實驗動物毒性病理判讀與量化評估
(The qualitative and quantitative evaluations of toxicopathology in the laboratory animal studies)

廖俊旺
Jiunn-Wang Liao D.V.M., Ph. D.

中興大學獸醫學院獸醫病理生物學研究所
Graduate Institute of Veterinary Pathobiology
National Chung Hsing University

Introduction

- In toxicity and carcinogenicity studies, pathology reports are written in order to convey information concerning the pathologic findings in a study.

- Such reports are read by pathologists and other scientists, and information from these reports is incorporated into documents that are subsequently submitted to regulatory agencies.

SHACKELFORD et al., TOXICOLOGIC PATHOLOGY, vol 30, no 1, pp 93–96, 2002

- The identification of effects in animals that may be predictive of adverse events in humans is the cornerstone of non-clinical safety testing of pharmaceuticals for human therapeutic use.

M.A. Dorato, J.A. Engelhardt / Regulatory Toxicology and Pharmacology 42 (2005) 265–274

International Harmonization of Toxicologic Pathology Nomenclature: An Overview and Review of Basic Principles

PETER C. MANN[1], JOHN VAHLE[2], CHARLOTTE M. KEENAN[3], JULIA F. BAKER[4], ALYS E. BRADLEY[5], DAWN G. GOODMAN[6], TAKANORI HARADA[7], RONALD HERBERT[8], WOLFGANG KAUFMANN[9], RUPERT KELLNER[10], THOMAS NOLTE[11], SUSANNE RITTINGHAUSEN[10], AND TAKUJI TANAKA[12]

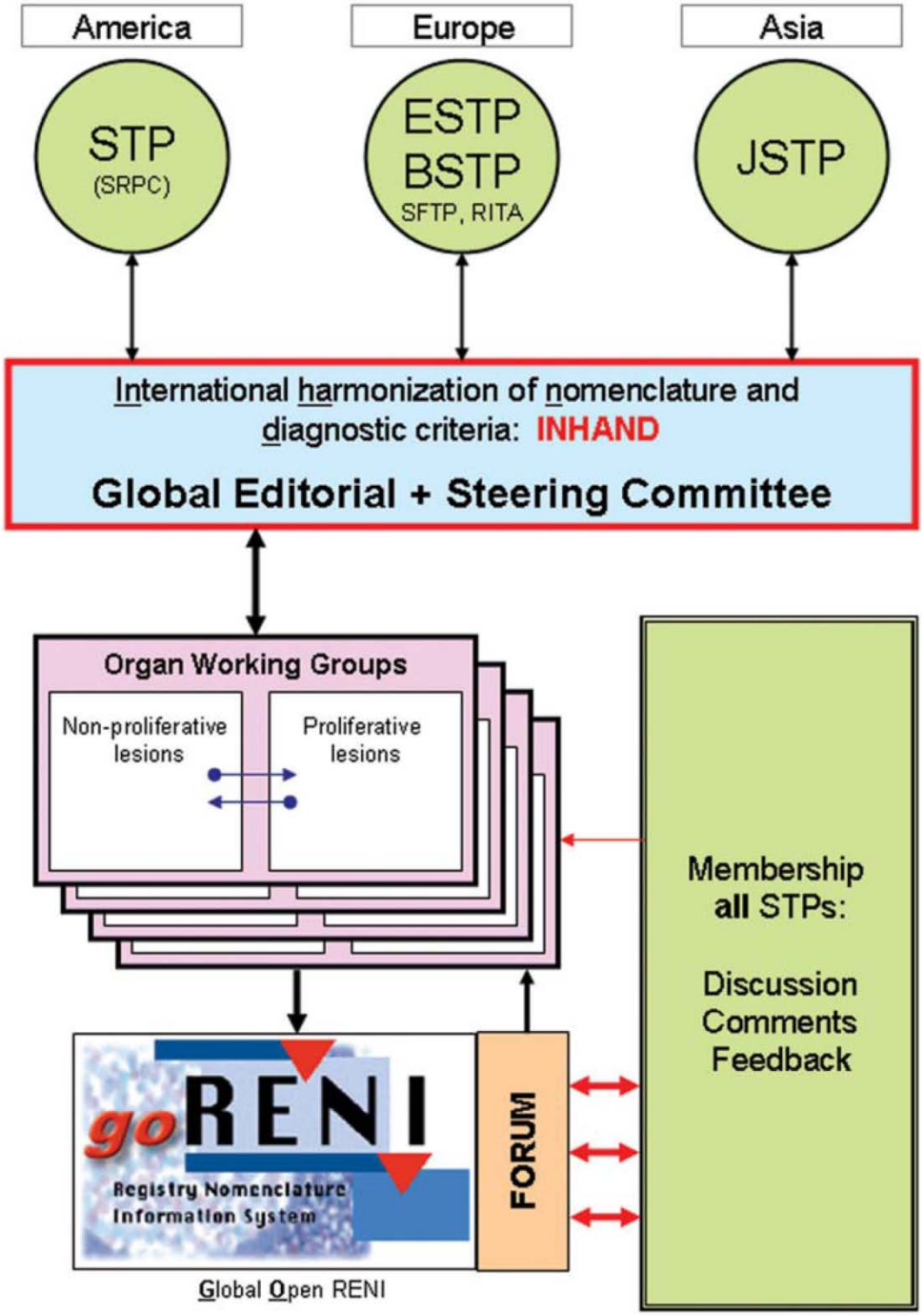
[1] *EPL Northwest, Seattle, Washington, USA*
[2] *Eli Lilly & Company, Indianapolis, Indiana, USA*
[3] *C. M. Keenan ToxPath Consulting, Doylestown, Pennsylvania, USA*
[4] *Charles River Pathology Associates, Frederick, Maryland, USA*
[5] *Charles River Laboratories, Tranent, Scotland, UK*
[6] *Private Consultant, Potomac, Maryland, USA*
[7] *The Institute of Environmental Toxicology, Ibaraki, Japan*
[8] *NIEHS, Research Triangle Park, NC, USA*
[9] *Merck Serono R&D, Darmstadt, Germany*
[10] *Fraunhofer ITEM, Hannover, Germany*
[11] *Boehringer Ingelheim Pharma, Biberach an der Riss, Germany*
[12] *Kanazawa Medical University, Ishikawa, Japan*

Toxicologic Pathology, 40: 7S-13S, 2012

INTERNATIONAL HARMONIZATION OF NOMENCLATURE AND DIAGNOSTIC CRITERIA FOR LESIONS IN RATS AND MICE NOMENCLATURE PROJECT

- The goal of the project is to produce publications for each organ system that provide a standardized nomenclature and differential diagnosis for classifying microscopic lesions observed in laboratory rats and mice in toxicity and carcinogenicity studies

- The project is referred to as the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice (INHAND)



Organ Working Groups:

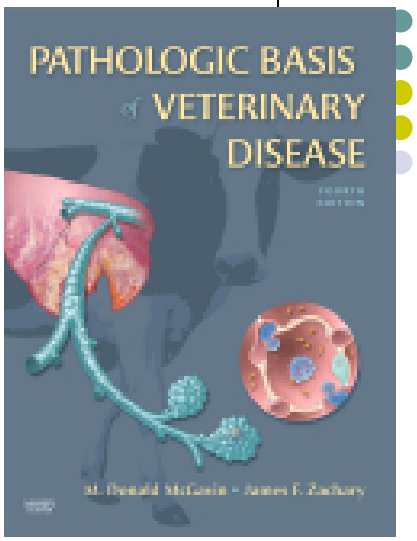
1. Toxicologic Pathology (STP)
2. British Society of Toxicologic Pathologists
3. Experimental and Toxicologic Pathology,
4. Journal of Toxicologic Pathology.

http://www.goreni.org/

- FIGURE 1.—Organization of INHAND


國立中興大學(Natio
「科技部全球資訊網
goRENI
British Society of To
www.goreni.org
應用程式
Home - PubM...
國立中興大學-...
網站管理系統 - ...
台灣高鐵網路訂...
教師網站管理系...
The Joint Path...
行政院國家科學...
其他書籤
goRENI
Registry Nomenclature Information System
global open RENI
The standard reference for nomenclature and diagnostic criteria in toxicologic pathology.
Login
Home
Nomenclature
Sponsors
Links + Infos
Contact us
Membership
Help
07-Mar-2016
Site Navigation
Login
Home
Nomenclature
Sponsors
Links + Infos
Contact us
Membership
Help
Welcome!
You have reached the homepage of goRENI.
goRENI is the standard reference for nomenclature and diagnostic criteria in toxicologic pathology and at the same time the Internet discussion platform for the global initiative "INHAND" - the International Harmonization of Nomenclature and Diagnostic criteria.
If you are interested to learn more about goRENI and INHAND, see the documents in the "Nomenclature" area. References to the Published INHAND Guides are also available there.
If you are a goRENI member, go to the Login page first and identify yourself before you proceed.
If you would like to become a member of goRENI, see this page for more details. You will be contacted by e-mail after submission of your data. If this is not the case, please contact us again. Perhaps, there was a typing error in your e-mail address.
Please do not forget to visit the home pages of our Sponsors: without their generous financial support, goRENI would not exists.
Please visit our Sponsors
Fraunhofer
ITEM
estp
European Society of Toxicologic Pathology
www.eurotoxpath.org
Society of Toxicologic Pathology
日本毒性病理学会
The Japanese Society of Toxicologic Pathology
JSTP
BSTP
The British Society of Toxicological Pathologists
Please visit our Sponsors
European Society of Toxicologic Pathology
estp
Fraunhofer
ITEM
global open RENI, Version 2.10.23 www.goRENI.org
Imprint
Contact us
Copyright © Fraunhofer ITEM 1991 – 2016
LOGOUT
下午 01:33
2016/3/7

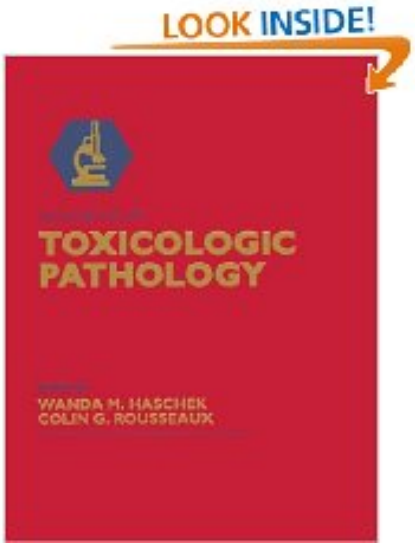
Introduction



1. In animal diagnostics, the pathologist takes in investigating the causes of diseases of individual patients and groups of animals and in determining their zoonotic or epidemic potential

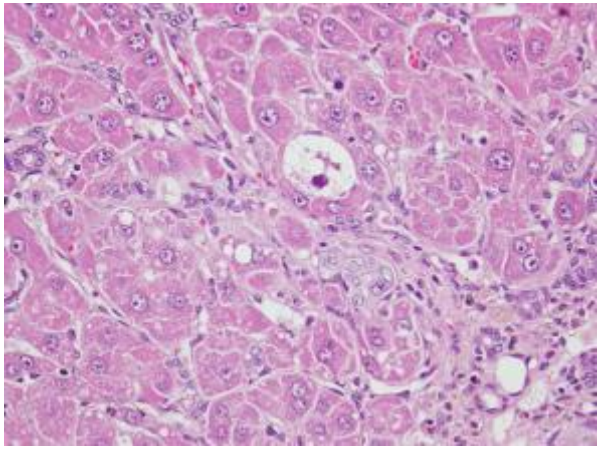
(McGavin, M.D., and Zachary, J.F. 2007. In: *Pathology Basis of Veterinary Disease*, 4th edition, Mosby and Elsevier, PA, USA.)

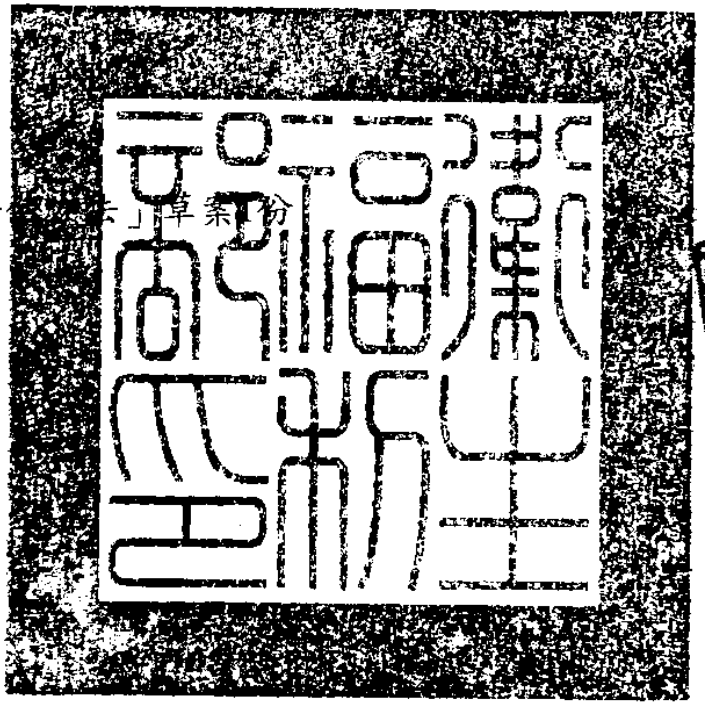
2. In the chemical and pharmaceutical industries, veterinary pathologists help ensure the safety of medicines, chemicals, and materials used in our daily lives



http://www.fda.gov.tw/upload/122/20130625141844306 31.jpg

發文日期：中華民國103年3月4日
發文字號：部授食字第1031300396號
附件：「健康食品之護肝功能(針對化學性肝損傷)評[illegible]」草案1份





（五）**組織病理切片觀察**：

3. 病理切片之判讀

本評估方法將肝組織進行H&E 染色，以方便觀察肝細胞的受損、脂肪堆積、壞死等慢性肝損傷之變化;

....**至於病理的半定量分析評估，則應由獸醫病理醫師 (病理專科獸醫師)**，在不清楚本實驗設計的情況下進行單盲封讀，對所有切片進行評分比較（評分表如表二），最後再以統計分析方法進行各組差異性的分析

Animals Used in the Toxicological Studies

Nude mice

Guinea pig

Hamster

Laboratory animals:

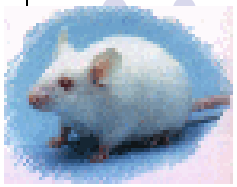
• mouse, rat, hamster, guinea pig, gerbil, rabbit, dog, cat...

Domestic animals:

• fish, chicken, quail, sheep, swine, cattle, horse...

Primate animals:

• monkey..



ICR mouse

SD rat

Wistar rat

Toxic Responses and Pathologic Evaluation

- **Introduction**

- **The Response of the Body to Injury**
  - ♣ Normal cell
  - ♣ Cell Injury
  - ♣ Healing and repair
  - ♣ Hyperplasia and neoplasm

- **Quality indicators of recoding observations**

Introduction

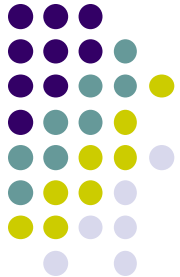
Toxicological pathology:

1. The study of structural and functional changes in cells, tissues, and organs that are induced by toxicants

2. Most toxicological pathologists are **veterinarians**, a smaller number are physicians or biologists

3. The ultimate goal is **ensure the safety and efficacy** of nutrients, drugs, chemicals , and to ensure the health of our environment

The Response of the Body to Injury



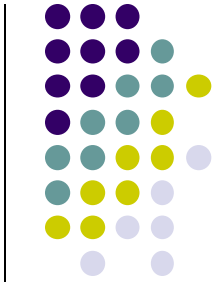
- **The normal cell**

1. **Cell has an outer membrane**, the **plasma membrane** as a **barrier**
2. **Cytoplasm** contains:

    1) Mitochondria ⇒ energy of ATP production and storage
    2) Golgi complex ⇒ for processing, concentration, and packaging for secretion from the cell
    3) Smooth endoplasmic reticulum ⇒ biosynthesis and metabolism of steroid hormones
    4) Lysosome ⇒ digest material, phagocytosis
    5) Ribosome ⇒ RNA location
    6) Cytoskeleton ⇒ connect the cell membrane to various organelles and enable to move and divide

3. **Nucleus** ⇒ genetic information, e.g., DNA, RNA

• Reaction of cell to chemicals



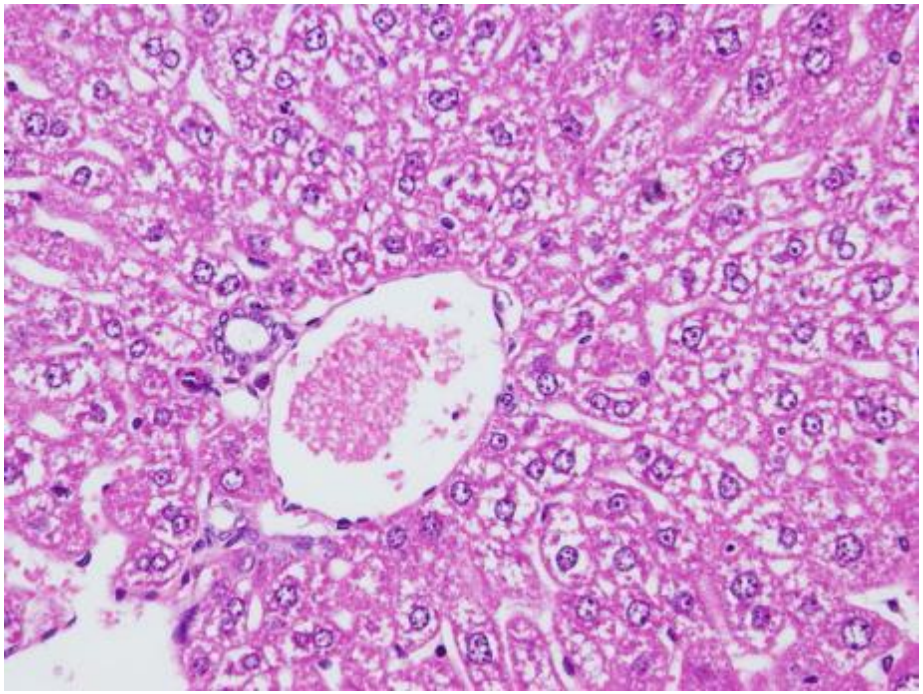
• **Chemical as stressors**

1. **Stress** can be defined as any disturbs the normal homeostasis of the cell
   ⇒ may involve alteration in cell structure and in function

2. **Cellular adaptation:**
   ⇒ adaptation to acetaminophen or phenobarbital by increasing cytochrome P-450 in liver
   ⇒ respiratory epithelium response to air pollutants can result in squamous metaplasia

3. **Cell injury:**
   ⇒ The more severe stress, cell injury may occur

2. **Cellular adaptation**



Adaptation of hepatocytes in the portal area after treated with acetaminophen

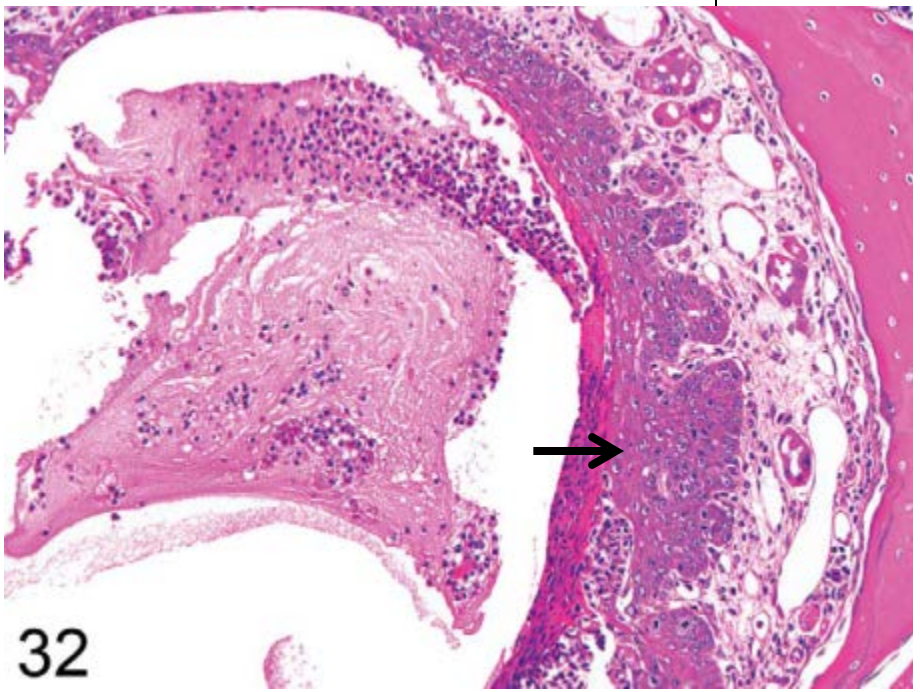


FIGURE 32.—Mouse. Nasal cavity, squamous cell metaplasia

Toxicologic Pathology, 37: 5S-73S, 2009

• Cell injury

1.Acute reversible cell injury:

Reversible cell injury includes:

1) **cell swelling** (hydropic swelling or degeneration)

2) **fatty change (vacuolization)**

3) **cell surface blebbing**

.

2. Irreversible or lethal cell injury

1) Necrosis

2) Apoptosis

•Cell swelling (細胞腫脹)

Etiology:

1) cell membrane injury and increase permeability
2) incapable maintain ionic and fluid homeostasis
   ⇒ hypoxia

Morphology changes:

1) an increase in cytoplasmic water content
2) has a large, pale cytoplasma with normal nucleus

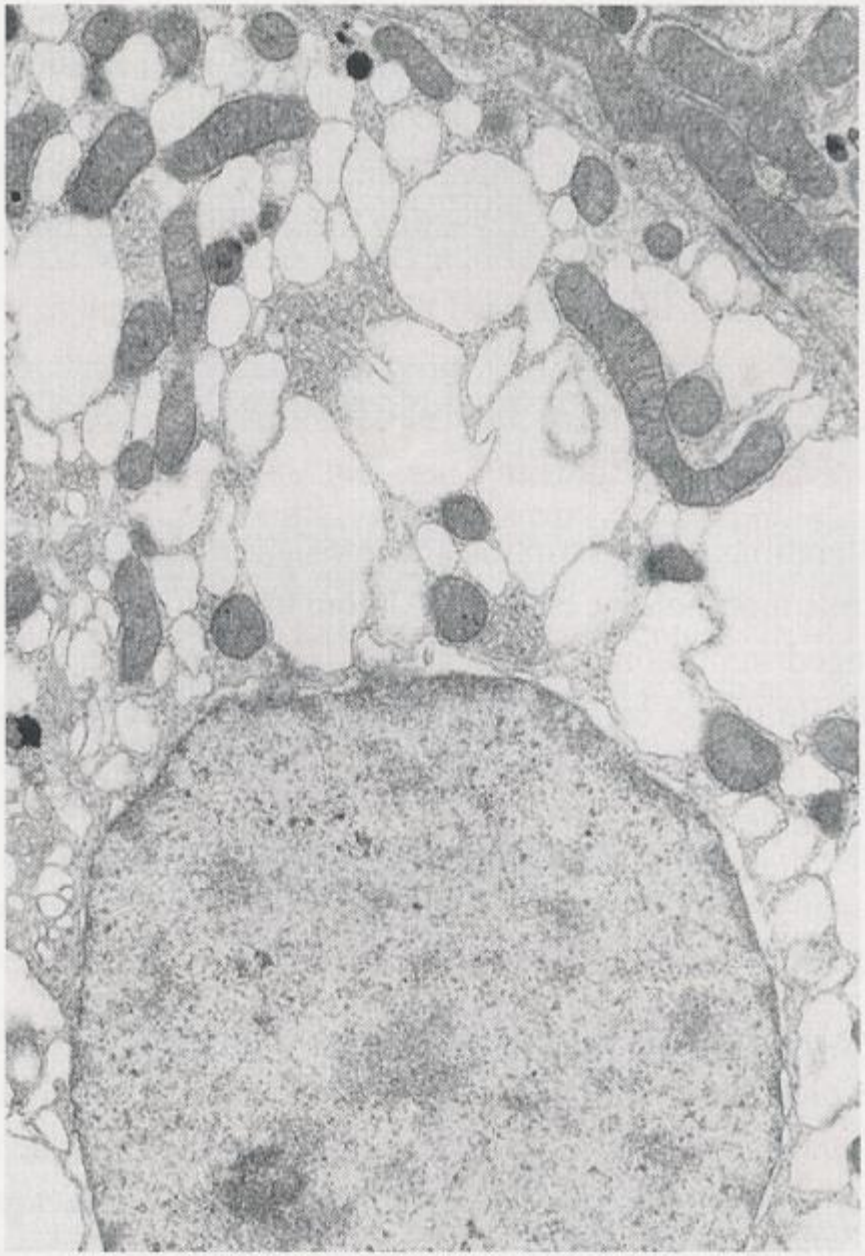
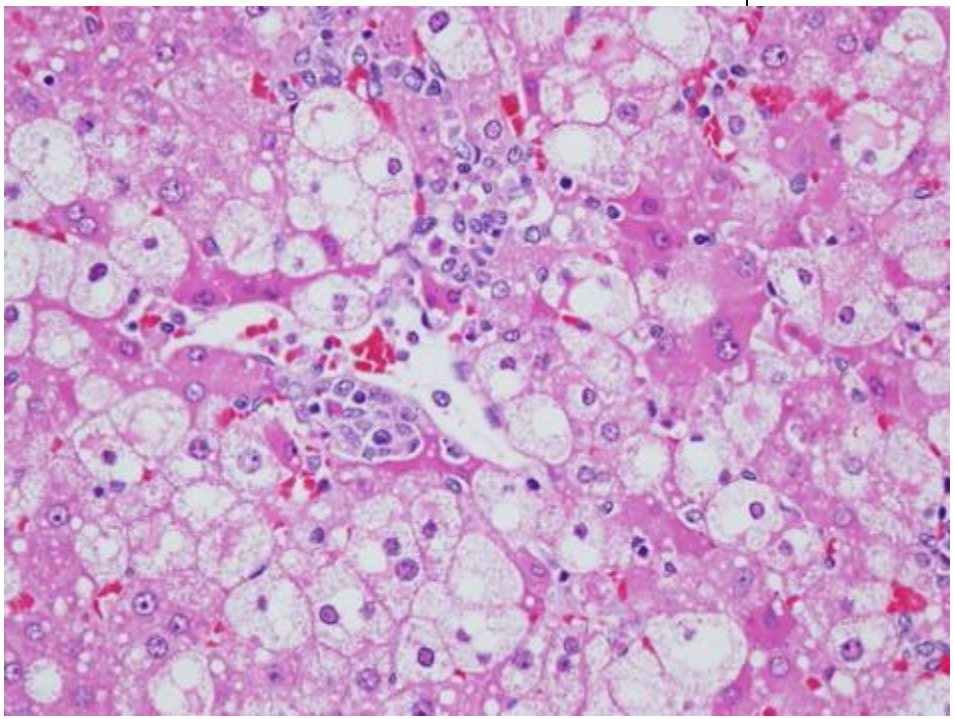


Figure 9. Hydropic degeneration characterized by dilated endoplasmic reticulum; rat.

• Fatty change (脂肪變性)

Etiology:

1) the abnormal accumulation of fat in a cell
2) most frequently found in hepatocytes or myocytes
   ⇒hypoxia and toxics (CCl_4) cause an imbalance in fat metabolism



Morphology changes:

1) various vacuoles characterized in the cytoplasma, with normal nucleus

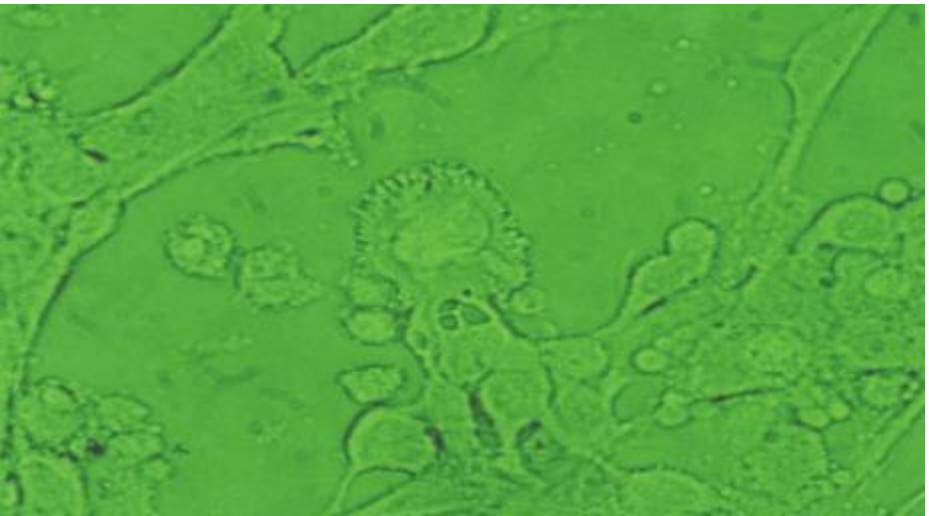
- **Cell surface blebs**

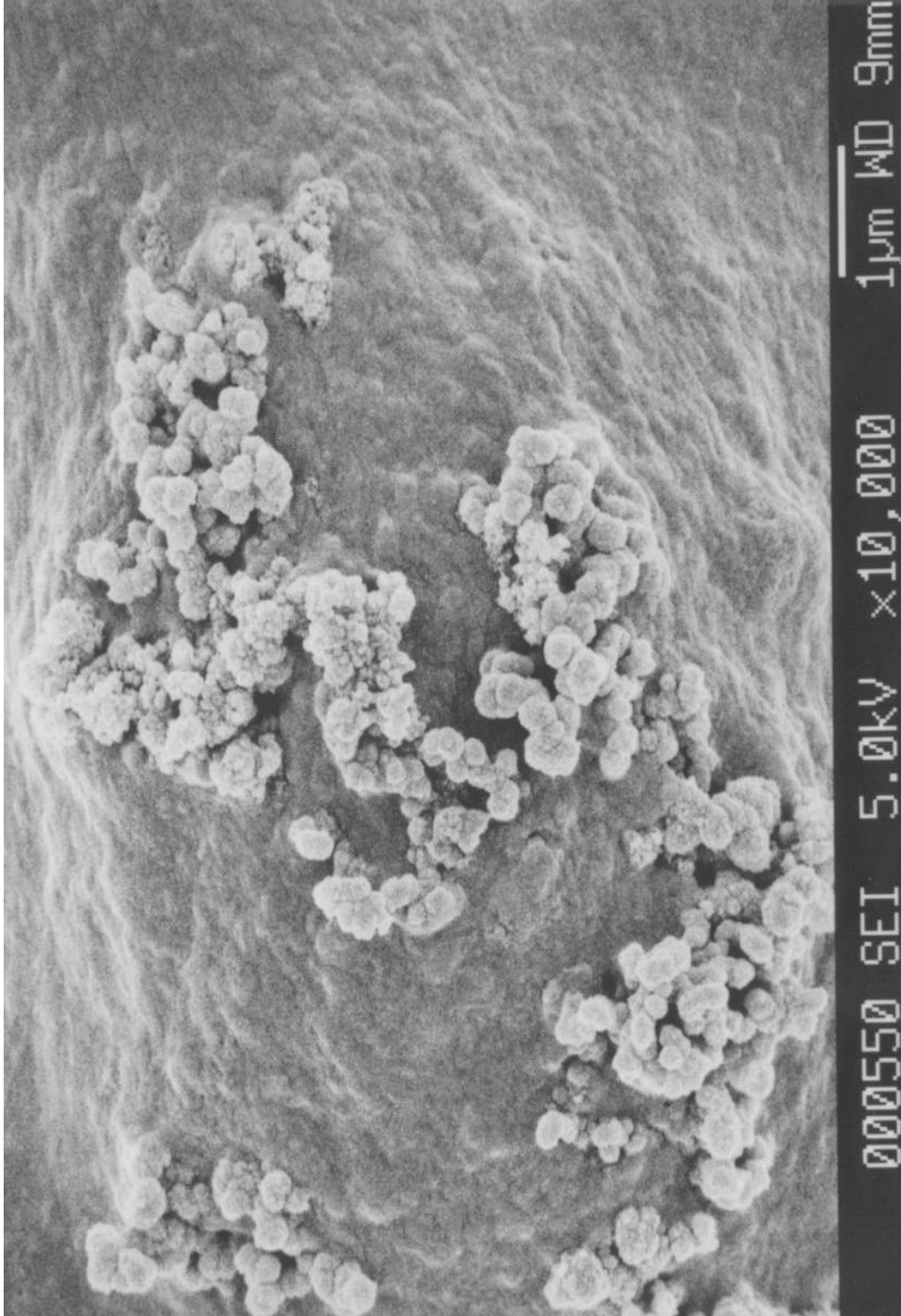
Etiology:

1) reverse cell injury
   ⇒ increase ammonia

Morphology changes:

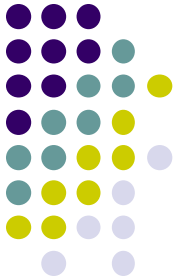
1) cell surface blebs formation
2) invagination of the plasma membrane





2. Irreversible or lethal cell injury

1) Necrosis
2) Apoptosis



- **Necrosis (壞死):**

Etiology:

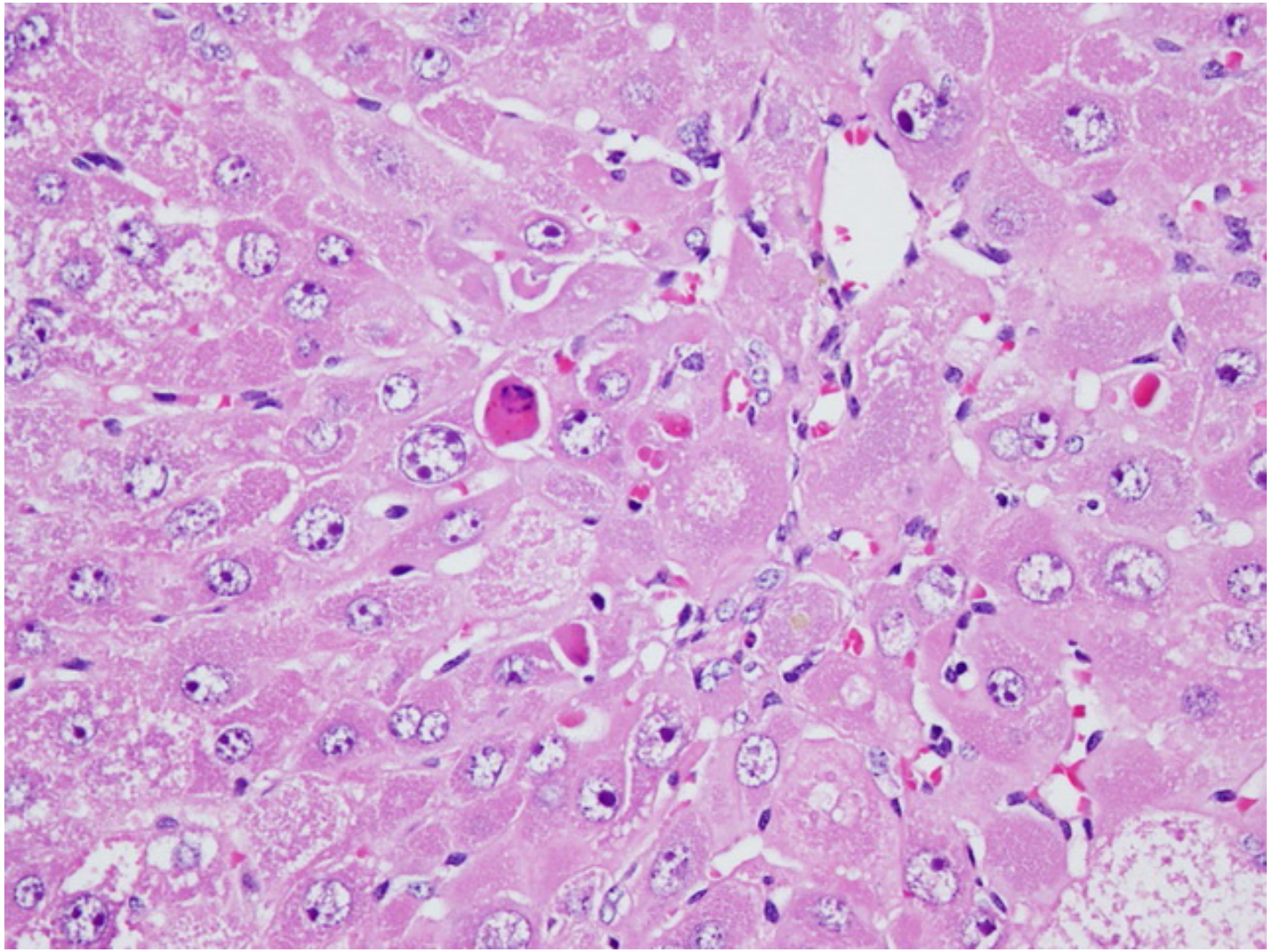
1. As a cell undergoes necrosis cause by various xenobiotics
2. Necrosis is followed by removal of necrotic cells by the inflammation, e.g., neutrophilic infiltration

Morphology changes:

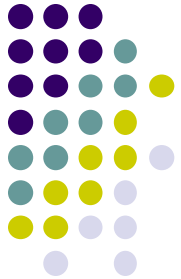
- Eosinophilic cytoplasma
  ⇒ due to eosin binding to denatured protein

- Basophilic granules
  ⇒ deposition of calcium phosphate in mitochondria

early necrosis vs
single cell necrosis vs
apoptosis



Three stages of nucleus change during necrosis



- **Pyknosis:**

⇒ as a shrunken nucleus with increase basophilic

- **Karyorrhexis (Karyolysis):**

⇒ as a fragmentation or lysis of nucleus

⇒ dissociation and disappearance of stainable chromatin

- **Loss of the nucleus**

⇒ lack a nucleus

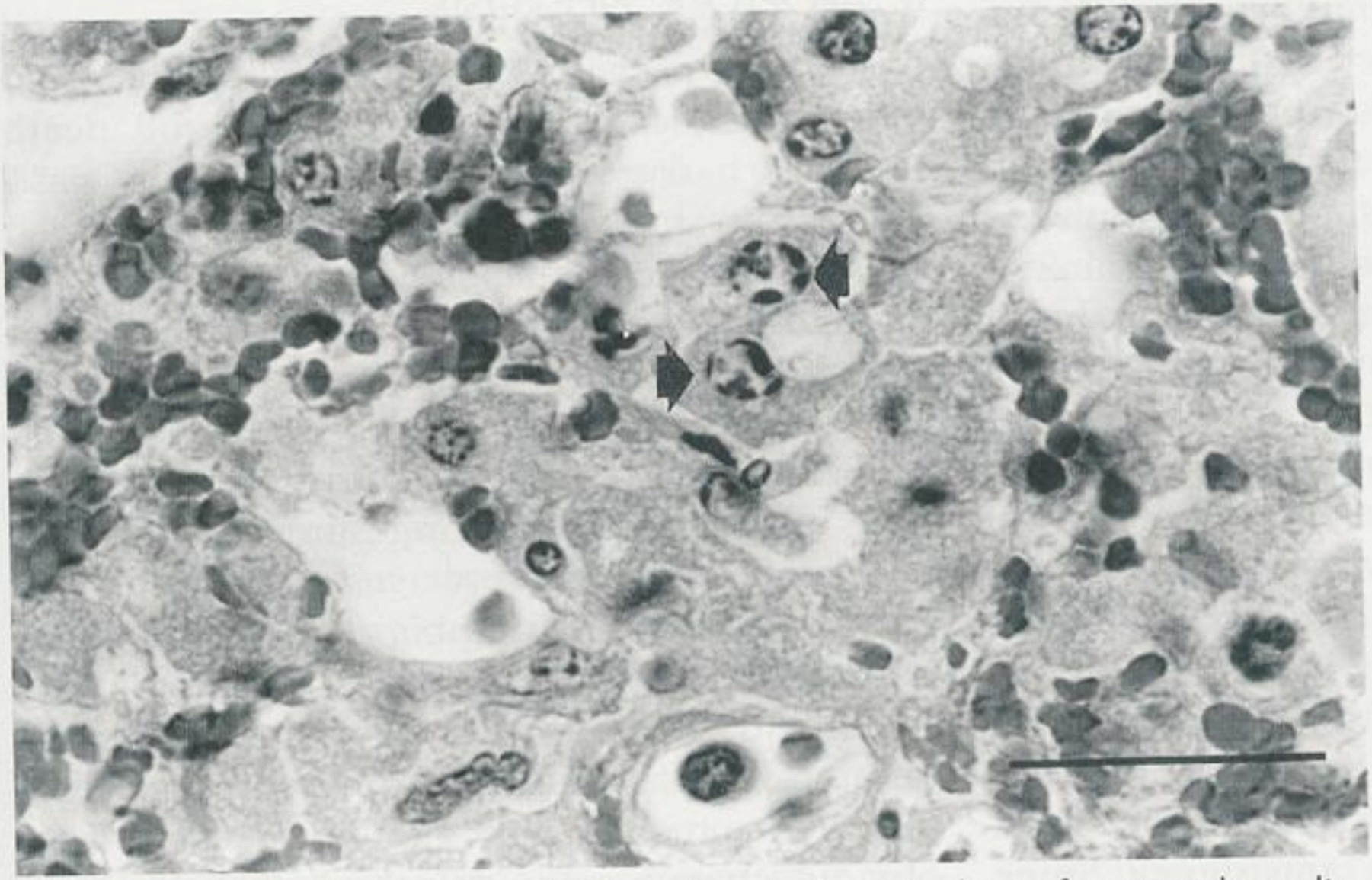


Figure 15. Karyorhexis (arrows) in hepatocytes from the liver of a cat with cardiomyopathy with congestive heart failure and subsequent hypoxia. Hematoxylin and eosin. Bar: 25 μm.

