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

A joint publication of the RITA*) and NACAD**) groups

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With 65 figures

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*) RITA: Registry of Industrial Toxicology Animal-data. Members: Abbott GmbH & Co KG, Ludwigshafen, Germany; ALTANA Pharma AG, Hamburg, Germany; AstraZeneca, Södertälje, Sweden and Macclesfield, England; Aventis Pharma Deutschland GmbH, Hattersheim, Germany; BASF AG, Ludwigshafen, Germany; Bayer HealthCare AG, Wuppertal, Germany; Boehringer Ingelheim Pharma GmbH & Co KG, Biberach, Germany; Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany; Hoffman-LaRoche AG, Basel, Switzerland; Merck KGaA, Darmstadt, Germany; Novartis Pharma AG, Basel, Switzerland; Pfizer, Amboise, France; Pharmacia, Nerviano, Italy; Syngenta CTL, Macclesfield, England

**) NACAD: North American Control Animal Database. Members: 3M Pharmaceuticals, St. Paul, MN, USA; Adolor Corporation, Malvern, PA, USA, Bayer CropScience, Stillwell, KS, USA; Pfizer, Inc., Groton, CT, USA; Pfizer, Inc., Ann Arbor, MI, USA; Pharmacia, Inc., Kalamazoo, MI, USA; R.W. Johnson Pharmaceutical Research Institute, Spring House, PA, USA; Schering-Plough Research Institute, Lafayette, NJ, USA

Summary

This is the second part of a series of three articles on trimming instructions of rat and mouse protocol organs and tissues in regulatory type toxicity studies, covering the respiratory, male and female genital, and the endocrine systems. The article is based on the experience of the European RITA and American NACAD working groups and is an extended revision of trimming guides published in 1995 (Bahnemann et al.). The optimum localization for tissue preparation, the sample size, the direction of sectioning and the number of sections to be prepared is described organ by organ. These descriptions are illustrated for each organ by a schematic drawing and/or a macro-photograph showing the plane of section as well as a low magnification of the H&E stained slide demonstrating the optimum "end-product".

The objectives of this work, as addressed in detail in the first part (Ruehl-Fehlert et al. 2003), are to stan-

dardize tissue sampling and trimming, to improve the comparability of historical data obtained from different studies and different laboratories, ensure the presence of all relevant target sites for histopathological evaluation and provide technical advice for preparatory techniques during necropsy, fixation and trimming.

Brief introduction to the use of the individual organ guides

For each organ the following information is usually presented (for more details see part 1: RUEHL-FEHLERT et al. 2003):

1. **Localization:** anatomical site or part of an organ from which a sample should be taken (i.e. lobe).
2. **Number of samples:** number of organs (i.e. both for bilateral organs) or organ pieces prepared for evaluation (not necessarily identical with the number of slides/blocks).
3. **Direction:** direction (plane of section) in which an organ should be cut at trimming or microtome sectioning. The proposed direction is shown in green color and optional sections (if defined) are shown in blue (see fig. 1 for an explanation of the symbols used).
4. **Sample size:** the size (area) of an organ or part of an organ which is sampled in a cassette for processing. The sample size is determined by the size of an organ or the cassette. For optimal fixation, sample thickness should not exceed 3–5 mm. In general, the examined area should be as large as possible and should contain the relevant anatomical structures. The tissue can be adapted to the size of cassettes by trimming the margins off.
5. Optional **remarks** are used to present additional information, as recommended by the RITA/NACAD groups, such as the instillation of fixative into the lung or the urinary bladder, reasons for optional recommended sections, placements of organs in cassettes, etc.
6. Schematic **drawings** and/or **gross photographs** are shown indicating the plane of section. Some of the gross photographs show the organ and trimming direction *in situ*. However, this is just for orientation purposes and it is recommended to remove the organ or tissue first. Trimming is performed as the next step, either on the fresh wet tissue or, in most cases, after fixation of the organ. Most of the gross photographs were taken from fresh unfixed organs; shape and color may be slightly different after fixation.
7. An image of a **Hematoxylin and Eosin (H&E) stained slide** is shown for the recommended section level (sometimes also for optional levels). Typical structures included in this section are indicated as necessary. If not otherwise specified, 10% buffered formalin is recommended as the **fixative.**

In the descriptions the following terms are used for the determination of the trimming directions (see also fig. 2 with a schematic presentation of the related cut levels):

- **transverse:** perpendicular to the long axis of an organ or part of an organ
- **longitudinal vertical:** in the direction of the long axis of the body, an organ or part of an organ in the dorsoventral axis or parallel to it (in the text also referred to in short as "longitudinal")
- **longitudinal horizontal:** in the direction of the long axis of the body, an organ or part of an organ, perpendicular to the dorsoventral axis (in the text also referred to in short as "horizontal")

By defining either the "body", the "whole organ" or a "part of an organ" (for example a liver lobe or a certain part of the brain), as a unit of reference, it is relatively simple to precisely characterize a trimming direction by using only the three above defined terms and avoiding therefore the vast amount of anatomical terms and confusing synonyms present in literature.

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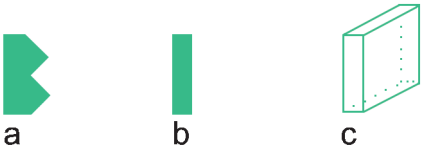

Fig. 1. Symbols used in the drawings and/or gross photographs to indicate the plane of section. **a:** cutting level parallel to the plane of the picture, **b:** cutting level perpendicular to the plane of the picture, **c:** cut level, 3-D.

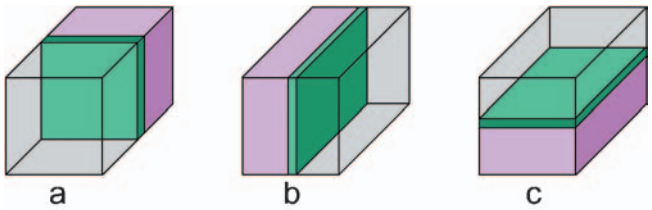

Fig. 2. Schematic presentation of the plane of section. **a:** transverse, **b:** longitudinal vertical, **c:** longitudinal horizontal.

3 Respiratory system

3.1 Nasal cavity, Nasopharynx and Paranasal sinus

Localizations:	1) Posterior part of upper incisors 2) Incisive papilla 3) Second palatine crest 4) First molar teeth
Number of sections:	1 (oral toxicity study: third level) 4 (inhalation study)
Direction:	Transverse
Remarks:	Embedded with the rostral faces down Decalcified

The structures of the palate and the teeth are used for orientation to achieve transverse sections through the nasal cavity at certain levels.

