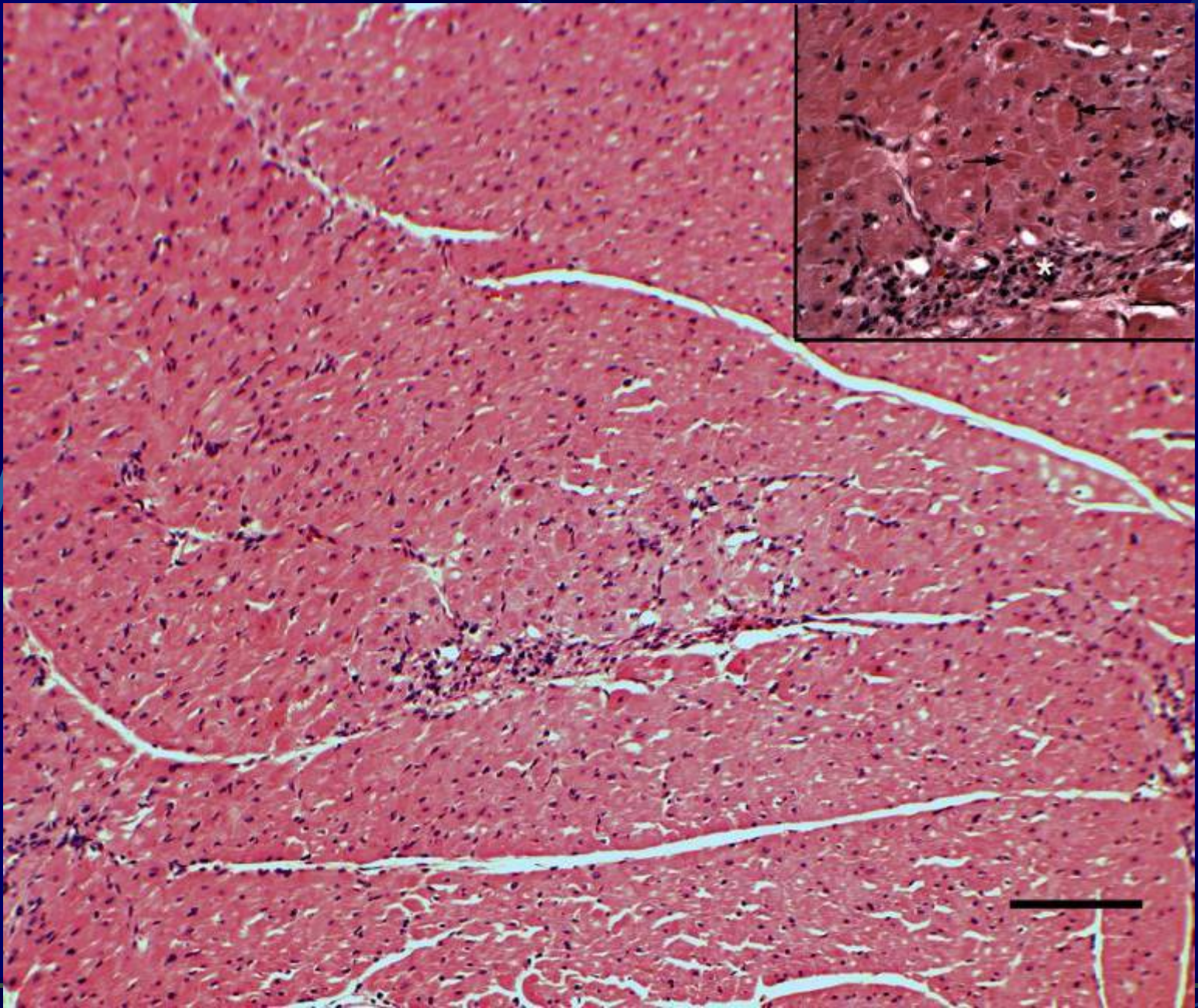




*Grindelia* spp.





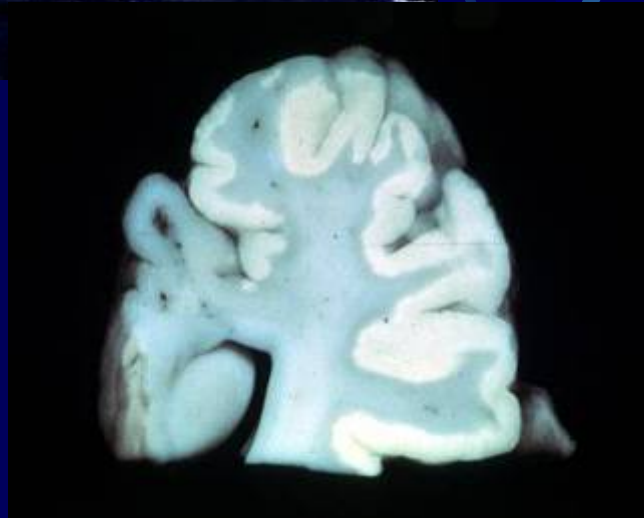
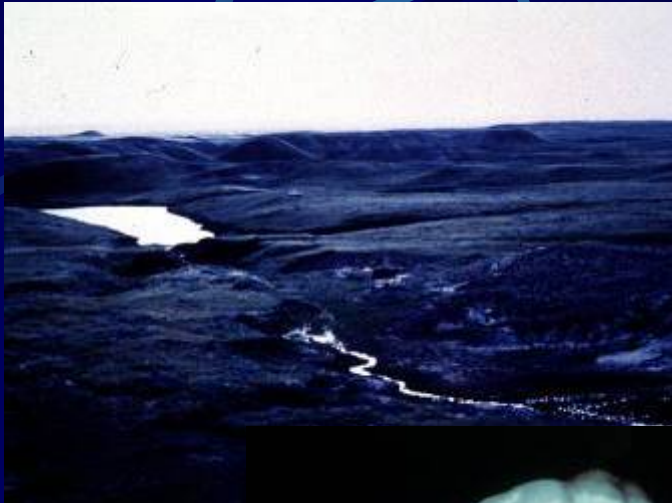


# Chronic Selenium Toxicity



- 5-50 ppm most often seen in cattle and horses (0.1-0.5 ppm nutrient requirement).
- loss of vitality, anemia, joint stiffness, lameness, loss of long hair from mane and tail, sore feet, inflamed coronary band, hoof and horn lesions, liver atrophy, cirrhosis and cardiomyopathy.

# Blind Staggers



- First thought to be caused by acute high Se poisoning-indicator poisoning.
- Most likely sulfate poisoning.

# Nitro-containing *Astragalus*



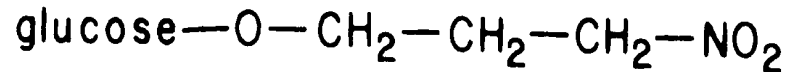
*A. praelongus*

263 Species

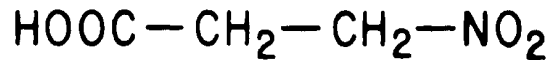
*A. miser*



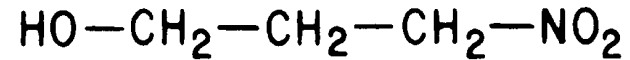
# Nitro- Toxins



Miserotoxin



3-nitropropionic acid



3-nitro-1-propanol

3-nitro propanol, 3-nitro propionic acid and their glycoside derivatives (miserotoxin is the  $\beta$ -D-glucoside of 3-nitro propanol).



# NPA Clinical Disease and Lesions



## Chronic poisoning:

“cracker heels”

Rear limb weakness

Staggers

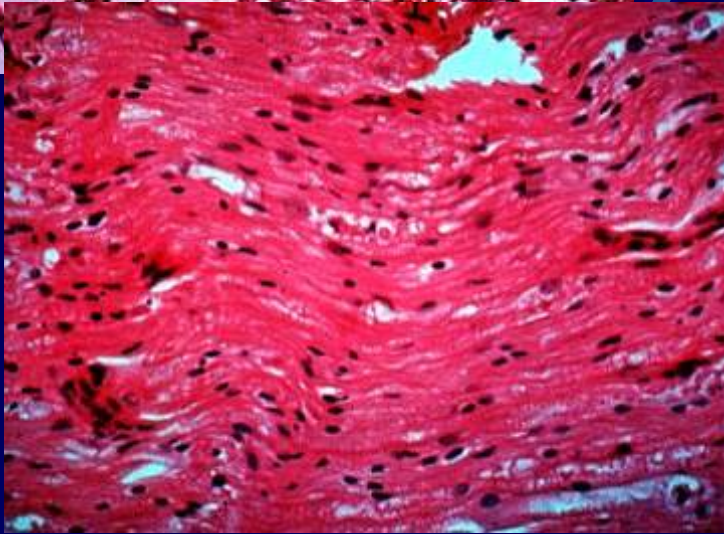
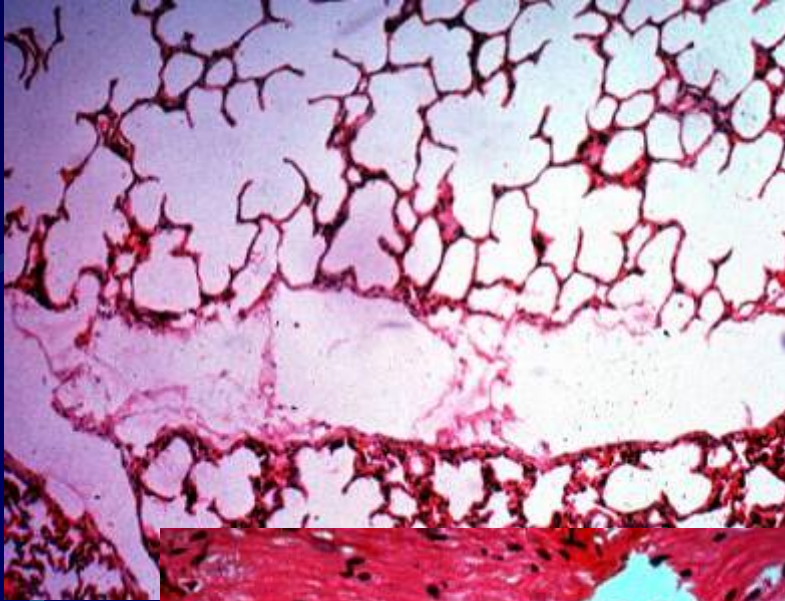
Weaving walk

