Practice 1：Animal Model of CO Poisoning and Morphological Techniques

morphological laboratory centre

Purposes and Requirements

- 1. Mastering basic morphological techniques and skills used in the experiments
- 2. Understanding the basic procedures of establishing animal model of carbon monoxide poisoning
- 3. Understanding how to write an experimental paper.
- 4. Knowing about photomicrography and image analysis technique with computers
- 5. Knowing about applications of laser scanning confocal microscope

Experimental Design

Inhaling CO

CO Poisoning

No treatment

control

biopsy

paraffin section

electron microscopy

HE Staining

Nissl staining

image analysis of experimental results

Practice Contents

- Establishing animal model : carbon monoxide poisoning of mice
- Paraffin sectioning techiques: biopsy , fixation , embedding and sectioning
- Nissl staining , HE staining and observing the slides
- Knowing about photomicrography and image analysis technique with computers
- Knowing about applications of laser scanning confocal microscop

1.Establishing animal model----
CO poisoning of mice

Experimental Principles

- **Tissue Hypoxia：**

  CO much more easily bind with hemoglobin (to form carboxyhemoglobin ,HbCO)and difficult to relaese, which effectively reduce the oxygen-carrying capacity of the blood.

- **Central nervous system** is **extremely sensitive** to h**ypoxia**

Materials

- animal: mice
- intruments: scalpels、surgical scissors、dissecting forceps , wide-mouth bottle
- reagents: carbon monoxide

Procedures

1.Mice are divided into two groups randomly.

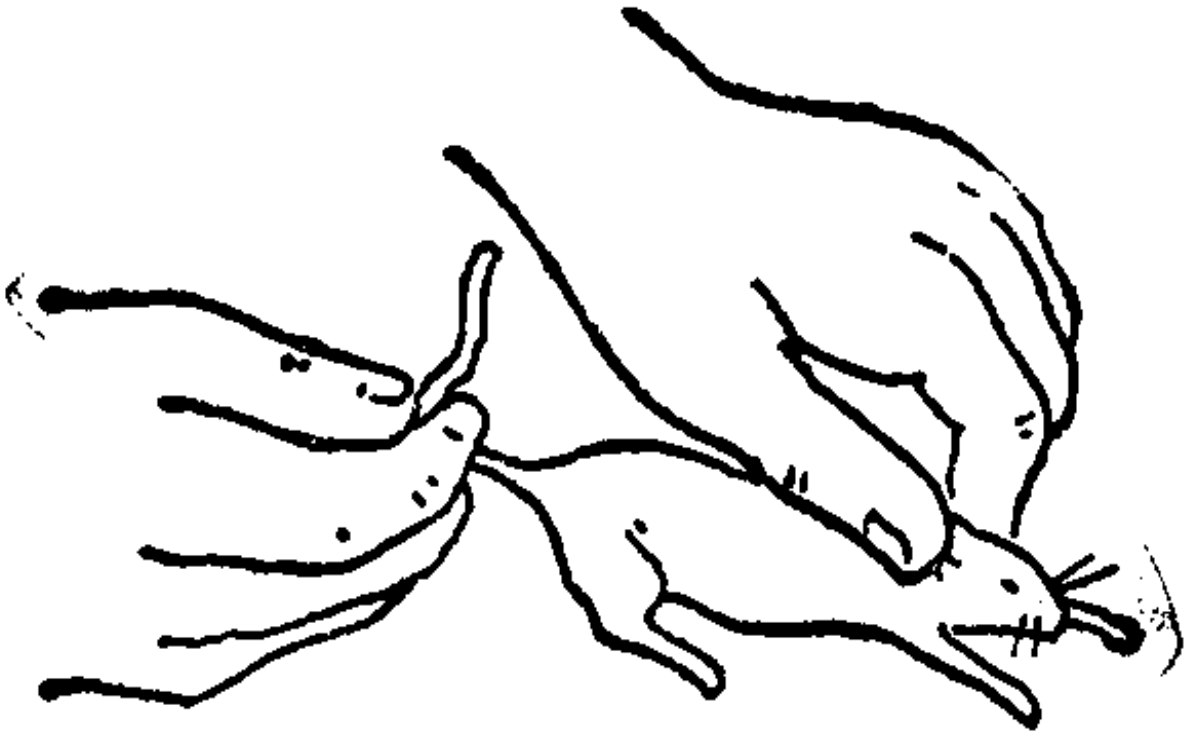
The experimental mice are kept into wide-mouth bottle,

they inhale CO until irritability and difficulties in breathing appear, then fresh air is given to recover them.