In inhalation studies, four transverse tissue levels should be taken, because the examination of these sections at defined levels assures consistent recognition of degenerative and proliferative lesions of all different epithelial cell types of the nasal cavity and paranasal sinus. Typically in oral toxicity studies, only the third level is examined. Neoplastic lesions occur more frequently in the anterior and middle portions of the nasal cavity, whereas some non-neoplastic and neoplastic lesions are observed exclusively in the olfactory epithelium. The third level includes respiratory and olfactory epithelial cells. The resulting slices of tissue are embedded with the rostral face down, because non-neoplastic lesions have been found to be most severe at the more rostral borders of the affected epithelium. Slight differences of the cut level may occur depending on anatomical variations in different strains.

If more squamous epithelium is required for examination, a section rostral to level 1 should be performed. For examination of the olfactory bulb, a section caudal to level 4 is recommended.

Relevant differences between rats and mice

Mice should be trimmed in the same manner as rats. However, in inhalation studies with very young or very small mice (e.g., transgenic strains), it can be difficult to cut the nose in four levels. For those exceptions, the following three level-procedure is recommended:

1) Immediately posterior to the incisors,
2) At the level of the incisive papilla,
3) Through the middle of the second molar tooth.

For non-inhalation studies level 3 is recommended for examination.

Related references

Brown 1990, Hardisty et al. 1999, Harkema 1990, Harkema and Morgan 1996, Morgan 1991, Popp and Monteiro-Riviere 1985, Uraih and Maronpot 1990, Young 1981

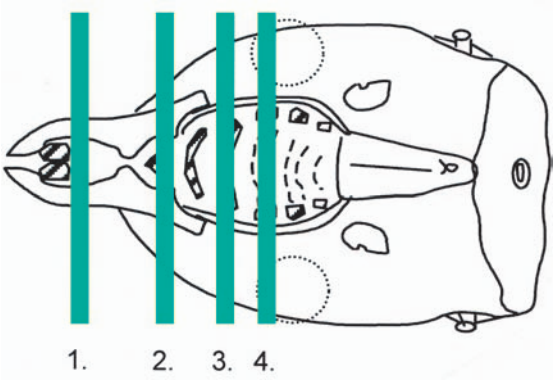

Fig. 3.1a. Nasal cavity, rat, 4 trimming locations.

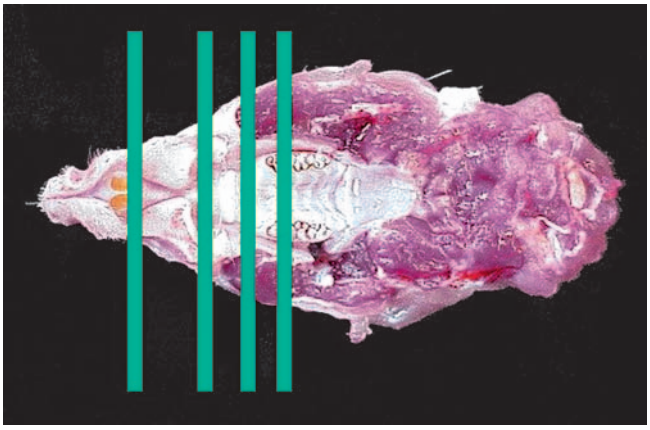

Fig. 3.1b. Nasal cavity, rat, 4 trimming locations.

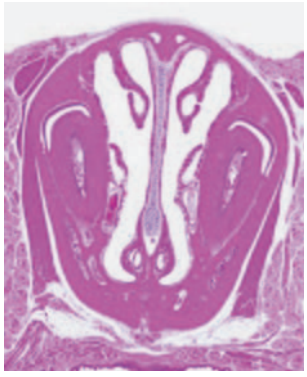

Fig. 3.1c. Nasal cavity, rat, location 1.

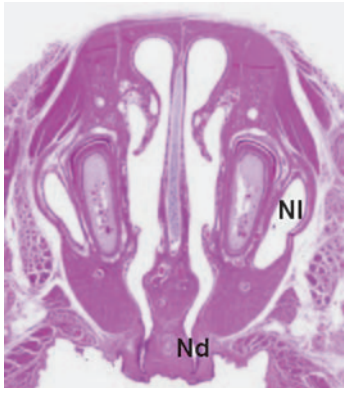

Fig. 3.1d. Nasal cavity, rat, location 2. Nd: nasopalatine (incisive) duct, Nl: Nasolacrimal duct.

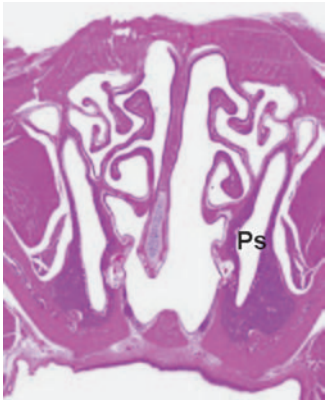

Fig. 3.1e. Nasal cavity, rat, location 3. Ps: paranasal sinus.

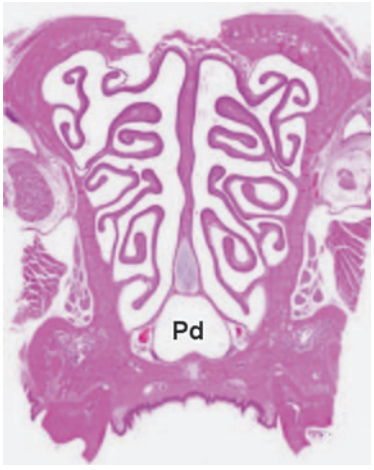

Fig. 3.1f. Nasal cavity, rat, location 4. Pd: pharyngeal duct (nasopharynx).

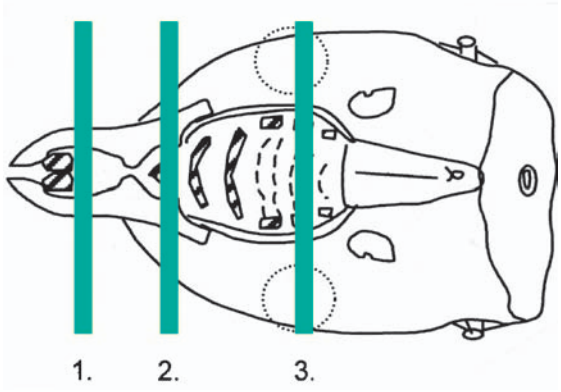

Fig. 3.1g. Nasal cavity, mouse, 3 trimming locations.

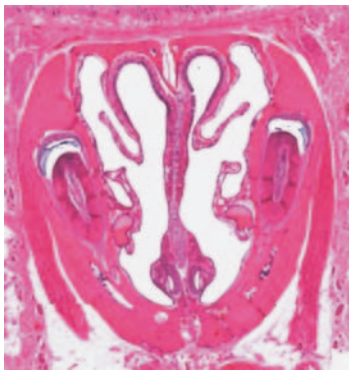

Fig. 3.1h. Nasal cavity, mouse, location 1.

