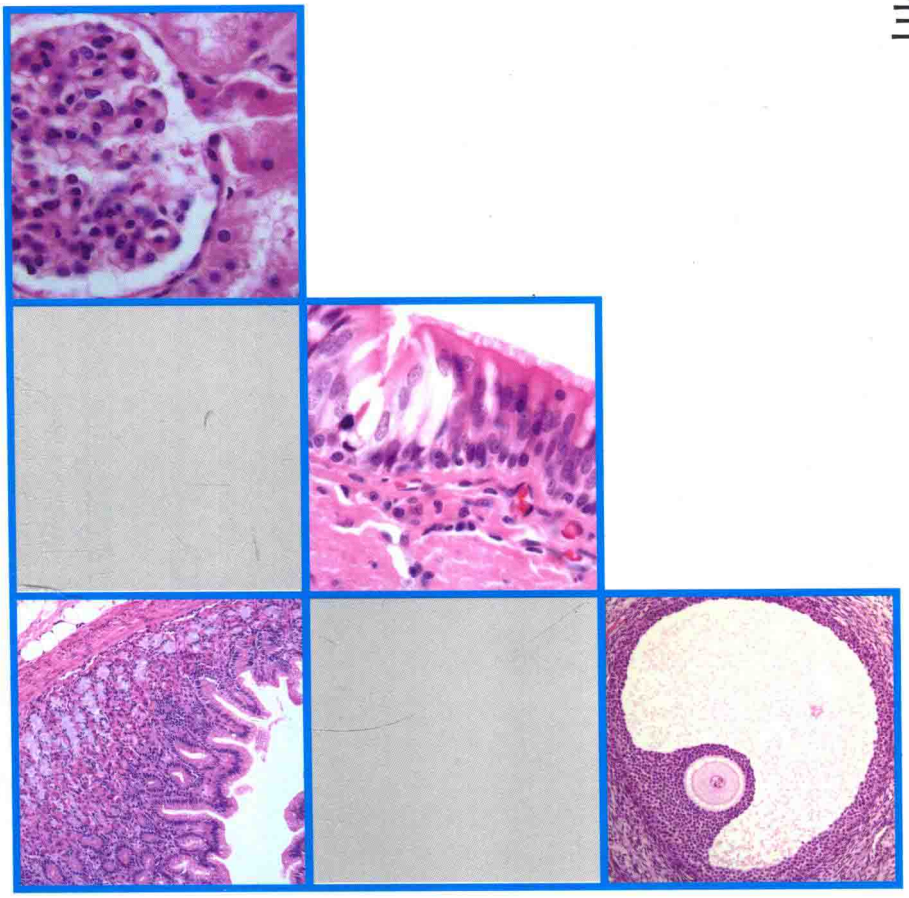
西安交通大学“十三五”规划教材

组织学彩色图谱

Color Atlas of Histology

主编　周劲松



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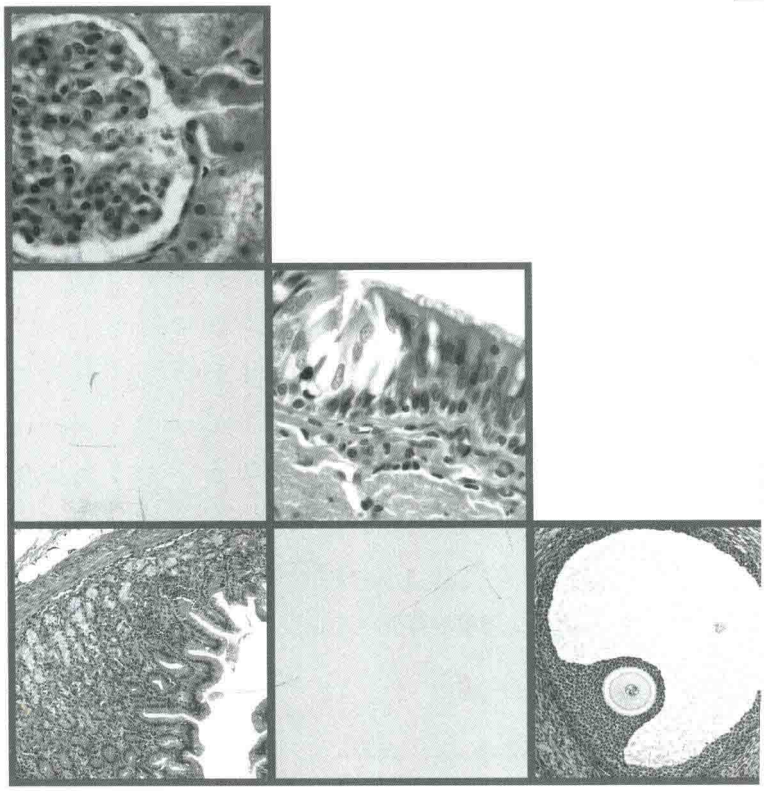

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图书在版编目(CIP)数据

组织学彩色图谱＝Color Atlas of Histology:汉英对照/
周劲松主编. —西安:西安交通大学出版社,2018.3
西安交通大学"十三五"规划教材
ISBN 978-7-5693-0508-1

Ⅰ.①组… Ⅱ.①周… Ⅲ.①人体组织学-图谱
Ⅳ. ①R329-64

中国版本图书馆 CIP 数据核字(2018)第 059624 号

书　　名　组织学彩色图谱
主　　编　周劲松
责任编辑　杜玄静

出版发行　西安交通大学出版社
（西安市兴庆南路 10 号　邮政编码 710049）
网　　址　http://www.xjtupress.com
电　　话　(029)82668357　82667874(发行中心)
(029)82668315(总编办)
传　　真　(029)82668280
印　　刷　陕西龙山海天艺术印务有限公司

开　　本　889mm×1194mm　1/16　**印张** 8.75　**字数** 259 千字
版次印次　2018 年 7 月第 1 版　2018 年 8 月第 1 次印刷
书　　号　ISBN 978-7-5693-0508-1
定　　价　46.00 元

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投稿热线:(029)82668526　(029)82668133
读者信箱:xj_rwjg@126.com

主　审：宋天保　西安交通大学医学部
Xi'an Jiaotong University Health Science Center
主　编：周劲松　西安交通大学医学部
副主编：张晓田　西安交通大学医学部
许　颖　西安交通大学外国语学院
School of Foreign Studies, Xi'an Jiaotong University
寇　博　西安交通大学医学部第一附属医院
First Affiliated Hospital, Xi'an Jiaotong University Health Science Center
参　编：田　宏　西安交通大学医学部
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序　言

