中華民國獸醫病理學會
Chinese Society for Veterinary Pathology

360th CSVP

動物毒性病理判讀及量化分析
(The qualitative and quantitative toxicopathology in the laboratory animal studies)

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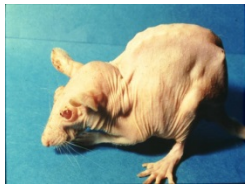
March 9, 2018

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

中文篇名：　試驗動物市場趨勢簡析
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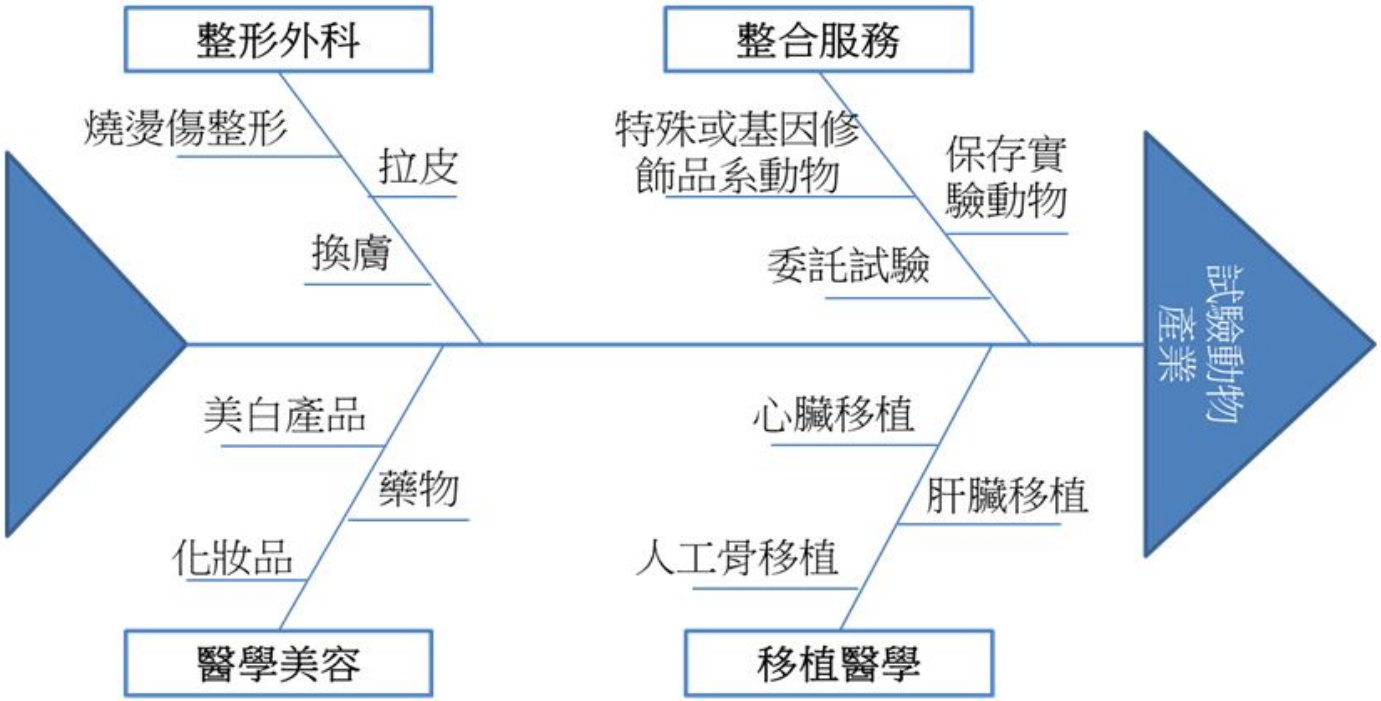
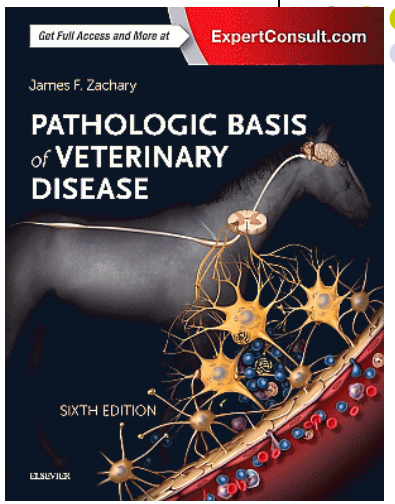


資料來源：農業科技研發成果產業化推動計畫。

圖一、試驗動物產業關聯圖

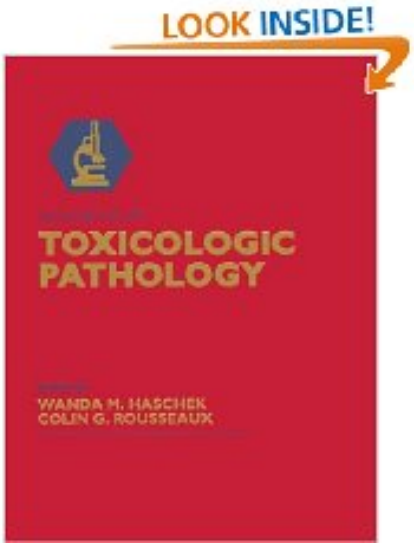
Introduction

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



Zachary JF (Editor). 2016. In: *Pathology Basis of Veterinary Disease*, 6th edition)

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



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

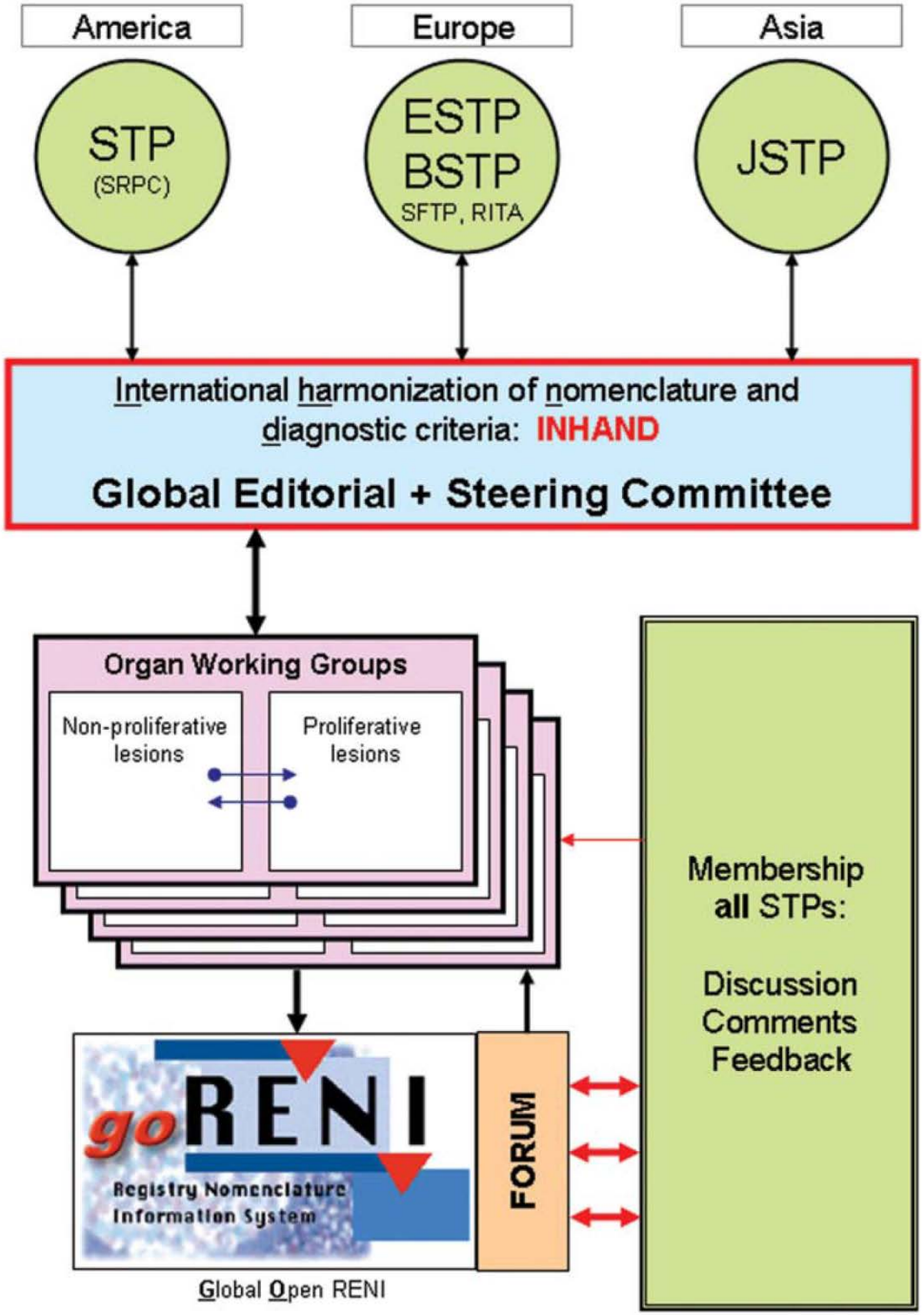
Peter C. Mann[1], John Vahle[2], Charlotte M. Keenan[3], Julia F. Baker[4], Alys E. Bradley[5], Dawn G. Goodman[6], Takanori Harada[7], Ronald Herbert[8], Wolfgang Kaufmann[9], Rupert Kellner[10], Thomas Nolte[11], Susanne Rittinghausen[10], and Takuji Tanaka[12]

[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*

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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www.goreni.org
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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.
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07-Mar-2016
Site Navigation
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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.
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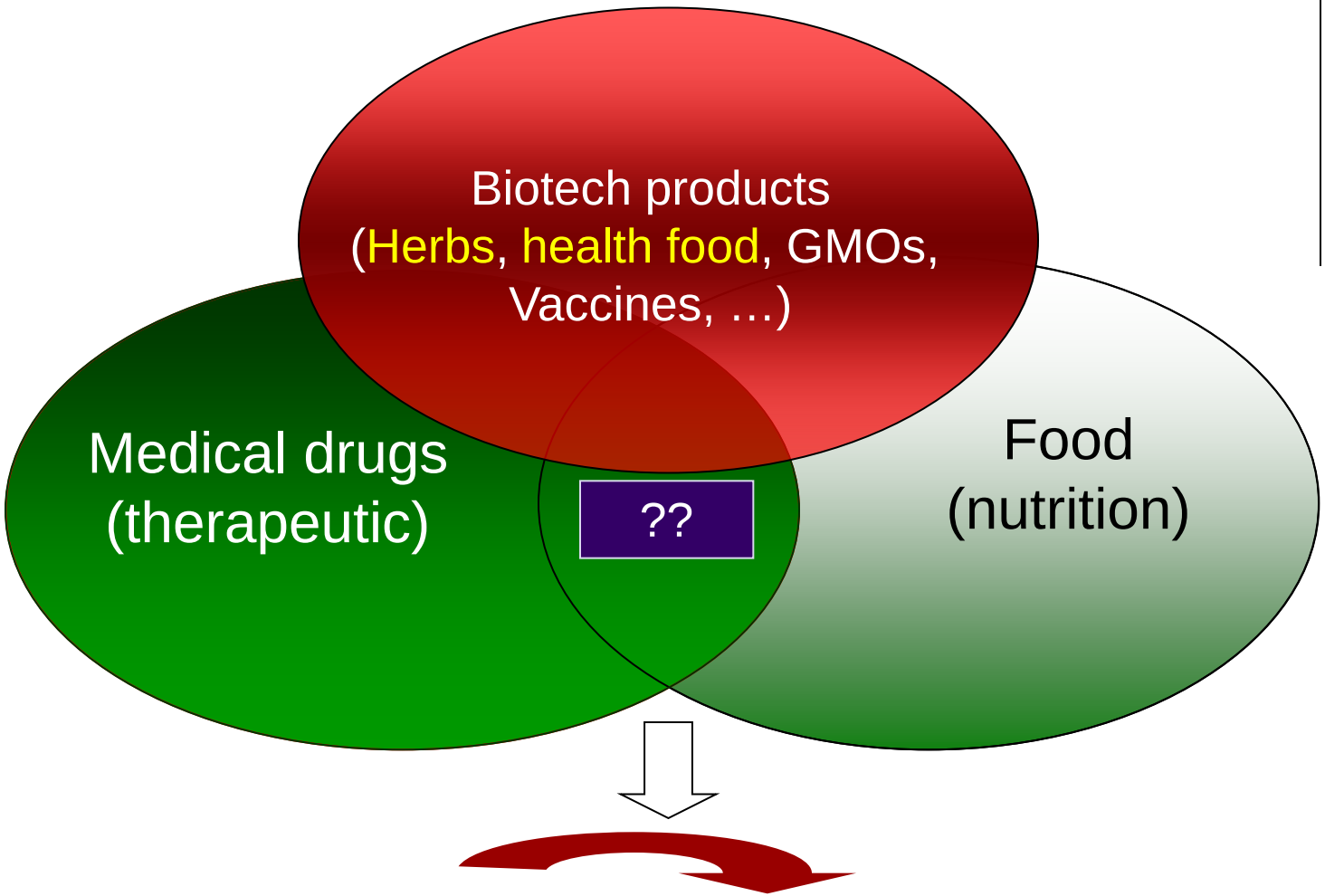


一般用詞

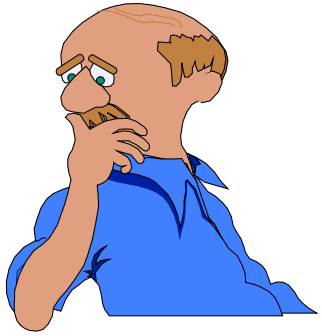
- 保健食品
- 機能性食品
- 生技產品

法定名詞

- 健康食品 (Health food, DOH, 1999)

陳樹功, 2003

Safety ??



Function ??

衛福部審核通過之健康食品一覽表

(1999/1-2018/2)

保健功效項目	評估指標	通過件數
1. 調節血脂	**TG, TC (HDL-C, LDL-C, Oxidation)...**	**155**
紅麴（規格標準）-調節血脂功能		**39**
魚油（規格標準）-調節血脂功能		**25**
2. 胃腸功能改善	促進消化; Probiotic; 胃黏膜細胞…	80
3. 免疫調節	Non-specific (Con-A) and specific OVA)..	45
4. 護肝功能	GOT, GPT, TG, TC, Antioxidant, Fibrosis…	42
5. 骨質保健	High serum Ca^{2+}; Bone density; low PTH…	20
6. 牙齒保健	Sucrose-induced; *Strep. Sorbrinus*….	6
7. 調節血糖	STZ-induced: Glucose level, Insulin, HbA_{1C}	18
8. 抗疲勞	運動能力; 生化值(BUN, glycogen, Lactate)…	15
9. 延緩衰老功能	Survival rate; D-galactose-induced ROS…	5
10.不易形成體脂肪功能	High energy food, BW, food intake, fat….	19
11.輔助調整過敏體質功能		21
12.輔助調節血壓功能		3
13.促進鐵吸收功能		5
總計		498

https://consumer.fda.gov.tw/Food/InfoHealthFood.aspx?nodeID=162#

(TFDA, 2018/2)

『健康食品』安全性毒理評估

- 第一類：(免提毒性測試資料)
  - 產品之原料為傳統食用且以通常加工食品形式供食者
  - 產品具有完整之毒理學安全性學術文獻報告及曾供食用之記錄

- 第二類：(產品之原料為傳統食用而非以通常加工食品形式供食者)
  - 基因毒性 (Ames with 5 strains, CA, MN assays)
  - 28天餵食毒性試驗

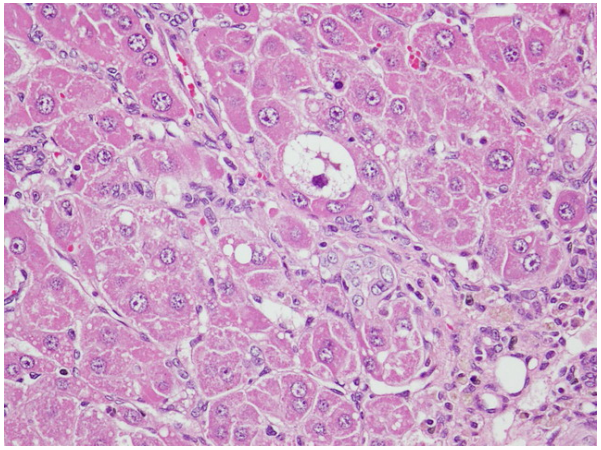
- 第三類：(產品之原料非屬傳統食用者)
  - 基因毒性
  - 90天餵食毒性試驗
  - 致畸胎毒性試驗

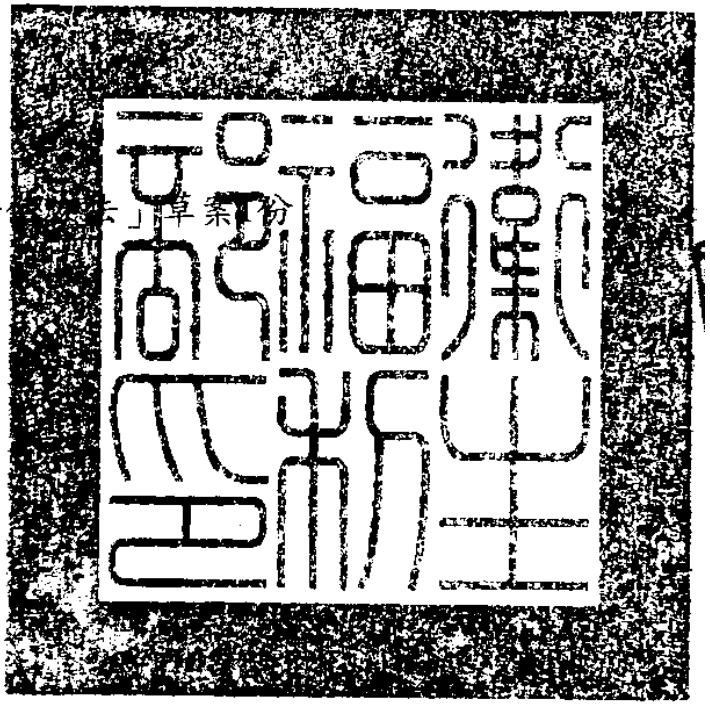
- 第四類：(產品之原料非屬傳統食用且成份含有致癌物之類似物者)
  - 基因毒性
  - 致畸胎毒性試驗
  - 致癌性試驗
  - 90天餵食毒性試驗
  - 後代繁殖試驗

(TFDA, 2002)

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)評估方法」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

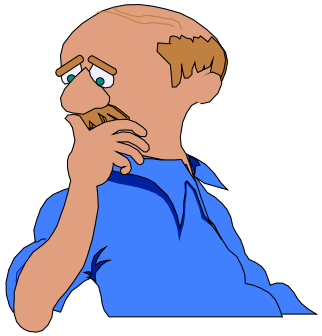
105年病理專科獸醫師甄審

商品說明:

提供皮膚及亮麗毛髮所需的頂級營養.均衡的礦物質.維生素維持最佳的健康狀態.狗狗最喜歡的口味.

希爾思成犬　　蛋白質21.5%脂肪13%纖維3%
耐吉斯羊肉幼犬 蛋白質25%脂肪17%纖維4.5%
博士巧思成犬　蛋白質28% 脂肪14%纖維3%
優格雞肉成犬　蛋白質26%脂肪16%纖維4%

Safety ??



Function ??

科技部 107 年度跨部會「動物保健產業及安全防護科技創新開發」專案研究計畫徵求公告

107/1

一、計畫說明與目的

臺灣地處熱帶、亞熱帶之交，環境溫度提升不僅影響禽畜動物的生長、生產和繁殖性能，同時也增加疫病的發生和感染。又由於我國禽畜生產空間狹小，在高密度飼養過程中，非治療型抗生素在疾病防治上之濫用，成為禽畜動物生產管理上的一大問題，對生態環境亦造成相當之衝擊。

此外，伴侶動物已成為家庭中重要的成員，也有益於銀髮族的身心健康，符合現今政府長照關懷政策。因此伴侶動物保健飼料添加物與疫苗之研發，亦有其重要性。

為增進經濟(禽畜)動物與伴侶動物的健康與生長性能，減少傳統化學藥物、化學色素和飼料中抗生素等添加，本計畫藉由新型疫苗或保健飼料添加劑之研發，提高動物對不良環境的抵抗/保護能力，提升飼料使用率與抗病免疫力，降低飼養與治療成本。

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

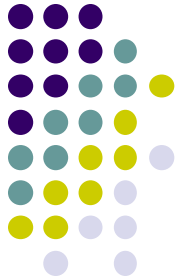
Toxicological pathology:

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

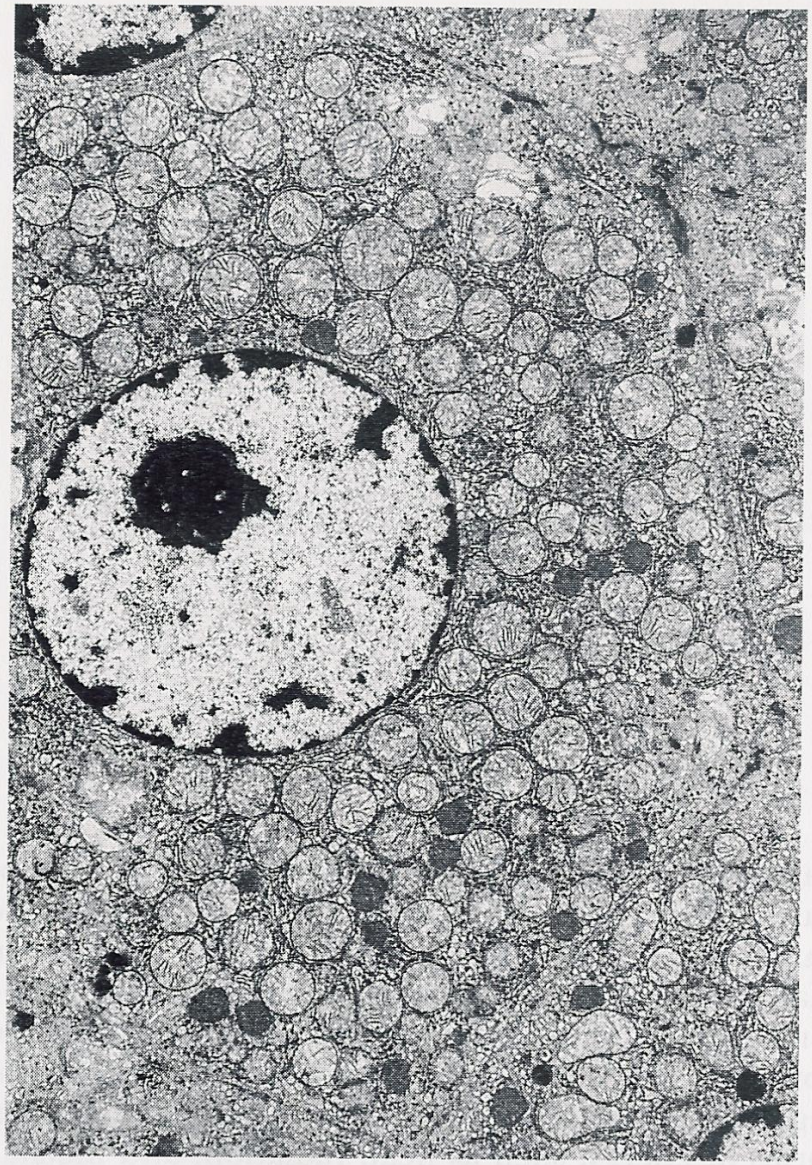


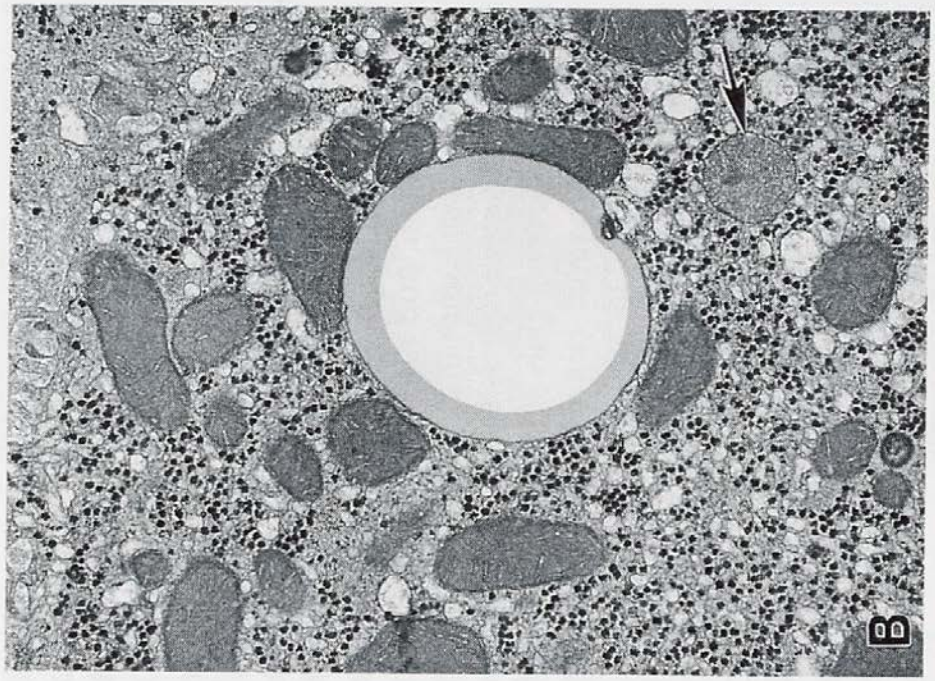
- **The normal cell**

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





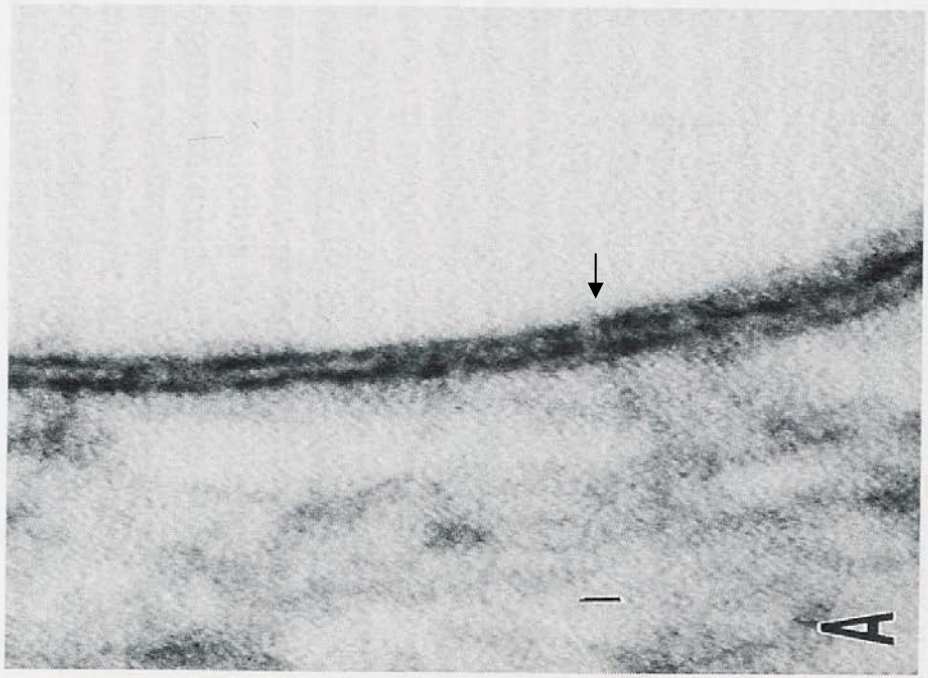
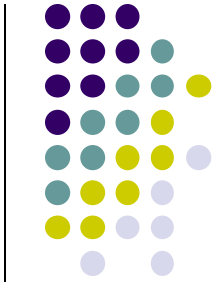


FIG. 1-1 The cell. Electron micrograph of portions of four liver cells from a cynomolgus monkey *(Macaca fascicularis).* Note nucleus containing a prominent nucleolus; cytoplasm containing numerous mitochondria; scattered darker staining peroxisomes; sparse lamellae of rough endoplasmic reticulum; and cell membranes separating adjacent cells ($\times$ 7000).