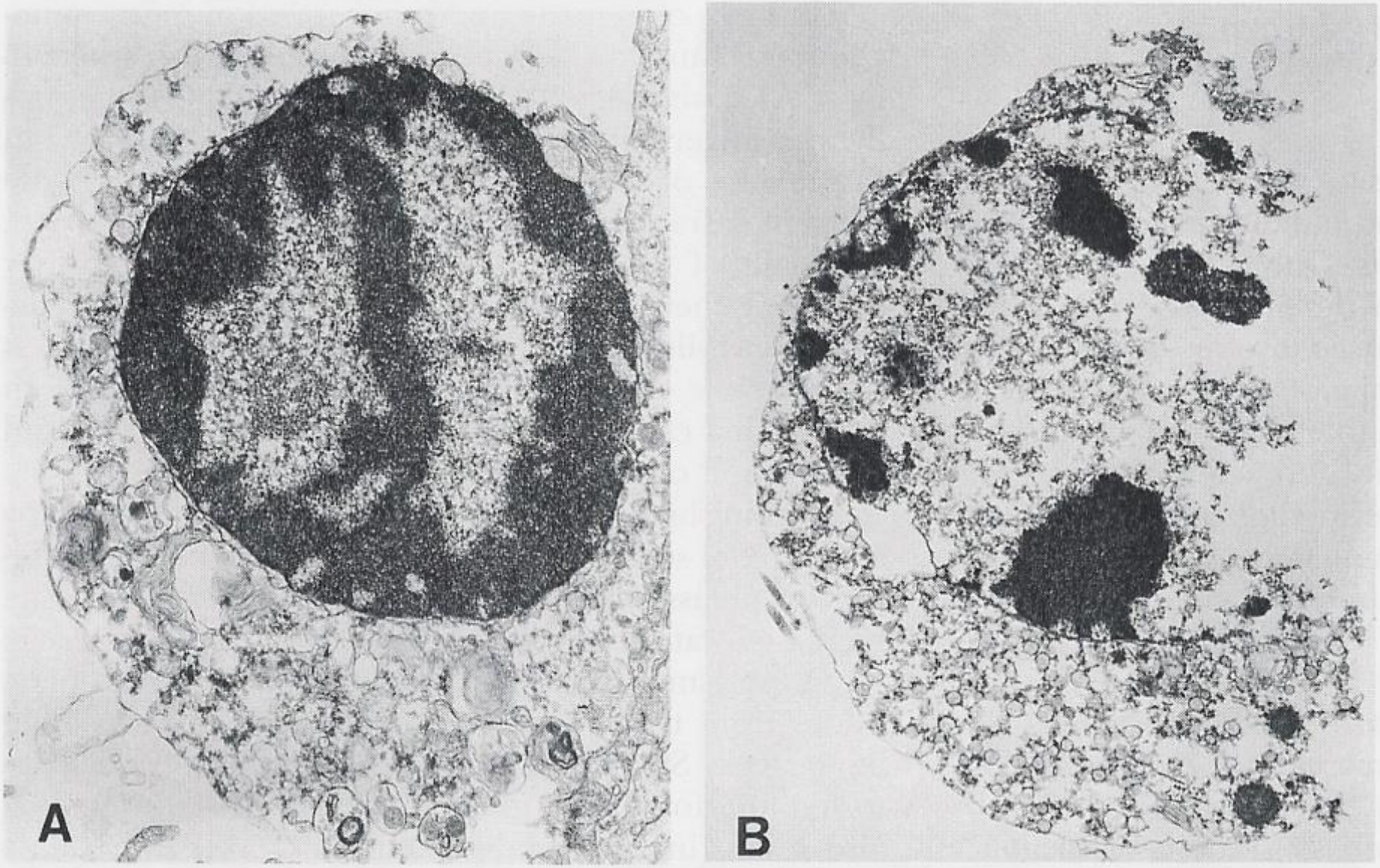


FIG. 1-8 Ultrastructural changes of necrosis. **A.** Pyknosis. Nucleus is shrunken, chromatin is more electron-dense than normal, and cytoplasm shows hydropic change and myelin figures (× 12,000). **B.** Karyorrhexis. Nuclear and cell membranes are disrupted. Note fragmentation of the chromatin into irregular electron-dense masses (× 12,000).

Apoptosis

Etiology:

1. Apoptosis or program cell death is a process in which cells die in a controlled manner or in response to specific stimuli
2. A individual or single cell necrosis
3. As a normal process in cell turnover
4. Toxicant-induced apoptosis may occur within minuets

Morphology changes:

1. Cell shrinkage and rounding, cytosolic blebbing
2. Chromatin condensation in nucleus as a apoptotic bodies
3. Phagocytosis by macrophges
4. Without inflammation and influx of inflammatory cells

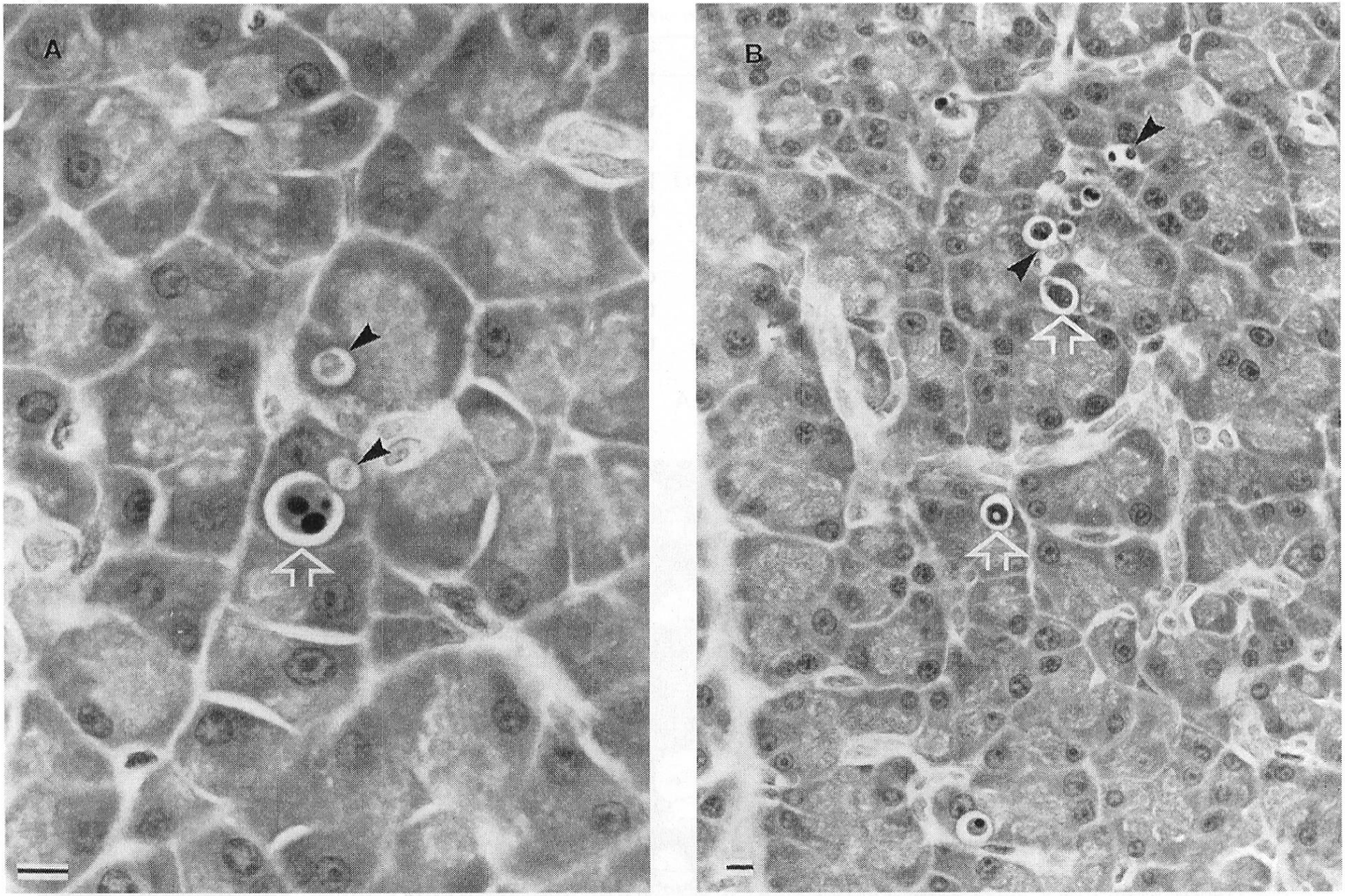
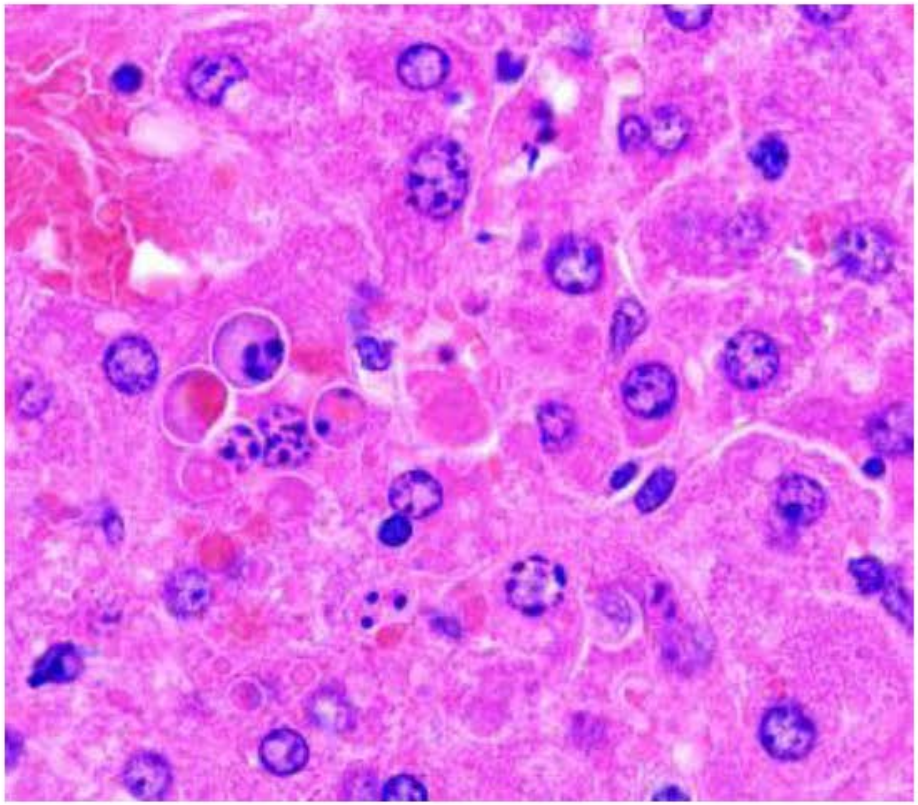


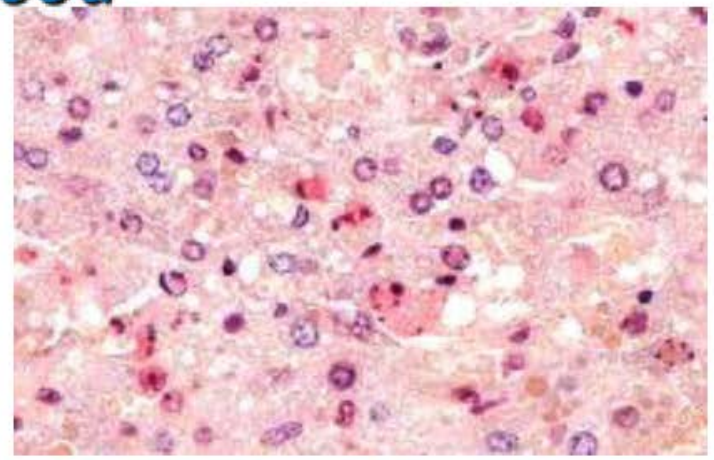
Figure 5. Pancreas of a rat treated 24 hr earlier with a single dose of camostate (FOY-305). Dense cytoplasmic inclusions (arrows) in acinar cells apparently represent autophagic vacuoles although heterophagy cannot be excluded. Apoptosis is also evident (open arrows). It is not always possible to distinguish large autophagic vacuoles and apoptotic cells by light microscopy. Hematoxylin and eosin stain. (A) Bar = 10 μm. (B) Bar = 10 μm.

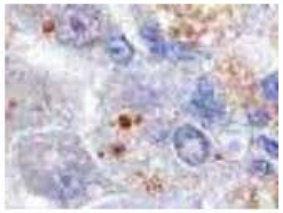
Hepatic Apoptosis

drug induced



TUNEL





Caspase 3

cited from Dr. Jerrold Ward, Phenotyping Workshop, Taipei, 2011

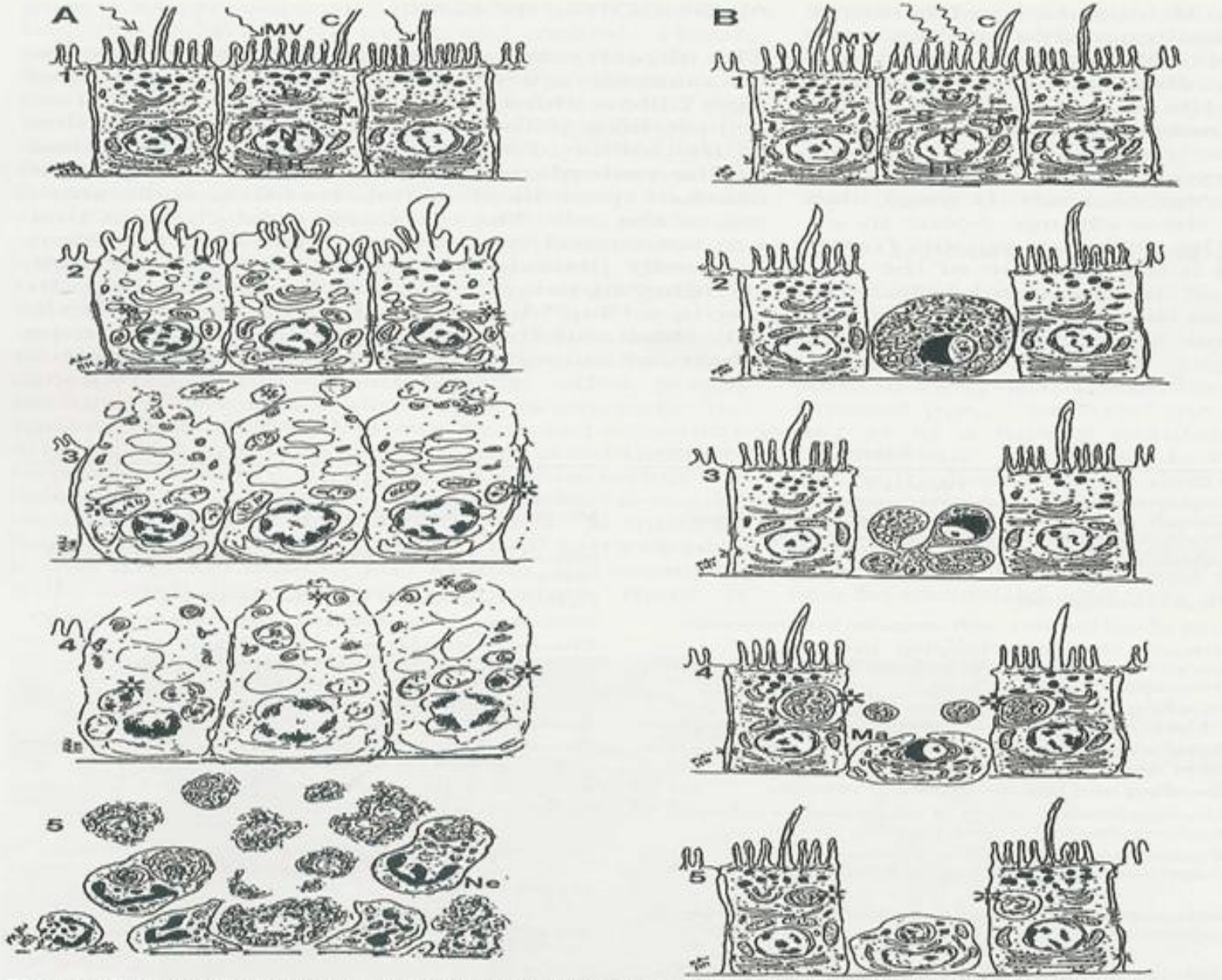
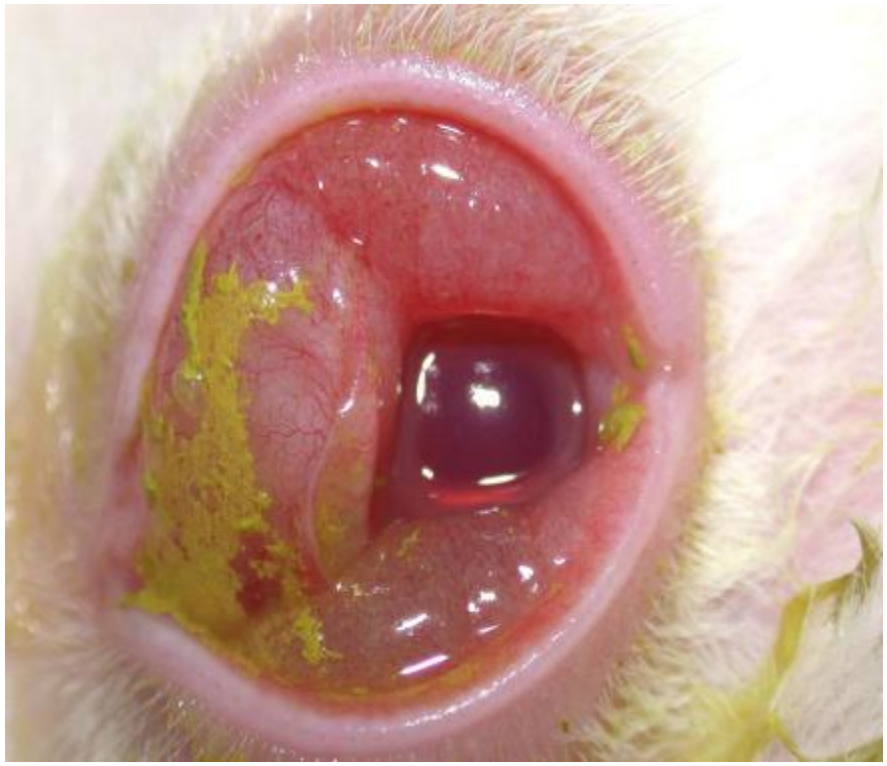


Figure 9. Morphologic changes associated with oncotic (A) and apoptotic (B) necrosis in a "prototypical" secretory epithelial cell. C, cilium; ER, rough endoplasmic reticulum; G, Golgi apparatus; M, mitochondrion; Ma, macrophage; MV, microvillous brush border; N, nucleus; Ne, neutrophil; and S, smooth endoplasmic reticulum. Changes represented in A: 1, toxic stimulus affecting the entire population of cells; 2, initial swelling with swelling of microvilli and cilia, low-amplitude swelling of mitochondria (*) clumping of chromatin

• Five cardinal signs of inflammation

1) **Red** ⇒ result from a dilation of vessels and increase blood flow
2) **Swelling** ⇒ result from exudate formation and transudation
3) **Heat** ⇒ represent an increased blood flow and dilation of vessels
4) **Pain** ⇒ result from release of chemical mediators and pressure on nerve ending
5) **Loss of function**



• Healing and repair following injury

The repair occurs when either tissue of:

1. the same type (regeneration)

2. fibrous tissue (granulation tissue)

3. results in scaring replaces injured tissue

• Healing and repair following injury

1. Healing by parenchymal regeneration

1) Stable cells⇒ retain the capacity for rapid division and cell proliferation, and able to reconstitute damage tissues.
⇒ hepatocytes, renal tubule cell

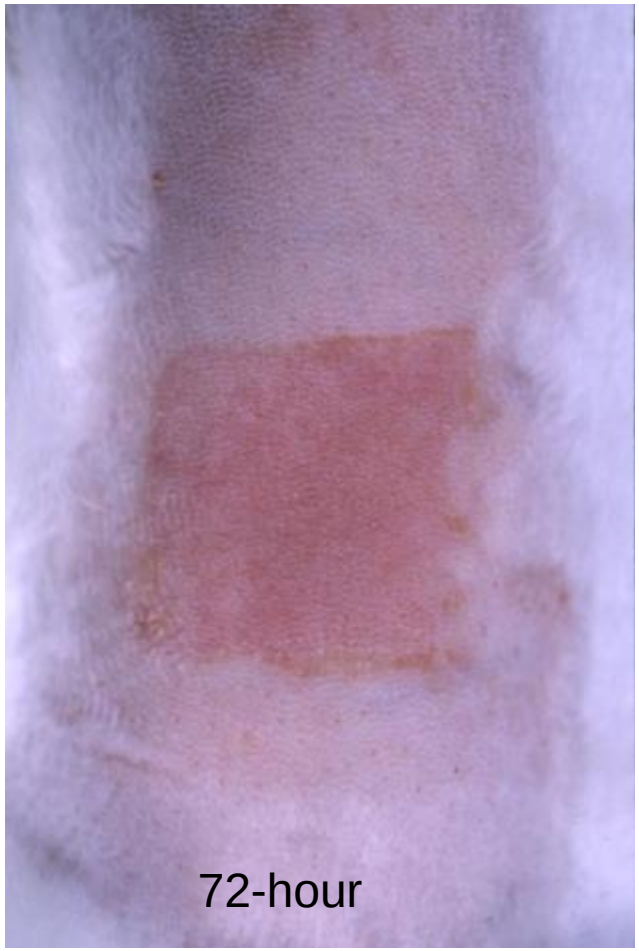
2. Healing by connective tissue replacement

1) Permanent cells ⇒ cannot regenerate, and these cells replaced by the supported tissue
⇒ neuron, muscle cells

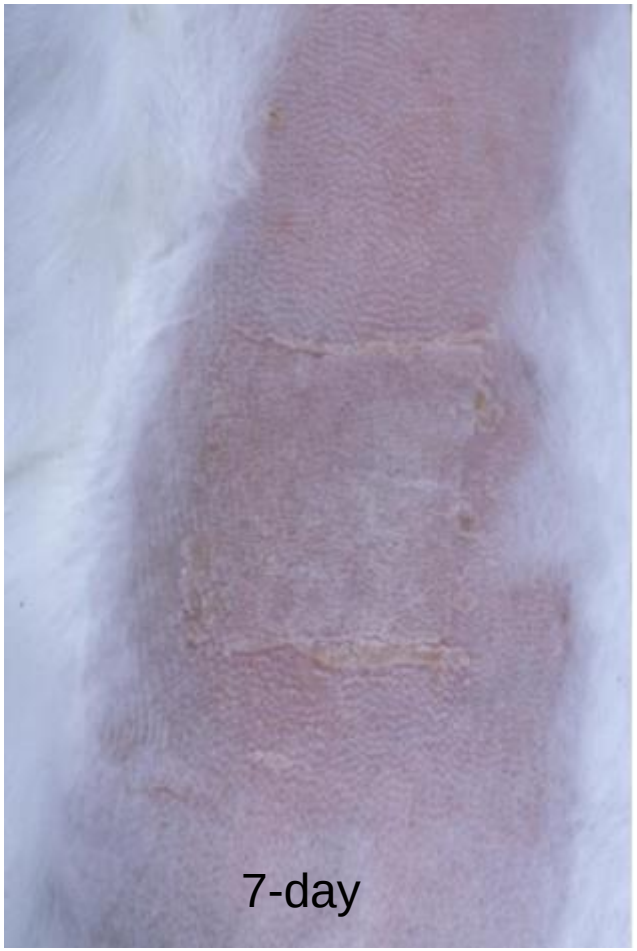
3. The inflammatory process

1) Inflammation can be caused by infectious agents, chemicals, mechanical or thermal injury, foreign bodies and immune-mediated mechanism

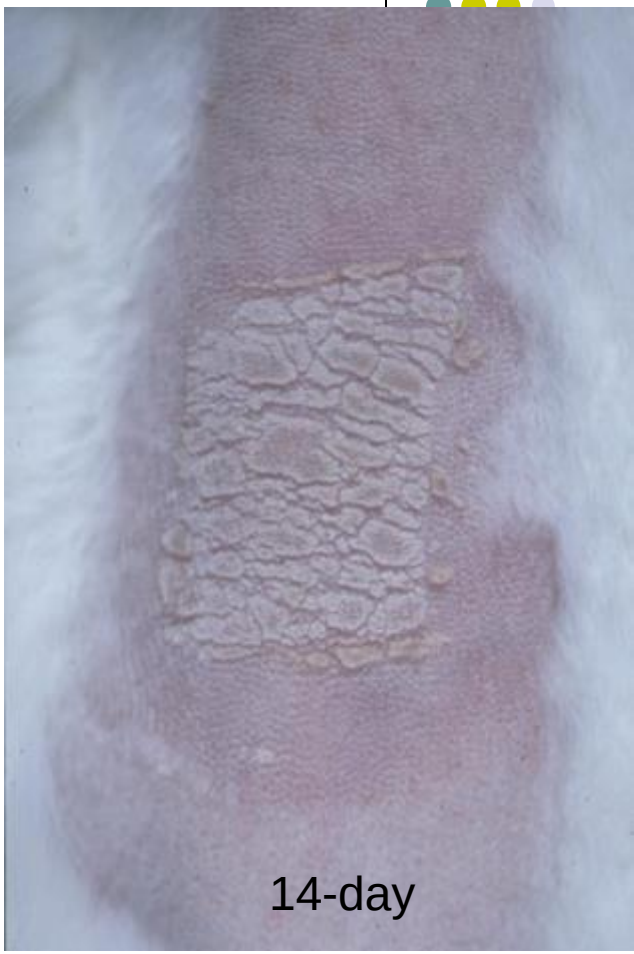
Solvent-induced skin irritation



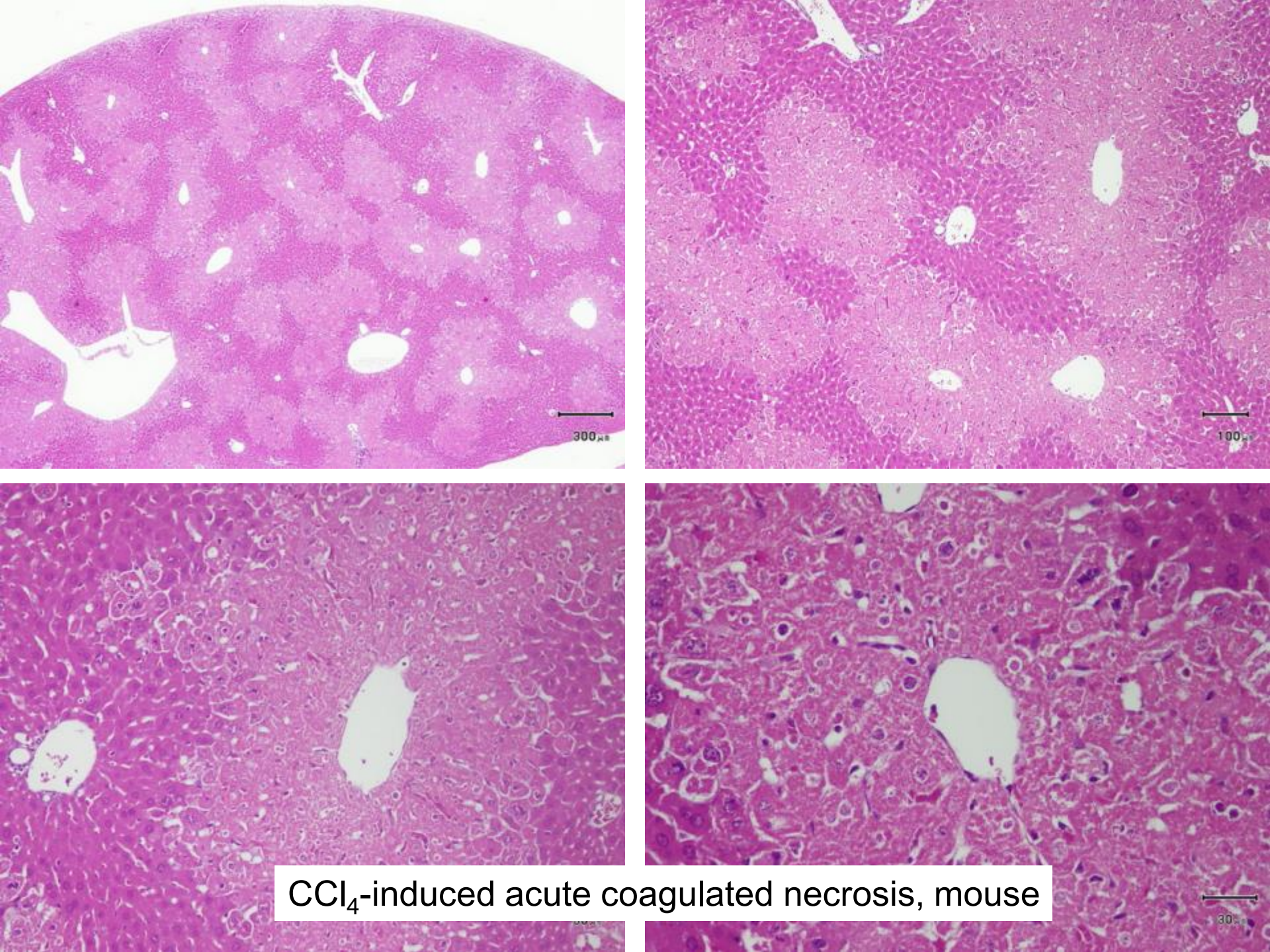
Erythema (2)
Edema (1)



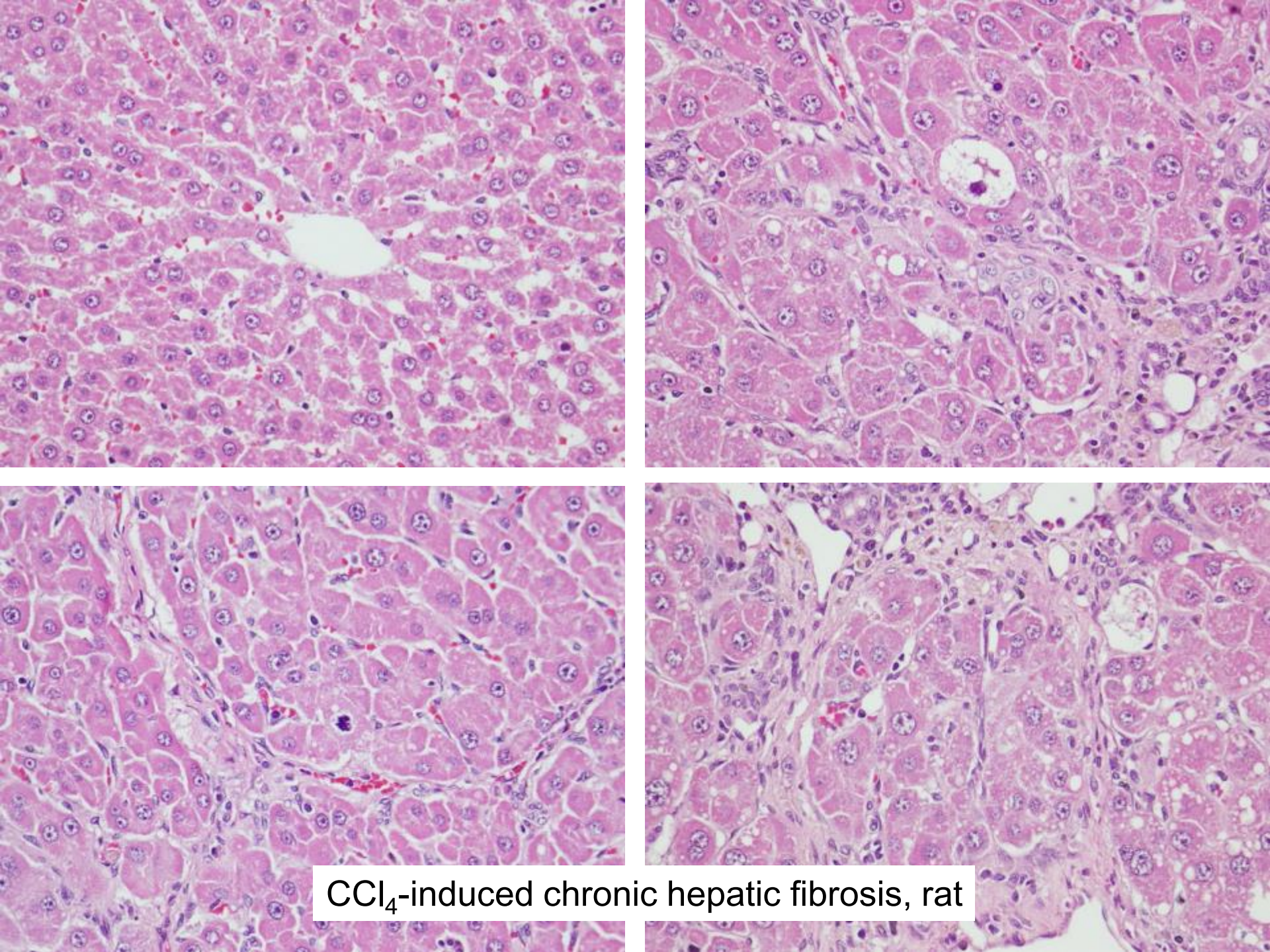
Eschar (4)
Edema (1)