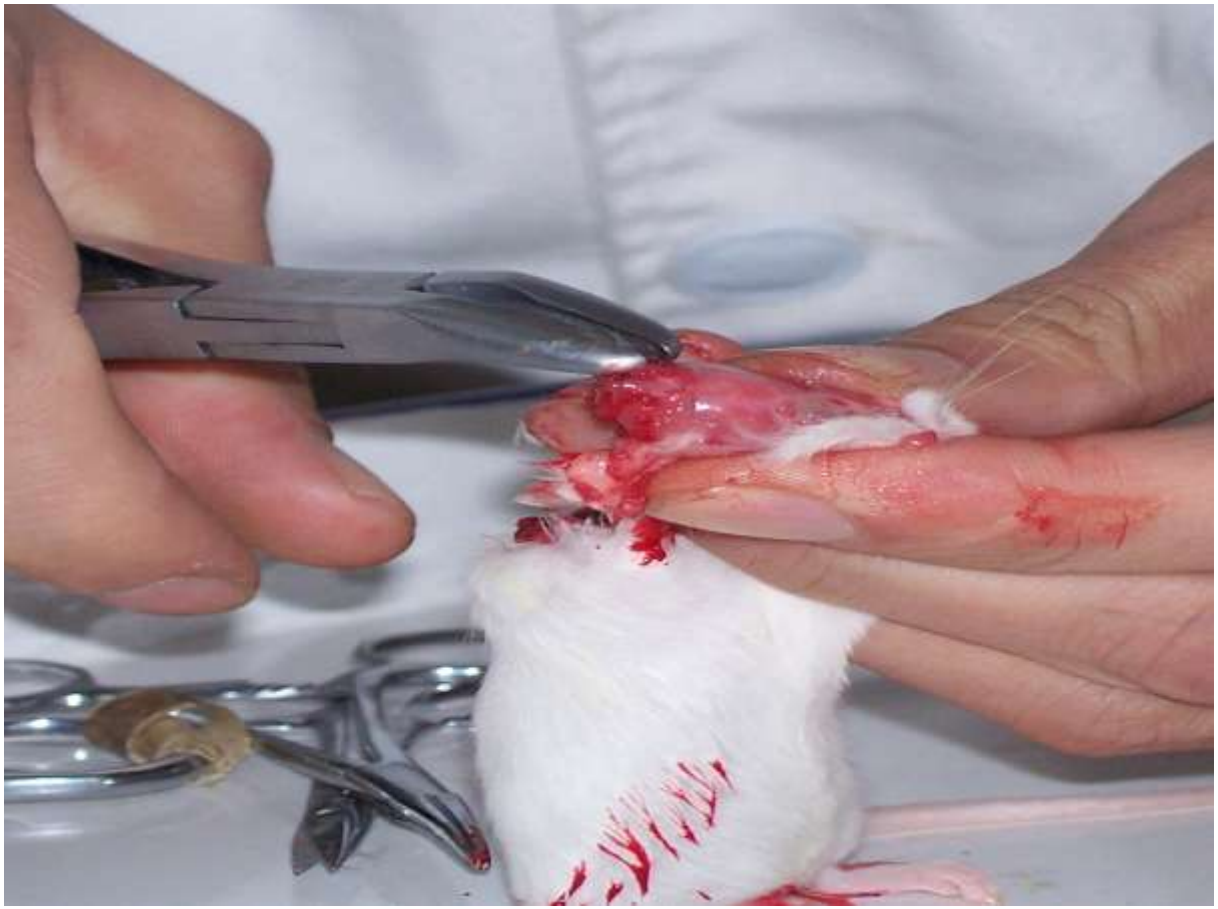
Control group： no treatment

2. **Repeated several times until mice are poisoned to death at the end of the experiment on the fourth day.**

Kill the control mice by the way of cervical vertebra dislocation



3. **Incise head skin , open the skull until brain tissues expose fully**



4. Remove intact brain tissue，

and observe gross anatomical alterations before it is immersed into fixatives.