• Reaction of cell to chemicals



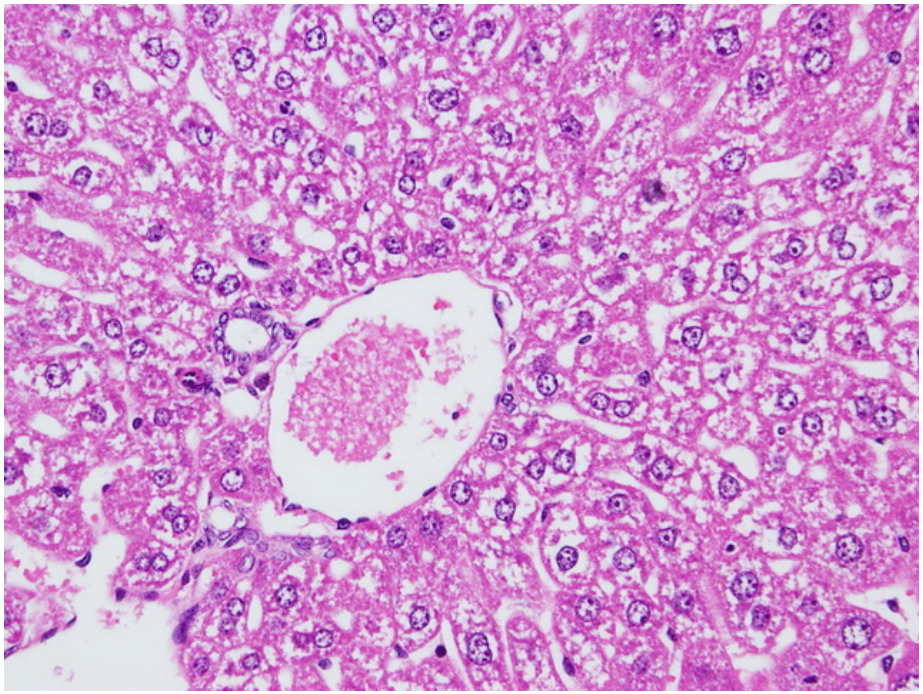
• **Chemical as stressors**

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

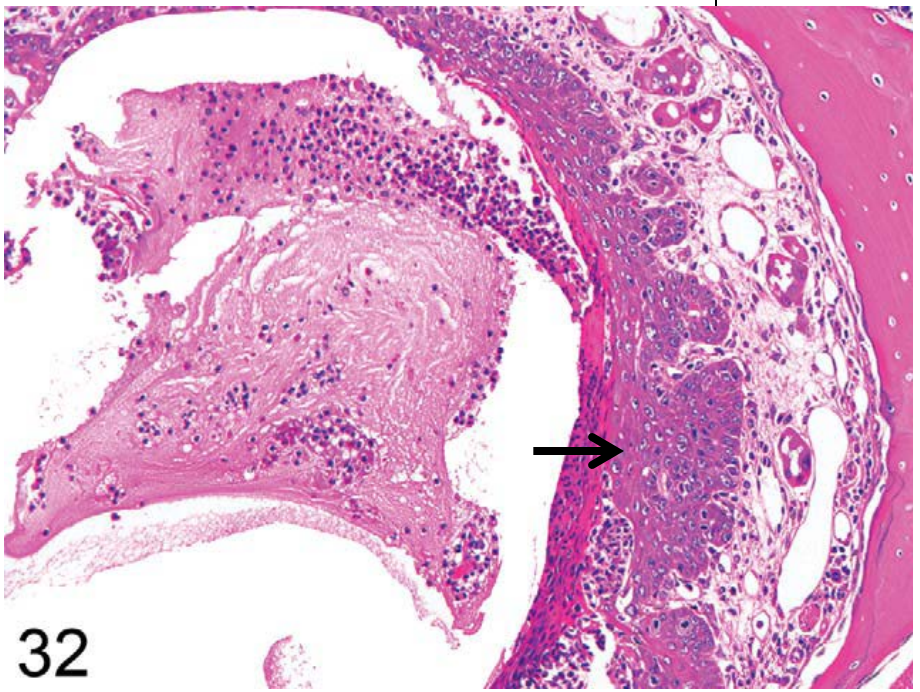


FIGURE 32.—Mouse. Nasal cavity, squamous cell metaplasia

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

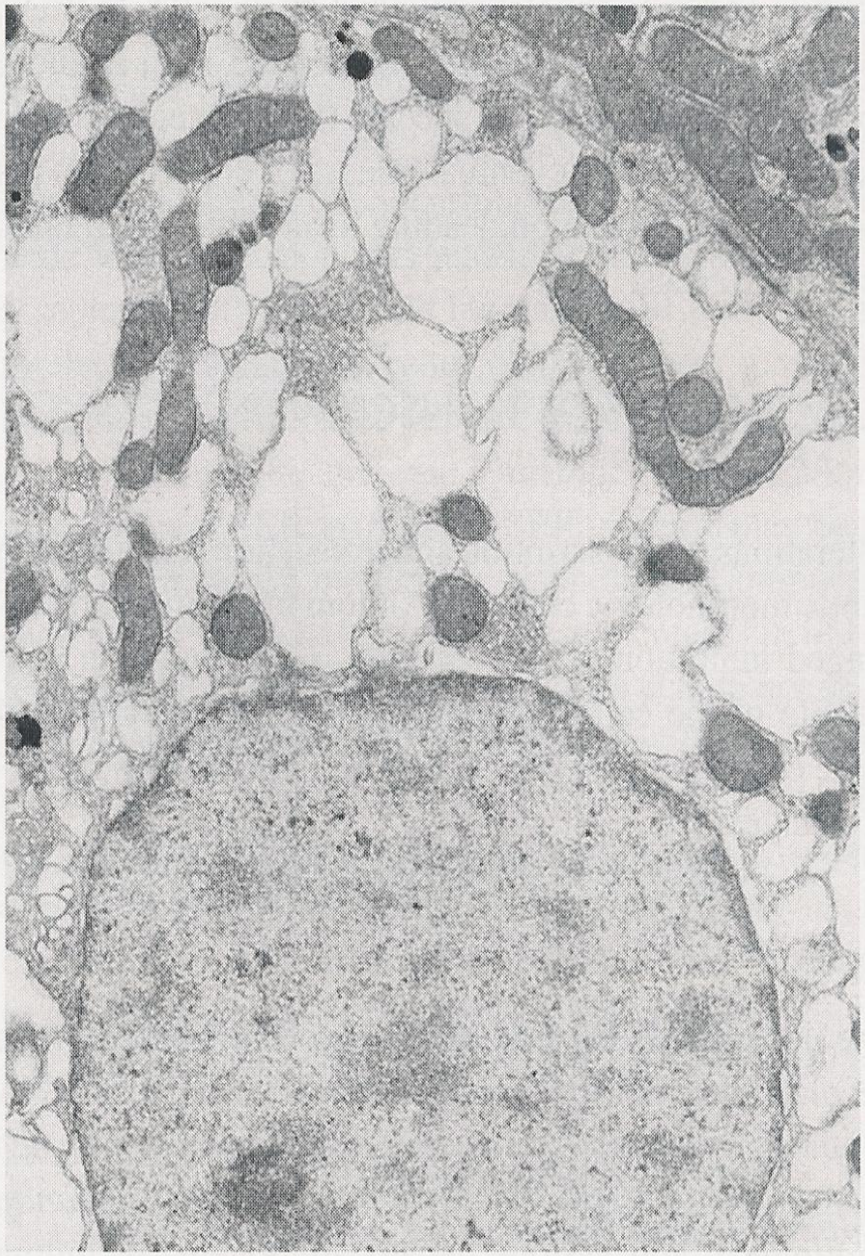
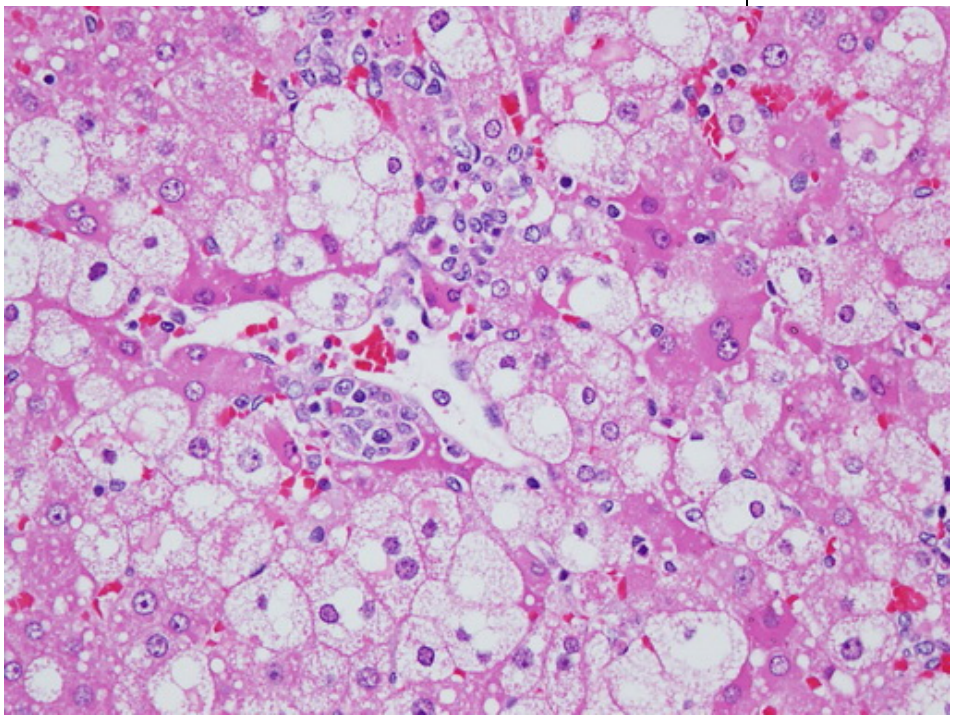


Figure 9. Hydropic degeneration characterized by dilated endoplasmic reticulum; rat.

- **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

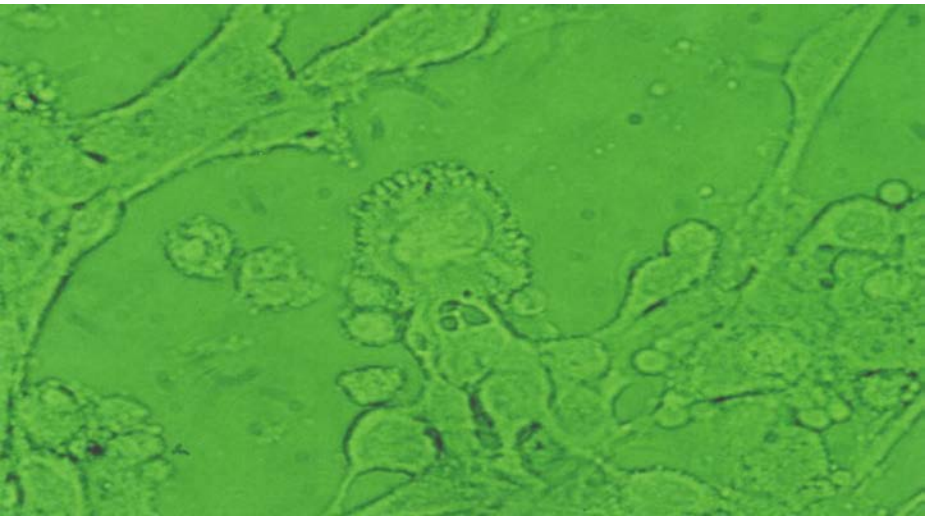
- **Cell surface blebs**

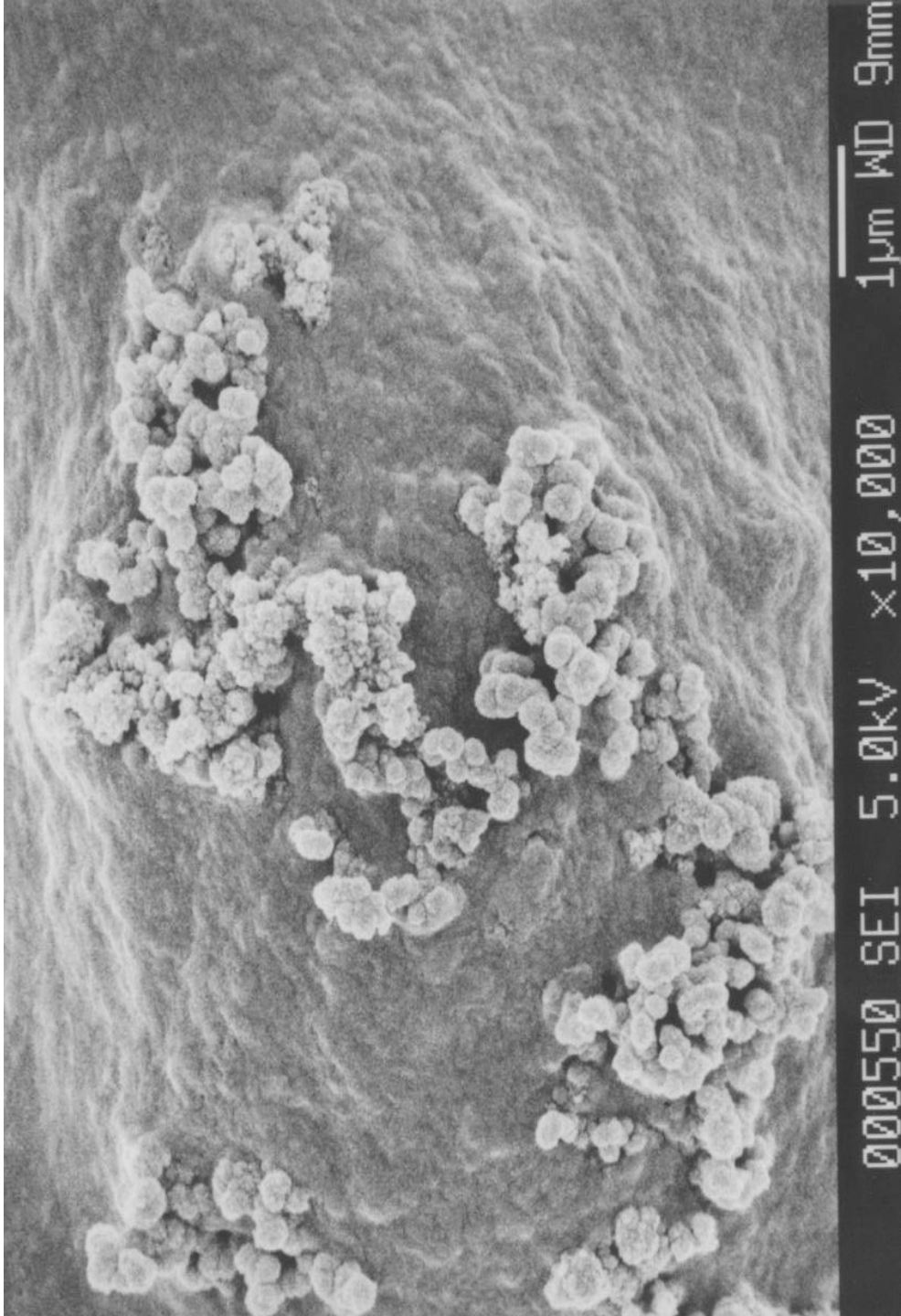
Etiology:

1) reverse cell injury
   ⇒ increase ammonia

Morphology changes:

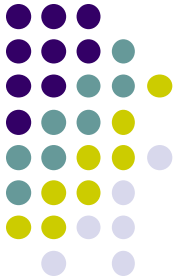
1) cell surface blebs formation
2) invagination of the plasma membrane





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

Three stages of nucleus change during necrosis

- **Pyknosis:**

⇒ as a shrunken nucleus with increase basophilic

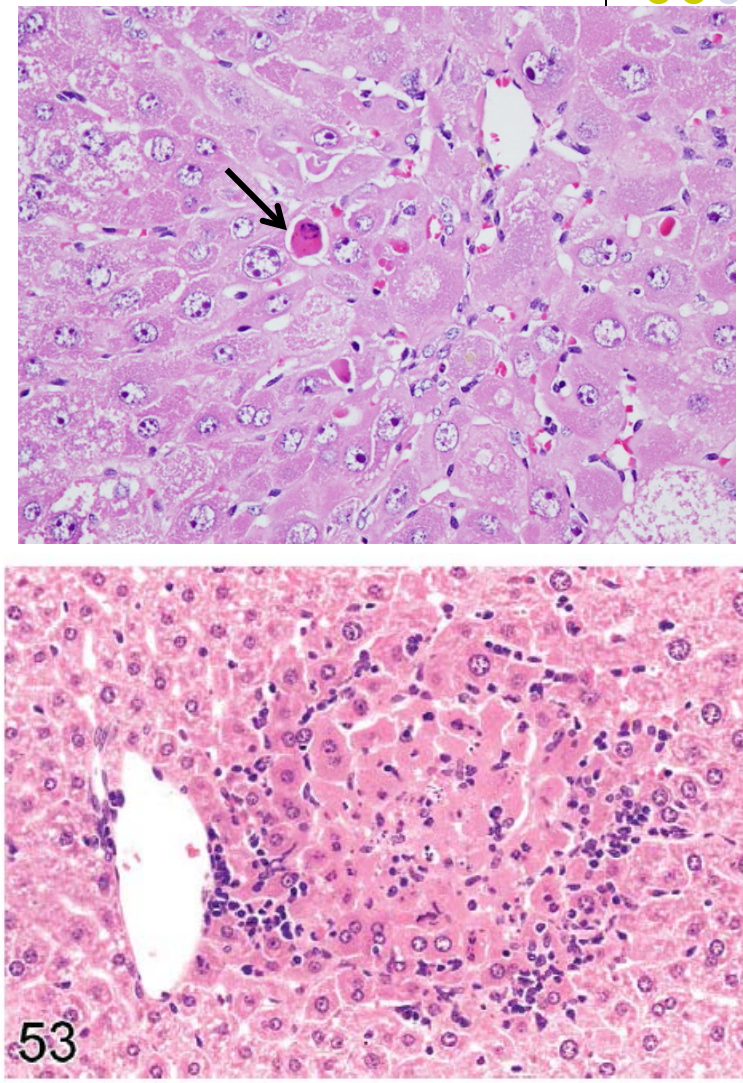
- **Karyorrhexis (Karyolysis):**

⇒ as a fragmentation or lysis of nucleus

⇒ dissociation and disappearance of stainable chromatin

- **Loss of the nucleus**

⇒ lack a nucleus



53 Mouse liver. Necrosis, focal.

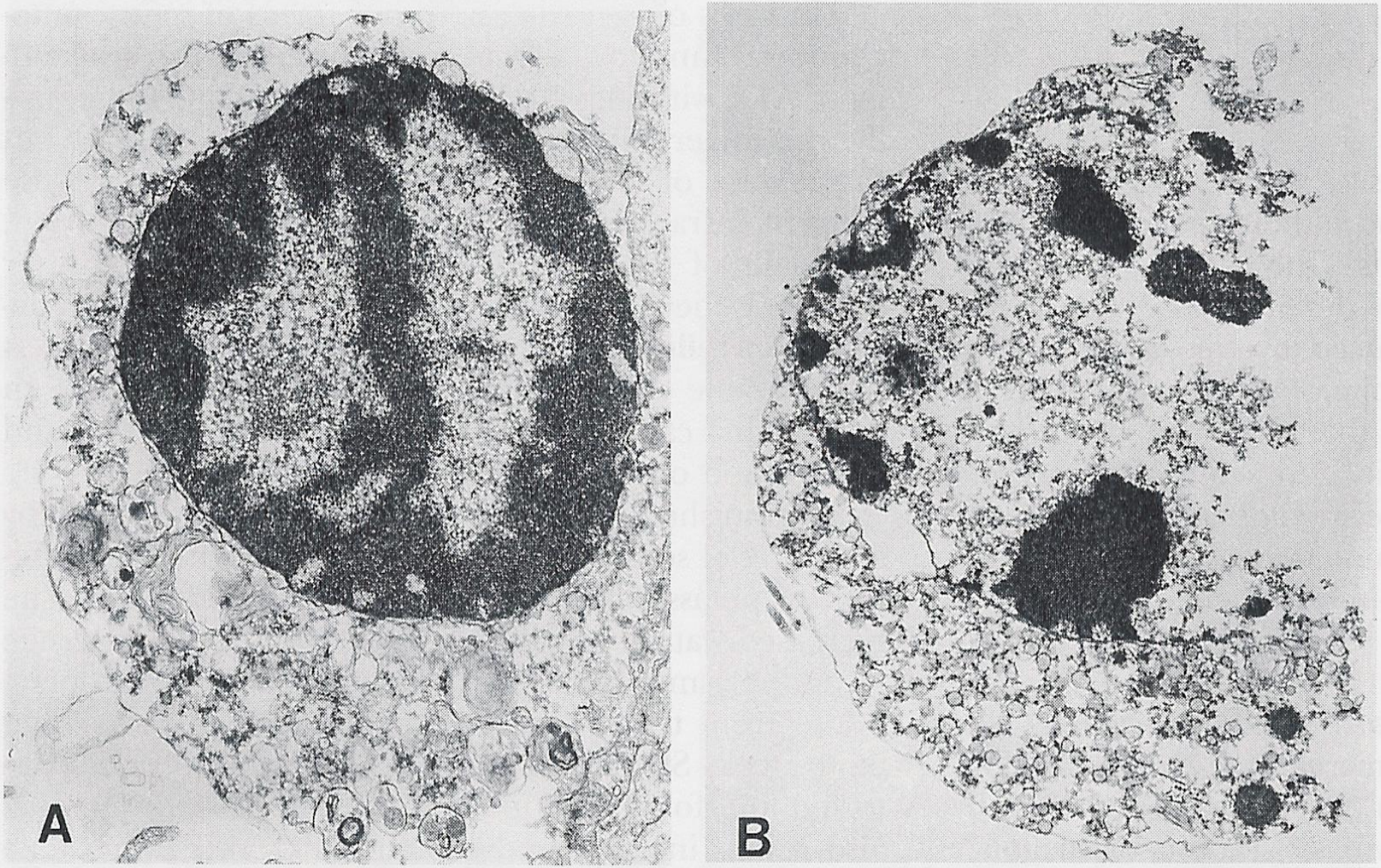


FIG. 1-8 Ultrastructural changes of necrosis. **A.** Pyknosis. Nucleus is shrunken, chromatin is more electron-dense than normal, and cytoplasm shows hydropic change and myelin figures (× 12,000). **B.** Karyorrhexis. Nuclear and cell membranes are disrupted. Note fragmentation of the chromatin into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

Hepatic Apoptosis

drug induced

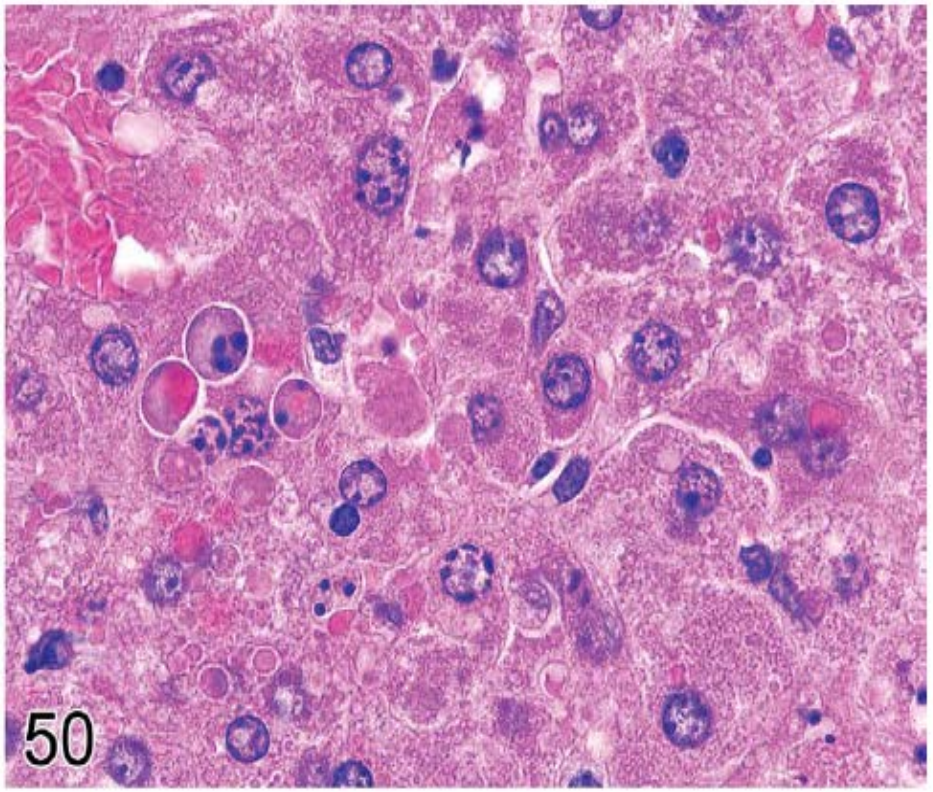
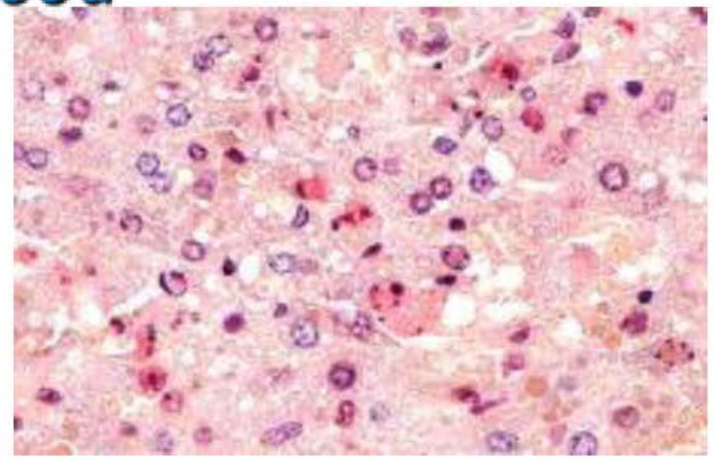
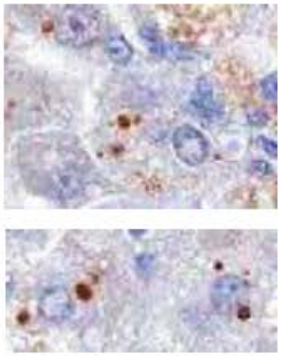


