



# Injection site histopathology - overview and interpretation



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# Disclaimer



**The ideas and thoughts expressed in this presentation are purely that of the presenter and do not necessarily reflect the thinking of the organization or represented by the presenter.**

# Behavioral reaction



# Factors need to be considered for evaluation of Injection Site

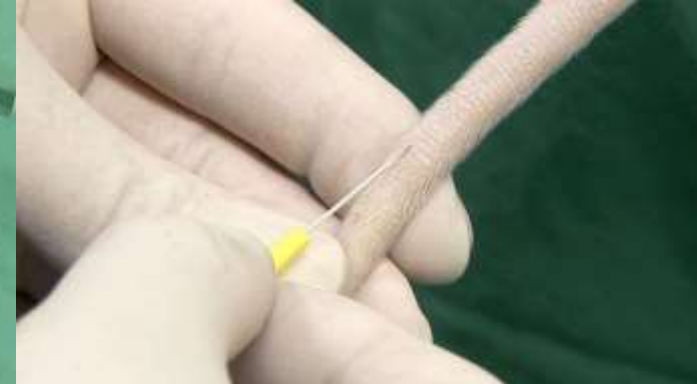


- **Test item/article and vehicle**
- **Route of administration**
- **Duration of treatment and recovery period**
- **Species**
- **Gross lesion present or absent**
- **Trimming of injection site**
- **Changes can be subtle or prominent**

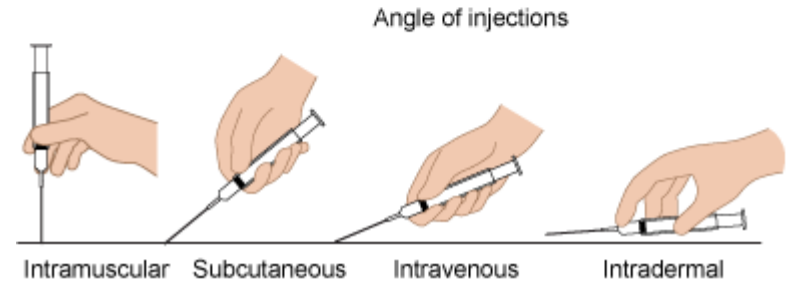
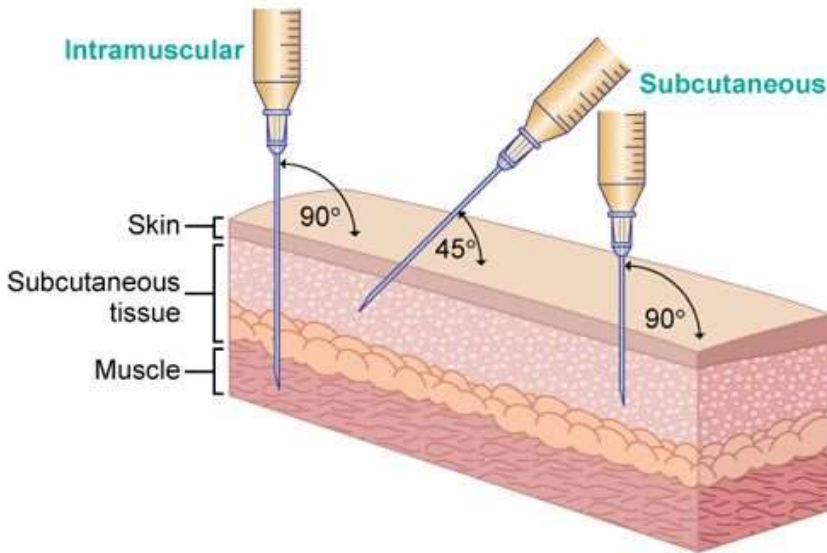
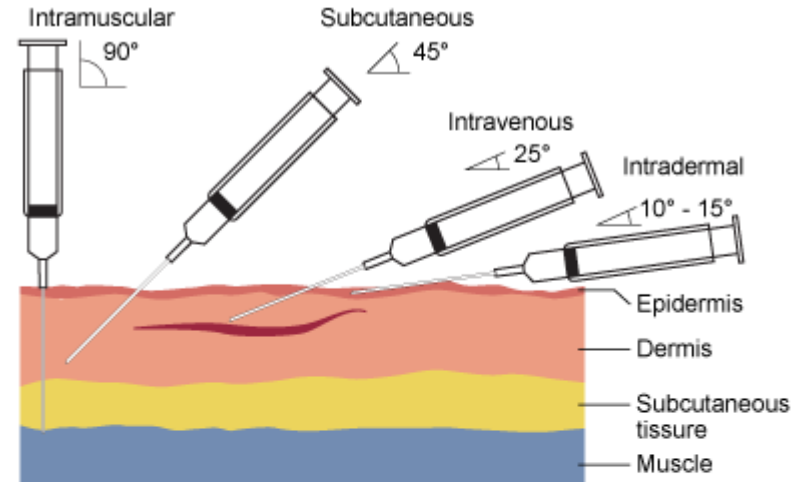
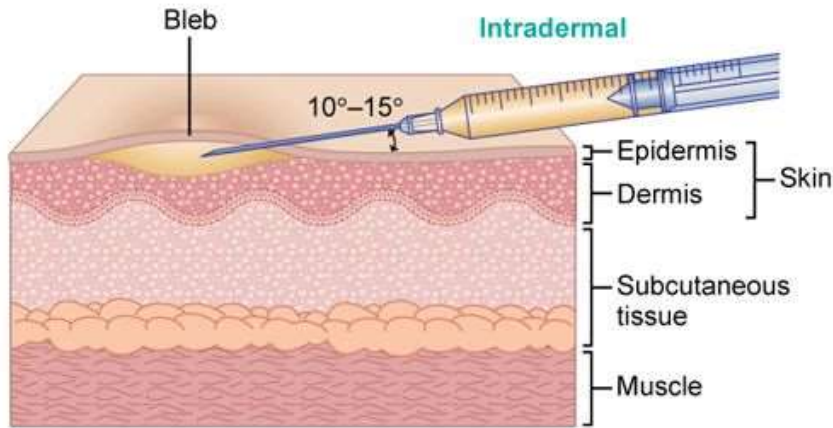
# Routes of Administration



- **Intramuscular**
- **Subcutaneous**
- **Intravenous**
- **Dermal Application**
- **Intraperitoneal**



# Needle insertion angles



# Local Reaction at the site of injection



The skin reaction is assessed according to the numerical scoring system of Draize *et. al.* (1944). Any other abnormalities recorded as they are observed.

<b>1. ERYTHEMA AND ESCHAR FORMATION</b>	<b>Score</b>
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to eschar formation (injuries in depth)	4
<b>MAXIMUM POSSIBLE SCORE – 4</b>	
<b>2. EDEMA FORMATION</b>	<b>Score</b>
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimetre)	3
Severe edema (raised more than 1 millimetre and extending beyond area of exposure)	4
<b>MAXIMUM POSSIBLE SCORE – 4</b>	

# Rabbit Skin Irritation Study



**Control**

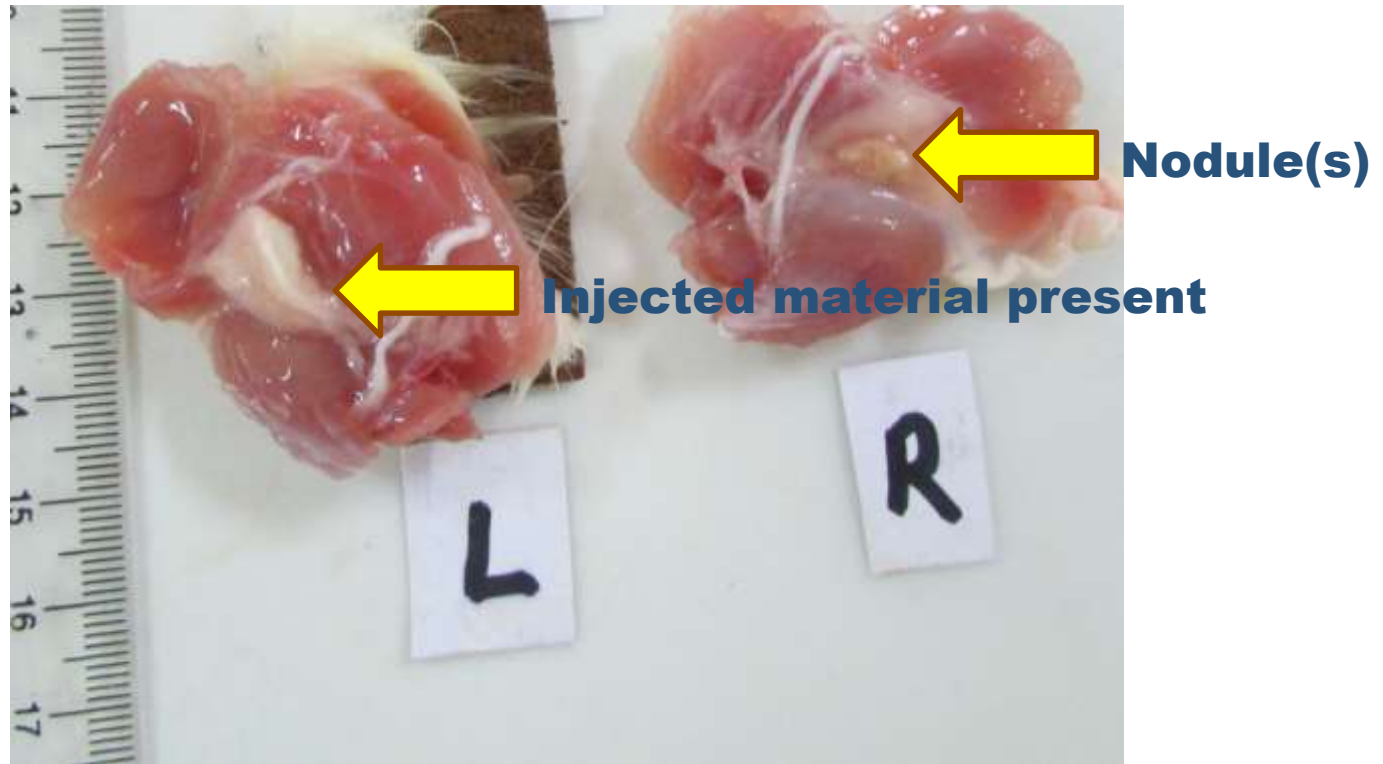


**Treated  
(erythema)**



# Gross Lesions

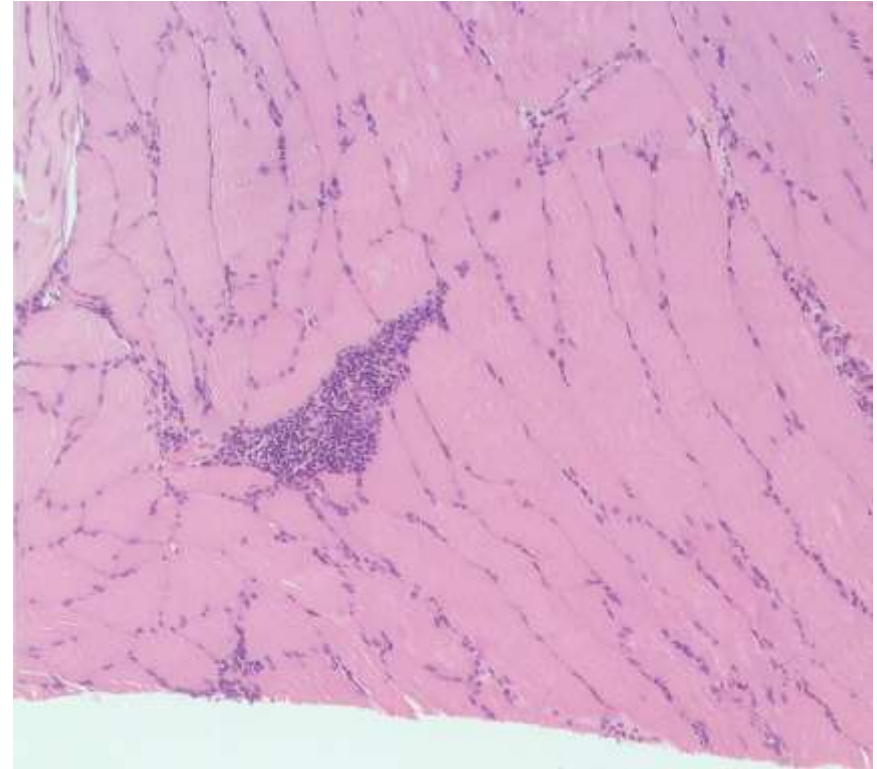
## Intramuscular Injection - Rat



# Microscopic Observation Intramuscular Injection - Rat



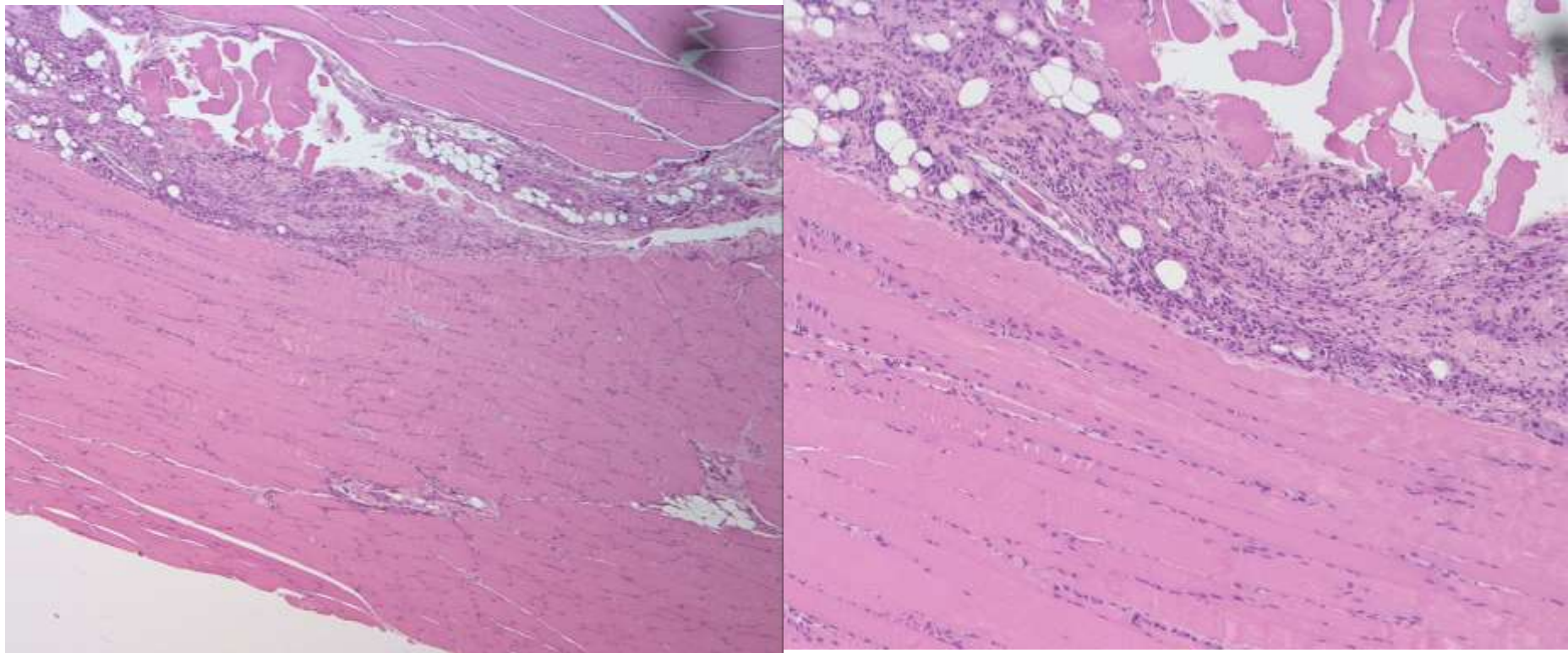
## Mononuclear cell infiltration; minimal



# Microscopic Observation Intramuscular Injection - Rat



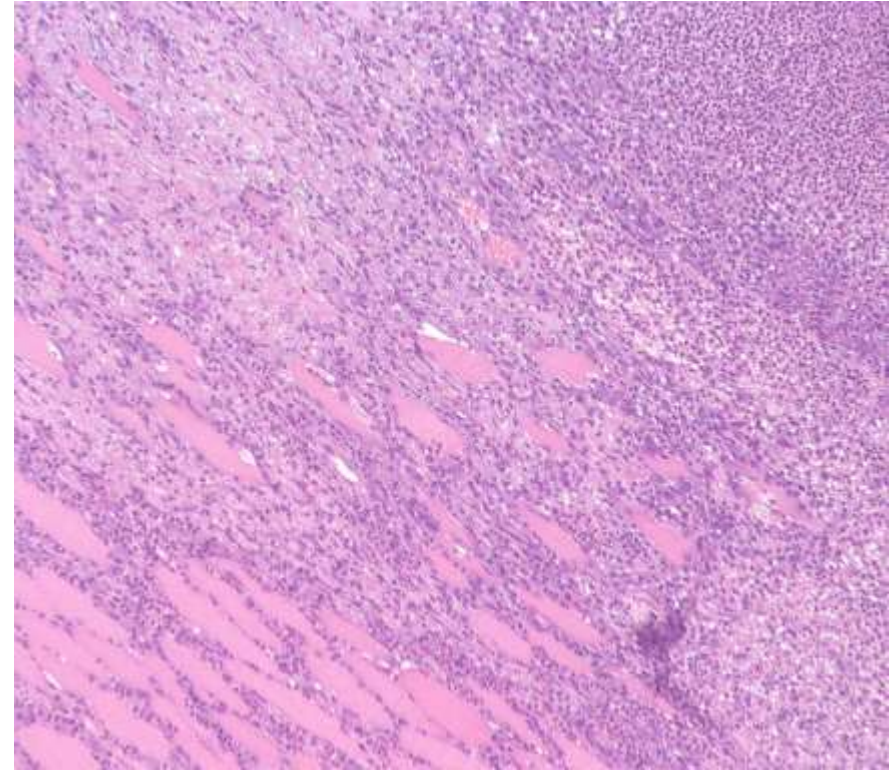
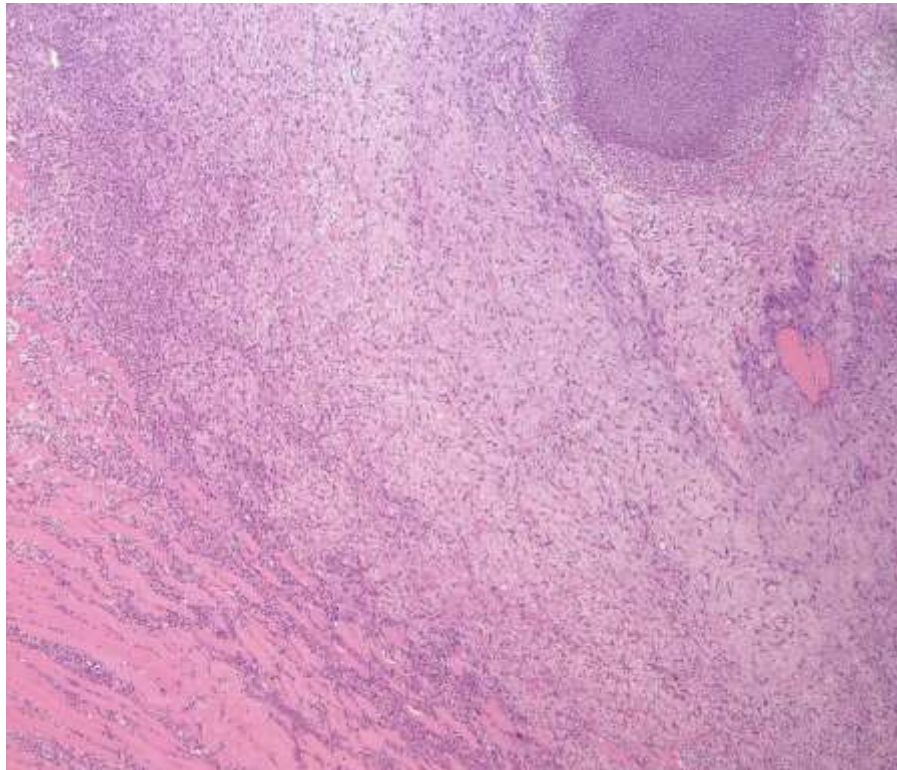
## Chronic inflammation; minimal