Blindness

Animals never  
recover

Death

# NPA Clinical Disease and Lesions



## Acute poisoning:

Weakness

Convulsions

Frequent urination

Tachycardia

Dyspnea

Cynosis

Methemoglobinemia

Coma

Death

# Locoweeds



*Astragalus lentiginosus*

*Oxytropis sericea*

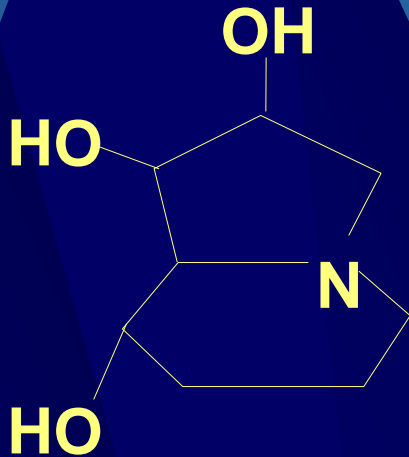




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# Locoweed Chemistry

Swainsonine



Inhibits

$\alpha$ -Mannosidase

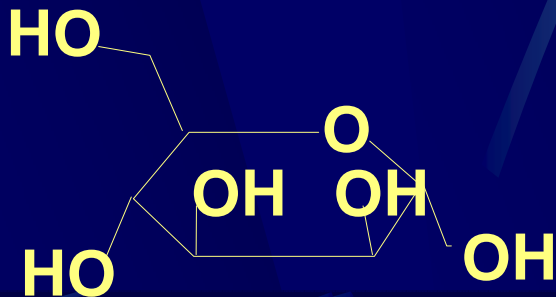
Mannosidase II

Lysosomal Storage

Disease

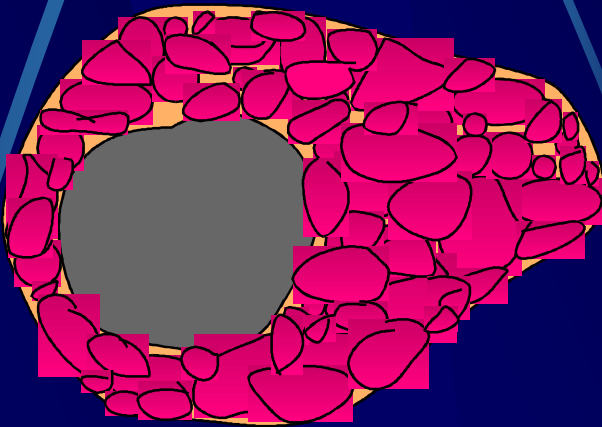
Altered Glycoprotein  
Synthesis

D-Mannose



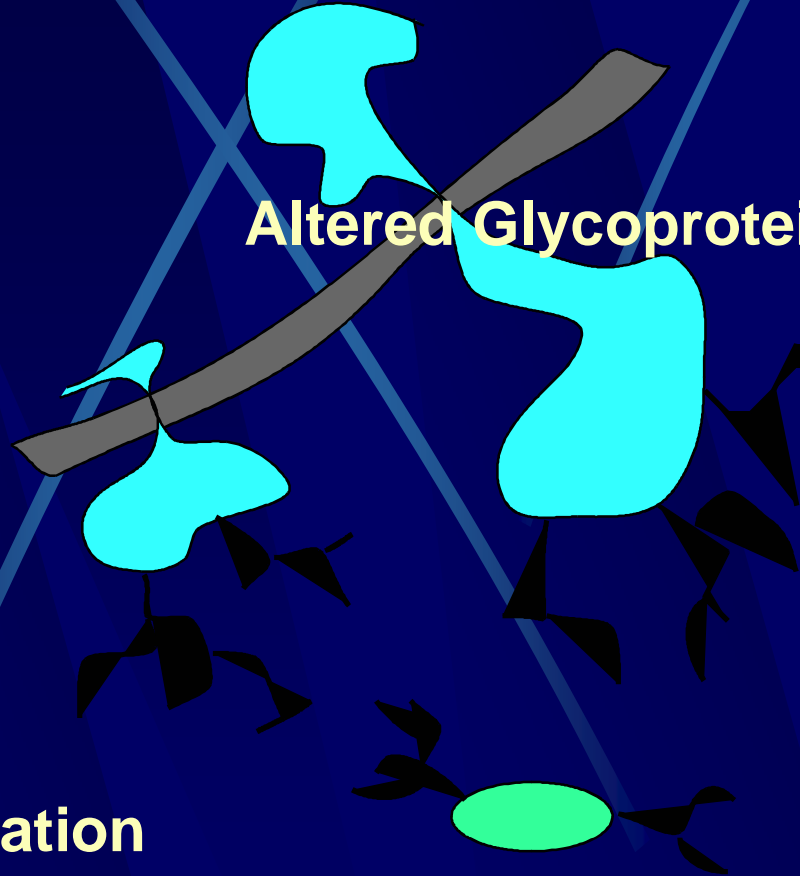
# Action of Swainsonine

$\alpha$ -Mannosidase Inhibition



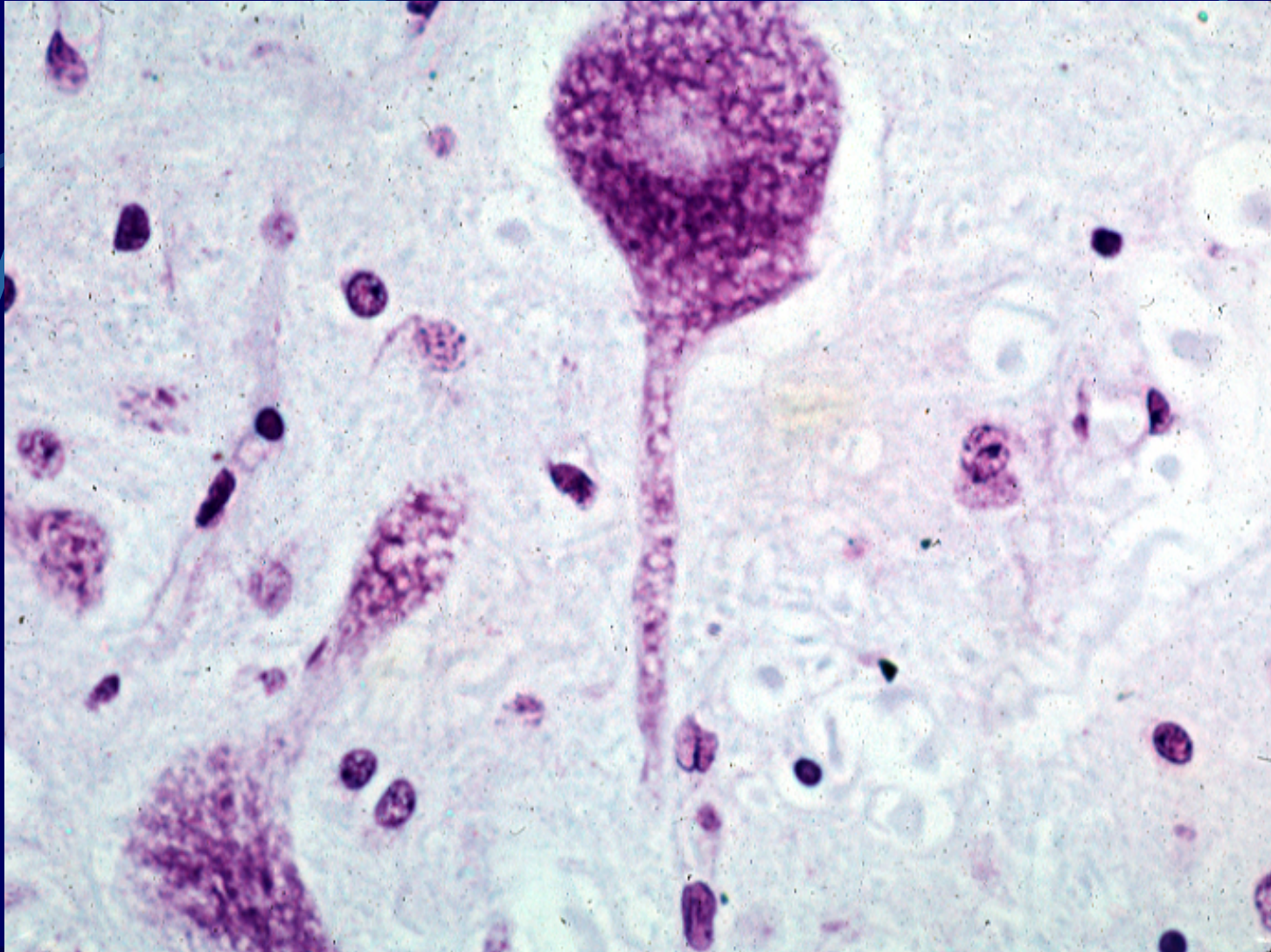
Cellular Constipation

Altered Glycoproteins



Mannosidase II Inhibition

# Cellular Constipation (neuron)



# Locoism



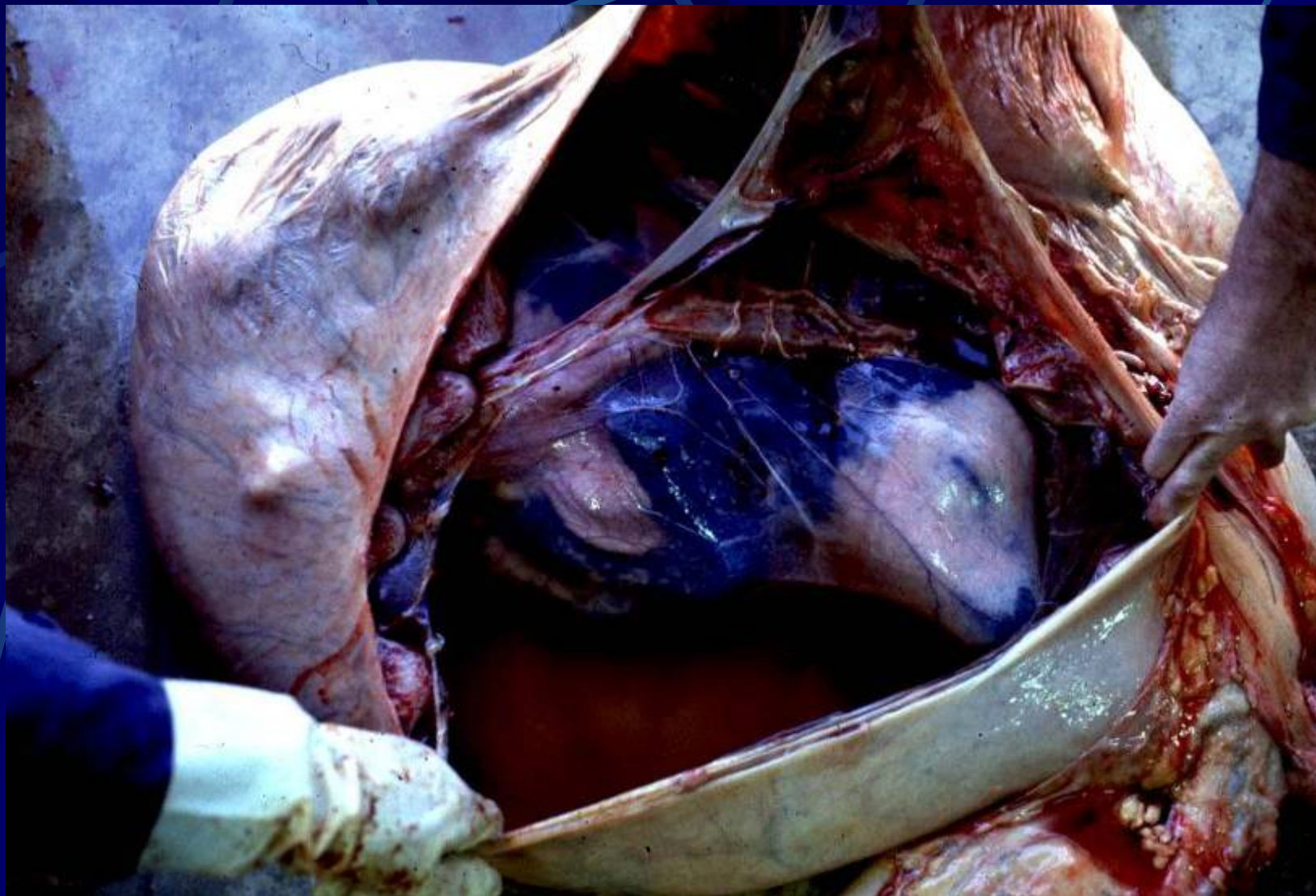
- Depression, anorexia, weight loss, dull hair coat, infertility, abortion
- Hyperexcitable, nervousness, frightened or violent
- Proprioceptive deficit, intention tremors, mild seizures, cardiovascular disease
- Ataxia, paresis, impaired vision