组织学与胚胎学是医学生学习的一门重要基础课。除了大量的理论知识外，在实验课中还要复习、记忆、讨论和理解人体组织器官的微细结构和相关功能，为以后学习病理学、生理学和病理生理学等其他医学课程打下坚实基础。

在多年教学过程中，我们发现因为组织切片不同批次和质量等问题，学生在实验课上观察到的微细结构并不能完全验证理论知识，也不能完全覆盖理论课中的重点和难点。在学校、组织学专家和同事等多方支持和鼓励下，我们从科室的切片库中精挑细选，并不断制备和补充新的组织切片，历经 4 年，终于建立了比较健全的组织学光学显微镜电子图库。本教材从图库中选择了组织学理论教学和实验观察中的重点和难点，共计 213 张（图注只显示物镜放大倍数，目镜放大倍数均为 10 倍），能更好地帮助学生学习和理解组织学理论知识和实验技术。因篇幅限制，本教材未讨论相关功能和电镜结构，这些内容可在实验教学中讨论完成。

本教材适用于生物医学、基础医学、临床医学、预防医学、法医学、口腔医学和护理专业等本科和研究生学习使用，也可作为医学研究工作者参阅使用。

衷心感谢宋天保教授通读整本教材，在图片选择、专业术语、文字内容和写作编排等方面给予的指导。感谢本科室实验技术人员李明和王丽蓉在切片制作和显微摄影中的无私帮助。感谢编委家属对本教材编写工作的理解与支持，感谢西安交通大学出版社编辑杜玄静热忱专业的出版服务。感谢西安交通大学教务处的资助（西安交通大学“十三五”规化教材），使得本书得以顺利出版。

书中难免有疏漏之处，请读者不吝批评指正。

西安交通大学医学部

周劲松

2018 年 3 月

Preface

Histology and Embryology is an important basic course for medical students. Beside the lecture knowledge, the students must go over, memorize, discuss and understand numerous microstructures and corresponding functions of tissues and organs of human body in the lab to ensure that they totally master the course knowledge, and will be prepared to study other medical courses in the future, such as Pathology, Physiology and Pathophysiology, etc.

In the past decades of teaching, we found that the microstructures observed in Histology lab can not completely demonstrate the knowledge or fully cover the important and key information learnt in Histology lectures, due to the different slide batches and quality. Thus, encouraged and supported by colleges, Histology expertise and the University, we spent about 4 years to select excellent Histology slides from department slide bank, prepared new Histology slides, and finally established a relatively intact light microscope Histology electronic atlas bank via photomicrography. This book selects 213 images from the bank (The image legend shows the objective magnification times, all the ocular magnification times are 10), which covers almost all the important and key information in Histology lecture and lab. It will greatly help students learn and understand knowledge and basic technology of Histology. Due to paper limitations, the corresponding functions and electronic microscope (EM) structures are not discussed in this book, which can be studied via discussions in lab.

This textbook is suitable for undergraduate and graduate students in specialty of Biomedicine, Basic Medicine, Clinic Medicine, Preventive Medicine, Forensic medicine, Oral Medicine and Nursing, which also can be used as references for medical researchers.

We sincerely thank Professor Tianbao Song, who critically read the entire book and gave us important suggestions on image selection, Histology terminology, text and writing pattern. We thank Ming Li and Lirong Wang, the department technicians, for their selfless help in slide preparation and photomicrography. We also extend our appreciation to family members of authors for their understanding and support for compiling work, and Xuanjing Du, the staff of Xi'an Jiaotong Universiy Press, for her editorial enthusiasm and expertise. We thank Dean's Office of Xi'an Jiaotong University who gave us financial support for the publishing the Thirteenth Five-year Plan programming textbook, Xi'an Jiaotong University.

Oversights and deficiencies may appear in the book, any corrections and suggestions are greatly appreciated.

Jinsong Zhou, PhD
Health Science Center
Xi'an Jiaotong University
March, 2018

目　录
catalogue

第一章　组织学标本制作和常用染色方法

一、组织学标本的制作

大部分组织学标本来源于人体，但也可来源于兔、猫、鼠、狗、羊和猪等动物，因其取材更加方便。取材时需要迅速精准而轻微，避免组织腐败或受损。

1. 固定：目的是将蛋白质等变性，尽量保持组织结构和成分与其存活状态时类似。大部分固定剂的溶剂是水。

2. 脱水、透明和包埋：通常用浓度梯度上升酒精等脱水剂将固定后组织中的水分完全置换，用二甲苯等透明剂将脱水剂完全置换，再用 56～60 ℃的液态石蜡等包埋剂完全置换透明剂。在室温时石蜡凝固，组织即成硬化的固态，利于切片。

3. 切片：用石蜡切片机或冷冻切片机等将组织块切成 7 μm 厚的组织切片，该厚度相当于人体细胞平均直径，能保障染料着色充分又利于观察时清晰聚焦。若组织为薄膜状，可制备组织铺片；如组织坚固，可制备组织磨片；如组织为液态，可制备涂片；必要时可将软化的组织块制成压片。

4. 裱片：用涂有黏附剂的洁净载玻片捞取温水表面漂浮的石蜡切片，或沾取冷冻切片机制备的冷冻切片。

5. 脱蜡：用二甲苯处理石蜡切片以除去石蜡，再经浓度梯度下降酒精处理入水，因染料常为水溶性。

二、常用染色方法

染色的目的是改变组织切片中不同结构的折光率，增加其光学对比性。组织学最常用的染料是苏木素和伊红，用它们来染色的方法称为苏木素-伊红（HE）染色。还可用甲苯胺蓝、银盐染色或 Wright's 染色法等。在研究组织中的化学成分时，PAS 反应、福尔根反应和四氧化锇可分别显示多糖、核酸和脂类；四唑盐法和 TUNEL 染色分别显示脱氢酶和细胞凋亡；免疫组织化学和原位杂交能分别精确地显示各类蛋白质和特定核苷酸序列的核酸片段。

1. HE 染色：苏木精（hematoxylin）是碱性化学物质，为蓝紫色染料，被苏木精着色的结构称嗜碱性结构；伊红（eosin）是酸性化学物质，为红色染料，被伊红着色的结构称嗜酸性结构；少数结构对苏木精或伊红均不敏感，称中性。

2. 过碘酸希夫（periodic acid Schiff，PAS）反应：是组织化学中显示多糖的方法之一。其原理是用过碘酸将糖分子中的 1，2 -二醇基氧化成游离二醛基，再用无色亚硫酸品红（希夫试剂）与醛基反应，从而在原位形成紫红色沉淀。