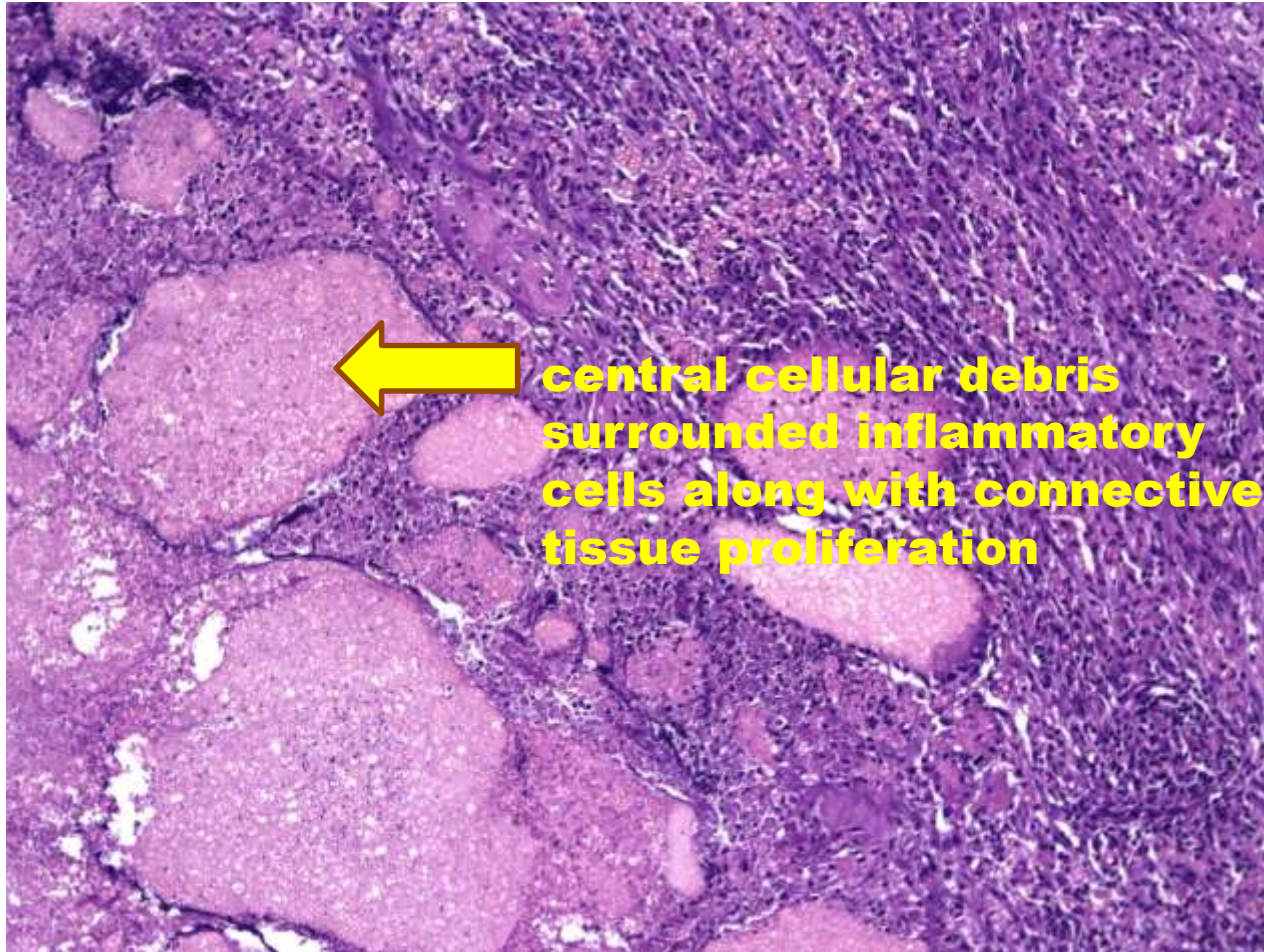
# Microscopic Observation Intramuscular Injection - Rat



**Chronic inflammation; moderate**

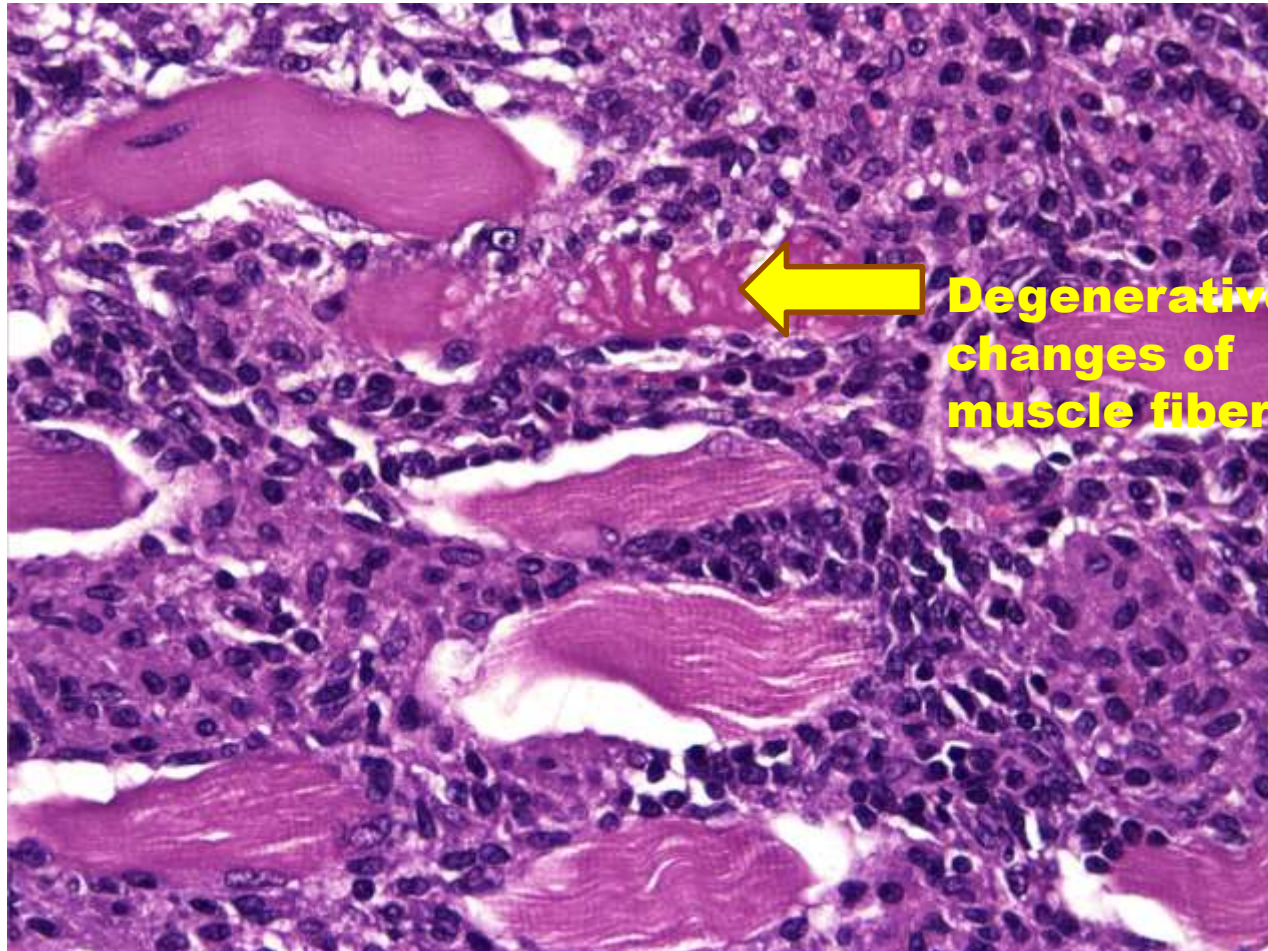


# Microscopic Observation Intramuscular Injection - Rat



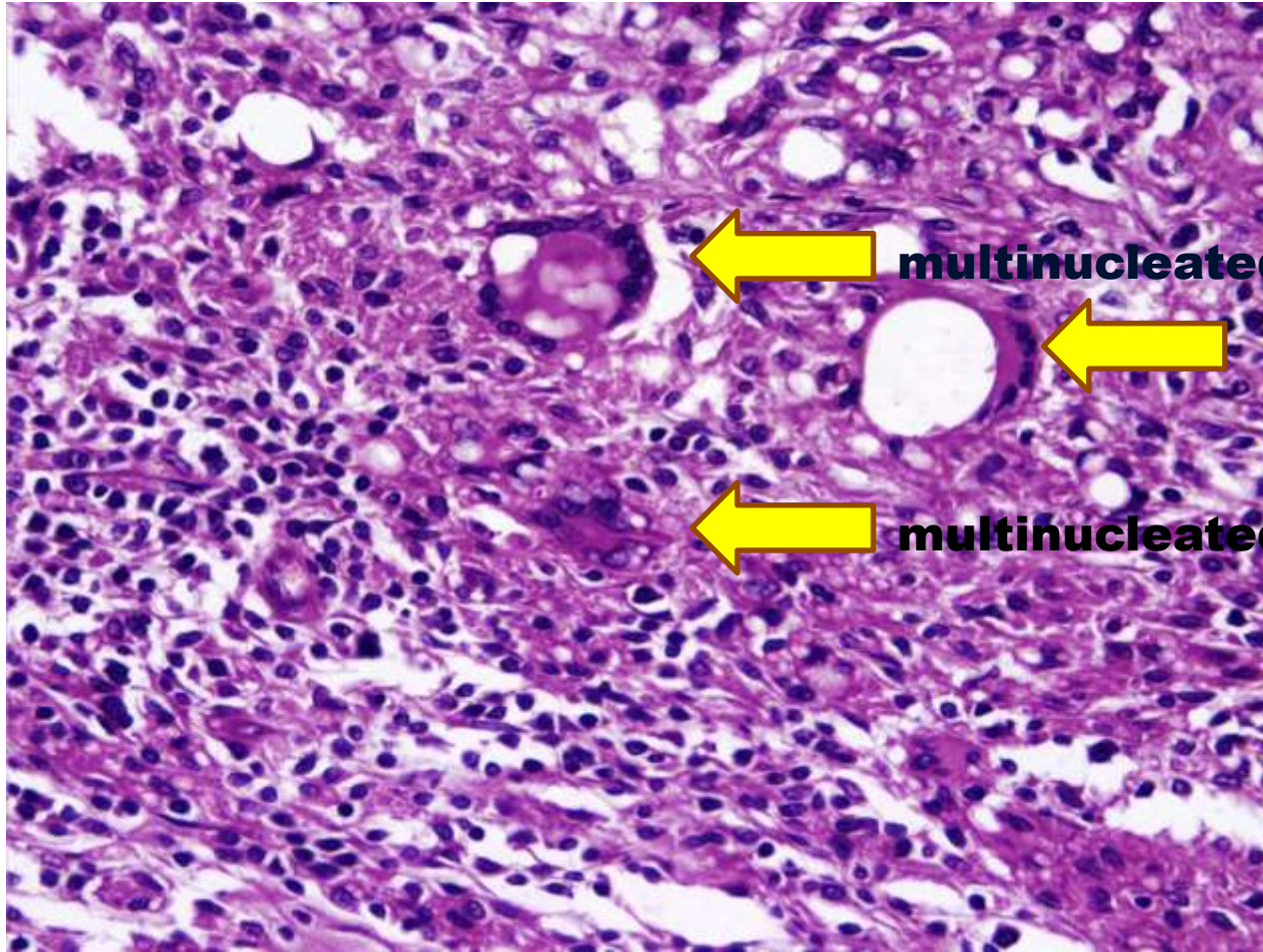
**central cellular debris  
surrounded inflammatory  
cells along with connective  
tissue proliferation**

# Microscopic Observation Intramuscular Injection - Rat



**Degenerative  
changes of  
muscle fibers**

# Microscopic Observation Intramuscular Injection - Rat

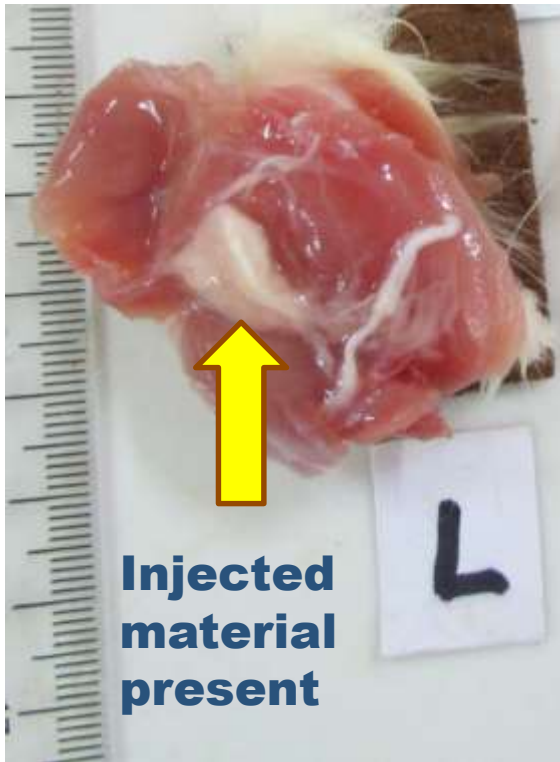


**multinucleated giant cell**



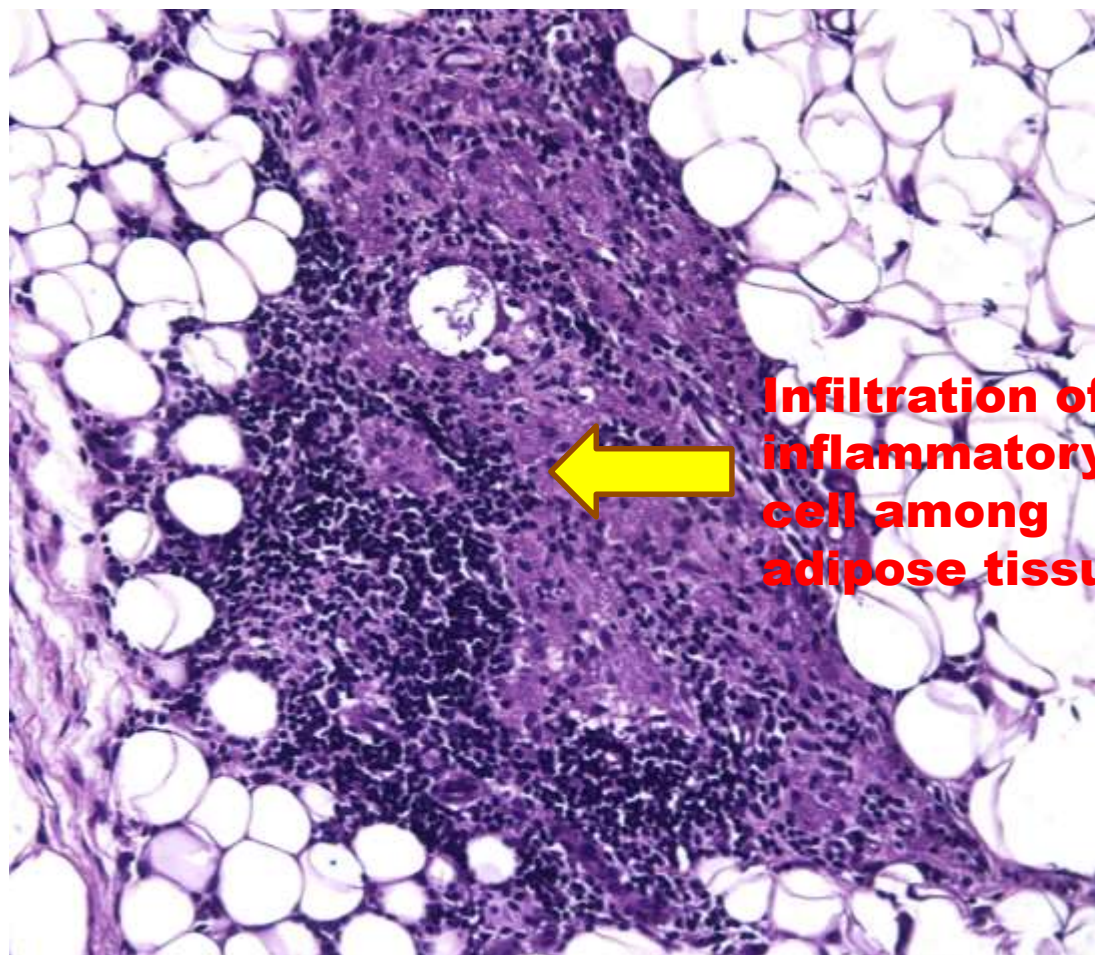
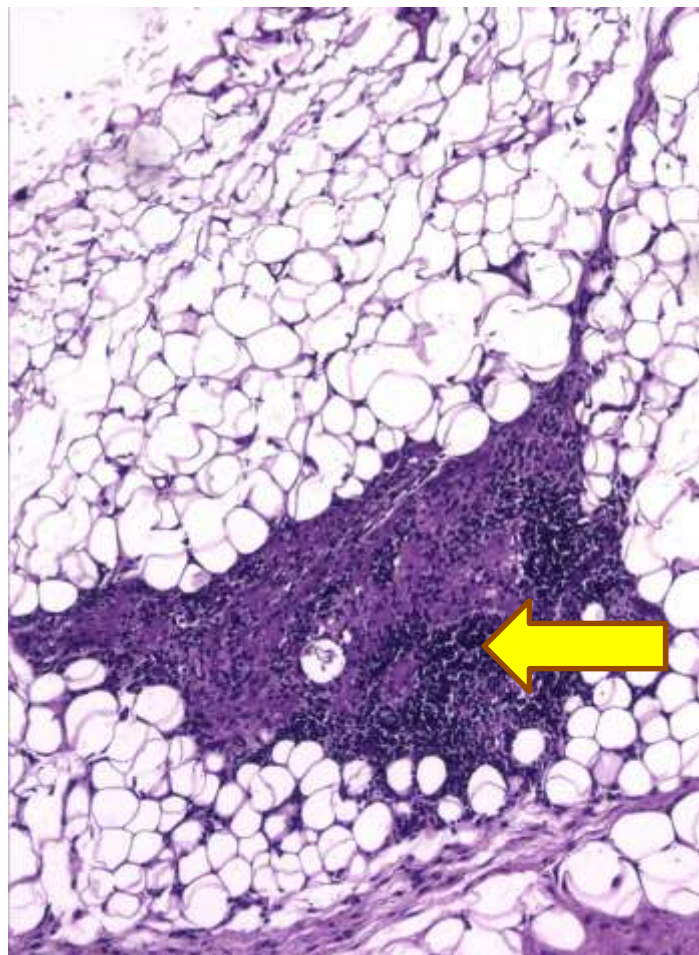
**multinucleated giant cell**