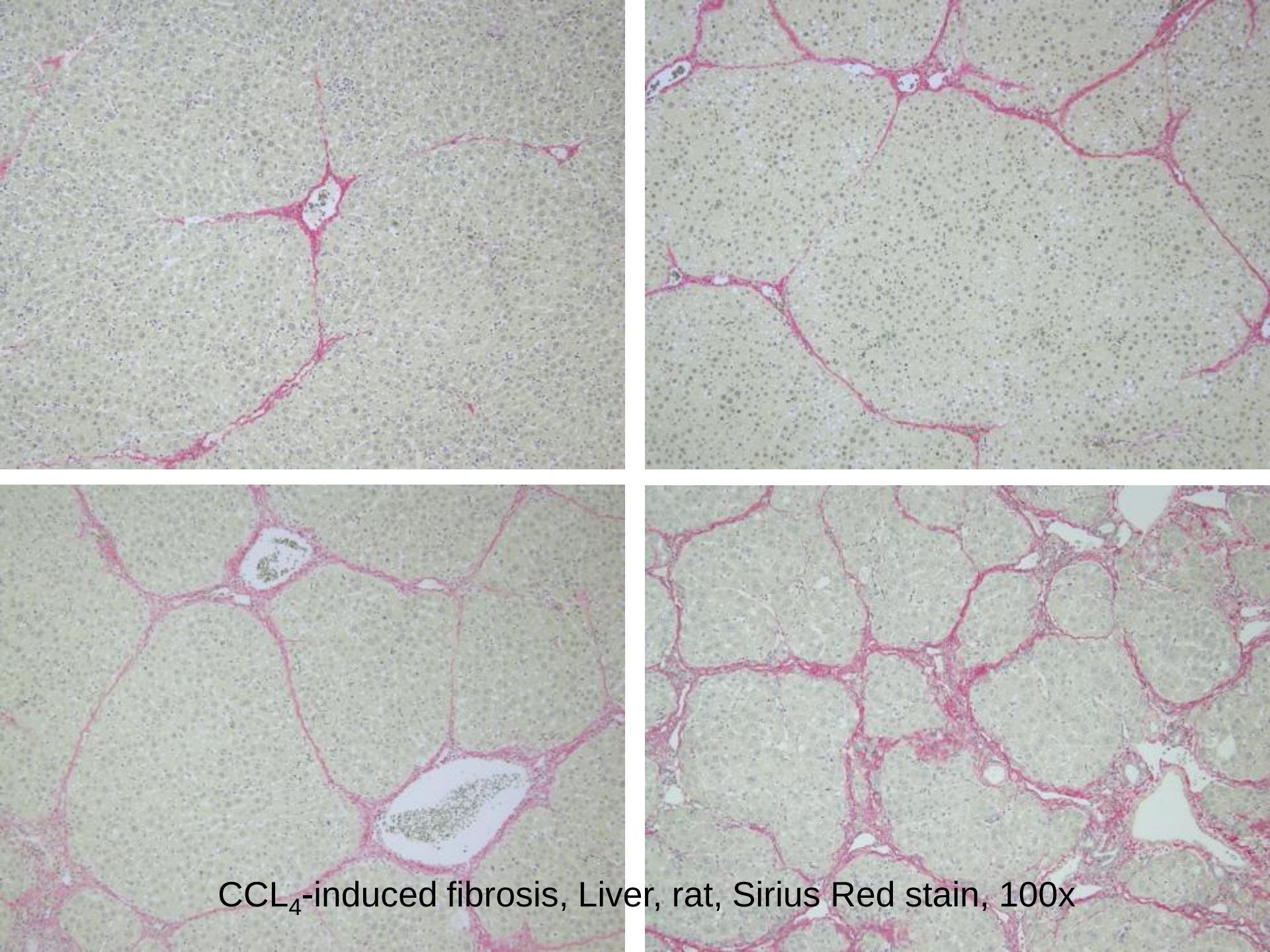
Eschar (4)
Edema (2)



CCl_4-induced acute coagulated necrosis, mouse



CCl_4-induced chronic hepatic fibrosis, rat



CCL_4-induced fibrosis, Liver, rat, Sirius Red stain, 100x

•Three types of inflammation

1. Acute inflammation ⇒ Abscess

1) The duration is from hours to several days

2) Edema, hyperemia, fibrin exudation, neutrophilic exudate

2. Subacute inflammation ⇒ Bronchopneumonitis

1) Range from days to weeks

2) Decrease vascular and cellular components

3) Polymorphorphic and mononuclear inflammatory cells

3. Chronic inflammation:

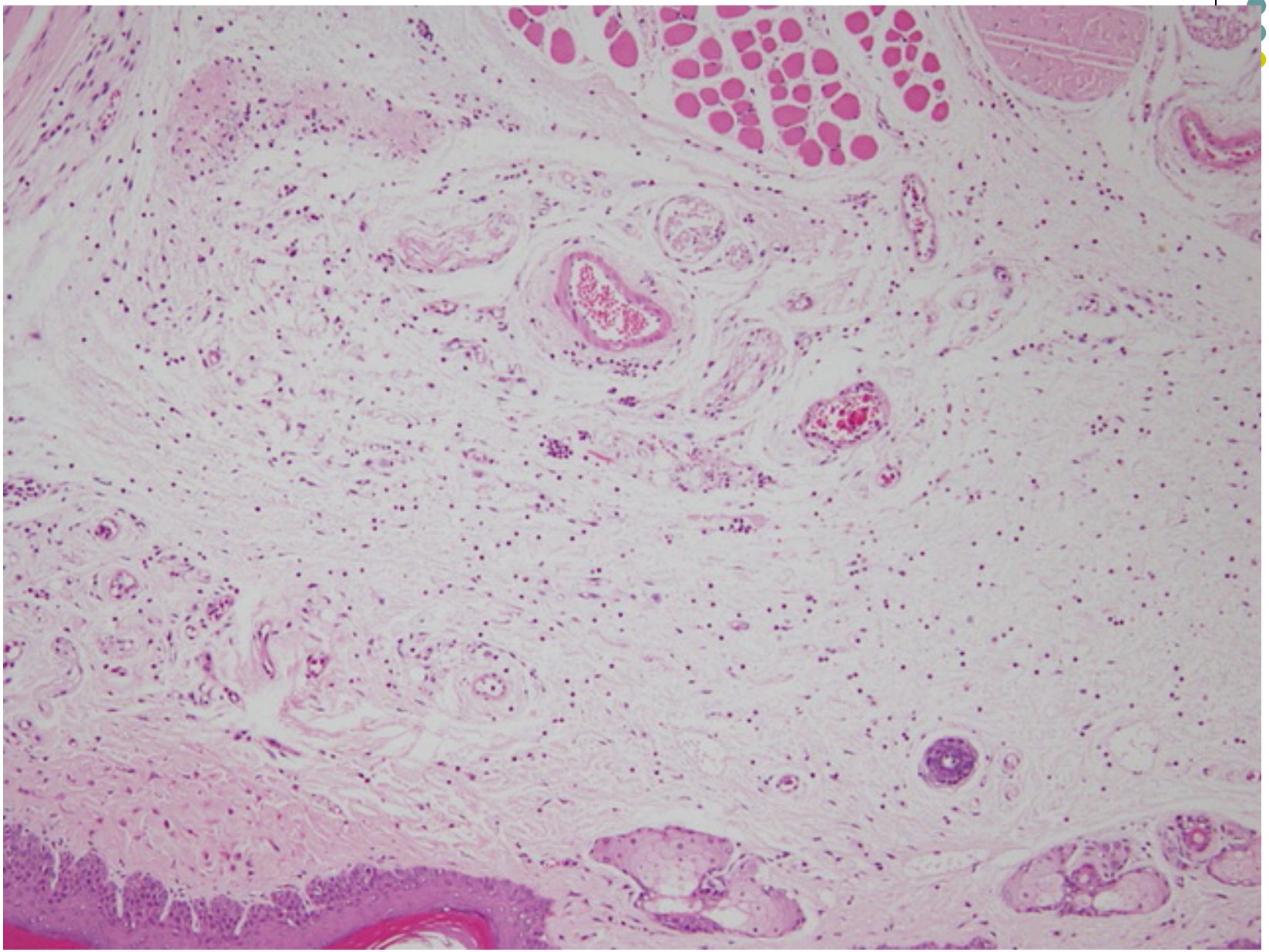
1) Persist for weeks to months, usually occur by fibrosis

2) Granulamatous inflammation ⇒ Tuberculosis

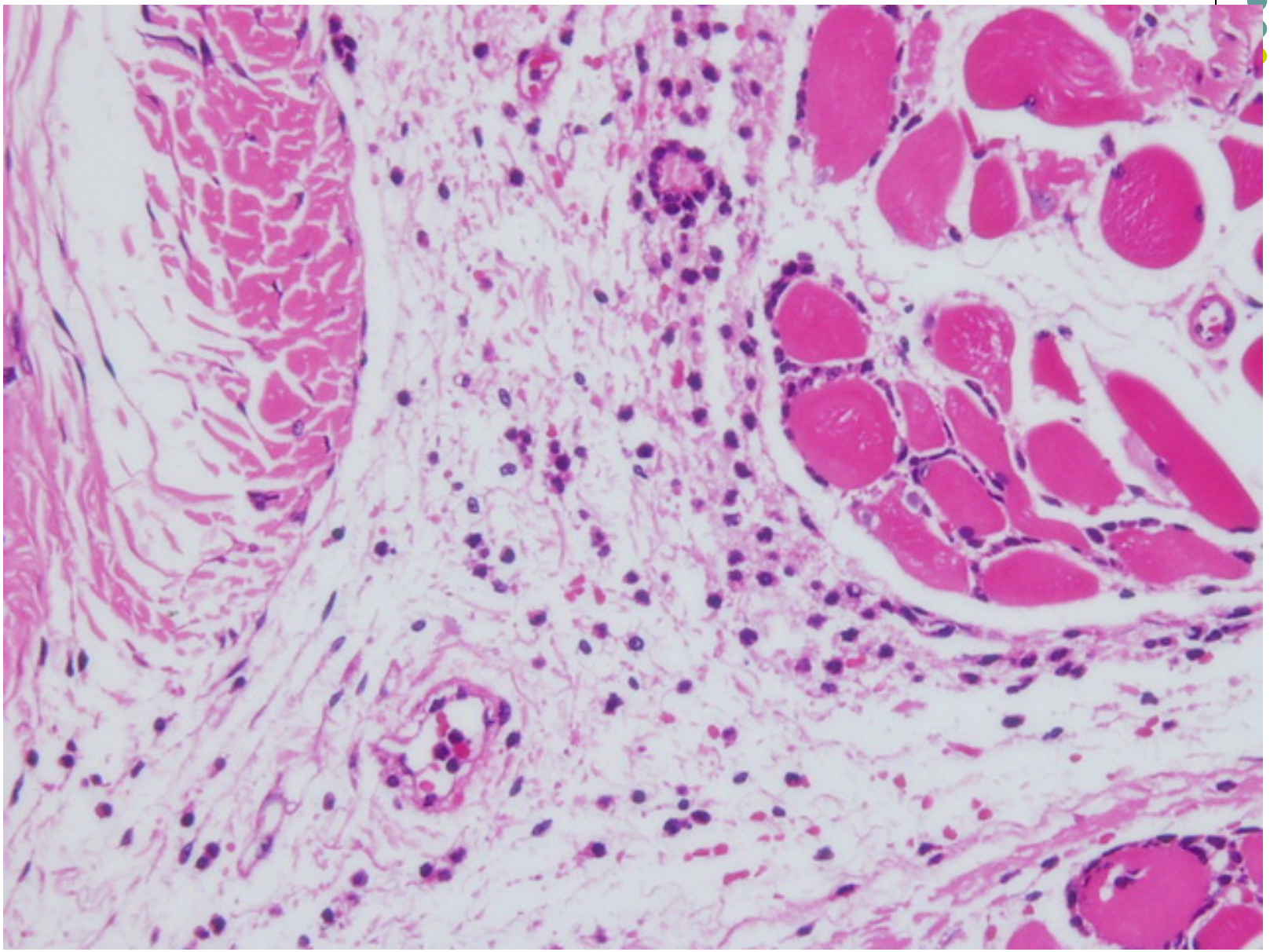
Central area⇒ aggregated macrophages, epithelioid cells,

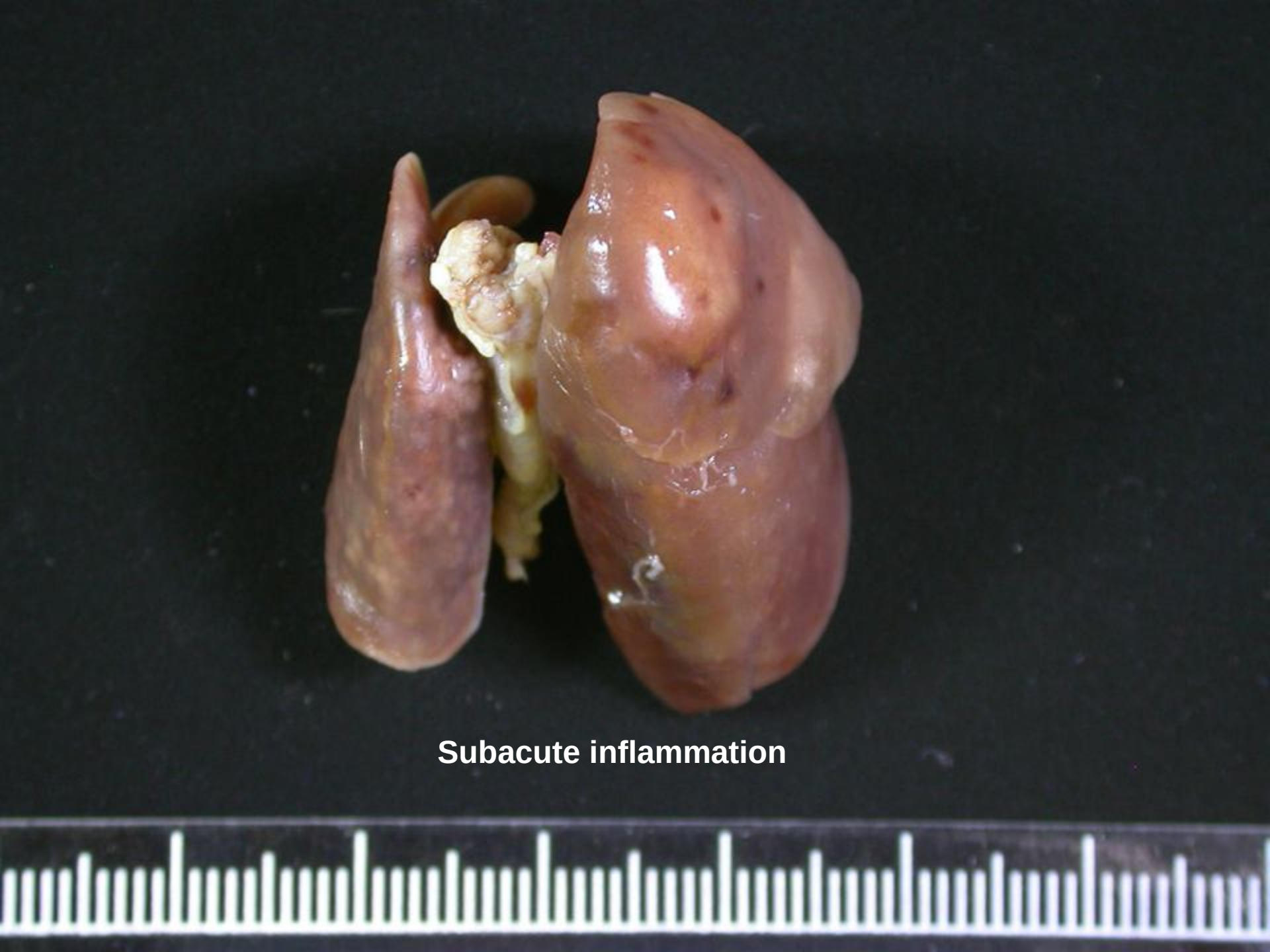
Outer layer ⇒ surrounded by mononulear cells, plasma cells, lymphocytes, multinucelar giant cells, fibroblasts, and collagen

Acute inflammation (4 hr)
Carrageen-Induced Paw Edema in a Mouse

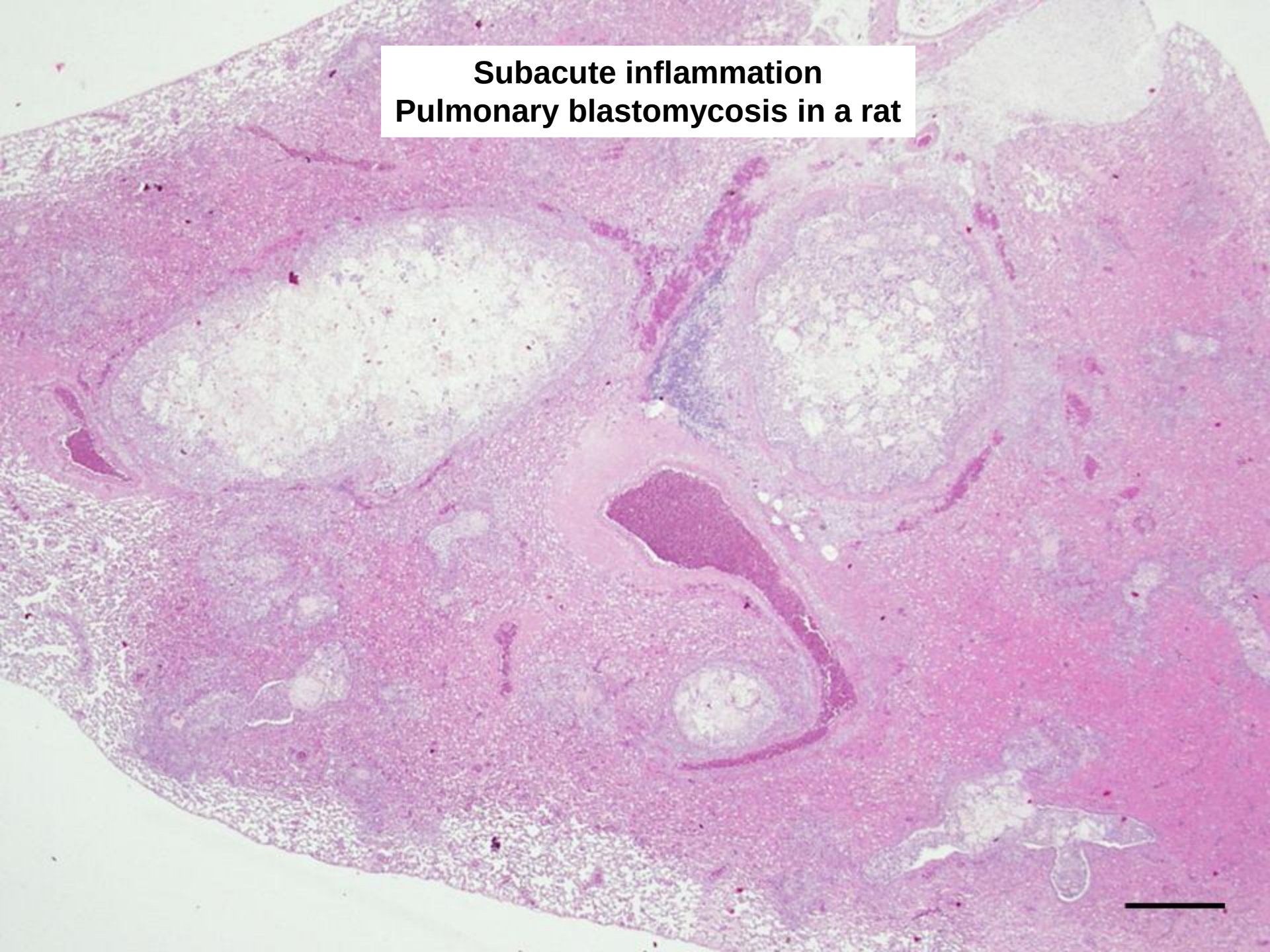


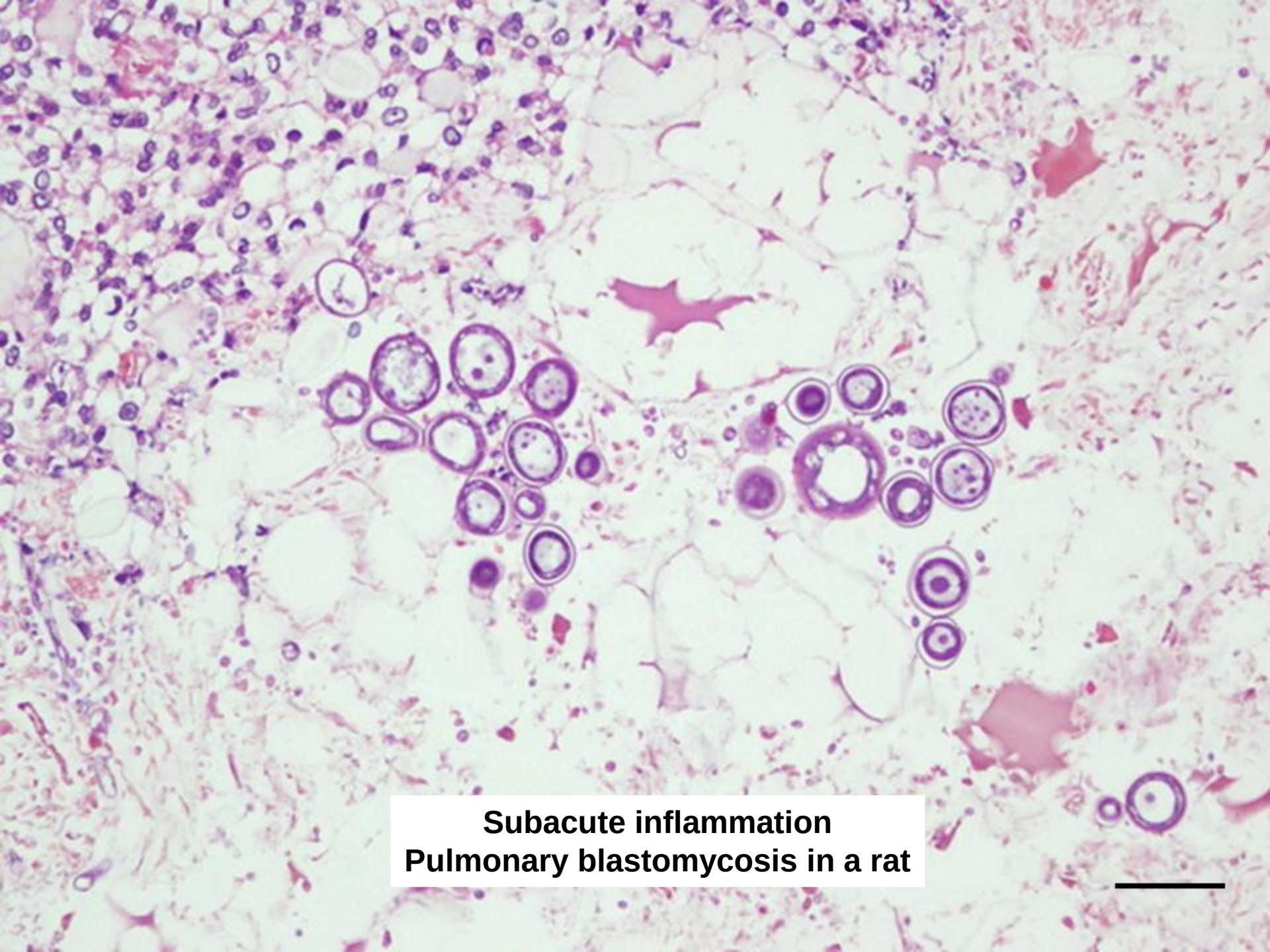
Acute inflammation (4 hr)
Carrageen-Induced Paw Edema in a Mouse



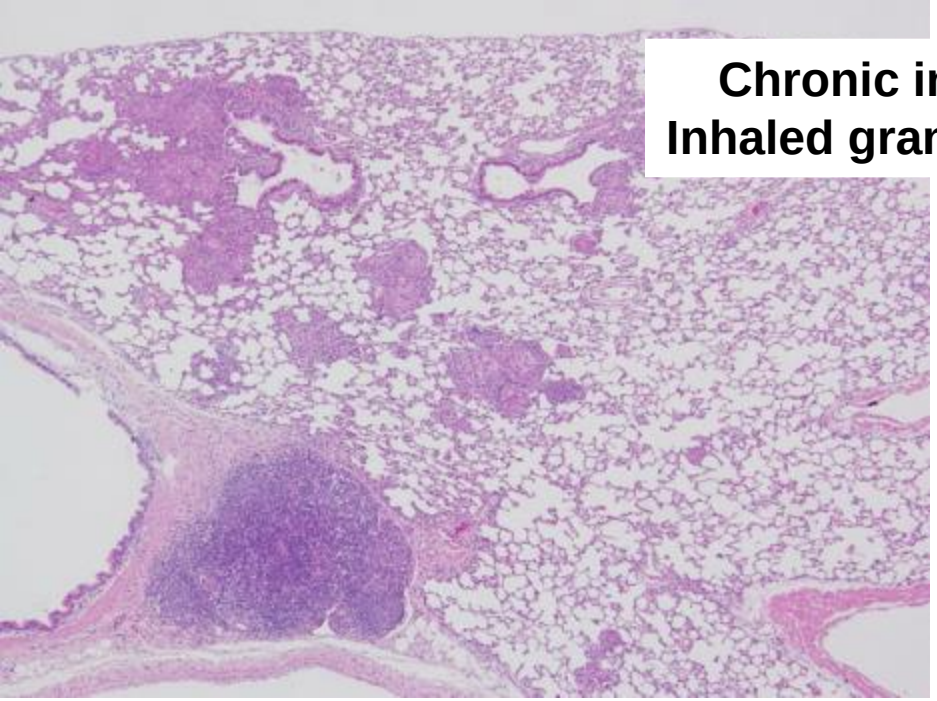


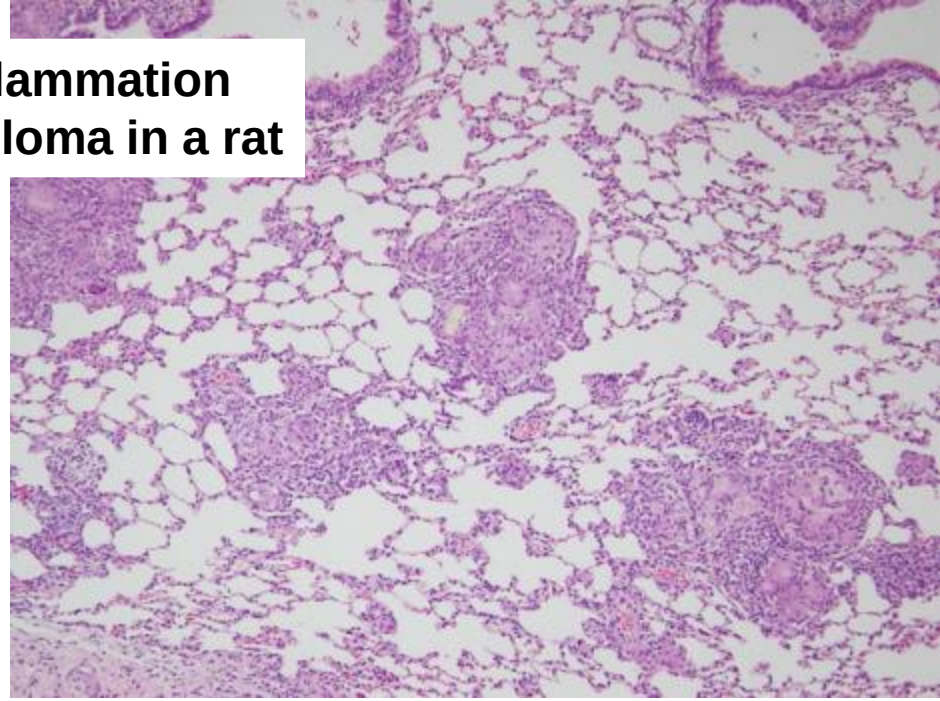
Subacute inflammation

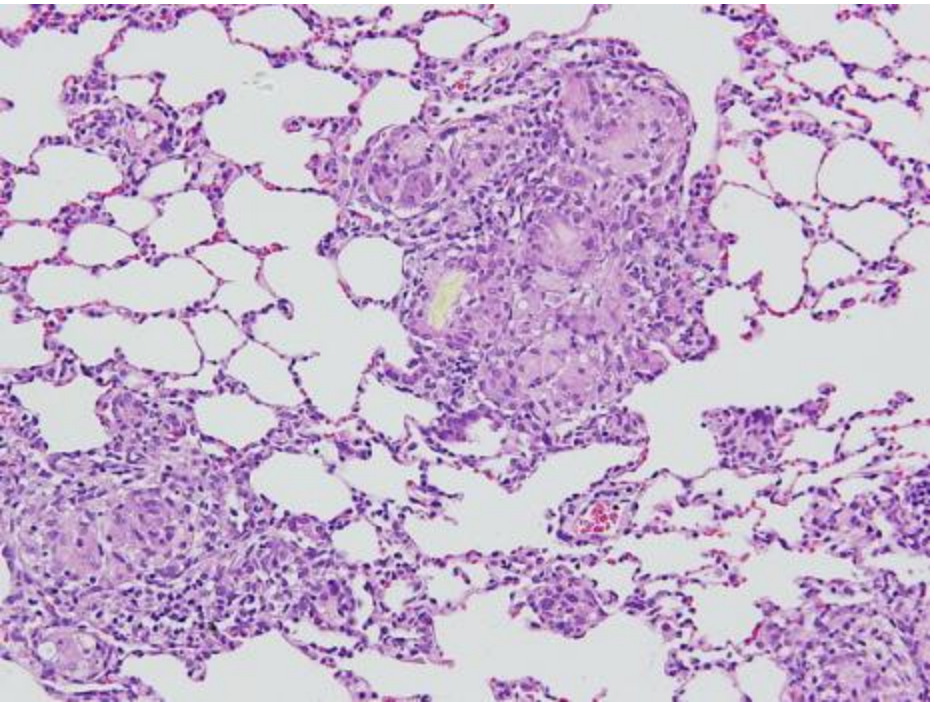

Subacute inflammation
Pulmonary blastomycosis in a rat

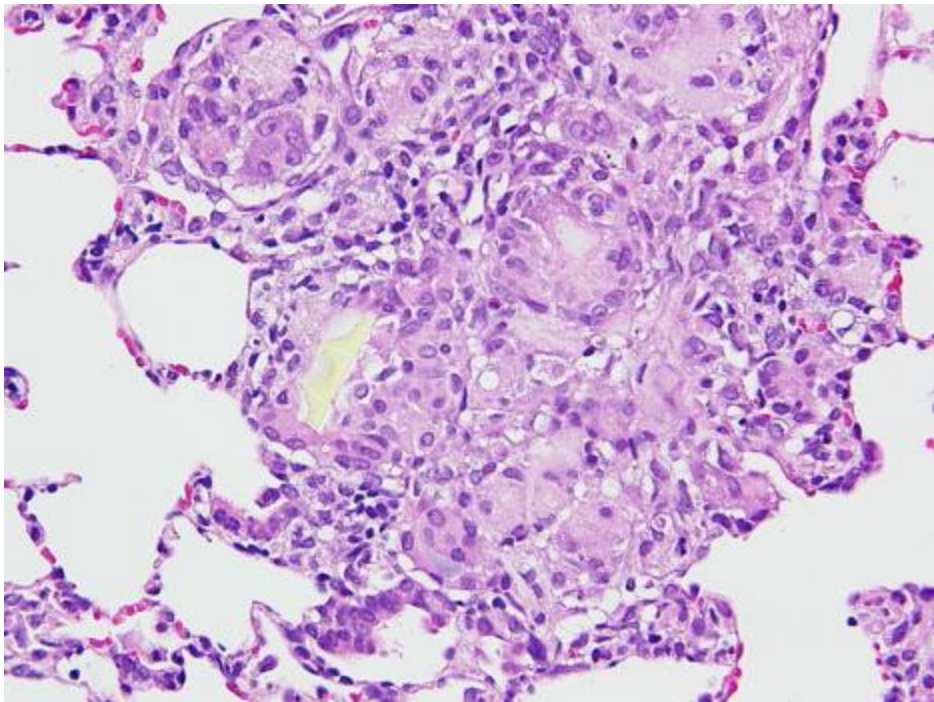

Subacute inflammation
Pulmonary blastomycosis in a rat

Chronic inflammation
Inhaled granuloma in a rat









• Changes in cell growth and cell size

• **Atrophy (萎縮)**

. Reduce cell in normal size

. Starvation or malnutrition causes liver cell atrophy

• **Hypertrophy (肥大)**

. An increase in size of an organ or tissue

. As an increase the amount of new cytoplasma and its constituent

. Adaptation response such as cardiac and skeletal muscles

• **Regeneration (再生)**

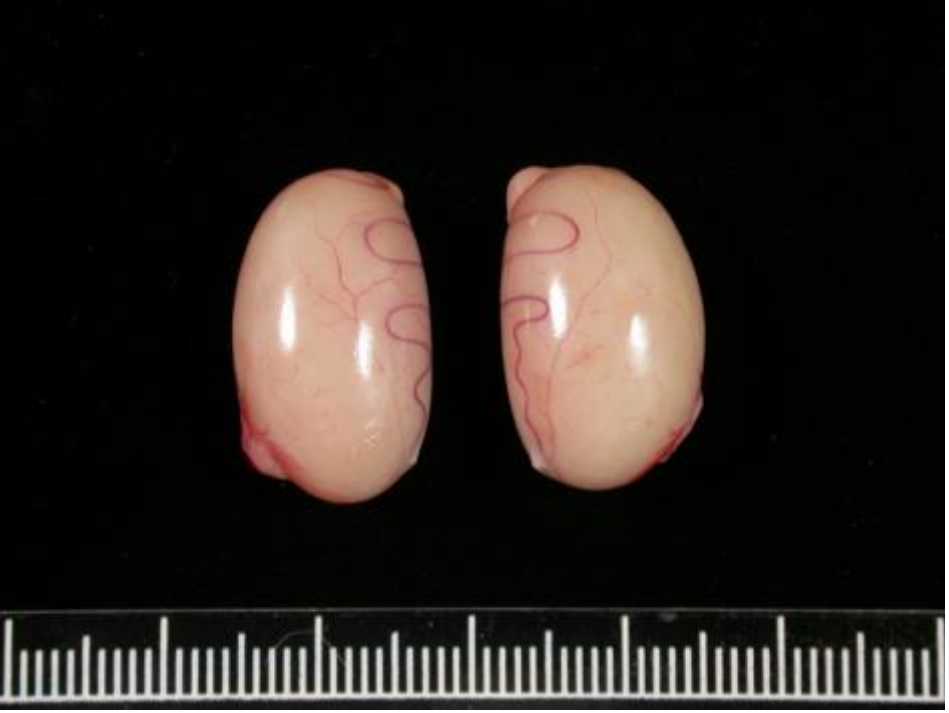
. The replacement of cells by new cells of the same type