3. 福尔根反应（Feulgen reaction）：是组织化学中显示 DNA 的方法之一。其原理是用稀盐酸水解 DNA，打开嘌呤碱基与脱氧核糖之间的糖苷键，形成醛基，再与希夫试剂反应，从而在原位形成紫红色沉淀。

4. 酶组织化学染色：酶是有催化功能的蛋白质，如乳酸脱氢酶。在特定条件下，乳酸脱氢酶将底物乳酸转变为丙酮酸，释放的氢将无色的氮蓝四唑（NBT）转变为蓝色沉淀。

5. 免疫组织化学染色：在免疫组织化学染色中，将待探测的蛋白质作为抗原，利用抗原-抗体反应，最终将带有荧光或其他标记的抗体原位结合于抗原处，再显示标记，从而显示抗原。

6. 原位末端标记法（TUNEL 染色）：细胞凋亡是程序性死亡，在凋亡的过程中细胞仍然有新陈代谢，但凋亡细胞的 DNA 双链会断裂或一条链出现缺口，产生一系列 3′-OH 末端。TUNEL 染色法中，在脱

氧核糖核苷酸末端转移酶作用下，将生物素等标记的 dUTP 连接到 3′-OH 末端，再通过组化或免疫组化等方法显示生物素，形成棕色沉淀。

染色后的标本经上升酒精脱水，二甲苯透明，滴加中性树胶后，盖玻片封固，称干封法。对于溶于酒精或二甲苯的染料或不能长期保存的染色，可滴加缓冲液等湿封短期保存。干封保存时间长久，因中性树胶折光率高而成像更清晰。

注意：制备组织标本过程中，会出现因人工操作不当而造成的结构假象，如组织脱落、脱水或透明不彻底、标本有刀痕、折叠或污染、染色不均或褪色以及组织自溶等。观察组织结构时需注意辨别。

Chapter 1 Commonly used methods in tissue preparation and staining

Ⅰ. Procedure for tissue preparation

Most specimens come from human body while some are from rabbit, cat, rat, dog, goat and pig, etc, because it is more convenient to obtain specimens from animals than from human being. The specimen collection must be rapid, accurate and gentle to avoid the decay and damages to tissue.

1 **Fixation** Fixatives are introduced to denature tissue proteins and preserve tissue structures as possible as it can. Water is the solvent of most fixatives.

2 **Dehydration, clearing and embedding** Usually, the concentration gradient ascending alcohol is used to totally replace water in tissue after fixation, and the xylene thoroughly replaces the alcohol. At 56～60℃, the melted paraffin replaces xylene and the tissue block becomes solid at room temperature for sectioning.

3 **Sectioning** Usually the regular or freezing microtome is used to cut the tissue block into 7-μm-thick sections, which is the average diameter of human body cells. The section containing 1 layer of the cells guarantees better staining, and is easy to be focused under microscope. The stretched preparation for membrane tissue, ground section for hard tissue, smear for fluid tissue and tableting for softened tissue are also common section methods.

4 **Adherence** Glue-coated glass slides are used to attach the paraffin sections on the surface of warm water or the frozen sections in freezing microtome.

5 **Dewaxing** The paraffin sections are treated by xylene to remove paraffin and then by concentration gradientdescending alcohol to water, because the dyes are usually water soluble.

Ⅱ. Commonly used staining methods

The purpose of staining is to change the refractive indices of different structures in sections and increase the optical contrast. The commonly used dyes in Histology are hematoxylin (H) and eosin (E), and the staining method using H and E is called HE staining. Toluidine blue, silver salts and Wright's staining can also be introduced. When studying tissue chemical compositions, PAS reaction, Feulgen reaction and OsO_4 are used to show polysaccharides, nucleic acids and lipids, respectively. Tetrazolium and TUNEL staining are used to demonstrate dehydrogenase and cell apoptosis, respectively. Immuno histochemical staining and in situ hybridization are introduced to detect proteins and nucleic acid fragments containing specific sequence of nucleotides, respectively.

1 **Hematoxylin-eosin staining** Hematoxylin is a basic blue dye, and the structure stained by hematox-

ylin is called basophilic structure. Eosin is an acid pink dye, and the structure stained by eosin is called acidophilic structure. Some structures are known as neutrophilic structures because they are insensitive to either hematoxylin or eosin.

2 **Periodic acid Schiff (PAS) reaction** PAS reaction is designed to detect polysaccharides in histochemistry. The periodic acid is used to oxidize 1,2-glycol groups in the sugar molecules into free aldehyde groups, which then react with colorless sulphurous acid fuchsin (Schiff reagent) to form magenta deposit.

3 **Feulgen reaction** Feulgen reaction is designed to detect DNA in histochemistry. The mild hydrochloric acid is used to cleave the glycosidic bonds between purine bases and deoxyribose to form aldehyde groups, which then react with Schiff reagent to form magenta deposits.

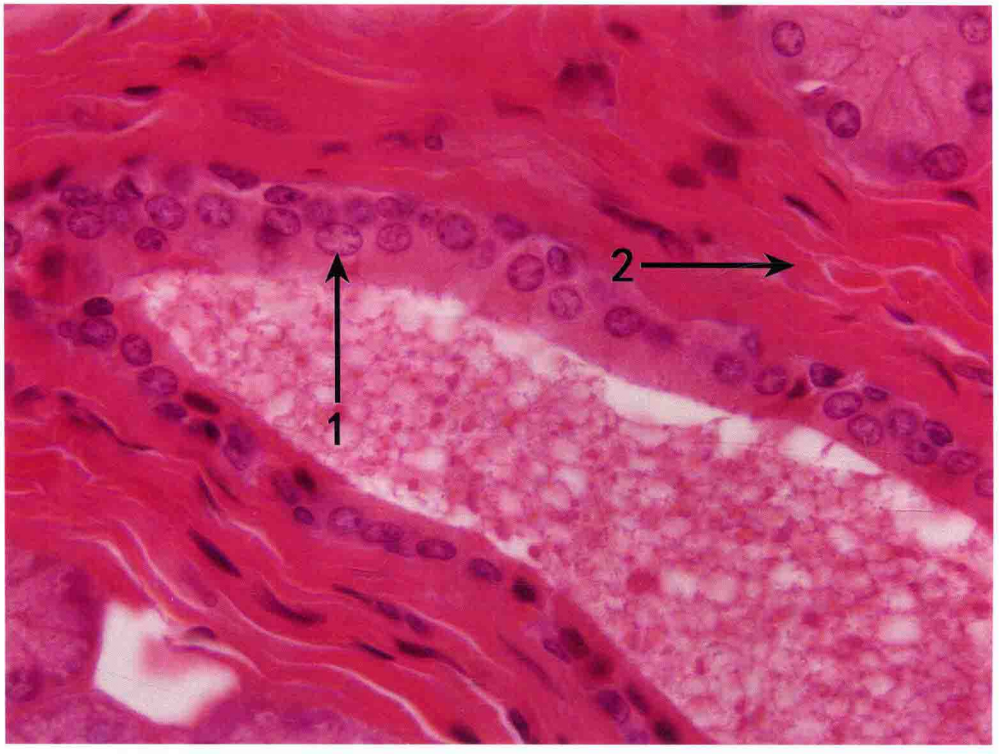
4 **Enzyme histochemistry** Enzymes are the proteins with catalytic power, such as lactate dehydrogenase (LDH). LDH catalyzes lactic acid into pyruvic acid under specific conditions and the released hydrogen combines with the nitroblue tetrazolium (NBT) to form blue deposits.