# Microscopic Observation Intramuscular Injection - Rat



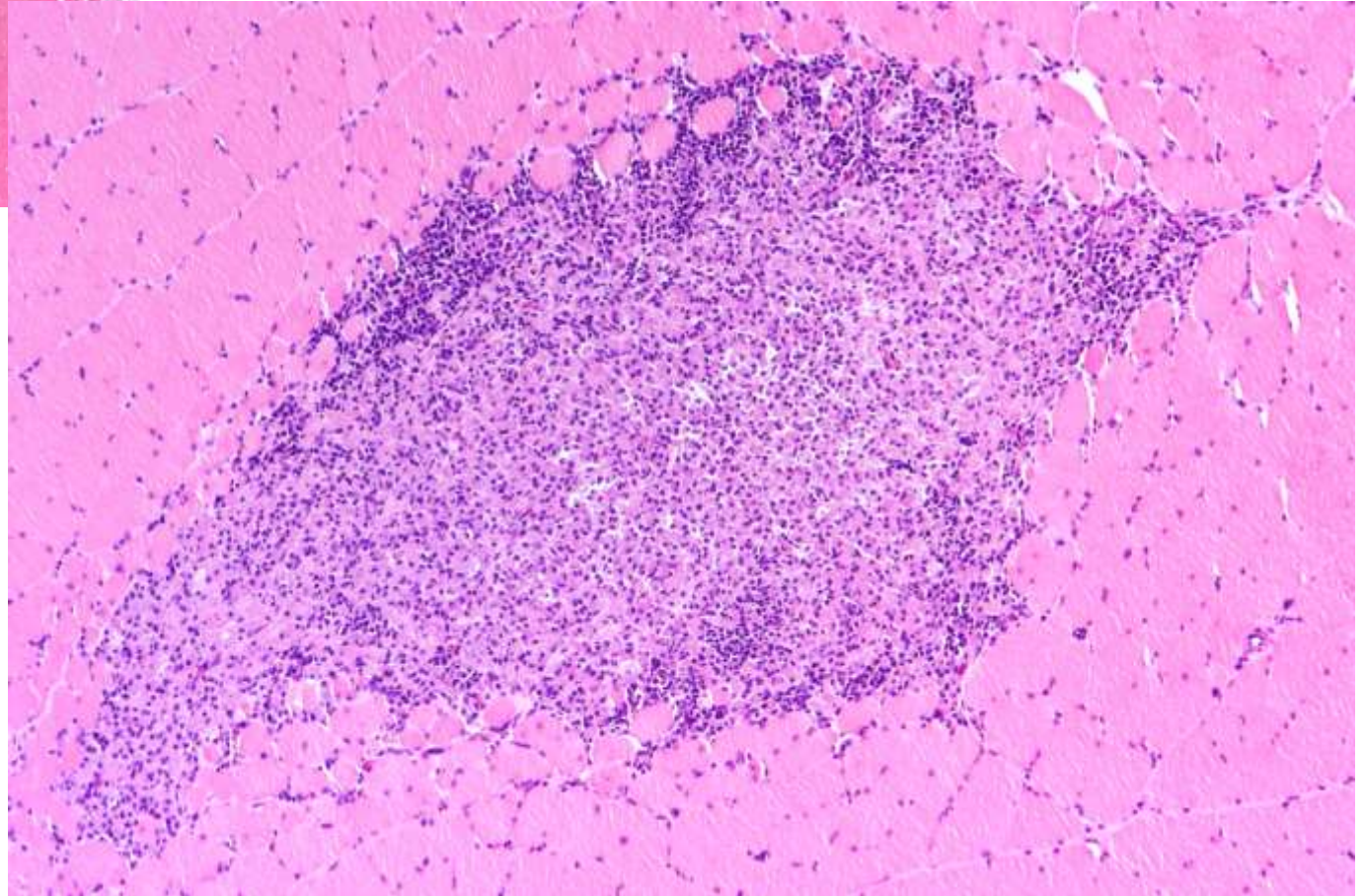
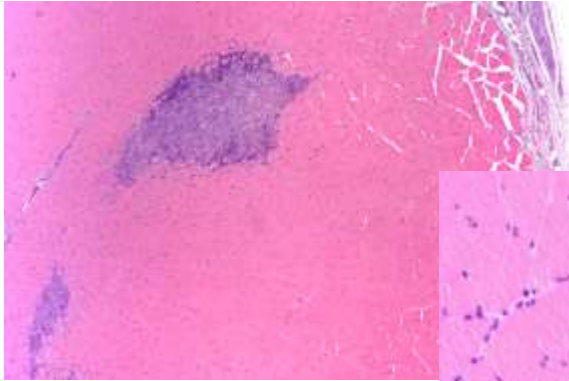
**Presence of central cavity (formed due to injected material deposits which was partially washed out during the histological processing) and/or injected material surrounded inflammatory cells**

# Microscopic Observation Intramuscular Injection - Rat

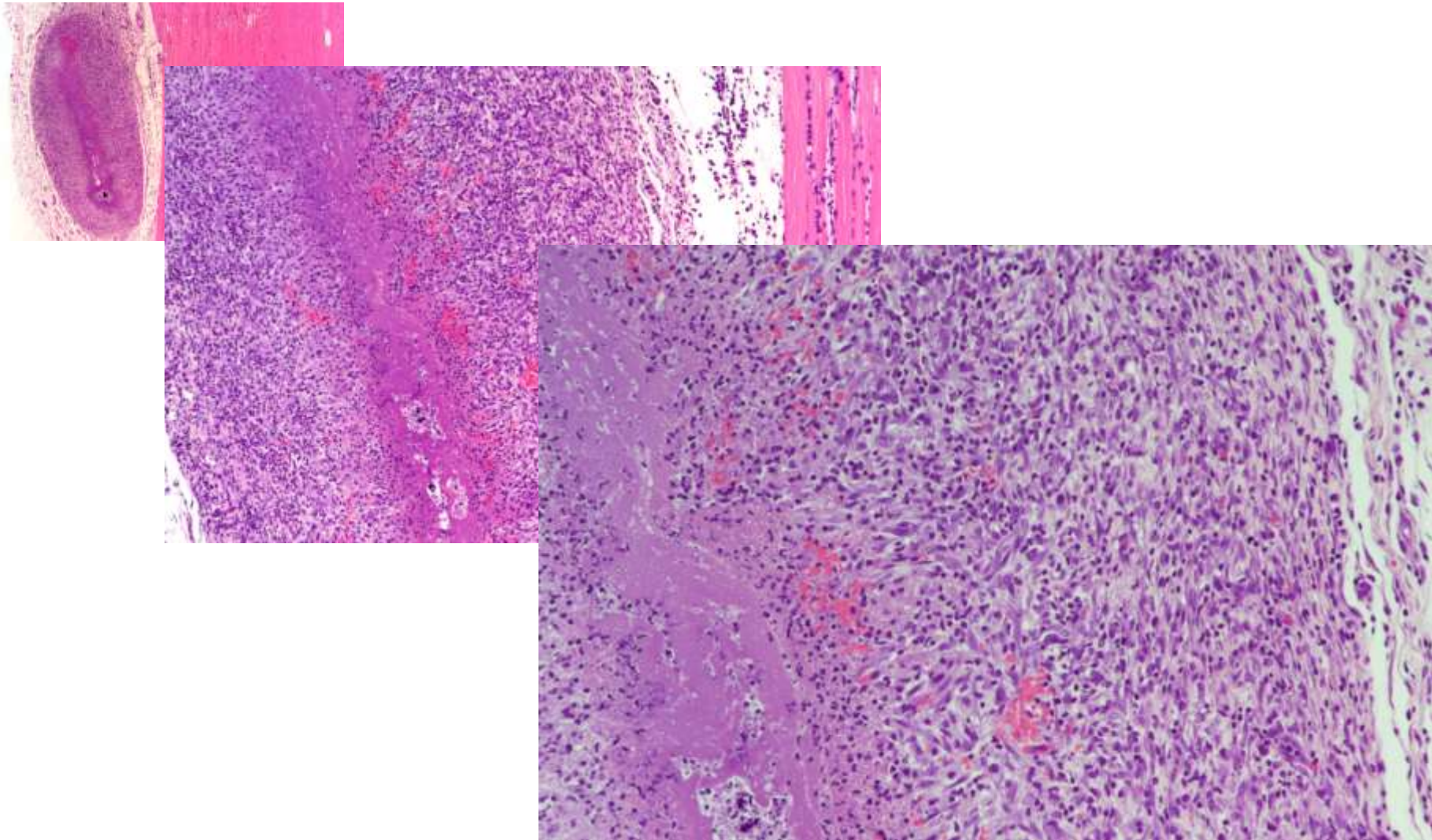


**Infiltration of  
inflammatory  
cell among  
adipose tissue**

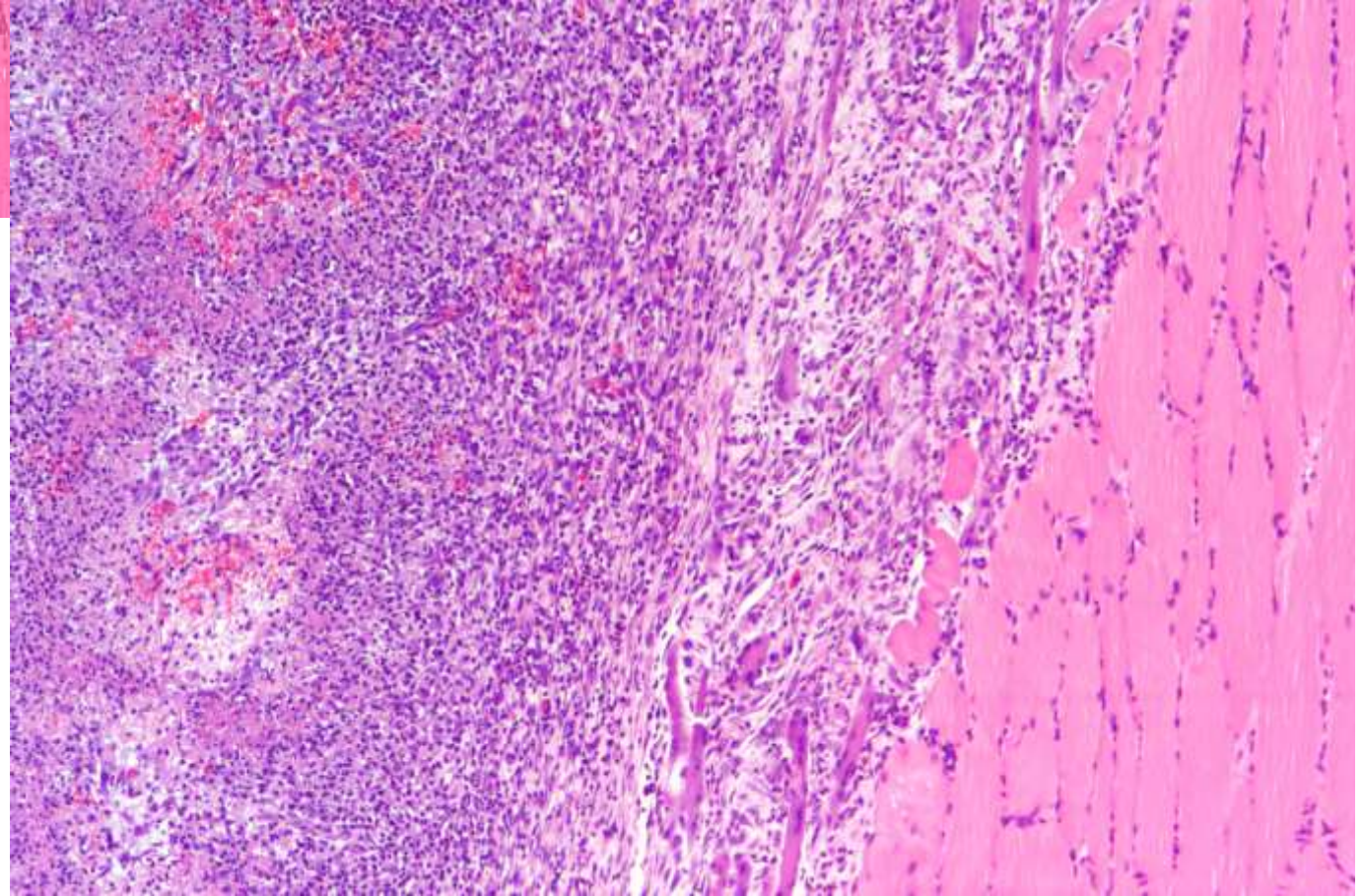
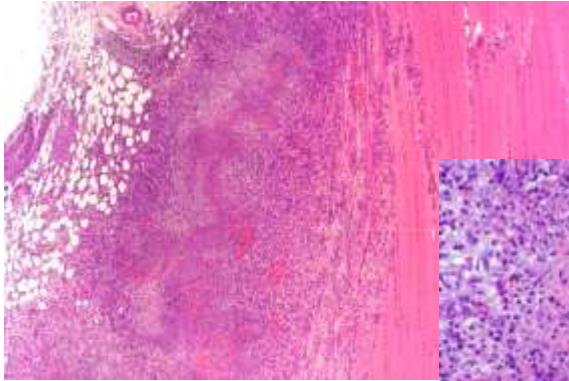
# Microscopic Observation Intramuscular Injection - Rat



# Microscopic Observation Intramuscular Injection - Rat



# Microscopic Observation Intramuscular Injection - Rat



# Gross and Microscopic Correlation

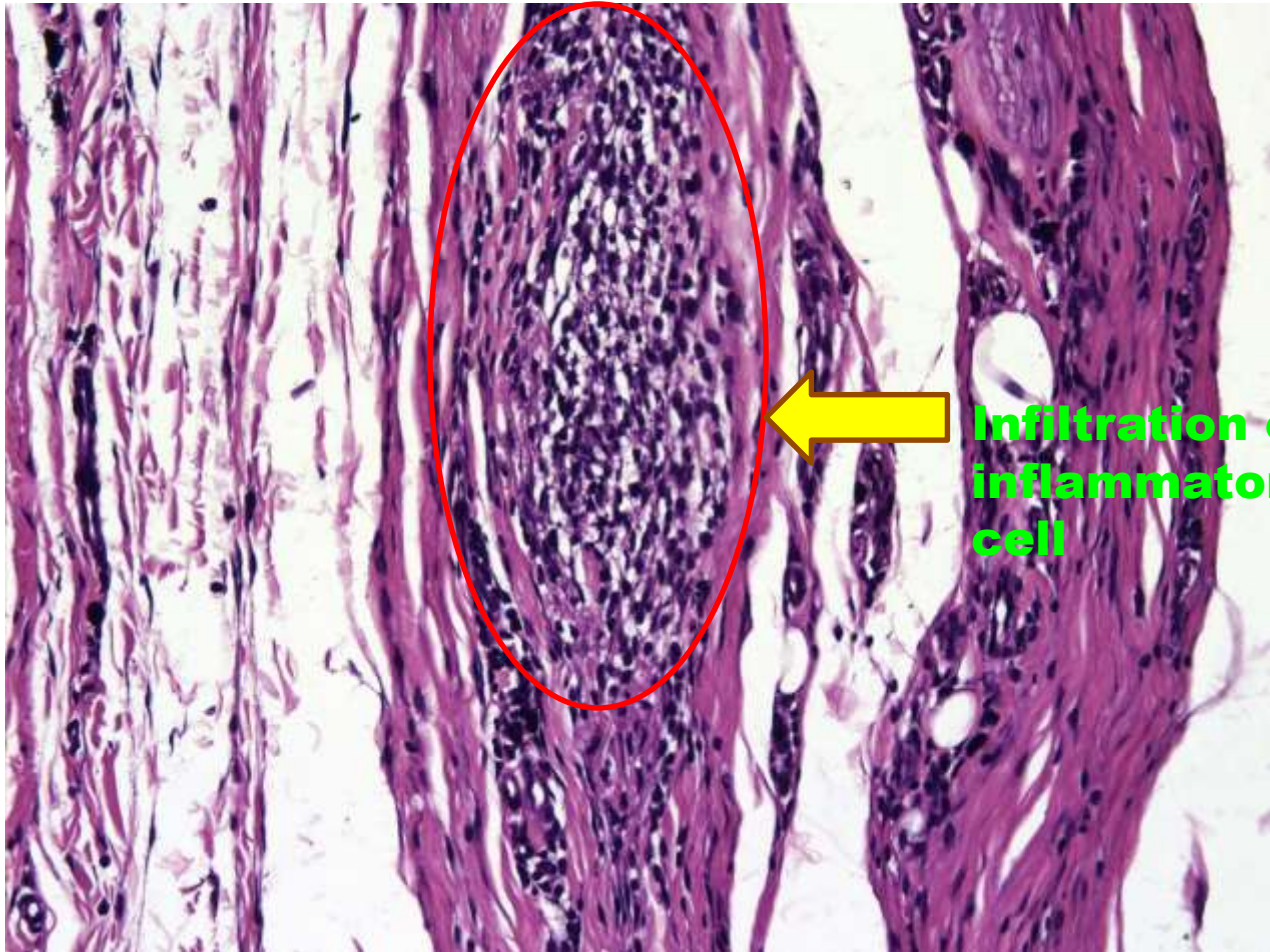
## Subcutaneous Injection - Rat



Gross and Histopathology	Gross and Histopathology
<b>SKIN (INJECTION SITE); abdominal;</b> <b>Discoloration; lateral; red; multifocal: left</b>	<b>SKIN (INJECTION SITE); abdominal;</b> <b>Wound; lateral: left, 0.8 cm</b>
<b>Inflammation</b> <b>Degenerative changes- muscularis</b> <b>Parakeratosis</b>	<b>Inflammation</b> <b>Necrosis- dermal/epidermal</b>