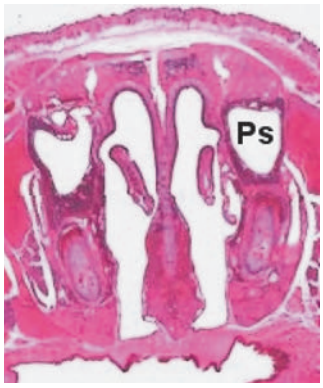

Fig. 3.1i. Nasal cavity, mouse, location 2. Ps: paranasal sinus.

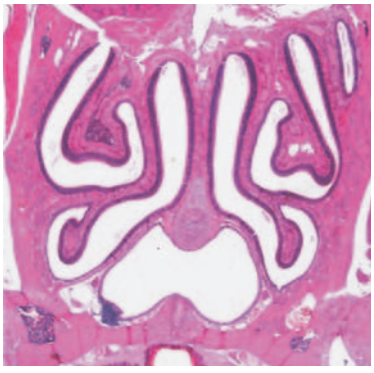

Fig. 3.1j. Nasal cavity, mouse, location 3.

3 Respiratory system
3.2 Larynx

Localizations:	1) Base of epiglottis 2) Ventral pouch 3) Cricoid cartilage (rats only)
Number of sections:	Inhalation studies: rats 3, mouse 2 *Optional for rats and mice:* if necessary, the larynx can also be embedded in one block and step sections are taken at the predilection sites. Oral studies: 1
Direction:	Transverse
Remarks:	Since the larynx of mice is very small, only two pieces (level 1 and 2) are trimmed. For oral studies the organ is cut at level 2 to include the most sensitive parts: ventral pouch and vocal processes (medial surfaces) of arytenoid cartilages.

Only the cranial portion of the epiglottis is removed to ensure inclusion of the major predilection site for induced lesions. This site is primarily represented by the epithelial lining of the ventral and ventrolateral luminal surface of the larynx (cranial to the ventral laryngeal pouch). The remaining larynx is trimmed according to the proposed scheme at three levels including base of epiglottis, ventral diverticulum and cricoid cartilage. The three pieces are embedded with the cranial cut surface downwards. The three levels assure recognition of all different epithelial cell types of the larynx and underlying seromucinous glands.

See also:
Thyroid gland

Related references
Lewis 1991, Renne et al. 1992, Sagartz et al. 1992

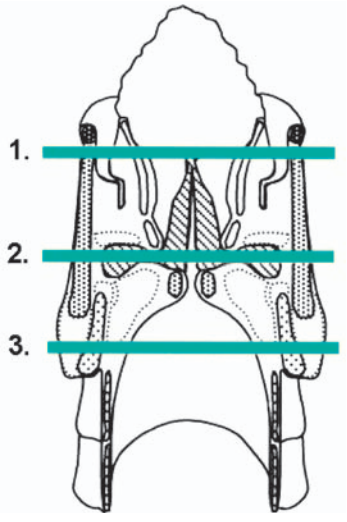

Fig. 3.2a. Larynx, inhalation studies. Rats: levels 1–3, mice: levels 1 and 2.

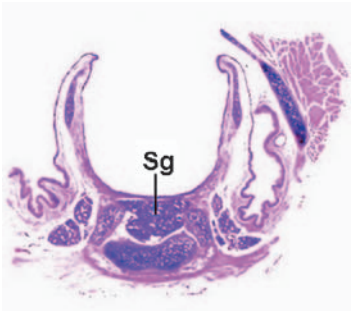

Fig. 3.2b. Larynx, level 1. Sg: seromucinous glands at the base of the epiglottis.

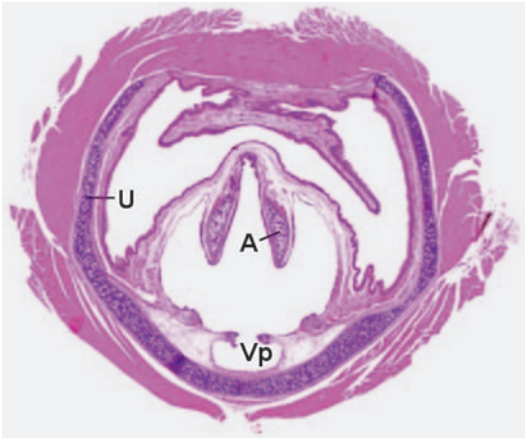

Fig. 3.2c. Larynx, level 2. Vp: ventral pouch, A: processes of the arytenoid cartilages, U: u-shaped cartilage.

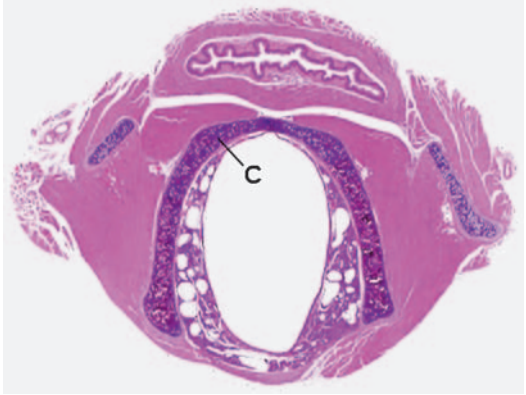

Fig. 3.2d. Larynx, level 3. C: cricoid cartilage.

3 Respiratory system
3.3 Trachea (inhalation study)

Localization:	Including the bifurcation
Number of sections:	1 (2)
Direction:	Longitudinal horizontal *Optional:* transverse
Remarks:	Embedded *in toto*; careful microtome sectioning until recommended cutting level is obtained.

In inhalation studies, tracheal epithelium including the epithelial lining of the bifurcation should be examined, because this is known as the most sensitive area to respond to inhaled particulate irritants. For this purpose, a longitudinal horizontal section should provide a long distance of the epithelial surface and the tip of the carina. For optimal estimation of e.g. mild hyperplasia, it can be helpful to have an optional transverse section (see alternative trimming technique for oral toxicity study under thyroid gland/trachea/parathyroid gland).

Related references
GOPINATH et al. 1987, SCHWARTZ et al. 1991

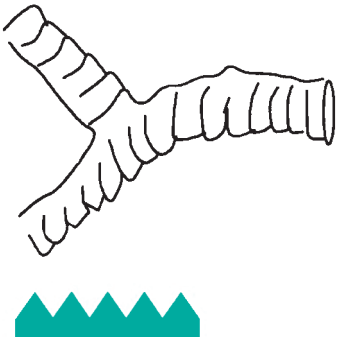

Fig. 3.3a. Trachea with bifurcation.