5 **Immunohistochemistry** In the immunohistochemical staining, the detected proteins are regarded as antigens, and the antibodies conjugated with fluorescence dye or other markers combine with antigens in situ, and then the markers are visualized.

6 **Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL)** Apoptosis is programmed cell death, during which the cells still metabolize. While cells undergo apoptosis, the DNA double-strands fracture or a single-strand breaks, resulting in a series of 3′-OH ends. With TUNEL staining, biotin labeled-dUTP are linked to 3′-OH ends by terminal deoxyribonucleotidyl transferase and then visualized by histochemical or immunohistochemical method.

Reservation: In the dry reservation, the sections undergo dehydration by ascending alcohol and clearance by xylene, and then are immersed in neutral balsam and covered by cover slips. In some cases, the final color can not tolerate alcohol or xylene, or the color fades quickly, so the sections have to be immersed in buffer for wet reservation for short term. The dry reservation keeps sections more clearly for a long time because the neutral balsam is highly refractive.

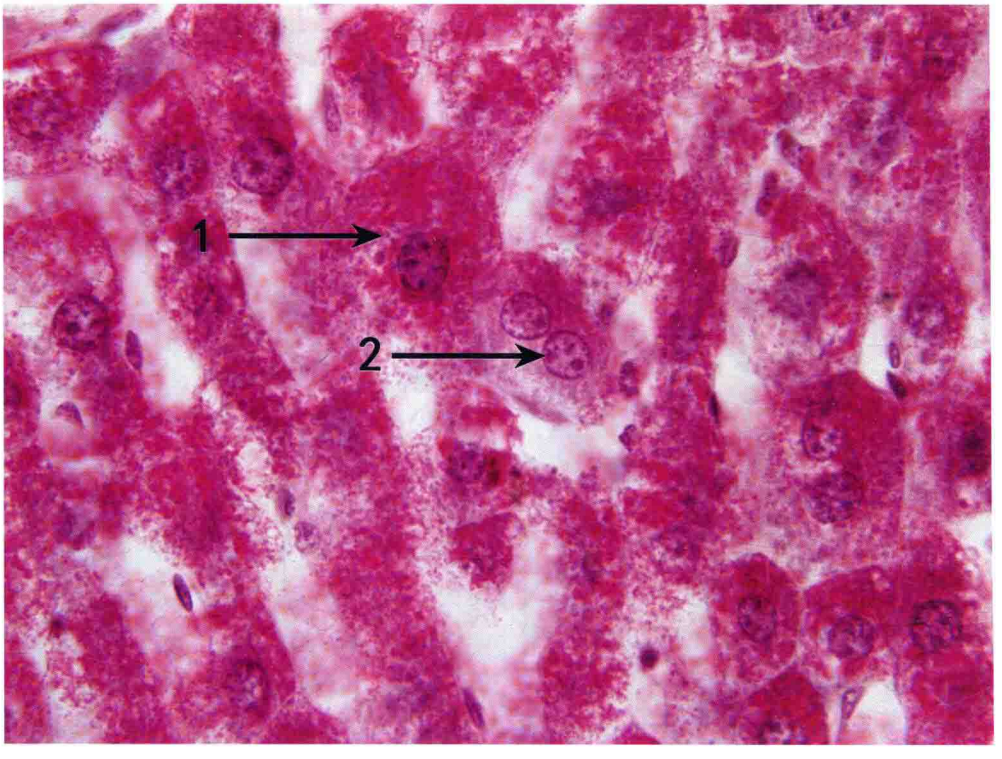
Artifacts may occur during tissue preparation, such as tissue shedding from the slide, incomplete dehydration or clearance, knife scratch, folding or contamination in tissue sections, nonuniform staining or color fading, and tissue autolysis, etc. These artifacts must be distinguished from normal structures.



1. 嗜碱性结构(basophilic structure) 2. 嗜酸性结构(acidophilic structure)

图 1-1 下颌下腺:人(HE 染色,×100)

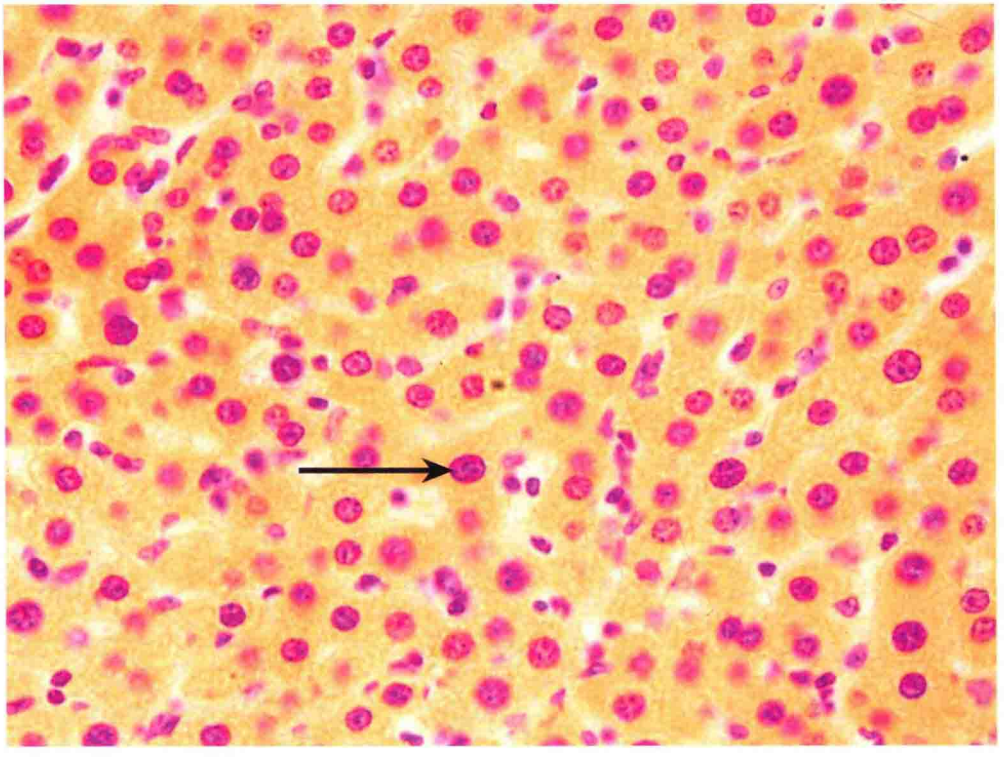
Pic. 1-1 Submandibular gland: Human. HE staining ×100