FIGURE 50.Mouse liver. Apoptosis

TUNEL





Caspase 3

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

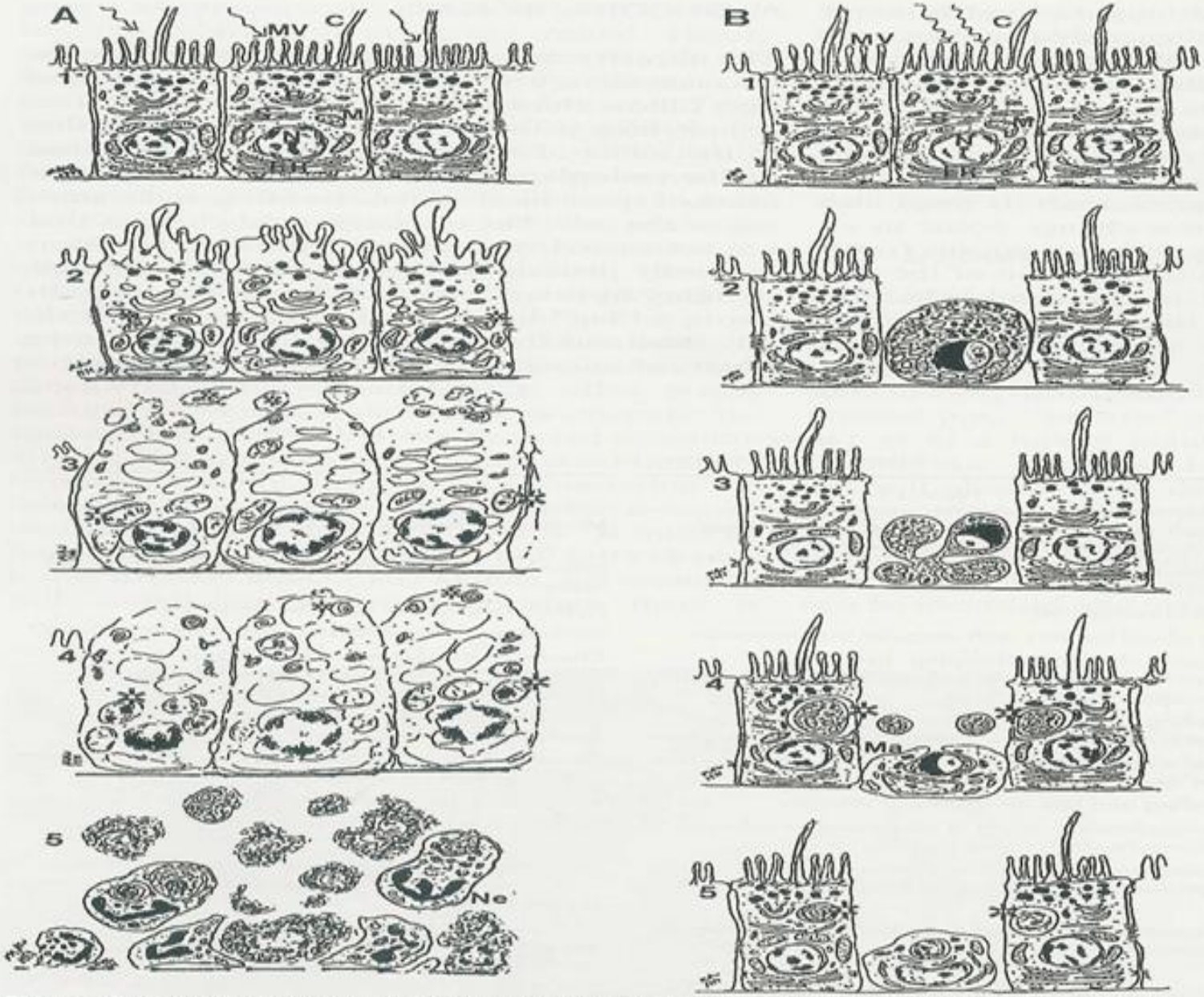


Figure 9. Morphologic changes associated with oncotic (A) and apoptotic (B) necrosis in a "prototypical" secretory epithelial cell. C, cilium; ER, rough endoplasmic reticulum; G, Golgi apparatus; M, mitochondrion; Ma, macrophage; MV, microvillous brush border; N, nucleus; Ne, neutrophil; and S, smooth 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,…

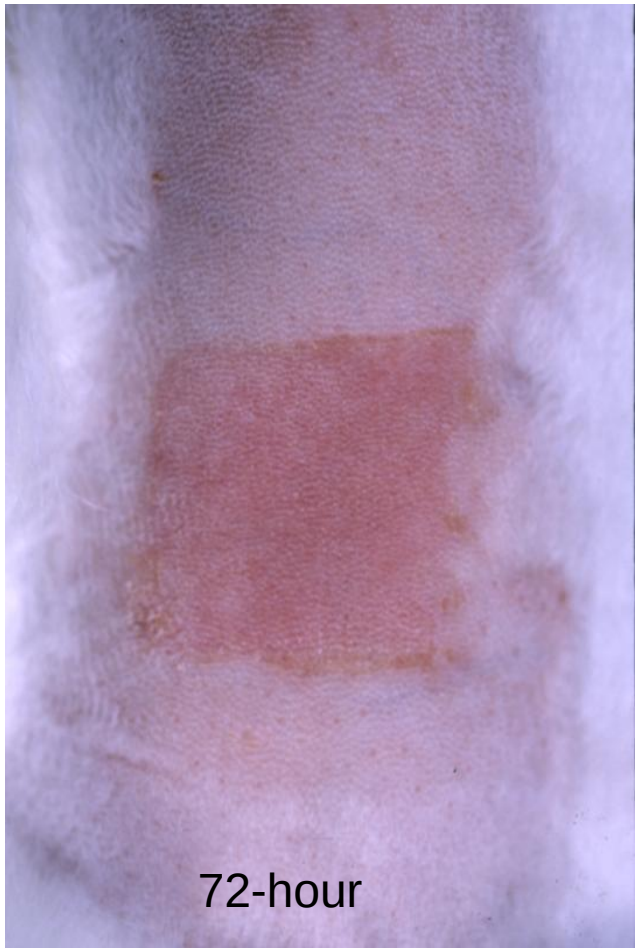
2. Healing by connective tissue replacement

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

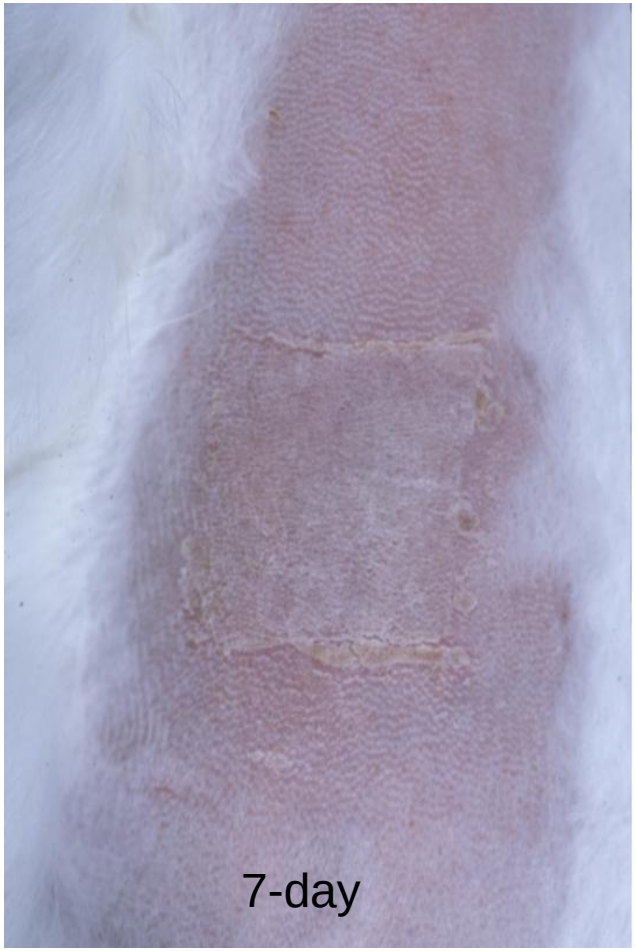
3. The inflammatory process

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

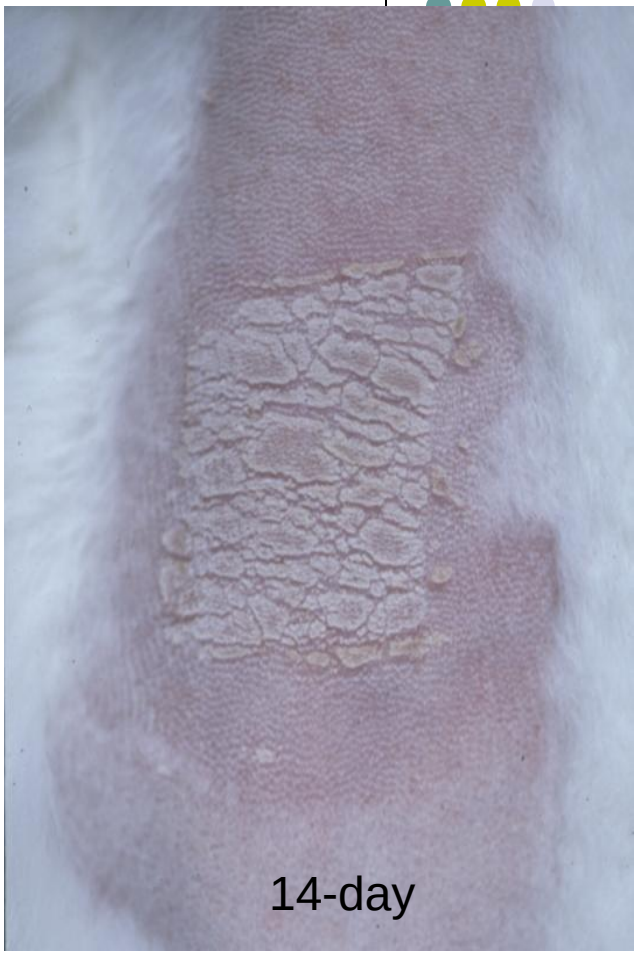
Solvent-induced skin irritation



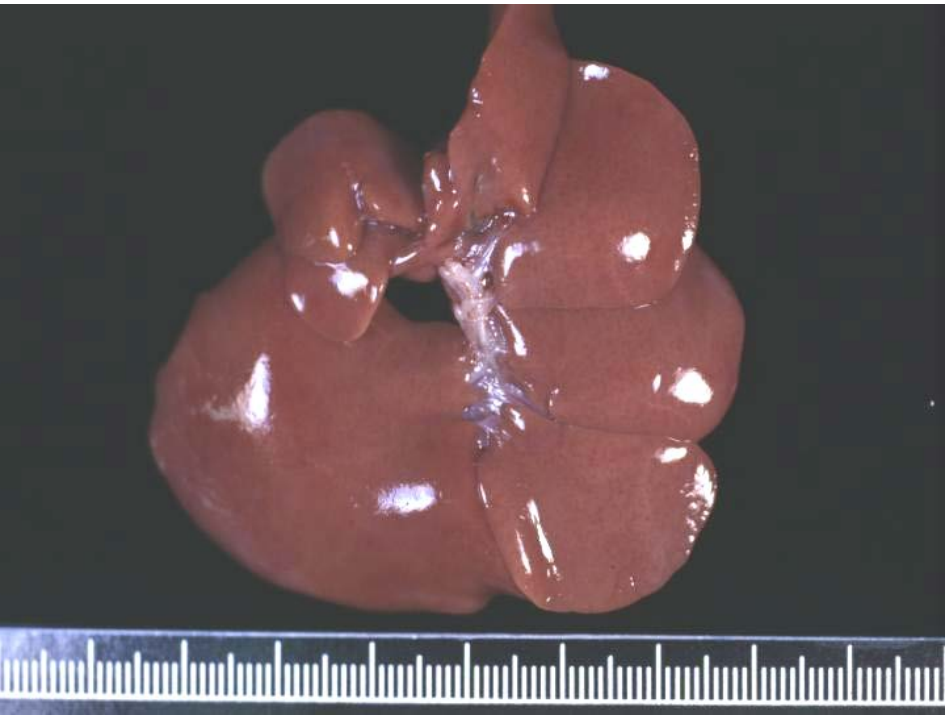
Erythema (2)
Edema (1)



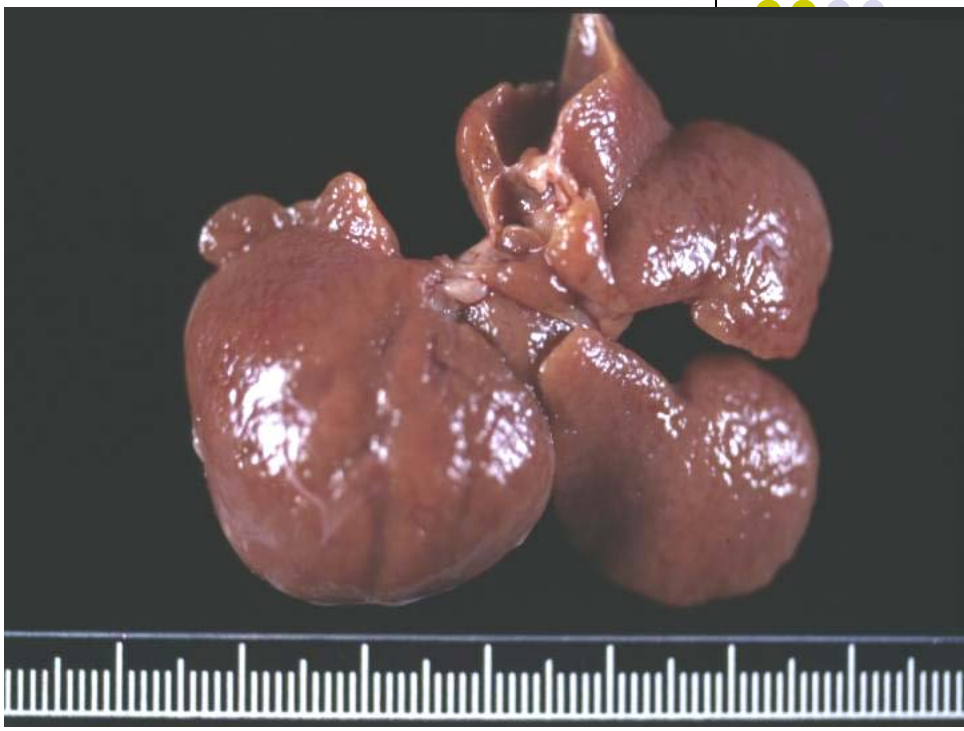
Eschar (4)
Edema (1)



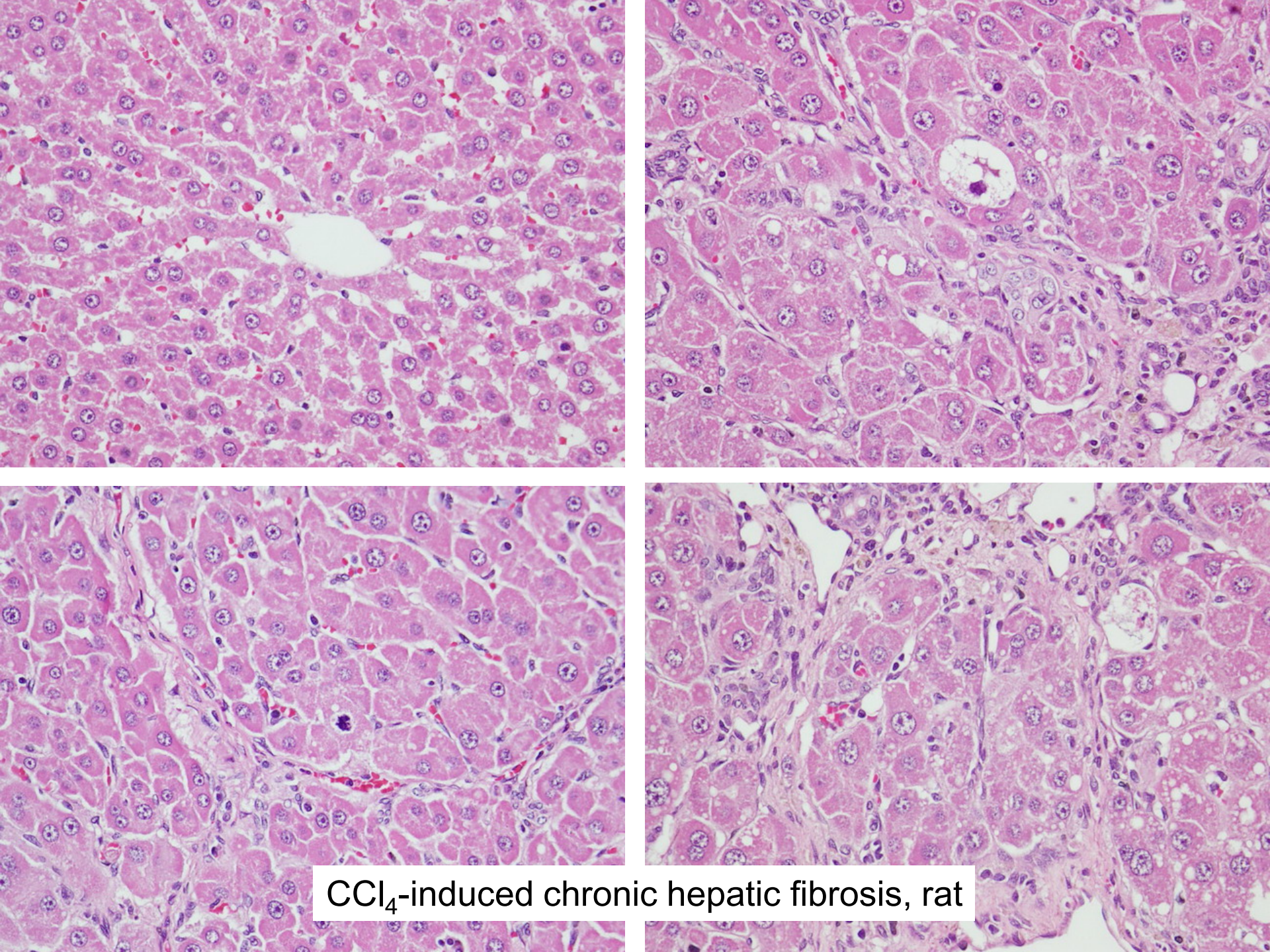
Eschar (4)
Edema (2)

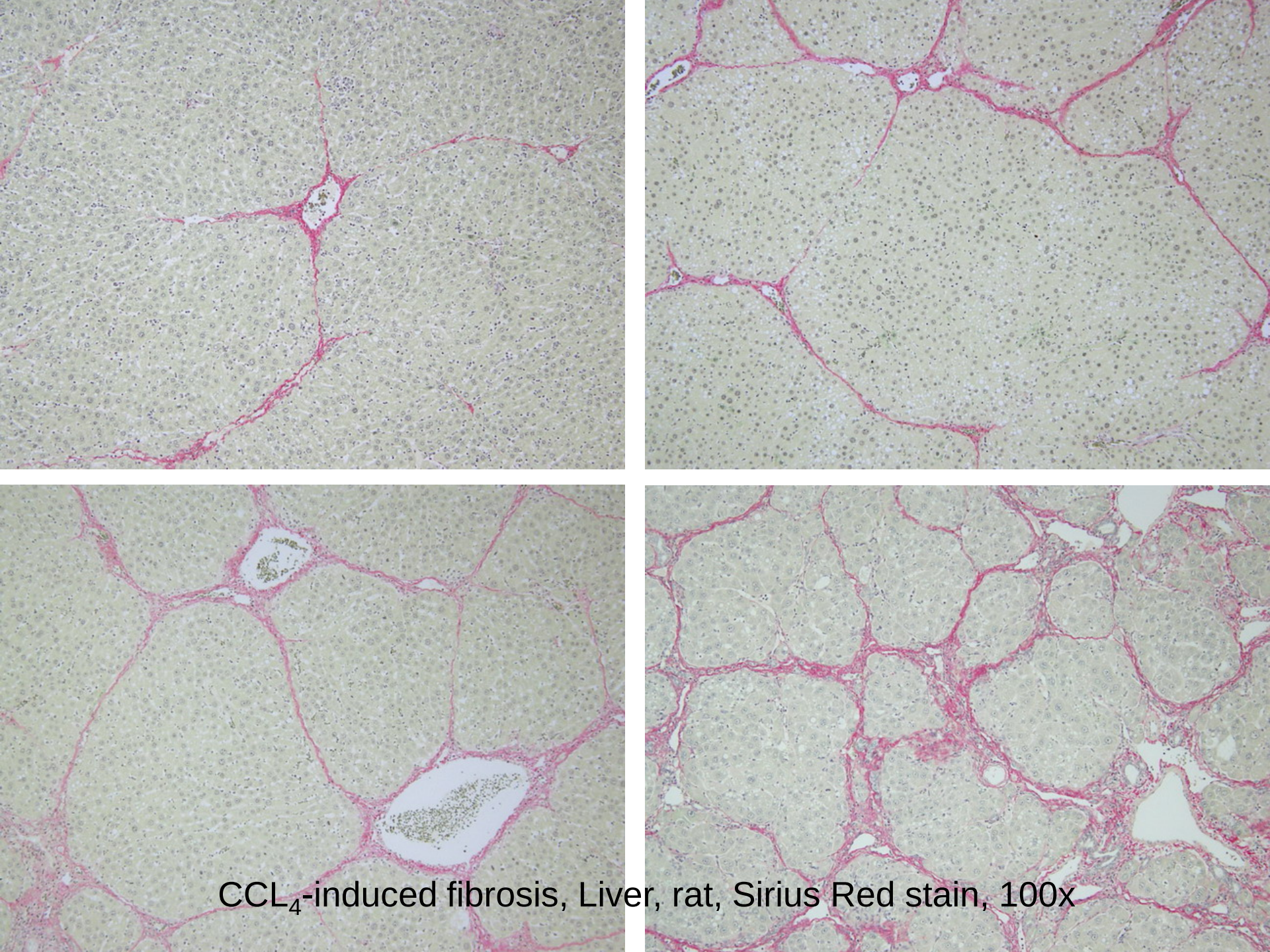


Control



Cirrhosis


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

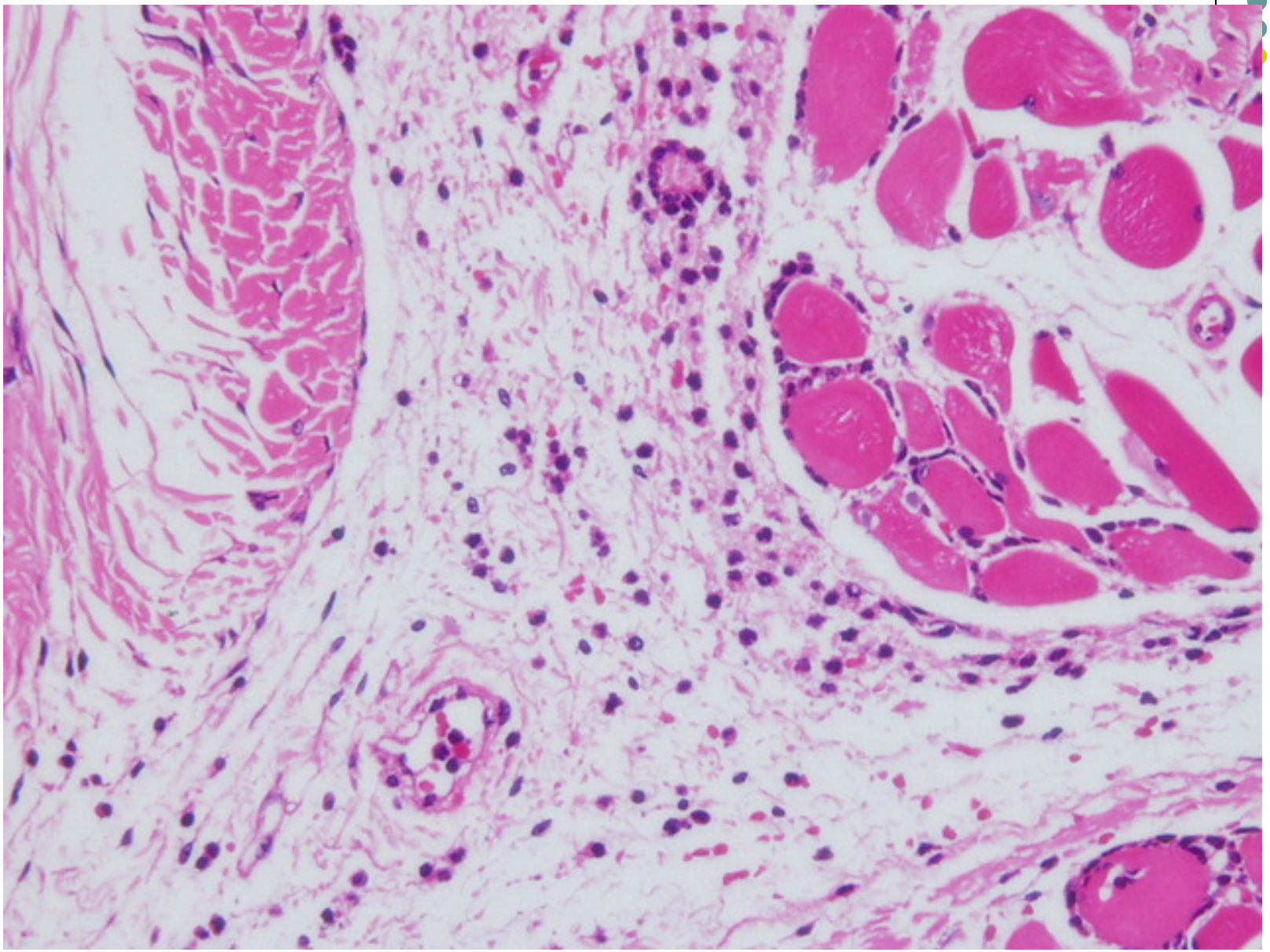
2. Subacute inflammation ⇒ Bronchopneumonitis

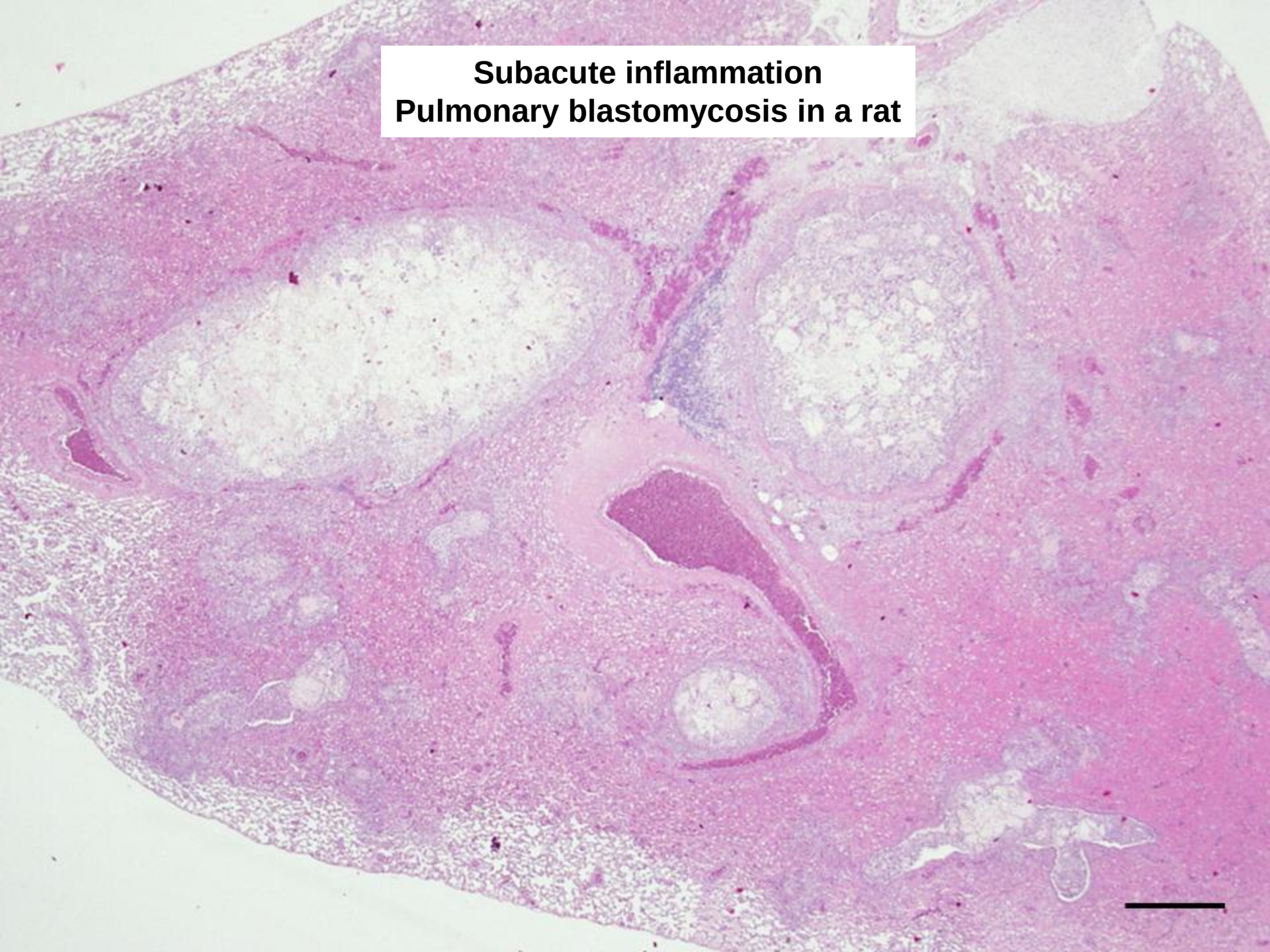
1) Range from days to weeks
2) Decrease vascular and cellular components
3) Polymorphorphic and mononuclear inflammatory cells