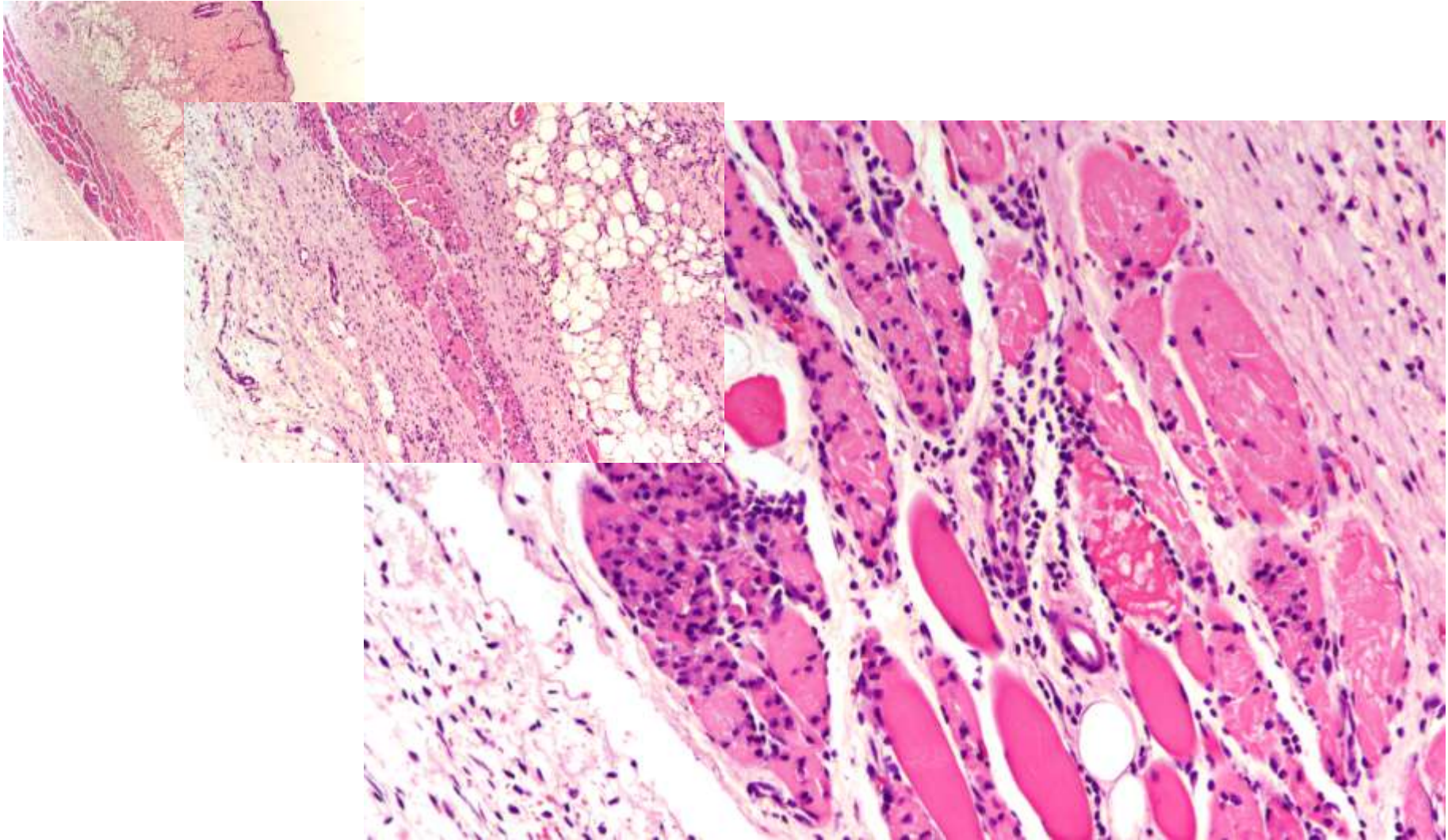


# Microscopic Observation Subcutaneous Injection - Rat

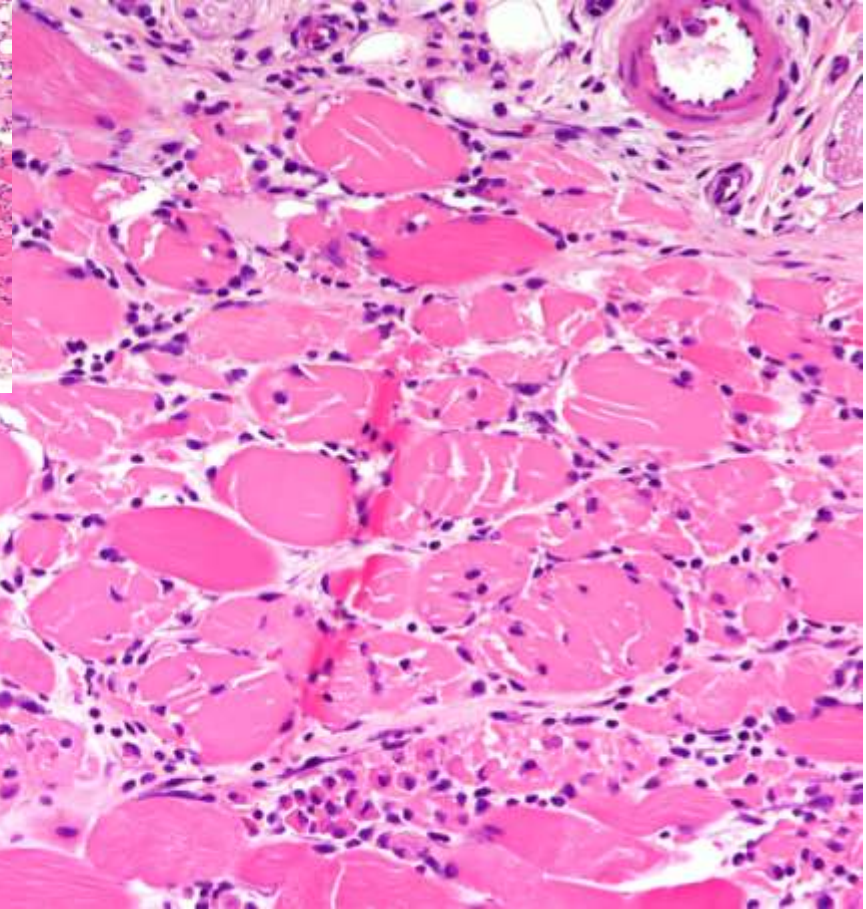
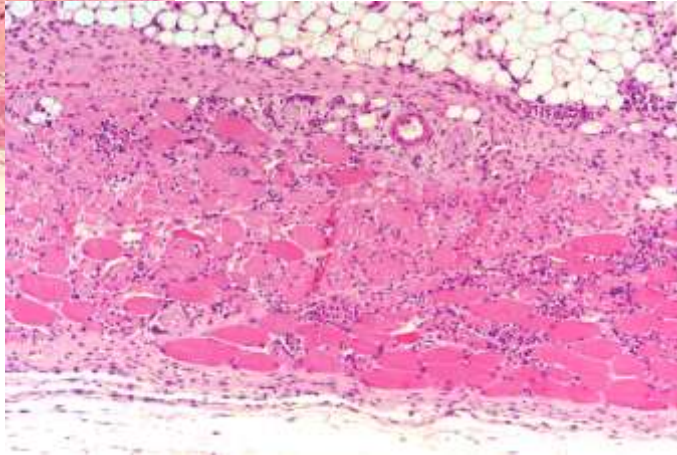
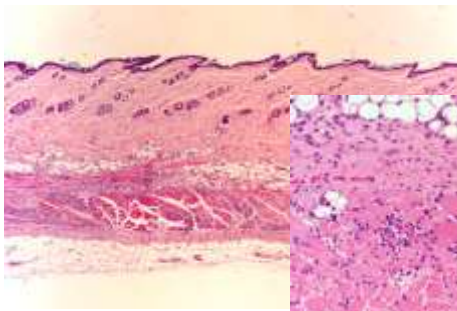


Infiltration of  
inflammatory  
cell

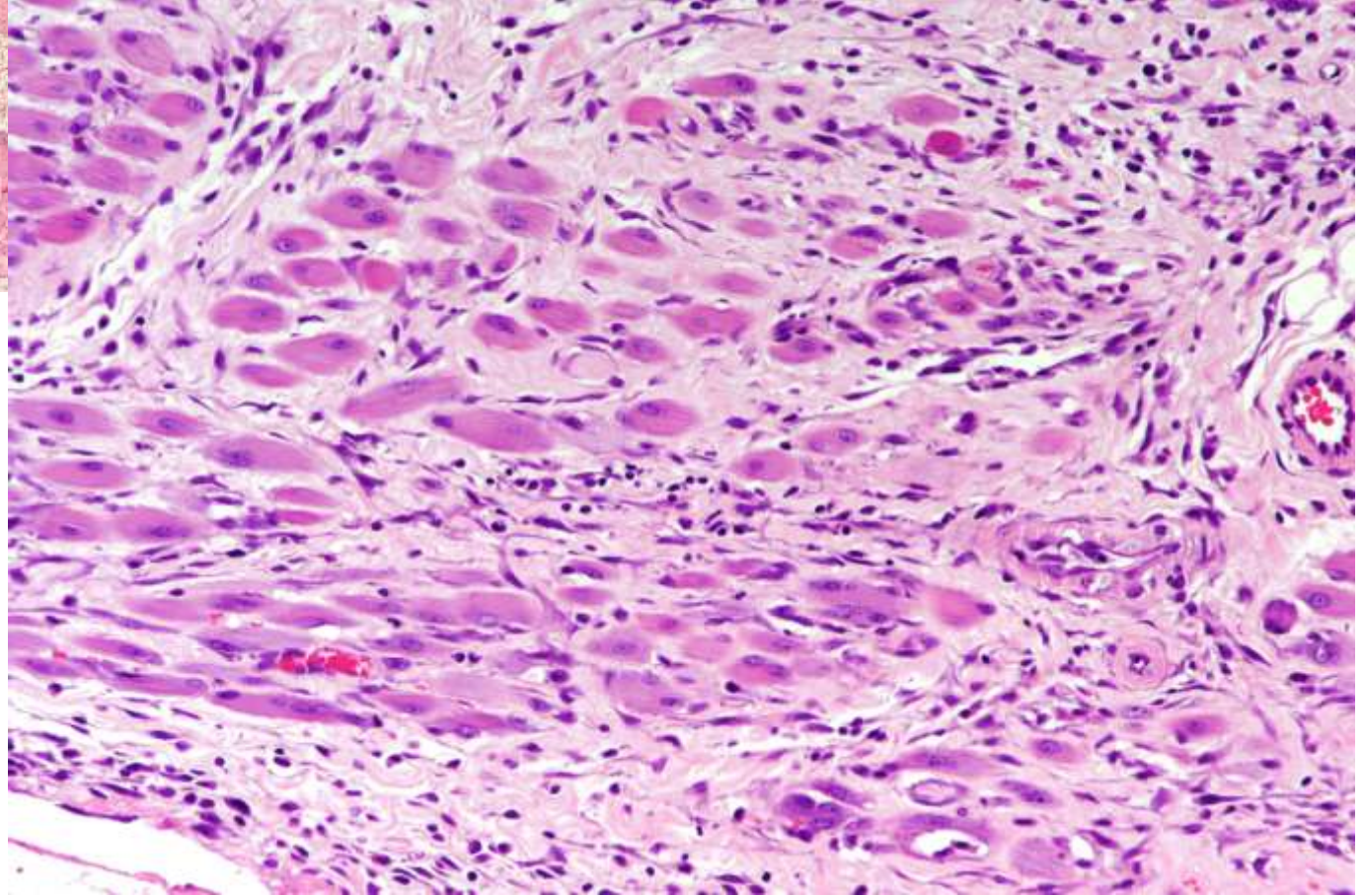
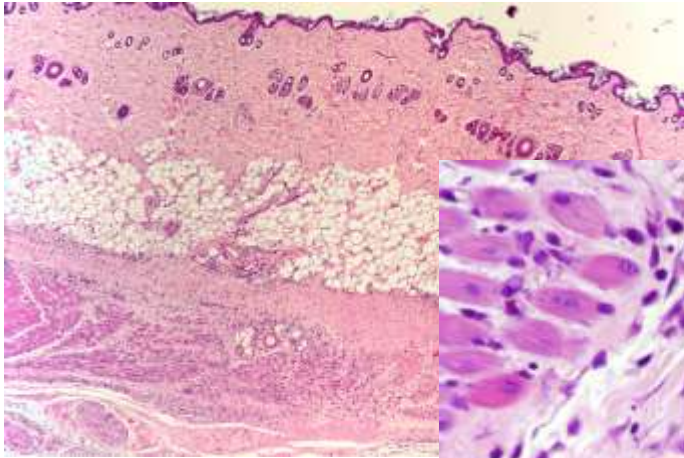
# Microscopic Observation Subcutaneous Injection - Rat



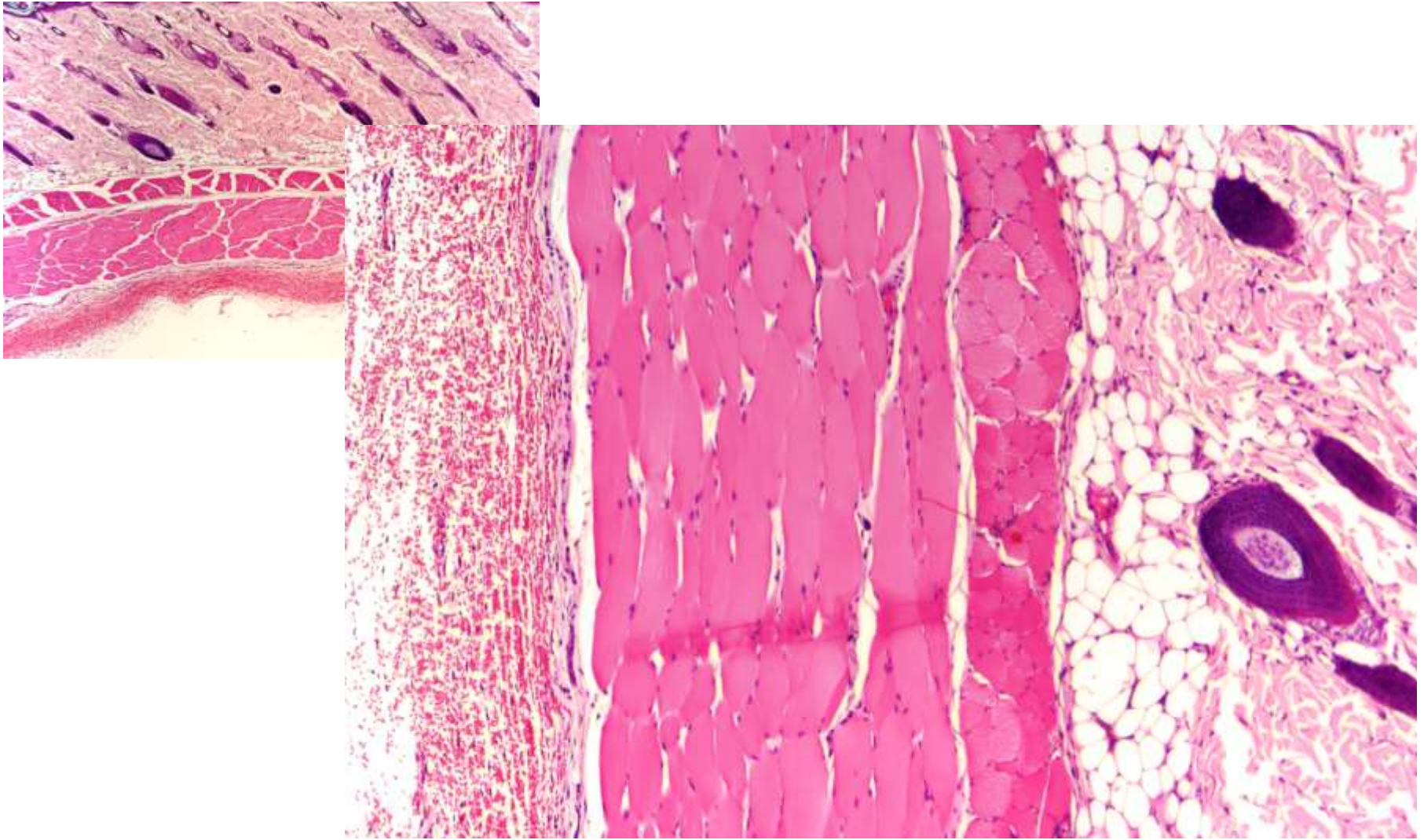
# Microscopic Observation Subcutaneous Injection - Rat