1. 胞质糖原(glycogens in cytoplasm) 2. 肝细胞核(nucleus of a hepatocyte)

图 1-2 肝脏:人(PAS 反应,×100)

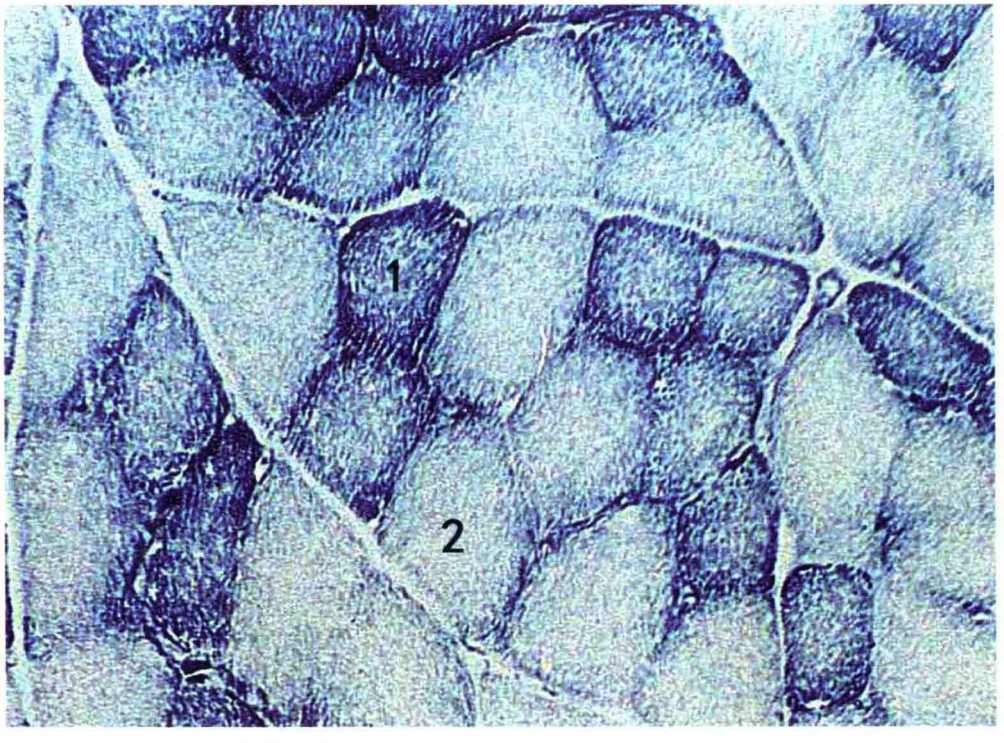
Pic. 1-2 Liver: Human. PAS reaction ×100



箭头示细胞核中的 DNA。The arrow shows DNA in nucleus.

图 1－3　肝脏：人（Feulgen 反应，×20）

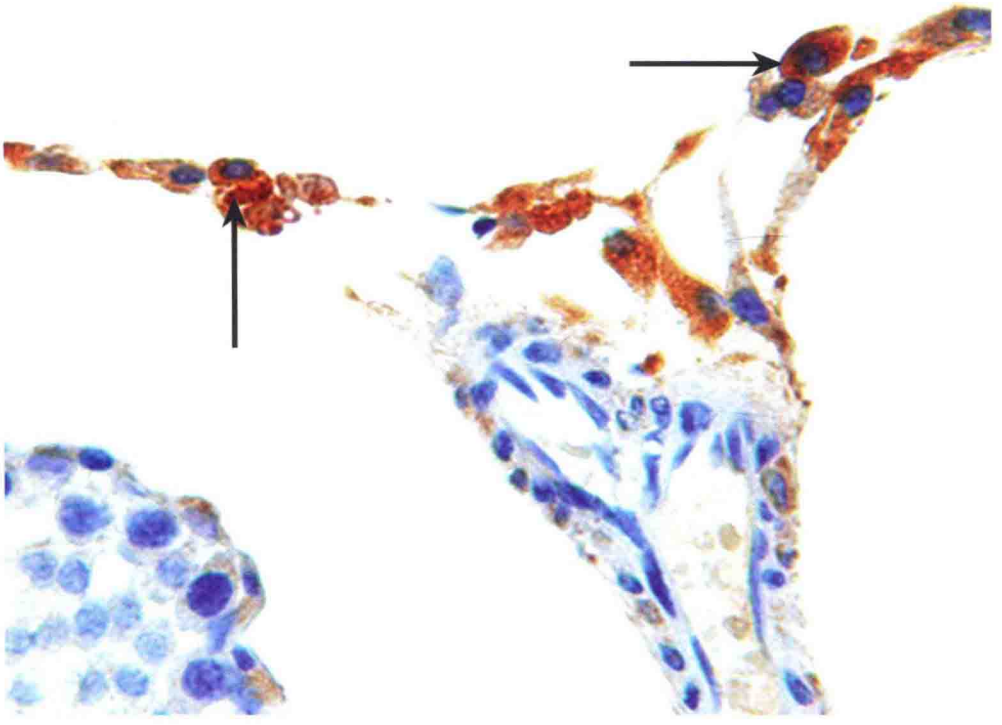
Pic. 1－3　Liver: Human. Feulgen reaction　×20