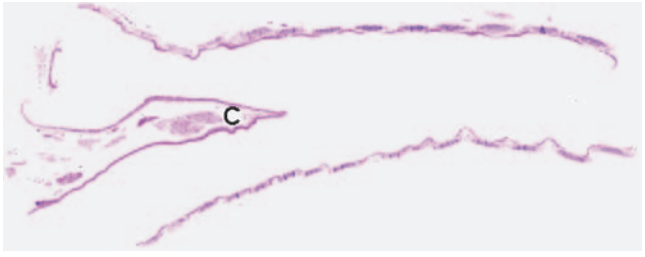

Fig. 3.3b. Trachea with bifurcation, C: carina.

3.4 Lung

Oral study: Rats and mice

Localizations:	Recommended procedure: 1) Left lobe 2) *Optional:* right caudal lobe 3) *Optional:* right cranial lobe
Number of sections:	1 (3)
Direction:	Longitudinal horizontal *Optional:* transverse
Remarks:	Instillation strongly recommended. Sectioning to the axis of the lobar bronchus. Longitudinal section comprising the lobar bronchus and its main branches. Sample size(s) adapted to the size of the cassette(s). Alternative procedure: Rat: right lobes embedded ventral surface down. Mouse: whole lung embedded, ventral surface down.

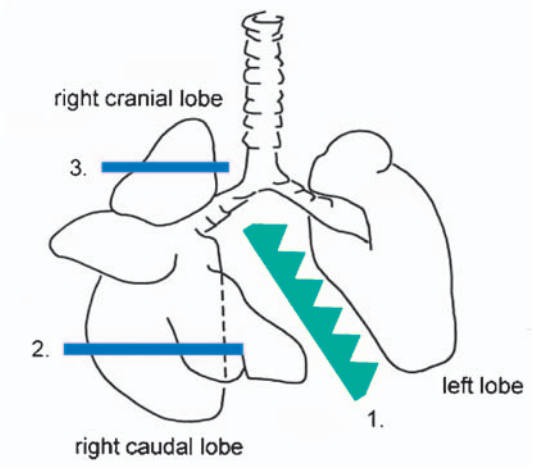

Fig. 3.4a. Lung, ventral aspect, oral study.

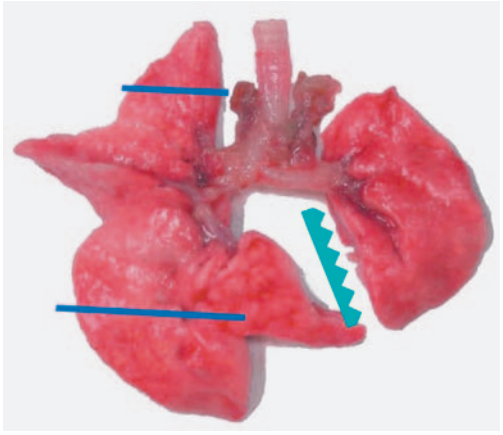

Fig. 3.4b. Lung, ventral aspect, oral study.

Inhalation study: Rats

Localizations:	1) Left lobe 2) Right caudal lobe 3) Right cranial lobe 4) Right middle lobe 5) Accessory lobe
Number of sections:	5
Direction:	Sections 1, 2: longitudinal horizontal Sections 3, 5: transverse Section 4: longitudinal vertical
Remarks:	Instillation obligatory. Longitudinal horizontal section comprising the lobar bronchus and its main branches. Sample size(s) adapted to the size of the cassette(s); preferentially, the diaphragmatic margin is trimmed off. Alternative procedure: right and left lobes (separate blocks) embedded ventral surface down.

Inhalation study: Mice

Localizations:	1) Left lobe 2) Right caudal lobe 3) Right cranial lobe 4) Right middle lobe 5) Accessory lobe
Number of sections:	5
Direction:	Sections 1, 2, 4, 5: longitudinal horizontal Section 3: transverse
Remarks:	Instillation obligatory. Similar procedure as in rats, but lobes are embedded *in toto*, ventral surface down and detached from the trachea. The five lobes normally fit into one cassette. *Option:* whole lung *in toto* (ventral surface down) without removal of the trachea. Microtome sectioning of left lobe and right caudal lobe until lobar bronchus and its main branches are visible (longitudinal-horizontal axis).

Spontaneous neoplastic pulmonary lesions are rare in rats and arise mostly in the lung periphery whereas regenerative hyperplasia and squamous metaplasia occur mainly in the centroacinar region. Therefore tissue of the lung including parenchyma, bronchiolo-alveolar junctions and main bronchi should be investigated. In oral toxicity studies, at minimum, one longitudinal section of the left lobe should be examined. Additionally, transverse sections of the right cranial and caudal lobes may be examined. In these sections, the epithelium of the major bronchioles, which is one important site of lesions,

can be examined at its widest diameter. In inhalation studies, sections of all five lobes should be examined according to the proposed scheme, which facilitates unambiguous identification of individual lung lobes. For histological identification of proliferative lesions in the lung, careful fixation by intratracheal instillation is recommended, even for oral studies.

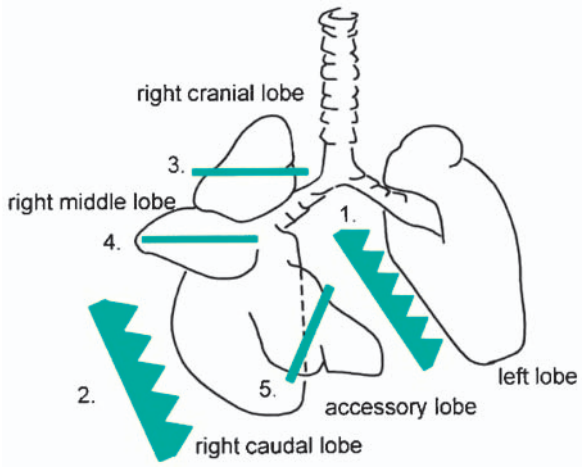

Fig. 3.4c. Lung, rat, ventral aspect, inhalation study.

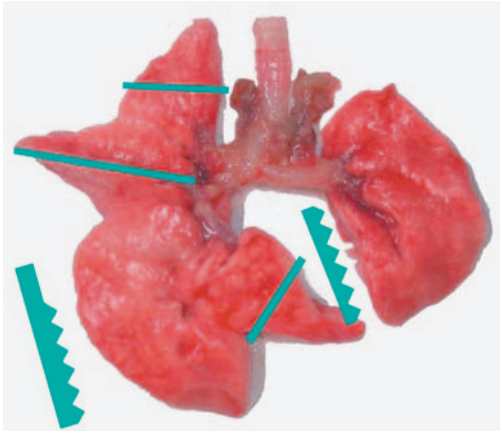

Fig. 3.4d. Lung, rat, ventral aspect, inhalation study.