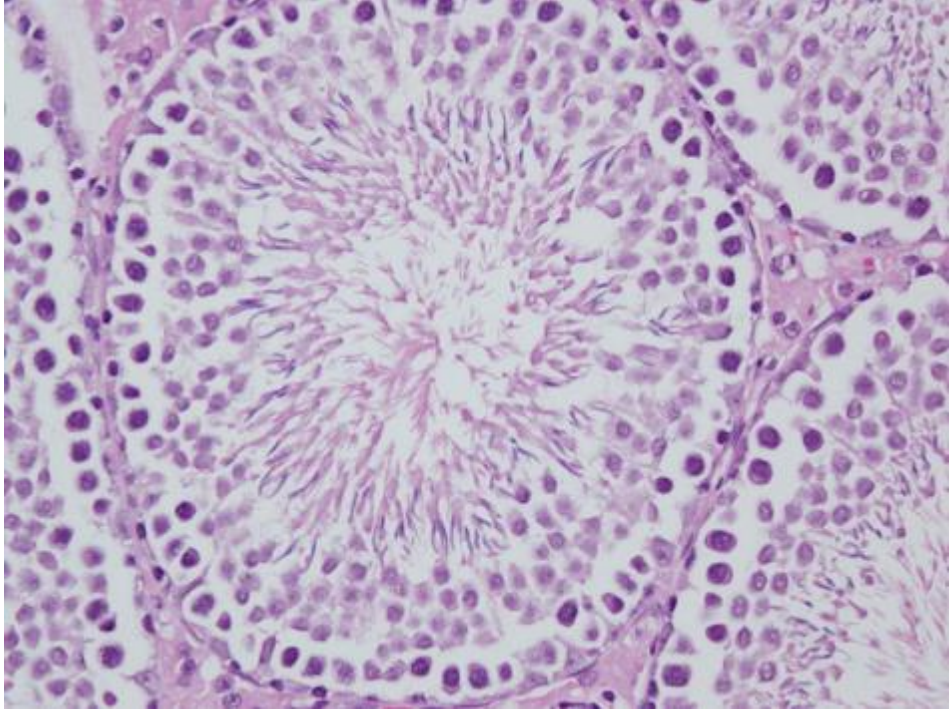
. Regenerated cells may become similar to the original cells

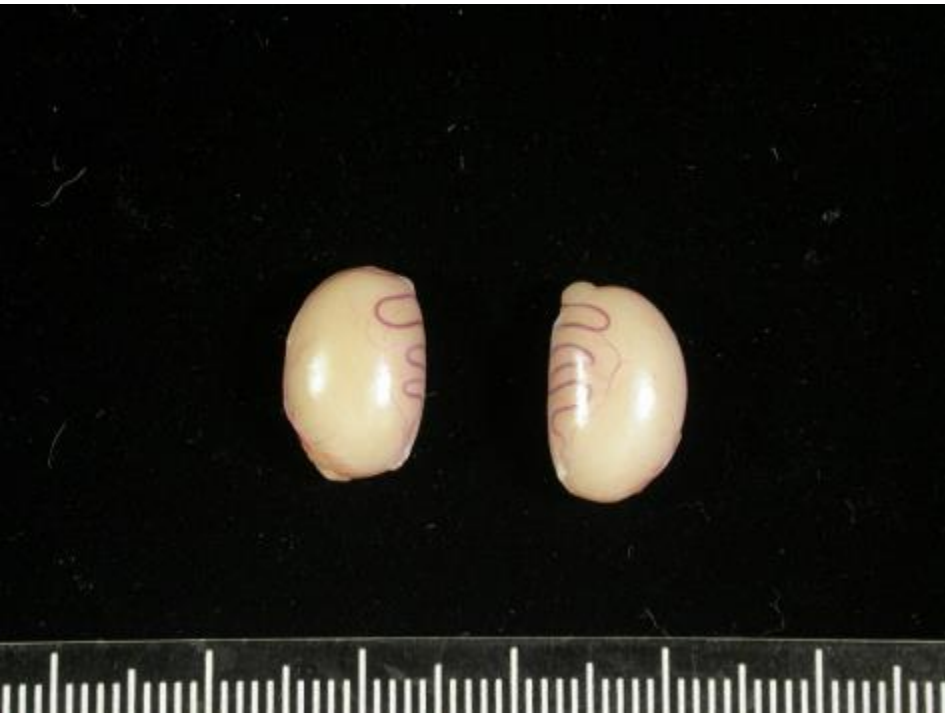
⇒ with a large amount of RNA to produce

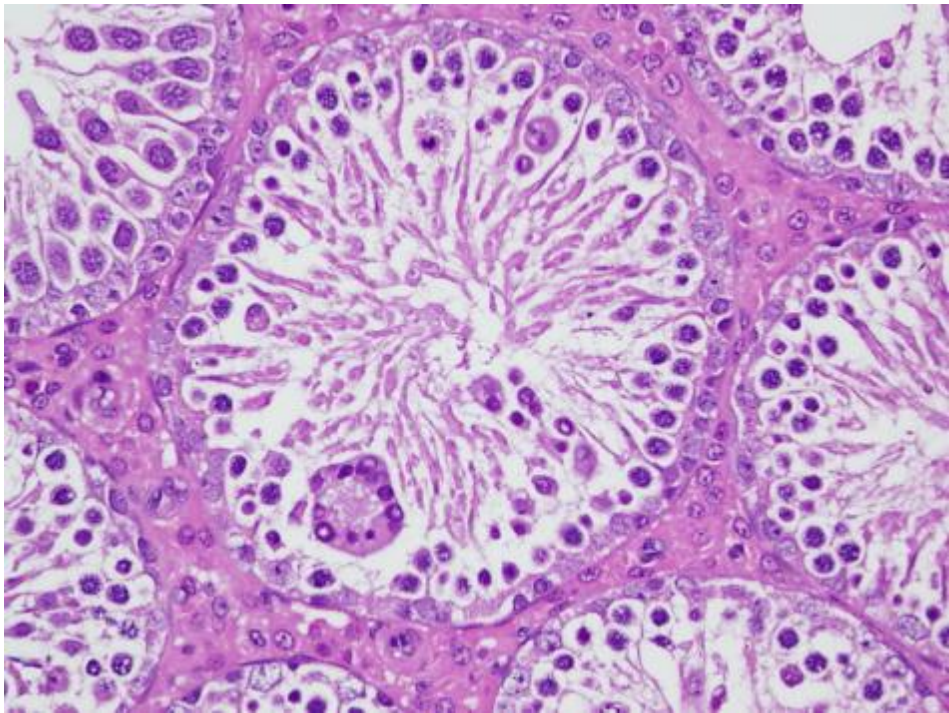
⇒ become blue staining by the H&E stain

. Hepatic regeneration (nodules) due to hepatic injury (20%)

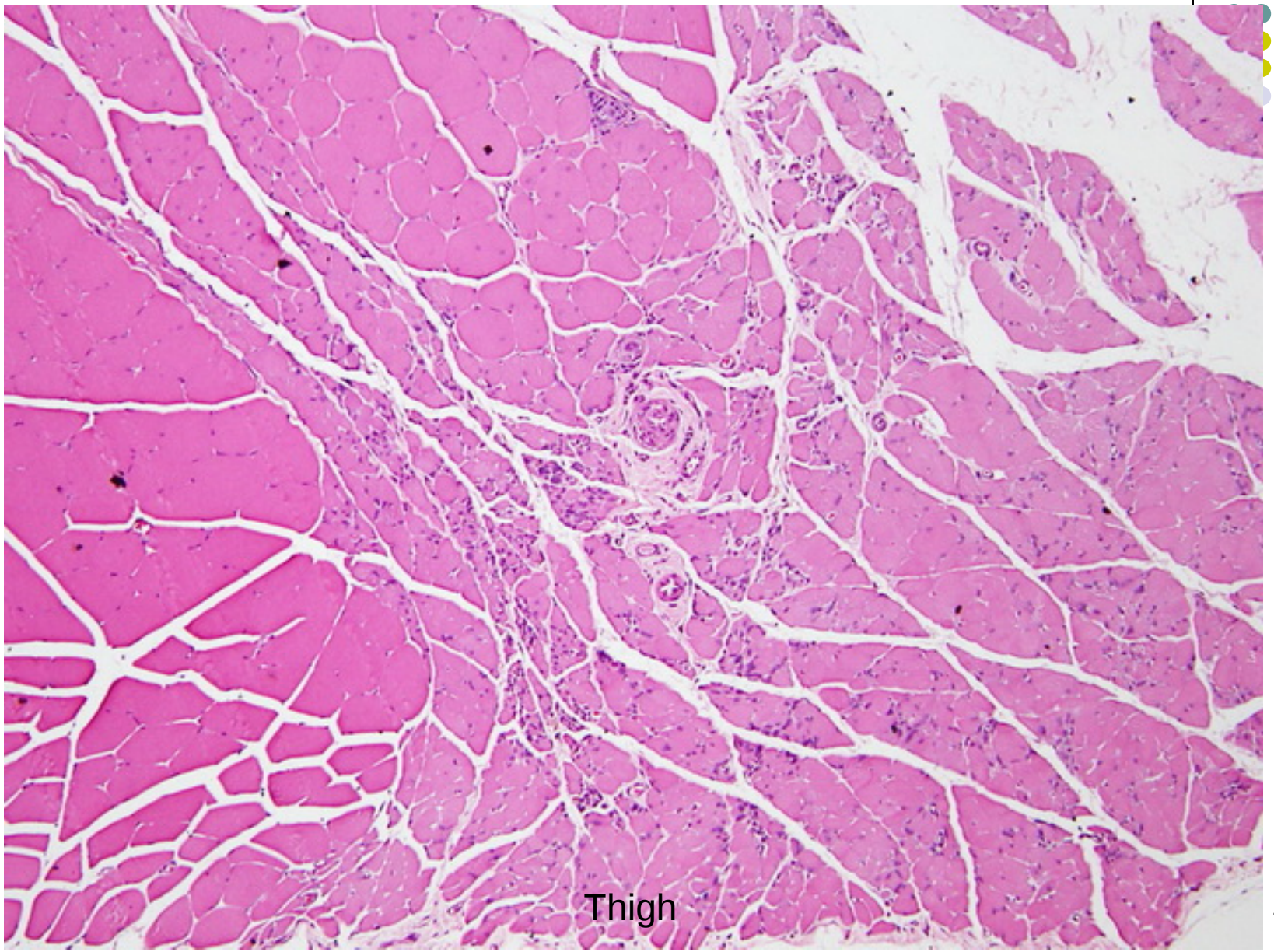




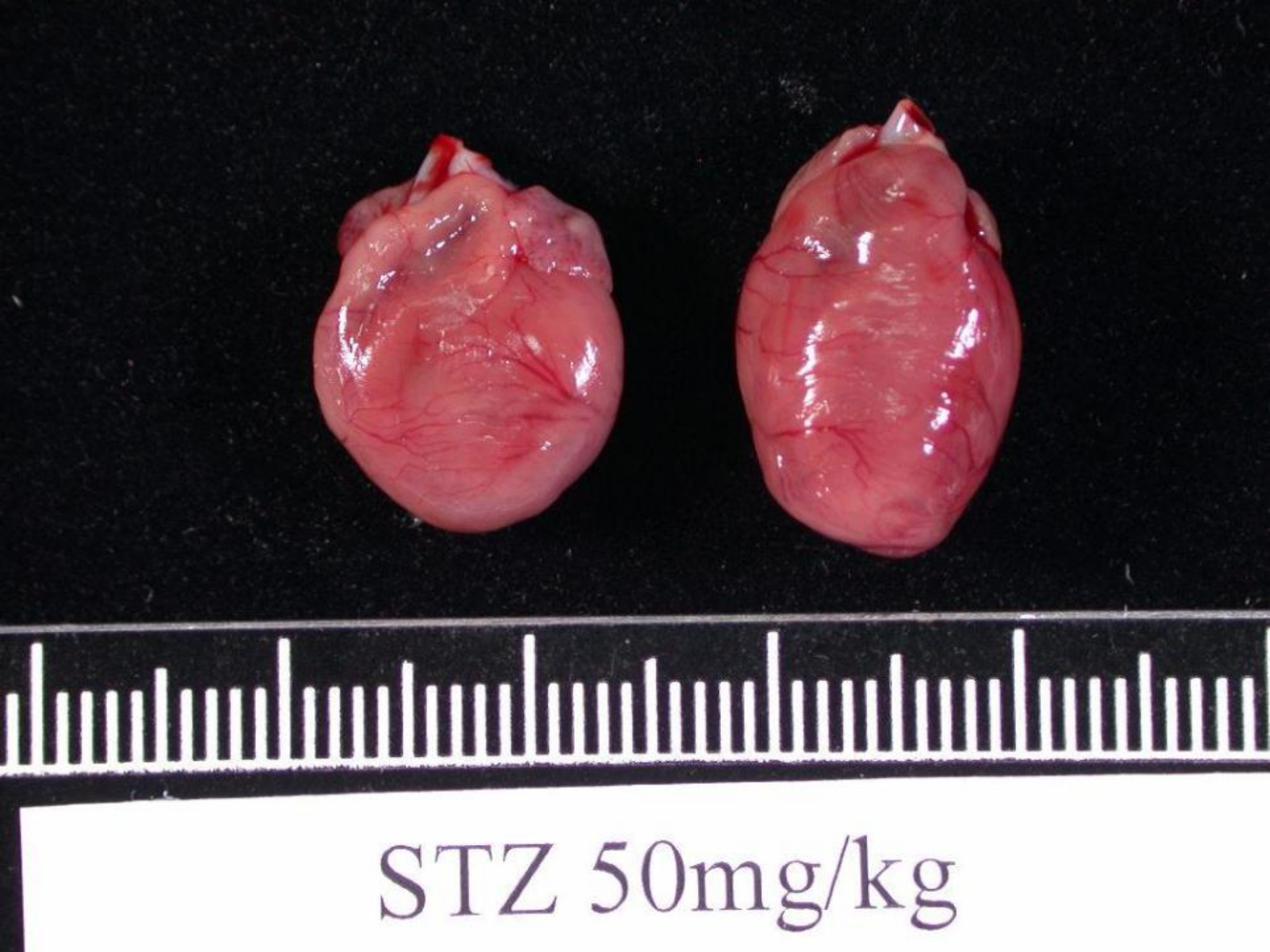


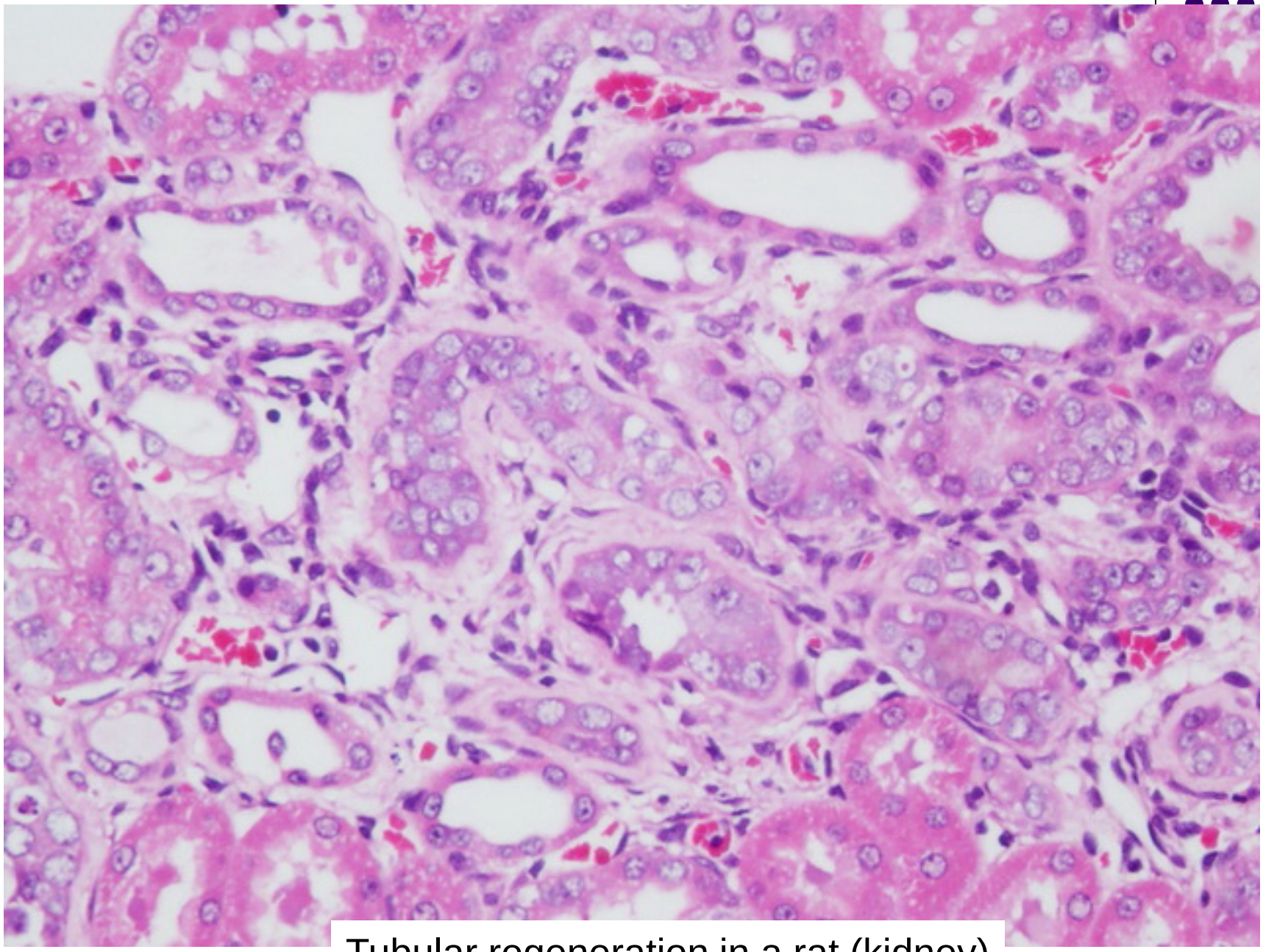


Botulinum toxin-induced paralysis leads to muscular atrophy



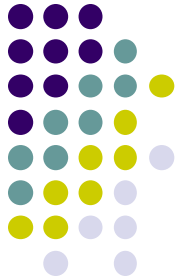
Thigh


STZ 50mg/kg



Tubular regeneration in a rat (kidney)

• Changes in cell growth and cell size



• Hyperplsia (增生)

.Increase numbers of normal cells in response to stimulation

.Injury response such as bile duct hyperplasia in liver

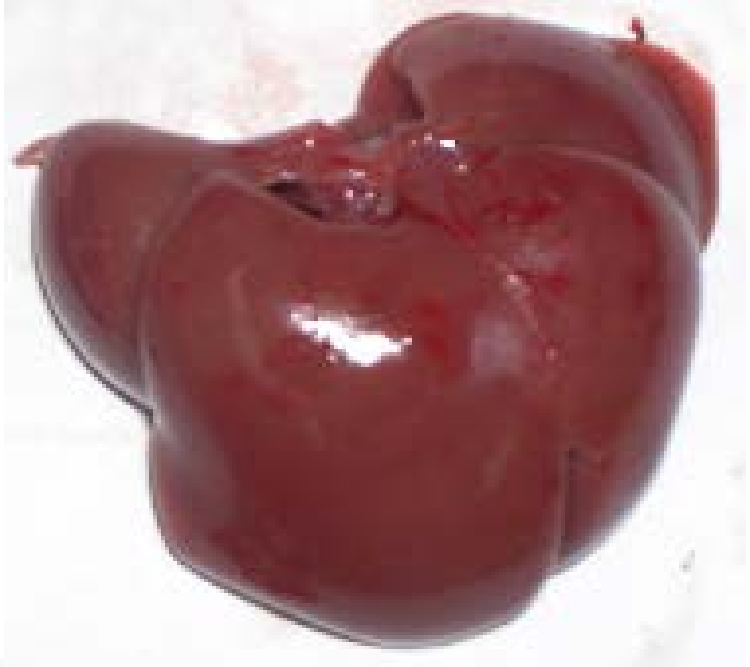
• Neoplasia or tumor, cancer (腫瘤)

.Implies new growth without normal control mechanism

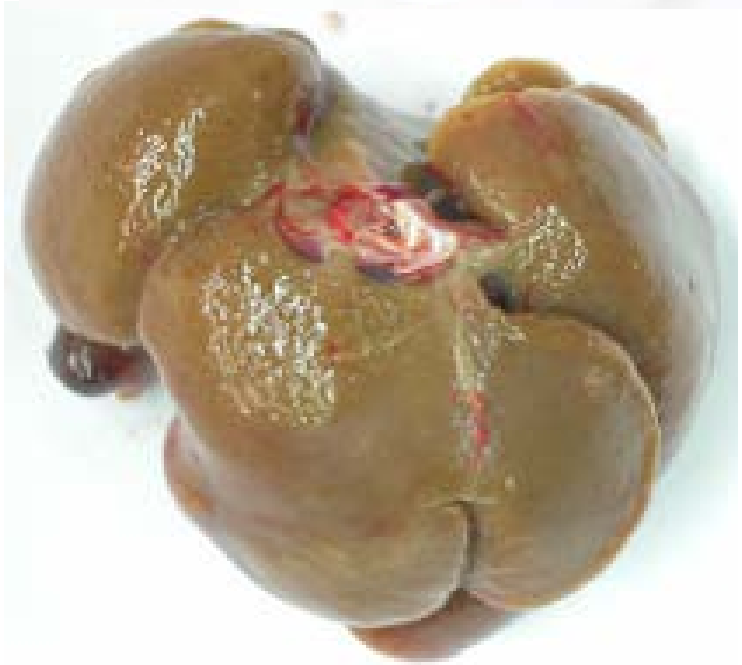
.Variation in size and shape of cells, hyperchromasia of nucleus, increase mitotic activity

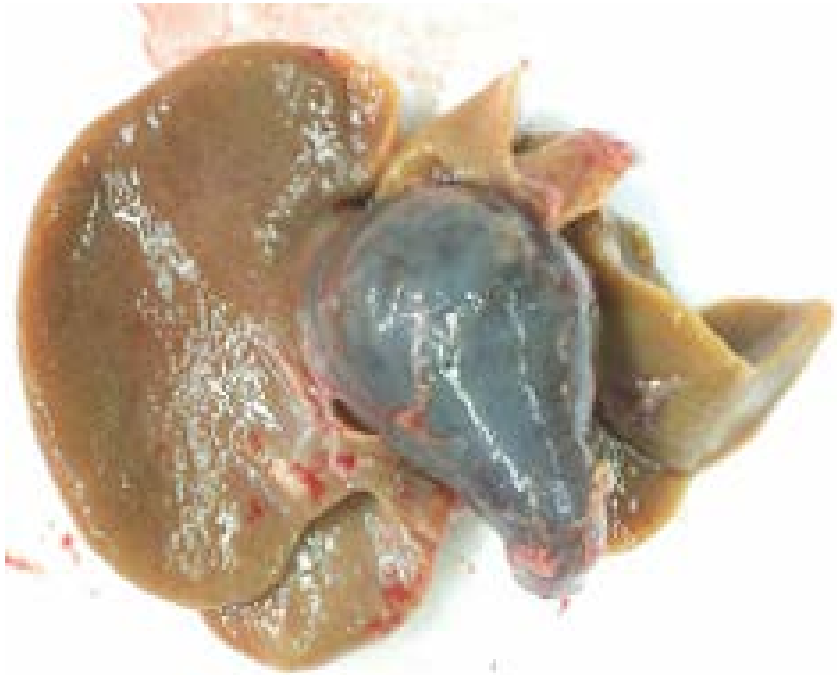
.Benign and malignant tumors classification

⇒ Papioloma, Heaptocellular carcinoma (HCC),
Squamous cell carcinoma,
Mammary gland tumor...



Bile duct ligation in rats after 4 weeks





W.-Y. Chen et al. / Life Sciences 84 (2009) 606–614

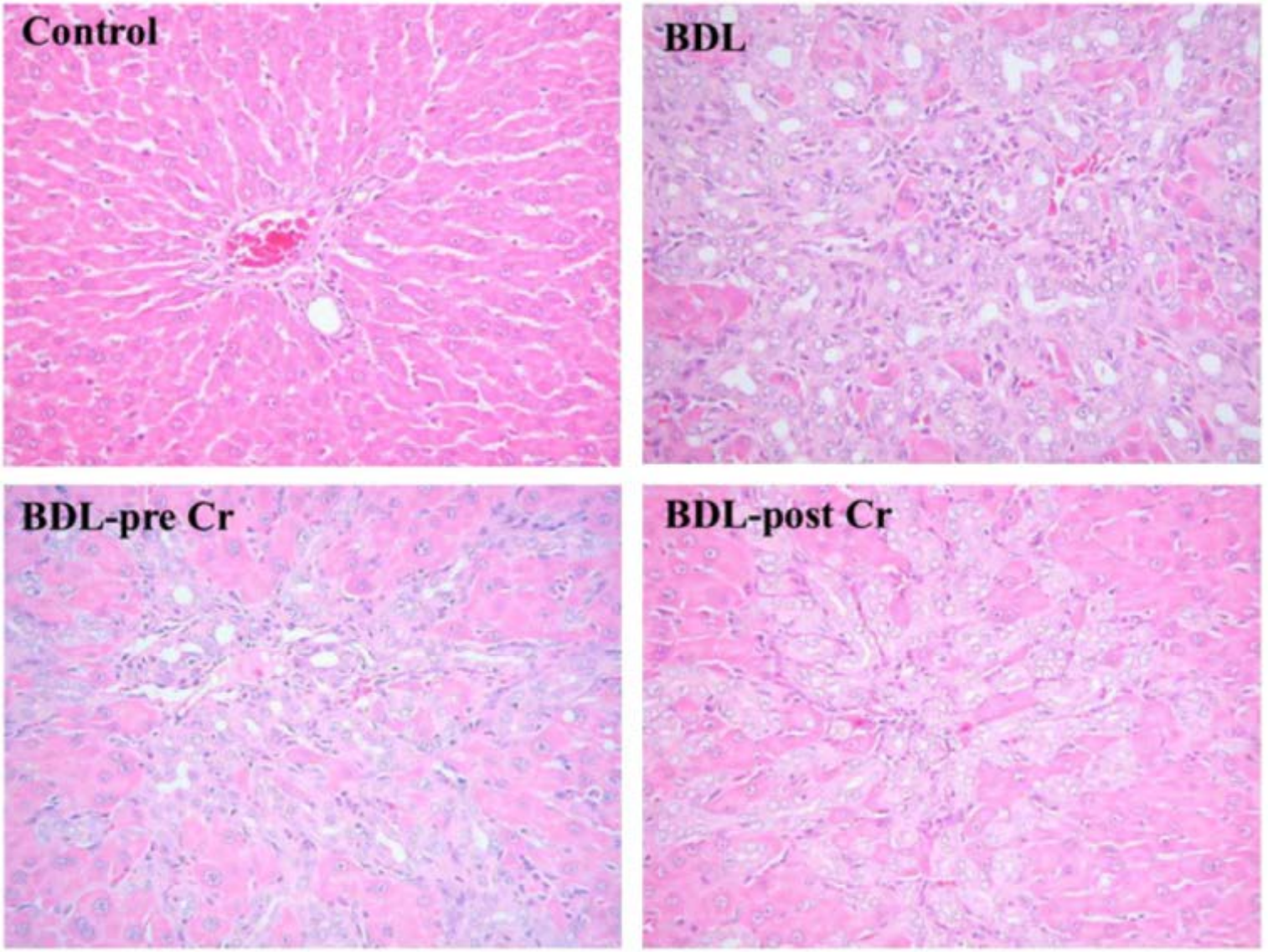


Fig. 1. Chromium attenuated BDL-induced liver injury. Severe bile duct hyperplasia in the portal area after bile duct ligation (BDL).

W.-Y. Chen et al. / Life Sciences 84 (2009) 606–614

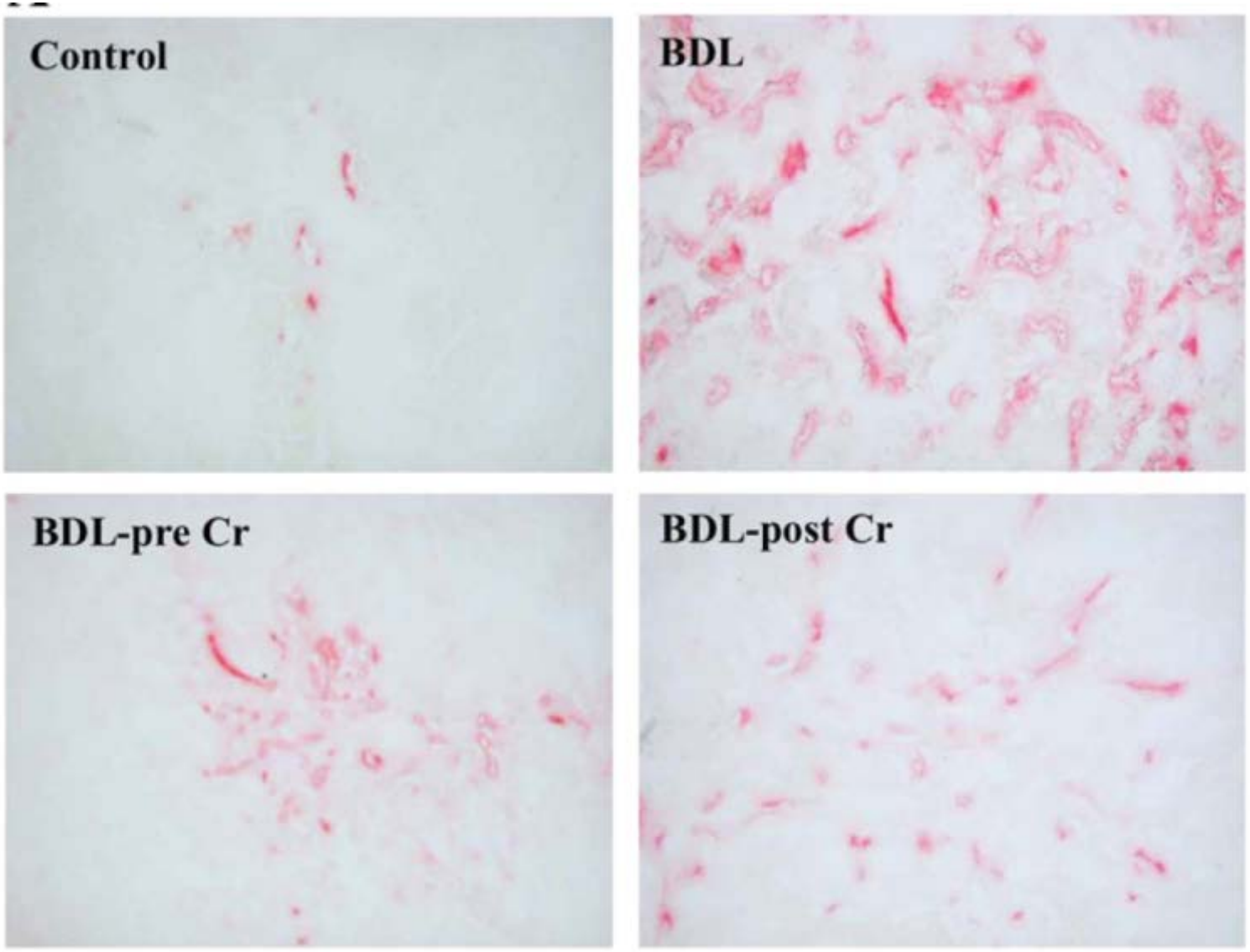
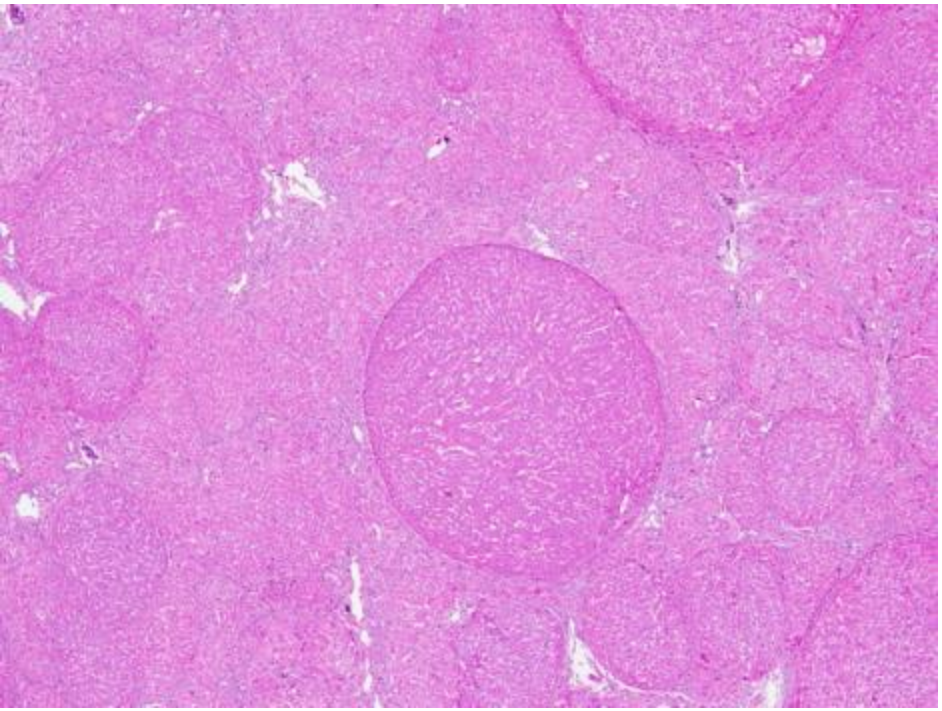
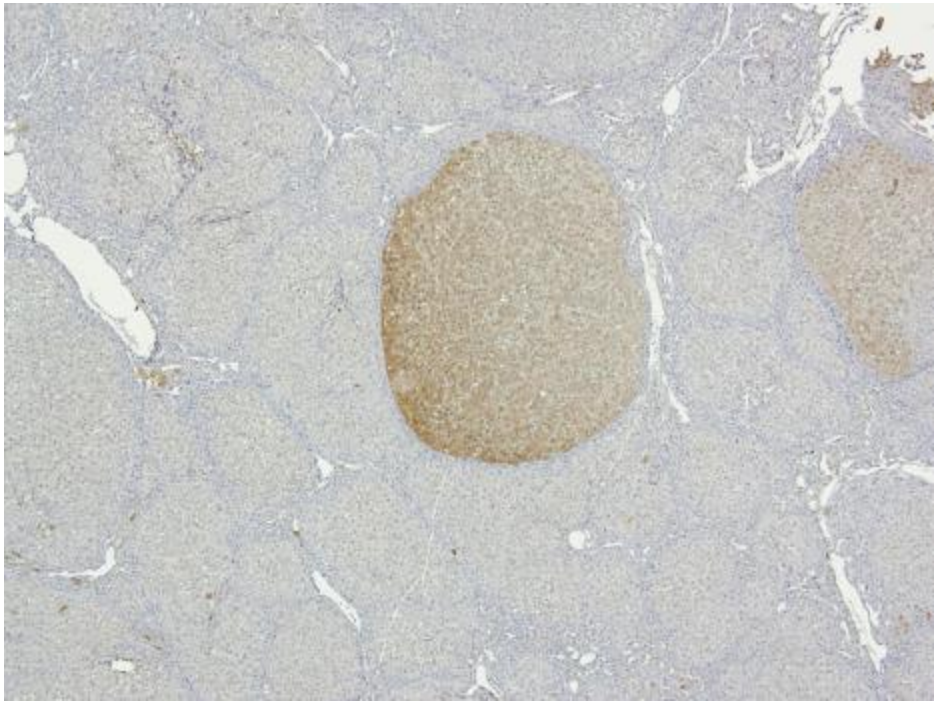


Fig. 2. Chromium attenuated BDL-induced bile duct proliferation. Positive reaction of bile ducts after staining with GGT.