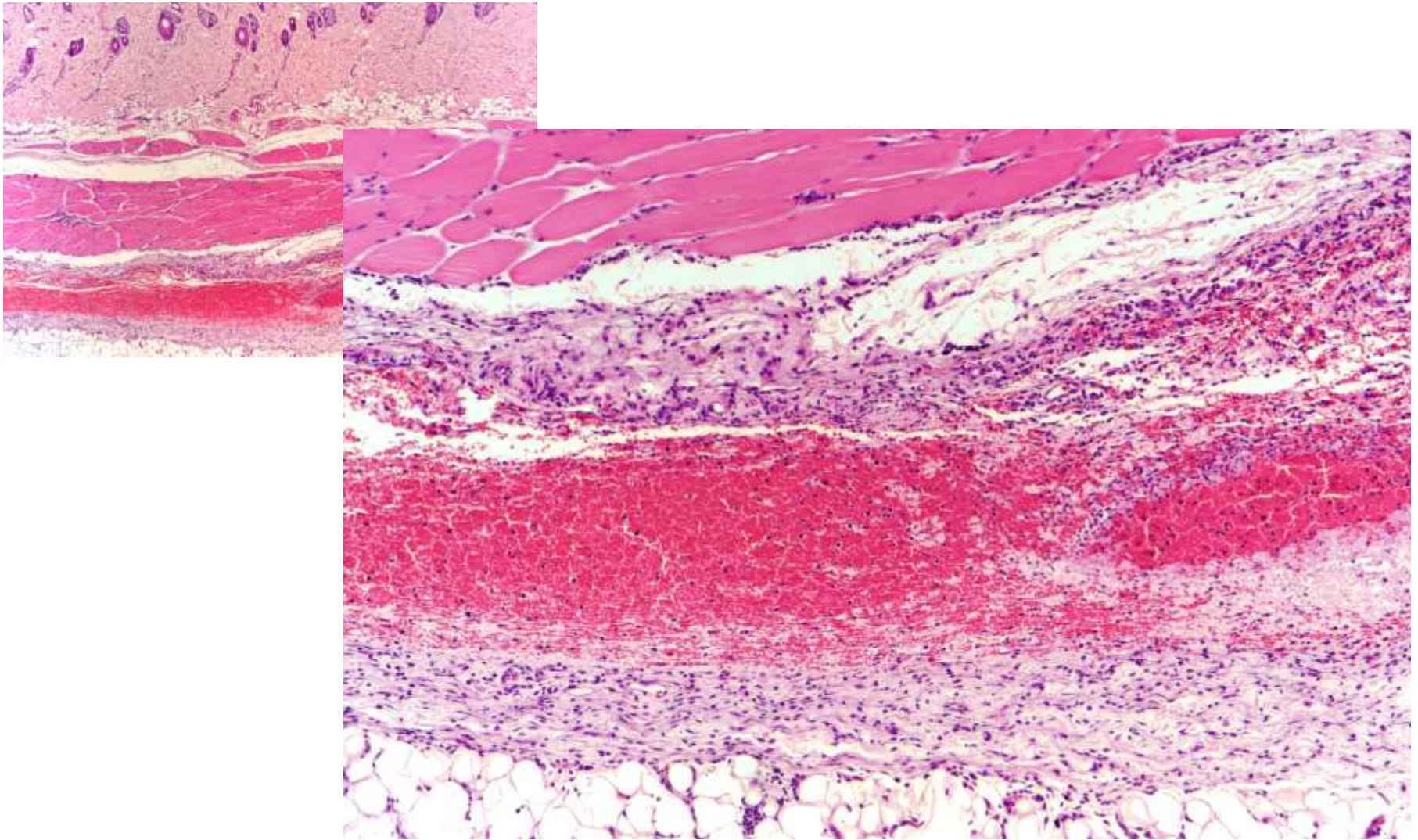
# Microscopic Observation Subcutaneous Injection - Rat



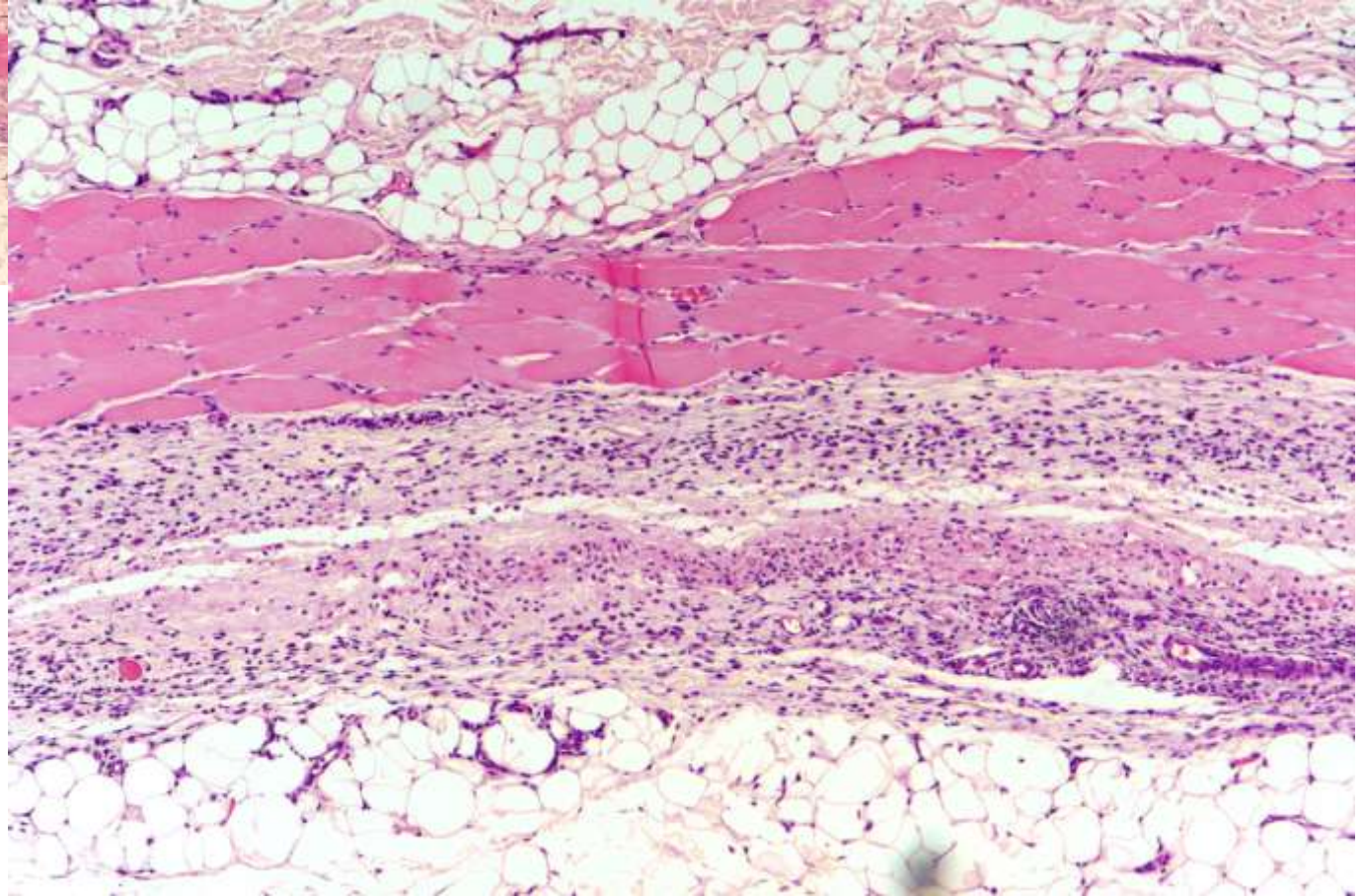
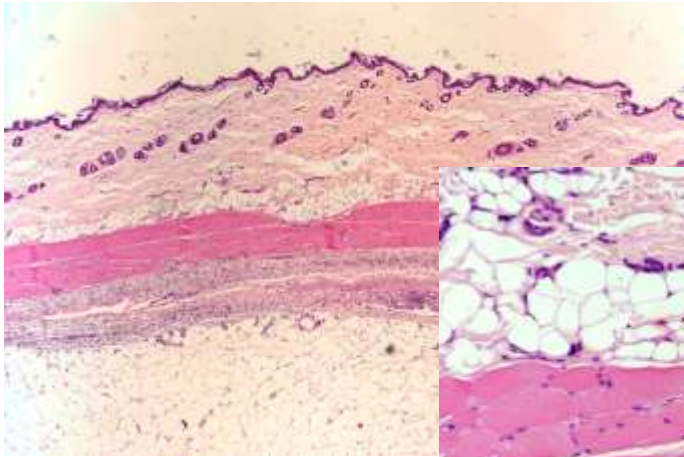
# Microscopic Observation Subcutaneous Injection - Rat



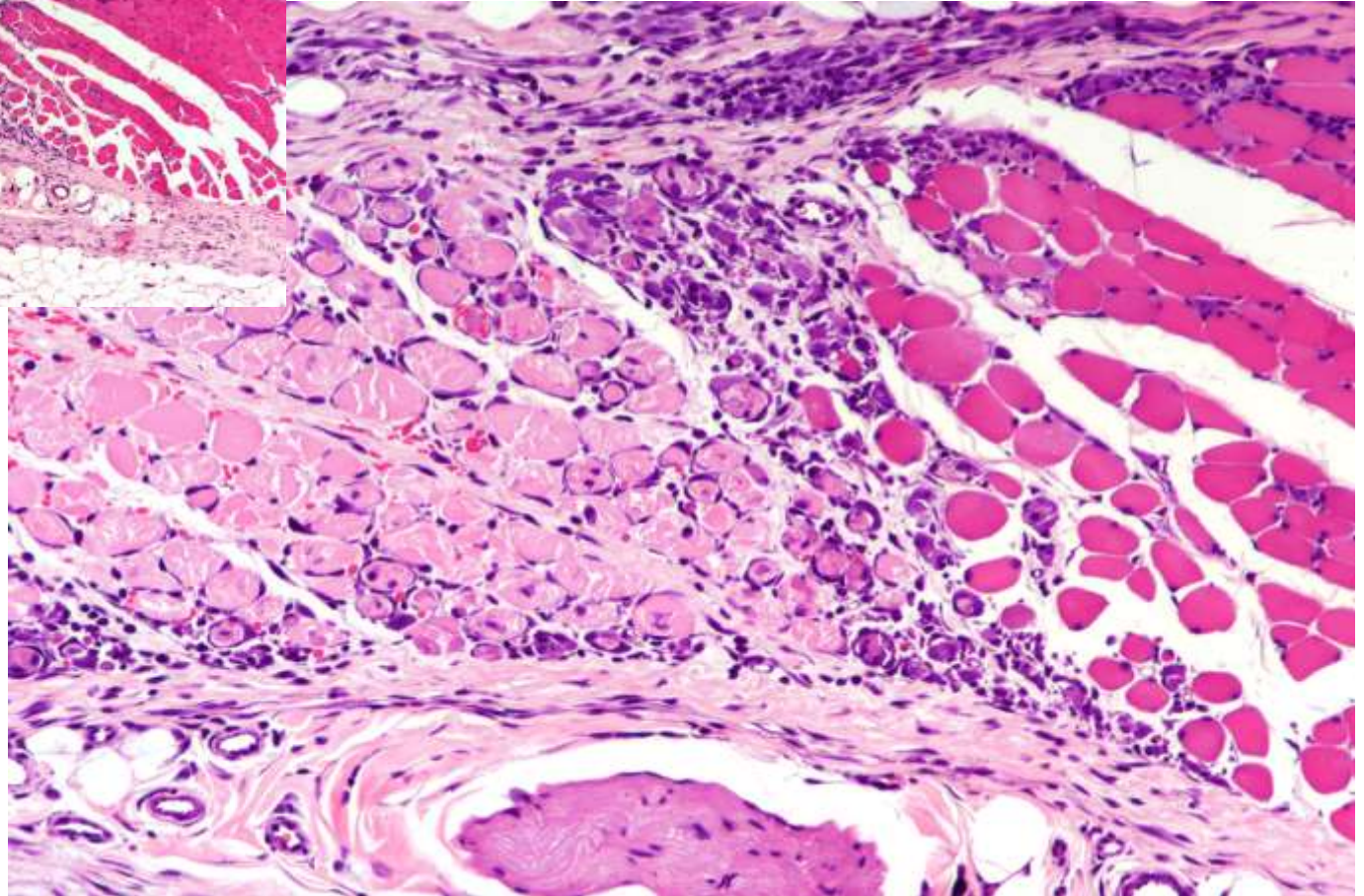
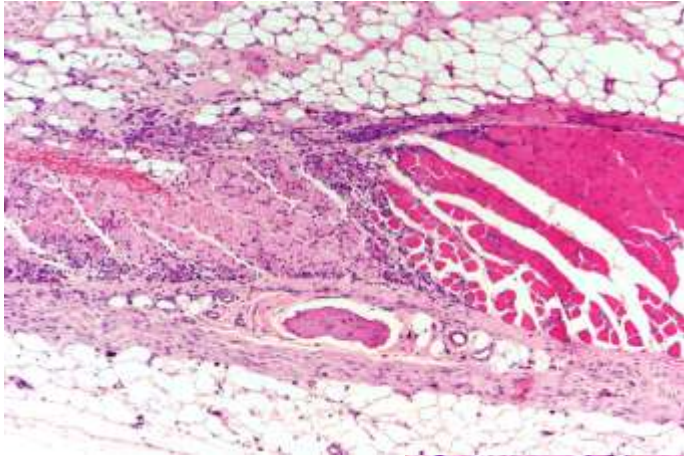
# Microscopic Observation Subcutaneous Injection - Rat



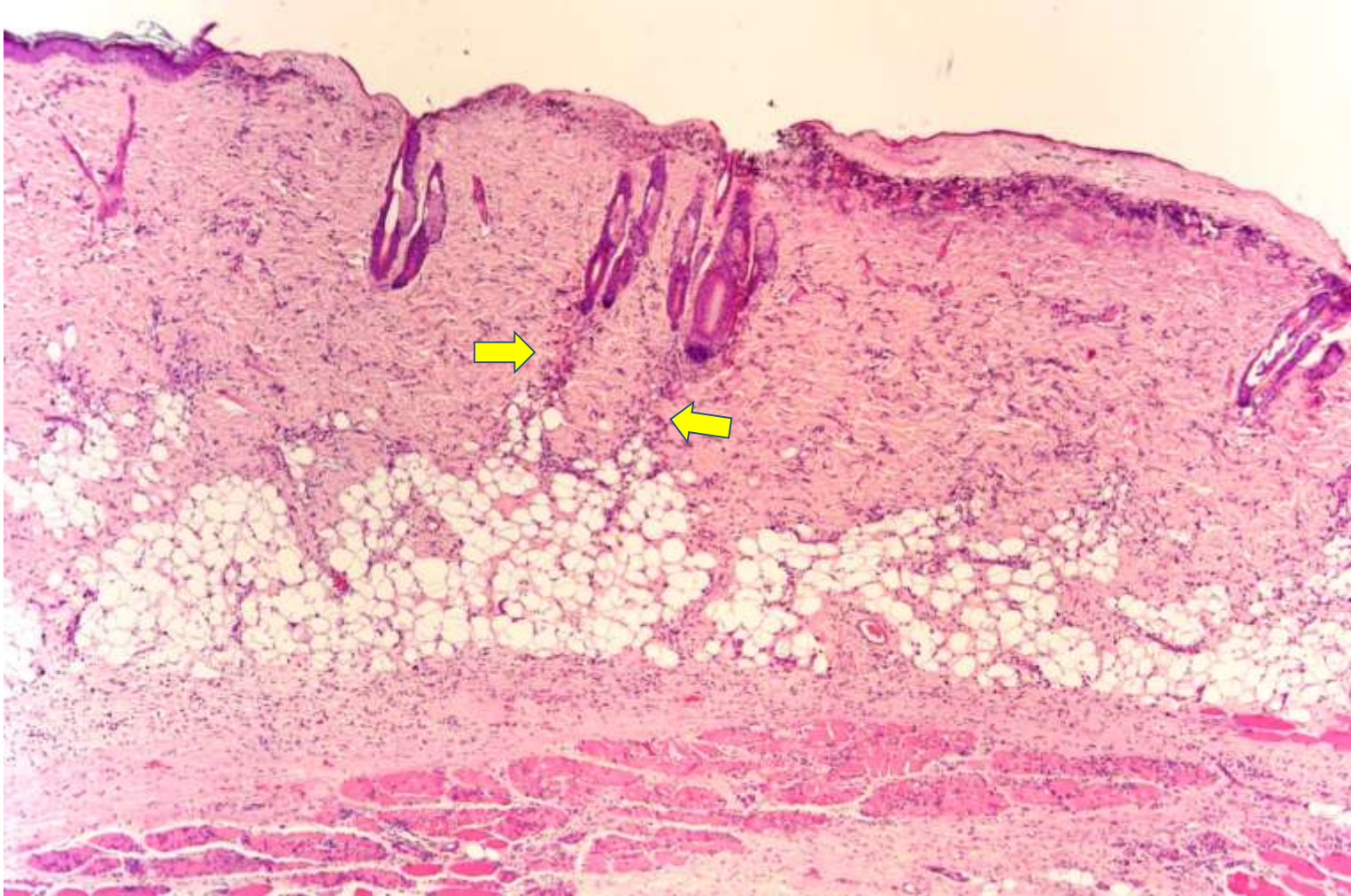
# Microscopic Observation Subcutaneous Injection - Rat