- ✓ **Remember to compare experimental group with control group:**
  - *** color**
  - *** anfractuosity**
  - *** gyrus**
  - *** hemorrhage**
- ✓ **Then brain tissue is kept in formalin fixative for future use**

2.Paraffin Technique

Primary Steps:

biopsy → fixation → dehydration →

clearing → embedding →

sectioning → staining

Aims of Experiment

- To know about the procedures of paraffin technique
- To master basic methods and skills in paraffin embeddding and sectioning

Technique Principles

- In this technique, tissues are fixed, and embedded in wax. This makes the tissue hard, and much easier to cut sections from.
- The sections are then stained, and examined with the light microscope to help distinguish the components of the tissue.
- But wax is not soluble in water or alcohol. So first the tissue has to be dehydrated and then the alcohol in the tissue needs to be replaced with xylene.

Experiment materials

1. Reagents

paraffin wax , formaldehyde(at neutral pH),

alcohol, xylene

2. Instruments

microtome、device for mounting

thermostat incubator、embedding frame,

glass slide and cover slip

Details in Procedures

1.Biopsy

- ✓Fresh tissue
- ✓Keep tissues and cells morphological intact
- ✓Prevent autolysis
- ✓Cut into square tissue blocks (about 1×0.5×0.2cm)
- ✓Immerse into fixatives soon

2.Fixation

- ✓**The fixative most commonly used is a 10% aqueous solution of formaldehyde, at neutral pH.**
- ✓**4 % paraformaldehyde**
- ✓**Volume: 1:4。**
- ✓**Proper time**
- ✓**Washing thoroughly after fixation**

4. Dehydration

Replacing water in the sample with alcohol. This is achieved by passing the tissue through increasing concentrations of ethyl alcohol (from 70 to 100%).

70% alcohol （2h）→80%（2h）→90%（1h）

→ 95% alcohol Ⅰ（1h）→95% alcohol Ⅱ（1h）

→100% alcohol Ⅰ（1h）→100% alcohol Ⅱ（1h）。

Automatic Dehydrator

5 Clearing

Replaced alcohol in the sample with xylene, wintergreen oil.

6 Paraffin- infiltration

Xylene is replaced by melted paraffin and the melted wax fills the spaces that used to have water in them.

paraffin wax Ⅰ (30min) → paraffin wax Ⅱ (1h) → paraffin wax Ⅲ (1h)

7 Embedding

The paraffin- infiltrated tissue is placed in fresh paraffin and the latter allowed to cool. After cooling, the tissue hardens, and can be used to cut slices.

Embedding Device

8、Sectioning

Use microtome to cut into thin sections, 3-10 μm in thickness.

9、Mounting

Spread slices in 45℃ water, then mount it on a microscope slide.

10、drying

Place the slide in oven 60 ℃, 3~5h， until it sticks firmly for future use.

Microtome(left) and Mounting Device(right)

Questions

- **What are basic principles of paraffin techniques?**
- **Are there any other ways of making sections for the light microscope?**

3.Nissl body staining techni

Outline

- Objective and Requirement
- Principle
- Material
- Operating steps
- Result

Objective and Requirement

- **Be familiar with the principle of Nissl body staining.**
- **Master the method of Nissl body staining.**

Principle

- Nissl bodies consist of abundent RER and Ri. They are the site of protein synthesis of neurons.
- Body tissue is colorless. In order to observe and identify under LM, they should be dyed for increasing the contrast of organizational structure. Toluidine blue o is basic dye which can make Nissl bodies purplish blue.
- The morphologic and quantitative observation of Nissl bodies can judge neurons function as a symbol of status.