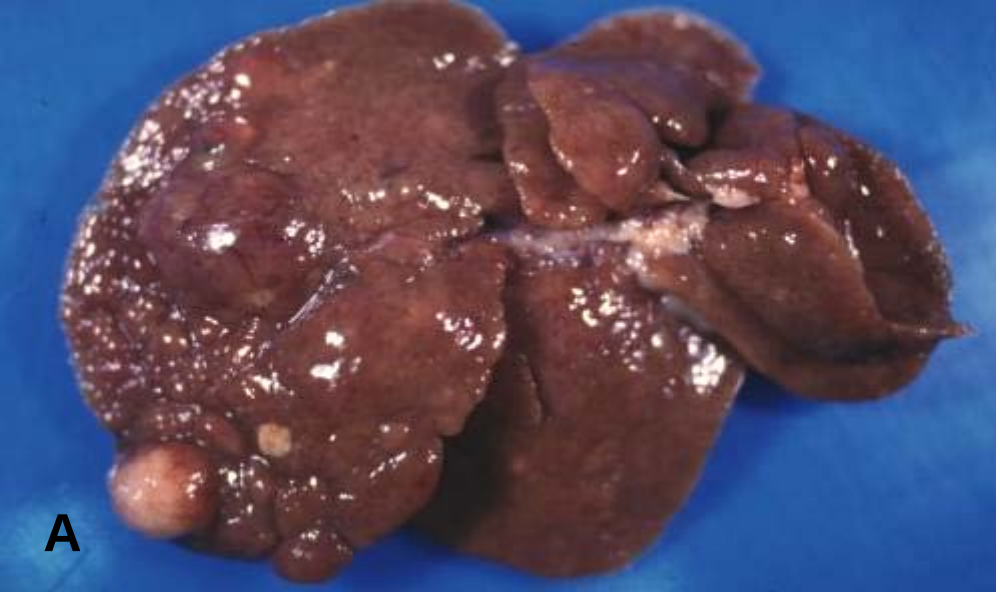
W.-Y. Chen et al. / Life Sciences 84 (2009) 606–614

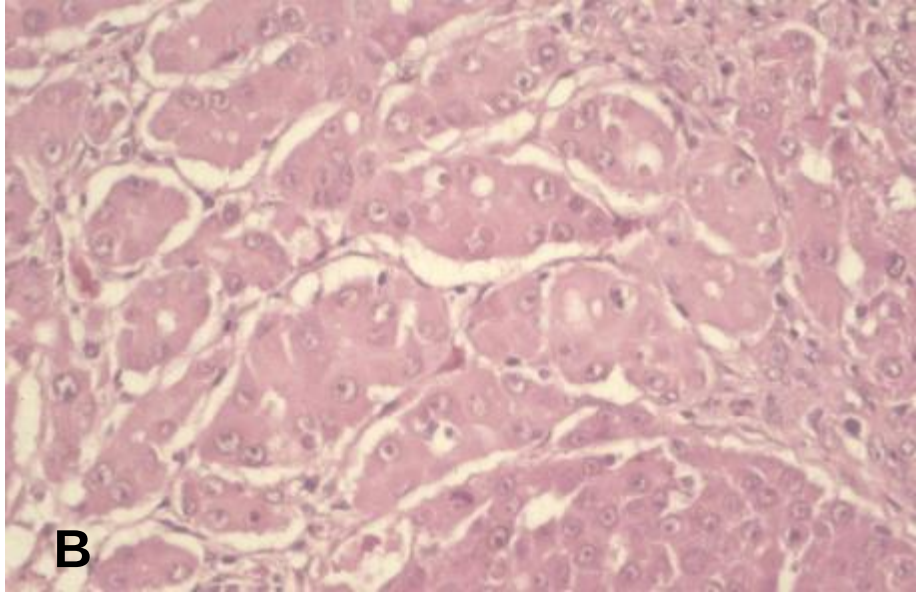


TAA induced nodular hyperplasia in rat’s liver

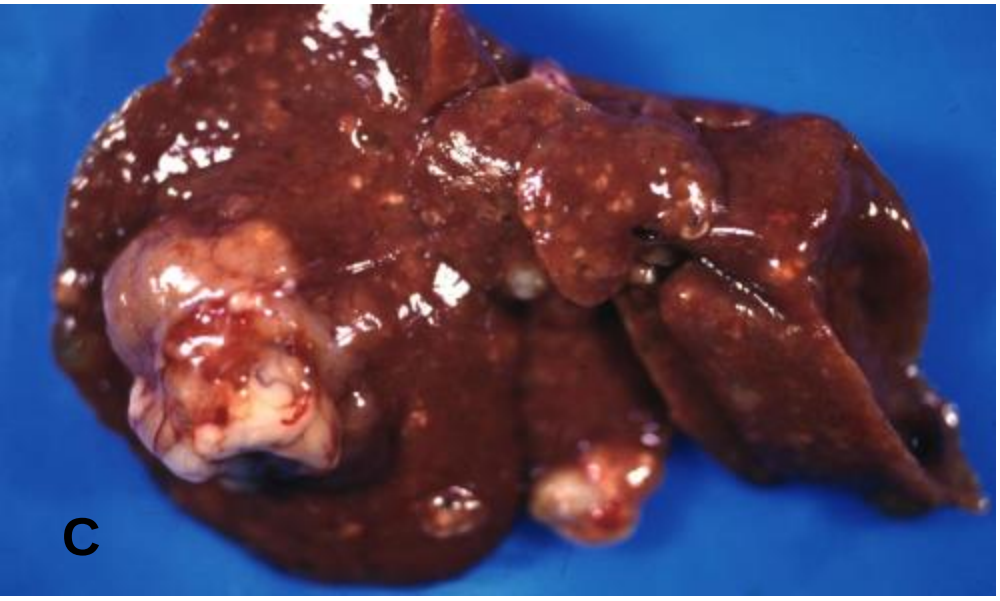


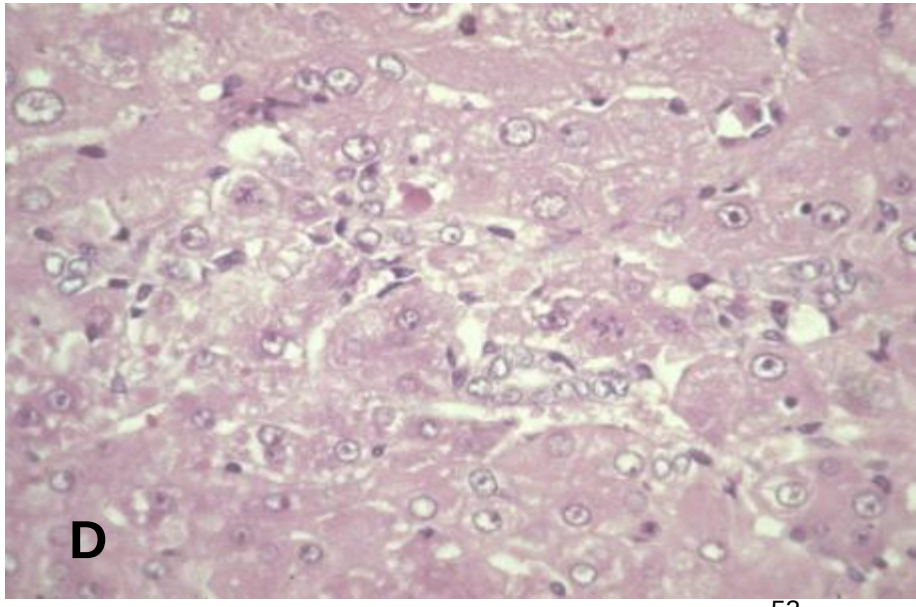
TAA induced nodular hyperplasia in rat’s liver, GST (+)



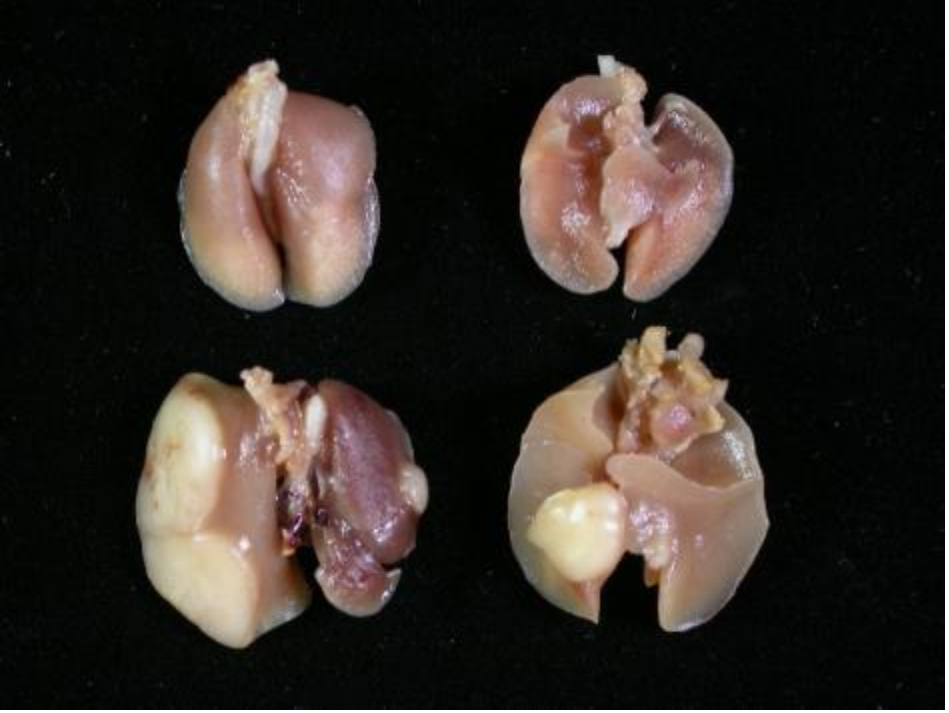


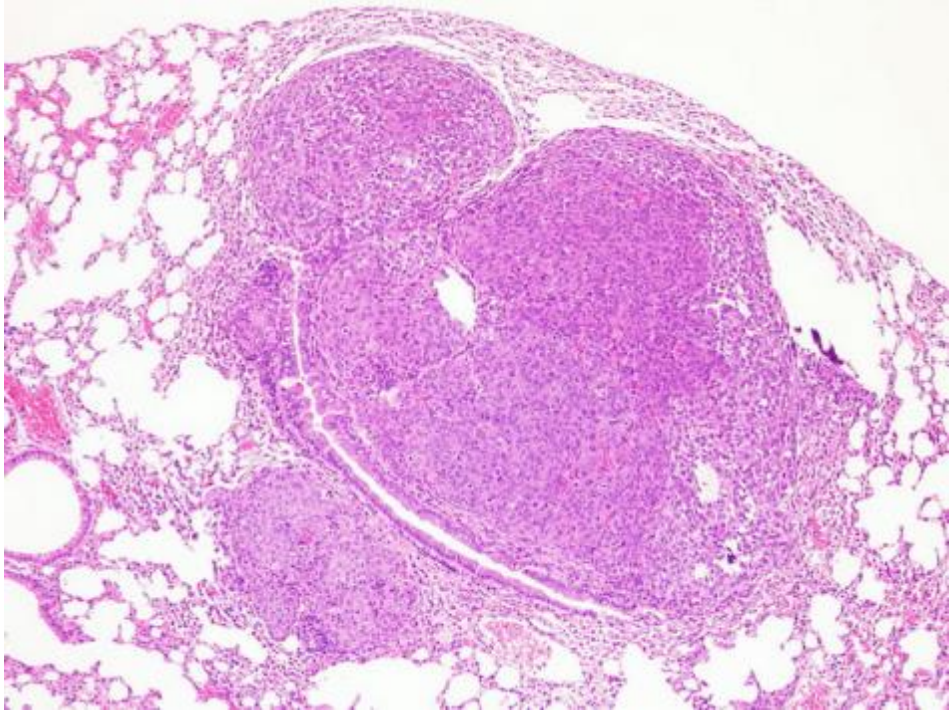
Aflatoxin B_1(1 ppm)-induced HCC in rats (12 months feeding)

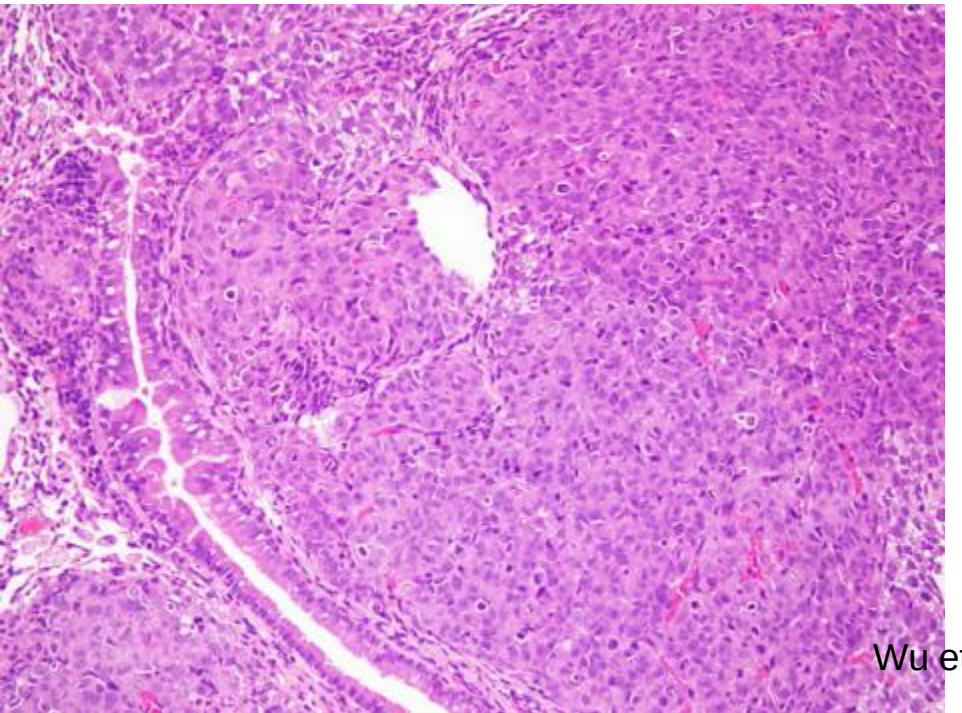


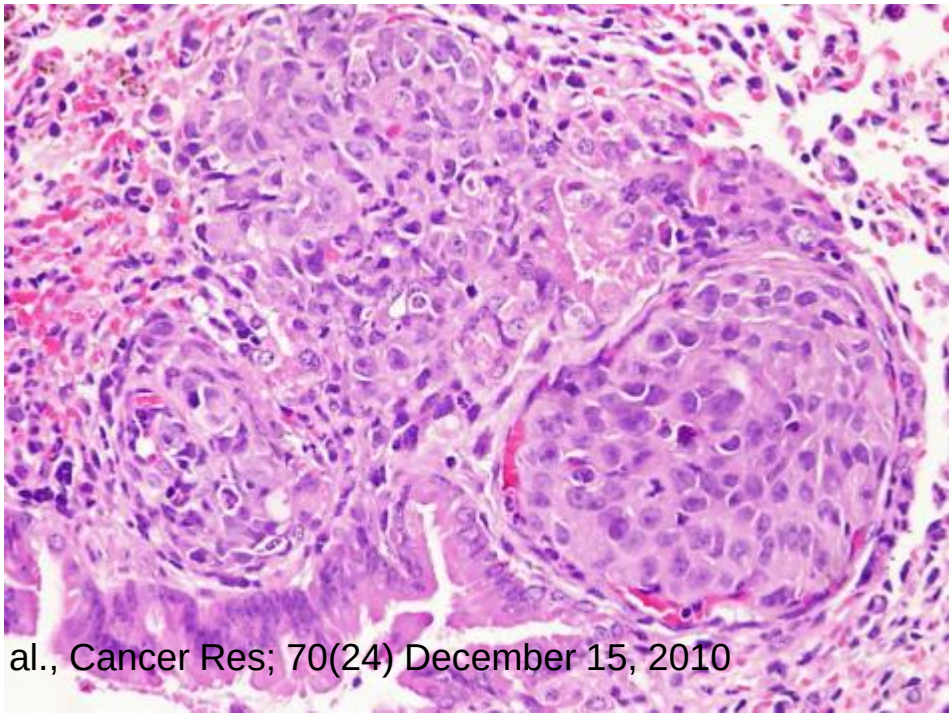


AAF(200 ppm)-induced HCC in rats (6 months feeding)









Wu et al., Cancer Res; 70(24) December 15, 2010

Recording Instruments

Microscopy

SEM &TEM

Multimedia e-learning environment

1. imaging system for *'virtual microscopy'* the later being the digital equivalent to conventional light microscopy.
2. The single images initially acquired during the scanning process are automatically stitched together to form a large seamless overview image (the *'virtual slide'*).
3. **This digital virtual microscopy image can be saved in a web-based database and is accessible for online conferencing, e.g. in pathology or histology.**

http://www.vm.ntu.edu.tw/dplab/index.htm

http://www.microscopy.olympus.eu/microscopes/Life_Science_Microscopes_dotSlide_-_Virtual_Slide_System.htm

獸醫病理生物學研究所
Graduate Institute of Veterinary Pathobiology, NCHU
國立中興大學
National Chung Hsing University

病理切片 - 儀器說明 Digital Lab Equipment

→ 廠牌型號

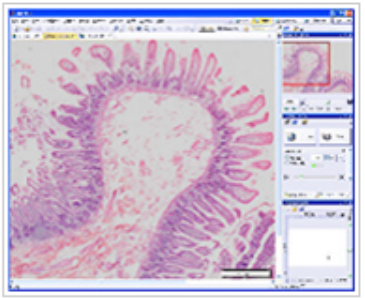
全自動病理掃描資料庫會議系統(DotSlide, Olympus, Germany)

→ 購置年月

獸醫病理生物學研究所購於2010年12月

→ 作業地點

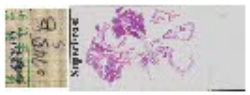
獸醫系館 4F



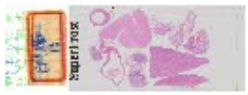
切片影像 Digital Slide Images


CSVP310-4


CSVP310-3


CSVP310-5


CSVP310-6

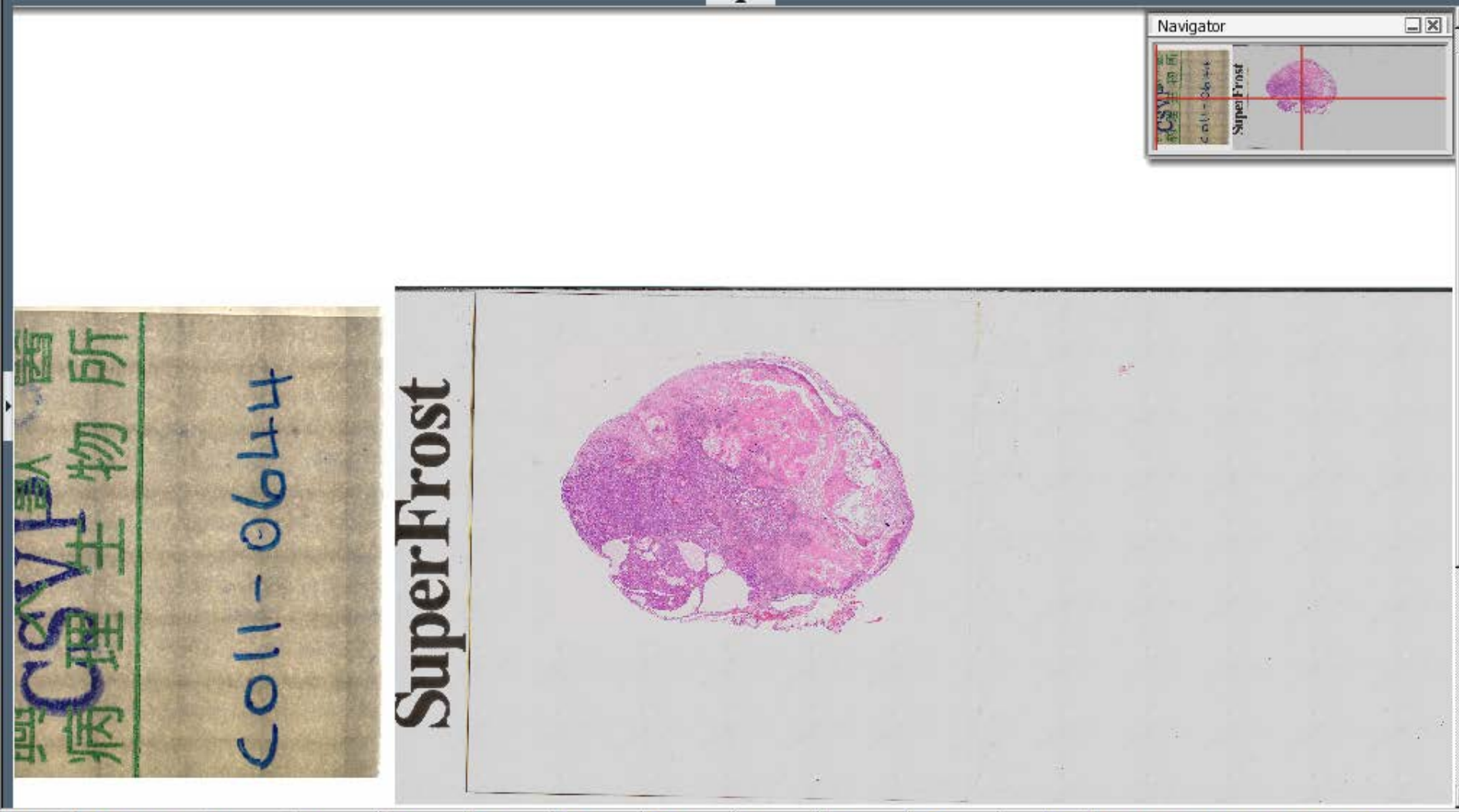

CSVP310-1b


CSVP310-1a


CSVP310-2

切片名稱：CSVP310-3

A mass measured 2.5 × 2 × 2 cm and weighed 3.0 g in the right abdomen was surgical removal from a 2.5 yr-old intact female hamster.



Quality indicators of recoding observations

- Three primary quality indicators of recording observations in toxicologic pathology reports have been identified:
  1. Thoroughness,
  2. Accuracy,
  3. Consistency

- The significance of **non-neoplastic lesions** can be recorded either semiqualitatively by applying defined **severity grades**

- or quantitatively by using **image analysis** and stereological techniques to provide numerical values for specific lesions

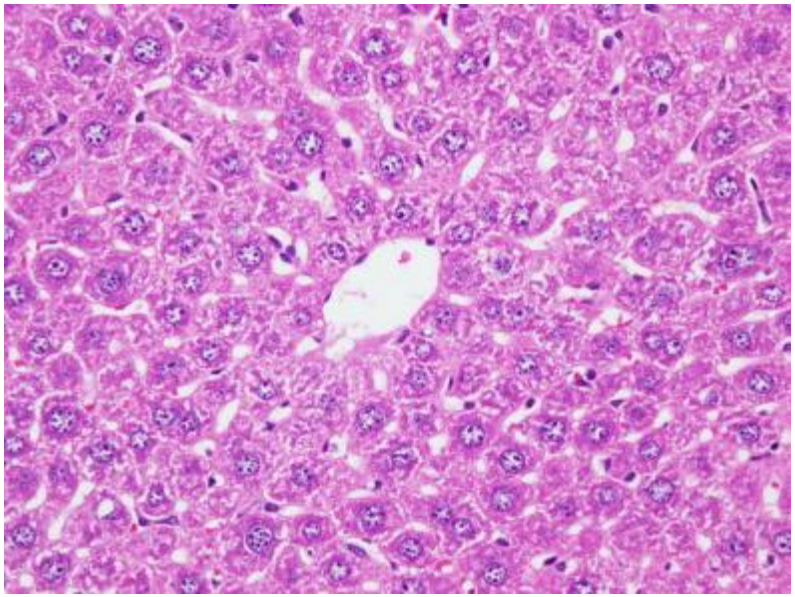
SHACKELFORD et al., TOXICOLOGIC PATHOLOGY, vol 30, no 1, pp 93–96, 2002

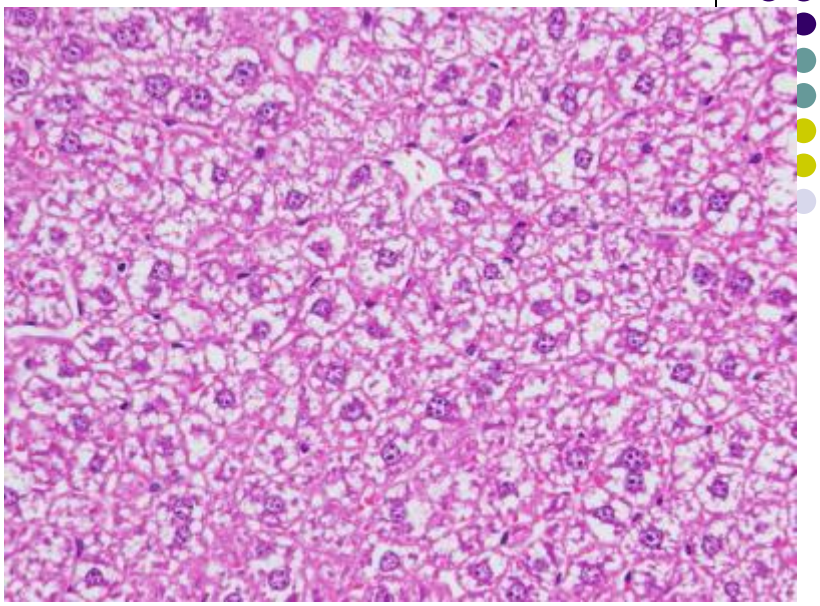
Quality indicators of recoding observations

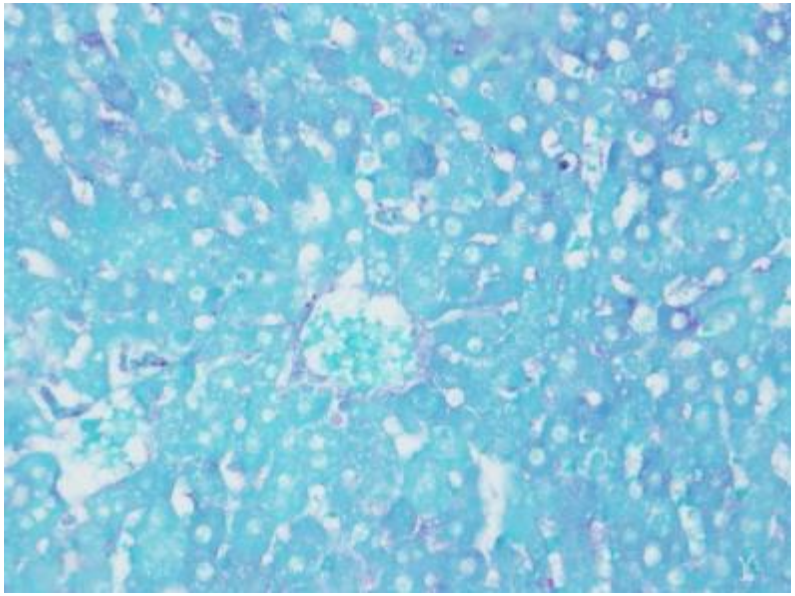
1. **Thoroughness:**
   can be defined as the recording of all lesions, including the frequently observed, usually spontaneous incidental background lesions, present in a particular organ or tissue.

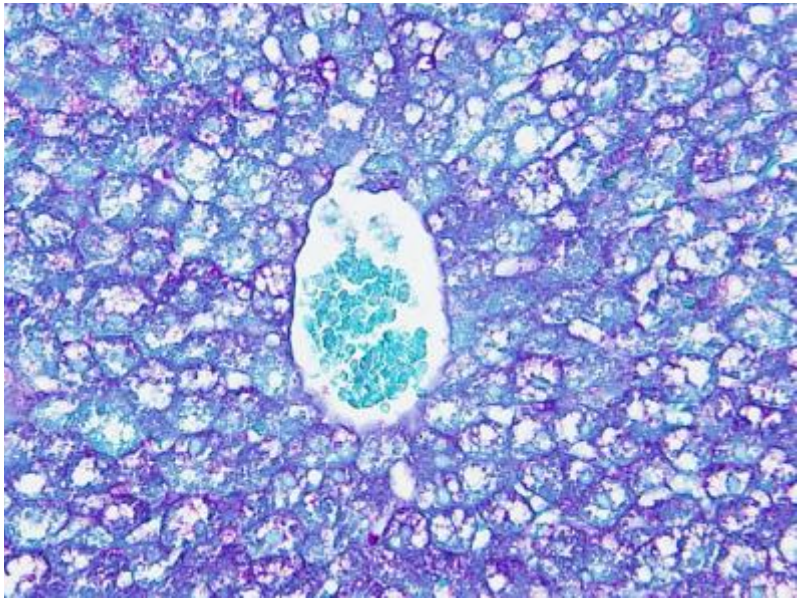
For example,
Hepatocellular cytoplasmic vacuolization is a commonly observed finding in untreated control mice, and the appearance of this finding may vary substantially from animal to animal

?? Many pathologists have the concern that their report will be reviewed **by a non-pathologist** who will not understand the nuances of pathological interpretation and will identify various lesions as significant when they are of no biological significance.



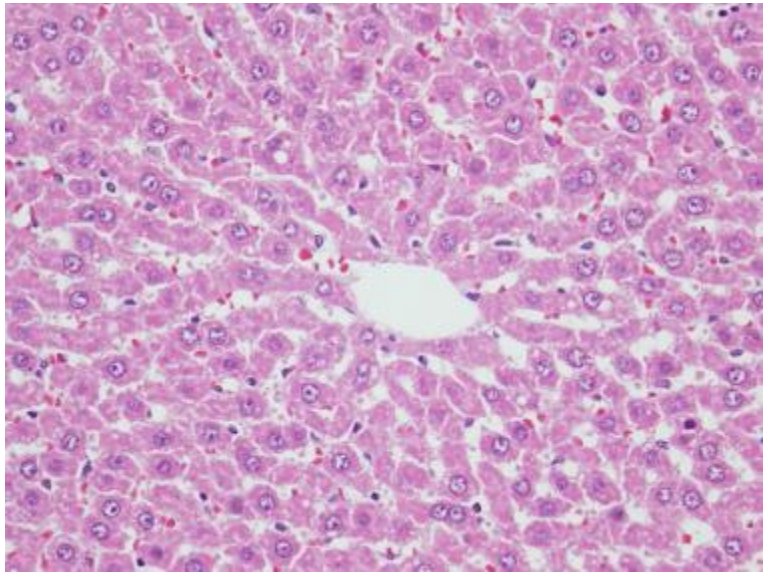




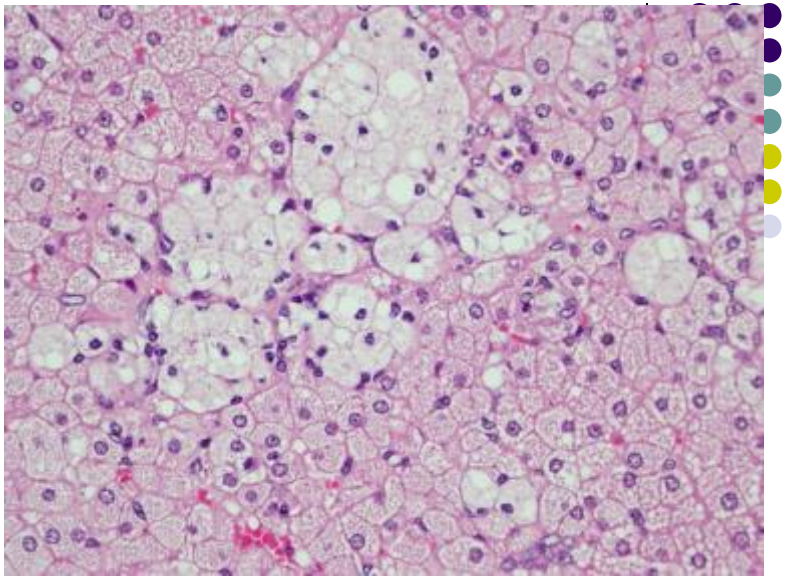


Normal fasted liver, mouse, H&E 400x

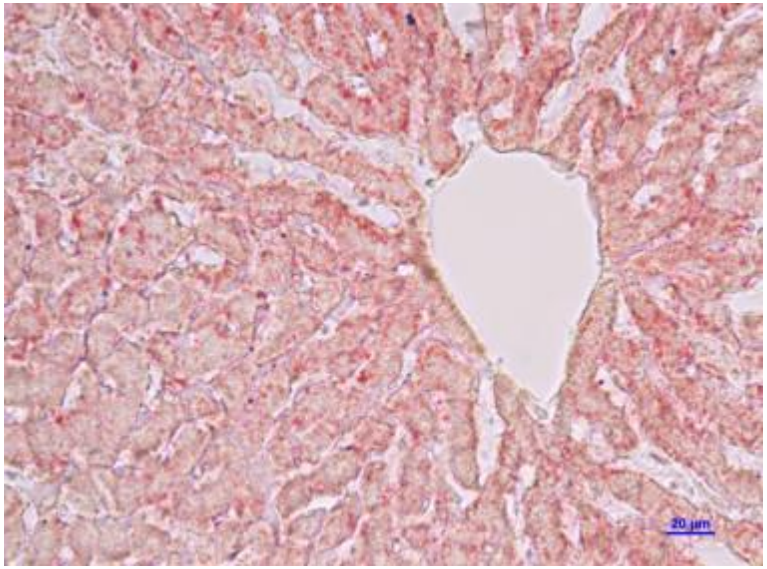
Un-fasted liver (Glycogen infiltration), mouse, H&E 400x

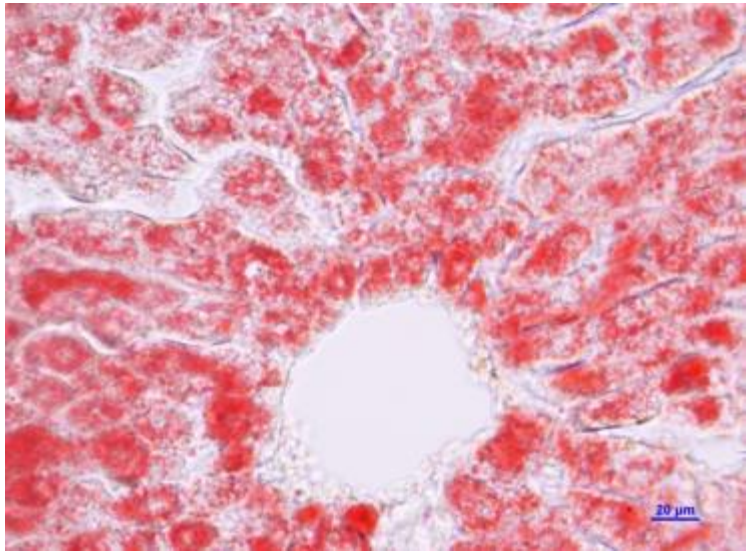


Liver, normal, rat



Fatty liver, severe, hamster





•Oil red-O stain, cryostat section

j. Synthetic-diet-induced nephrocalcinosis

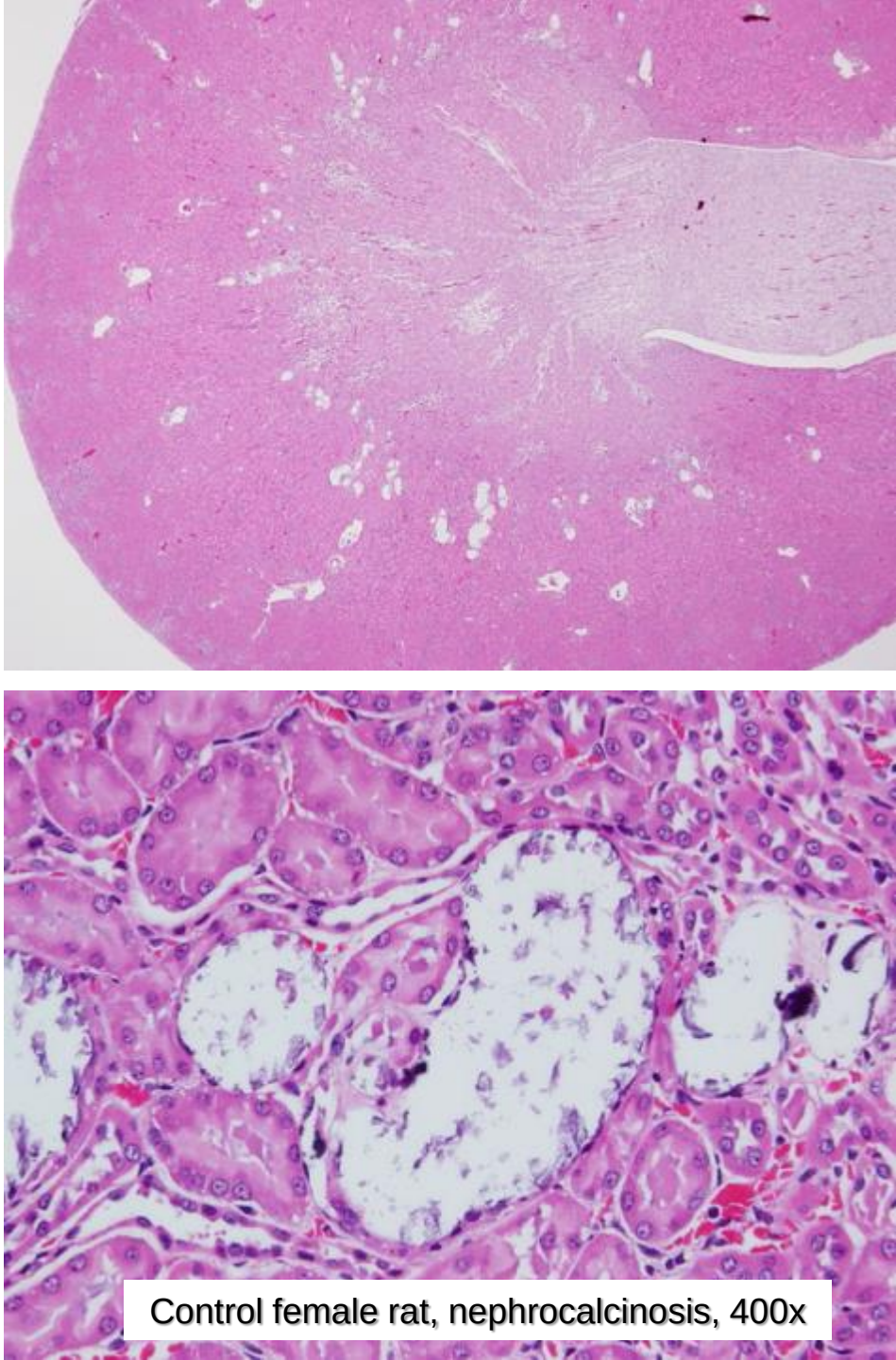
1. **Commercialized diet, AIN-76**

2. intratubular mineral deposits of calcium and phosphate salt in the pars recta

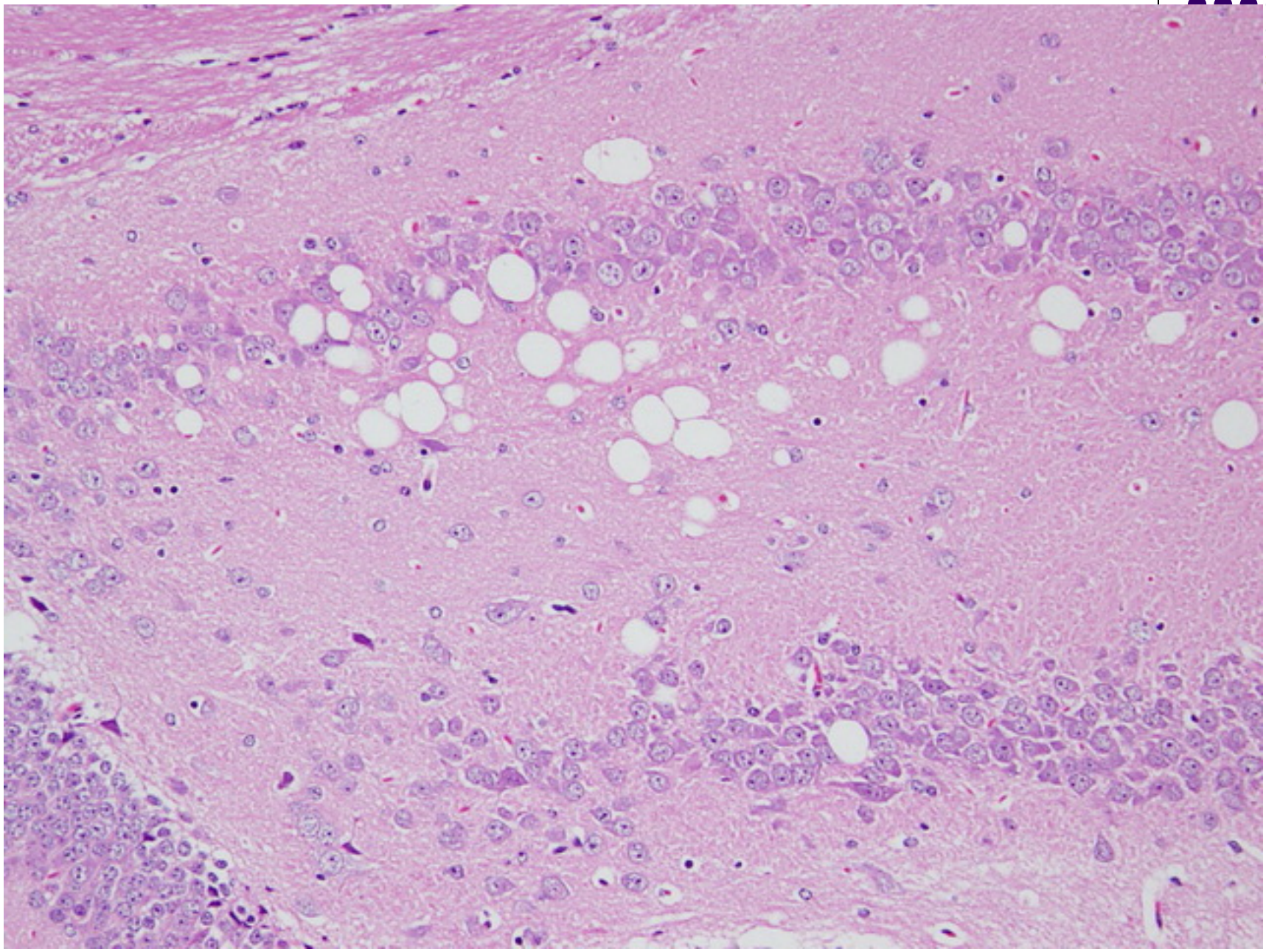
3. Change **Ca: P ratio**, increase Ca, Mg deceased or prevents mineral precipitation

4. alternating water and pH

5. associated with **estrogen**



Control female rat, nephrocalcinosis, 400x



Control male mice, vacuolization, 200x

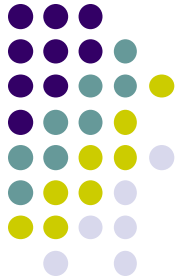
Quality indicators of recoding observations

2. "Accuracy" :

is the application of **correct terminology** when recording observed lesions. The evaluation of how accurate a pathologist was in applying terminology to a specific lesion can be rather subjective in many cases because the assignment of specific terminology may be a matter of **professional opinion**

- A diagnosis is made by consecutively defining the:
  1) **organ** topography (eg, stomach, forestomach),
  2) **site** qualifier when necessary (eg, epithelium),
  3) **morphology** (microscopic appearance or type of lesion)
  4) **severity** grade when necessary
  5) beneficial in some instances to include a distribution qualifier (eg, focal) and an indicator of chronicity (eg, acute, subacute, …).