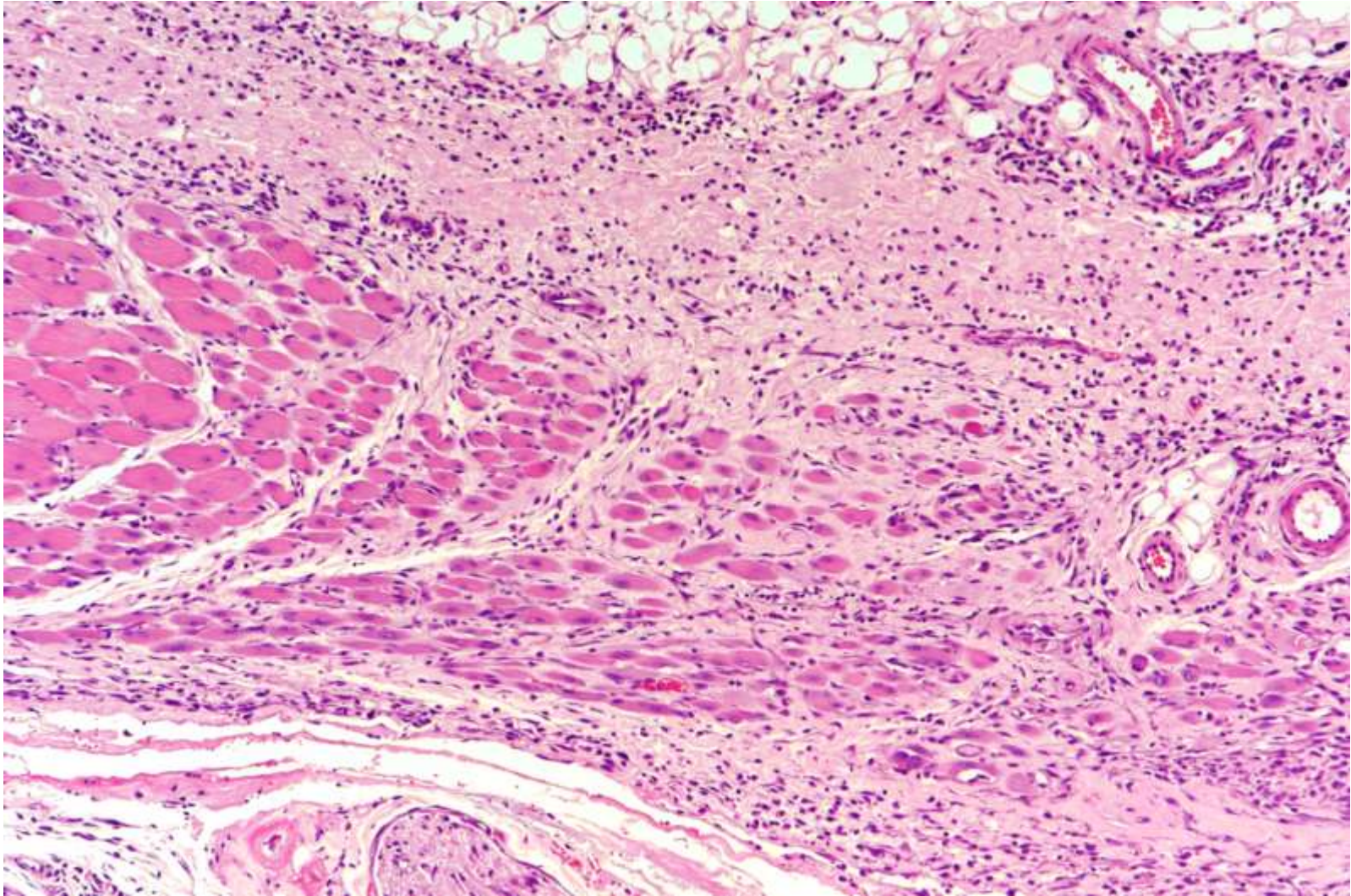
# Microscopic Observation Subcutaneous Injection - Rat



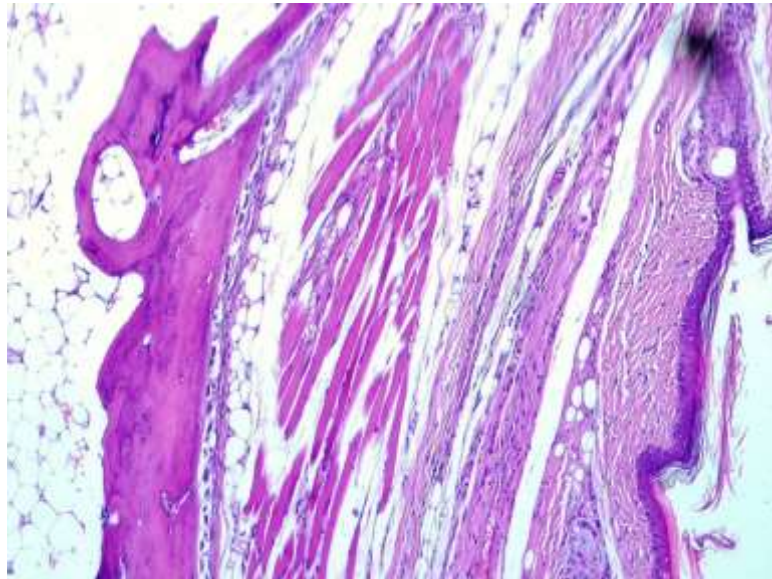
# Microscopic Observation Subcutaneous Injection - Rat



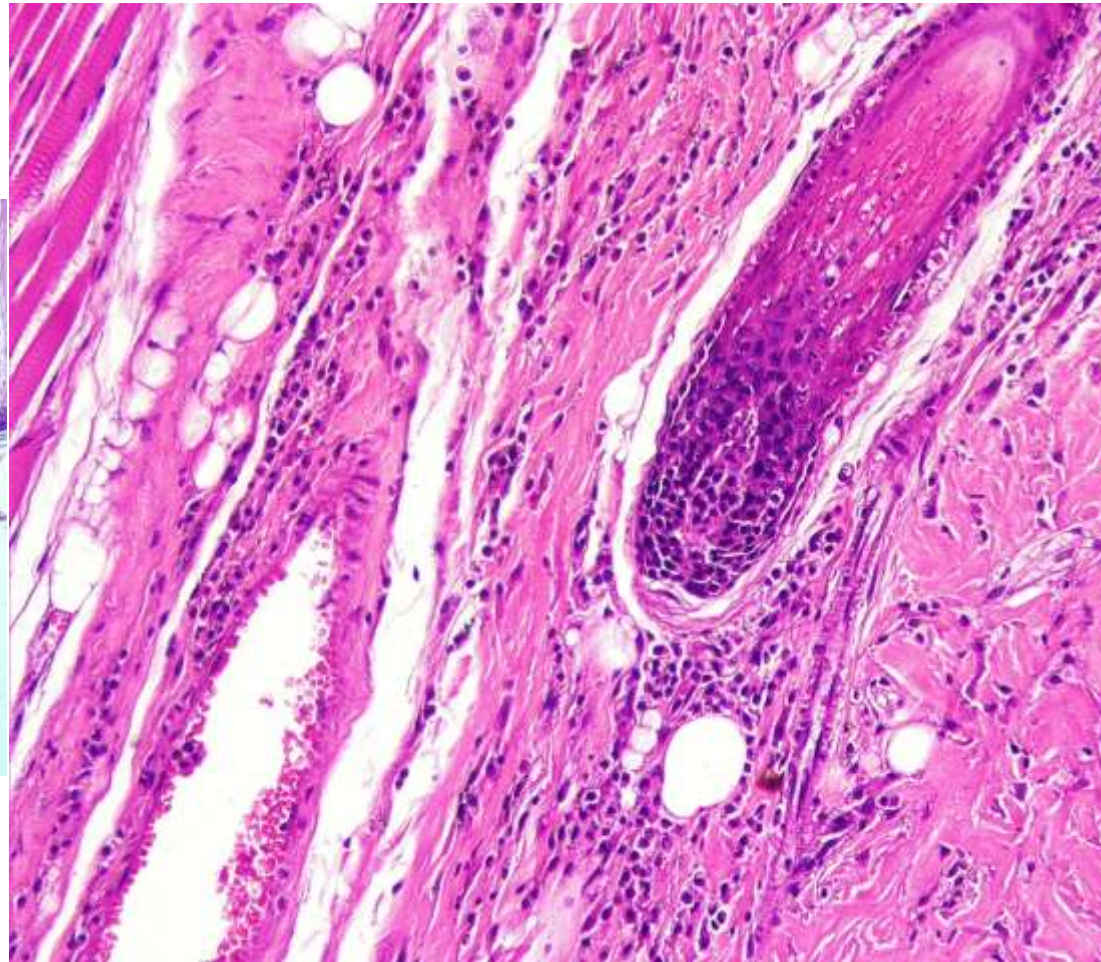
# Microscopic Observation Subcutaneous Injection - Rat



# Microscopic Observation Intravenous Injection - Rat



**Normal**



**Leukocytic Infiltration; Minimal:  
Perivascular**

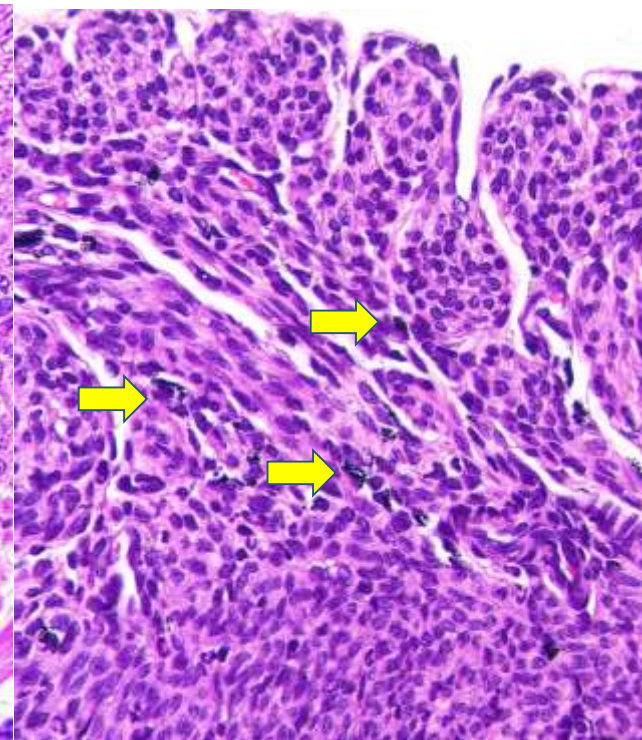
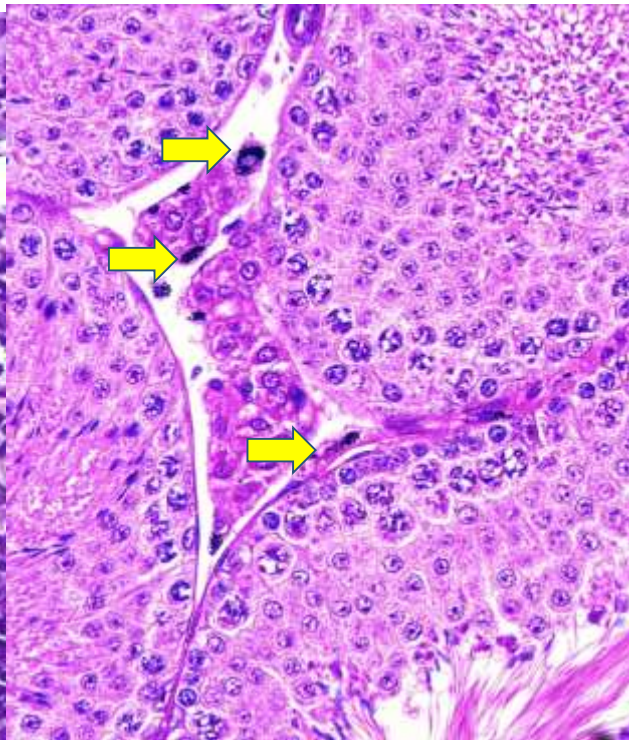
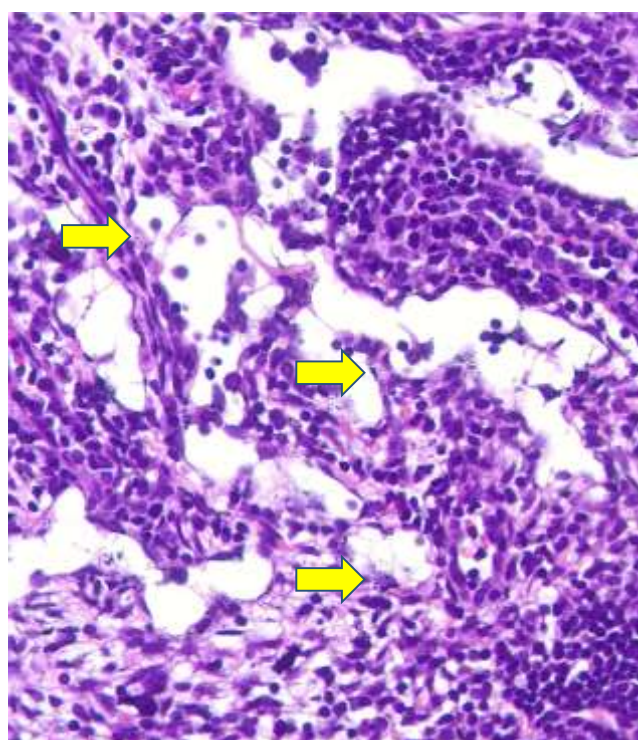
# GOLD NANO-PARTICLES (GNP): SINGLE DOSE INTRAVENOUS (IV) TOXICITY STUDY IN BALB/C MICE



**INGUINAL LYMPH NODES:  
Pigmentation**

**TESTES: Pigmentation**

**UTERUS WITH CERVIX:  
Pigmentation**



# Gross Lesions

## Dermal Application- Rat



**Normal**



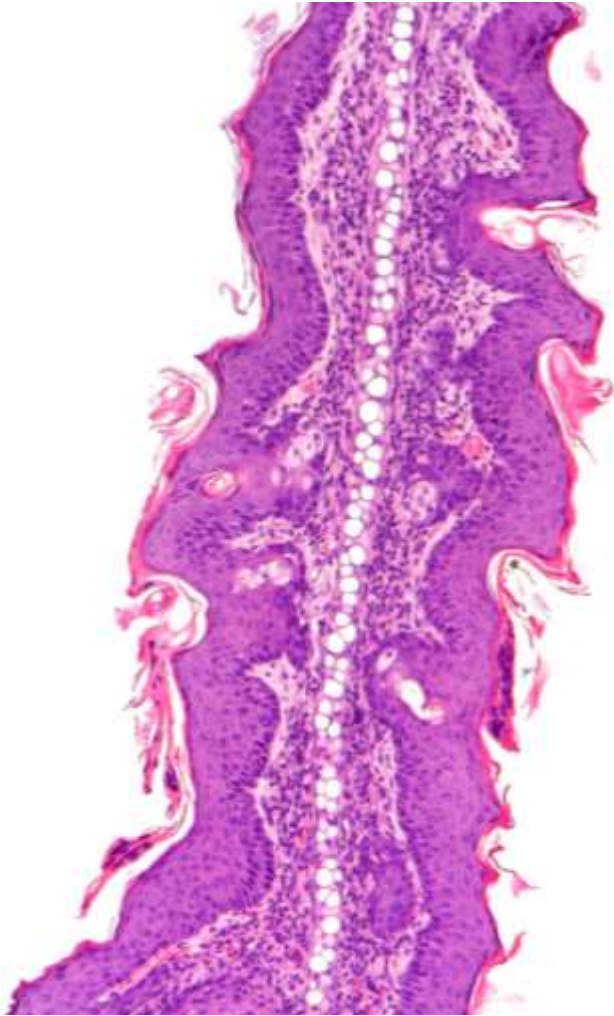
**SKIN-TREATED; Thickening;  
multifocal; minimal**



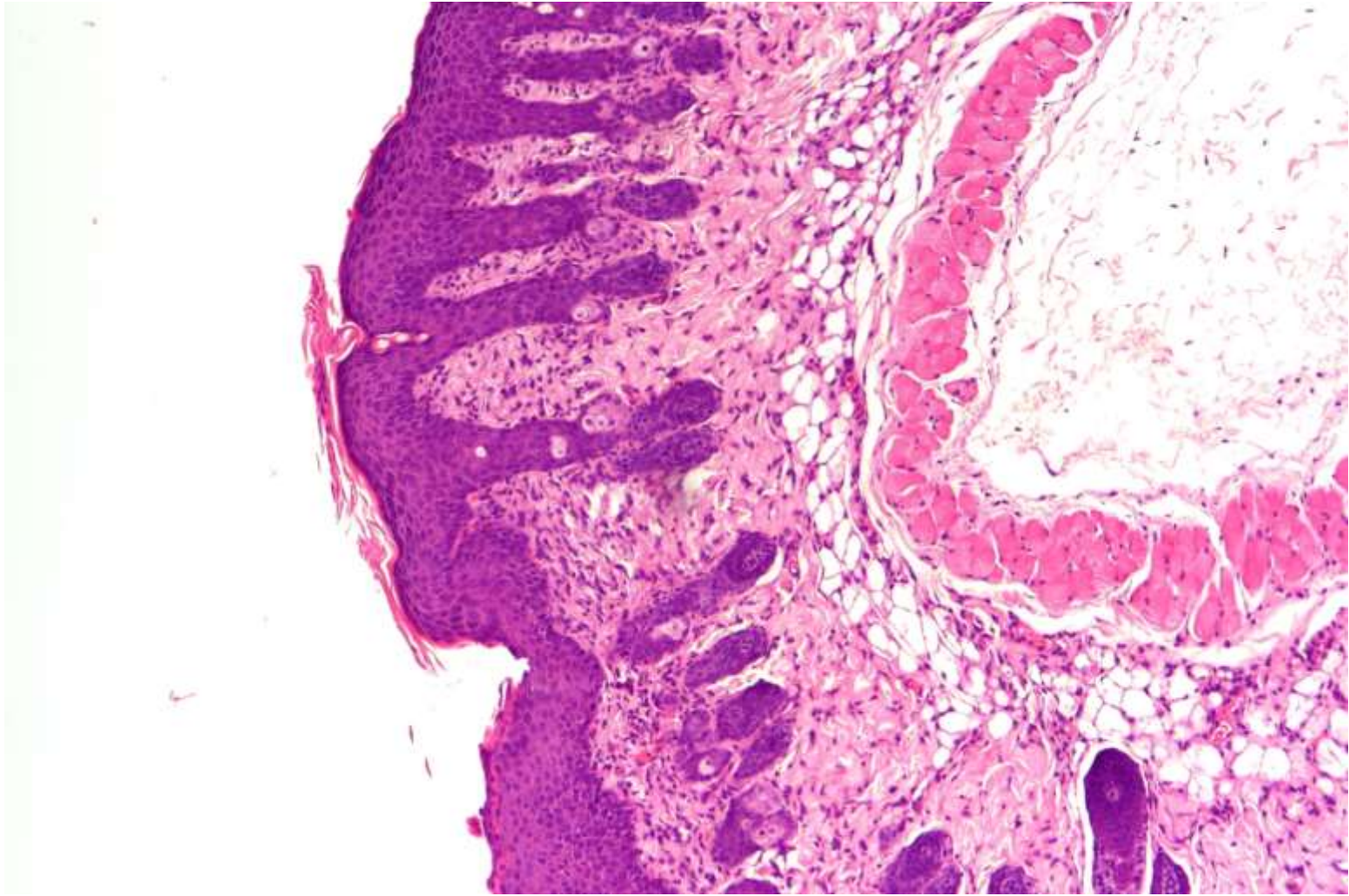
**SKIN-TREATED; Thickening;  
multifocal, severe**