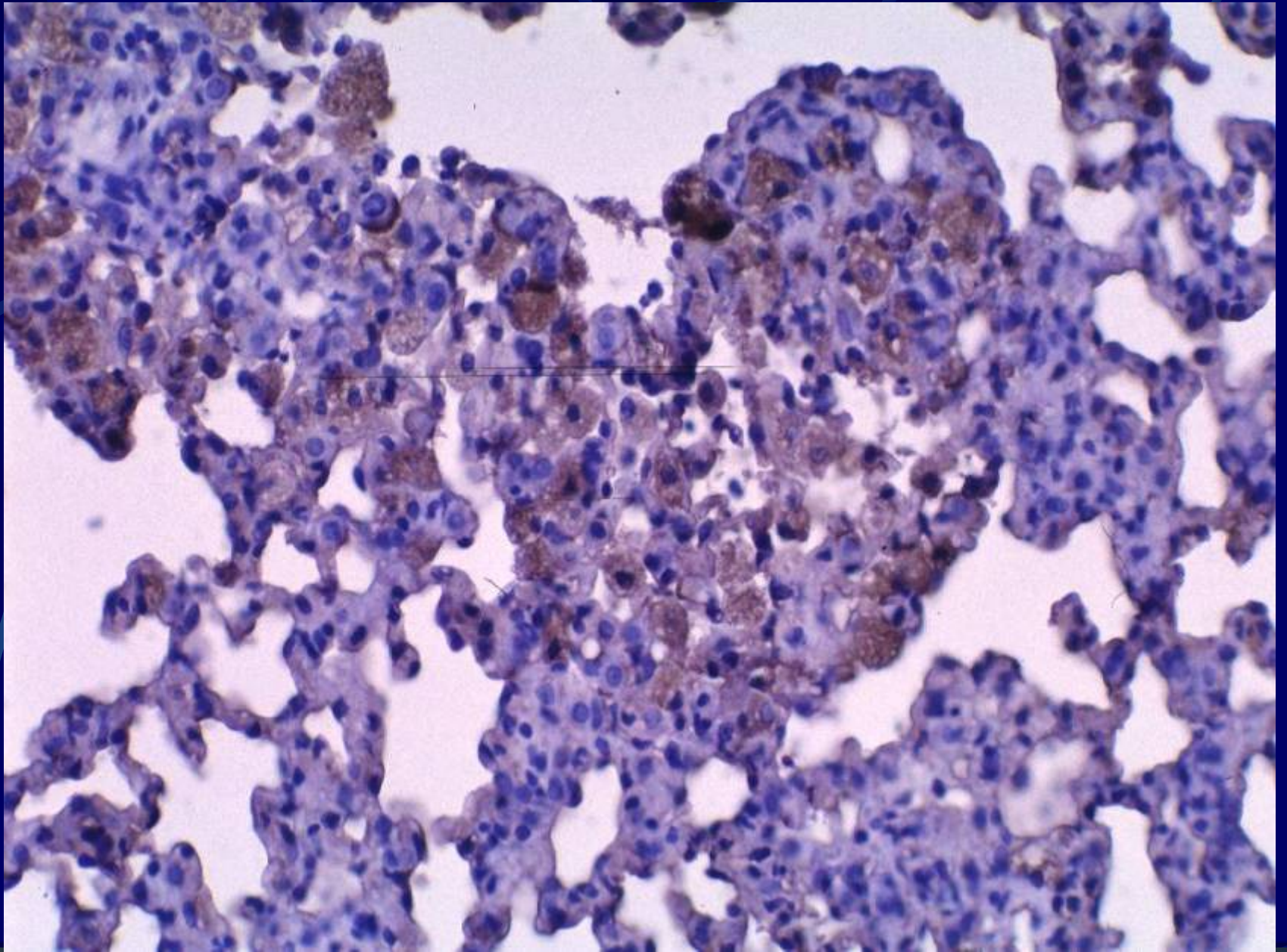


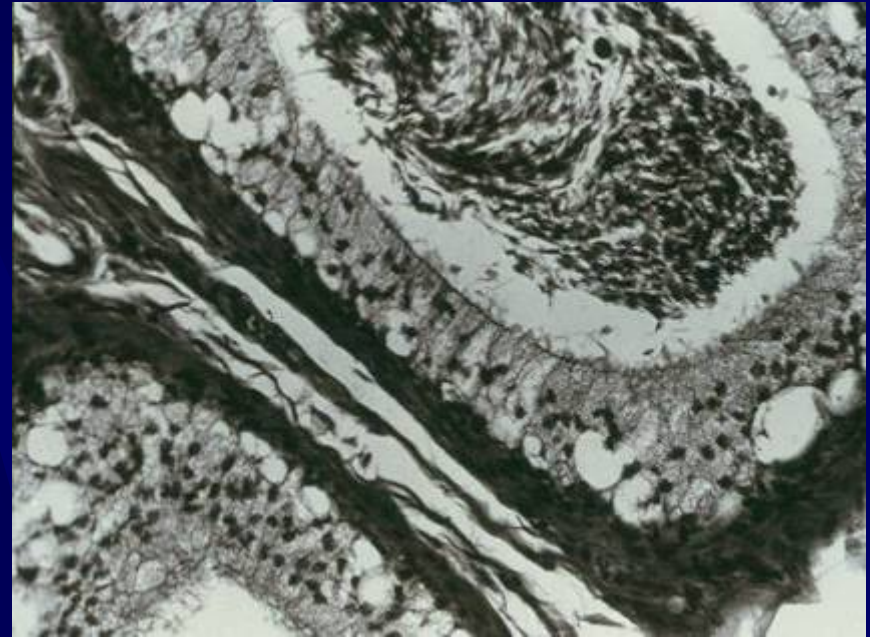
# Effects of Abnormal Glycoproteins

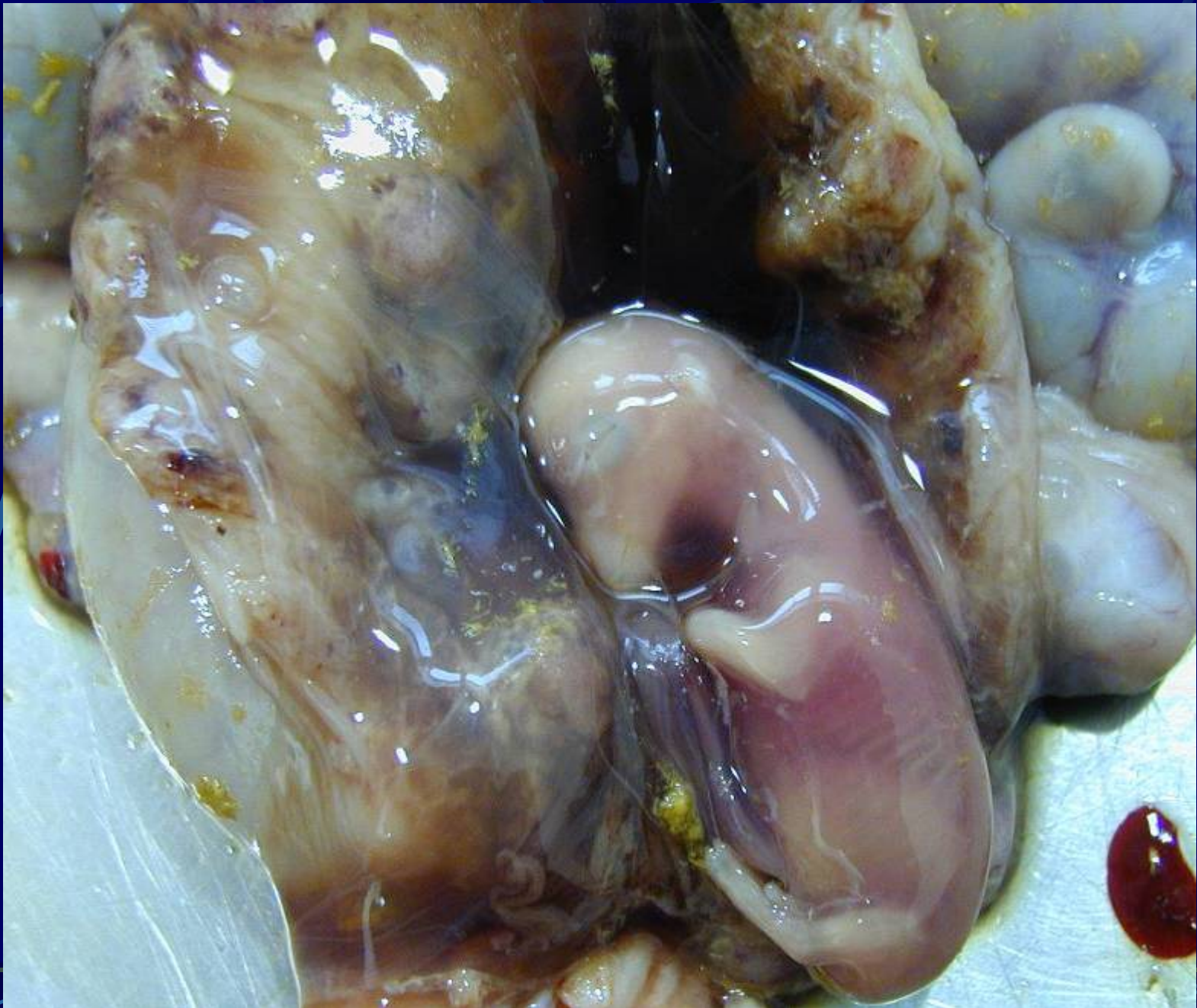


- Reproduction
- Thyroid function
- Micronutrient homeostasis
- Cardiovascular function
- Immunologic function