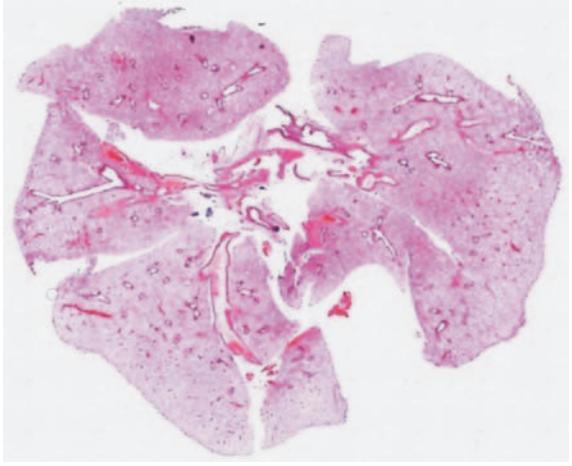

Fig. 3.4e. Lung, mouse, in toto (option).

Related references

DUNGWORTH et al. 1992, GOPINATH et al. 1987, PLOPPER 1996, RENNE et al. 2001, RITTINGHAUSEN et al. 1992, SCHWARTZ et al. 1991, SMINIA et al. 1990

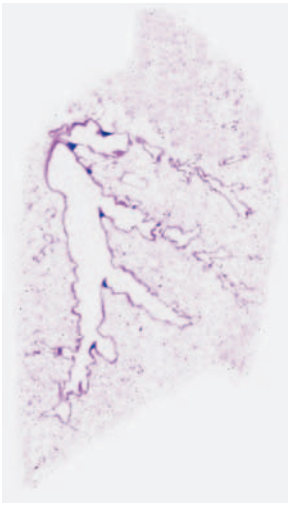

Fig. 3.4f. Lung, rat, location 1, left lobe.

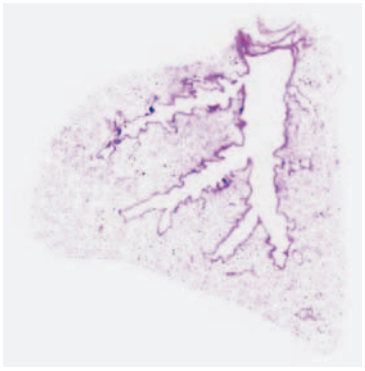

Fig. 3.4g. Lung, rat, location 2, right caudal lobe.

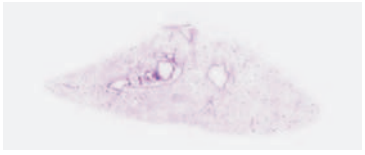

Fig. 3.4h. Lung, rat, location 3, right cranial lobe.

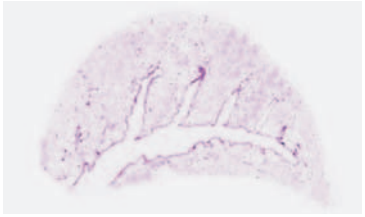

Fig. 3.4i. Lung, rat, location 4, right middle lobe.

Fig. 3.4j. Lung, rat location 5, accessory lobe.

4 Male genital system

4.1 Testis and Rete testis

Localization:	Transverse, close to the rete testis
Number of sections:	2 (1 per side)
Direction:	Transverse
Remarks:	A transverse section containing the area of the rete testis provides good histology of seminiferous tubules as well as of the rete testis. *Optional:* longitudinal vertical section containing the rete testis.

Near the capsule, focal tubules with flat or incomplete epithelium can be found. These should not be confused with atrophic or degenerating tubules, as they represent tubules of the rete testis. Generally, the rete testis is very small in rodents and not well visible in the histological section. In mice it is slightly more prominent than in rats and hyperplasia of the rete testis can be observed in older animals. For inclusion of the rete testis in the histological section orientation is given by the vasculature.

In short-term studies, fixation with Davidson's or Bouin's solutions is highly recommended to detect less extensive toxicity.

Leydig cells (interstitial cells) are present in small groups in the interstitium between the seminiferous tubules. They are found in a similar distribution in all sections proposed.

Related references

Boorman et al. 1990a, Chapin and Heindel 1993, Creasy and Foster 2002, Ferm 1987, Foley 2001, Lanning et al. 2002, Latendresse et al. 2002, Russell et al. 1990

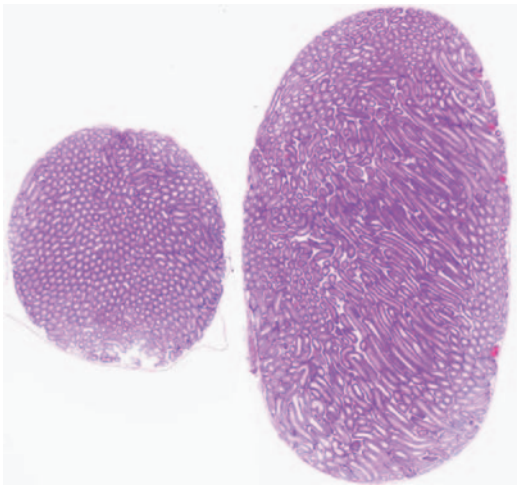

Fig. 4.1b. Testis, transverse section left, optional longitudinal section right.

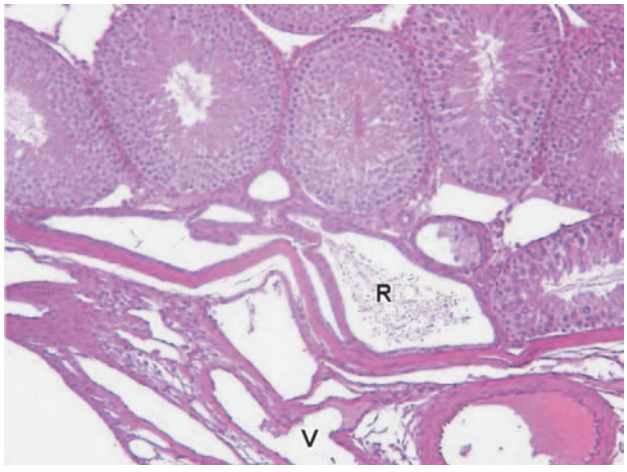

Fig. 4.1c. Rete testis (R), rat (V: vasculature).