1. 阳性纤维（positive fiber）　2. 阴性纤维（negative fiber）

图 1－4　骨骼肌：人（乳酸脱氢酶组织化学染色，×40）

Pic. 1－4　Skeletal muscle: Human. Lactic dehydrogenase histochemical staining　×40

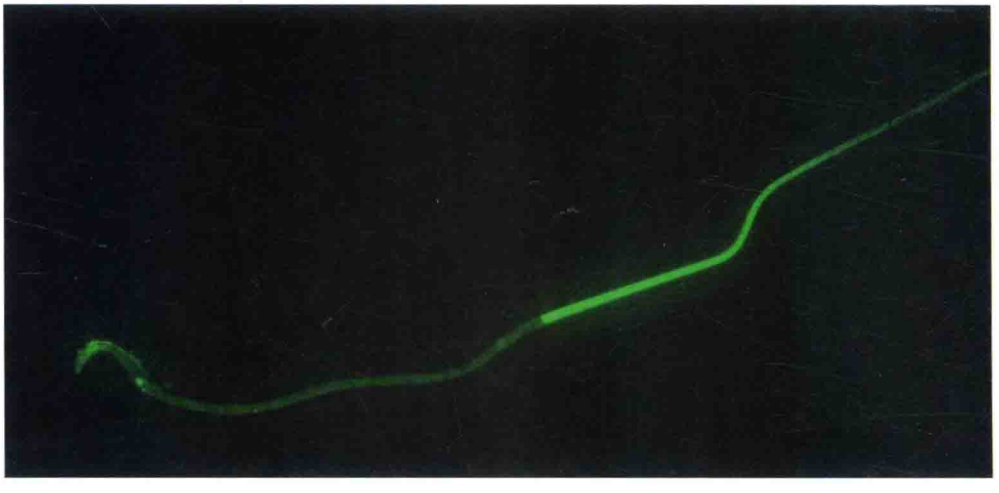


箭头示间质细胞棕色胞质含 P 物质样物质。

The brown Leydig cell cytoplasm marked by arrows indicates that there are Substance P-like substance.

图 1-5　睾丸：大鼠（免疫酶染色，DAB 显色，×40）

Pic. 1-5　Testis: Rat. Immunoenzyme staining, DAB visualization　×40

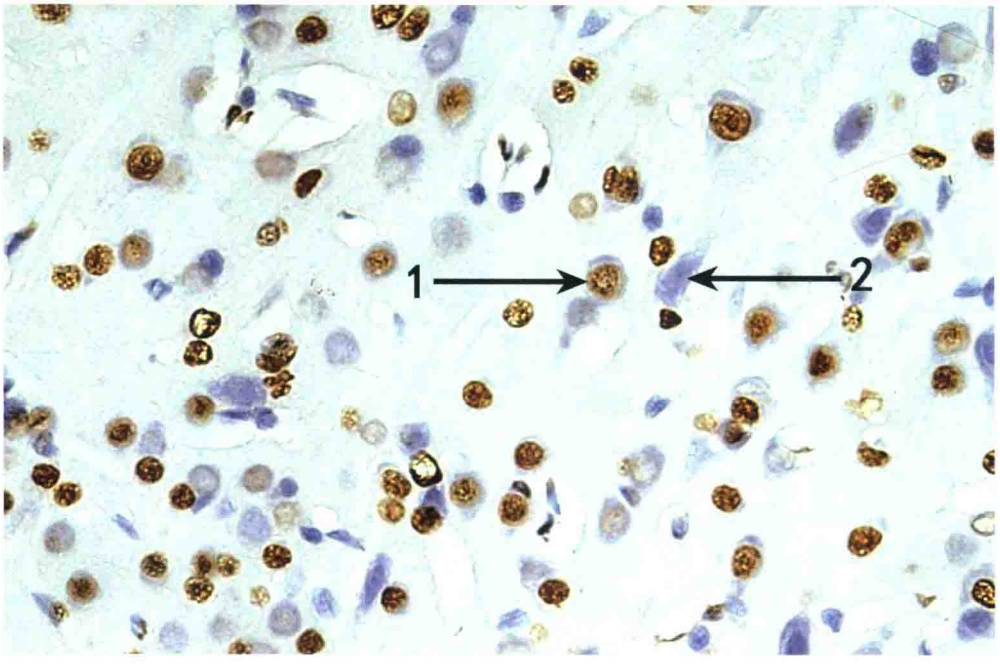


精子尾部亮绿色示其含精子相关抗原样物质。

The bright green color of the tail shows that it contains Sperm associated antigen11C-like substance.

图 1-6　精子：小鼠（免疫荧光染色，×40）

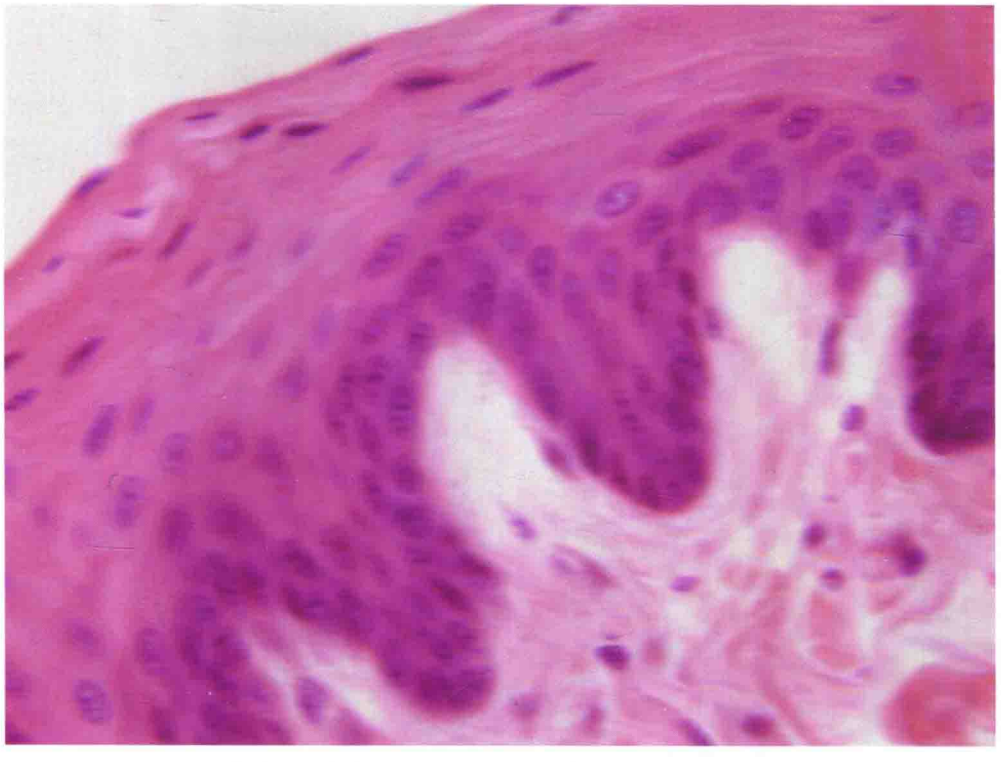
Pic. 1-6　Spermato zoon: Mouse. Immunofluorescence staining　×40



1. 凋亡细胞(apoptotic cell) 2. 正常细胞(normal cell)

图 1-7　脑:兔(TUNEL 染色,×40)