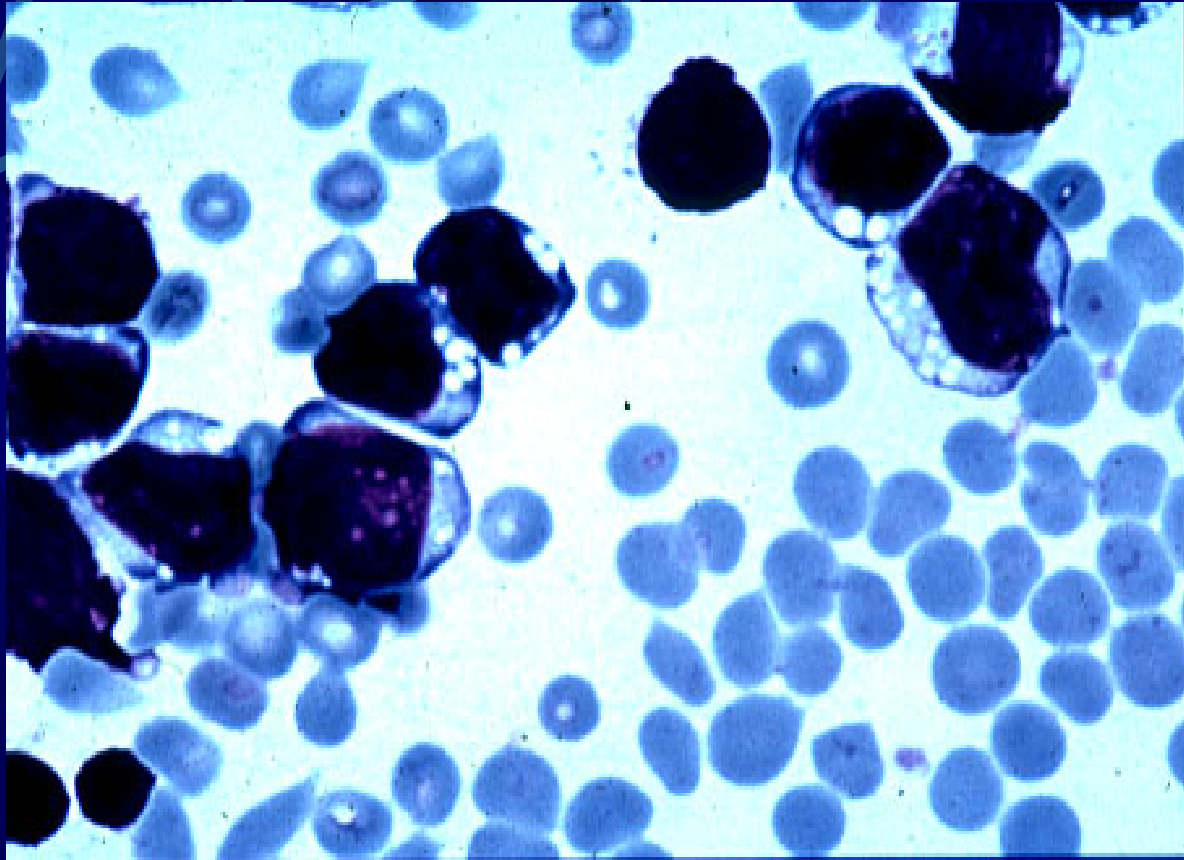






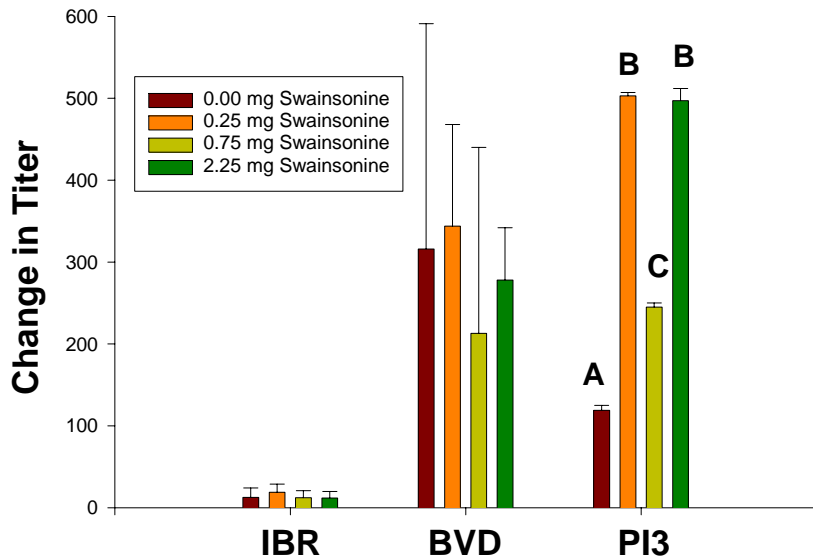
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# Immune Study Findings

Change in Viral Neutralization Titer 10 Days After Vaccination in Locoweed Poisoned Cattle

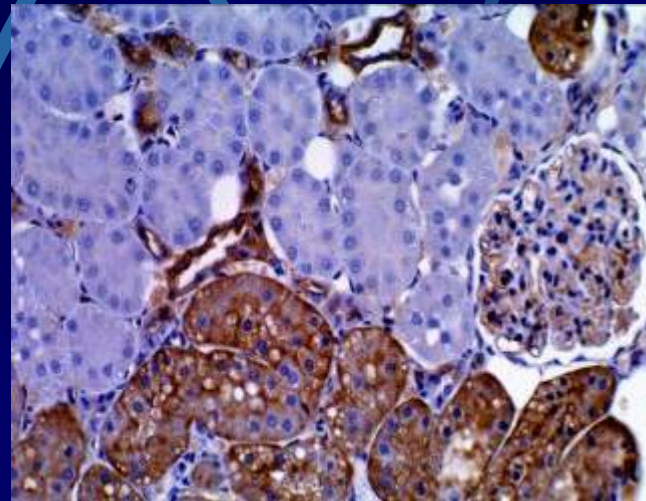
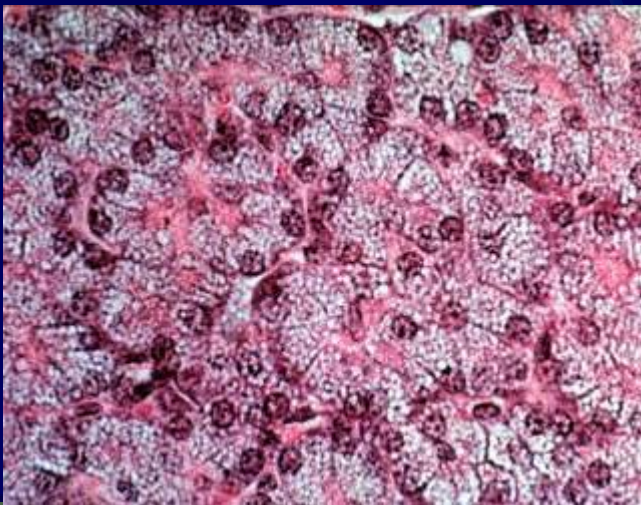
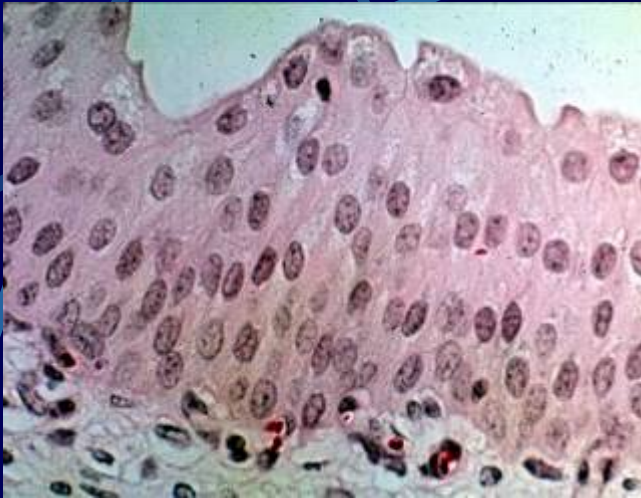


Initially locoweed promotes IgA production, increases viral neutralization titers and lymphocyte blastogenesis

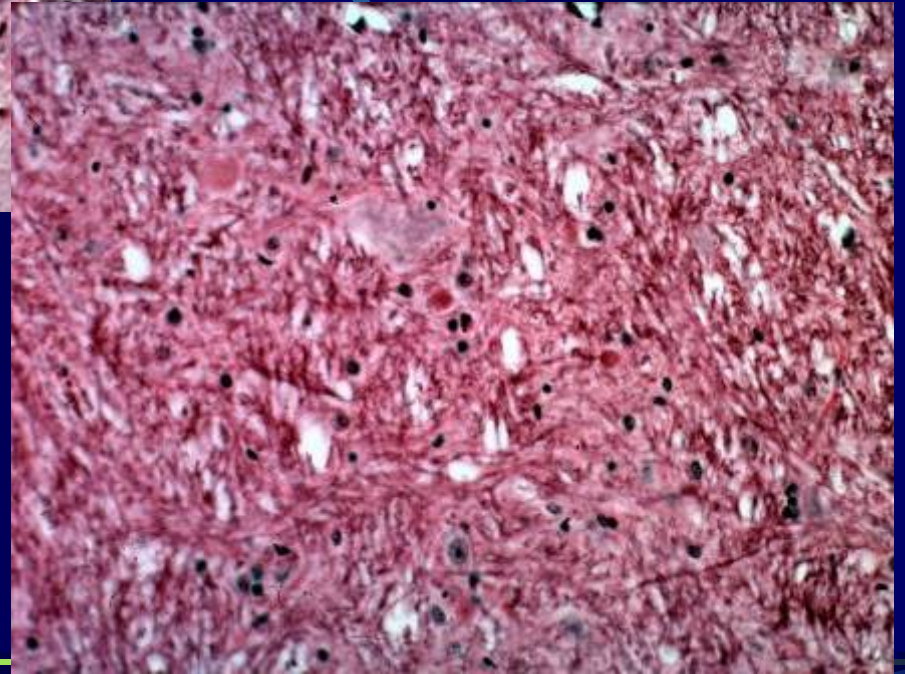
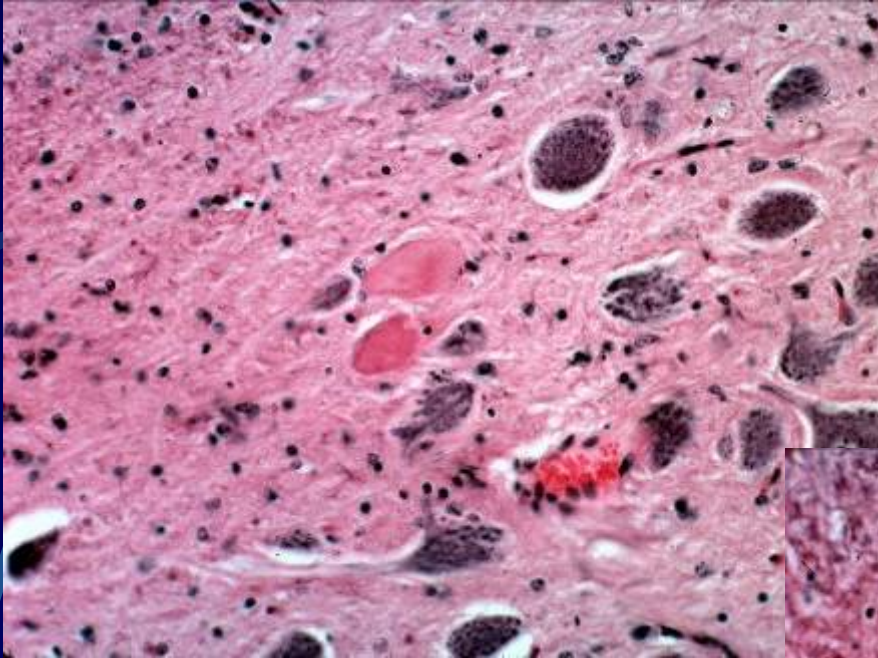
Chronic poisoning depresses neutrophil bacterial killing, neutrophil superoxide anion production