Pathological nomenclatures



Gross finding:

No abnormalities (NA)
Left (L); Right (R)
Bilateral (B)
Slight, +
Mild, ++
Moderate, +++
Severe, ++++

Histopathological nomenclatures: (MDDDE)

Modification: Inflammation (pneumonia), tumor (fibrosarcoma), degeneration/necrosis, …
Distribution: Focal, Multifocal, Local Extensive and Diffuse
Degree: Minimal, Slight, Moderate, Moderate/Severe and Severe/High
Duration: Acute, Subacute, and Chronic
Exudate: Serous, Fibrinous, and Purulent

TABLE 1.—Some commonly used severity grading schemes.

Grading scheme I

0 = Not present
1 = Minimal (< 1%)
2 = Slight (1–25%)
3 = Moderate (26–50%)
4 = Moderately Severe/high (51–75%)
5 = Severe/high (76–100%)

Grading scheme II

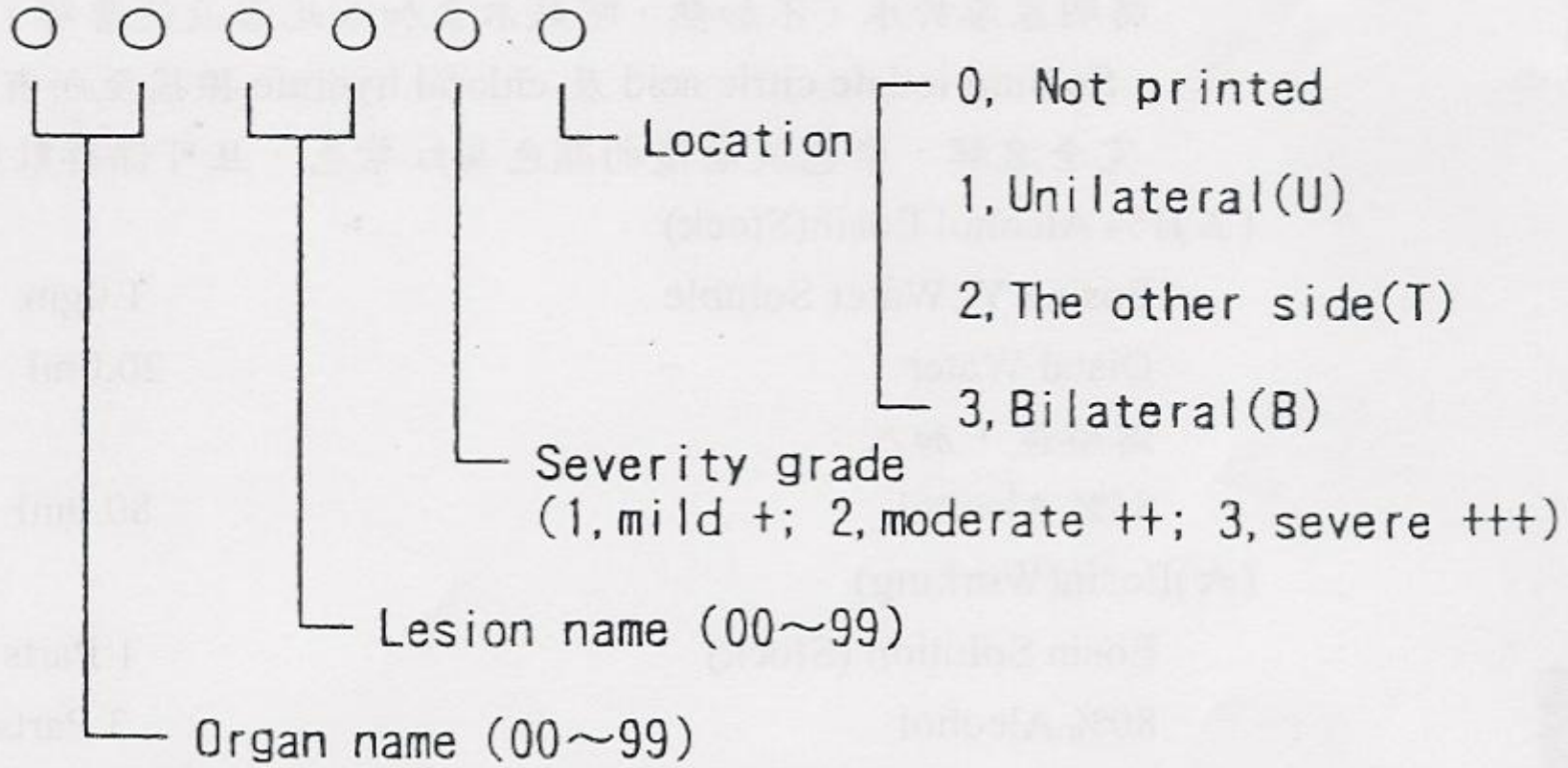
	A	B
Grade 1 = Minimal	(< 10%)	(0–25%)
Grade 2 = Mild	(10–39%)	(26–50%)
Grade 3 = Moderate	(40–79%)	(51–75%)
Grade 4 = Marked	(80–100%)	(76–100%)

Grading scheme III

Grade 1 = Minimal
Grade 2 = Slight (same as mild)
Grade 3 = Moderate
Grade 4 = Marked (same as severe)
Grade 5 = Massive (same as very severe)

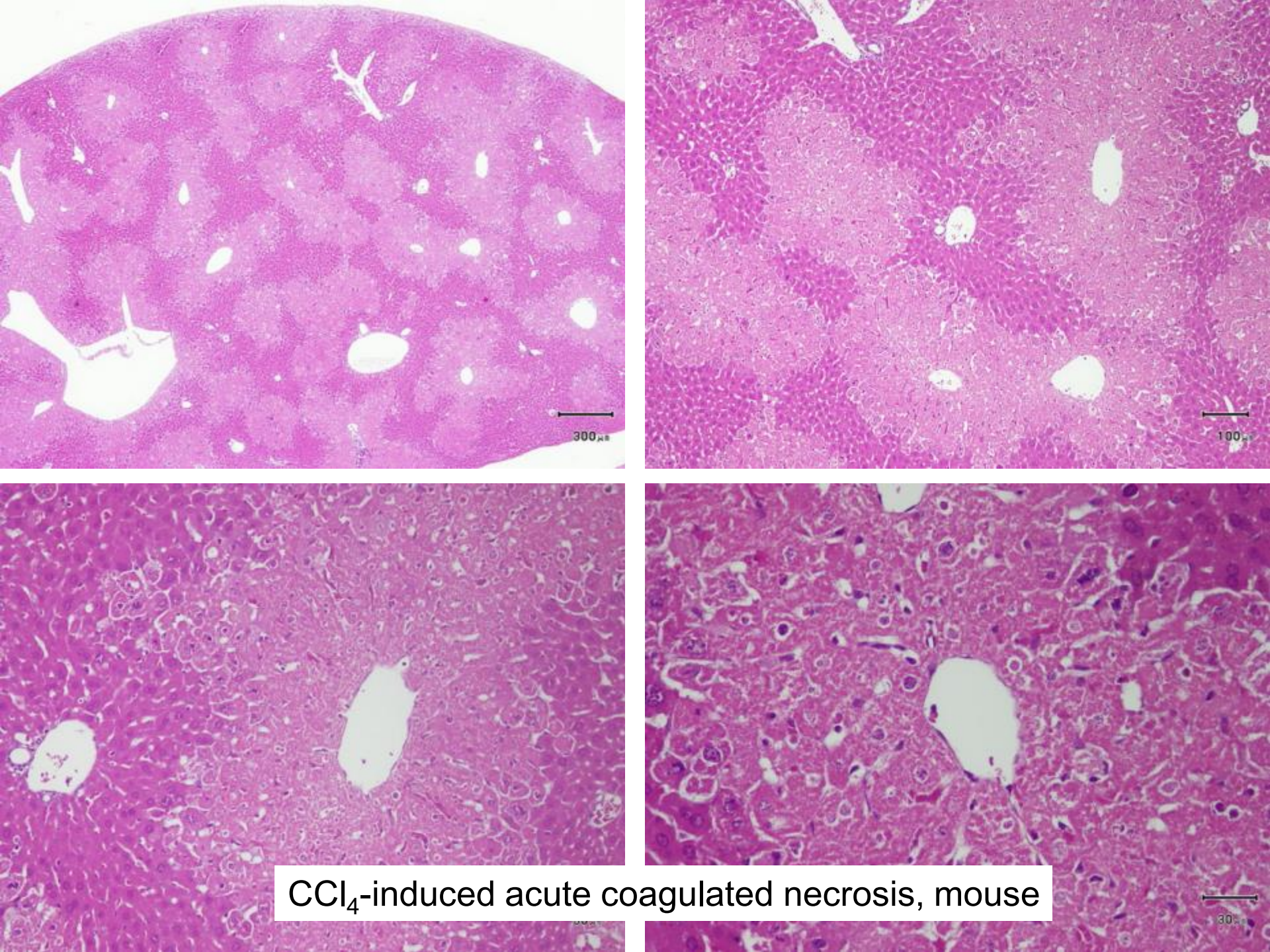
SHACKELFORD et al., TOXICOLOGIC PATHOLOGY, vol 30, no 1, pp 93–96, 2002

2) Microscopic findings (6 figures)



e.g., Liver, hepatocellular carcinoma, severe, multiple, media lobe

IET



CCl_4-induced acute coagulated necrosis, mouse

附錄1. S, ML, MM, MH, 組個別鼠之組織病理變化

Appendix 1. Pathology – individual micro findings in mice (S, ML, MM, MH)

Group	Histopathological findings	Animal code							
		S				ML			
		1	2	3	4	1	2	3	4
Liver									
	Vacuolization	3[1]	3	2	3	1	1	2	2
	Inflammation	2	2	1	2	1	1	2	2
	Necrosis	3	3	2	2	3	3	3	3

Group	Histopathological findings	Animal code							
		MM				MH			
		1	2	3	4	1	2	3	4
Liver									
	Vacuolization	1	1	2	3	2	1	2	1
	Inflammation	1	1	1	2	2	2	2	1
	Necrosis	1	1	2	2	2	2	2	2

[1]The histological indices of hepatic inflammation and necrosis were quantified based on Knodell et al. (1981) method. The liver damage was graded 0-4 as following: 1 = slight (1-25%); 2 = moderate (26-50%); 3 =moderate/severe (51-75%); 4 = severe/high (76-100%).

Table 3. Summary of pathological scores of liver injury in mice

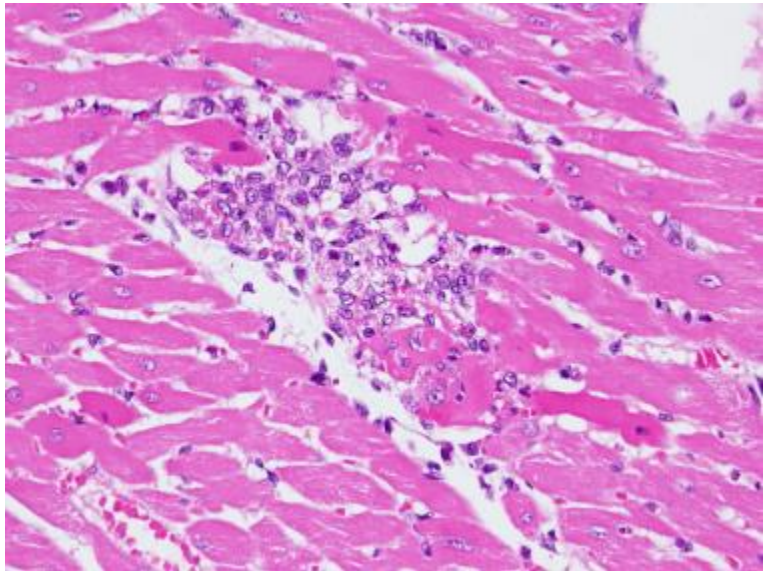
Block code	Organ	Histopathological scoring		
		Vacuolization	Inflammation	Necrosis
C	Liver	0	0	0
CCL_4	Liver	1.8±0.4*	2.0±0.0*	5.0±0.5*
S	Liver	2.7±0.5*, a	1.7±0.5*	2.7±0.5*, a
ML	Liver	1.5±0.5*	1.5±0.5*	3.0±0.0*, a
MM	Liver	1.8±0.8*	1.3±0.4*	1.5±0.5*, a
MH	Liver	1.5±0.5*	1.8±0.4*	2.0±0.0*, a

The liver damage was graded 0-4 as following: 1 = slight (1-25%); 2 = moderate (26-50%); 3 =moderate/severe (51-75%); 4 = severe/high (76-100%). The final numerical score was calculated by dividing the sum of the number per grade of affected mice by the total number of examined mice.

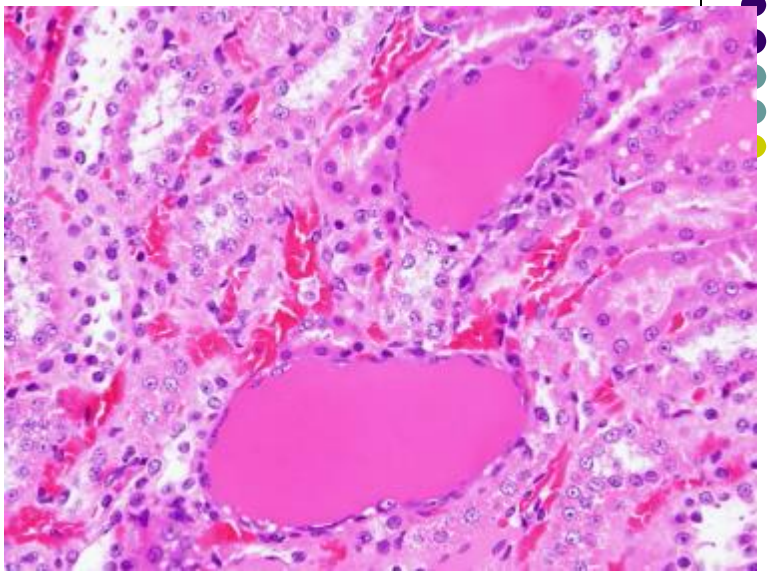
* Statistically significant difference between control and treated groups at $p<0.05$.

[a] Statistically significant difference between negative C and treated groups at $p<0.05$.

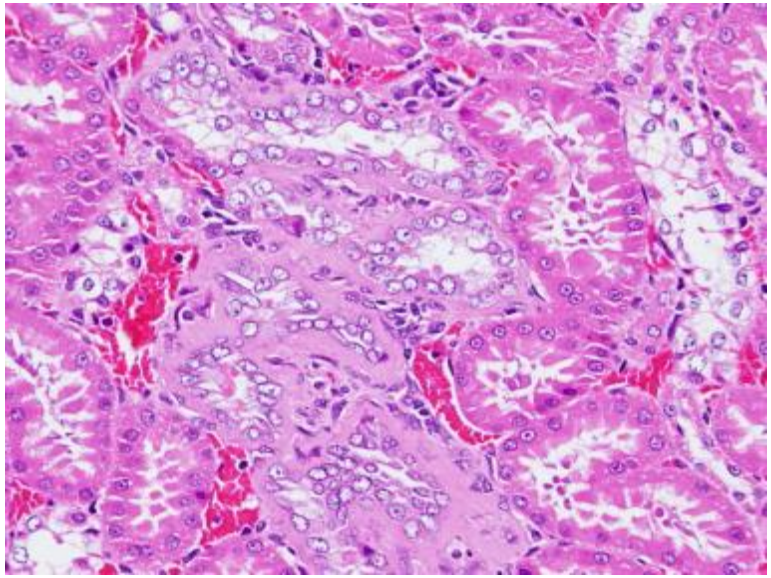
Non-specific lesions of rats in a 13-week feeding study



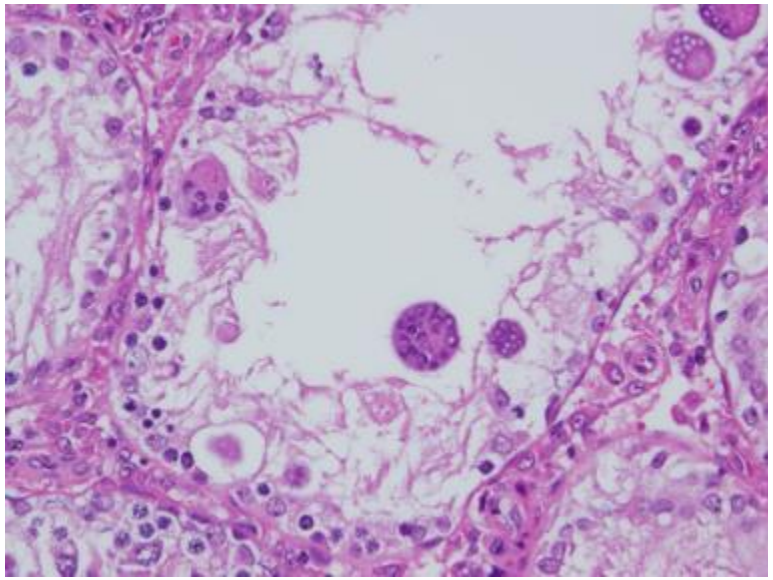
Heart, mono, 1, 400x



Kidney, cast , 2, 400x



Kidney, reg, 2, 400x



Testis, azoospermia, 5, 400x

Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn

B. Hammonda,*, R. Dudekb, J. Lemena, M. Nemetha

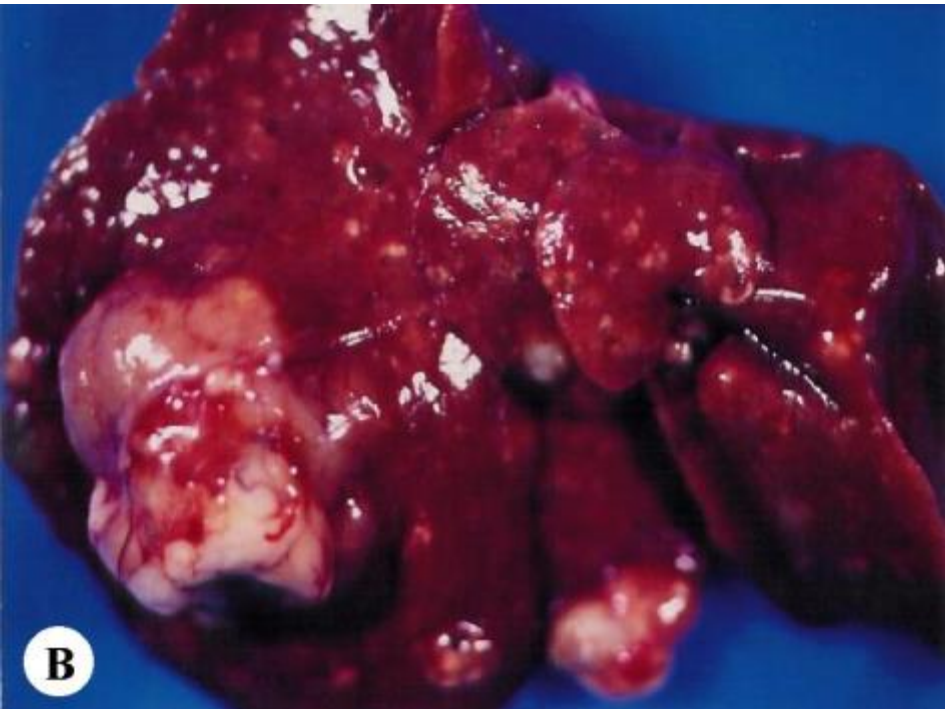
Table 7
Summary incidence microscopic findings in male and female Sprague–Dawley rats following 13 weeks of exposure to high dose (33%) test and control corn grain in the diet

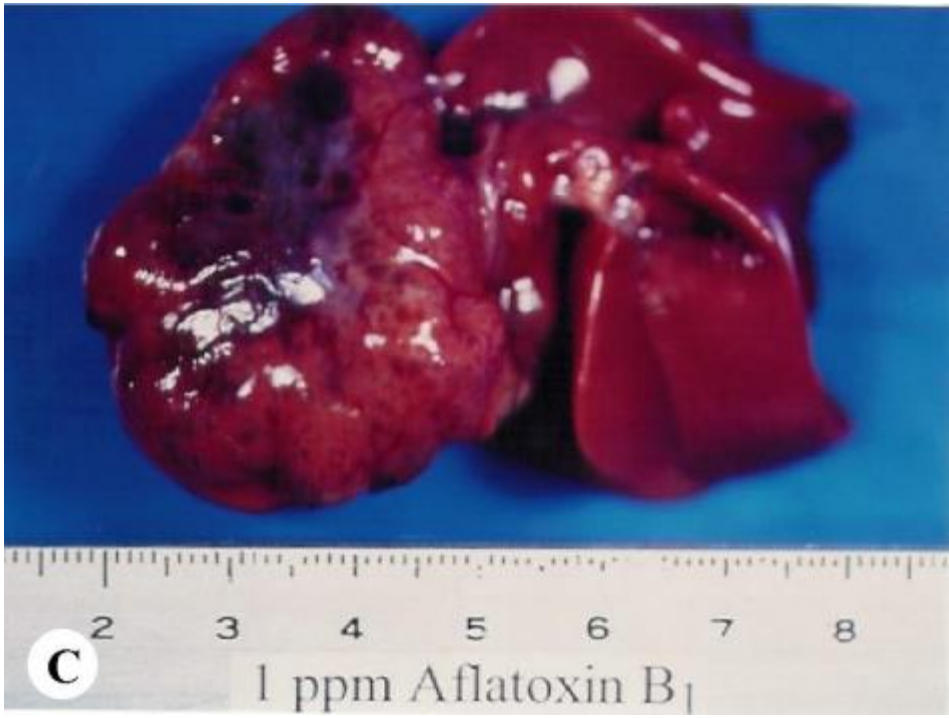
Tissue	Microscopic finding	Control Males $N=20$	RR Males $N=20$	Control Females $N=20$	RR Females $N=20$
Heart	Cardiomyopathy	4	6	3	3
Kidney	Casts, proteinaceous	5	9	3	2
	Infiltrate, mononuclear cell	14	10	4	7
	Cystic tubules	2	2	1	1
	Dilation, pelvic, unilateral	0	2	0	0
	Mineralization, tubular	0	2	6	5
	Regeneration, tubular epithelium	17	17	3	2
Liver	Infiltrate, mononuclear cell	8	8	6	7
	Inflammation, chronic, multifocal	16	17	15	17
Pancreas	Infiltrate, mononuclear cell	2	2	2	1
	Inflammation, chronic, focal	2	1	0	0
Thyroid	Cyst, ultimobranchial	3	2	5	3

Food and Chemical Toxicology 42 (2004) 1003–1014

Aflatoxin B1 and 2-acetylaminofluorene induced hepatic carcinogenicity and gamma-glutamyltranspeptidase expression via chronic feeding in rats

Liao, et al., 2002, Bull. Plant Protect. 44: 37 - 50, 2002





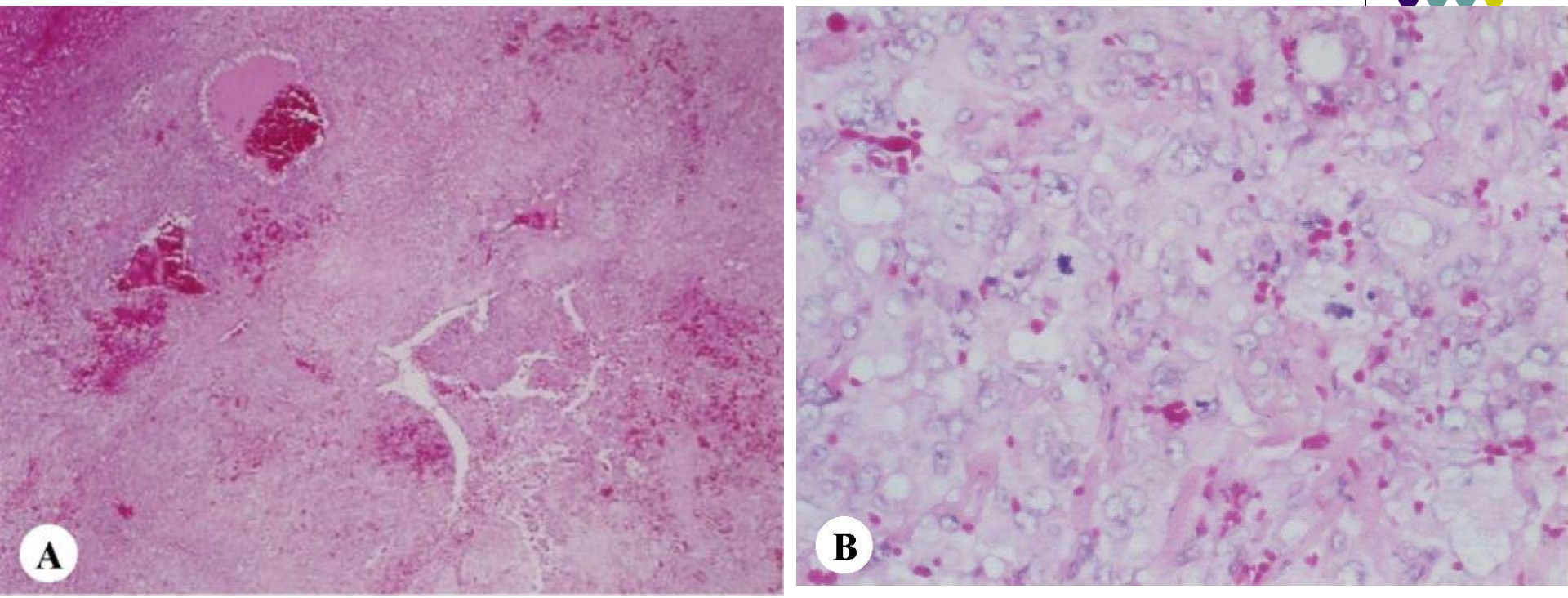


Fig. 3. Photomicrograph from Fig. 2. B. (A) Note the massive occupying and pressing to the normal hepatic cells by neoplastic cells (arrow) and massive hemorrhage (arrow head) in a 2-acetylaminofluorene treated rat. (H&E stain, 40×). (B) Higher magnification from A. Note the highly cellular mitotic figures (arrow) in the tumor masses (H&E stain, 400×).

Table 3. Histopathological incidence of rats fed continuously on a diet containing 200 ppm 2-acetylaminofluorene for 24 weeks or 1 ppm aflatoxin B_1 for 40 weeks

Organ/Lesion	Male			Female		
	Control	2-AAF	AFB_1	Control	2-AAF	AFB_1
Liver						
Preneoplastic foci	0/10[1)]	10/10	6/10	0/10	7/10	10/10
Bile duct proliferation	0/10	7/10	3/10	0/10	7/10	4/10
Cyst formation	0/10	7/10	1/10	0/10	7/10	0/10
Hepatocellular carcinoma	0/10	10/10	10/10	0/10	0/10	5/10
Lung						
Metastatic carcinoma	0/0	2/10	1/10	0/0	0/0	0/10
Skin						
Squamous cell carcinoma	0/10	1/10	0/10	0/10	0/10	0/10
Mammary gland						
Adenoma	0/10	0/10	0/10	0/10	2/10	0/10

[1)] Data presented as number of effect animals (included dead rats)/total number of examine animals.

Quantity indicators of recoding observations

Image analysis: approaches and systems

Commercially Available Automated Analysis Systems		
System	Manufacturer	Information Source
Bliss	Bacus Laboratories, Inc	http://www.bacuslabs.com
ACIS	Clarient, Inc	http://www.clarientinc.com
IVision and GenoMx	BioGenex	http://www.biogenex.com
ScanScope Systems	DakoCytomation	http://www.aperio.com
Ariol SL-50	Applied Imaging Corporation	http://www.aicorp.com
LSC	CompuCyte Corporation	http://www.compucyte.com
AQUA	HistoRx Inc	http://www.historx.com

Q500, Lieca HistoQuest
Olympus, DP20, 72
Image Pro Plus
Nikon

Taylor and Levenson, 2006

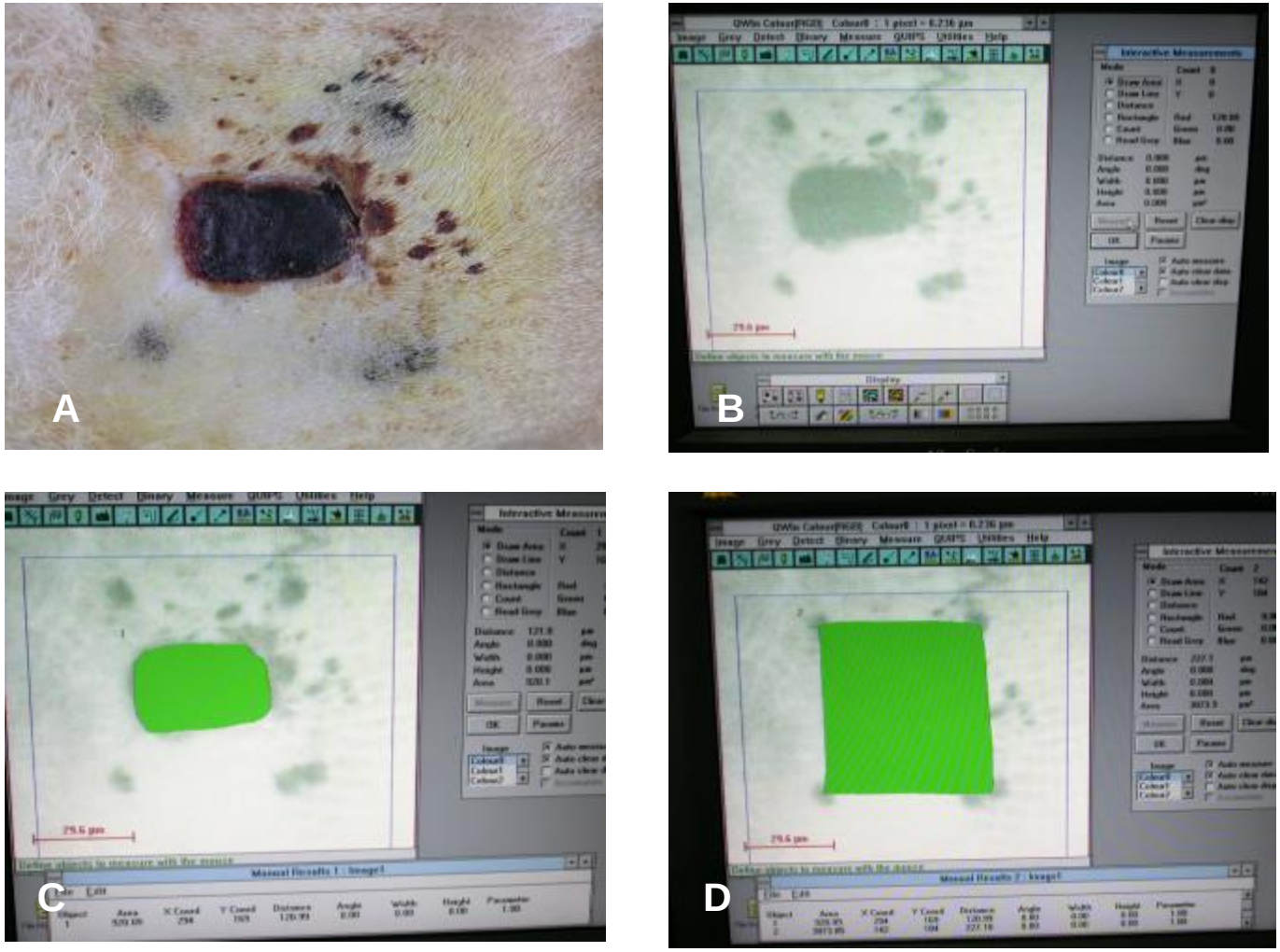


Fig. Measurement of residual wound area wound of each test material in a full thickness skin wound in rats. A. Gross finding of wound area of 1N at D-14 for example. B. Wound residual was acquired by CCD camera. C. Wound residual area (%) was calculated by the area of wound and divided with total excised area (2 x 2 cm) and then multiplied with 100.