Material

- staining fluid, alcohol, xylene, etc
- staining jar, forceps, filter paper, etc

Operating steps

1. dewax: section dewax to water

xylene 20min * twice

100% alcohol 10min

90% alcohol 10min

80% alcohol 10min

70% alcohol 10min

distilled water 5 min

2. dye：20~30min（60^0C incubator）

- dye liquor preparation: toluidine blue o 0.5g
  50% alcohol 100ml

- Wash with distilled water after dyeing.

3. colour separation： 95% alcohol, 1-2 seconds，under LM

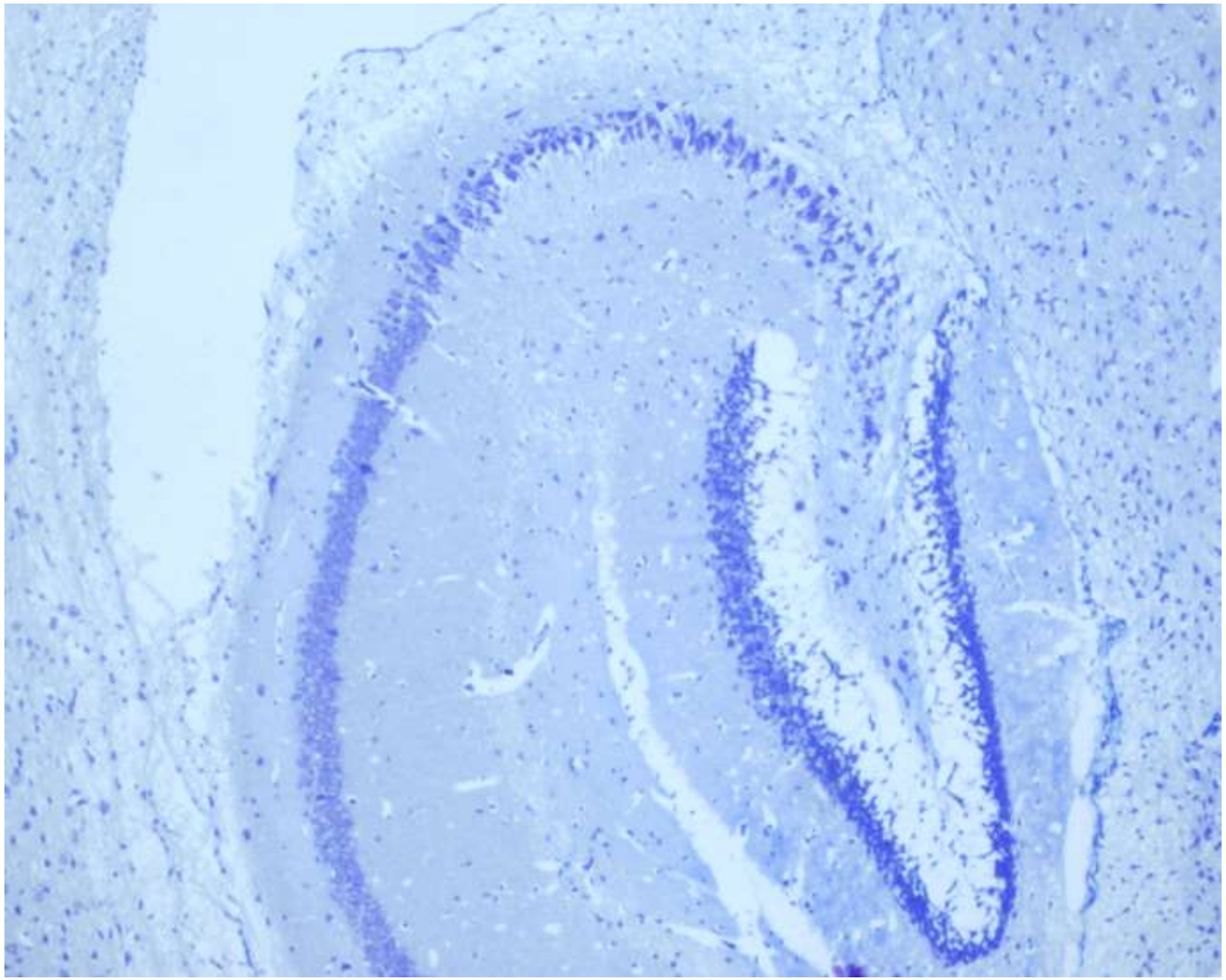
4. dehydration： 100% alcohol, 10 seconds, twice