3. Chronic inflammation:

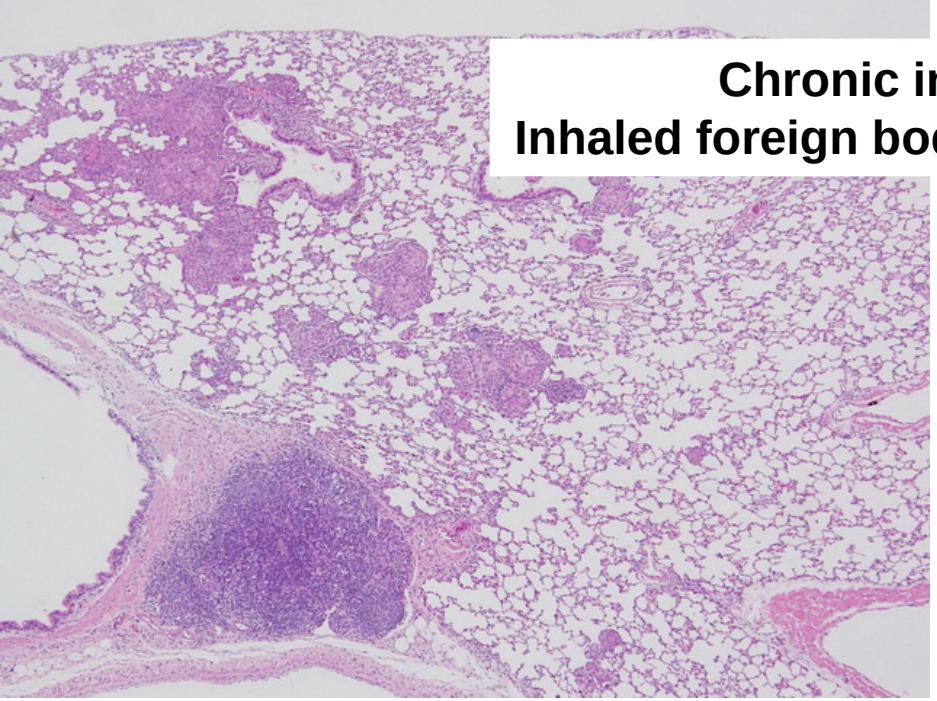
1) Persist for weeks to months, usually occur by fibrosis
2) Granulomatous inflammation ⇒ Inhaled granuloma in the lungs
   Central area⇒ aggregated macrophages, epithelioid cells,
   Outer layer ⇒ surrounded by mononulear cells, plasma cells, lymphocytes, multinucelar giant cells, fibroblasts, and collagen

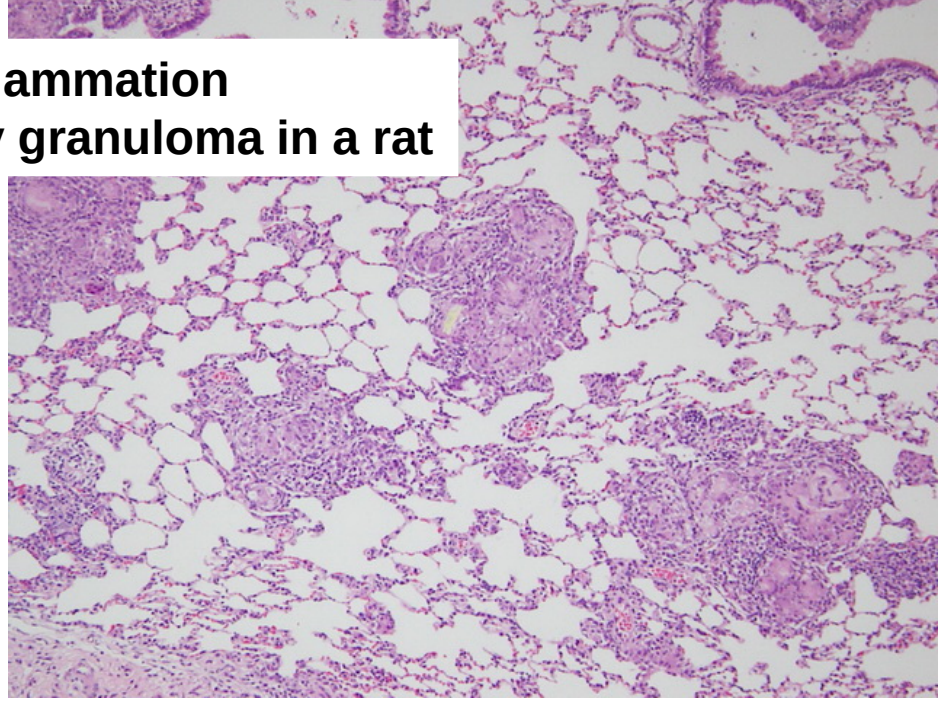
Acute inflammation (4 hr)
Carrageenan-Induced Paw Edema in a Mouse

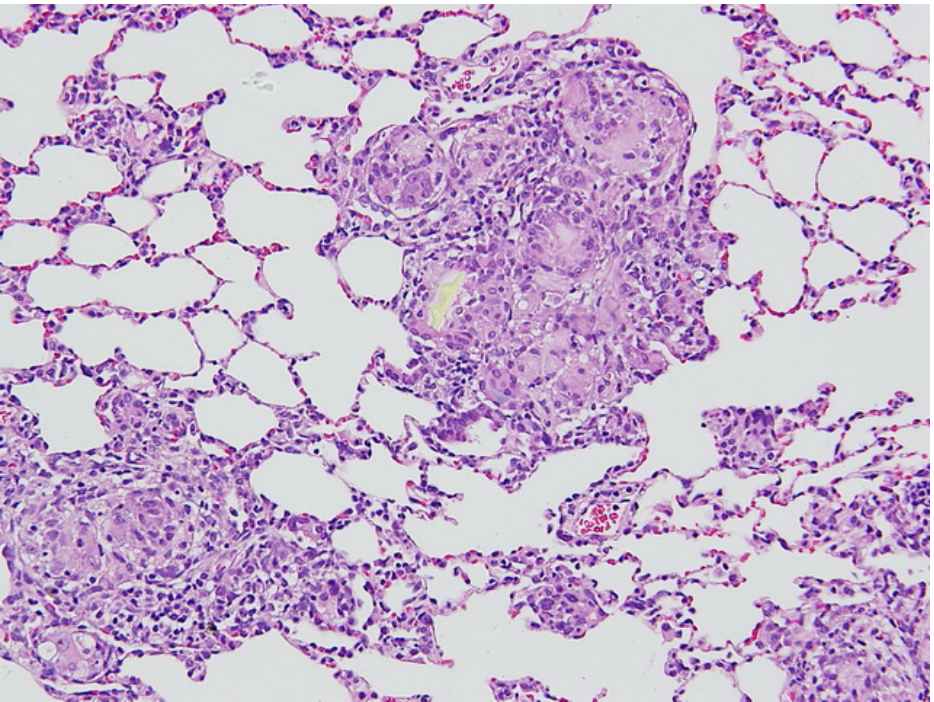


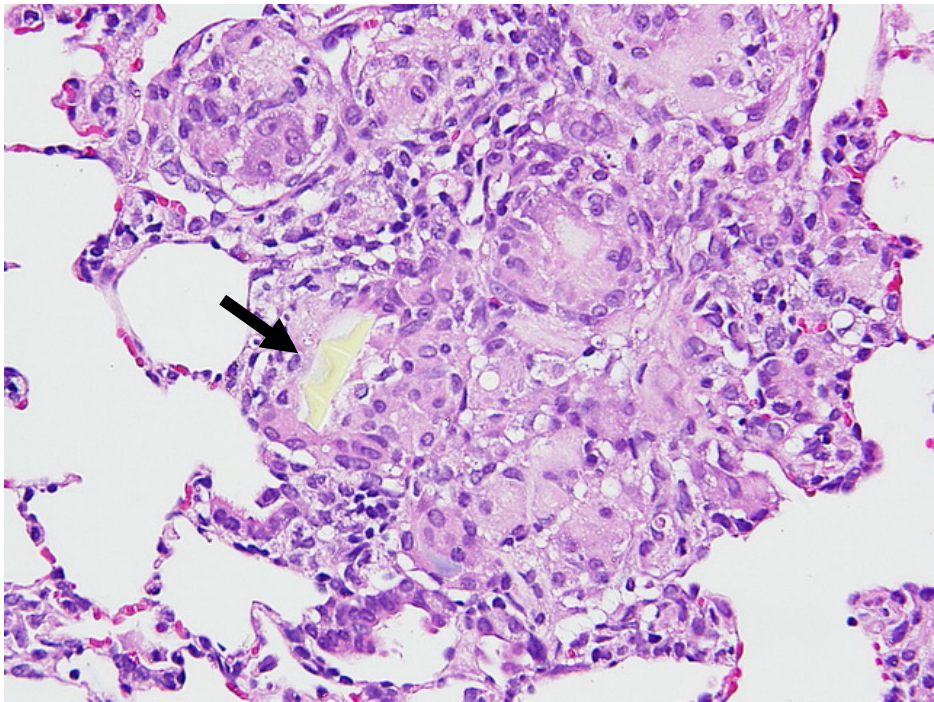

Subacute inflammation
Pulmonary blastomycosis in a rat

Chronic inflammation
Inhaled foreign body 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)**

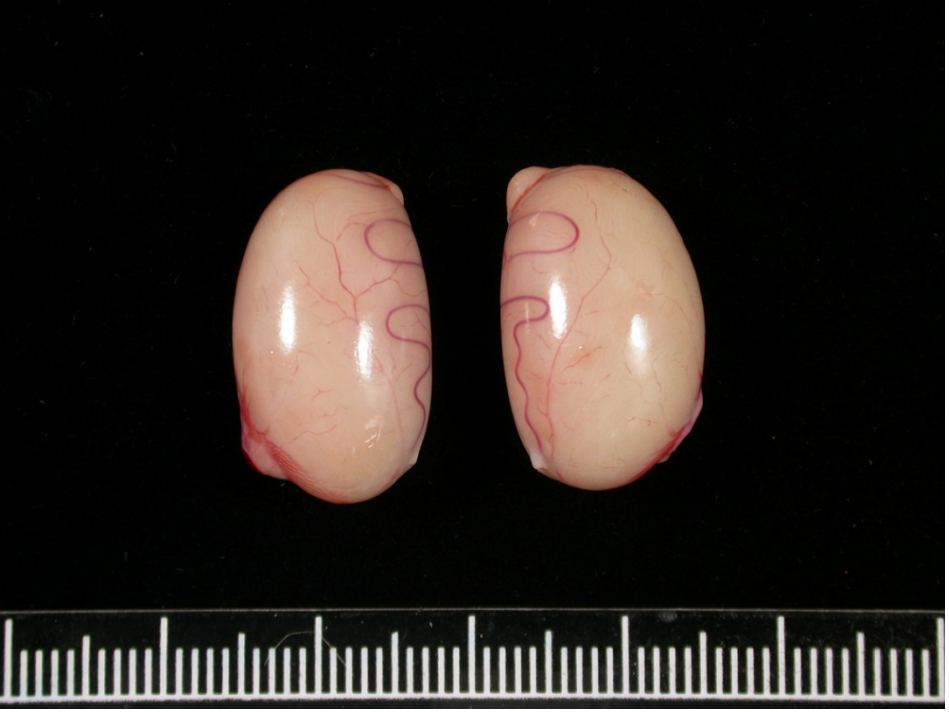
. The replacement of cells by new cells of the same type

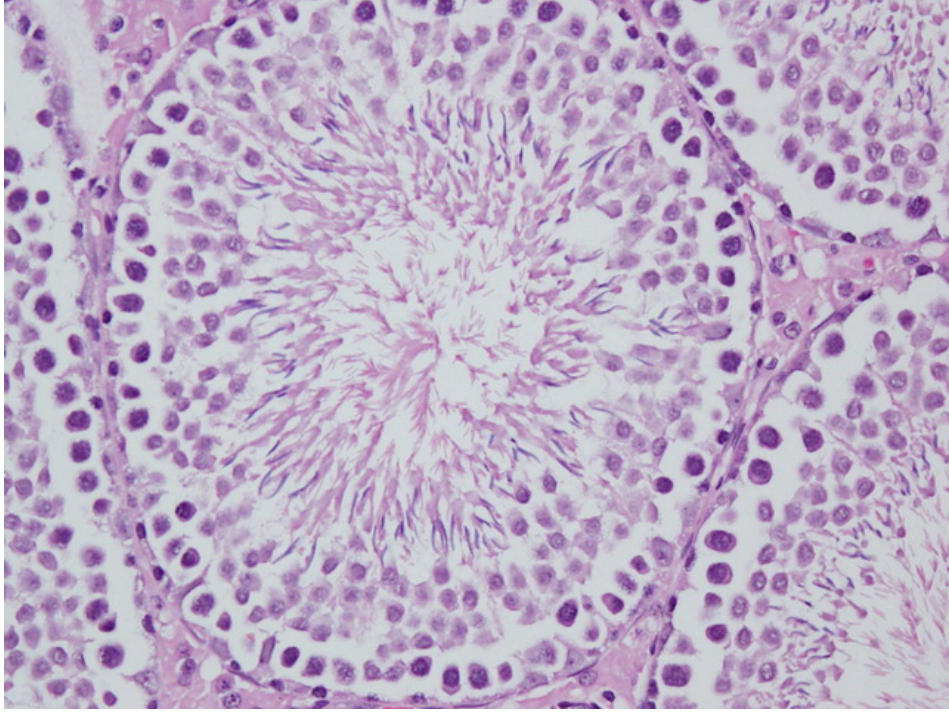
. Regenerated cells may become similar to the original cells

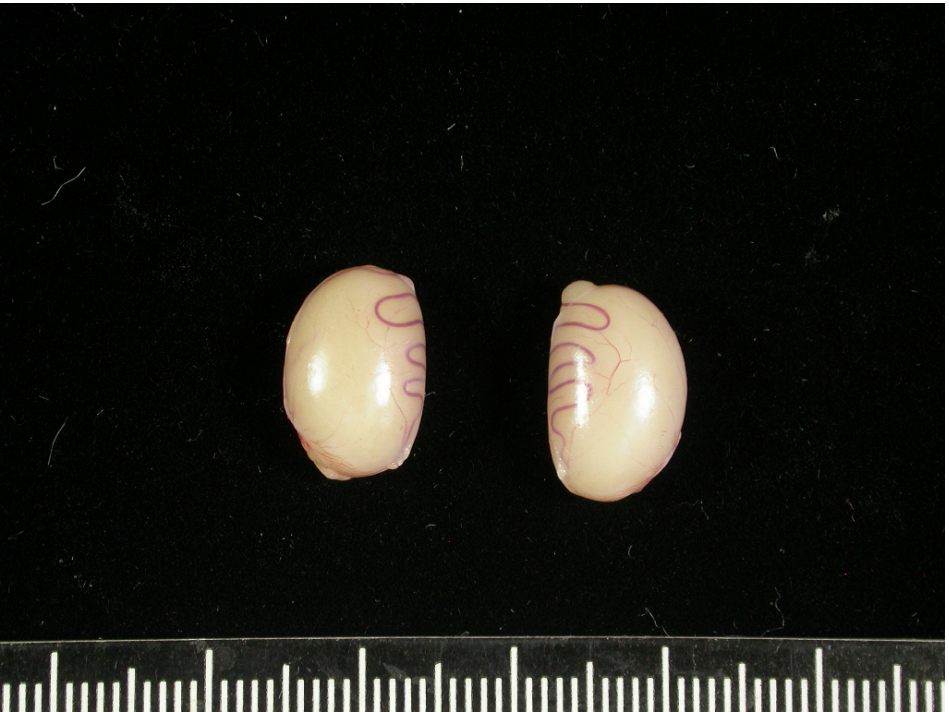
⇒ with a large amount of RNA to produce

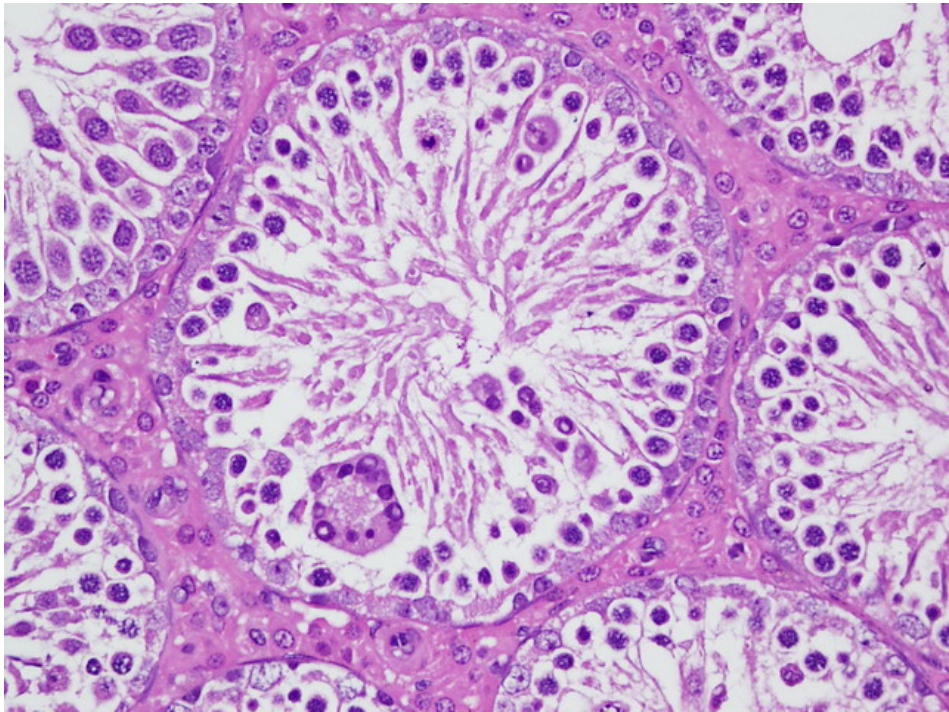
⇒ become blue staining by the H&E stain

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

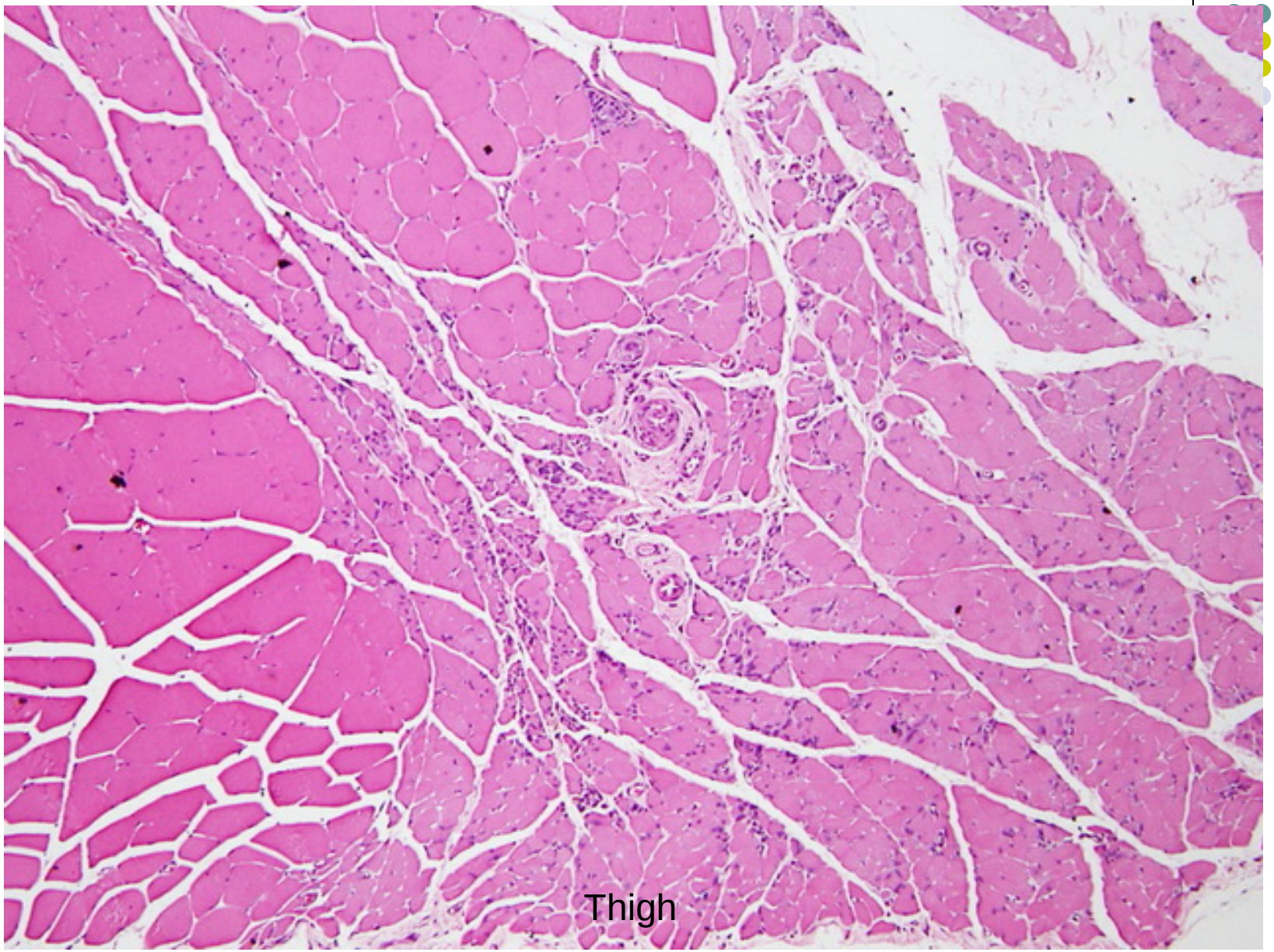




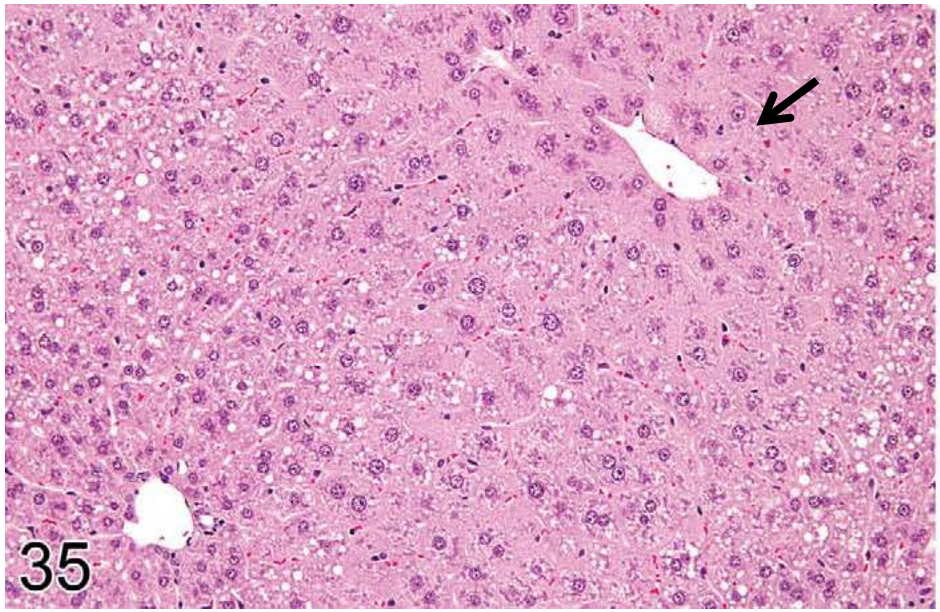




Botulinum toxin-induced paralysis and leads to muscular atrophy in rats



Thigh



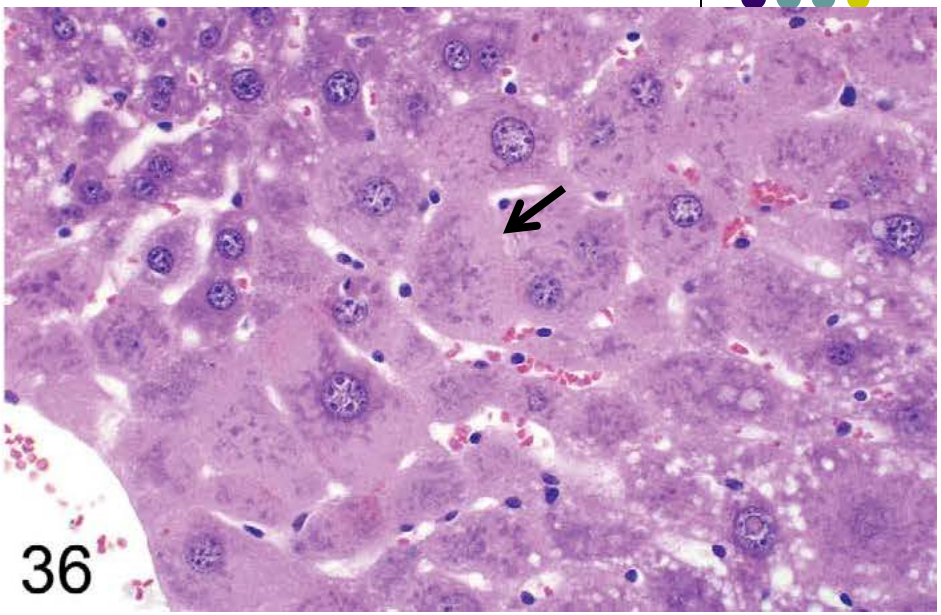
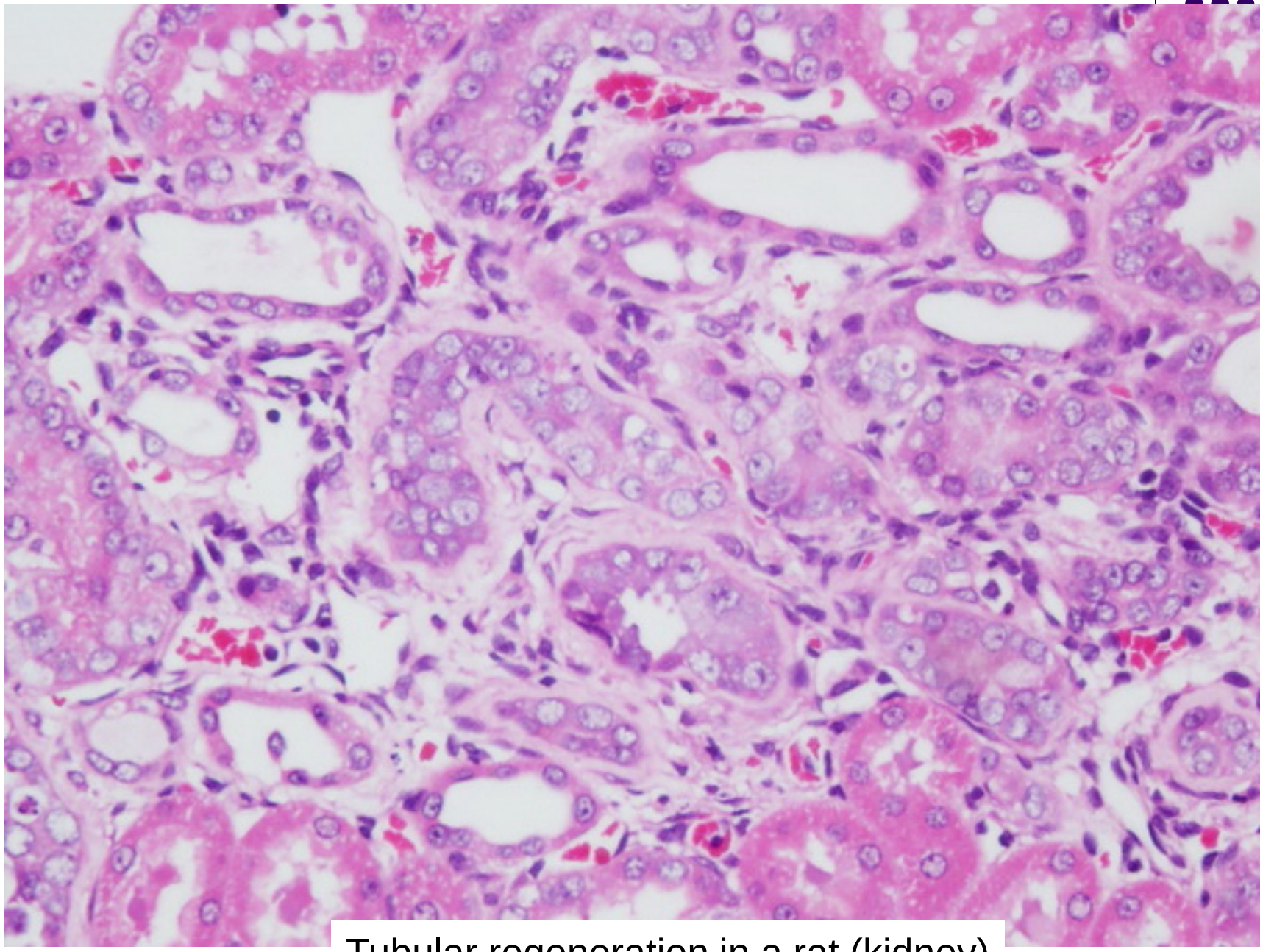


FIGURE 35.—Mouse liver. Higher magnification of centrilobular hepatocellular hypertrophy.