- **Q500, Lieca**

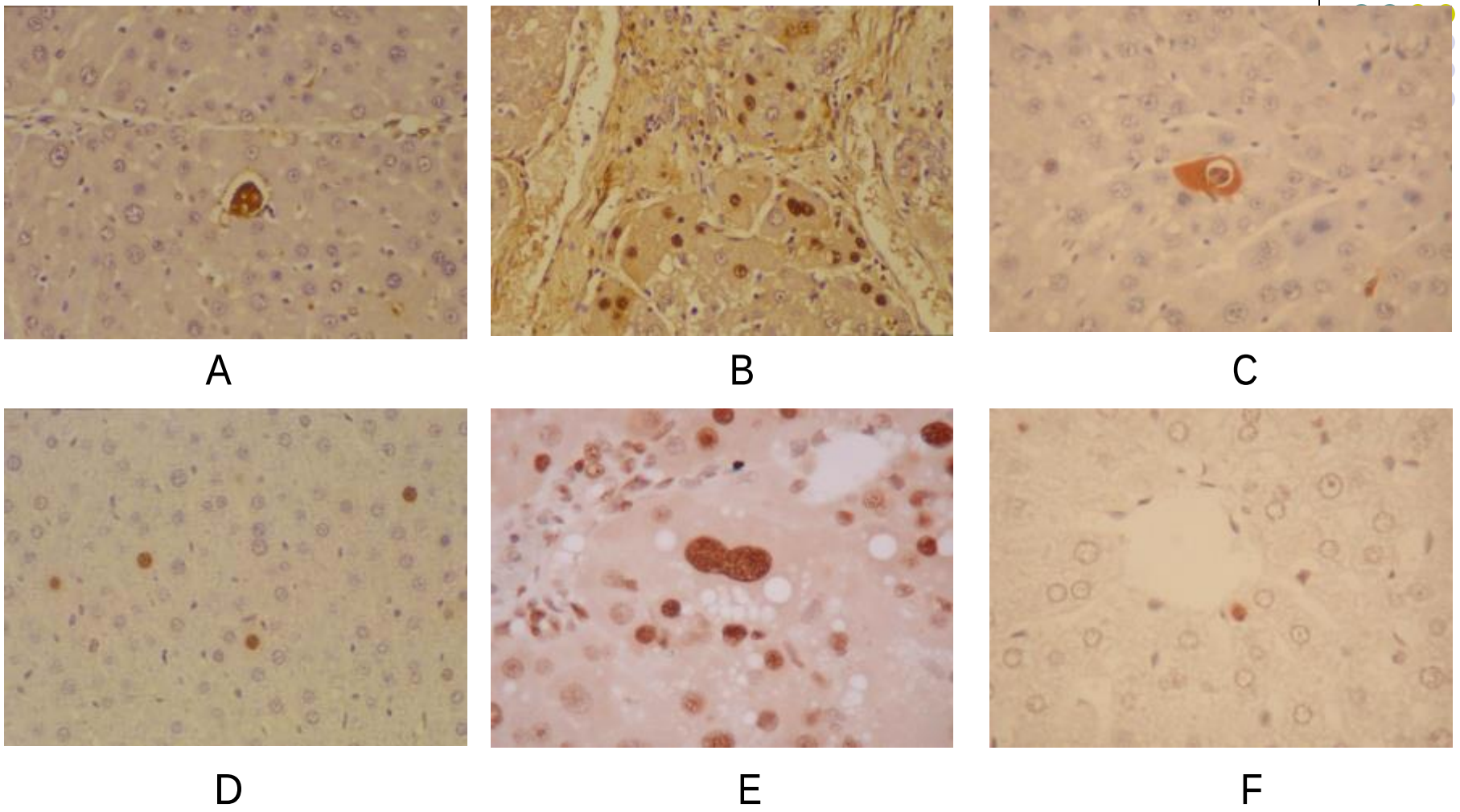


Figure 1. Photographs of immunohistochemical staining of PCNA (A, B, and C, 400x), and apoptosis (D, E, and F, 400x) of livers in the CCl_4 and yam treated rats.

Immunohistochemical counts of apoptosis and PCNA cells of rat's livers treated with silymarin or yam for 8 weeks in the CCl_4-induced cirrhosis

Group	TdT-positive cells	PCNA-positive cells
Control	0.9±0.5	3.9±1.9[1]
CCl_4 only	2.1±1.1*	11.9±5.3*
Silymarin	1.2±0.5	5.3±4.1[a]
Yam		
Low	2.2±0.6*	4.2±4.6[a]
Middle	1.7±1.1	4.0±5.3[a]
high	1.5±0.5	3.7±5.2[a]

1) Number of positive cells were counted per field.
2) Data were expressed as mean±SD (n=10).
* Significant difference between treated groups and control groups at $p<0.05$.
[a] Significant difference between treated groups and 40% CC_{l4}/i.pl groups at $p<0.05$.

- **Image analysis**

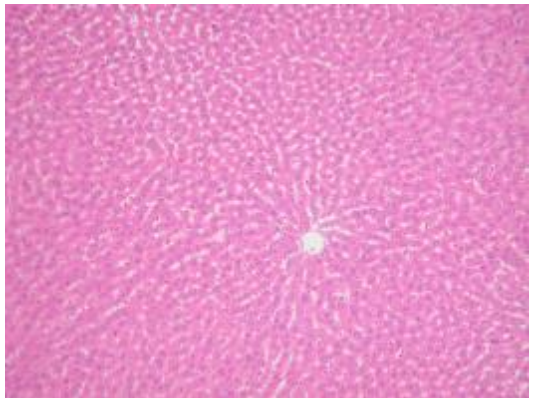
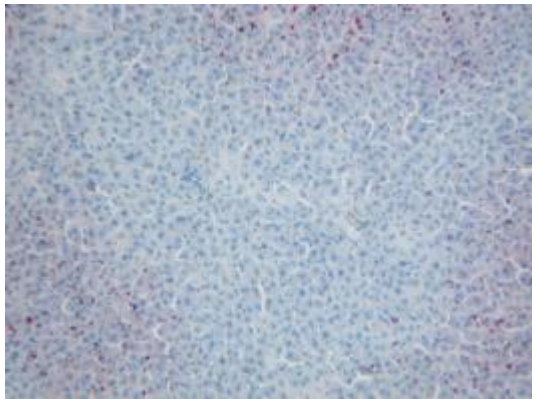
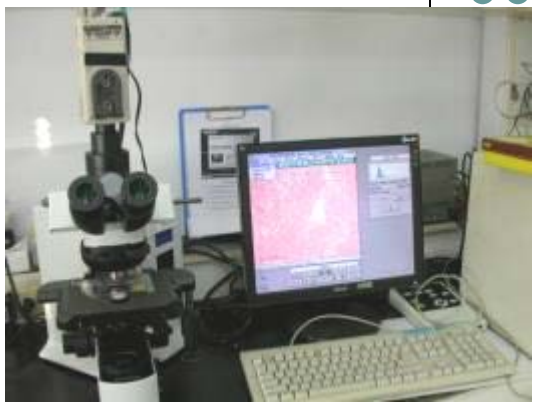
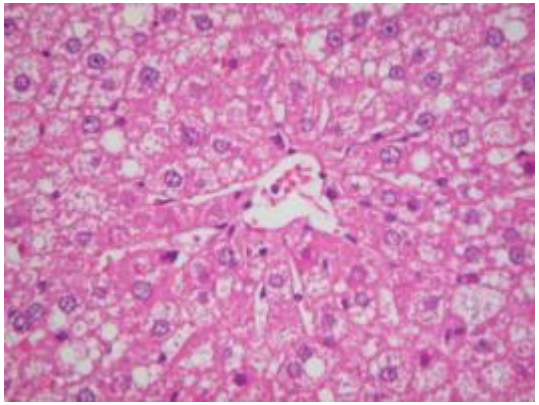
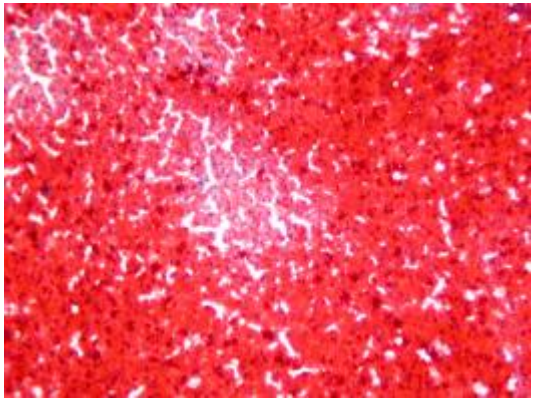
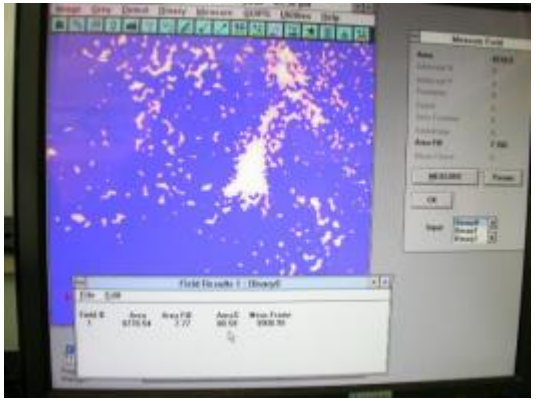







Fig. 1. Image analysis system. Fat cells were positive stained by Oil red staining in cryostat liver section. Liver sections were acquired under light microscope with 80x magnification, and then detected the positive areas, following measurement and analysis, data of positive areas (%) were counted.

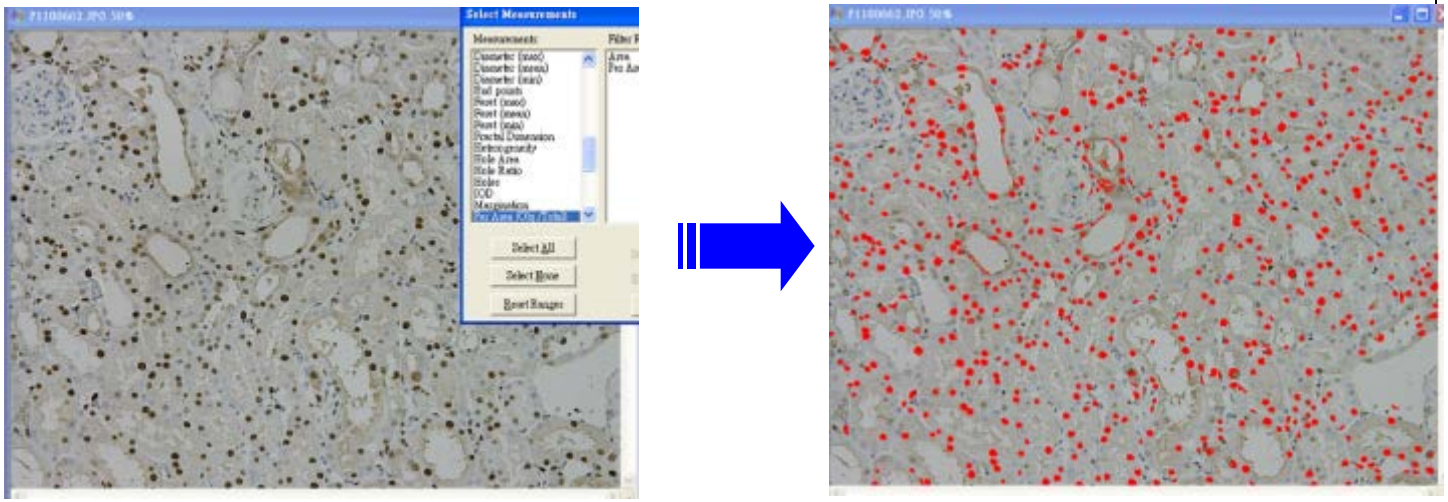
Table 2. Effect of xxx on the incidence and percentage of fatty livers in rats

Organ	Histopathological finding	Group			
		Control	High fat diet	A	B
Liver					
	Infiltration, fat droplet, diffuse, moderate to severe/high[1]	**0/5**	**5/5**	**5/5**	**5/5**
	Histopathology score of fatty liver[2]	**0**	**4.6±0.8***	**4.6±0.8***	**4.6±0.8***
	Positive area (%) of oil red stained fat cells[3]	**6.4±0.8**	**73.5±4.3***	**69.1±2.9***	**71.2±10.1***

[1] Incidence: Affected rats/ Total examined rats.
[2] Score of fatty change = Mean score of livers /Examined livers
[3] The oil red-positive areas were measured at 40x optical magnification under light microscopy, and the percentage (%) of oil red-positive area was calculated by using computerized image analyzer (Lieca, Q500MC, Nussloch, Germany). Positive area (%) of fat cell infiltration = (Positive area/Total section of liver) x 100.
* Significant difference between control and treated groups at $p < 0.05$.

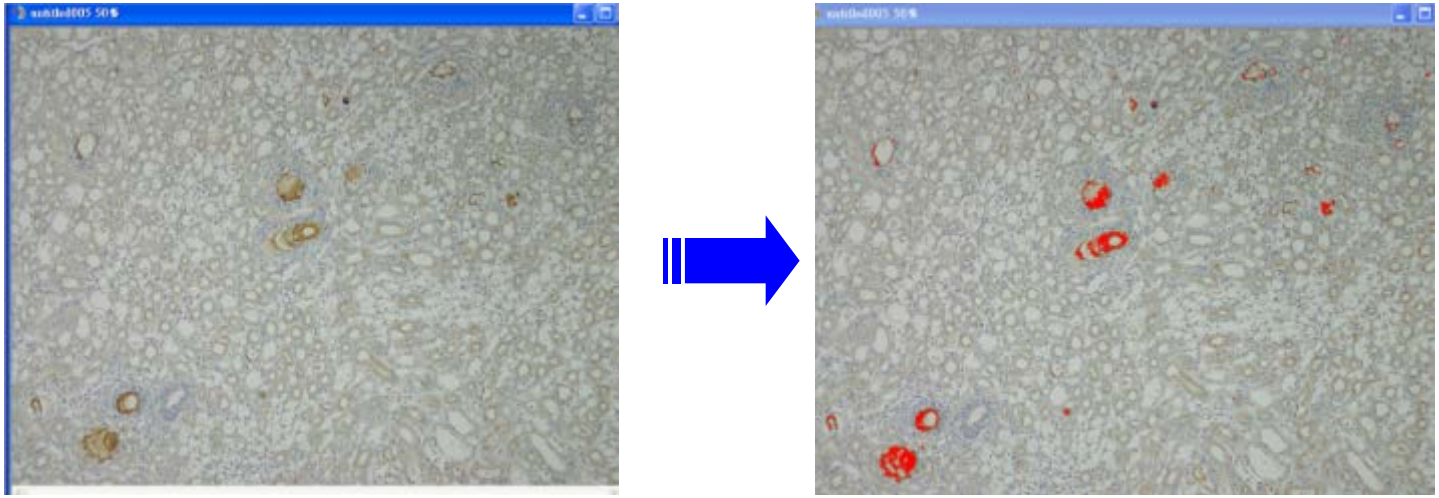
Image Pro Plus
(PCNA & OPN count)

PCNA



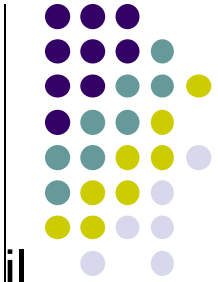
Calculation area contains 20 fields in cortex and 15 fields in medulla under 200X magnification

OPN



Calculation area contains 10 fields in cortex and medulla under 100X magnification

Interpretation of observations

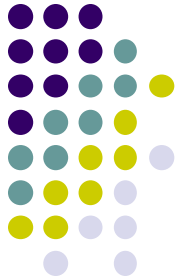


- **All lesions that occur** because of treatment, regardless of the severity of the lesions, should be identified and described in detail.
- The **majority of treatment-related lesions** must not only be identified, but must be put into a context that a scientist not familiar with pathology can understand.
- **Hypothesis**, however, should be clearly labeled such, to not mislead the reader regarding the factual nature of something that has not been tested.
- The pathologist has the **responsibility to define these points** and to put these lesions into proper context.
- **Comments** should be made when the pathologist feels that lesions **might be statistically significant, or statistically significant but not biologically relevant.**

Reasons For Pathology Peer Review

- Ensure data meets requirements of regulatory agencies
- Increase accuracy of data
- Increase confidence in data
- Confirm target organs
- Confirm “No Observed Adverse Effect Level (NOAEL)”

Experimental Pathology Laboratories Inc. (EPL, Research Triangle Park, 27709, USA)



Services

1. EPL services are delivered by experienced scientific and technical personnel.
2. These innovations include:
   Toxicologic Pathology, Pathology Consultation, Special Pathology, Toxicology Service, Service Alliances

案例研析

A repeated dose **28-day oral toxicity** study
with the GM **newly expressed proteins**

Regulatory Toxicology and Pharmacology 54 (2009) 154–163

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Acute and repeated dose (28 day) mouse oral toxicology studies with Cry34Ab1 and Cry35Ab1 Bt proteins used in coleopteran resistant DAS-59122-7 corn

Daland R. Juberg [a,*], Rod A. Herman [a], Johnson Thomas [b], Keith J. Brooks [b], Bryan Delaney [c]

[a] Dow AgroSciences, LLC, Indianapolis, IN 46268, USA
[b] Dow Chemical Company, Midland, MI, USA
[c] Pioneer Hi-Bred, International, Inc., Ankeny, IA, USA

案例研析

A repeated dose **90-day oral toxicity** study with **the stacked trait** GM feed

Food and Chemical Toxicology 53 (2013) 417–427

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Thirteen week rodent feeding study with grain from molecular stacked trait lepidopteran and coleopteran protected (DP-ØØ4114-3) maize

Bryan Delaney [a,*], Sule Karaman [a], Jason Roper [a], Denise Hoban [b], Greg Sykes [b], Pushkor Mukerji [b], Steven R. Frame [b]

[a] Pioneer Hi-Bred International, Inc., Johnston, IA, USA
[b] DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, DE, USA

Introduction

- Genetically modified (GM) **stacked trait** maize DP-OO4114- 3 (4114) containing **multigene** DNA construct that drives expression of transgenic Cry1F, Cry34Ab1/Cry35Ab1, and PAT proteins

1. resistant to certain *Lepidopteran* pests, including the European corn borer (*Ostrinia nubilalis*)

2. resistant to certain coleopteran pests, including western corn rootworm (*Diabrotica virgifera virgifera*)

3. tolerant to glufosinate herbicide

蔡伊婷, 2013

Experimental Design

- This study was conducted in accordance with OECD guidelines (No. 408; OECD, 1998) for 13 week repeated dose toxicology studies

Male and female (SD) rats
(n = 12/sex/group)
144 in total

G1: Control diet: ground 091 maize
G2: DP-OO4114-3 maize grain from glufosinate-treated plants (4114GLU)
G3: untreated plants (4114)
~~G4: reference diets 32D78~~
~~G5: reference diets 33N29~~
~~G6: reference diets 34P88~~

32% (wt/wt)
in PMI 5002

Weekly examined
body weight, food consumption, …

The last week

Blood:
Hematology and biochemical indices

91–96 day
Sacrifice

Histopathologic analysis was conducted on all rats from the 091, 4114 and 4114GLU maize grain groups.

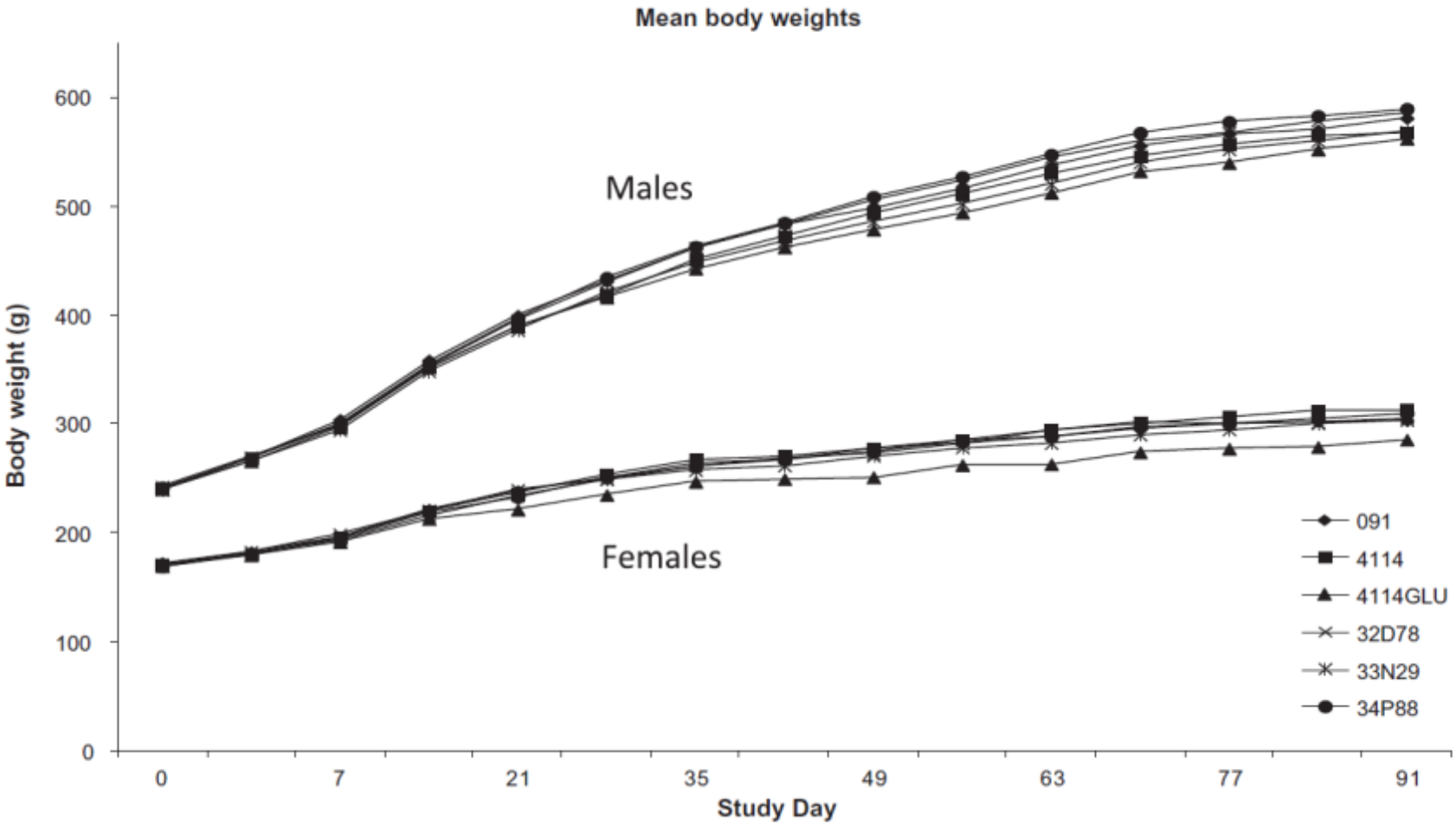
蔡伊婷, 2013

Compositional Analysis

Table 1
Nutritional composition of diets.

Nutritional analysis of rodent diets						
Analyte[a,b]	091	4114	4114GLU	32D78	33N29	34P88
Proximate analytes						
Moisture (%)	11.4	10.9	10.8	11.1	10.8	11.2
Dry matter (%)	88.6	89.1	89.2	88.9	89.2	88.8
Crude protein (% FW)	20.55	20.55	20.64	20.67	20.31	20.3
Carbohydrate (% FW)	57.5	58.0	58.2	57.6	58.3	58.1
Crude fat (% FW)	5.39	5.46	5.36	5.34	5.40	5.40
Ash (% FW)	5.20	5.07	4.98	5.30	5.13	4.98
Crude fiber (% FW)	3.83	4.27	4.39	4.22	4.39	4.28
Neutral detergent fiber (% FW)	11.3	12.8	12.7	12.5	12.4	13.0
Acid detergent fiber (% FW)	5.50	6.15	6.69	5.85	6.09	6.19
Gross energy (kcal/100 g of FW)	401	405	404	402	406	405
Amino acids (%FW)						
Arg	1.12	1.10	1.11	1.15	1.06	1.14
Cys	0.291	0.309	0.305	0.280	0.288	0.283
Gly	0.977	0.928	0.927	0.971	0.896	0.962
His	0.530	0.511	0.517	0.526	0.491	0.527
Ile	0.818	0.808	0.812	0.839	0.818	0.832
Leu	1.63	1.59	1.60	1.65	1.64	1.65
Lys	1.11	1.13	1.12	1.18	1.20	1.14
Met	0.446	0.411	0.435	0.413	0.454	0.398
Phe	0.965	0.929	0.949	0.964	0.898	0.960
Tyr	0.496	0.482	0.490	0.498	0.470	0.500
Thr	0.774	0.763	0.763	0.785	0.757	0.782
Trp	0.218	0.215	0.217	0.212	0.217	0.212
Val	0.964	0.950	0.948	0.985	0.955	0.976
Ser	0.975	0.955	0.962	0.992	0.953	0.984
Asp	1.86	1.98	1.98	2.03	2.04	1.90
Glu	3.74	3.81	3.79	3.96	4.01	3.80
Ala	1.03	1.03	1.03	1.07	1.07	1.05
Pro	1.33	1.25	1.25	1.33	1.30	1.30

Body Weight Gain



Feed Consumption

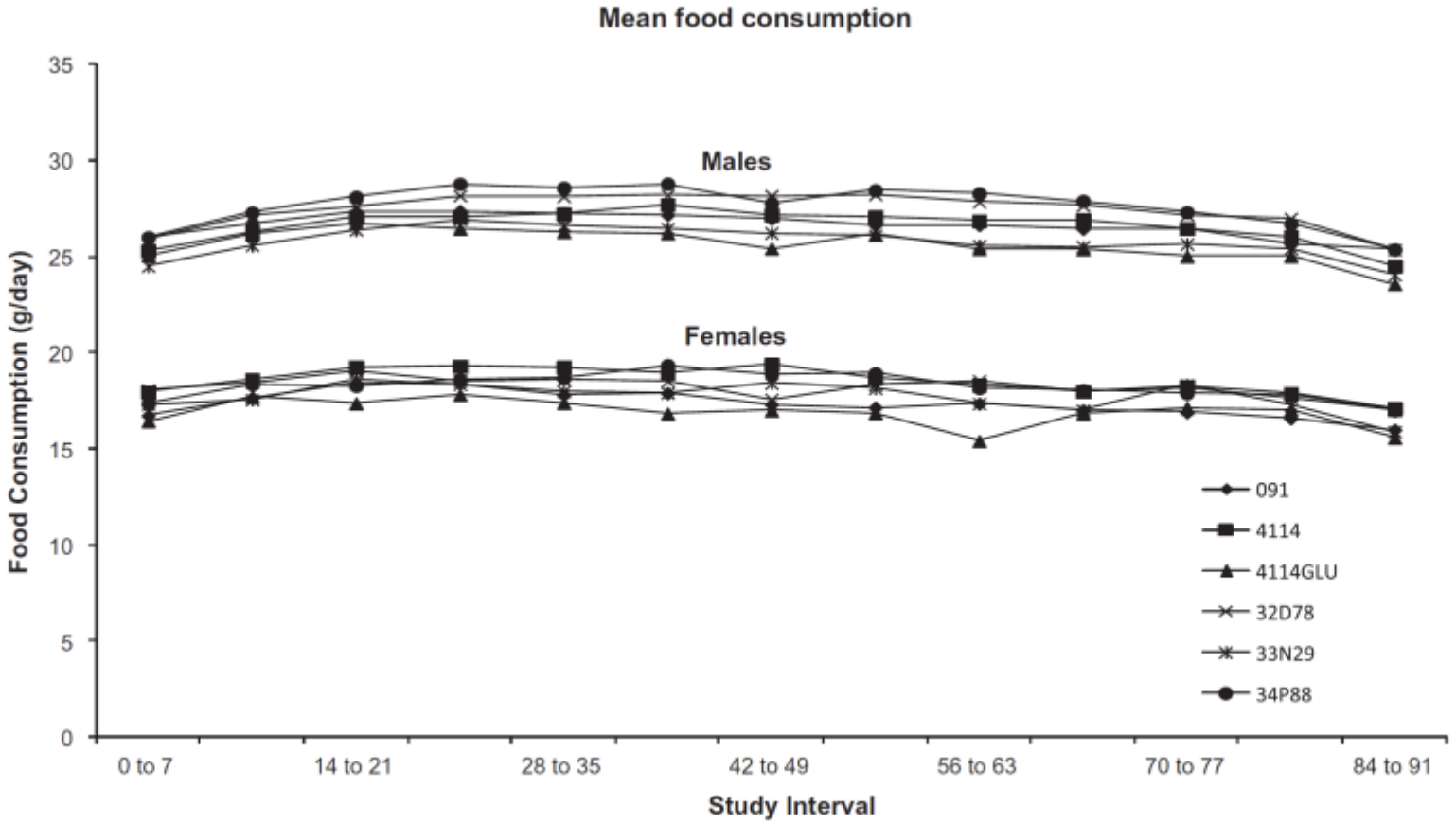


Table 2

Hematology and coagulation values for male rats (Day 92–93; mean ± SD).

Hematology

	091 *n* = 12	4114 *n* = 12	4114GLU *n* = 12	32D78 *n* = 12	33N29 *n* = 12	34P88 *n* = 12
RBC (×10^6/μL)	8.61 ± 0.27	8.81 ± 0.31	8.54 ± 0.36	8.88 ± 0.39	8.76 ± 0.27	8.88 ± 0.35
HGB (g/dL)	15.8 ± 0.4	16.3 ± 0.5*	15.8 ± 0.4	16.0 ± 0.5	15.7 ± 0.5	15.9 ± 0.6
HCT (%)	45.8 ± 1.1	47.7 ± 1.7*	46.2 ± 1.5	46.9 ± 1.6	46.0 ± 1.5	46.2 ± 1.9
MCV (fL)	53.3 ± 2.1	53.9 ± 2.0	54.1 ± 1.7	52.8 ± 1.4	52.5 ± 1.8	52.1 ± 1.2
MCH (pg)	18.4 ± 0.8	18.5 ± 0.6	18.6 ± 0.6	18.0 ± 0.6	18.0 ± 0.8	17.9 ± 0.5
MCHC (g/dL)	34.4 ± 0.6	34.3 ± 0.9	34.3 ± 0.9	34.1 ± 0.4	34.2 ± 0.7	34.4 ± 0.5
RDW (%)	12.7 ± 0.5	12.6 ± 0.7	12.9 ± 1.1	12.9 ± 0.7	12.8 ± 0.8	12.5 ± 0.5
ARET (×10^3/μL)	175.53 ± 41.97	164.40 ± 24.52	199.19 ± 72.48	186.52 ± 33.64	159.29 ± 33.47	169.99 ± 52.01
PLT (×10^3/μL)	1079 ± 71	1112 ± 261	1089 ± 76	1046 ± 87	1096 ± 91	1069 ± 95
WBC (×10^3/μL)	9.71 ± 2.50	9.12 ± 1.76	8.56 ± 1.70	10.05 ± 2.47	8.96 ± 2.12	9.36 ± 1.79
ANEU (×10^3/μL)	1.25 ± 0.31	1.20 ± 0.33	1.18 ± 0.38	1.47 ± 0.52	1.29 ± 0.45	1.11 ± 0.33
ALYM (×10^3/μL)	8.00 ± 2.36	7.45 ± 1.66	7.03 ± 1.37	8.12 ± 2.36	7.24 ± 1.92	7.81 ± 1.70
AMON (×10^3/μL)	0.20 ± 0.05	0.22 ± 0.10	0.16 ± 0.04	0.22 ± 0.06	0.22 ± 0.07	0.22 ± 0.06
AEOS (×10^3/μL)	0.16 ± 0.05	0.16 ± 0.08	0.13 ± 0.06	0.16 ± 0.04	0.14 ± 0.05	0.15 ± 0.06
ABAS (×10^3/μL)	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
ALUC (×10^3/μL)	0.08 ± 0.04	0.06 ± 0.03	0.05 ± 0.02	0.06 ± 0.03	0.06 ± 0.03	0.05 ± 0.02
PT (s)	11.2 ± 0.9	11.3 ± 0.6	11.2 ± 0.8	11.1 ± 0.4[a]	11.2 ± 0.9	11.1 ± 0.7
APTT (s)	16.3 ± 1.5	16.8 ± 1.6	16.1 ± 1.6	17.1 ± 1.0[a]	16.4 ± 1.4	16.0 ± 1.6

Hematology and coagulation values for female rats (Day 93–97; mean ± SD).

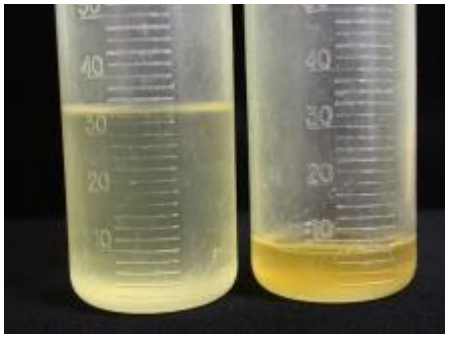
	091 *n* = 10	4114 *n* = 12	4114GLU *n* = 9	32D78 *n* = 10	33N29 *n* = 12	34P88 *n* = 10
RBC (x10^6/μL)	8.29 ± 0.32	8.23 ± 0.33	8.23 ± 0.25	8.32 ± 0.32	8.29 ± 0.37	8.32 ± 0.24
HGB (g/dL)	15.7 ± 0.6	15.7 ± 0.4	15.7 ± 0.4	16.0 ± 0.6	15.7 ± 0.5	15.9 ± 0.4
HCT (%)	45.5 ± 1.3	45.2 ± 1.2	44.9 ± 1.3	46.1 ± 1.8	45.2 ± 1.2	45.5 ± 1.2
MCV (fL)	55.0 ± 1.4	55.0 ± 2.5	54.5 ± 1.5	55.5 ± 2.7	54.6 ± 2.1	54.7 ± 1.7
MCH (pg)	19.0 ± 0.4	19.1 ± 0.6	19.1 ± 0.5	19.3 ± 0.7	19.0 ± 0.6	19.1 ± 0.6
MCHC (g/dL)	34.6 ± 0.6	34.7 ± 0.7	34.9 ± 0.5	34.8 ± 0.7	34.8 ± 0.9	34.9 ± 0.5
RDW (%)	11.3 ± 0.3	11.2 ± 0.5	11.2 ± 0.5	11.4 ± 0.3	11.4 ± 0.5	11.1 ± 0.4
ARET (×10^3/μL)	164.77 ± 28.89	136.97 ± 28.44	151.68 ± 44.40	148.05 ± 35.67	163.48 ± 37.12	144.19 ± 23.24
PLT (×10^3/μL)	1038 ± 101	932 ± 85	982 ± 106	944 ± 156	1015 ± 156	992 ± 118
WBC (×10^3/μL)	5.69 ± 1.48	6.35 ± 1.54	7.11 ± 1.59	6.79 ± 1.30	6.67 ± 2.23	6.31 ± 1.31
ANEU (×10^3/μL)	0.72 ± 0.35	0.92 ± 0.40	0.95 ± 0.37	0.82 ± 0.24	0.98 ± 0.38	0.92 ± 0.23
ALYM (×10^3/μL)	4.72 ± 1.25	5.11 ± 1.21	5.87 ± 1.44	5.65 ± 1.37	5.35 ± 1.93	5.08 ± 1.28
AMON (×10^3/μL)	0.12 ± 0.04	0.15 ± 0.09	0.13 ± 0.06	0.16 ± 0.04	0.16 ± 0.06	0.14 ± 0.05
AEOS (×10^3/μL)	0.09 ± 0.04	0.12 ± 0.04	0.10 ± 0.02	0.11 ± 0.04	0.12 ± 0.06	0.11 ± 0.03
ABAS (×10^3/μL)	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
ALUC (×10^3/μL)	0.04 ± 0.01	0.04 ± 0.03	0.06 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.05 ± 0.02
PT (s)	10.0 ± 0.3[b]	9.8 ± 0.4	9.9 ± 0.3[a]	10.0 ± 0.2[c]	9.9 ± 0.2	9.9 ± 0.2[a]
APTT (s)	13.9 ± 0.6[b]	14.1 ± 0.9	14.1 ± 0.8[a]	14.2 ± 0.8[c]	13.9 ± 0.6	14.3 ± 0.9[a]

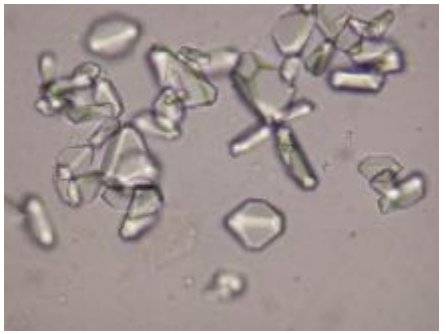
Urinalysis

Table 6
Urinalysis values (mean ± SD).