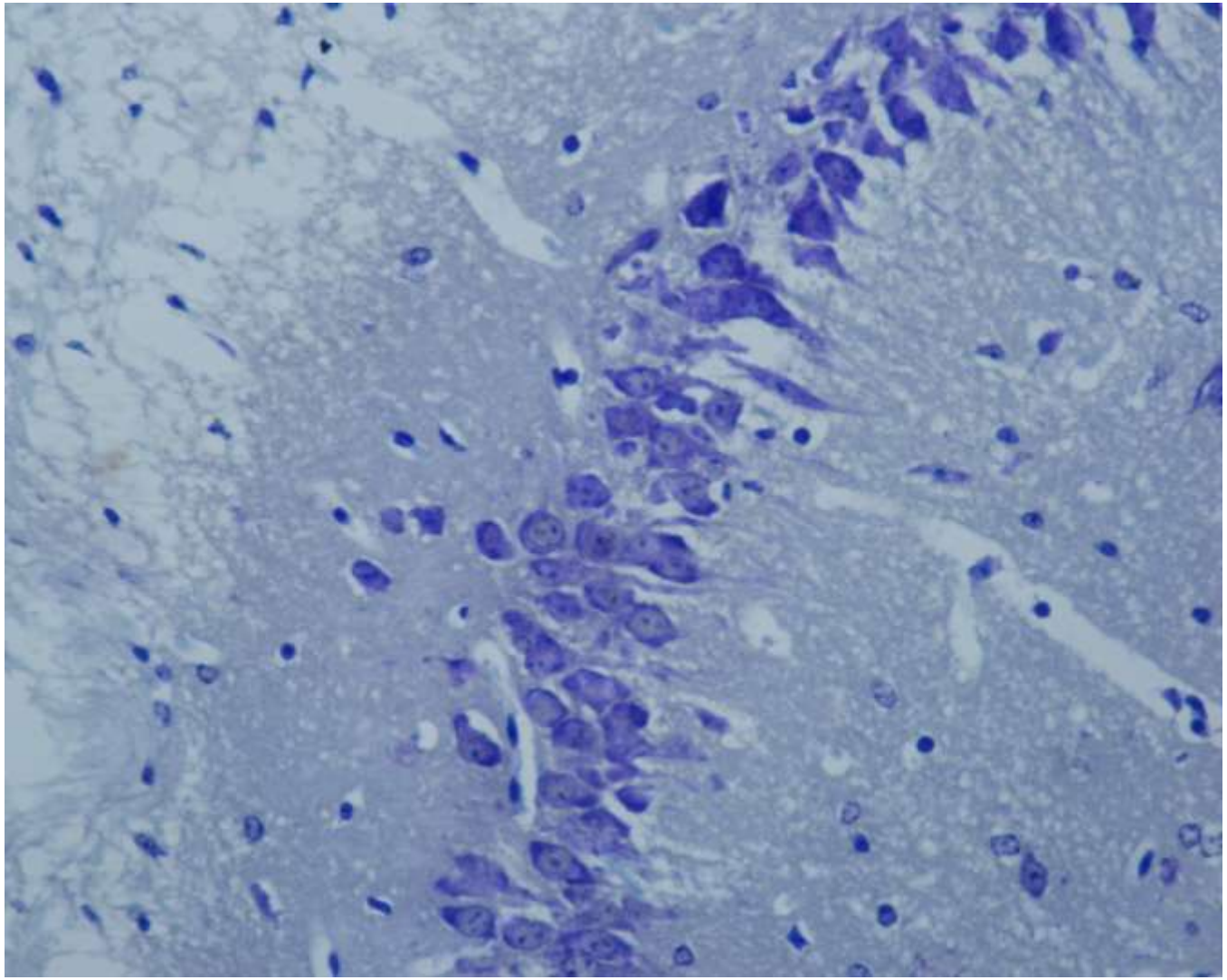
5. transparentizing： xylene, 5 min, twice