# Locoweed-induced Lesions



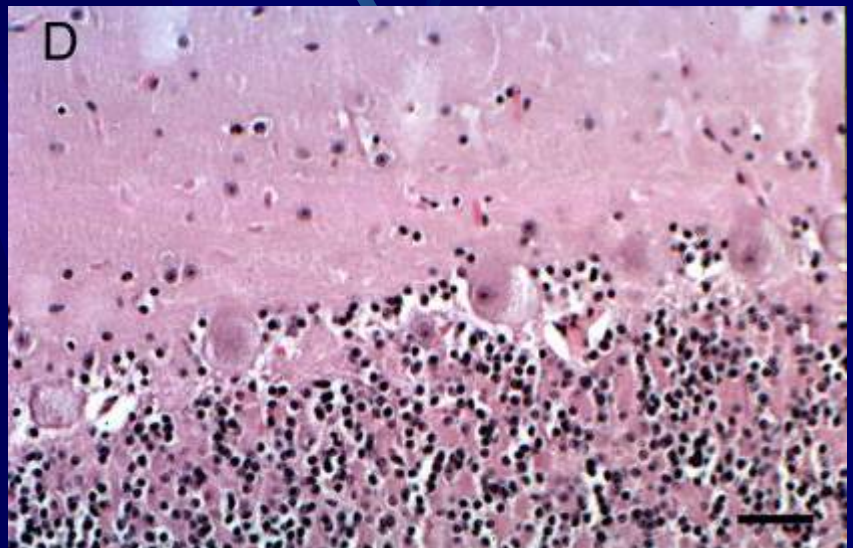
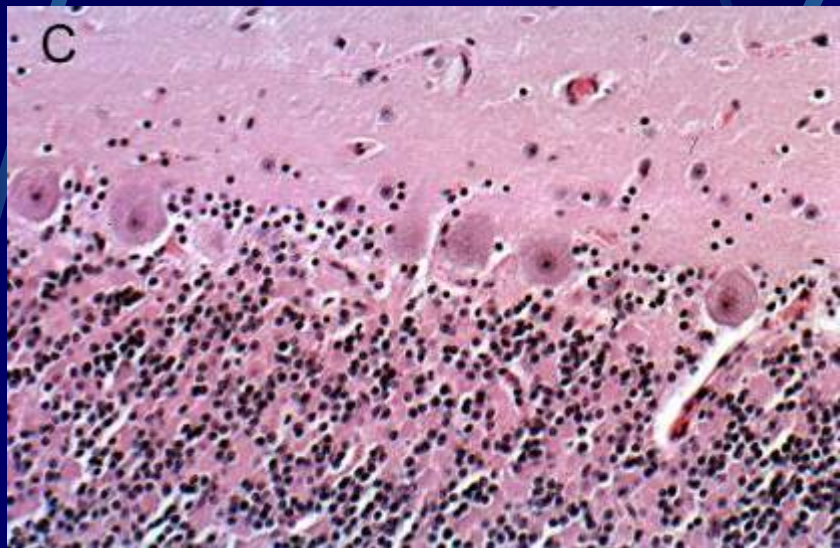
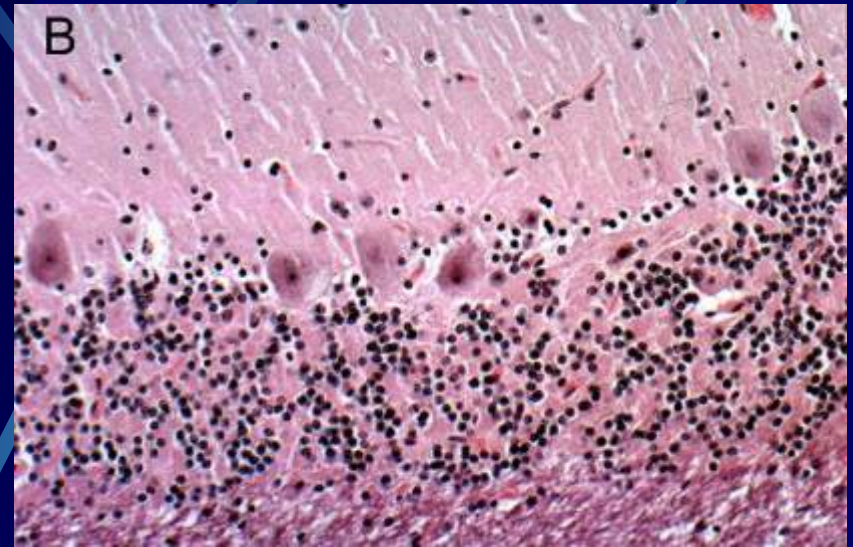
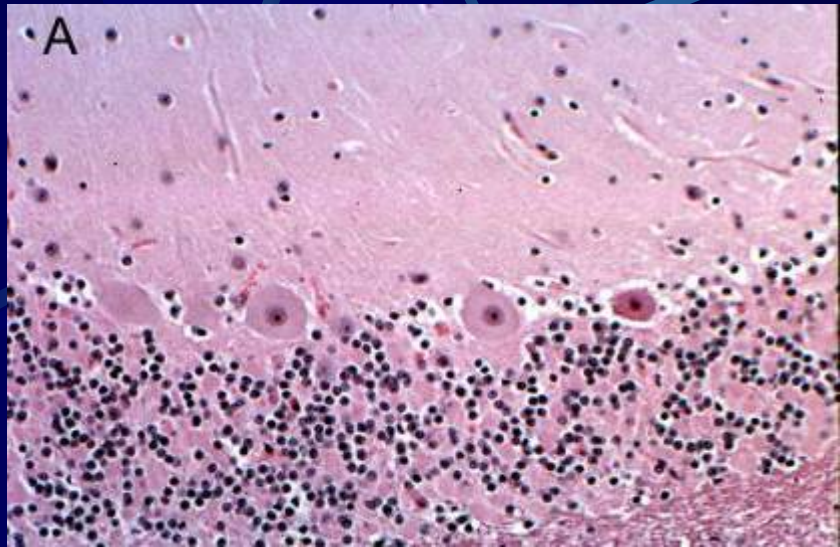
# Lesion Resolution



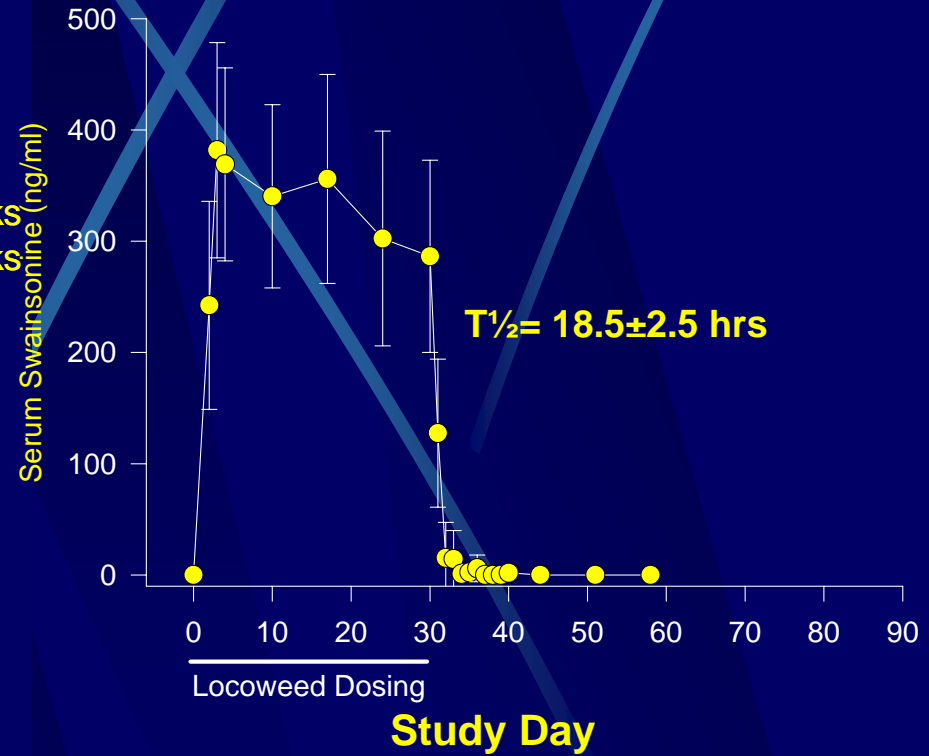
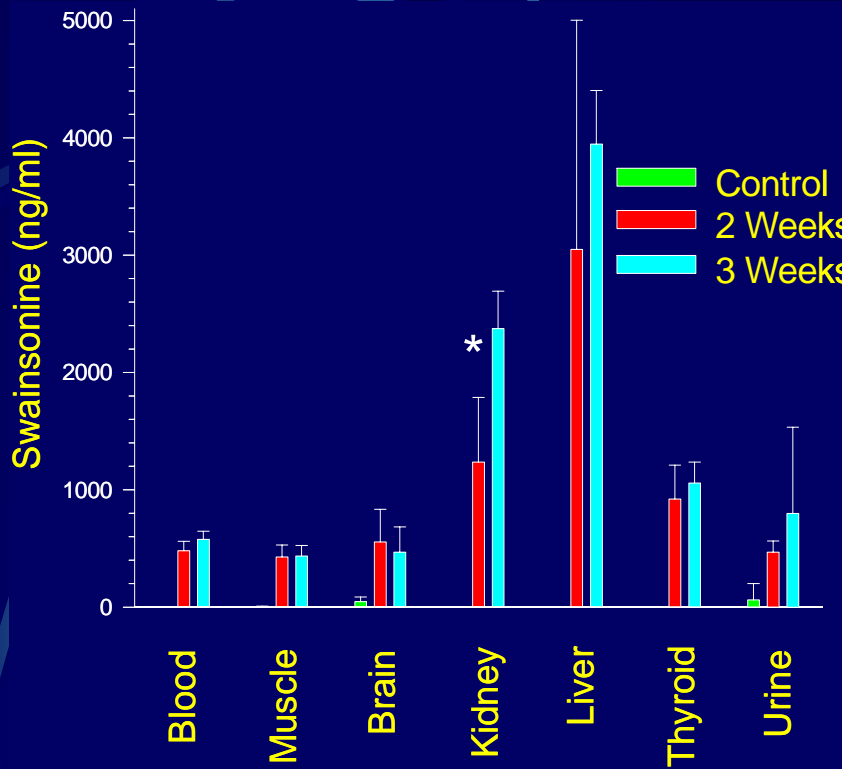
# Intermittent Intoxication

- 9 groups of 4 wethers dosed BID with *Oxytropis sericea* at 1.0 mg swainsonine kg<sup>-1</sup> body weight day<sup>-1</sup> for a total of 45 days interrupted by varying length recovery periods.
- 1 negative control group was dosed with alfalfa.
- Monitored daily, bled and weighed weekly for clinical and biochemical responses
- Necropsied 7 days following the final dosing period.

Photomicrograph of cerebellar Purkinje cells from locoweed-poisoned sheep with dosing durations of (A) 5 days, (B) 9 days, (C) 15 days and (D) 45 days. H&E. Bar=50 microns.



# Toxicokinetics



*Clearance:*

*Blood/Muscle/Brain/Thyroid/Pancreas/Intestine~20 hrs,  
Kidney/Liver~60 hrs*

# Species Susceptibility

- Horse (~0.1 mg/kg)
- Sheep (0.2 mg/kg)
- Cow (0.25 mg/kg)
- Rat (2.0 mg/kg)



## Prions in Skeletal Muscles of Deer with Chronic Wasting Disease

Rachel C. Angers,<sup>1\*</sup> Shawn R. Ewing,<sup>1,2\*</sup> Tanja S. Seward,<sup>3</sup> Christina J. Sigurdson,<sup>2</sup> Michael W. Miller,<sup>4</sup> Edward A. Hoover,<sup>5</sup> Glenn C. Telling<sup>1,2,3,6</sup>

Prions are transmissible proteinaceous agents of neurodegenerative diseases of the central nervous system (CNS). The presence of infectivity in skeletal muscle of experimentally infected mice raised the possibility that dietary exposure to prions might occur through meat consumption (1). Chronic wasting disease (CWD), an prionemic and contagious prion disease of North American cervids, is of particular concern. The emergence of CWD in an increasingly wide geographic area and the transspecies transmission of bovine spongiform encephalopathy (BSE) to humans as variant Creutzfeldt-Jakob disease (vCJD) have raised concerns about zoonotic transmission of CWD.