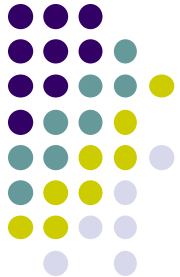
FIGURE 36.—Mouse liver. Hepatocellular hypertrophy.

Thoolen et al., 2010. Proliferative and non- proliferative lesions of the rat and moues hepatobiliary system. Toxicol Pathol. 38 (7 Suppl):5S-81S



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

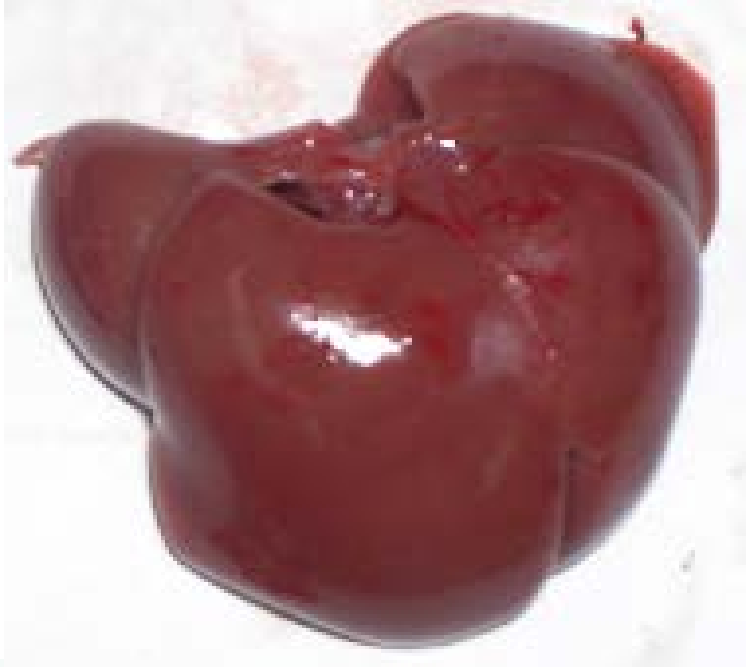
• Neoplasia or tumor, cancer (腫瘤)

.Implies new growth without normal control mechanism

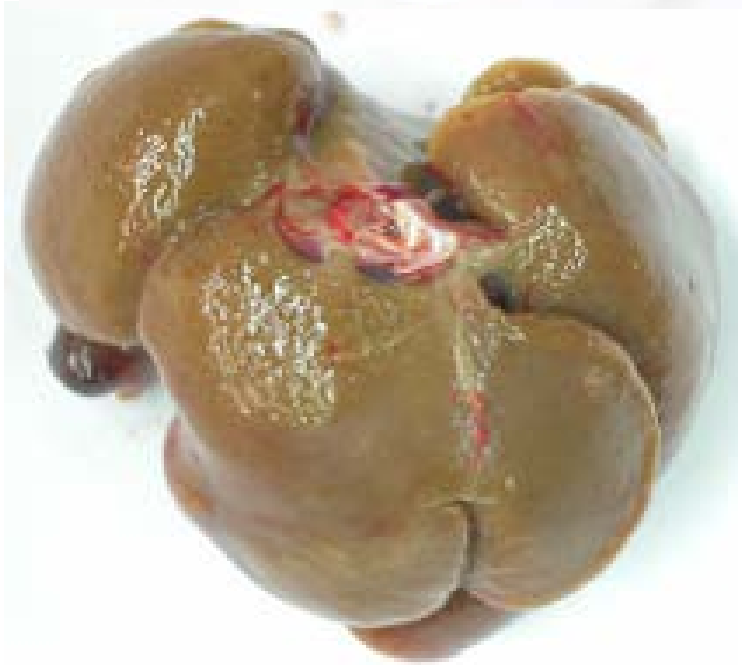
.Variation in size and shape of cells, hyperchromasia of nucleus, increase mitotic activity

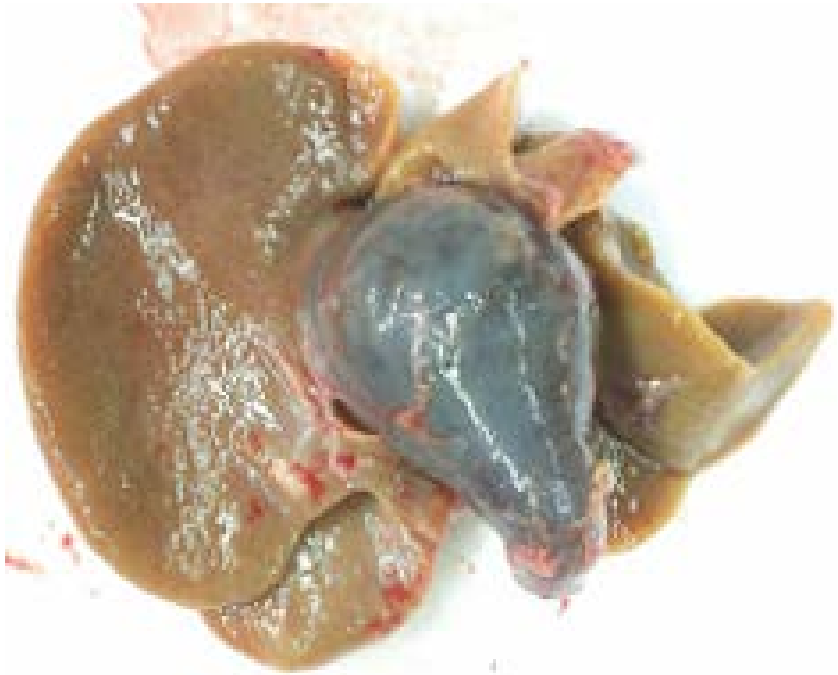
.Benign and malignant tumors classification

⇒ 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

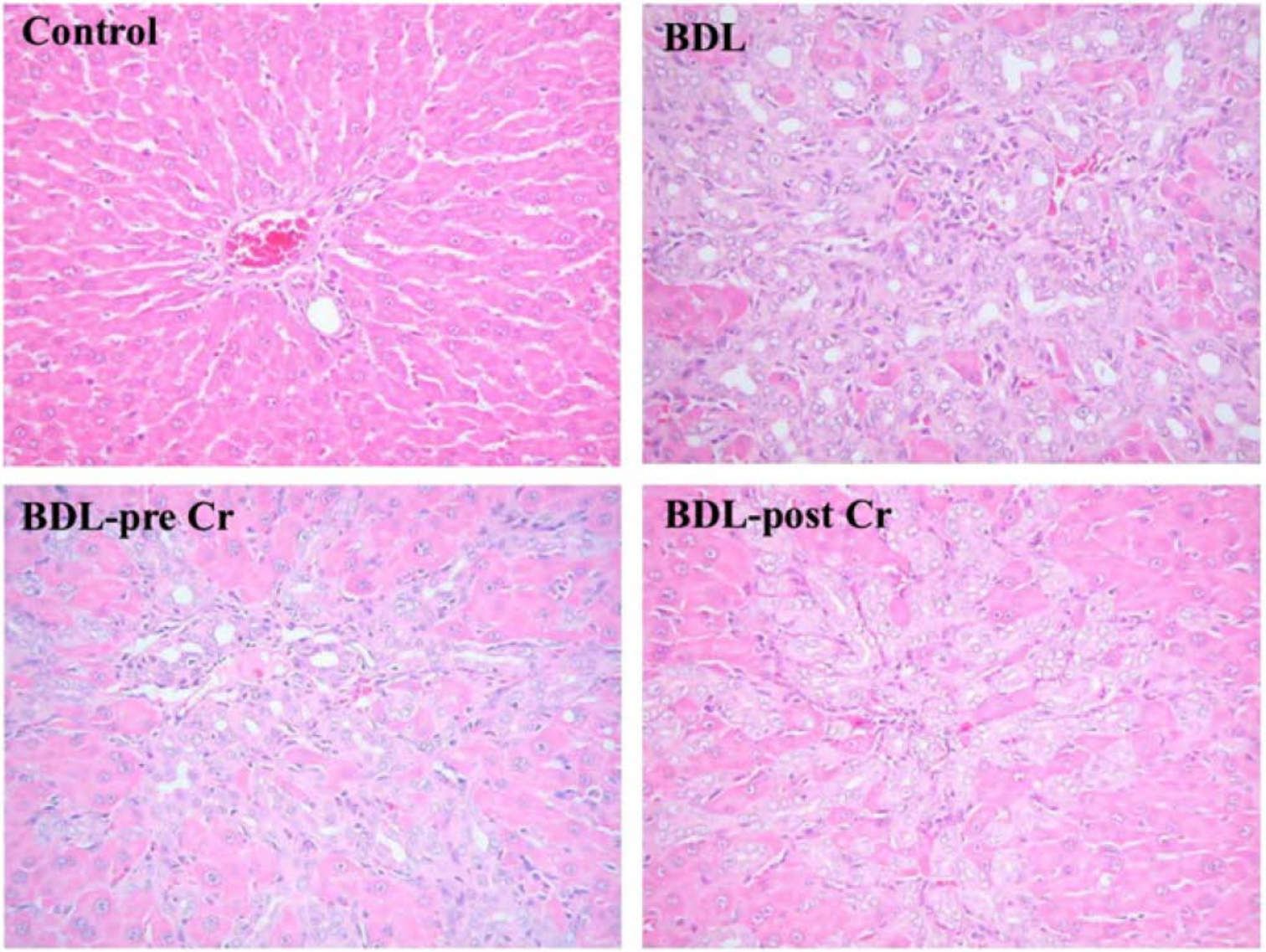


Fig. 1. Chromium attenuated BDL-induced liver injury. Severe bile duct hyperplasia in the portal area after bile duct ligation (BDL).

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

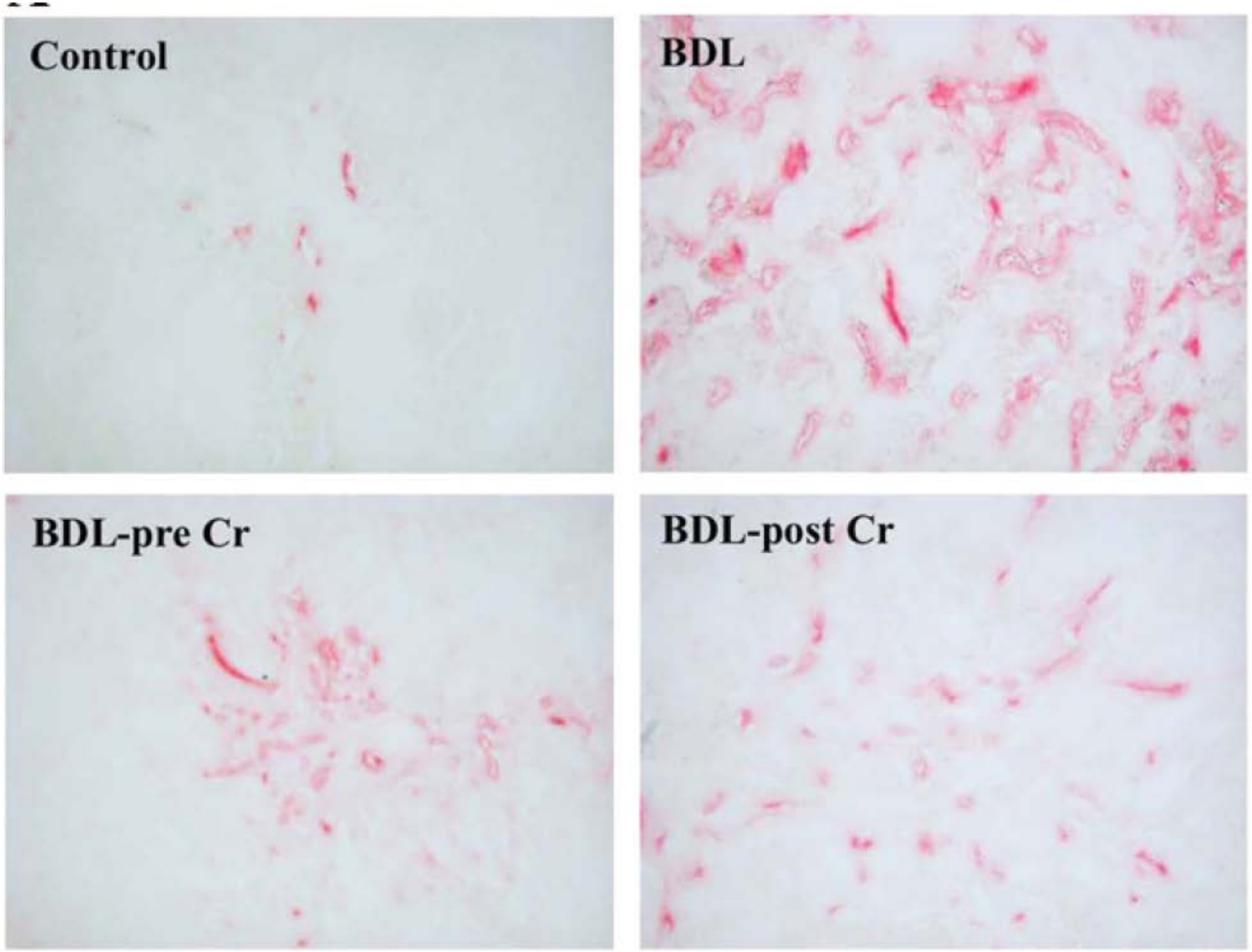
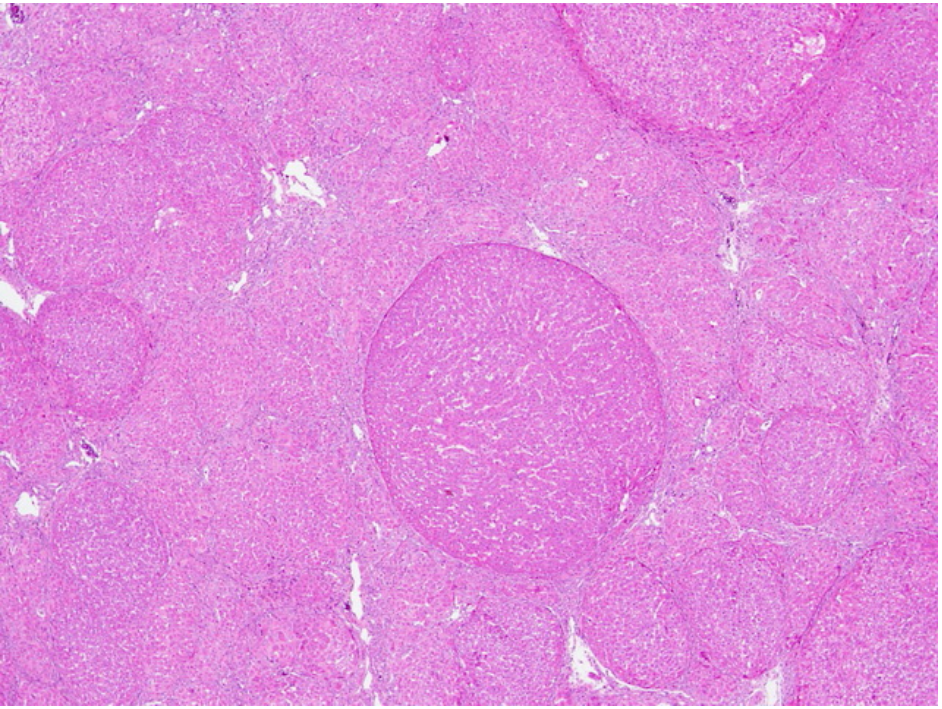
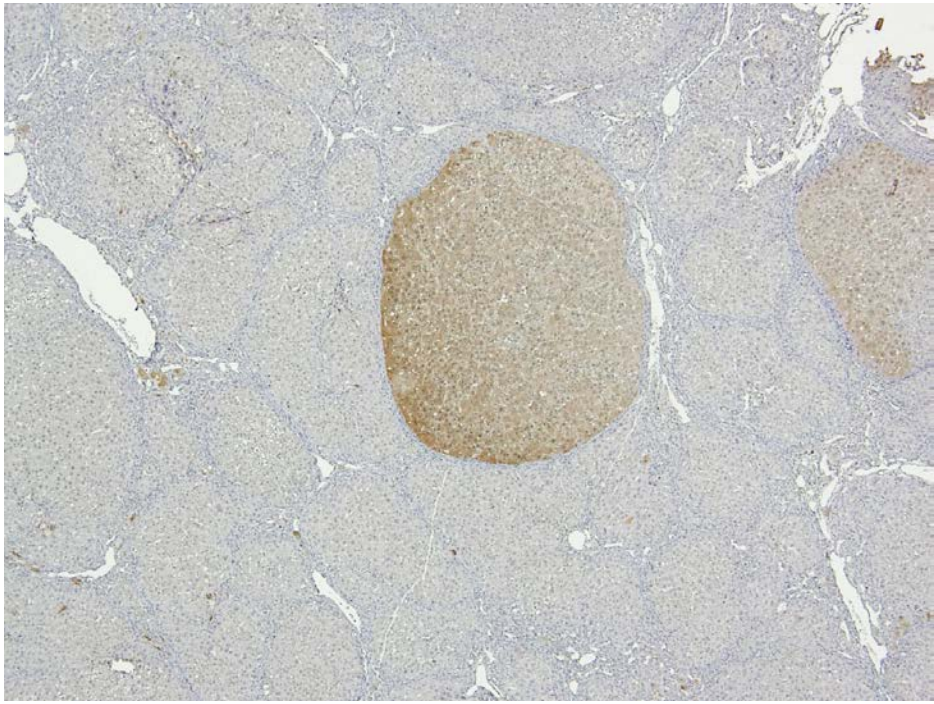


Fig. 2. Chromium attenuated BDL-induced bile duct proliferation. Positive reaction of bile ducts after staining with GGT.

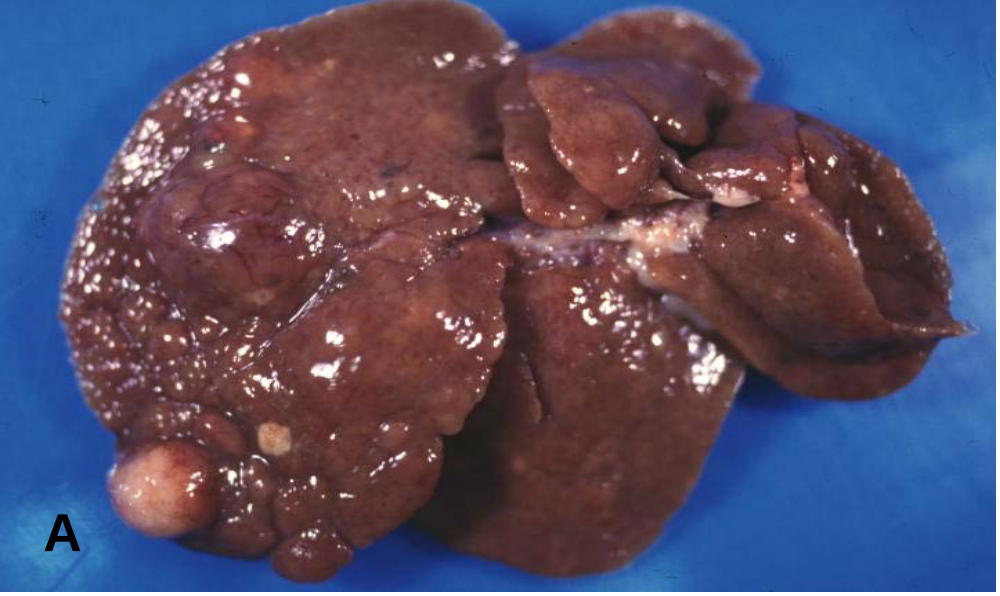
W.-Y. Chen et al. / Life Sciences 84 (2009) 606–614

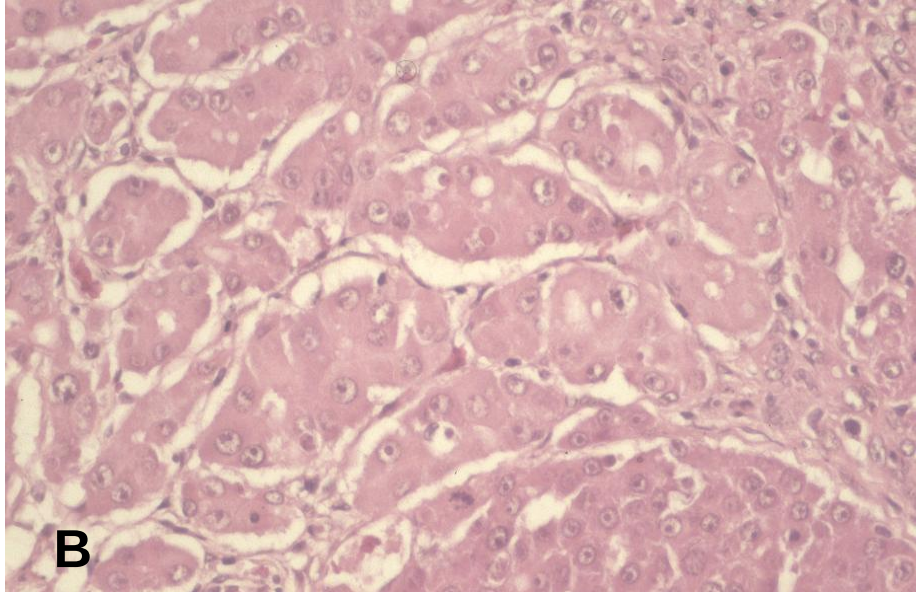


TAA induced nodular hyperplasia in rat’s liver

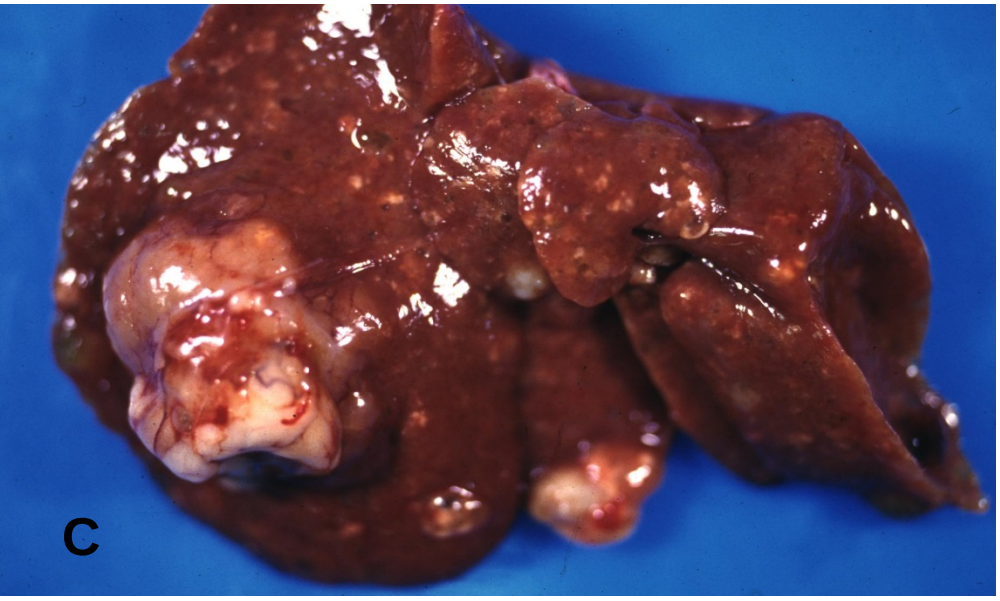


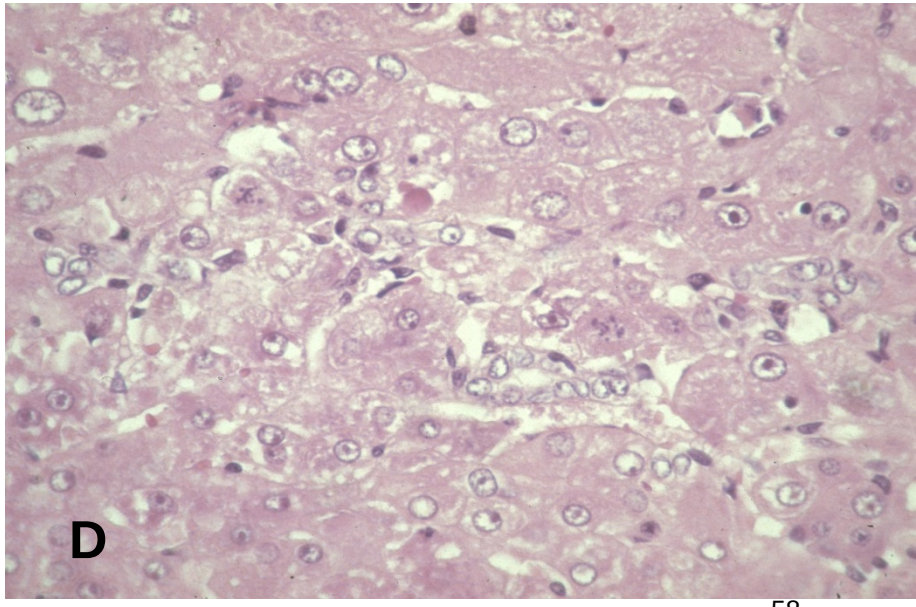
TAA induced nodular hyperplasia in rat’s liver, GST (+)