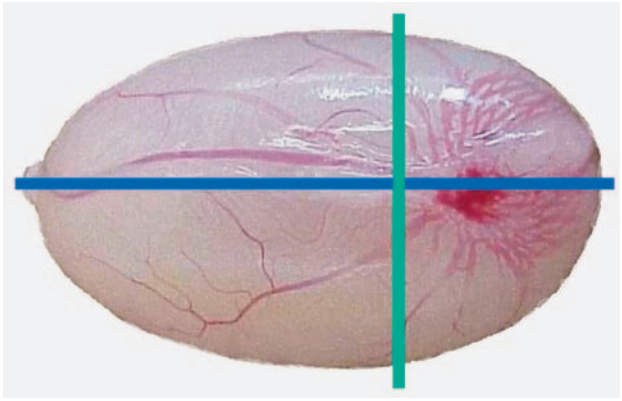

Fig. 4.1a. Testis, recommended transverse section (green), optional section (blue).

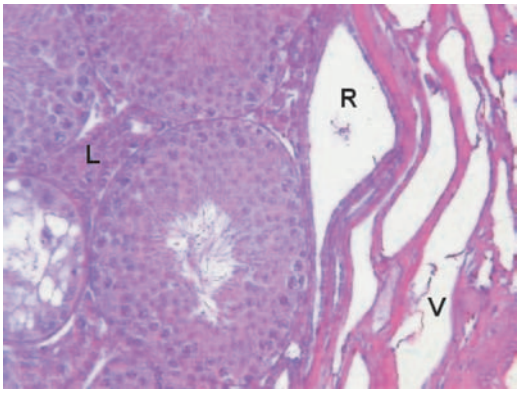

Fig. 4.1d. Rete testis (R), mouse (V: vasculature, L: Leydig cells).

4 Male genital system
4.2 Epididymis

Number of sections: 2 (1 per side)
Direction: Longitudinal vertical
Sample size: Whole organ

It appears that the epithelium in the body of the organ is sometimes more sensitive than in the head and tail. Therefore, the body should not be excluded from the investigation. The whole organ should be fixed and embedded. It should be noted that some toxicants affect in particular the efferent ducts, which are located between testis and epididymal head. Care should be taken that these structures are not destroyed during preparation.

Related references
Boorman et al. 1990a, Cardy 1987, Ferm 1987, Hess 1998

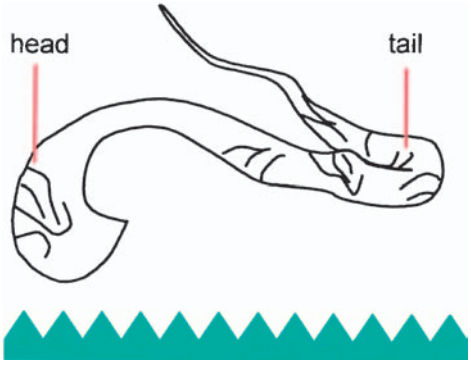

Fig. 4.2a. Epididymis.

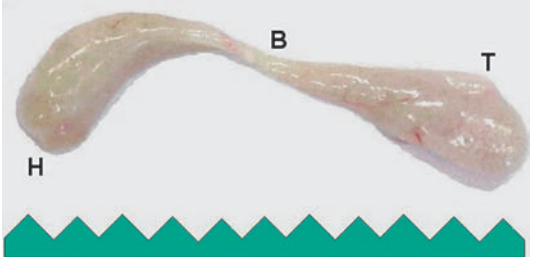

Fig. 4.2b. Epididymis (B: body, H: head, T: tail).

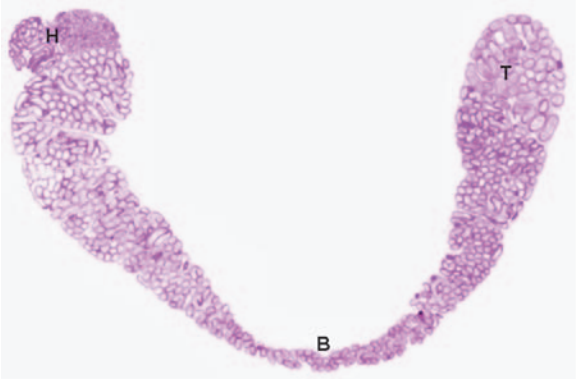

Fig. 4.2c. Epididymis (B: body, H: head, T: tail).

4 Male genital system
4.3 Seminal vesicle and Coagulating gland

Localization: In the mid portion
Number of sections: 2 (1 per side)
Direction: Transverse
Remarks: Together with coagulating gland

The coagulation gland represents the dorsocranial part of the prostate.

A transverse section should be made through the widest part of seminal vesicle together with coagulating gland.

Related references
Boorman et al. 1990b, Creasy and Foster 2002, Ferm 1987, Suwa et al. 2002

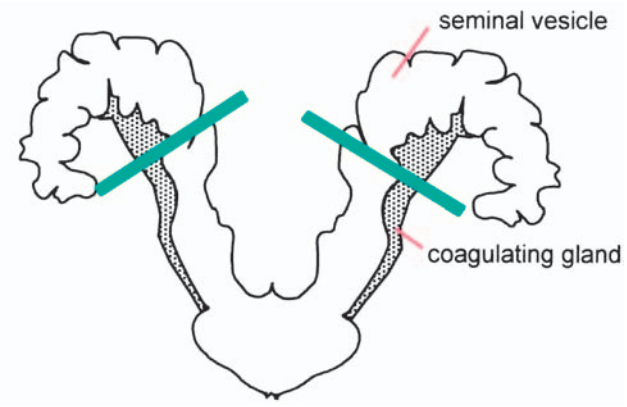

Fig. 4.3a. Seminal vesicle and coagulating gland.

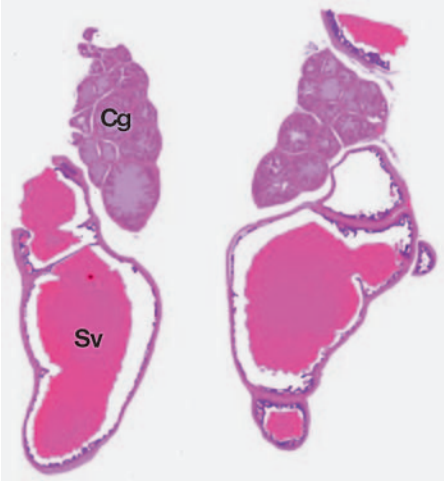

Fig. 4.3b. Seminal vesicle (Sv) and coagulating gland (Cg).

4 Male genital system
4.4 Prostate

Localization:	Dorsolateral and ventral lobe
Number of sections:	1
Direction:	Longitudinal horizontal after special preparation (see below).