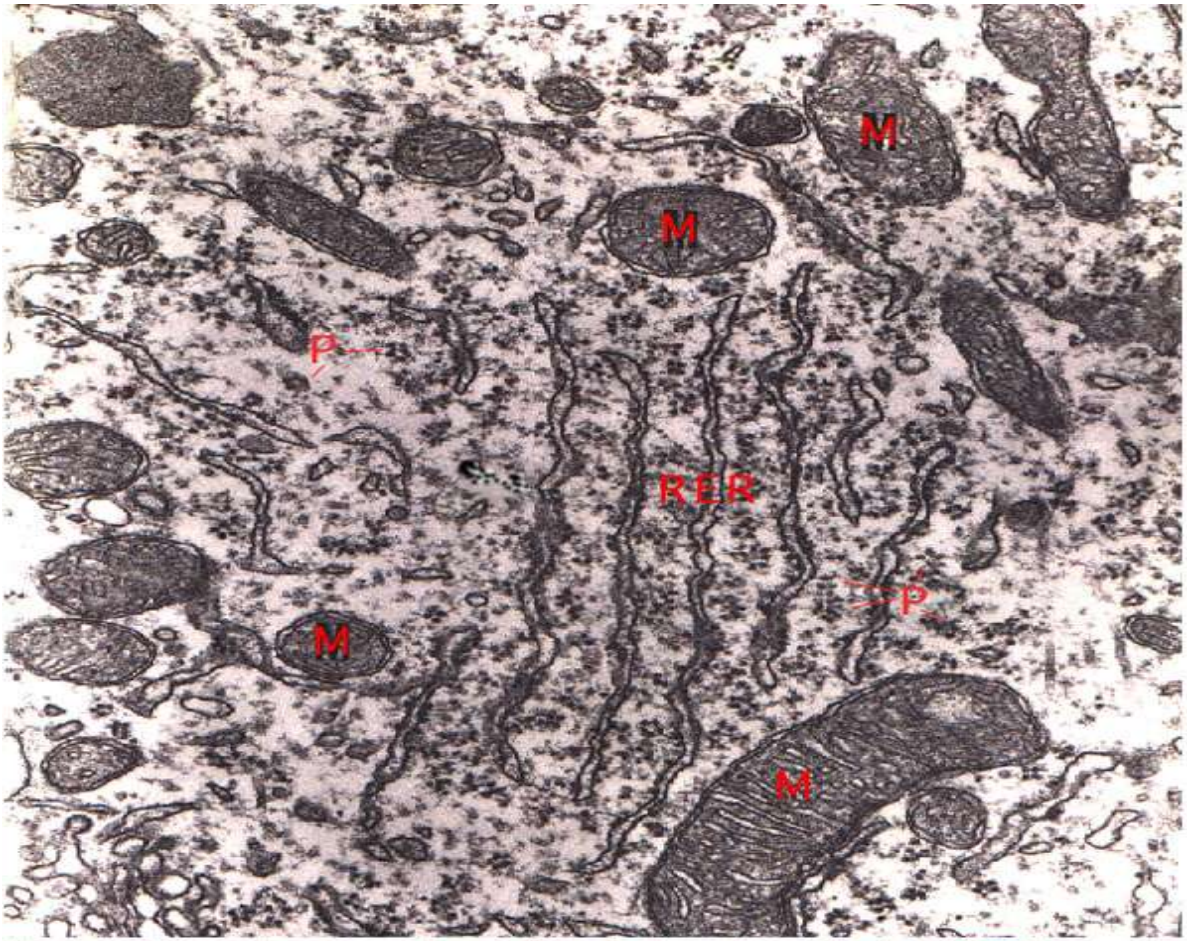
6. slide sealing： by neutral gum

7. observation

Result







Neuronal cytoplasm with rough endoplasmic reticulum (RER) which corresponds to Nissl substance in the light microscope.