To test whether skeletal muscle of diseased cervids contained prion infectivity, Tg(CwPr)<sup>0</sup> mice (2) expressing cervid prion protein (CwPrP) were inoculated intracerebrally with extracts prepared from the semitendinosus/semimembranosus muscle group of CWD-affected male deer or from CWD-negative deer. The stability of CNS materials also allowed for direct comparisons of prion infectivity in skeletal muscle and brain. All skeletal muscle extracts from CWD-affected deer induced progressive neurological dysfunction in Tg(CwPr)<sup>0</sup> mice, with mean incubation times ranging between 269

and ~490 days, whereas the incubation times of prions from the CNS ranged from ~230 to 280 days (Table 1). For each inoculation group, the diagnosis of prion disease was confirmed by the presence of disease-associated, proteinase-resistant PrP (PrP<sup>Sc</sup>) in the brains of multiple infected Tg(CwPr)<sup>0</sup> mice (see (3) for examples). In contrast, skeletal muscle and brain material from CWD-negative deer failed to induce disease in Tg(CwPr)<sup>0</sup> mice (Table 1), and PrP<sup>Sc</sup> was not detected in the brains of asymptomatic mice as late as 523 days after inoculation (3).

Our results show that skeletal muscle as well as CNS tissue of deer with CWD contains infectious prions. Similar analyses of skeletal muscle from BSE-affected cattle did not reveal high levels of prion infectivity (4). It will be important to assess the cellular location of PrP<sup>Sc</sup> in muscle. Although PrP<sup>Sc</sup> has been detected in muscle of scrapie-infected sheep (5), previous studies failed to detect PrP<sup>Sc</sup> by immunohistochemical analysis of skeletal muscle from deer with natural or experimental CWD (6, 7). Because the time of disease onset is inversely proportional to prion dose (8), the longer incubation times of prions from skeletal muscle extracts compared with those from matched brain samples indicated that prion sizes were lower in muscle than in the CNS,

when infectivity titres are known to reach high levels. Although possible effects of CWD strains or strain mixtures on host incubation times cannot be excluded, the variable 269- to ~490-day incubation times suggested a range of prion titres in skeletal muscles of CWD-affected deer. Muscle prion titres at the high end of the range produced the fastest incubation times, which were ~30% longer than the incubation times of prions from the CNS of the same animal. Because all mice in each inoculation group developed disease, prion sizes in muscle samples producing the longest incubation times were higher than the end point of the Noakes, defined as the infectious dose at which half the inoculated mice develop disease. Although the risk of exposure to CWD infectivity after consumption of prions in muscle is mitigated by relatively inefficient prion transmission via the oral route (9), our results show that semitendinosus/semimembranosus muscle, which is likely to be consumed by humans, is a major source of prion infectivity. Therefore, consuming or handling meat from CWD-affected deer are activities at risk to prion exposure.

### References and Notes

1. P. J. Angers et al., *Proc Natl Acad Sci U S A* 96, 9222 (1999).
2. S. R. Ewing et al., *J Biol Chem* 274, 12345 (1999).
3. Materials and methods are available as supporting material on Science Online.
4. S. M. Barlow et al., *Neurology* 53, 1992 (1999).
5. S. M. Barlow et al., *Neurology* 53, 1992 (1999).
6. S. R. Ewing et al., *Am J Pathol* 162, 1242 (2003).
7. H. M. Borchers et al., *Neurology* 53, 1992 (1999).
8. T. S. Seaward et al., *Neurology* 53, 1992 (1999).
9. S. R. Ewing et al., *Neurology* 53, 1992 (1999).
10. This work was supported by grants from the U.S. Public Health Service (grant NS017602) and the National Institute of Neurological Disorders and Stroke (contract H01-01-1545) from the National Institute of Health and American Prion Research Center.

### Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5764/1117

Materials and Methods

Fig. S1

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Include this information when citing this paper:

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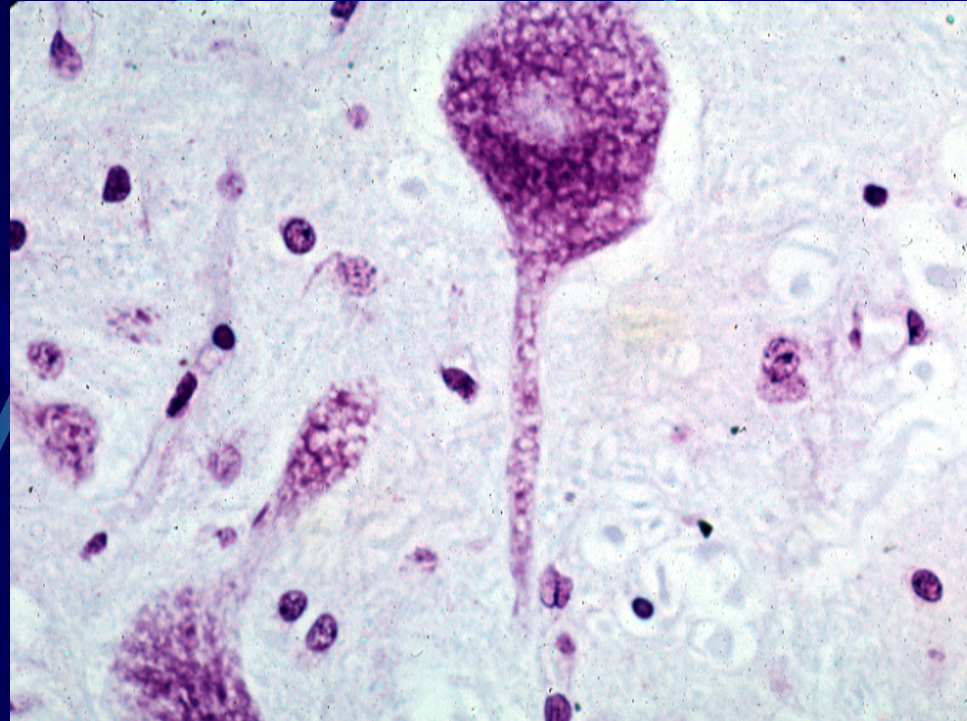
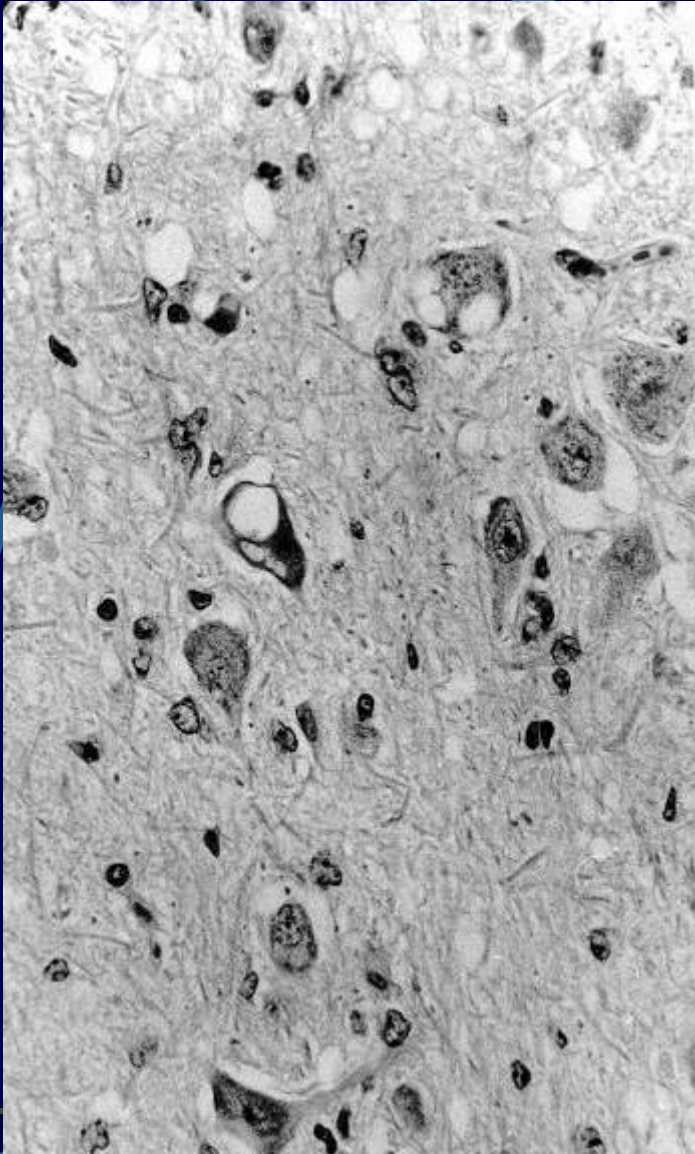


**Table 1.** Incubation times after inoculation of Tg(CwPr)<sup>0</sup> mice with prions from skeletal muscle and brain samples of CWD-affected deer. PrP, prion protein; buffer, buffer.

Inocula	Incubation time, mean days ± SEM (n) <sup>a</sup>	
	Skeletal muscle	Brain
	CWD-affected deer	
HR2	362 ± 2 (60)	283 ± 7 (60)
SR96	367 ± 8 (60)	278 ± 11 (60)
SR61	427 ± 18 (10)	
DS1	483 ± 8 (60)	231 ± 17 (10)
DS2	452 ± 4 (10)	
Averages	426	264
	Brain-affected deer	
PS 6-23	>523 (2)	
PS 1-11	>454 (2)	>454 (2)
PS 6	>490 (2)	
PS 5	>509 (2)	

<sup>a</sup>The number of mice developing prion disease (D) divided by the original number of inoculated mice (N) is shown in parentheses. Mean times of incubation (SD) were calculated.