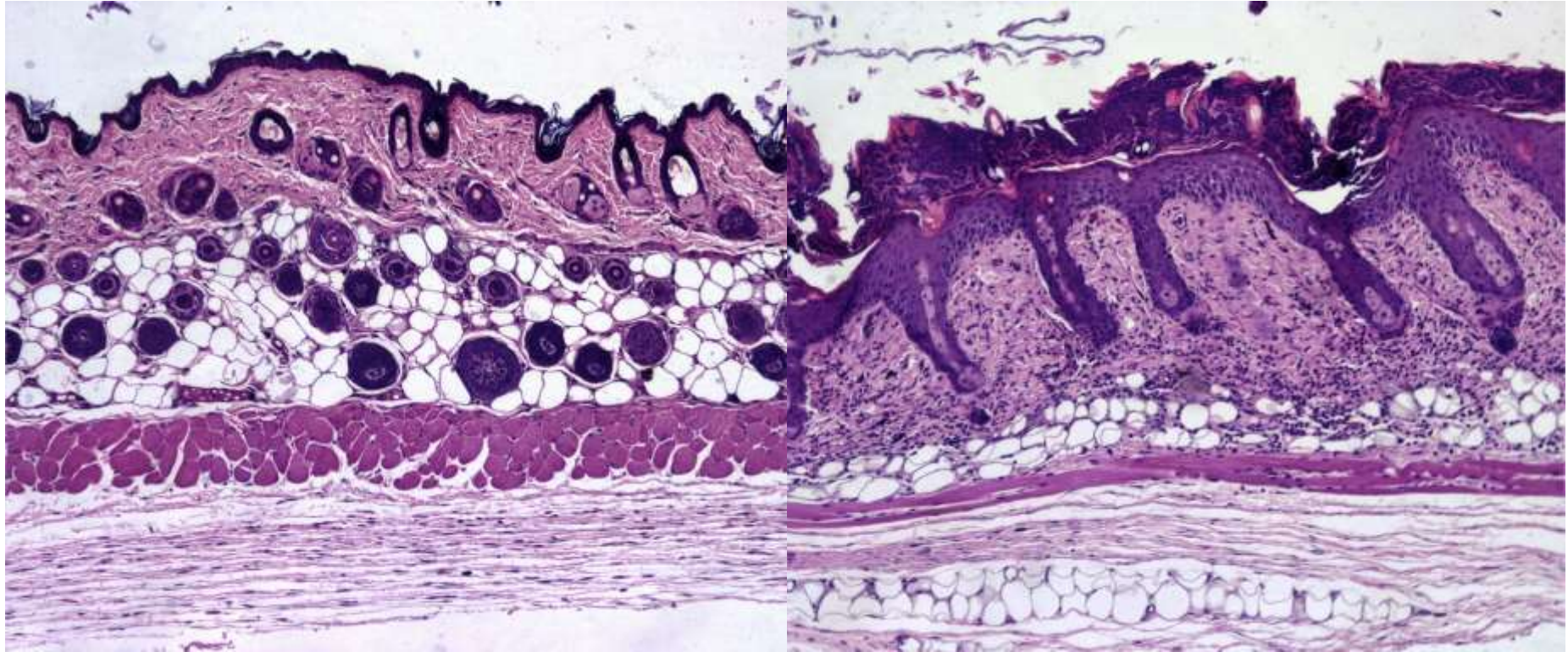
# Microscopic Observation Psoriasis mice model -Ear



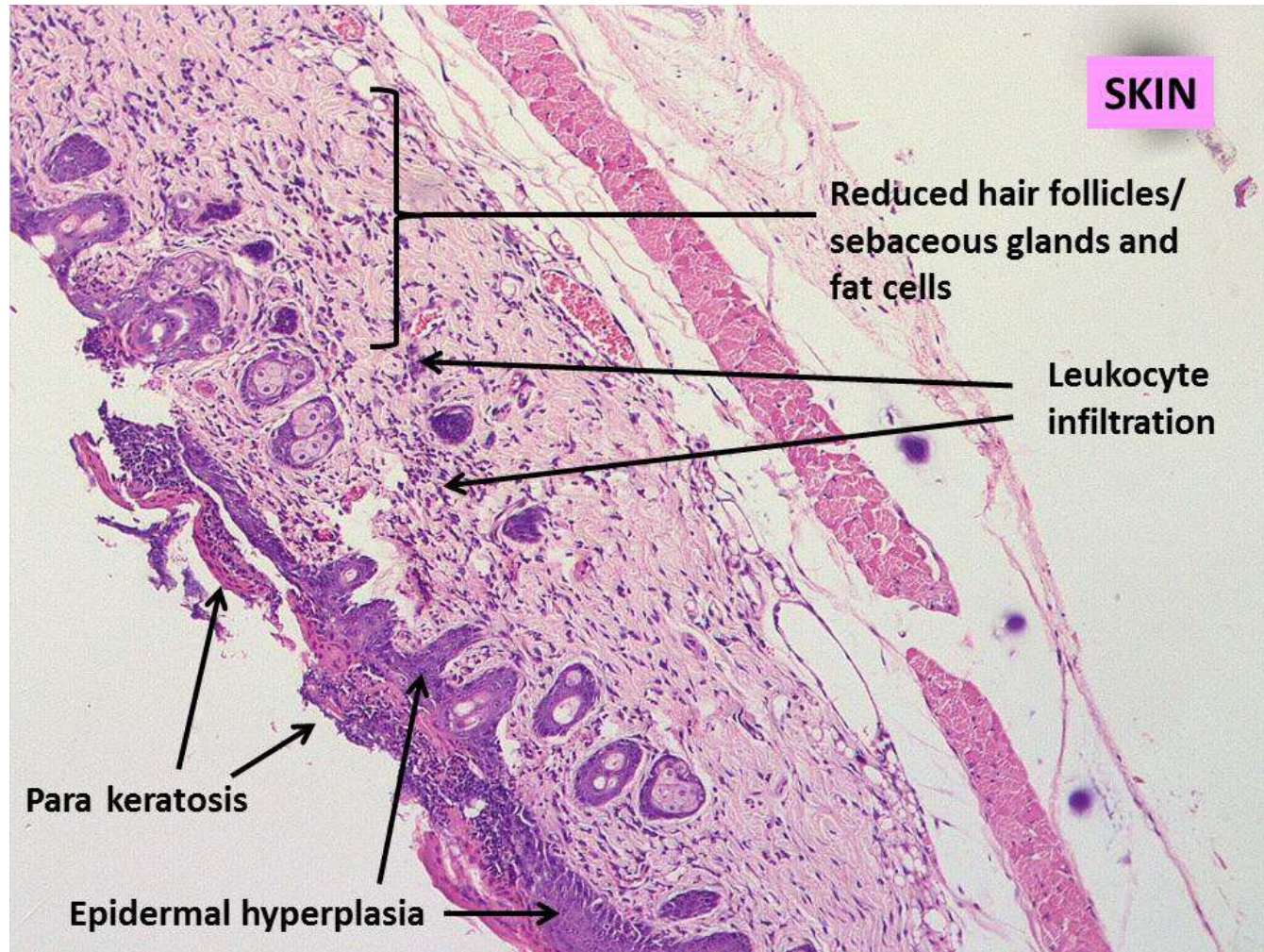
# Microscopic Observation Psoriasis mice model - Skin



# Microscopic Observation Dermal Application- Rat



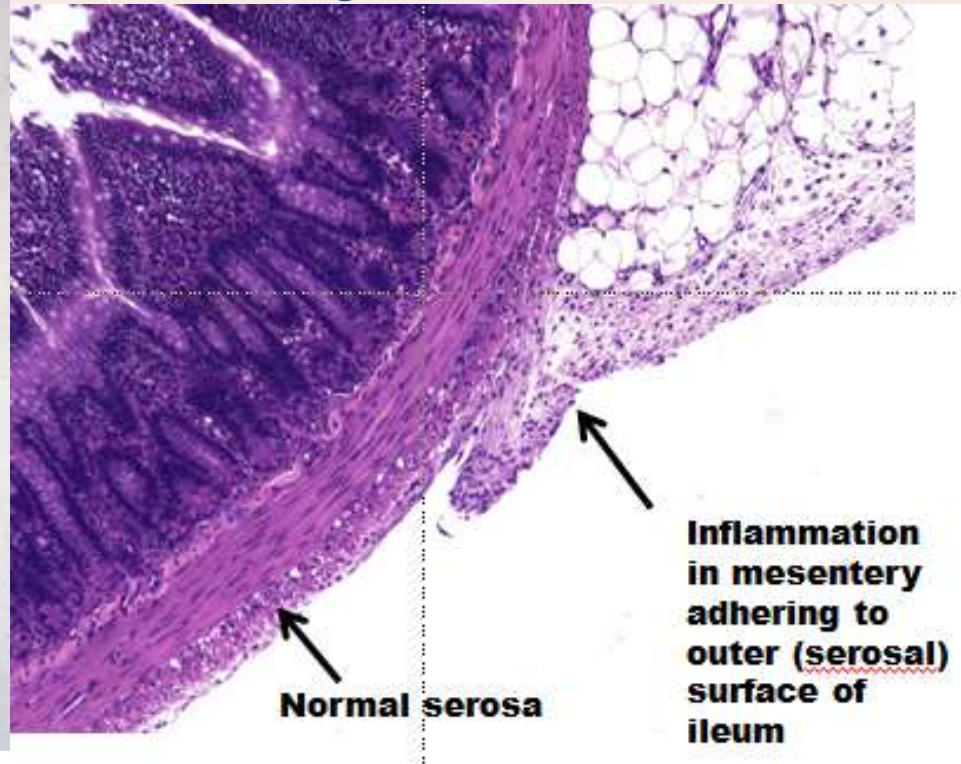
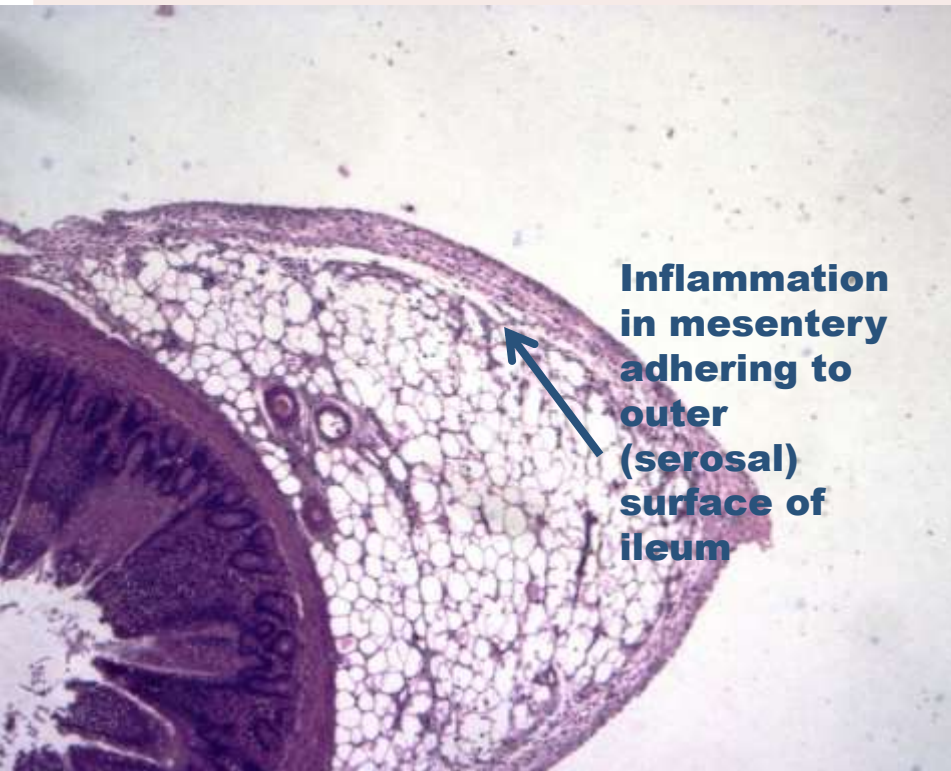
# Microscopic Observation Dermal Application - Rat



# Microscopic Observation Intraperitoneal injection- Rat



<b>ABDOMINAL CAVITY: Fluid-filled</b>	
<b>INTESTINE: Discoloration; red</b> <b>ABDOMINAL WALL: Discoloration; red</b>	<b>Hemorrhage</b>
<b>ABDOMINAL WALL; Focus (i)</b>	<b>Inflammation of serosa or capsule of various organs</b>



# Clinical Pathology

(may or may not be present)



## Hematology:

- **Increased WBC, neutrophil, leukocyte and monocyte counts**

## Clinical Biochemistry (Vaccine):

- **Increased total protein, globulin concentration and albumin globulin ratio, fibrinogen or acute phase proteins (ACP). These changes were likely due to antigenic stimulation induced by vaccine/adjuvant.**
- **Increased CK, LDH and AST (Case by case basis)**

## Local effects



**Grossly discoloration, nodule, thickening etc correlated with infiltration of various inflammatory cells and/or Inflammation at the site of injection and in surrounding tissues**

**Grossly enlarged draining lymph node correlated with lymphoid hyperplasia or histiocytosis/plasmacytosis**

# Systemic Effects: Topical corticosteroids



## ■ Haematology

Group	Main Group	Recovery Group
<b>Dose (mg/kg/day)</b>	<b>500</b>	<b>500</b>
<b>No. of rats</b>	<b>10</b>	<b>5</b>
<b>WBC</b>	↓(55)	↓(58)
<b>Platelet</b>	↓(23)	—
<b>Neutrophil %</b>	↑(3.3 folds)	↑(≤1.4 fold)
<b>Lymphocyte %</b>	↓(45)	↓(7)
<b>Monocyte %</b>	↑(4.1 folds)	—
<b>Basophils %</b>	↓(52)	—
<b>Neutrophil Absolute</b>	↑(32)	—
<b>Lymphocyte Absolute</b>	↓(72)	↓(60)
<b>Monocyte Absolute</b>	↑(1.8 folds)	↓(≤1 fold)
<b>Eosinophils Absolute</b>	↓(63)	—
<b>Basophils Absolute</b>	↓(74)	↓(71)
<b>Reticulocytes %</b>	↓(≤1 fold)	↑(1.7 folds)
<b>Reticulocytes Absolute</b>	↓(33)	↑(53)

## Clinical Chemistry

Group	Main Group	Recovery Group
<b>Dose (mg/kg/day)</b>	<b>500</b>	<b>500</b>
<b>No. of rats</b>	<b>10</b>	<b>5</b>
<b>AST</b>	↑(1.9 folds)	↓(0.8 folds)
<b>ALT</b>	↑(3.0 folds)	—
<b>Total Cholesterol</b>	↑(1.8 folds)	↑(1.3 folds)
<b>Triglyceride</b>	↑(2.4 folds)	—
<b>Total Protein</b>	↑(7)	—
<b>Albumin</b>	↑(12)	—

# Systemic Effects: Topical corticosteroids



## Terminal Fasting Body Weights, Organ Weights and Organ to Body Weight Ratios

Group	Main Group	Recovery Group
<b>Dose of formulation (mg/kg/day)</b>	<b>500</b>	<b>500</b>
<b>No. of rats</b>	<b>10</b>	<b>5</b>
<b>Terminal fasting Bwt</b>	↓(33)	↓(18)
<b>Spleen</b> – absolute wt	↓(53)	–
– relative to Bwt	↓(30)	–
<b>Thymus</b> – absolute wt	↓(78)	↓(47)
– relative to Bwt	↓(67)	↓(36)
<b>Adrenals</b> – absolute wt	↓(50)	–
– relative to Bwt	↓(25)	–
<b>Liver</b> – absolute wt	↓(11)	–
– relative to Bwt	↑(34)	–
<b>Heart</b> – absolute wt	↓(12)	–
– relative to Bwt	↑(32)	–
<b>Kidneys</b> – absolute wt	↓(13)	–
– relative to Bwt	↑(29)	↑(12)
<b>Epididymides</b> – absolute wt	↓(6)	↓(9)
– relative to Bwt	↑(40)	↑(12)
<b>Prostate</b> – absolute wt	↓(20)	↓(27)
<b>Seminal vesicles &amp; coagulating glands</b> – absolute wt	↓(33)	↓(28)
<b>Brain</b> – absolute wt	↓(3)	–
– relative to Bwt	↑(45)	↑(12)

# Systemic Effects: Topical corticosteroids



## ■ Gross Pathology

<b>Group</b>	<b>Main Group</b>	<b>Recovery Group</b>
<b>Dose (mg/kg/day)</b>	<b>500</b>	<b>500</b>
<b>No. of rats</b>	<b>10</b>	<b>5</b>
<b>SKIN-TREATED</b>		
<b>Discoloration; red</b>	<b>1</b>	—
<b>Thickening; multifocal</b>	<b>2</b>	—
<b>SPLEEN</b>		
<b>Small</b>	<b>10</b>	—
<b>THYMUS</b>		
<b>Small</b>	—	<b>4</b>
<b>Not prominent</b>	<b>10</b>	—
<b>ADRENALS</b>		
<b>Small</b>	<b>10</b>	—

# Systemic Effects: Topical corticosteroids



## ▪ Histopathology

Group		Main Group	Recovery Group
<b>Dose (mg/kg/day)</b>		<b>500</b>	<b>500</b>
<b>No. of rats</b>		<b>10</b>	<b>5</b>
<b>Treated skin</b>	<b>No. examined</b>	<b>10</b>	<b>5</b>
<b>Atrophy</b>		<b>10</b>	<b>0</b>
	<b>Mild</b>	<b>9</b>	<b>—</b>
	<b>Moderate</b>	<b>1</b>	<b>—</b>
<b>Thymus</b>	<b>No. examined</b>	<b>9</b>	<b>5</b>
<b>Lymphoid depletion</b>		<b>9</b>	<b>4</b>
	<b>Minimal</b>	<b>—</b>	<b>4</b>
	<b>Mild</b>	<b>—</b>	<b>—</b>
	<b>Moderate</b>	<b>6</b>	<b>—</b>
	<b>Severe</b>	<b>3</b>	<b>—</b>
<b>Bone-femur with joint</b>	<b>No. examined</b>	<b>10</b>	<b>5</b>
<b>Bone marrow hypocellularity</b>		<b>10</b>	<b>0</b>
	<b>Mild</b>	<b>7</b>	<b>—</b>
	<b>Moderate</b>	<b>3</b>	<b>—</b>
<b>Sternum with marrow</b>	<b>No. examined</b>	<b>10</b>	<b>5</b>
<b>Bone marrow hypocellularity</b>		<b>10</b>	<b>0</b>
	<b>Minimal</b>	<b>2</b>	<b>—</b>
	<b>Mild</b>	<b>6</b>	<b>—</b>
	<b>Moderate</b>	<b>2</b>	<b>—</b>
<b>Spleen</b>	<b>No. examined</b>	<b>10</b>	<b>5</b>
<b>Lymphoid depletion</b>		<b>10</b>	<b>0</b>
	<b>Minimal</b>	<b>3</b>	<b>—</b>
	<b>Mild</b>	<b>7</b>	<b>—</b>
<b>Increased extramedullary hematopoiesis</b>		<b>0</b>	<b>5</b>
	<b>Mild</b>	<b>—</b>	<b>5</b>