P = polyribosomes

M = mitochondria

high mag ~ 70,000 X

4.H E staining technique

Outline

- Objective and Requirement
- Principle
- Material
- Operating steps
- Result

Objective and Requirement

- Master the principle of HE staining.
- Master the method of HE staining.

Principle

- **Body tissue is colorless. In order to observe and identify under LM, they should be dyed for increasing the contrast of organizational structure.**

- **HE staining：hematoxylin-eosin staining**

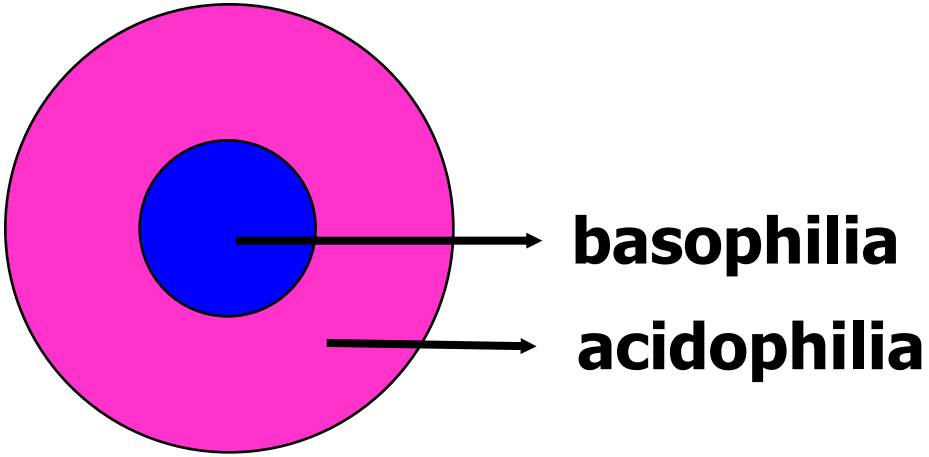
Principle

（H）Hematoxylin： basic dye

nucleus, RER, Ri ⟶ purplish blue

（E）Eosin： acid dye

cytoplasm, extracellular matrix ⟶ red



Material

- formaldehyde, alcohol, xylene, paraffins, hematoxylin, eosin, etc
- microtome, slicing drift drying temperature control, incubator, embedding box, glass slide, cover glass, staining jar, etc

Operating steps

1. dewax: section dewax to water

xylene 20min (10min * twice)

100% alcohol 10min

100% alcohol 5min

90% alcohol 5min

80% alcohol 5min

70% alcohol 5min

distilled water

2. hematoxylin staining： 20min（58^0C incubator）

- dye liquor preparation: hematoxylin 2g
  100%alcohol 250ml
  aluminum sulfate 17.6g
  double distilled water 750ml
  sodium iodate 0.2g
  glacial acetic acid 20ml

- Wash away the floating color by tap water after dyeing.

3. colour separation： 0.5% hydrochloric acid alcohol,
1-2 seconds, under LM

4. blue： wash about 10 min by tap water.

5. dehydration： 70% alcohol 15 min →
80% alcohol 15 min

6. eosin replication solution staining:

about 1 min

- dye liquor preparation: eosin replication solution 0.5g
  double distilled water 5ml
  glacial acetic acid a few drops