# Histologic Lesions

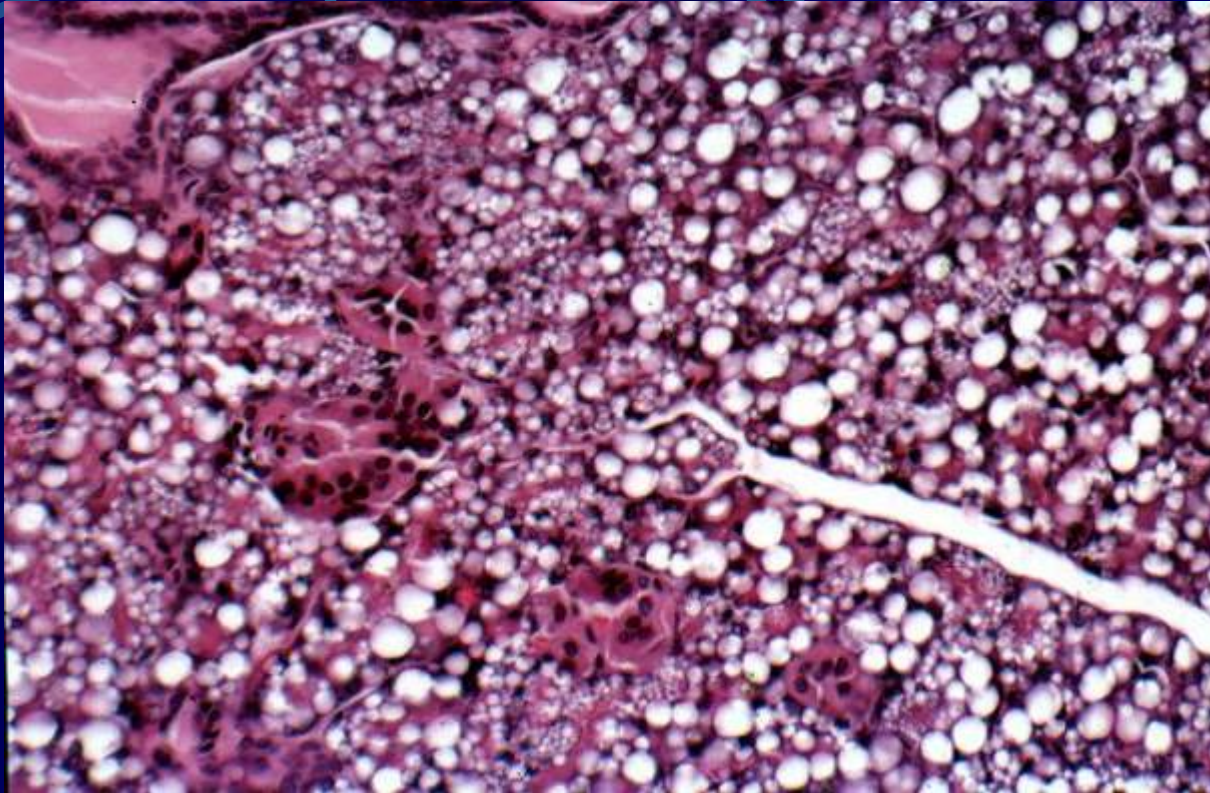


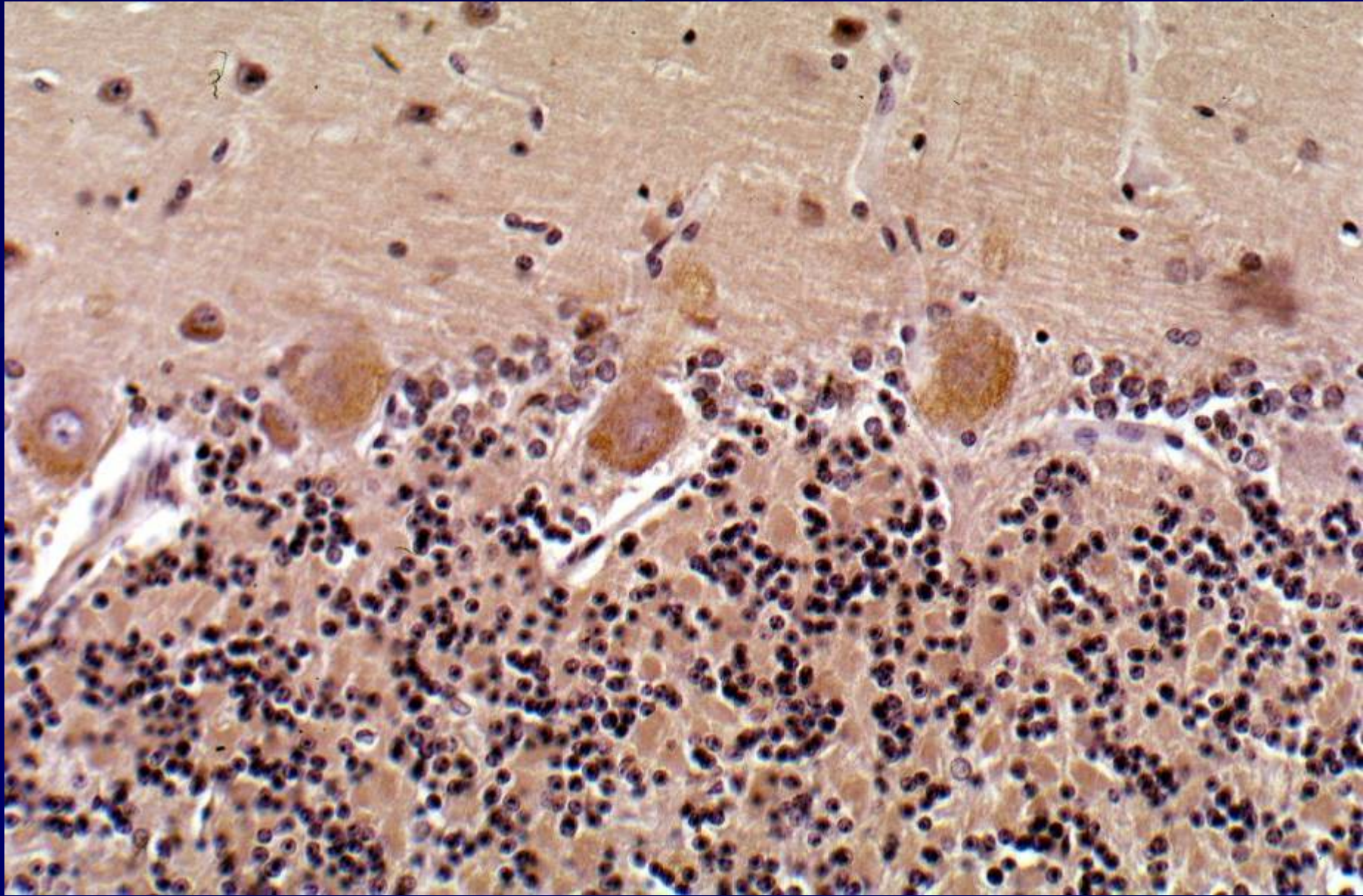


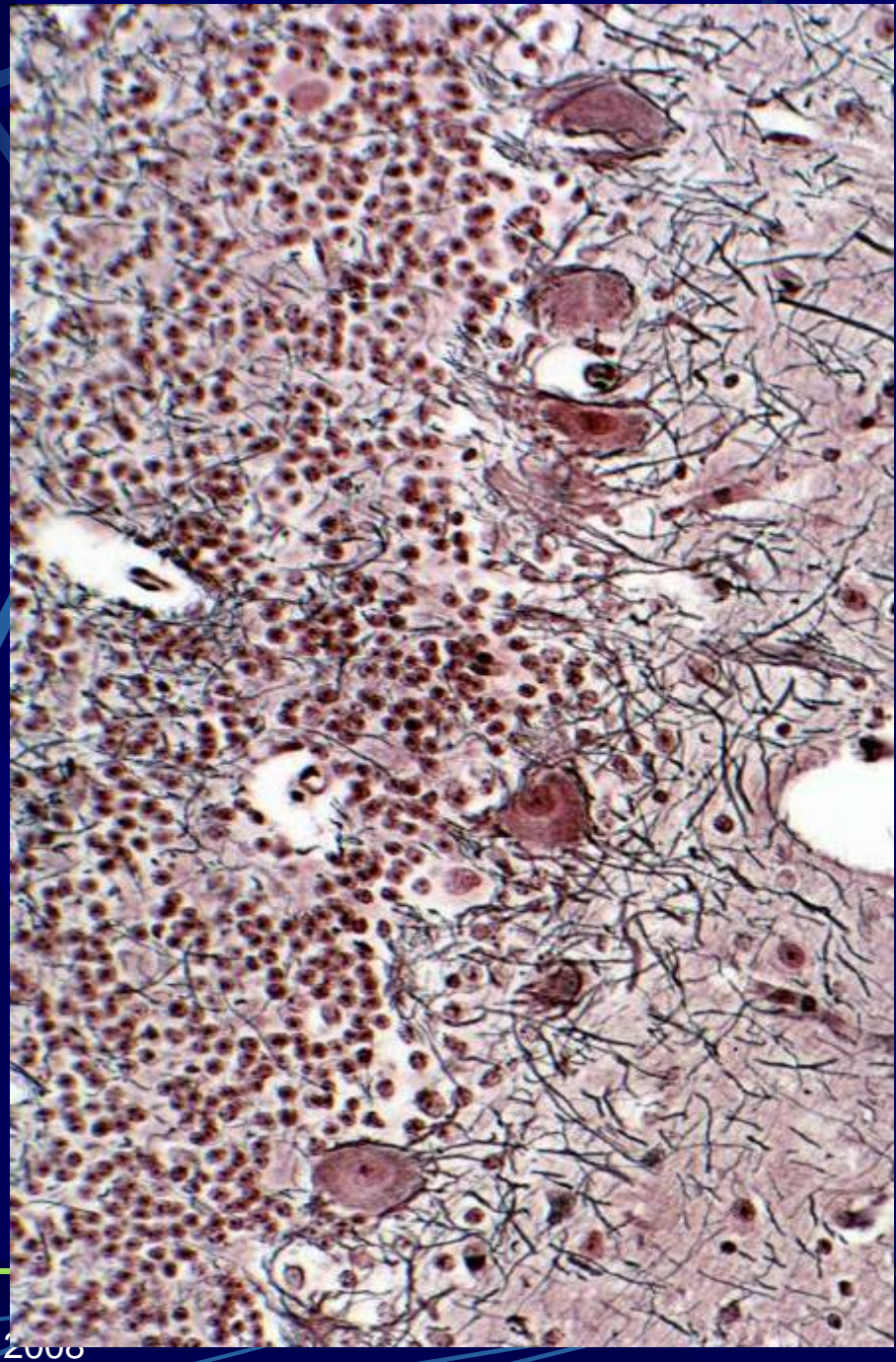
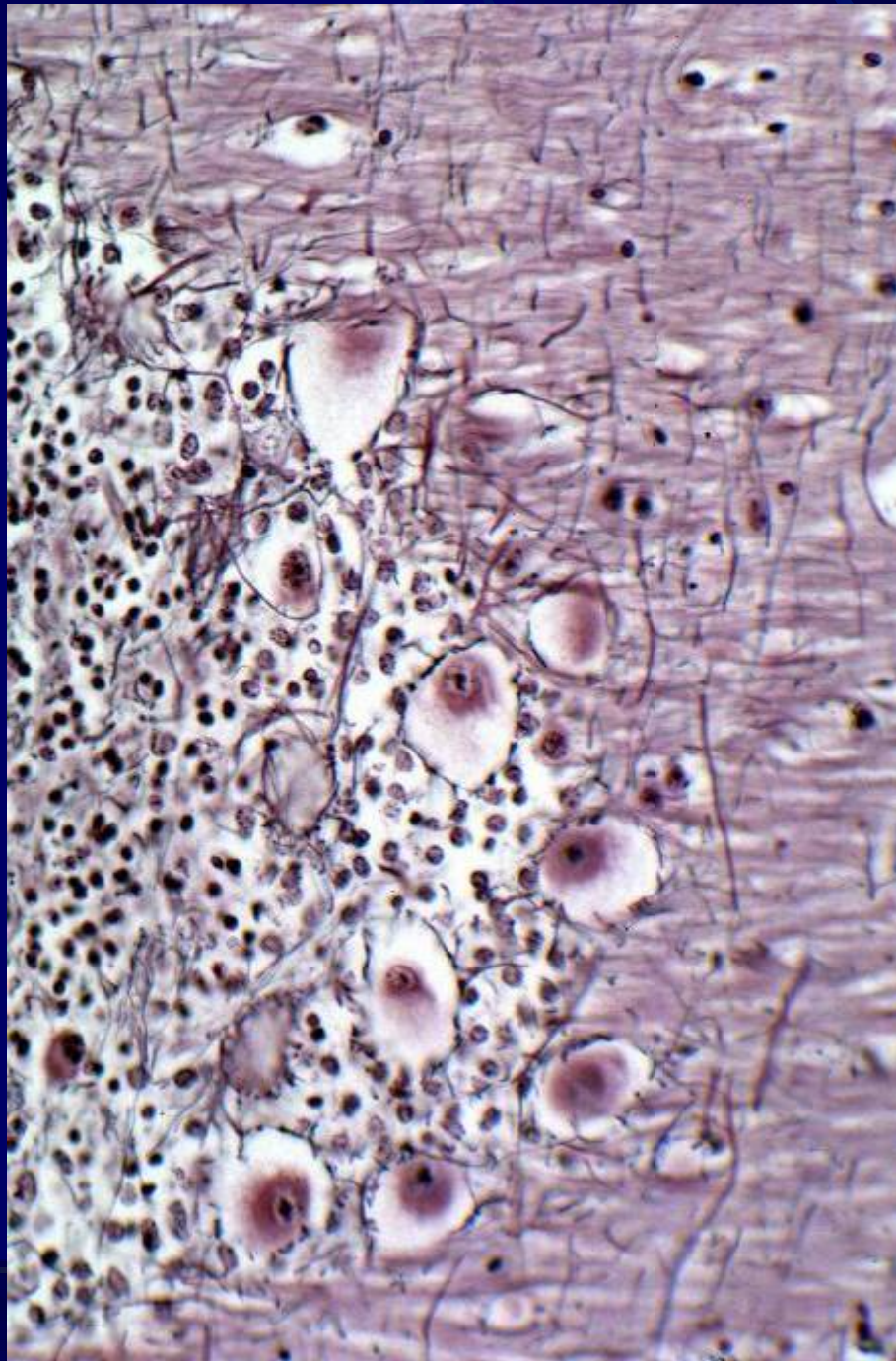
# Gross Signs and Lesions