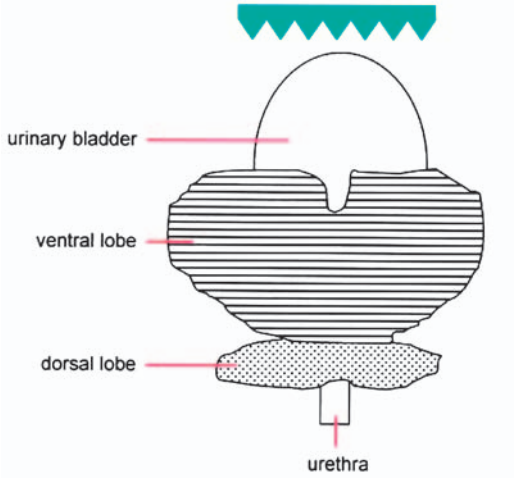

Fig. 4.4a. Prostate, rat, ventral aspect. Lateral lobes not visible, dorsal lobe: only caudal part visible.

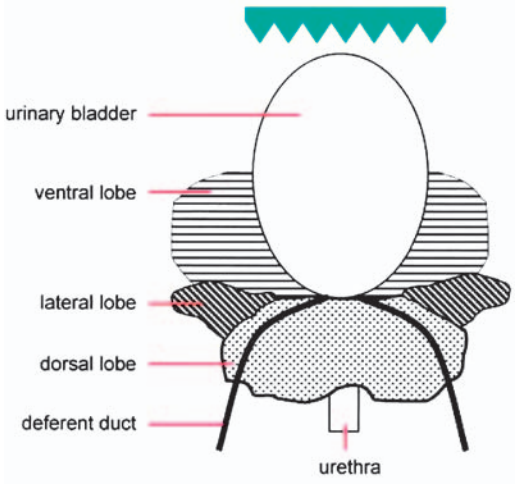

Fig. 4.4b. Prostate, rat, dorsal aspect.

The dorsolateral and ventral lobes that normally lie in a vertical axis above each other (with urinary bladder and seminal vesicle in between) are spread in a horizontal axis and embedded with the "outer" aspect down into the cassette.

Preparation: The group of adjacent organs consisting of prostate, urinary bladder, seminal vesicles and coagulation glands is removed (see figures 4.4.d through 4.4.f) and (if weights are not required) fixed *in toto* to prevent leakage of the glandular secretions.

After fixation, the ventral lobe is detached from the urinary bladder and is flipped back. The urinary bladder and seminal vesicles with coagulation glands are removed. The two ventral lobes are separated from each other, but are left attached to the dorsolateral parts. The dissected prostate is put into a cassette with the "outer" surfaces down; i.e. ventral face of the ventral lobes down and dorsal face of the dorsolateral lobes down (see figures 4.4.g through 4.4.i). After histotechnical processing, a section at the mid level of the ventral lobes is made.

The dorsocranial lobe of the prostate (i.e. coagulating gland) is processed with the seminal vesicle.

Chemically induced or spontaneous proliferative lesions of the rat prostate can be found in all three lobes. The dorsal and lateral lobes exhibit the same spectrum of proliferative lesions. These differ from spontaneous and induced lesions in the ventral lobe. Additionally, some strain specific deviations in the interlobular distribution of benign and malignant neoplasms consequently require the assessment of all compartments. Accordingly, a longitudinal-horizontal section through the prostate complex, including dorsolateral and ventral lobes, urethra and, optionally, ureter and ductus deferens represents a less time consuming method, applicable to routine histological processing and examination.

Related references

Boorman et al. 1990b, Ferm 1987, Lee and Holland 1987, Mitsumori and Elwell 1988, Suwa et al. 2001, Suwa et al. 2002

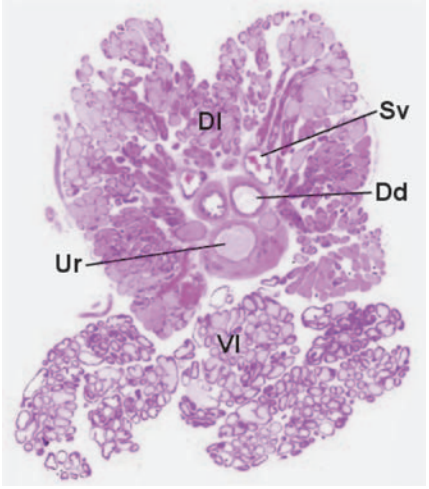

Fig. 4.4c. Prostate.

Abbreviations used in figures 4.4c to 4.4h:

Cg: Coagulation gland
Dd: deferent duct
Dl: dorsolateral lobe of prostate
Dsv: Duct of seminal vesicle
Sv: Seminal vesicle
Ub: Urinary bladder
Ur: Urethra
Vl: ventral lobe of prostate

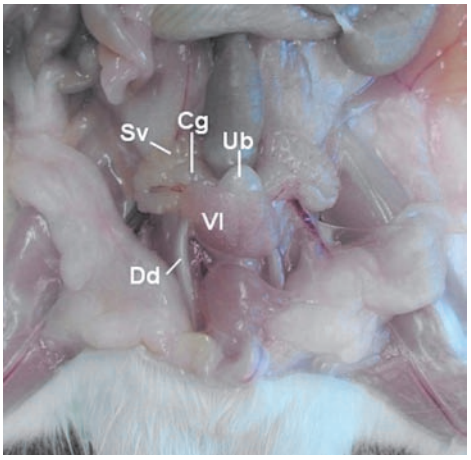

Fig. 4.4d. Rat, abdominal cavity, ventral aspect. In-situ localization of prostate and attached organs.

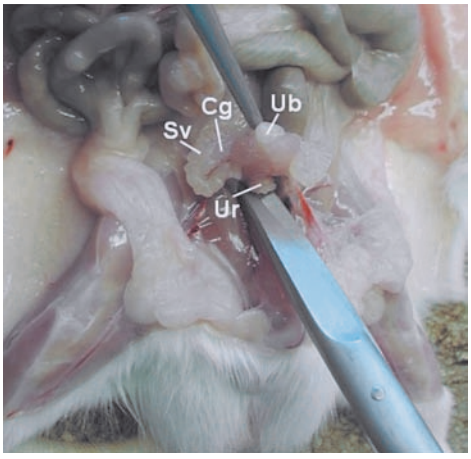

Fig. 4.4e. The urethra is dissected.

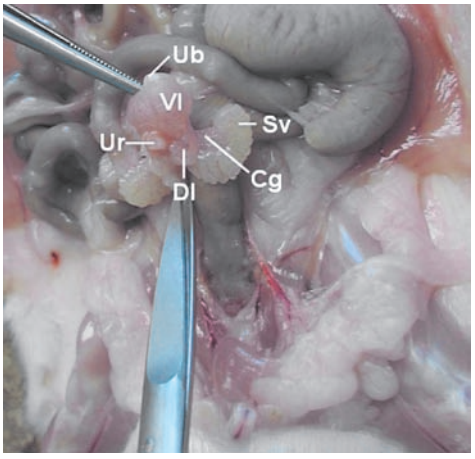

Fig. 4.4f. Removal of prostate, urinary bladder, and seminal vesicles as a unit.