7. colour separation： 95% alcohol, 2min, under LM

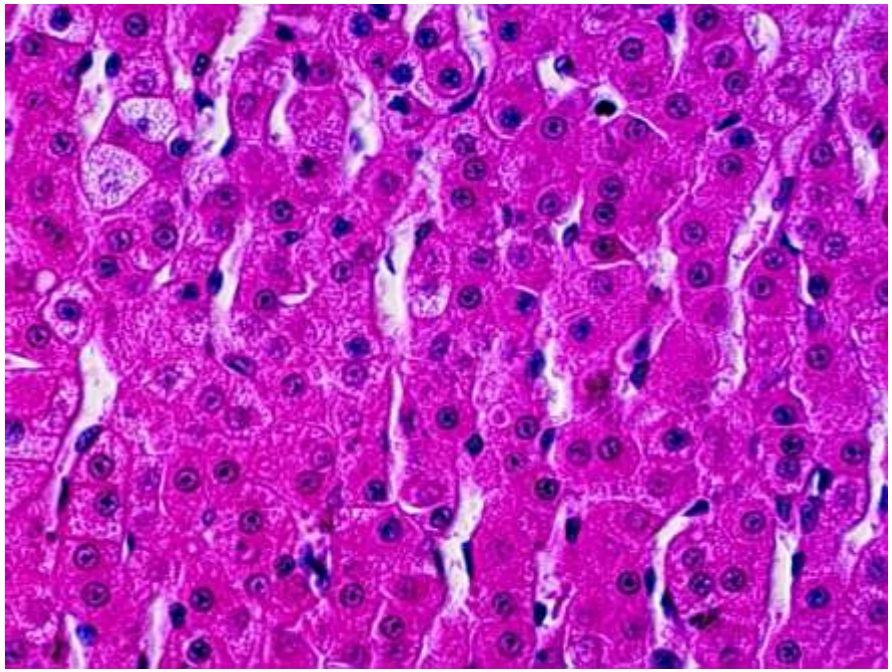
8. dehydration： 95% alcohol （5min）→
100% alcohol I（10min）→
100% alcohol II（10min）

9. transparentizing： xylene I（10min）→
xylene II（10min）

10. slide sealing： by neutral gum, under LM

Result

- **Nucleus is dyed blue and cytoplasm is dyed red.**



Result analysis

- **control group：**

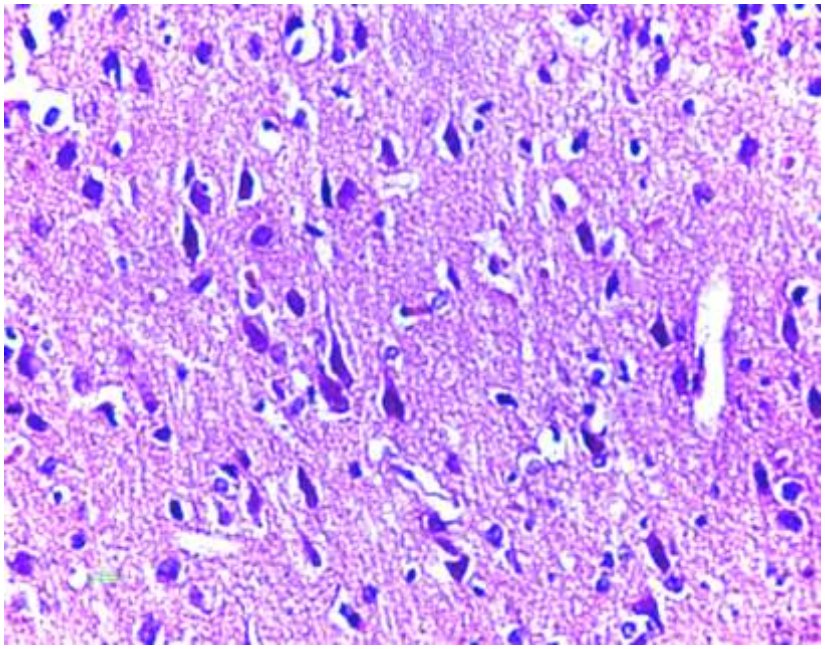
cortex neurons are multipolar neurons. They can divide into 3 types according to their shape.

1. pyramidal cells：

the most neurons

2. granule cells

3. spindle cells



Result analysis

- **experimental group：**
  - **neurons in cerebral cortex degeneration and necrosis**
  - **hyperplasia，swelling and cystose appearance with devouring lipid of microglia**
  - **cerebrovascular expansion and hyperemia**
  - **cuff lymphocytic infiltration around the small blood vessels**