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





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

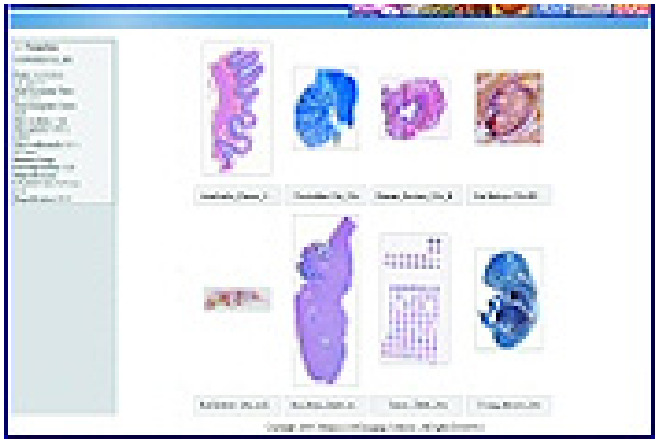
Recording Instruments

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

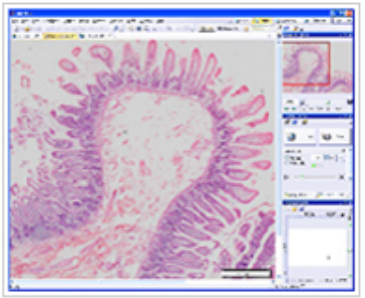
全自動病理掃描資料庫會議系統(DotSlide, Olympus, Germany)

→ 購置年月

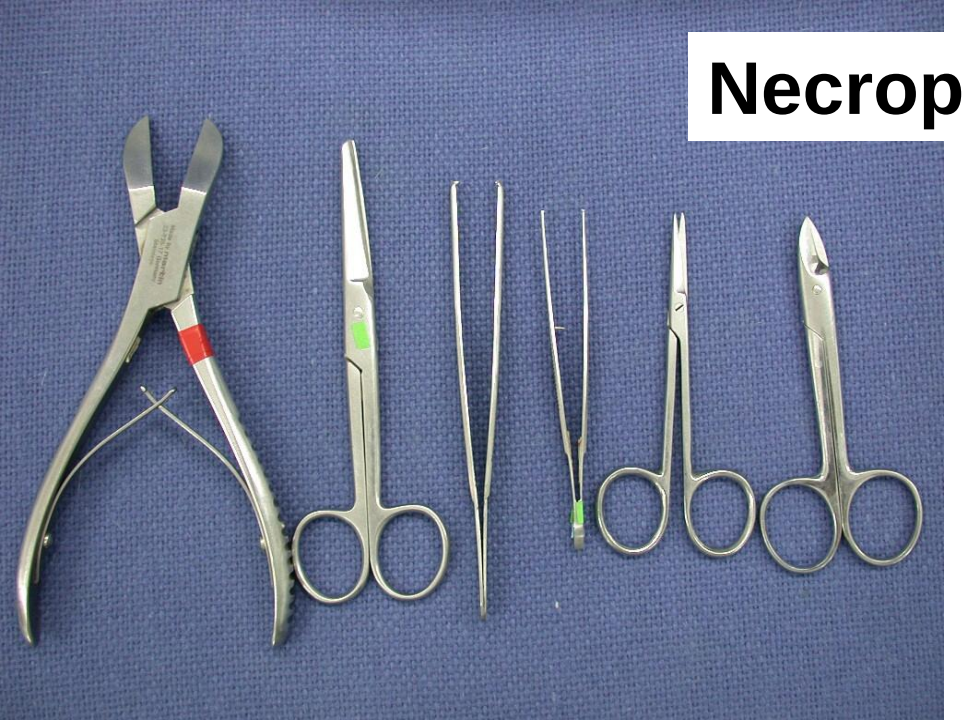
獸醫病理生物學研究所購於2010年12月

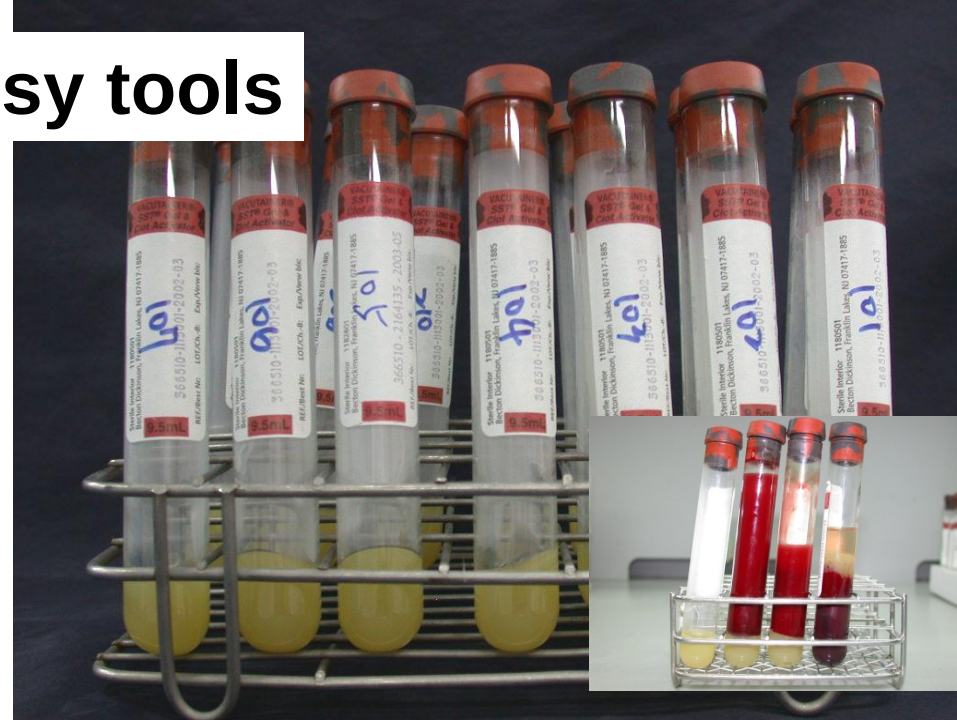
→ 作業地點

獸醫系館 4F



Necropsy tools



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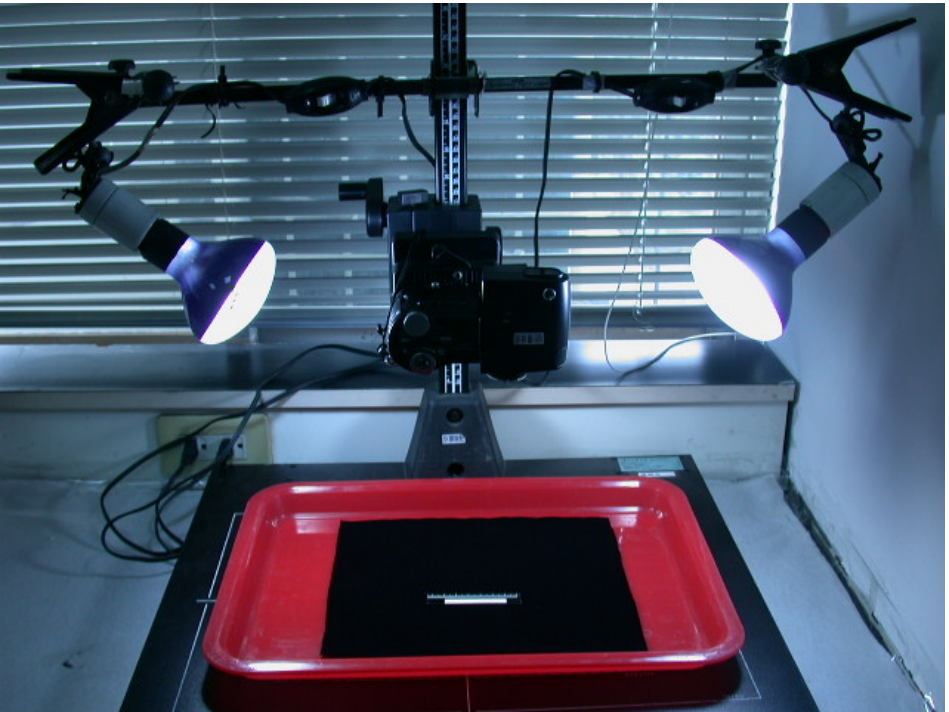

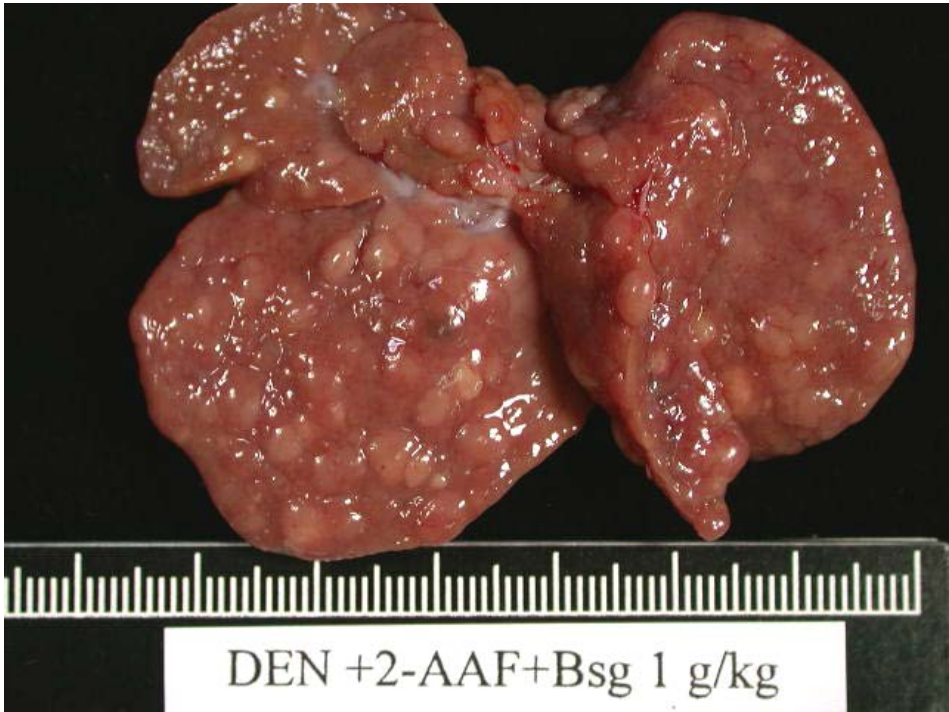
DEN +2-AAF+Bsg 1 g/kg

Revised guides for organ sampling and trimming in rats and mice – Part 1, 2 and 3

CHRISTINE RUEHL-FEHLERT[1], BIRGIT KITTEL[2], GERD MORAWIETZ[3], PAUL DESLEX[4], CHARLOTTE KEENAN[5], CHARLES R. MAHRT[6], THOMAS NOLTE[7], MERVYN ROBINSON[8], BARRY P. STUART[9], andULRICH DESCHL[7]

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8Department of Regulatory Toxicology, Syngenta CTL, Alderley Park, Macclesfield, England
9Toxicology, Pathology and Veterinary Services Department, Bayer CropScience, Stillwell, KS, USA

Exp Toxic Pathol 2003; **55**: 91–106

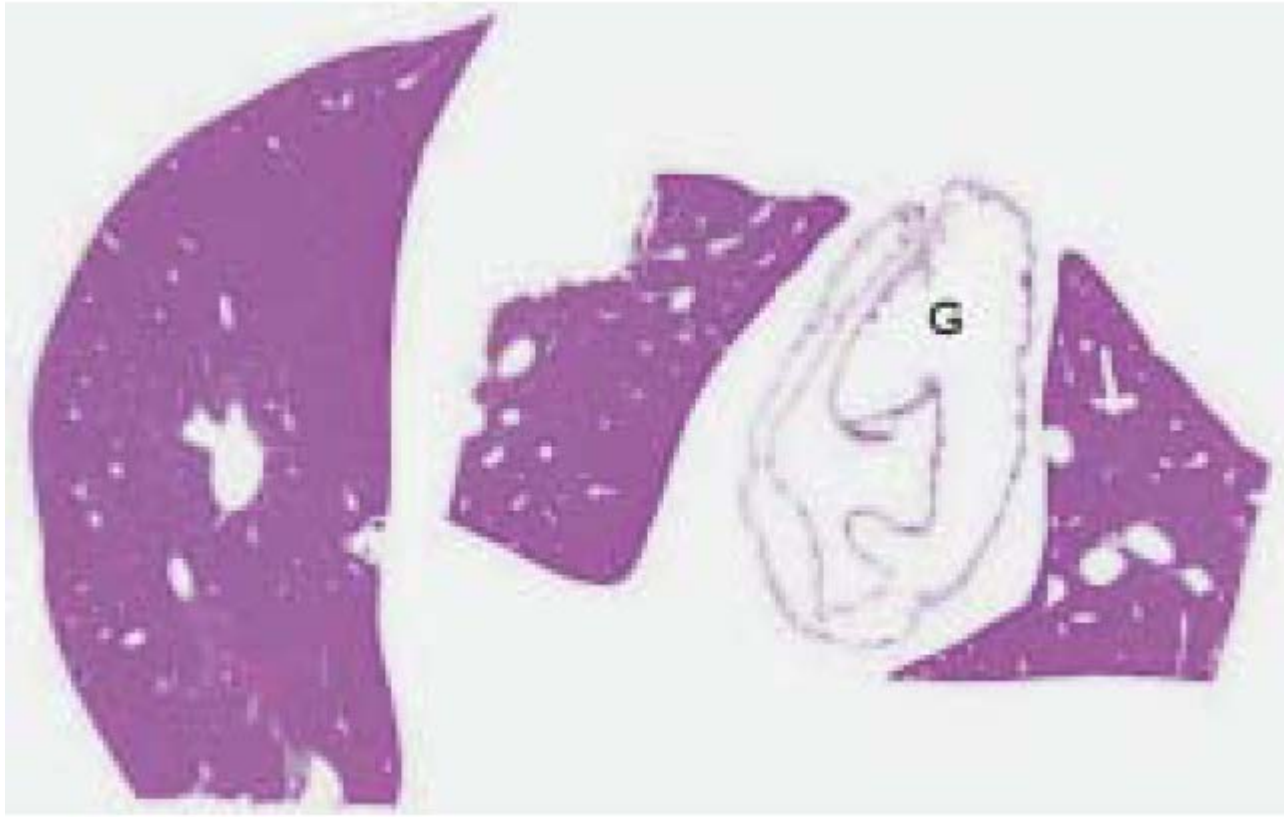
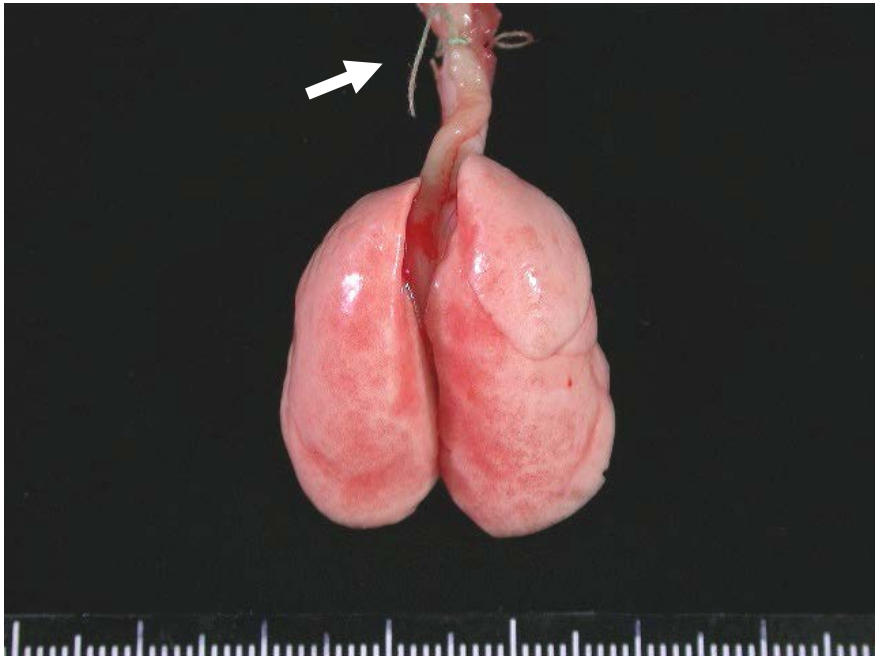
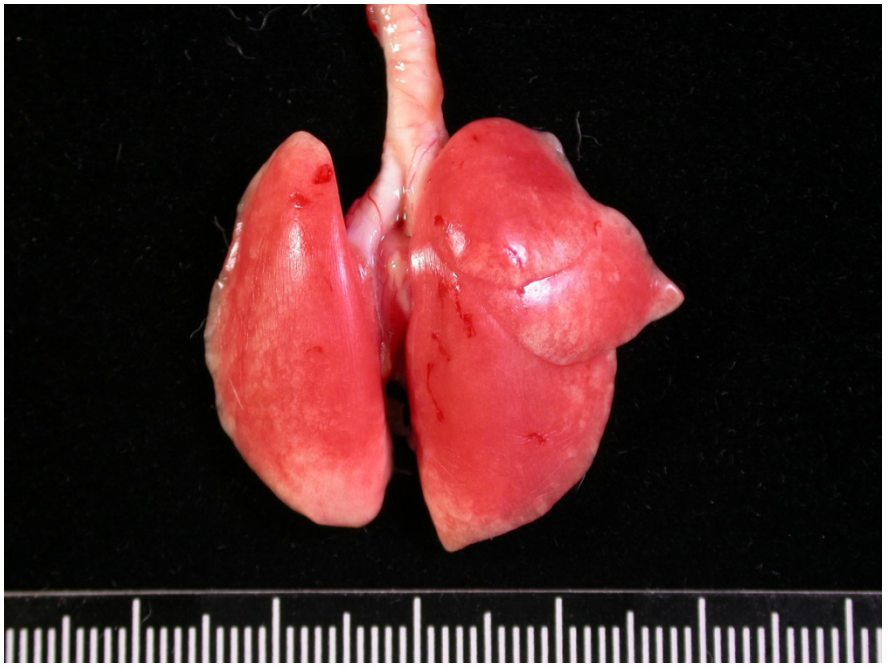


Fig. 2.7d. Mouse: liver and gall bladder (G), sections 1 and 2b.

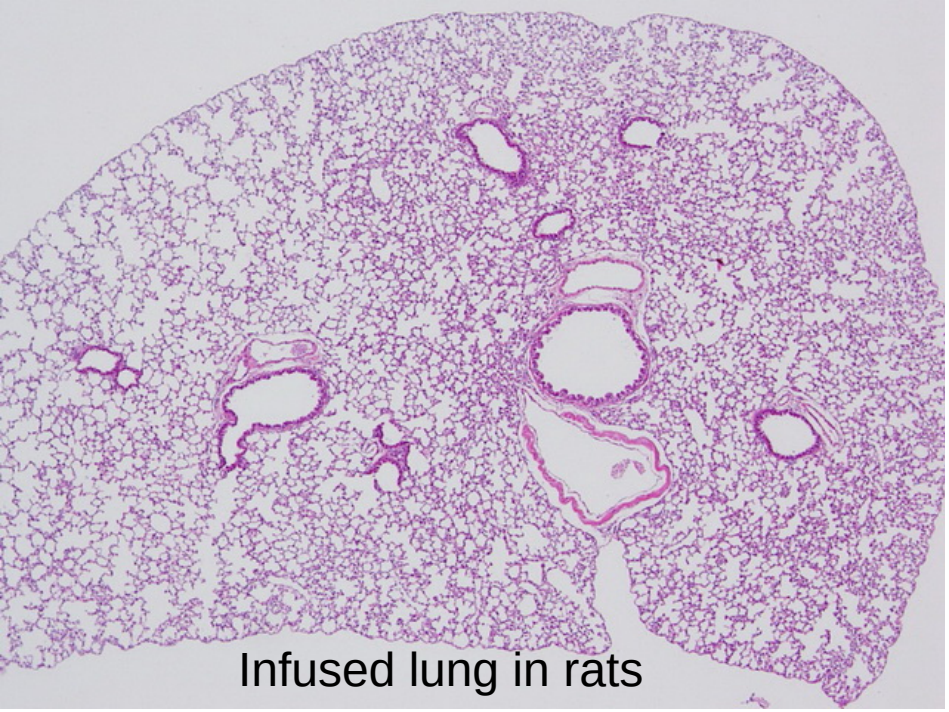
Instillation of 2 ml (rat), 1 ml (mouse) of 10% formalin sol. In lungs is strongly recommended



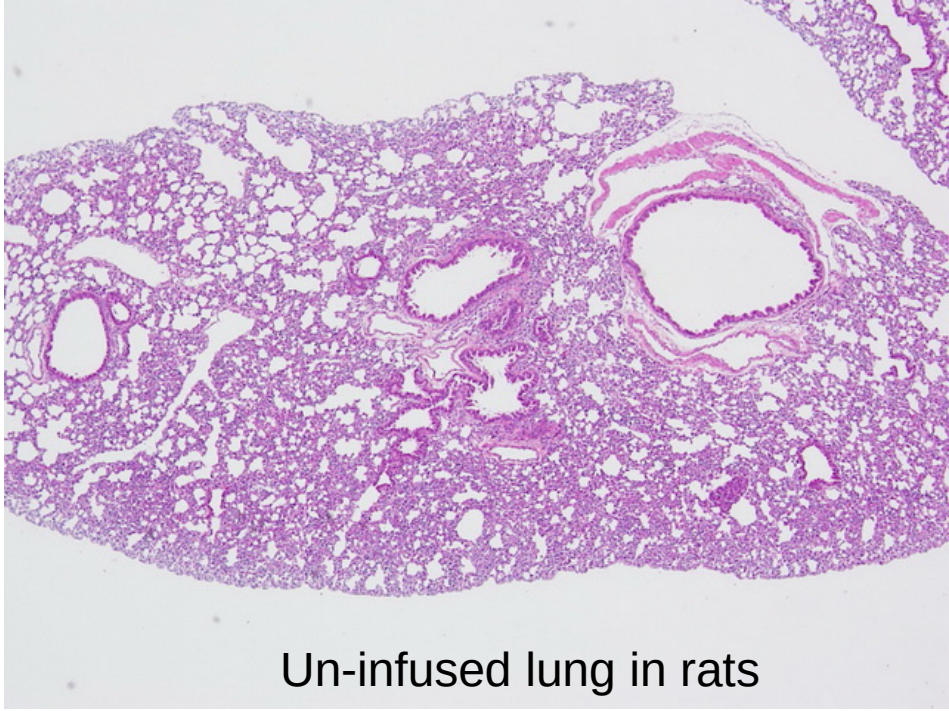
Lung before infuse in rats



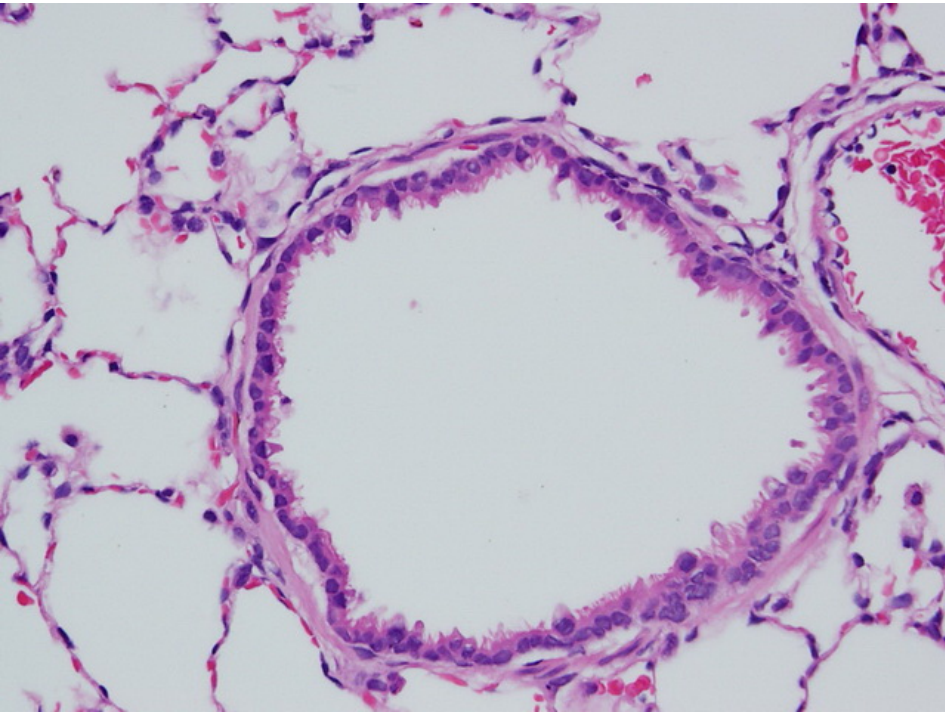
Lung after infusing with 2 ml 10% formalin solution

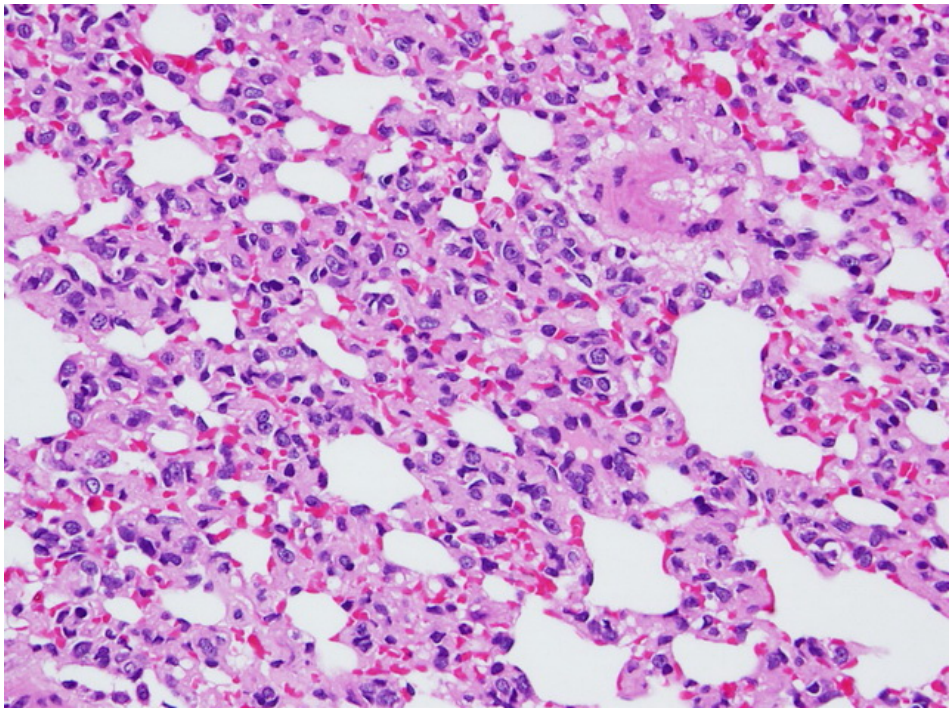


Infused lung in rats



Un-infused lung in rats





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

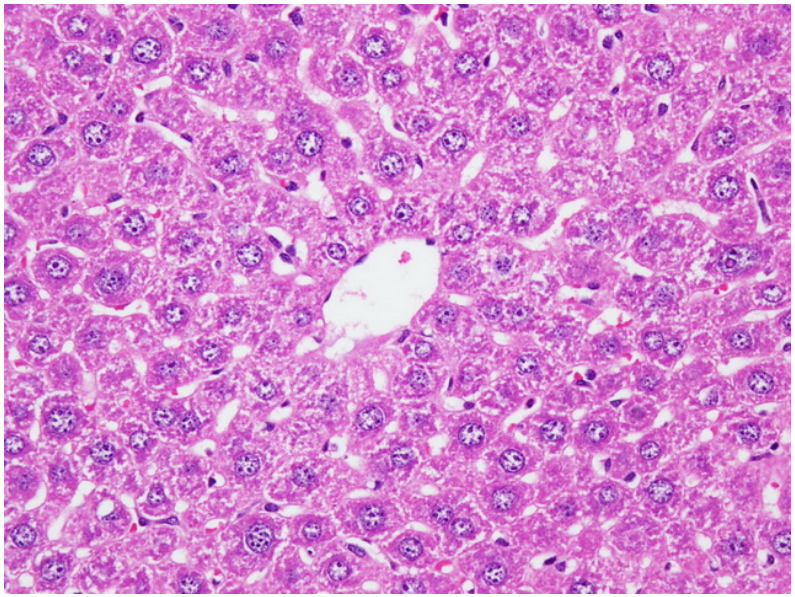
SHACKELFORD et al., TOXICOLOGIC PATHOLOGY, vol 30, no 1, pp 93–96, 2002

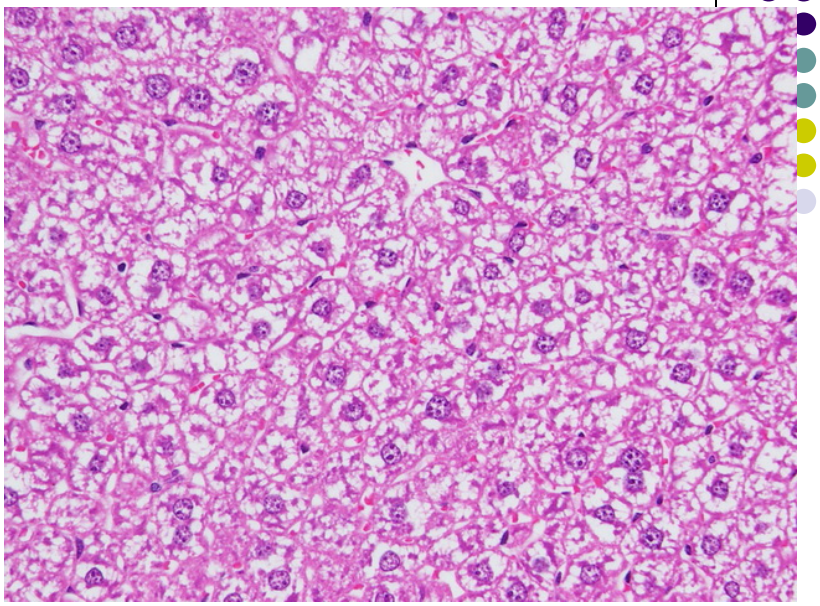
Quality indicators of recoding observations

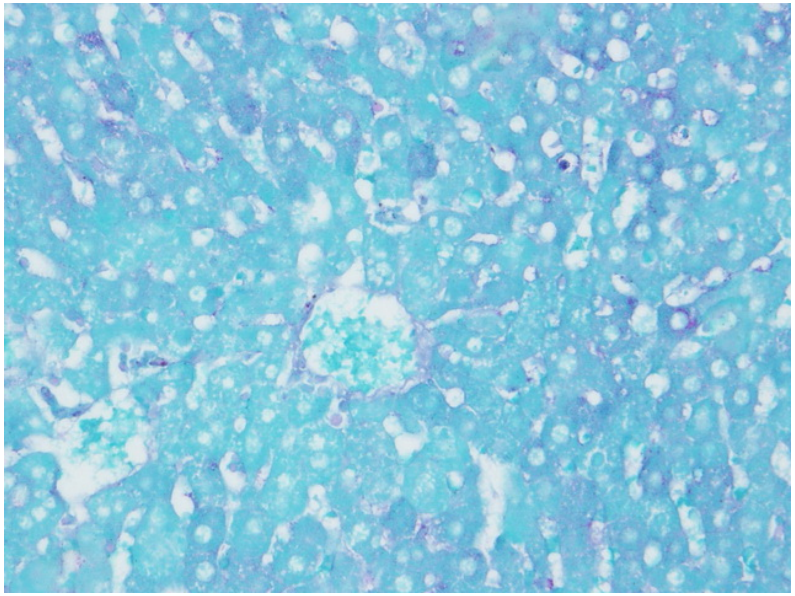
1. **Thoroughness:**
   can be defined as the recording of all lesions, including the frequently observed, usually spontaneous incidental background lesions, present in a particular organ or tissue.