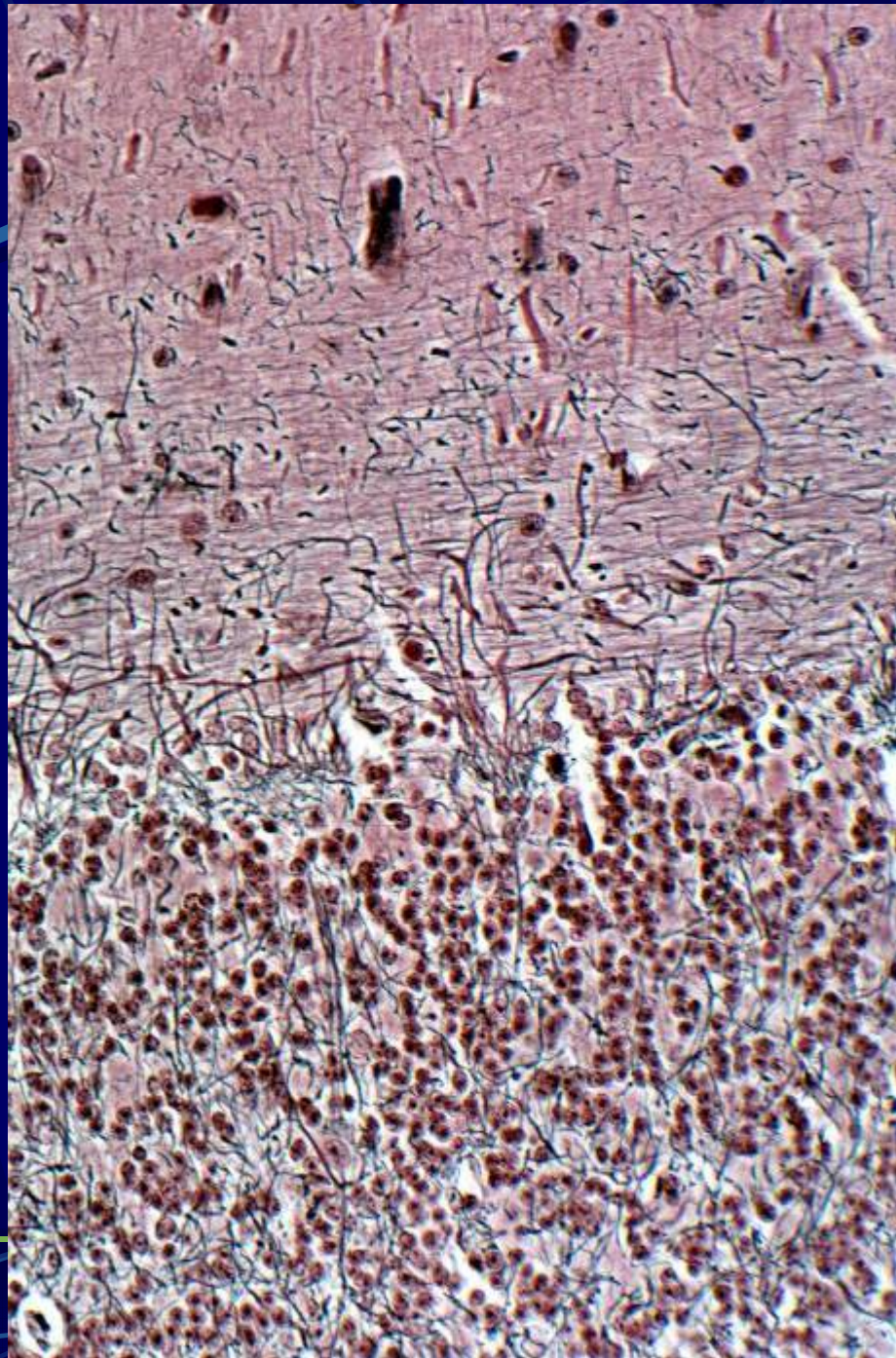


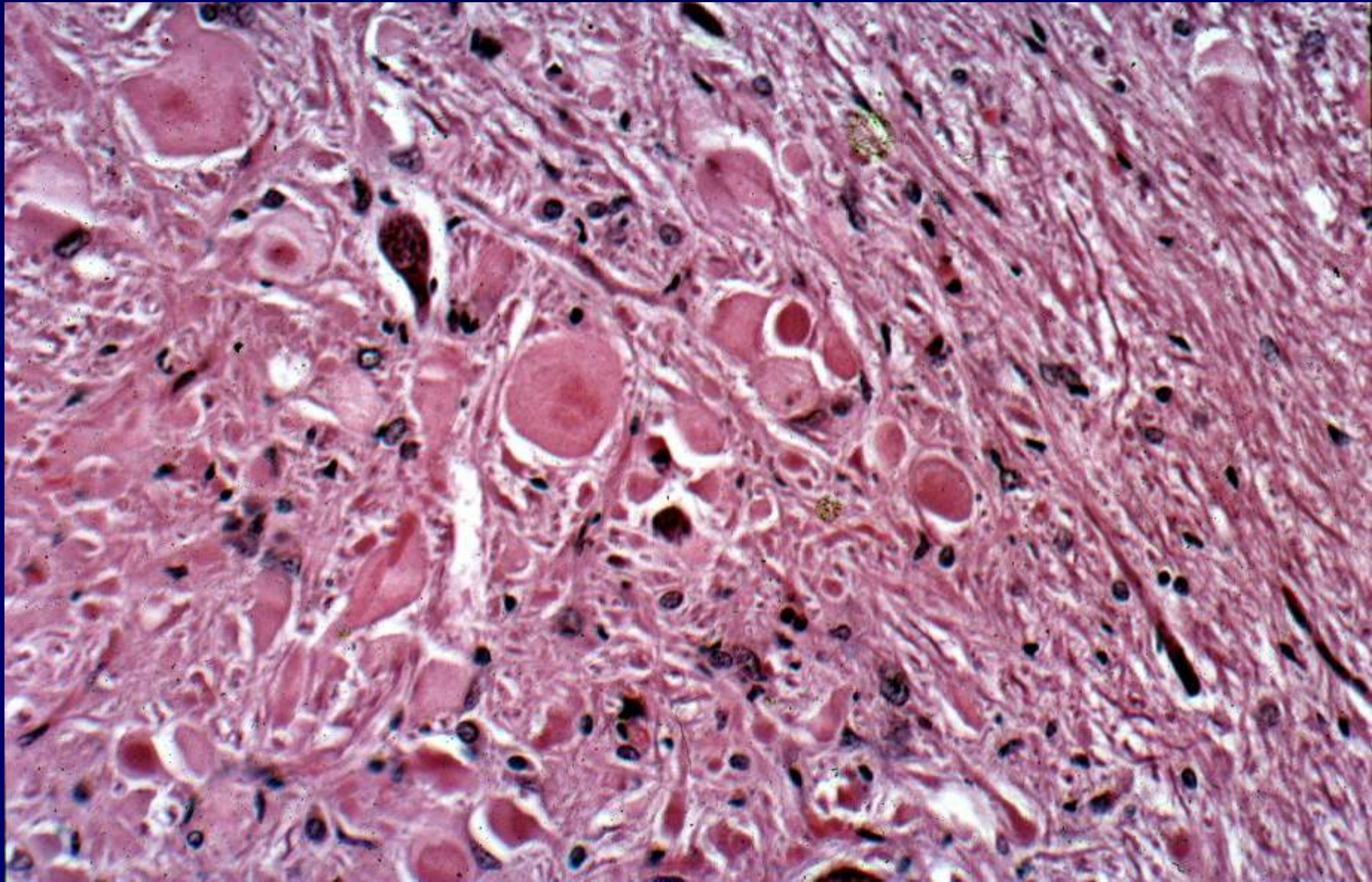
# Histologic Lesions



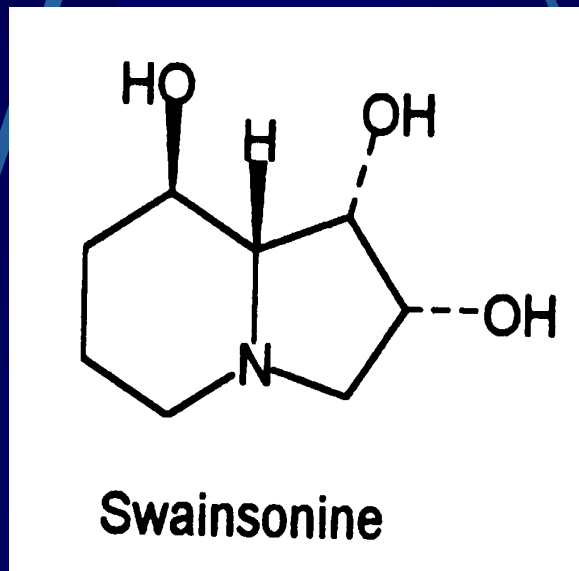






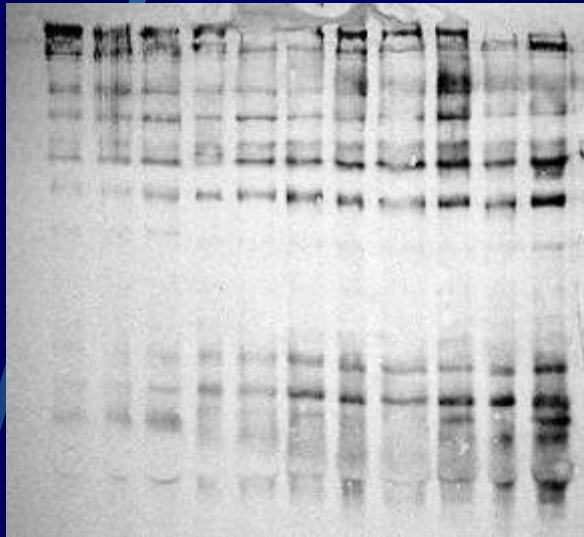


# Diagnostic Technique Development



- Mannosidase inhibition assay
- ELISA and Vaccine Development
- Serum protein glycosylation
  - Lectin colorimetric assay
  - Transferrin glycosylation

# Diagnostic Indicators of Toxicity



Indicator	Develop	Resolve
Clinical Signs	24 days	15 days
Swainsonine	1 day	3 days
AST	12 days	20 days
ALP	10 days	10 days
Lymphocytes	4 days	7 days
Transferrin	14 days	28 days
Lymph nodes	7 days	5 days
Kidney tubules	2 days	5 days
Purkinje cells	14 days	30 days



# Locoweed Summary

- Locoweed poisoning is a chronic disease that develops in livestock grazing certain *Astragalus* and *Oxytropis* spp. for several weeks.
- Diagnosis made by documenting exposure, identifying the neurologic signs and histologic lesions, and analyzing serum.
- Many lesions resolve after poisoning; however, some neurologic changes are irreversible.
- Prognosis is poor for animals used for draft, riding or competition.