Pic. 1-7　Brain: Rabbit. TUNEL staining　×40

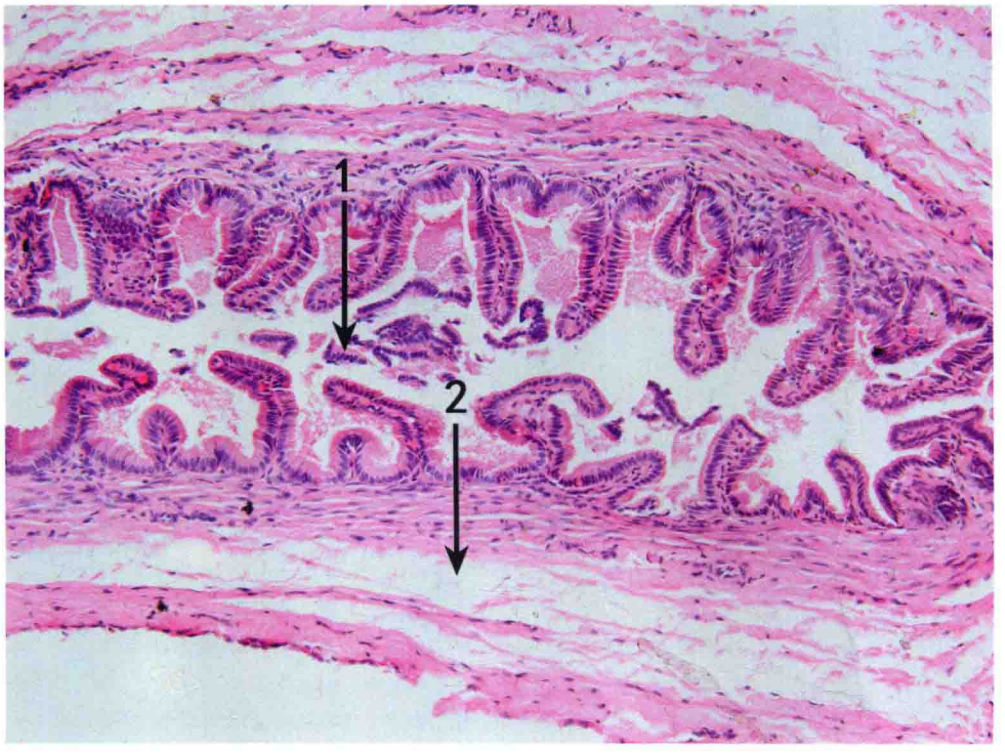


结构模糊提示染色后封存时脱水透明不彻底。此为人工假象。

The obscure structure suggests that the dehydration and clearance are not thorough after staining. It is an artifact.

图 1-8　舌:猫(HE 染色,×40)

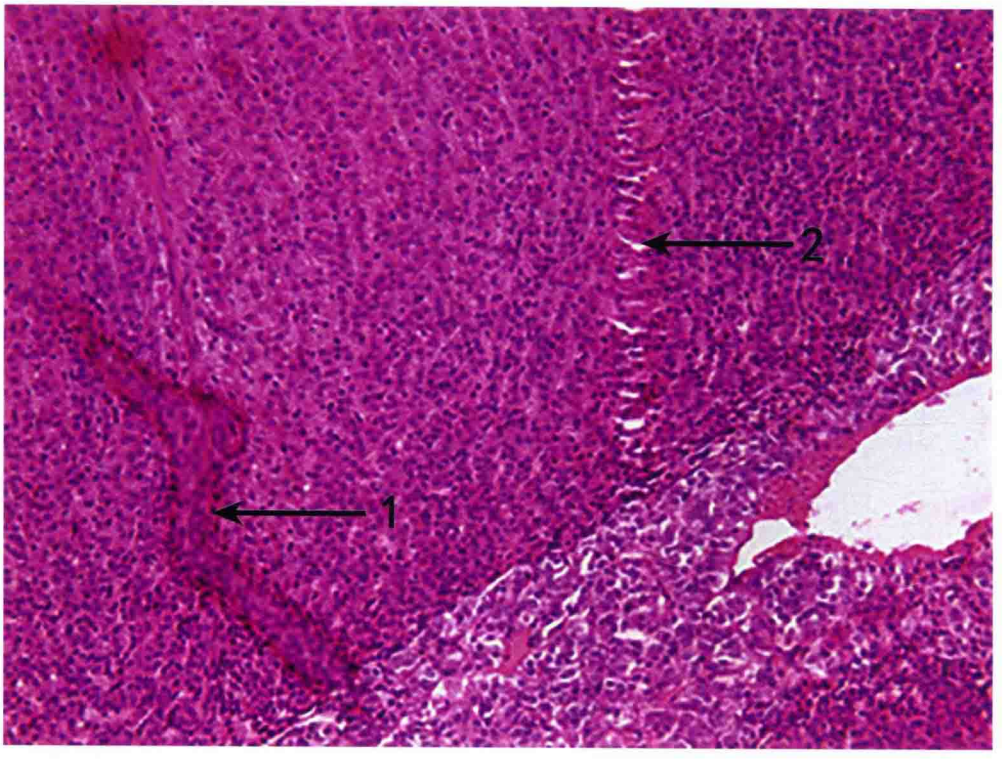
Pic. 1-8　Tongue: Cat. HE staining　×40



1. 脱落结构(shedding structure) 2. 组织制备造成的裂隙(tissue fissure caused by sample preparation) 均为人工假象。These are artifacts.

图 1-9 胆囊:人(HE 染色,×20)