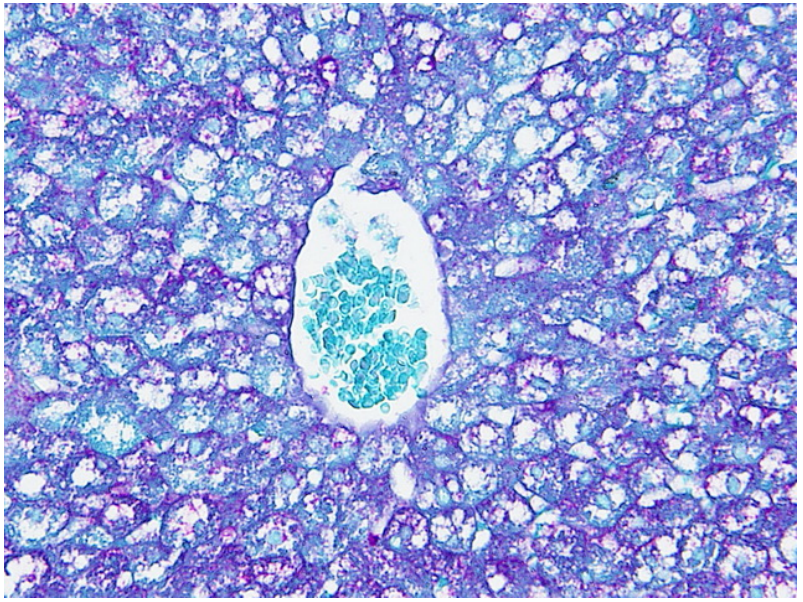
For example,
Hepatocellular cytoplasmic vacuolization is a commonly observed finding in untreated control mice, and the appearance of this finding may vary substantially from animal to animal

?? 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

Quality indicators of recoding observations

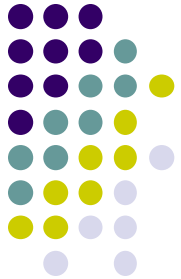
2. "Accuracy" :

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, …).

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

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

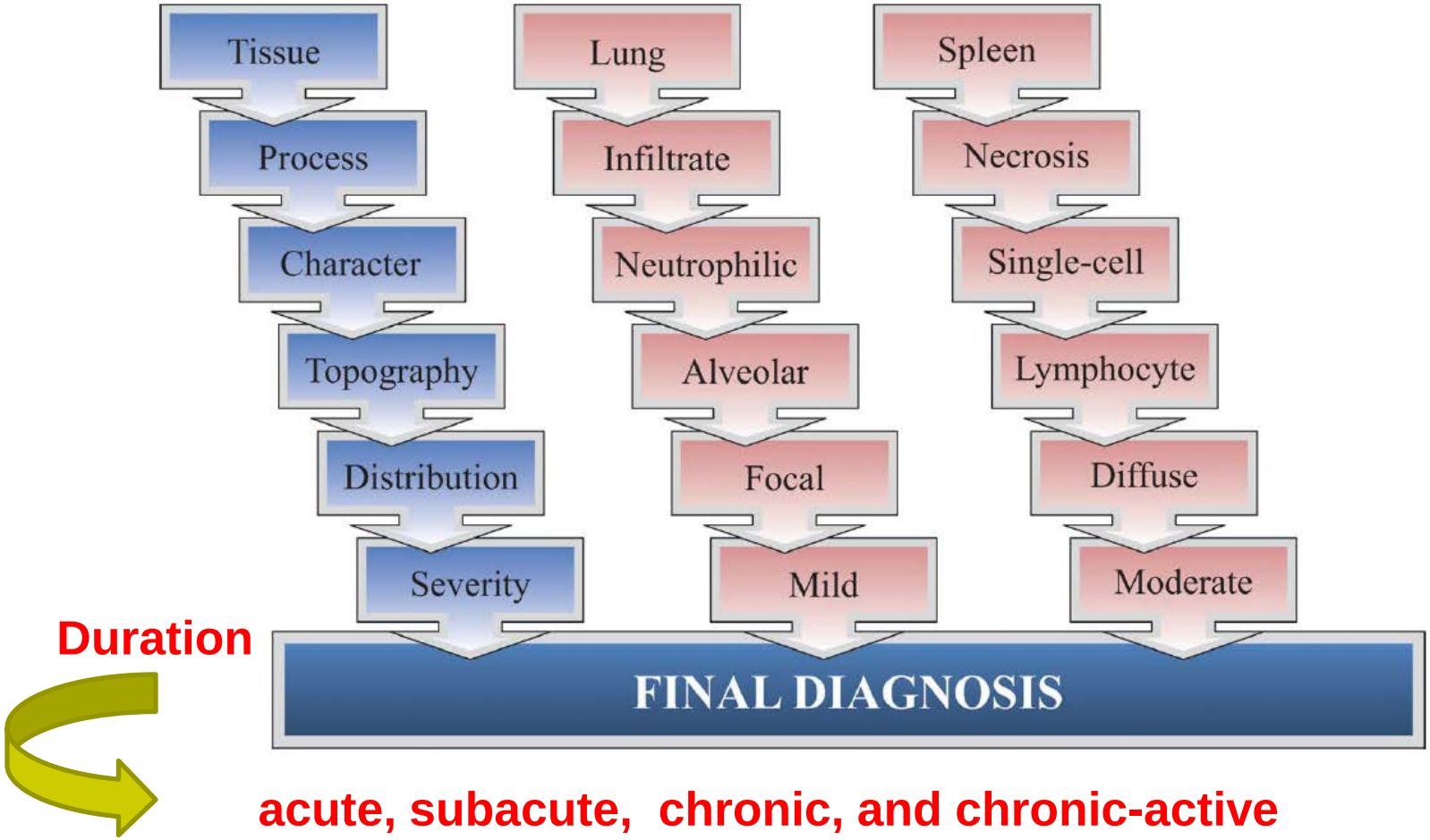


FIGURE 2.—Organization of a final diagnosis. Various modifiers and severity grades can be added to a basic key term or diagnosis to describe and categorize microscopic observations. In blue is an example of the fields possible in a diagnostic term, and examples are in red

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%)

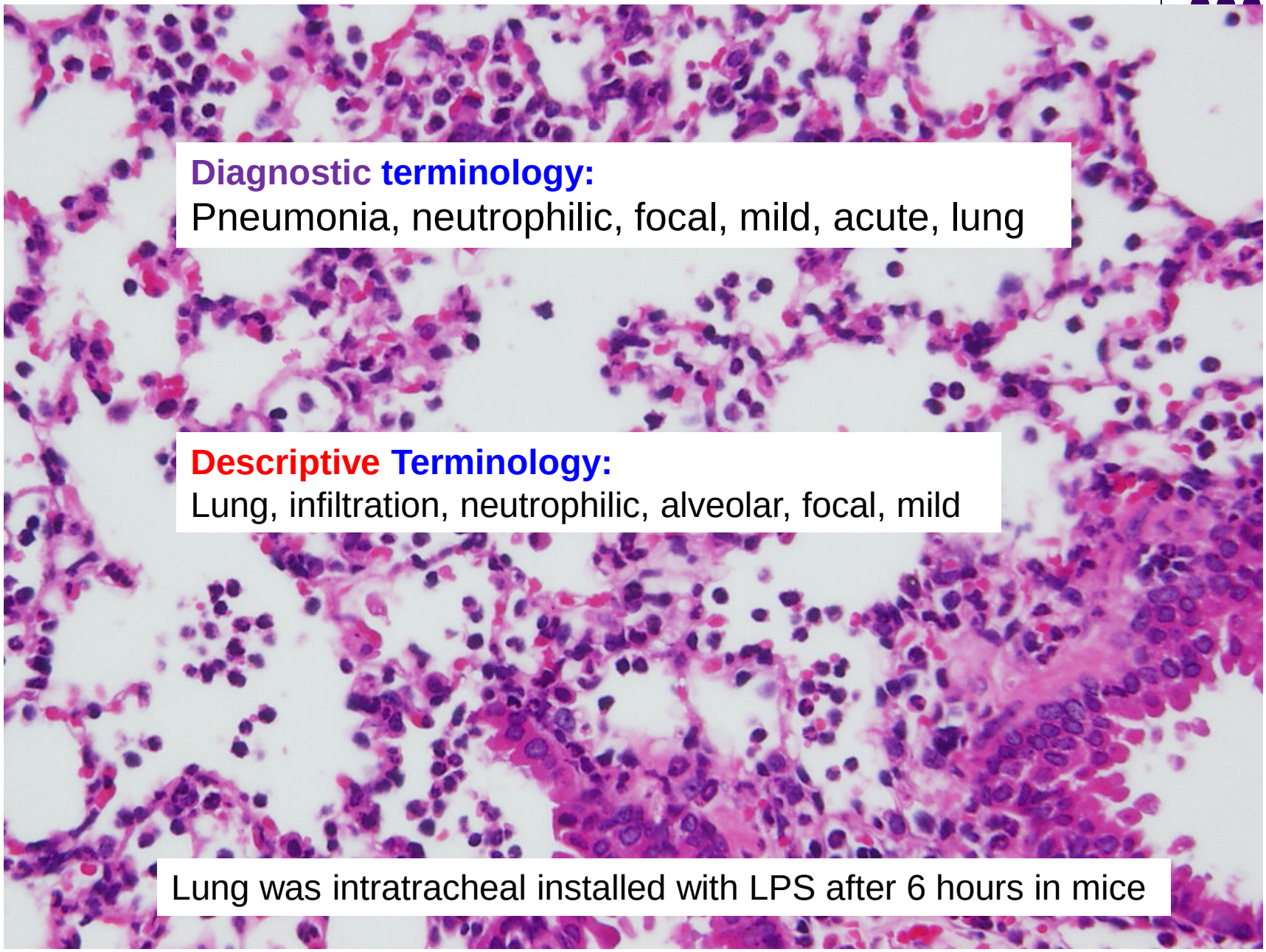
Grading scheme II

	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)

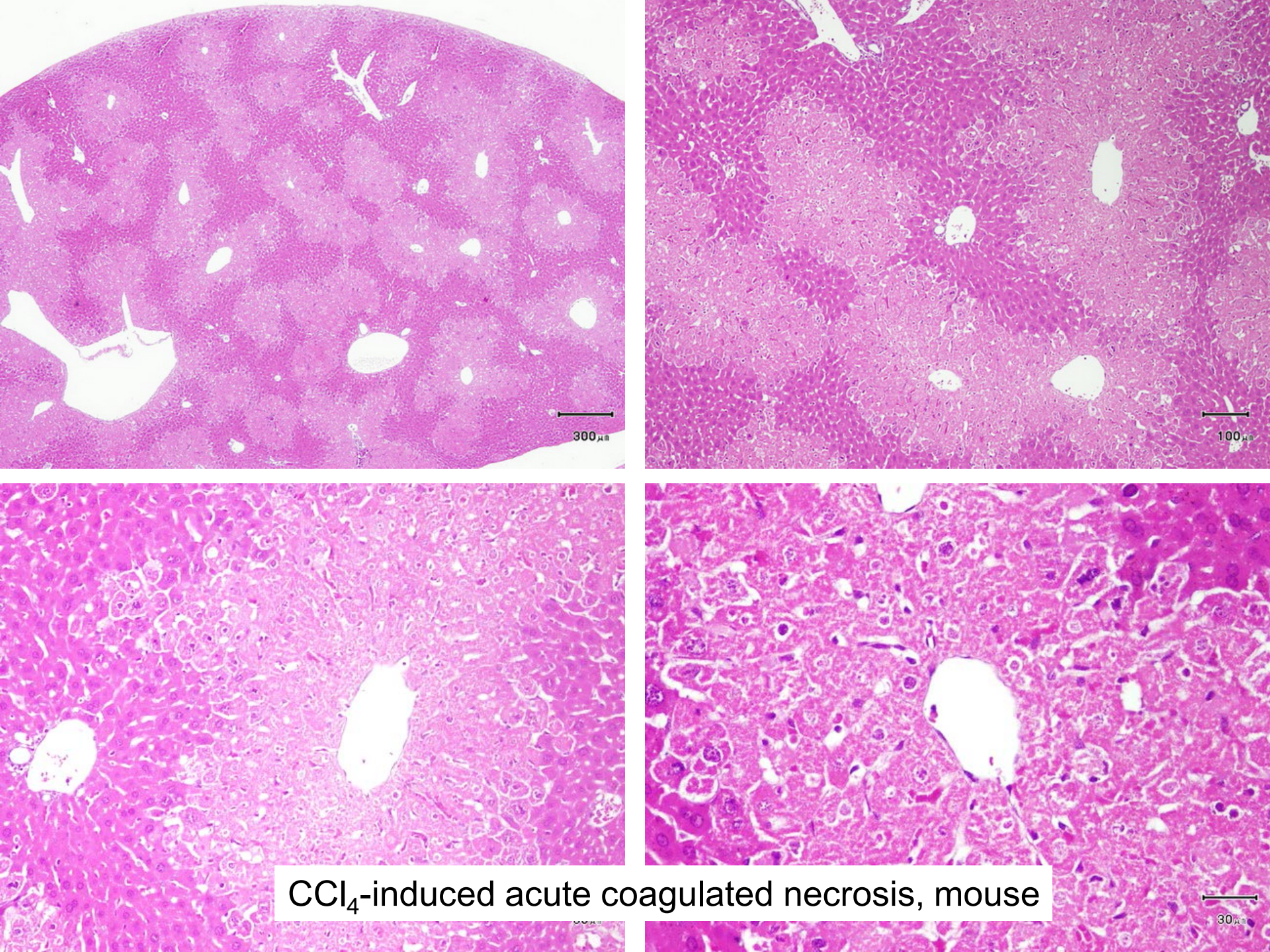
SHACKELFORD et al., TOXICOLOGIC PATHOLOGY, vol 30, no 1, pp 93–96, 2002



Diagnostic terminology:
Pneumonia, neutrophilic, focal, mild, acute, lung

Descriptive Terminology:
Lung, infiltration, neutrophilic, alveolar, focal, mild

Lung was intratracheal installed with LPS after 6 hours in mice



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%).

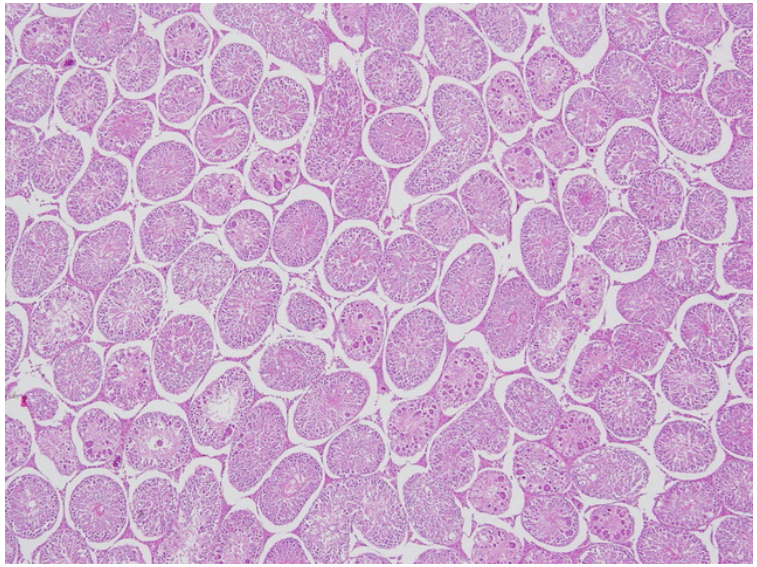
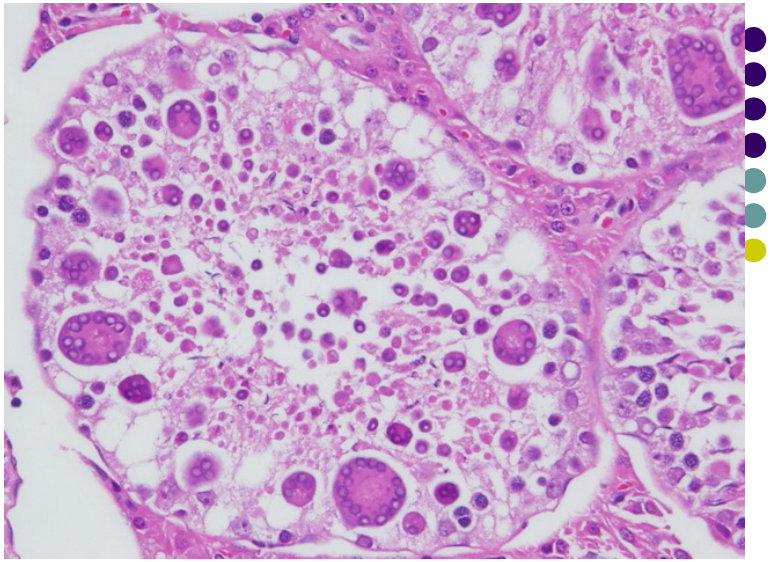
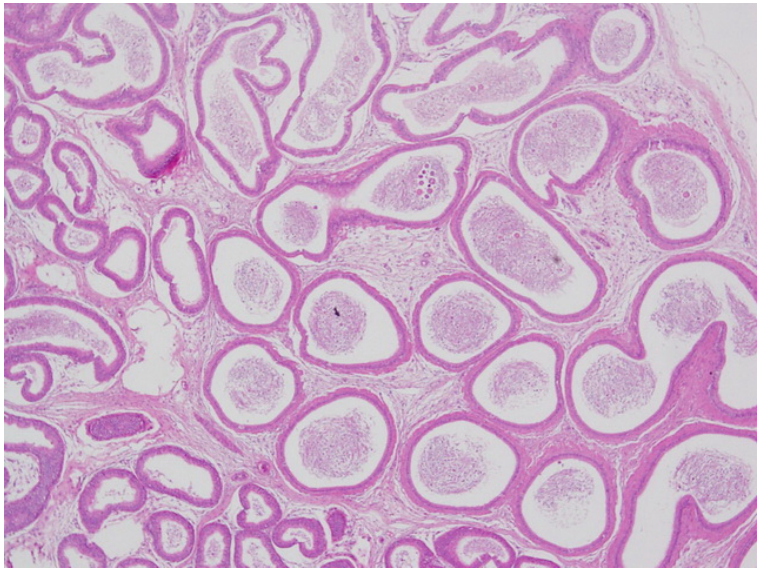
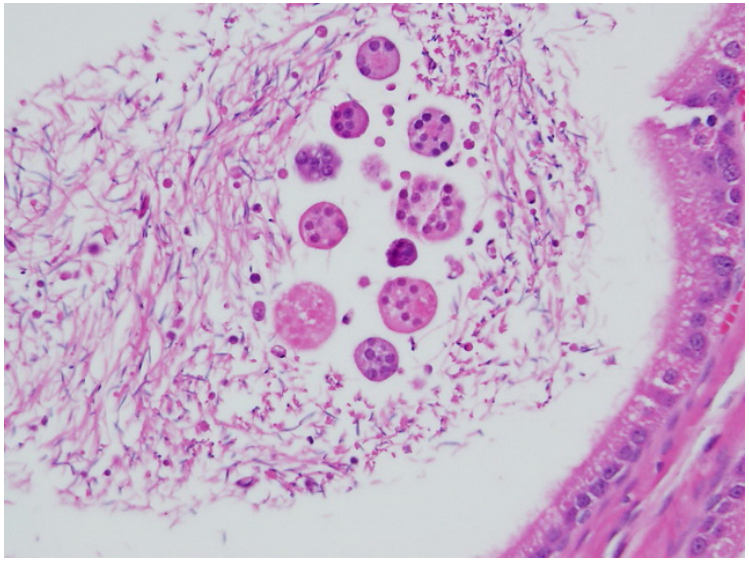
Table 3. Summary of pathological scores of liver injury in mice

Block code	Organ	Histopathological scoring		
		Vacuolization	Inflammation	Necrosis
C	Liver	0	0	0
CCL_4	Liver	1.8±0.4*	2.0±0.0*	4.0±0.0*
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.

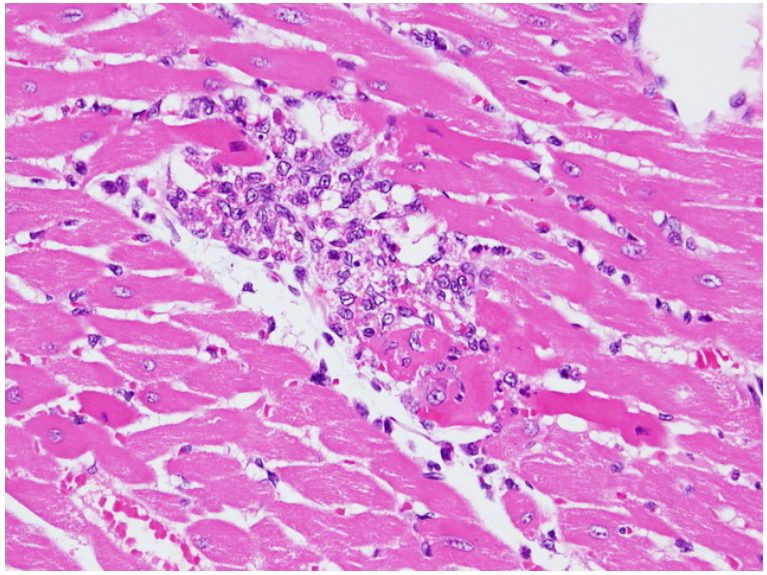
* Statistically significant difference between control and treated groups at $p<0.05$.