# Systemic Effects: Topical corticosteroids

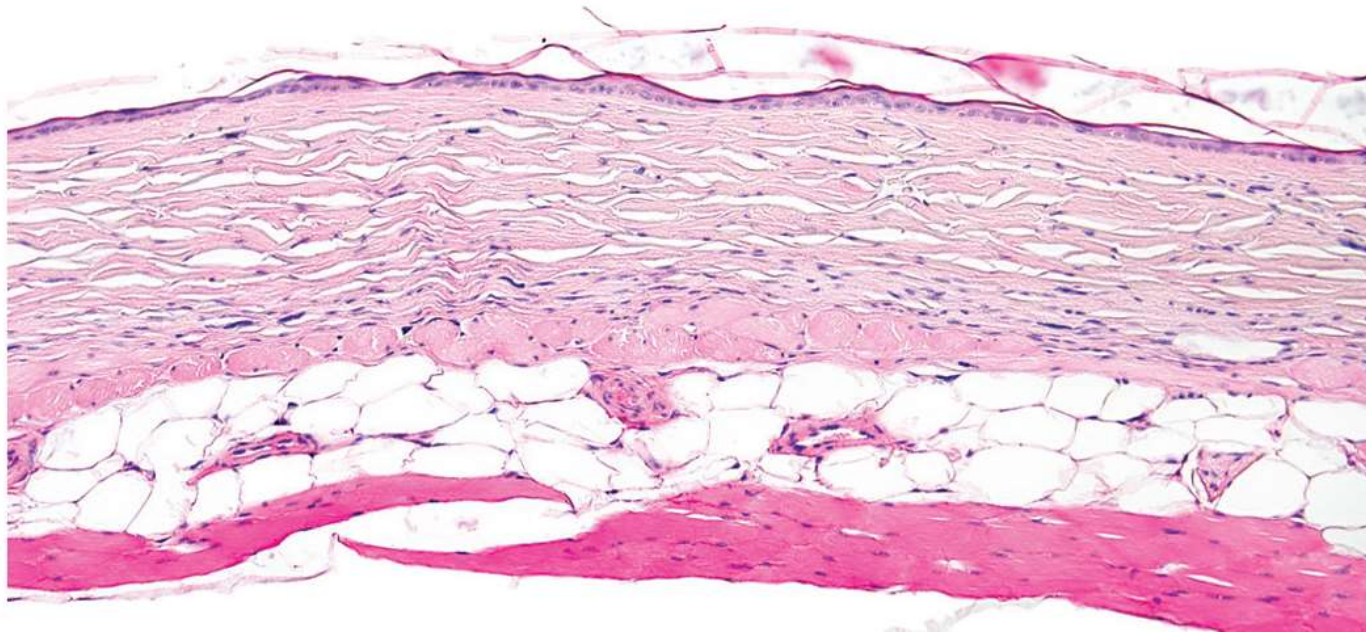


<b>Group No.</b>	<b>Main Group</b>	<b>Recovery Group</b>
<b>Adrenal glands                      No. examined</b>	<b>10</b>	<b>5</b>
<b>Cortical cell hypertrophy-zona glomerulosa</b>	<b>10</b>	<b>5</b>
<b>Minimal</b>	<b>1</b>	<b>5</b>
<b>Mild</b>	<b>9</b>	<b>—</b>
<b>Cortical cell atrophy-zona fasciculata, zona reticularis</b>	<b>10</b>	<b>0</b>
<b>Minimal</b>	<b>2</b>	<b>—</b>
<b>Mild</b>	<b>8</b>	<b>—</b>
<b>Mesenteric LN                      No. examined</b>	<b>10</b>	<b>5</b>
<b>Lymphoid depletion</b>	<b>3</b>	<b>0</b>
<b>Minimal</b>	<b>3</b>	<b>—</b>
<b>Mandibular LN                      No. examined</b>	<b>8</b>	<b>5</b>
<b>Lymphoid depletion</b>	<b>7</b>	<b>0</b>
<b>Minimal</b>	<b>4</b>	<b>—</b>
<b>Mild</b>	<b>3</b>	<b>—</b>
<b>Axillary LN                          No. examined</b>	<b>10</b>	<b>5</b>
<b>Lymphoid depletion</b>	<b>6</b>	<b>0</b>
<b>Minimal</b>	<b>2</b>	<b>—</b>
<b>Mild</b>	<b>4</b>	<b>—</b>
<b>Liver                                      No. examined</b>	<b>10</b>	<b>5</b>
<b>Hepatocellular hypertrophy</b>	<b>9</b>	<b>0</b>
<b>Minimal</b>	<b>3</b>	<b>—</b>
<b>Mild</b>	<b>6</b>	<b>—</b>

# Systemic Effects: Topical corticosteroids



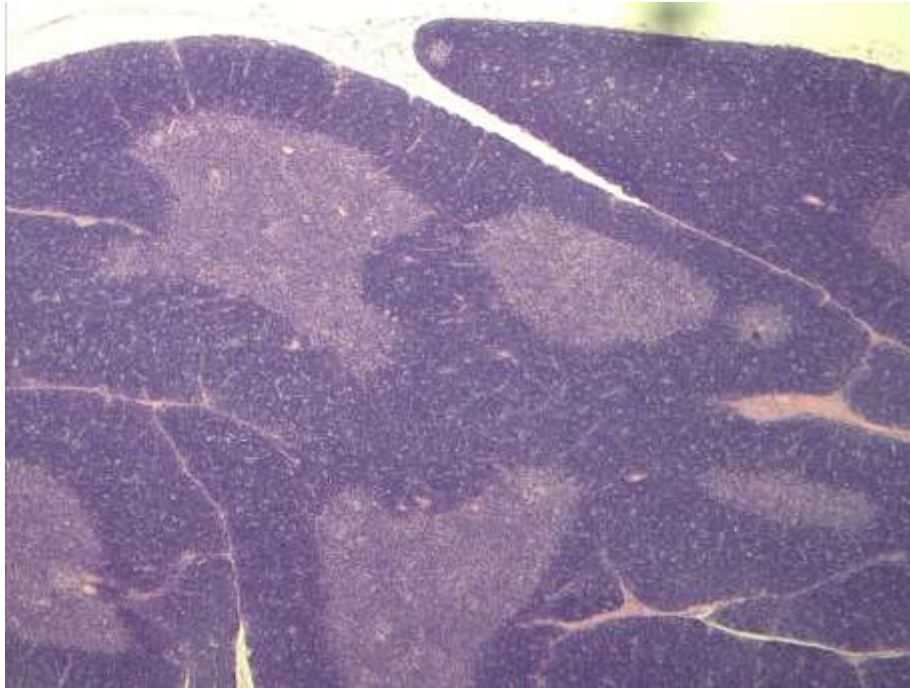
## Skin: Atrophy



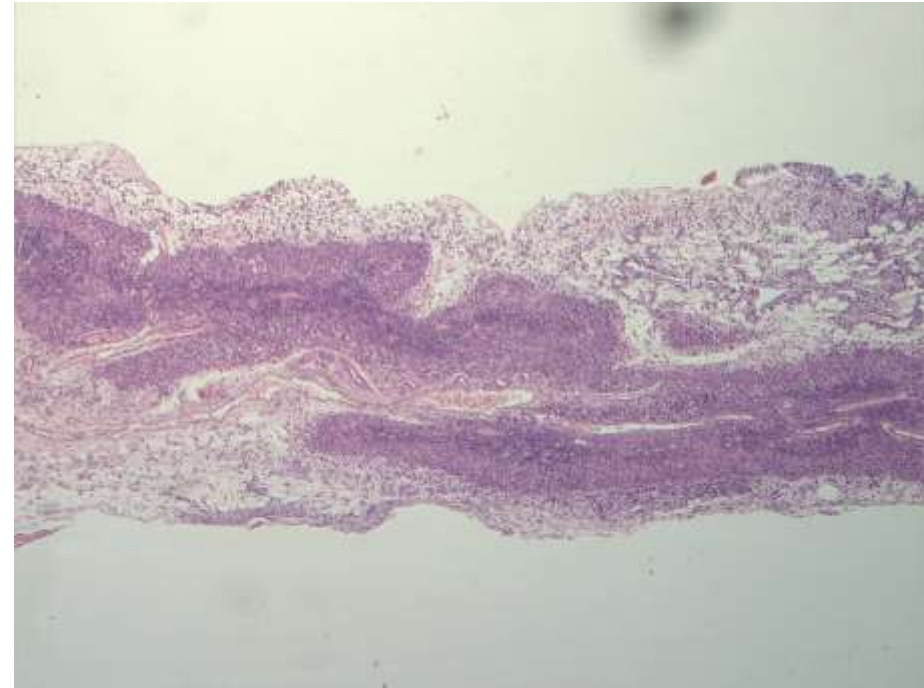
# Systemic Effects: Topical corticosteroids



**Thymus – Normal**



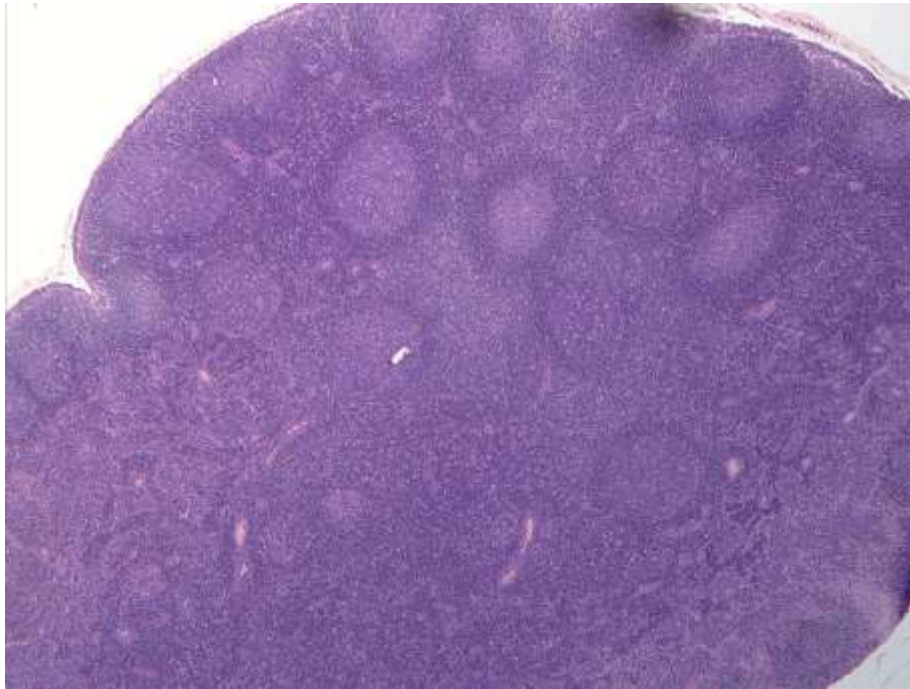
**Thymus – Lymphoid depletion, severe**



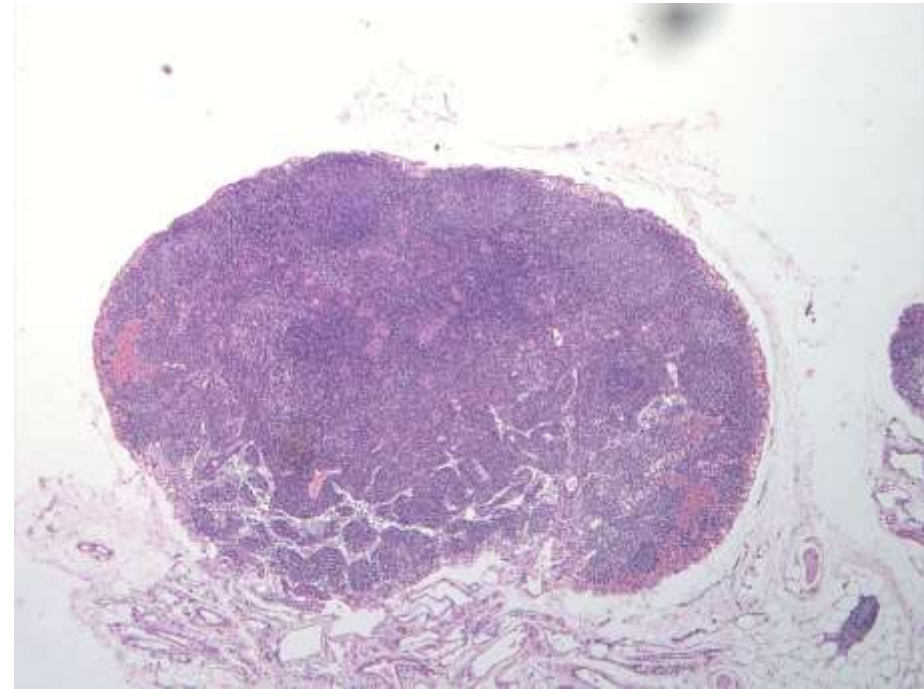
# Systemic Effects: Topical corticosteroids



**Mandibular LN – Normal**



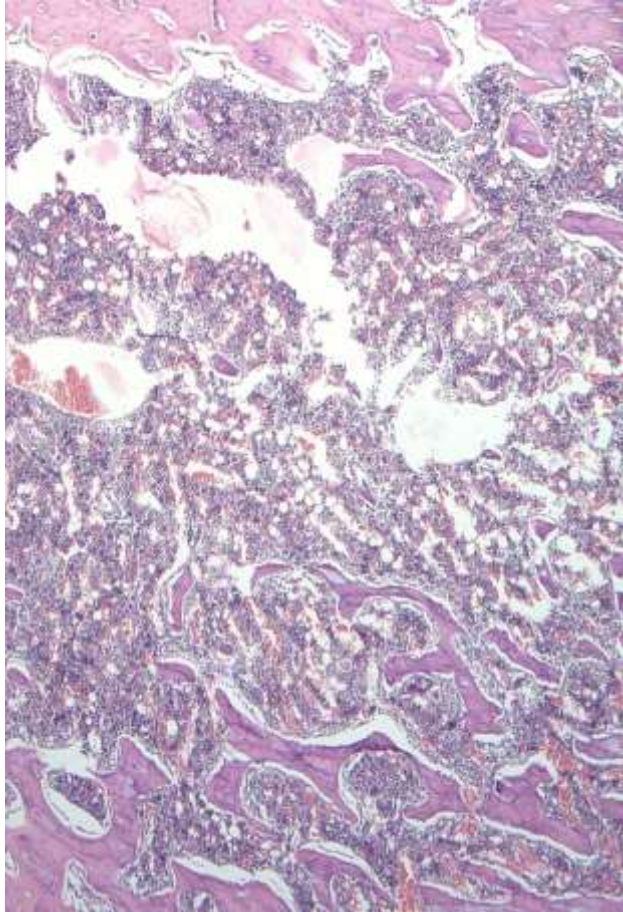
**Mandibular LN – Lymphoid depletion, mild**



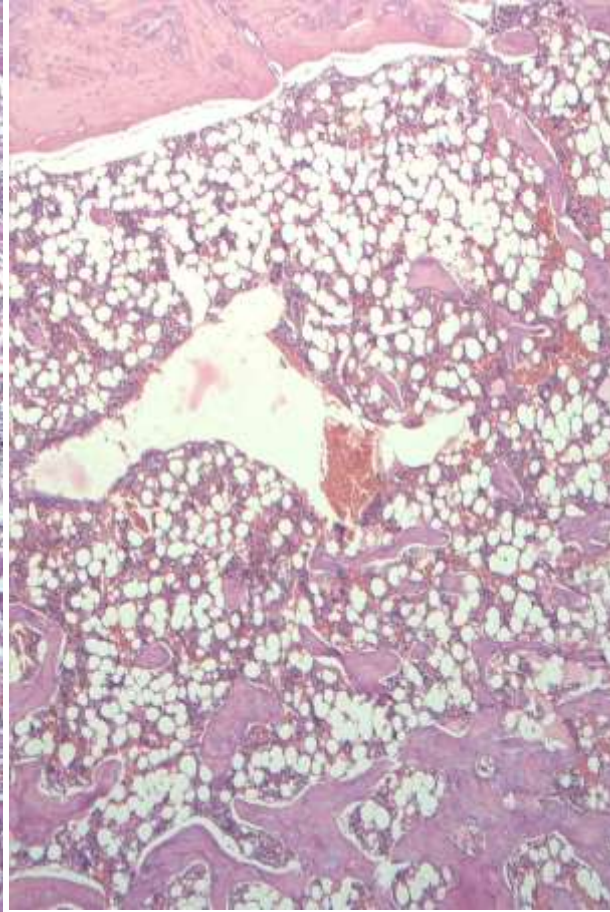
# Systemic Effects: Topical corticosteroids



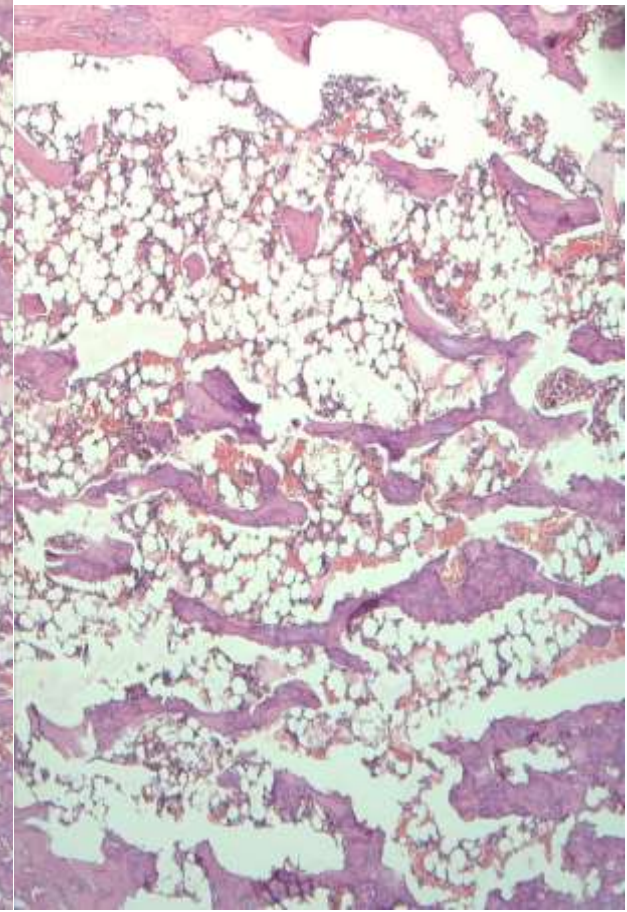
**Femur bone (shaft) – Normal**



**Femur bone (shaft) - Hypocellularity, minimal**



**Femur bone (shaft)- Hypocellularity, mild**





# Thank You !