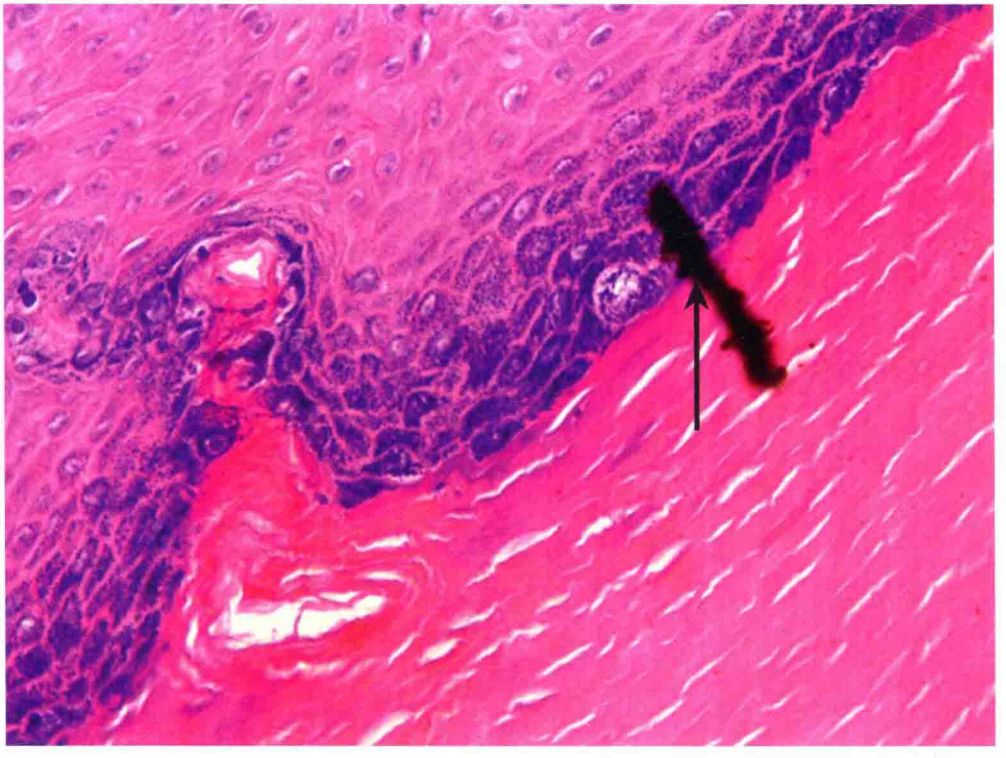
Pic. 1-9 Gall bladder: Human. HE staining ×20



1. 组织折叠(tissue folding) 2. 组织划痕(knife scratch) 均为人工假象。These are artifacts.

图 1-10 肾上腺:兔(HE 染色,×40)

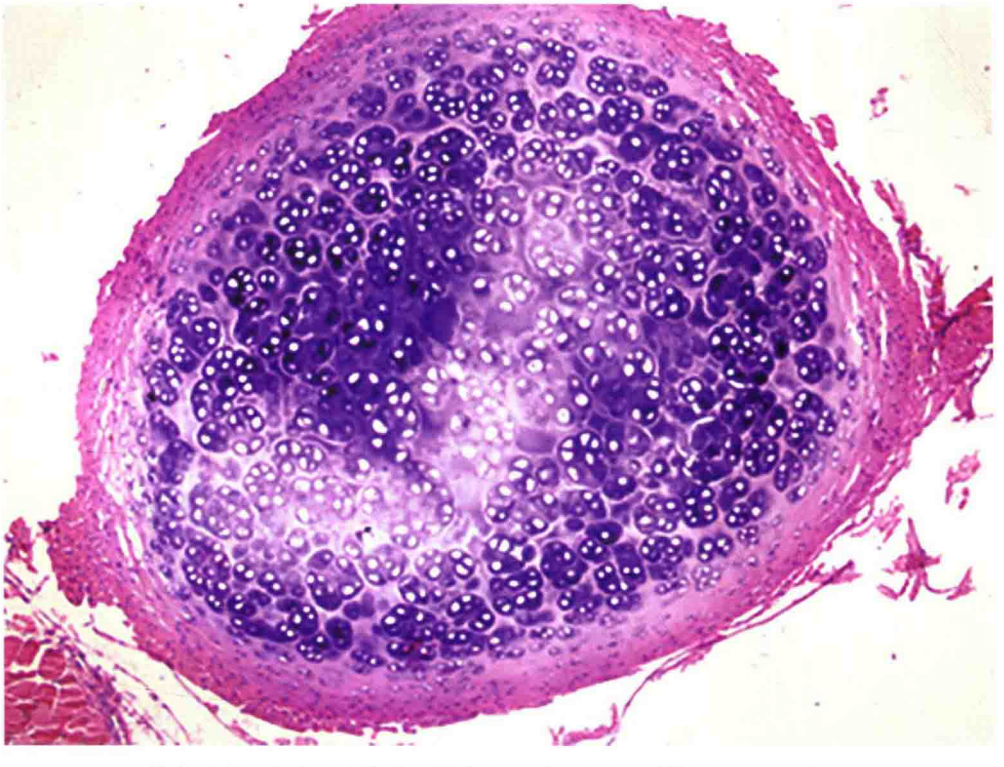
Pic. 1-10 Adrenal gland: Rabbit. HE staining ×40



箭头示污染物。此为人工假象。The arrow shows the contaminant. This is an artifact.

图 1－11　指皮：人(HE 染色，×40)

Pic. 1－11　Finger skin: Human. HE staining　×40



染色不均。此为人工假象。Unbalanced staining. This is an artifact.

图 1－12　肋软骨：兔(HE 染色，×10)

Pic. 1－12　Costal cartilage: Rabbit. HE staining　×10

第二章　上皮组织

实验基本要求：

1. 了解腺体的分类。多细胞外分泌腺的结构和分类。

2. 熟悉蛋白质分泌细胞、糖蛋白分泌细胞和类固醇分泌细胞的光学显微镜(LM)和电镜(EM)结构特点。

3. 掌握上皮组织的分类和一般特点。单层扁平上皮、单层立方上皮、单层柱状上皮、假复层纤毛柱状上皮、复层扁平上皮和变移上皮的结构、主要分布和功能。

4. 掌握上皮组织各种特殊结构的 LM 和 EM 结构和功能：微绒毛、纤毛、紧密连接、中间连接、桥粒、缝隙连接、连接复合体、基膜、质膜内褶和半桥粒。

5. 光镜下识别单层扁平上皮、单层立方上皮、单层柱状上皮、假复层纤毛柱状上皮、复层扁平上皮和变移上皮。

6. 相关超微结构和功能可通过课堂讨论等完成。

Chapter 2　Epithelial tissue

Basic requirements for the experiment

1. Know the classifications of gland, the structures and classifications of multi-cell exocrine glands.

2. Familiar with the structural characteristics of protein, glycoprotein and steroid-secreting cell under LM and EM.

3. Familiar with the classifications and general features of the epithelial tissues. The structures, distributions and functions of the simple squamous epithelium, simple cuboidal epithelium, simple columnar epithelium, pseudostratified ciliated columnar epithelium, stratified squamous epithelium and transitional epithelium.

4. Master the structures and functions of epithelial specializations: microvillus, cilium, tight junction, intermediate junction, desmosome, gap junction, junctional complex, basement membrane, plasma membrane infolding, hemidesmosome under LM and EM.

5. Identify simple squamous epithelium, simple cuboidal epithelium, simple columnar epithelium, pseudostratified ciliated columnar epithelium, stratified squamous epithelium and transitional epithelium under LM.

6. The corresponding EM structures and functions will be discussed in lab.