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

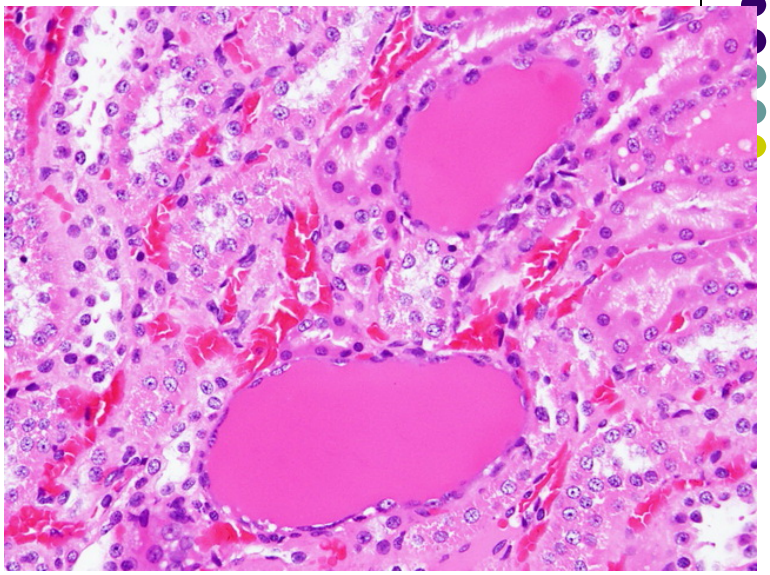





Toxicopathological changes of testes and epididymis of high dose-treated male rats in the 28-day oral toxicity test

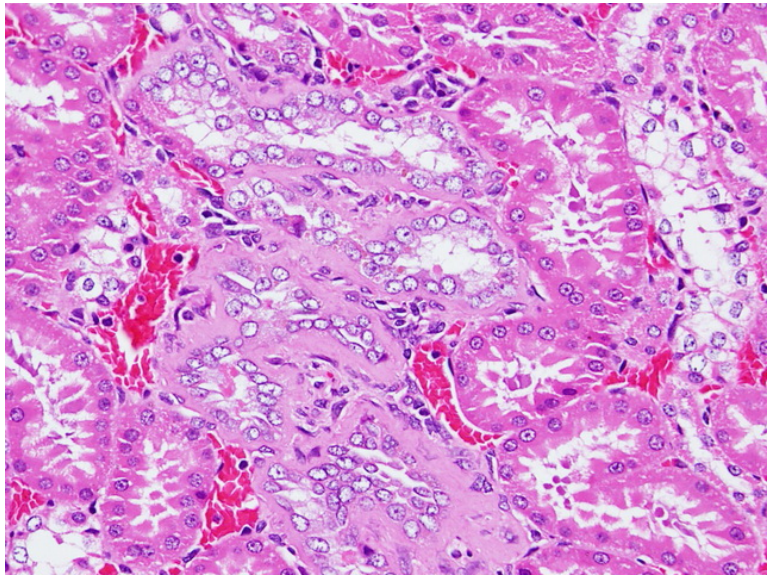
Non-specific lesions of rats in a 13-week feeding study



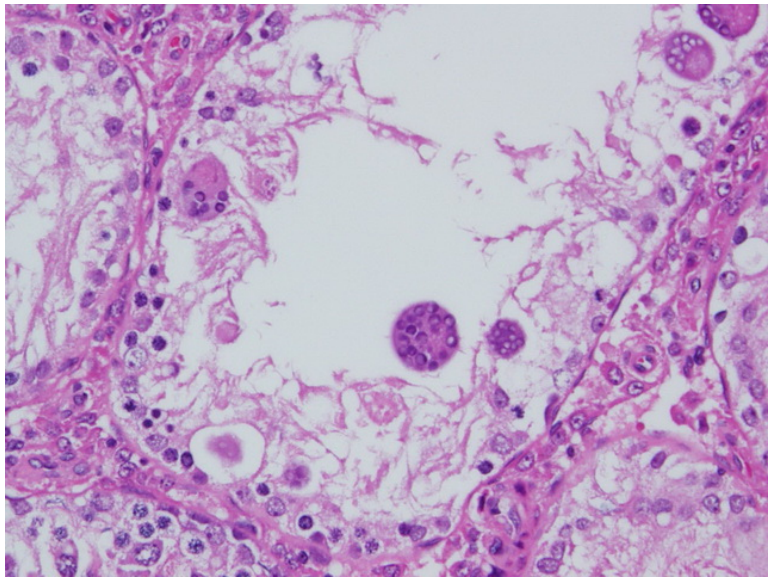
Heart, mono, 1, 400x



Kidney, cast , 2, 400x



Kidney, reg, 2, 400x



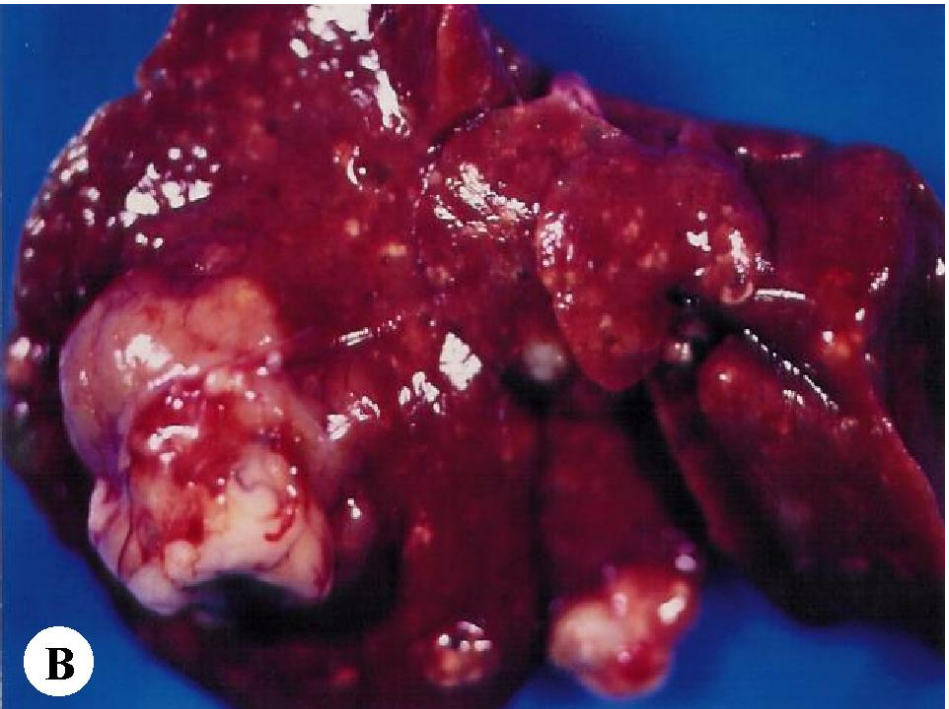
Testis, azoospermia, 5, 400x

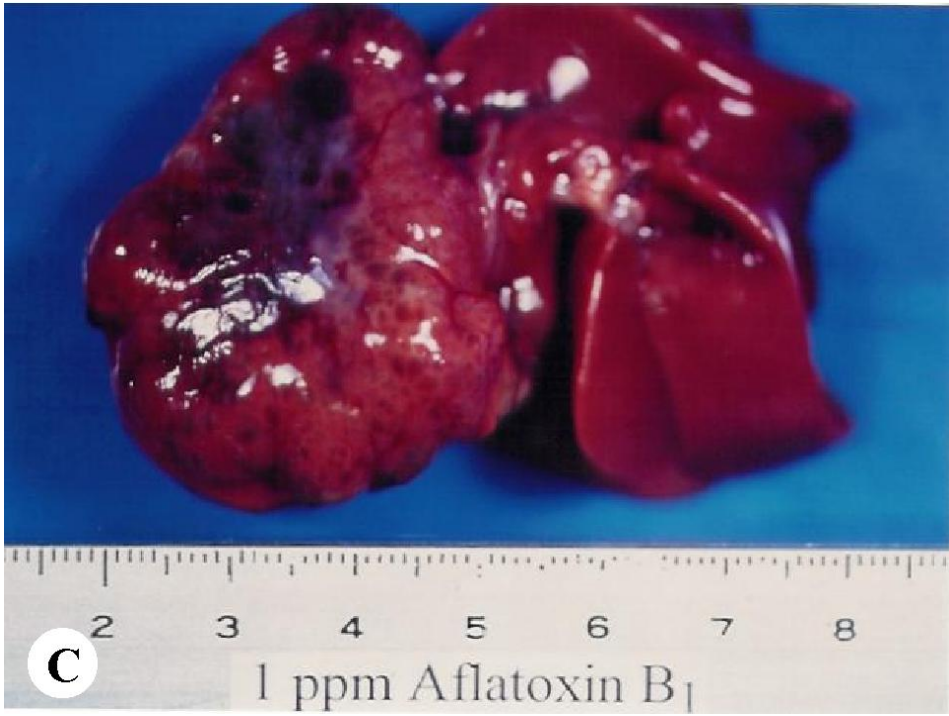
Table 3. Summary of pathological incidence of rats in the 28 days oral subacute toxicity

Organ	Lesions	Group					
		Male			Female		
		Control	Middle	High	Control	Middle	High
Testes							
	Degeneration/necrosis, seminiferous tubule, focal, minimal to moderate	-	-	8/10	N	N	N
Epididymis							
	Granuloma, spermatic, focal, moderate	-	1/10	-	N	N	N
	Degeneration/necrosis, sperm, focal, minimal to moderate	-	-	8/10	N	N	N
Adrenal		-		-	-	-	-
Cervix		N	N	N	-	-	-
Heart							
	Infiltration, mononuclear cell, focal, minimal	1/10	-	1/10			
Kidney							
	Hydronephrosis, pelvis, focal, slight to moderate	1/10	-	1/10	-	-	-
	Pyelonephritis, pelvis, focal, slight to moderate	-	-	-	1/10	1/10	-
	Infiltration, mononuclear cell, focal	1/10	1/10	-	-	-	-
	Regeneration, tubule, ,focal	2/10	1/10	2/10	-	-	-
Liver		-	-	-	-	-	-
Lung							
	Aggregation, macrophage, focal, minimal to slight	-	2/10	-	1/10	1/10	-
Ovary		N	N	N	-	-	-
Oviduct		N	N	N	-	-	-
Spleen		-		-	-		-
Thymus		-	-	-	-	-	-
Uterine		N	N	N	-	-	-
Vagina		-	-	-	-	-	-

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





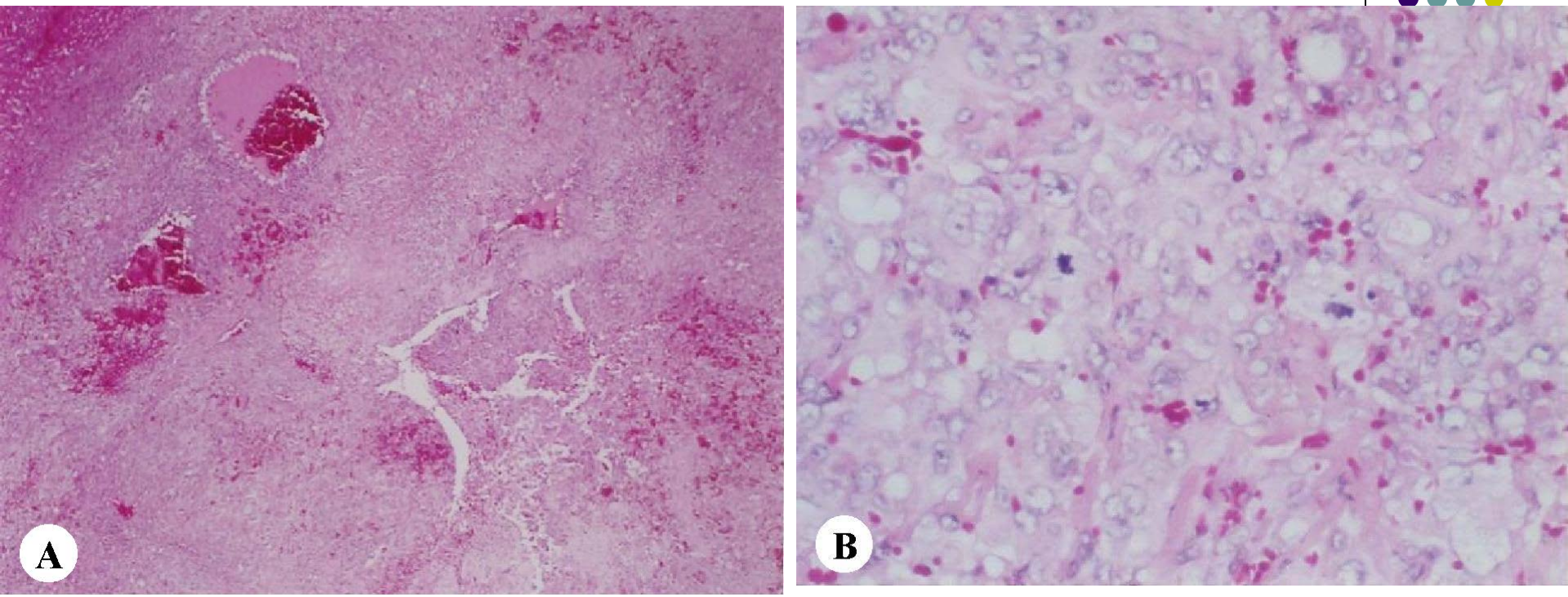


Fig. 3. Photomicrograph from Fig. 2. B. (A) Note the massive occupying and pressing to the normal hepatic cells by neoplastic cells (arrow) and massive hemorrhage (arrow head) in a 2-acetylaminofluorene treated rat. (H&E stain, 40×). (B) Higher magnification from A. Note the highly cellular mitotic figures (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
Olympus, DP20, 72
Image Pro Plus
Nikon

HistoQuest

Taylor and Levenson, 2006

- **Image analysis**

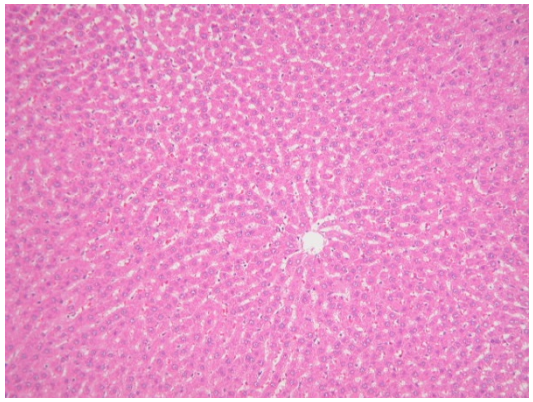
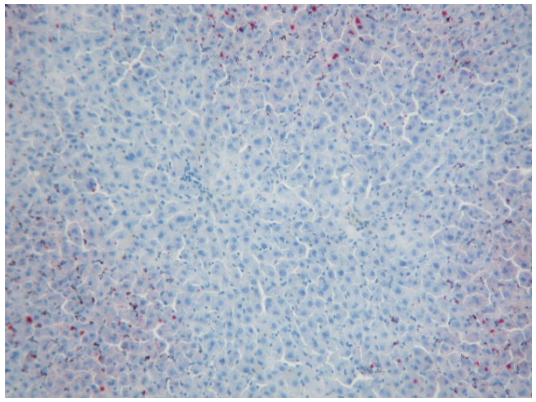
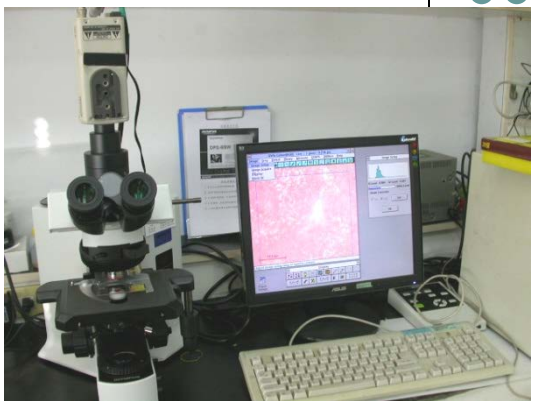
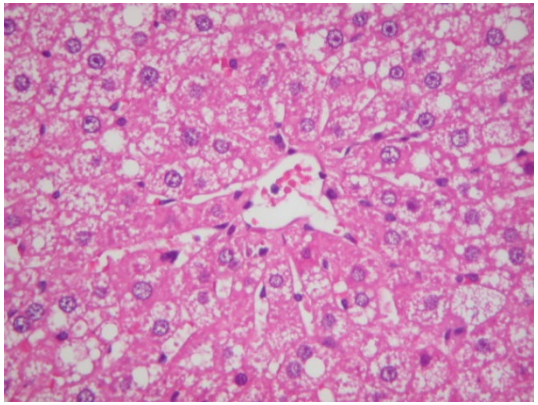
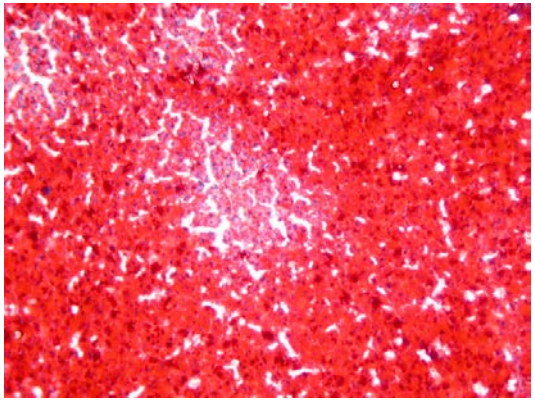
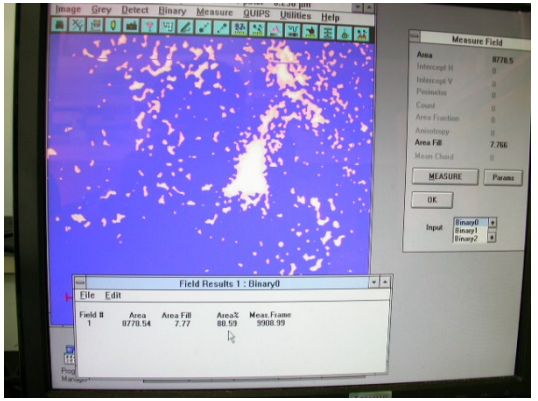







Fig. 1. Image analysis system. Adipose tissue was positive stained by Oil red staining in cryostat liver section. Liver sections were acquired under light microscope with 80x magnification, and the positive areas, following measurement and analysis, data of positive areas (%) were counted.

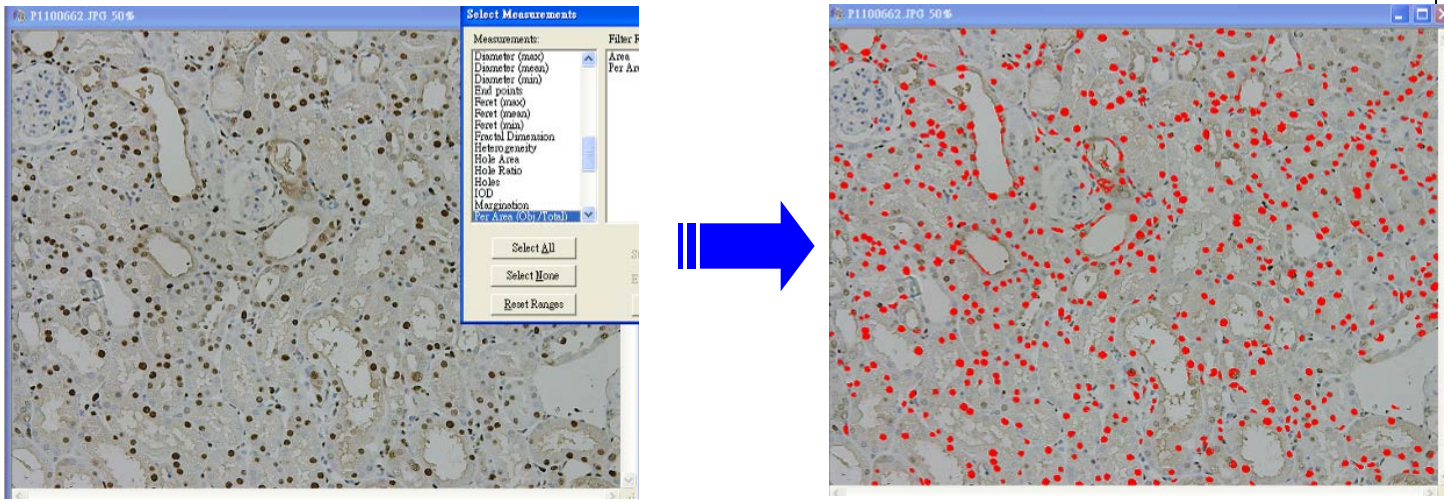
Table 2. Effect of xxx on the incidence and percentage of fatty livers in rats

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 $p < 0.05$.

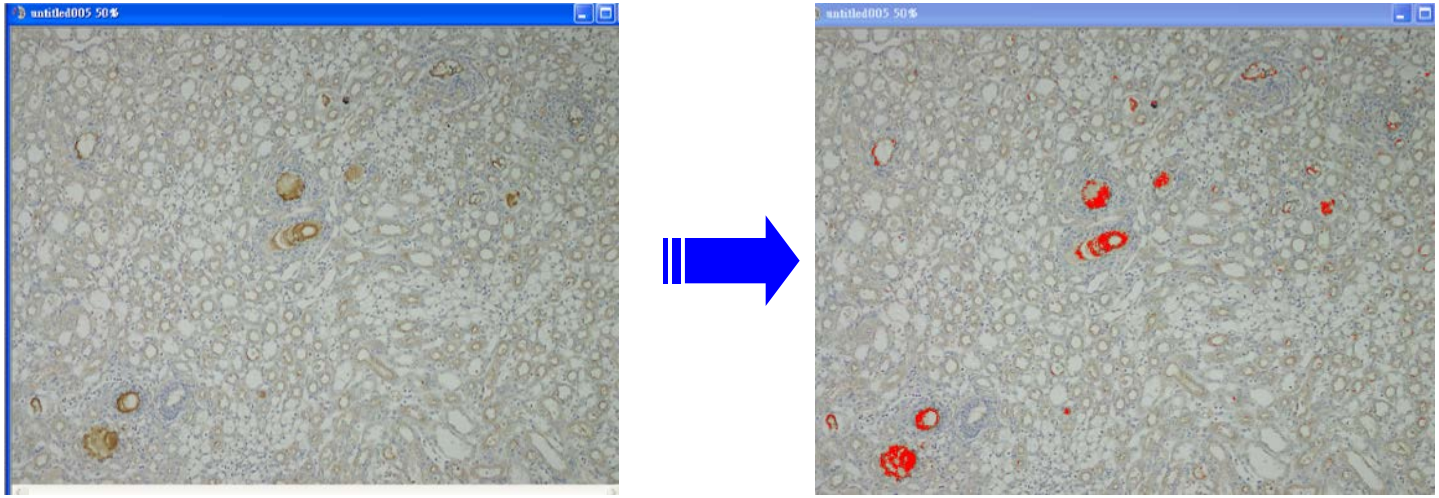
Image Pro Plus
(PCNA & OPN count)

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

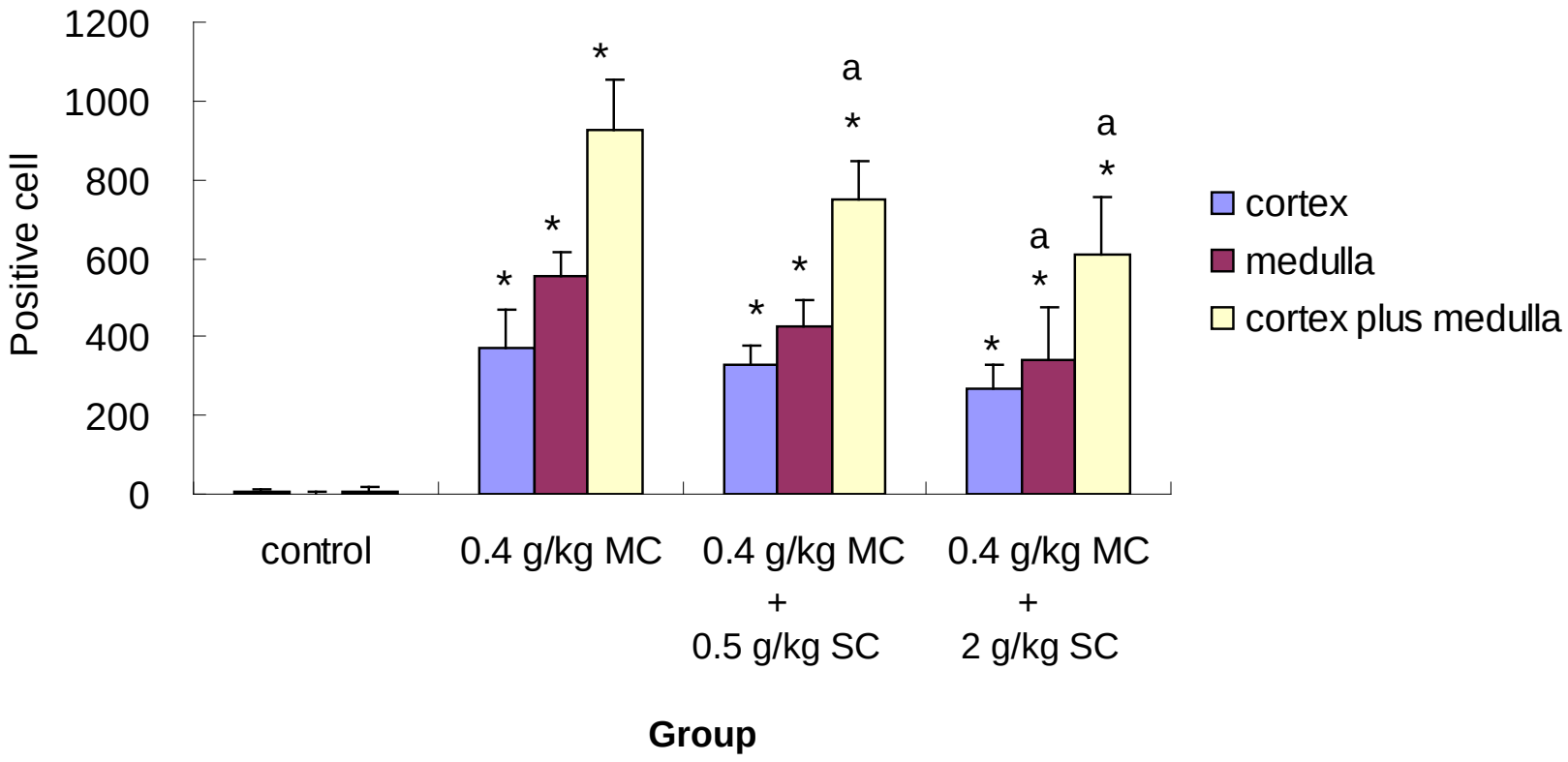


Fig. Counting of PCNA positive cells in MC +SC (treated rat kidney. *p< 0.05 compared to control group; ^{a}p< 0.05 compared to 0.4 g/kg MC group

Global Engage

4TH DIGITAL PATHOLOGY CONGRESS: ASIA

Development of Healthcare Through DP and AI for Improved Patient Management & Outcome

TOKYO, JAPAN

— 9-10 May 2018 —

- Current Trends and Movement in Digital Pathology **- Artificial Intelligence (AI) in digital pathology**
- Computational Pathology: Applications, Technologies and Prospects
- Digital Image Analysis
- **Transitioning to Telepathology for Accurate Diagnosis**
- Digital Pathology Application and Research Case Studies

DAY 1 - TRACK 1

Current Trends and Movement in Digital Pathology

- Contemporary issues and barriers of adopting digital pathology
- Emerging technology revolving digital pathology
- Regulatory overview in digital pathology
- Quality assurance, control and improvement
- Implementation of digital pathology in training and research
- Artificial Intelligence (AI) in digital pathology
- Digital pathology: benefits, barriers and future implications

DAY 1 - TRACK 2

Computational Pathology: Applications, Technologies and Prospects

- Computational methodologies in digital pathology
- Approaches and scientific challenges in computational pathology
- Development of tools in computational pathology
- AI and machine learning
- Whole slide imaging: acquisition, processing, archiving and retrieval
- Cloud computing / storage solutions

DAY 2 - TRACK 1

Digital Image Analysis

- Overcoming challenges in image analysis
- Computer aided diagnoses
- User interfaces and image registration
- Image quality and scanning speed
- Quantitative image analysis research
- Visualisation methods for diagnosis and prognosis
- Image processing
- Pattern recognition and annotation tools
- Algorithm development / Image analysis algorithms

DAY 2 - TRACK 2

Transitioning to Telepathology for Accurate Diagnosis

- Current status and future trends in telepathology
- Workflow integration
- Teleconsultation
- Legal and regulatory issues in telepathology
- Challenges in implementation
- E-learning and e-training
- Telemedicine and consultation
- Virtual pathology

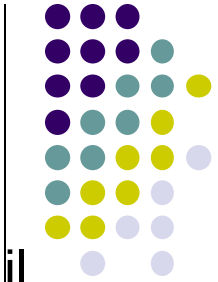
CASE STUDIES

Digital Pathology Application and Research Case Studies

- Diagnostic studies
- Clinical studies
- Personalised targeted therapy
- Tissue based research
- Artificial intelligence
- Machine learning
- Deep learning

http://www.global-engage.com/event/digital-pathology-congress-asia/

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)”

PATHOLOGY WORKING GROUP (PWG)

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 risk

1. **An acceptable daily intake (ADI)** for human is based on the no observed adverse effect level **(NOAEL) in chronic toxicity in rodent 2-yr test**

2. **ADI** = NOAEL/UF x MF (mg/kg/day)
   ⇒ Species difference (10) x Individual viability (10) x Magnified factor (target organ, 10)

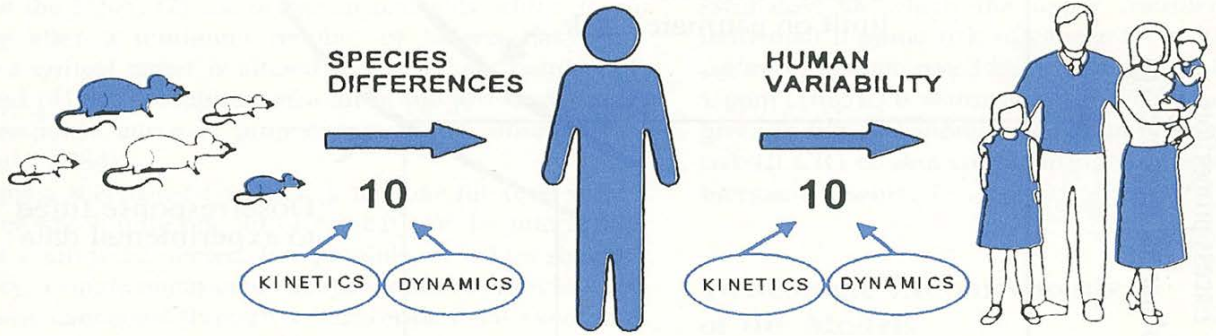


Figure 4-4. Toxicokinetic (TK) and toxicodynamic (TD) considerations inherent in interspecies and inter-individual extrapolations.

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.)