	091 *n* = 12	4114 *n* = 12	4114GLU *n* = 12	32D78 *n* = 12	33N29 *n* = 12	34P88 *n* = 12
Males						
UVOL (mL)	12.4 ± 11.8	12.2 ± 9.0	11.9 ± 9.4	13.7 ± 9.2	12.1 ± 8.3	11.5 ± 7.9
SG	1.040 ± 0.024	1.032 ± 0.015	1.035 ± 0.018	1.031 ± 0.018	1.033 ± 0.018	1.037 ± 0.020
pH	6.8 ± 0.4	6.8 ± 0.4	6.7 ± 0.3	7.0 ± 0.6	6.7 ± 0.2	6.9 ± 0.4
URO (EU/dL)	0.3 ± 0.2	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.2	0.2 ± 0.0	0.3 ± 0.2
UMTP (mg/dL)	140 ± 83	132 ± 66	144 ± 66	132 ± 89	160 ± 95	147 ± 79
Females						
UVOL (mL)	6.6 ± 4.1	6.4 ± 4.8[a]	3.9 ± 4.1[a]	6.9 ± 3.7[a]	8.1 ± 9.4	5.9 ± 4.3
SG	1.032 ± 0.013	1.036 ± 0.014[a]	1.052 ± 0.032[a]	1.031 ± -0.015[a]	1.034 ± 0.021	1.036 ± 0.024
pH	6.4 ± 0.4	6.4 ± 0.2[a]	6.6 ± 0.4[a]	6.5 ± 0.4[a]	6.5 ± 0.3	6.7 ± 0.5
URO (EU/dL)	0.2 ± 0.0	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.2 ± 0.0	0.2 ± 0.0
UMTP (mg/dL)	30 ± 15	37 ± 17[a]	66 ± 51[a]	30 ± 14[a]	33 ± 22	40 ± 33

[a]*n* = 11.
There were no statistically significant differences from 091 control at $p < 0.05$.
Results are presented as means ± SD.





Serum chemistry

Table 4

Serum chemistry values for male rats (Day 92–93; mean ± SD).

	091 *n* = 12	4114 *n* = 12	4114GLU *n* = 12	32D78 *n* = 12	33N29 *n* = 12	34P88 *n* = 12
AST (U/L)	105 ± 23	97 ± 31	104 ± 31	102 ± 25	102 ± 33	106 ± 24
ALT (U/L)	36 ± 8	34 ± 7	35 ± 3	39 ± 8	46 ± 20	36 ± 7
SDH (U/L)	7.3 ± 4.6	8.9 ± 4.5	8.5 ± 6.8	10.3 ± 6.1	13.8 ± 7.6	7.9 ± 6.0
ALKP (U/L)	80 ± 21	77 ± 18	96 ± 13@	93 ± 21	90 ± 20	82 ± 19
BILI (mg/dL)	013.±0.02	0.12 ± 0.02	0.12 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.12 ± 0.02
BUN (mg/dL)	16 ± 2	16 ± 2	15 ± 2	16 ± 2	15 ± 2	15 ± 1
CREA (mg/dL)	0.37 ± 0.04	0.38 ± 0.04	0.44 ± 0.07*	0.41 ± 0.06	0.41 ± 0.05	0.37 ± 0.05
CHOL (mg/dL)	70 ± 15	68 ± 14	62 ± 11	70 ± 13	65 ± 10	73 ± 19
TRIG (mg/dL)	70 ± 12	69 ± 23	68 ± 23	82 ± 34	66 ± 22	68 ± 19
GLUC (mg/dL)	126 ± 16	125 ± 10	134 ± 16	127 ± 14	135 ± 15	129 ± 12
TP (g/dL)	6.5 ± 0.4	6.6 ± 0.2	6.5 ± 0.3	6.7 ± 0.3	6.7 ± 0.2	6.5 ± 0.2
ALB (g/dL)	3.5 ± 0.2	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.2	3.5 ± 0.1	3.4 ± 0.1
GLOB (g/dL)	3.1 ± 0.2	3.1 ± 0.2	3.0 ± 0.3	3.2 ± 0.2	3.2 ± 0.2	3.1 ± 0.2
CALC (mg/dL)	9.8 ± 0.4	9.7 ± 0.2	9.6 ± 0.3	9.9 ± 0.3	9.8 ± 0.2	9.7 ± 0.3
IPHS (mg/dL)	6.6 ± 0.4	6.4 ± 0.8	6.5 ± 0.8	6.8 ± 0.5	6.8 ± 0.7	6.7 ± 0.6
NA (mmol/L)	145.5 ± 5.1	144.5 ± 2.6	144.5 ± 1.8	146.0 ± 4.1	147.0 ± 4.9	146.5 ± 5.3
K (mmol/L)	5.25 ± 0.41	5.35 ± 0.63	5.03 ± 0.65	5.33 ± 0.48	5.31 ± 0.43	5.22 ± 0.45
CL (mmol/L)	107.7 ± 3.1	107.1 ± 1.9	106.9 ± 1.8	107.5 ± 3.0	108.4 ± 3.0	107.9 ± 4.1

Serum chemistry values for female rats (Day 93–97; mean ± SD).

	091 *n* = 12	4114 *n* = 12	4114GLU *n* = 12	32D78 *n* = 11	33N29 *n* = 12	34P88 *n* = 12
AST (U/L)	117 ± 33[a]	109 ± 22	114 ± 35	104 ± 12	99 ± 13	123 ± 59
ALT (U/L)	42 ± 18[a]	36 ± 9	37 ± 12	34 ± 6	32 ± 6	41 ± 41
SDH (U/L)	13.2 ± 5.5[b]	8.4 ± 5.1	10.5 ± 5.1	9.1 ± 5.1	14.1 ± 7.4	14.3 ± 8.5
ALKP (U/L)	62 ± 17[a]	54 ± 17	59 ± 20	58 ± 18	64 ± 19	52 ± 24
BILI (mg/dL)	0.15 ± 0.02[a]	0.15 ± 0.03	0.15 ± 0.03	0.15 ± 0.03	0.14 ± 0.02	0.16 ± 0.03
BUN (mg/dL)	18 ± 2	17 ± 2	18 ± 2	18 ± 2	18 ± 2	18 ± 2
CREA (mg/dL)	0.49 ± 0.06[a]	0.46 ± 0.04	0.46 ± 0.06	0.46 ± 0.07	0.50 ± 0.05	0.47 ± 0.07
CHOL (mg/dL)	77 ± 15[a]	83 ± 13	79 ± 20	77 ± 10	86 ± 17	82 ± 11
TRIG (mg/dL)	60 ± 20[a]	59 ± 16	59 ± 17	56 ± 11	75 ± 23	63 ± 15
GLUC (mg/dL)	116 ± 15[a]	117 ± 7	121 ± 15	114 ± 11	120 ± 14	121 ± 15
TP (g/dL)	7.0 ± 0.4[a]	7.1 ± 0.4	7.1 ± 0.4	7.2 ± 0.5	7.0 ± 0.4	7.5 ± 0.5
ALB (g/dL)	4.0 ± 0.3[a]	4.0 ± 0.2	4.0 ± 0.2	4.0 ± 0.3	3.9 ± 0.2	4.2 ± 0.3
GLOB (g/dL)	3.0 ± 0.2[a]	3.1 ± 0.3	3.1 ± 0.2	3.2 ± 0.3	3.1 ± 0.3	3.3 ± 0.3
CALC (mg/dL)	10.3 ± 0.3[a]	10.2 ± 0.3	10.1 ± 0.3	10.3 ± 0.3	10.2 ± 0.4	10.4 ± 0.4
IPHS (mg/dL)	5.5 ± 1.3	5.0 ± 0.8	4.8 ± 0.5	5.6 ± 0.5	5.3 ± 0.6	5.2 ± 0.6
NA (mmol/L)	146.2 ± 0.33	142.7 ± 4.5	147.0 ± 6.8	148.9 ± 9.1	144.9 ± 3.8	145.1 ± 5.8
K (mmol/L)	5.09 ± 0.33	4.80 ± 0.27*	4.70 ± 0.52*	5.27 ± 0.32	4.90 ± 0.31	4.77 ± 0.25
CL (mmol/L)	108.5 ± 4.4	107.0 ± 3.4	110.2 ± 5.4	110.4 ± 6.9	107.5 ± 1.9	107.5 ± 4.8

Organs Weights and Pathology

Table 7
Organ/body weight ratios (mean ± SD).

	091 *n* = 12	4114 *n* = 12	4114GLU *n* = 12	32D78 *n* = 12	33N29 *n* = 12	34P88 *n* = 12
Males						
Body weight (final, g)	552.7 ± 57.0	540.4 ± 35.4	532.5 ± 65.6	557.8 ± 61.5	542.3 ± 49.3	560.6 ± 55.1
Adrenals	0.012 ± 0.002	0.011 ± 0.001	0.012 ± 0.002	0.011 ± 0.001	0.012 ± 0.002	0.011 ± 0.002
Brain	0.387 ± 0.041	0.395 ± 0.024	0.400 ± 0.050	0.378 ± 0.037	0.401 ± 0.038	0.381 ± 0.033
Epididymides	0.292 ± 0.030	0.284 ± 0.030	0.272 ± 0.032	0.272 ± 0.050	0.284 ± 0.021	0.280 ± 0.039
Heart	0.293 ± 0.029	0.285 ± 0.014	0.302 ± 0.029	0.295 ± 0.021	0.290 ± 0.022	0.294 ± 0.027
Kidneys	0.595 ± 0.051	0.586 ± 0.047[a]	0.584 ± 0.055	0.598 ± 0.059	0.592 ± 0.051	0.616 ± 0.058
Liver	2.594 ± 0.293	2.603 ± 0.175	2.540 ± 0.248	2.678 ± 0.190	5.594 ± 0.180	2.605 ± 0.180
Spleen	0.165 ± 0.036	0.154 ± 0.019	0.147 ± 0.018	0.144 ± 0.015	0.145 ± 0.026	0.156 ± 0.024
Testes	0.647 ± 0.082	0.682 ± 0.083	0.664 ± 0.090	0.626 ± 0.050	0.652 ± 0.039	0.657 ± 0.059
Thymus	0.074 ± 0.012	0.067 ± 0.014	0.086 ± 0.014	0.081 ± 0.022	0.077 ± 0.020	0.078 ± 0.015
Females						
Body weight (final, g)	288.3 ± 29.8	291.6 ± 33.6	265.1 ± 17.1	285.0 ± 35.0[a]	286.0 ± 26.6	289.0 ± 25.6
Adrenals	0.023 ± 0.004	0.023 ± 0.004	0.023 ± 0.004	0.026 ± 0.004[a]	0.023 ± 0.004	0.023 ± 0.004
Brain	0.674 ± 0.058	0.655 ± 0.062	0.708 ± 0.047	0.687 ± 0.069[a]	0.672 ± 0.061	0.653 ± 0.065
Heart	0.348 ± 0.025	0.358 ± 0.053	0.367 ± 0.018	0.354 ± 0.037[a]	0.352 ± 0.040	0.367 ± 0.040
Kidneys	0.631 ± 0.059	0.648 ± 0.067	0.681 ± 0.050	0.659 ± 0.071[a]	0.638 ± 0.038	0.636 ± 0.053
Liver	2.483 ± 0.189	2.536 ± 0.175	2.656 ± 0.209	2.569 ± 0.221[a]	2.553 ± 0.201	2.581 ± 0.137
Ovaries	0.049 ± 0.007	0.047 ± 0.010	0.052 ± 0.007	0.057 ± 0.006[a]	0.049 ± 0.008	0.048 ± 0.010
Spleen	0.179 ± 0.026	0.173 ± 0.026	0.195 ± 0.030	0.171 ± 0.014[a]	0.180 ± 0.024	0.180 ± 0.023
Thymus	0.105 ± 0.027	0.115 ± 0.018	0.120 ± 0.024	0.115 ± 0.026[a]	0.112 ± 0.019	0.106 ± 0.023
Uterus	0.273 ± 0.095	0.241 ± 0.072	0.233 ± 0.070	0.235 ± 0.100[a]	0.224 ± 0.066	0.243 ± 0.073

[a] *n* = 11.
There were no statistically significant differences from 091 Control at $p < 0.05$.
Results are presented as means ± SD.

Pathological Findings

Table 8
Summary of microscopic incidence findings.

Tissue	Finding	Male			Female		
		091	4114	4114GLU	091	4114	4114GLU
Cecum	Inflammation, mucosal	0	0	0	1	1	2
	Minimal	[0]	[0]	[0]	[1]	[0]	[1]
	Mild	[0]	[0]	[0]	[0]	[1]	[1]
Eyes	Degeneration/atrophy, retinal, multifocal	1	0	1	0	0	0
	Minimal	[1]	[0]	[0]	[0]	[0]	[0]
	Mild	[0]	[0]	[1]	[0]	[0]	[0]
	Fold/rosette, retinal, minimal	1	0	1	0	0	0
Heart	Cardiomyopathy, minimal	3	2	2	0	1	1
Kidney	Aggregates, lymphoid	5	5	6	4	4	4
	Minimal	[5]	[5]	[5]	[4]	[4]	[3]
	Mild	[0]	[0]	[1]	[0]	[0]	[1]
	Atrophy, focal tubular, minimal	1	2	0	0	0	2
	Chronic progressive nephropathy, minimal	9	11	8	1	4	3
	Adenoma, tubular, amphophilic-vacuolar, multiple, bilateral, benign, primary, incidental	0	2	0	0	0	0
	Carcinoma, tubular, amphophilic-vacuolar, multiple, unilateral, malignant without metastasis, primary, incidental	0	1	0	0	0	0
	Mild	[1]	[0]	[0]	[0]	[0]	[0]
	Hyperplasia, atypical, multifocal, bilateral	0	2	0	0	0	0
	Mild	[0]	[1]	[0]	[0]	[0]	[0]
	Moderate	[0]	[1]	[0]	[0]	[0]	[0]
	Pyelonephritis, unilateral, minimal	0	0	0	0	2	1
	Adenoma, tubular, amphophilic-vacuolar, multiple, bilateral, benign, primary, incidental	0	2	0	0	0	0
	Carcinoma, tubular, amphophilic-vacuolar, multiple, unilateral, malignant without metastasis, primary, incidental	0	1	0	0	0	0

Spontaneous renal tumors in two rats from a thirteen week rodent feeding study with grain from molecular stacked trait lepidopteran and coleopteran resistant (DP-ØØ4114-3) maize

Jerry F. Hardisty [a,*], Deborah A. Banas [a], Chirukandath Gopinath [b], William C. Hall [c], Gordon C. Hard [d], Michihito Takahashi [e]

[a] Experimental Pathology Laboratories, Inc., Research Triangle Park, NC 27709, United States
[b] Consultant in Toxicology/Pathology, 53 High Street, Alconbury, Cambridgeshire PE28 4DP, United Kingdom
[c] Hall Consulting, Inc., 12337 Sherwood Forest Drive, Mount Airy, MD 21771, United States
[d] Private Consultant, 203 Paku Drive, Tairua 3508, New Zealand
[e] Pathology Peer Review Center, Hatanodai 6-23-2, Shinagawa-ku Tokyo 142-0064, Japan

Food and Chemical Toxicology 53 (2013) 428–431

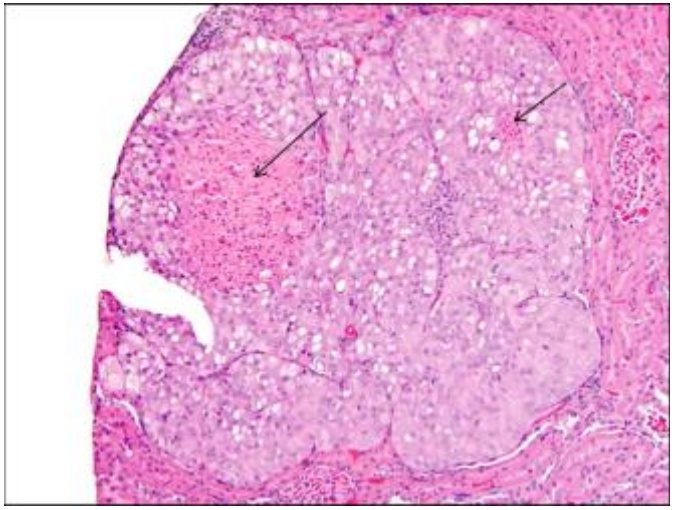


Fig. 1. Amphophilic vacuolated renal tubule adenoma containing lobules of eosinophilic to amphophilic vacuolated cells separated by a fine fibrovascular stroma. Focal necrosis is present in the center of two of the lobules(arrows).

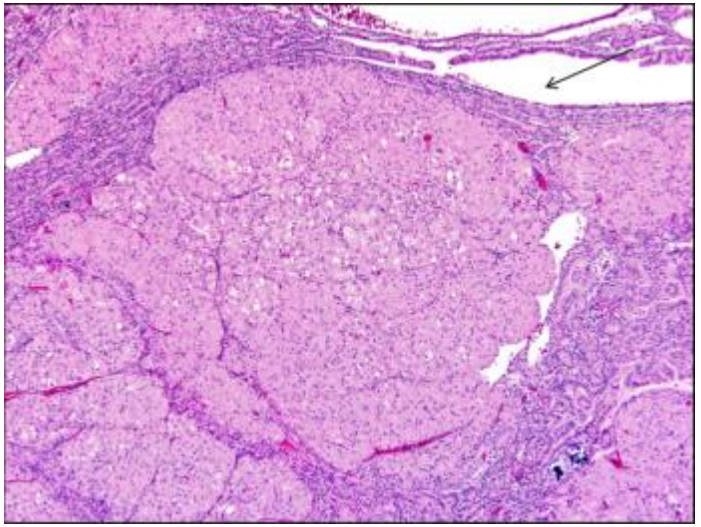
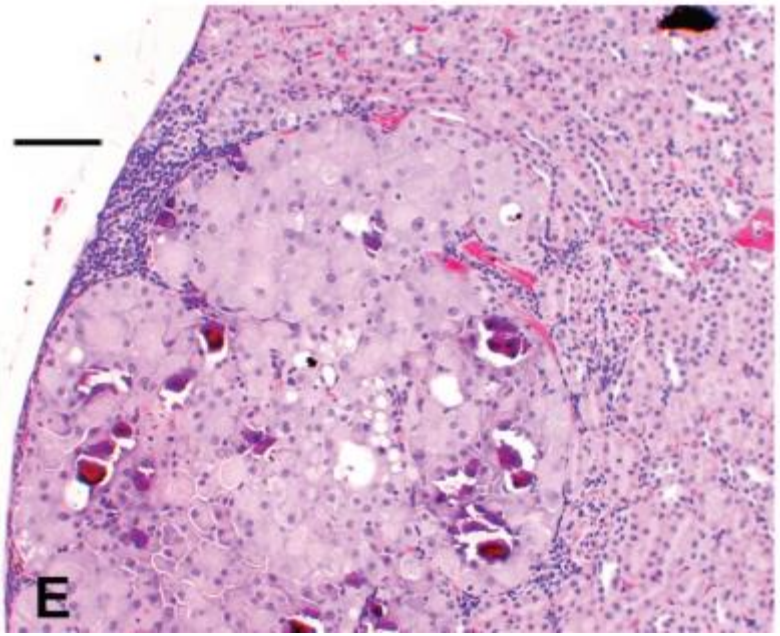


Fig. 3. Amphophilic vacuolated renal tubule carcinoma containing solid lobular proliferations of amphophilic vacuolated cells adjacent to smaller basophilic cells in a tubular pattern. Portions of the neoplasm are cystic (arrow).

Spontaneous Renal Tubular Hyperplastic and Neoplastic Lesions in Three Sprague-Dawley Rats from a 90-Day Toxicity Study

WILLIAM C. HALL,[1] BRUCE ELDER,[2] CHERYL LYN WALKER,[3] SHENG-LI CAI,[3]
DAVID G. PETERS,[4] DAWN G. GOODMAN,[4] BORGE M. ULLAND,[4] AND JOSEPH F. BORZELLECA[5]

[1]*Hall Consulting, Inc., Mount Airy, MD 21771, USA*
[2]*Rodent Genetics, Charles River Laboratories, Troy, NY 12180, USA*
[3]*Department of Carcinogenesis, University of Texas MD Anderson Cancer Center, Smithville, TX 78957, USA*
[4]*Covance Laboratories, Inc., Vienna, Virginia 22182, USA (DGG, BMU) and Madison, WI 53704 (DGP)*
[5]*Professor Emeritus, Dept. of Pharmacology and Toxicology, VA Commonwealth University, Richmond, VA 23229, USA*



(e) Animal 293. Solid neoplasm from the high-dose animal with cell degenerative changes and mineralization. The cytoplasm is lightly eosinophilic and finely granular

TABLE 2.—Incidence of renal tubular cell adenomas and hyperplasias.

	Control	Low Dose	Mid Dose	High Dose
Female Rats	0/20	0/20	1/20 (bilateral adenomas and hyperplasia)	2/20 (single or bilateral adenomas and bilateral hyperplasia)
Male Rats	0/20	0/20	0/20	0/20

Toxicologic Pathology, 35:233–241, 2007

Atypical Tubule Hyperplasia and Renal Tubule Tumors in Conventional Rats on 90-Day Toxicity Studies*

GORDON C. HARD,[1] PHILIP H. LONG,[2] JAMES W. CRISSMAN,[3] JEFFREY I. EVERITT,[4] BARRY L. YANO,[3] AND TIMOTHY A. BERTRAM[2]

[1]*American Health Foundation, Valhalla, New York 10595,*
[2]*The Procter & Gamble Co., Miami Valley Laboratories, Cincinnati, Ohio 45239,*
[3]*The Dow Chemical Company, Midland, Michigan 48674, and*
[4]*Chemical Industries Institute of Toxicology, Research Triangle Park, North Carolina 27709*

Toxicol Pathol. 1994 Sep-Oct;22(5):489-96.

TABLE I.—Study design and treatment of the 4 tumor-bearing rats in the 3 90-day studies.

Case	Strain	Sex	Test compound	Group	Route of exposure	Exposure time (wk)	Age at termination (wk)	No. of rats per group	Total rats in study
1	F-344	F	Vehicle for Compound A	Control	Inhalation	13	21	10	120
2	F-344	M	Compound B	High dose	Diet	13	19.5	10	120
3	S-D	F	Compound C	Mid-dose	Diet	13	19	20	160
4	S-D	F	Compound C	High dose	Diet	13	19	20	

Abbreviations: S-D = Sprague-Dawley; F = female; M = male.

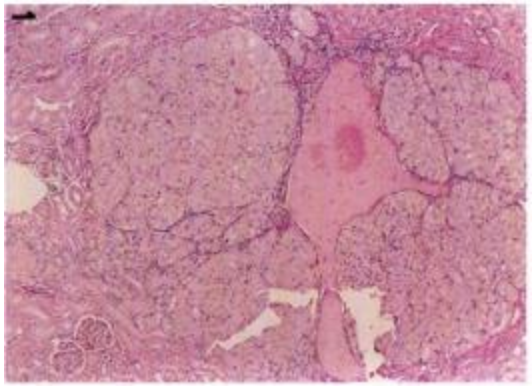


TABLE II.—Enumeration of proliferative renal lesions in the 4 tumor-bearing rats from 3 90-day studies.

Case	Foci of atypical hyperplasia	Adenomas	Carcinomas	Adenomas and carcinomas combined
1	6	2	1	3
2	18	2	2	4
3	11	2	1	3
4	13	2	0	2

Interpretation and Conclusion

- No biologically significant, treatment related differences in body weight, food consumption, clinical pathology parameters (hematology, blood chemistry, urinalysis, or organ weight) were observed in rats consuming the diets containing 4114 maize grain compared with rats fed conventional maize diets.

- At the end of the in-life phase, **renal tubule tumors** were reported **in two male rats** consuming diets containing 4114 maize grain.

1. An expert panel of pathologists was convened as a Pathology Working Group (PWG) to review coded kidney histology sections from control (091) and treated (4114 and 4114GLU) male rats.
2. The PWG concluded unanimously that the kidney tumors were characteristic of **amphophilic vacuolar (AV) tumors** and AV atypical tubular hyperplasia which represent a distinctive phenotype that has been reported to occur **sporadically in young Sprague Dawley Rats**.

Table 4-3

EPA/FIFRA Requirement for Hazard Evaluation of Pesticide and toxicants (Toxicological studies)

GUIDELINE NO.	REVISED 870 GUIDELINE	TYPE OF TOXICITY STUDY	TEST SYSTEM	OBJECTIVE	APPROXIMATE COST/STUDY (US$)
81-1	1100	Acute oral	Rats	Define toxic dose by ingestion	2000
81-2	1200	Acute dermal	Rabbits	Define toxic dose by absorption through skin	1500
81-3	1300	Acute inhalation	Rats	Define toxic dose by inhalation	5000
81-4	2400	Ocular	Rabbits	Assess eye irritation/injury	1500
81-5	2500	Skin irritation	Rabbits	Assess skin irritation/injury	1000
81-6	2600	Sensitization	Guinea pigs	Assess allergic potential	3000
81-7	6100–6855	Neurotoxicity*†	Hens/rats	Assess nervous system injury	25,000†
84-2	5100–5915	Mutagenicity‡	In vivo/in vitro	Determine genotoxic potential; screen for carcinogenicity	5,000§
82-1	3050–3465	Range-finding‡	Rats	Determine effects following	70,000
		Subacute (28- to 90-day§)	Mice	repeated doses; set dose level	70,000
			Dogs	for longer studies	100,000
			Rabbits		75,000
			Rats	Identify target organs; set dose	190,000
			Mice	levels for chronic studies	190,000
83-5	4200–	Carcinogenicity/	Rats	Determine potential to induce	1, 400,000
83-2	4300	Chronic toxicity	Mice	tumors; define dose-response relationships (lifetime)	800,000
83-1			Dogs	Determine long-term toxic effects (1 year)	400,000
83-3	3550–	Reproduction and	Rats	Determine potential to cause fetal	505,000
83-4	3800	teratogenicity	Rabbits	abnormalities and effects on development, fertility, pregnancy, and development of offspring over at least two generations	
85-1	7485	Toxicokinetics	Rats	Determine and quantitate the	100,000
			Mice	metabolic fate of a pesticide	

NT$:141,487,500

Dietary contaminants

Base on subacute, subchronic, and chronic toxicity studies:

*** No observed effect level (NOEL):**

is defined as the highest dose where there are no differences between the control and the treated group.

*** No observed adverse effect level (NOAEL):**

is defined as the highest dose where the effects observed in the treated group do not imply an adverse effect to the subject

1. The problem of pesticides and other contaminants in the diet is a complex and controversial for human and domestic animals

2. **An acceptable daily intake (ADI)** for human is based on the no observed adverse effect level **(NOAEL) in rodents tests**

3. **ADI** = NOAEL/ species difference (10) x individual viability (10) x Magnified factor (target organ, 10) (mg/kg/day)

Vol. 1, p.318-319

每日可攝取劑量
(Acceptable Daily Intake, ADI)

- ADI = NOAEL/UF (100-1000), mg/kg body weight/day)

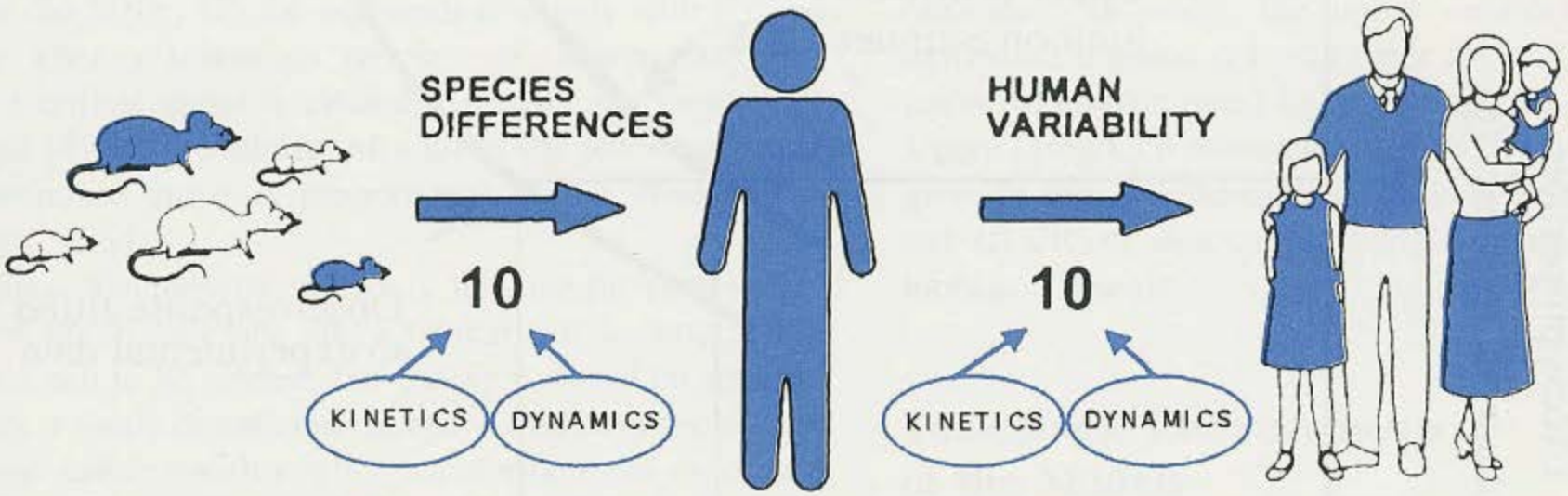


Figure 4-4. Toxicokinetic (TK) and toxicodynamic (TD) considerations inherent in interspecies and inter-individual extrapolations.

Toxicokinetics refers to the processes of absorption, distribution, elimination, and metabolism of a toxicant. *Toxicodynamics* refers to the actions and interactions of the toxicant within the organism and describes processes at organ, tissue, cellular, and molecular levels. This figure shows how uncertainty in extrapolation both across and within species can be considered as being due to two key factors: a kinetic component and a dynamic component. Refer to the text for detailed explanations. (Adapted from Renwick, 1999, 1998.)

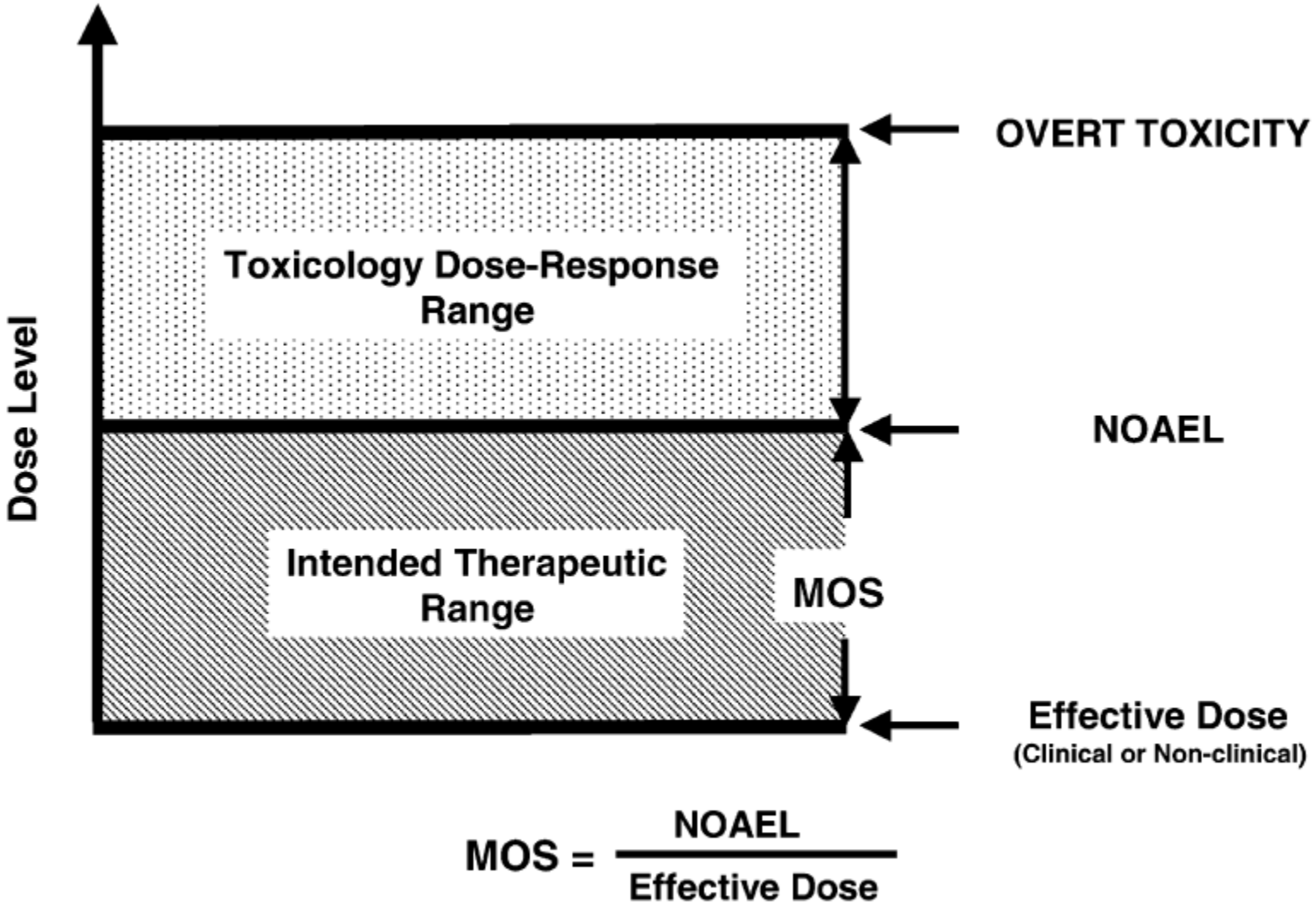


Fig. 3. Relationship of margin of safety to toxicity profile.

M.A. Dorato, J.A. Engelhardt / Regulatory Toxicology and Pharmacology 42 (2005) 265–274

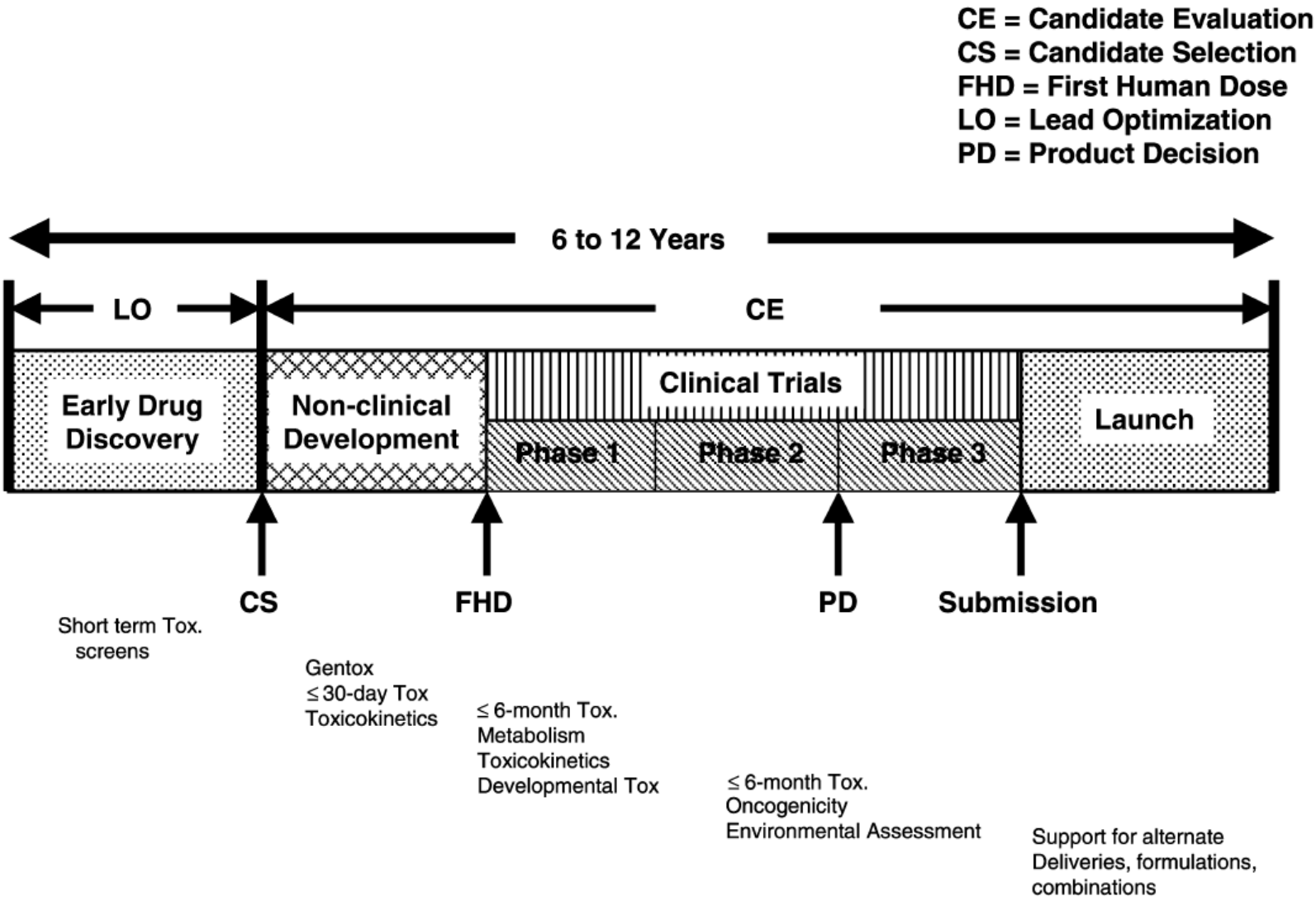


Fig. 2. High-level relationship of toxicology profile to phases of drug development.