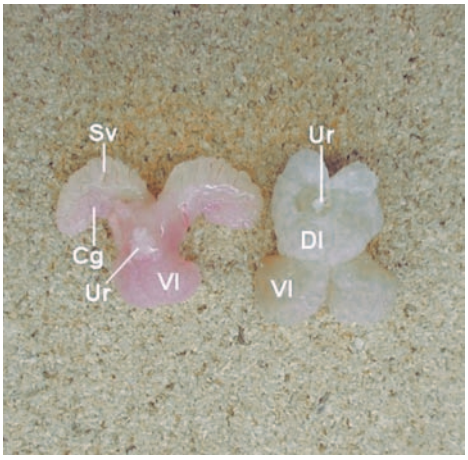

Fig. 4.4g. "Outer" aspects of freshly dissected (left) and fixed (right) prostate.

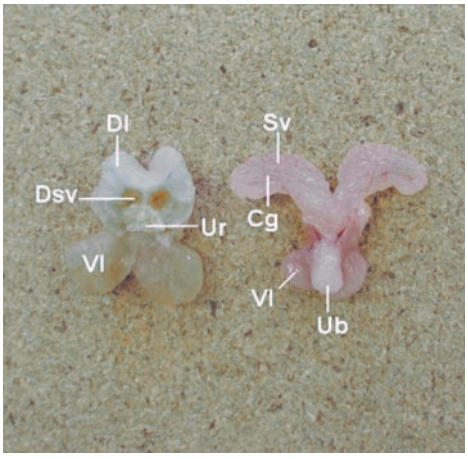

Fig. 4.4h. "Inner" aspects of fixed (left) and freshly dissected (right) prostate. Compared to the situation in the living animal (in situ), the ventral prostate is flipped back.

Fig. 4.4i. The prostate lobes are embedded with the "outer" aspects down, i.e. the dorsolateral lobes with the dorsal surface down, and the ventral prostate with the ventral surface down, because this part was flipped back.

5 Female genital system

5.1 Ovary and Oviduct

Number of sections:	2 (1 per side and organ)
Direction:	Ovary: longitudinal
Oviduct:	transverse
Remarks:	**If ovaries are not weighed:** Ovary processed along with the oviduct. Longitudinal sections are made, resulting in multiple transverse sections of the oviducts.
	If ovaries are weighed: Ovaries and oviducts are separated at necropsy.

In rats, the ovary is embedded together with the oviduct and a central section is cut. Larger ovaries or ovaries with masses are halved longitudinally or a slice from the middle of the organ is taken at trimming. In mice, the ovary is very small and therefore removed and fixed together with the bursa to avoid preparation artifacts. This procedure allows the detection of bursa cysts and cystadenomas in mice. Ovarian cysts should remain intact if possible.

If the oviduct is prepared attached to the ovary, the longitudinal cut through the ovary will result in multiple transverse sections through the oviduct.

If the ovaries are weighed, attached tissues (ovarian bursa, oviduct) have to be removed. In this case, the oviduct together with the tip of the uterine horn is dissected from the ovary during necropsy. The whole oviduct in conjunction with the tip of the uterine horn is fixed and embedded. Longitudinal sections through the tip of the uterine horns are made, resulting in transverse sections of the oviduct.

If the uterus is also weighed, the oviducts are detached from the horns and put into a cassette with the ovaries.

Related references

Ferm 1987, Heindel and Chapin 1993, U.S. Food and Drug Administration 2000, Yuan and Foley, 2002

Fig. 5.1a. Ovary and oviduct, if ovaries are not weighed.

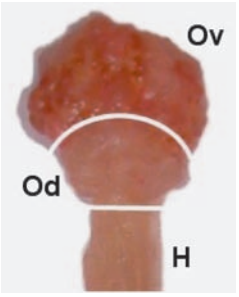

Fig. 5.1b. Ovary (Ov), oviduct (Od) and uterine horn (H), if ovaries are weighed.

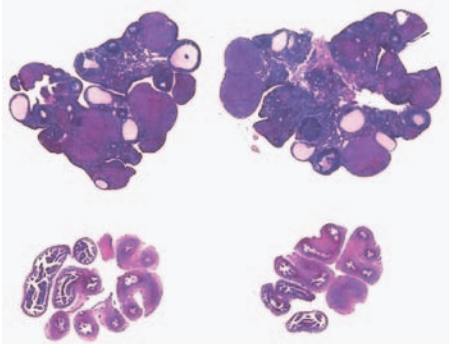

Fig. 5.1c. Ovaries (top) and oviducts (bottom). Ovarian bursa removed, oviducts separated from ovaries and uterine horns.

5 Female genital system
5.2 Uterus and Vagina

Localizations:	1 + 2) Middle region: uterine horns 3) Whole organ: uterine body and cervix 4) Whole organ or anterior portion: vagina
Number of sections:	3, 4 if uterus is separated from vagina
Direction:	1 + 2) Transverse: uterine horns 3) Longitudinal horizontal: uterine body (corpus), cervix and vagina
Remarks:	*Optional:* oviducts together with the tip of the uterine horns; other options see ovary.

The uterine body (fused part of the uterus) together with the vagina should be placed with its dorsal aspect on cardboard before fixation. If uterus is weighed, uterine cervix and vagina are two separate specimens which are cut longitudinally.

A horizontal section is made through the cervix and vagina. A transverse section is made through the middle portion of both uterine horns. These sections cover the relevant anatomical and functional structures of these organs. In most cases, the uterine body and most of the vagina will fit in one cassette, facilitating the interpretation of findings in the female genital tract.

The oviducts are fixed attached to the uterus when the ovaries are weighed.

Related references

Ferm 1987, Hebel and Stromberg 1986, Heindel and Chapin 1993, U.S. Food and Drug Administration 2000, Yuan and Carlson 1987, Yuan and Foley, 2002

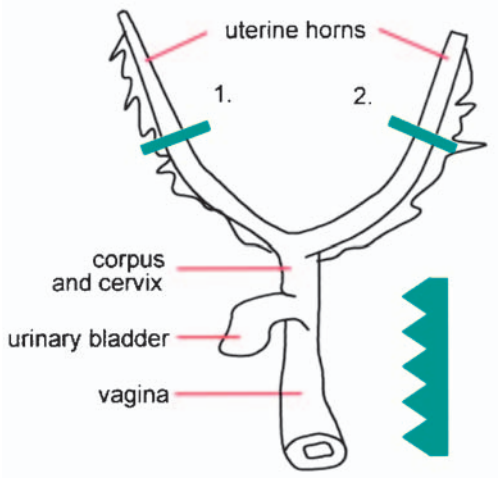

Fig. 5.2a. Uterus and vagina, ventral aspect.

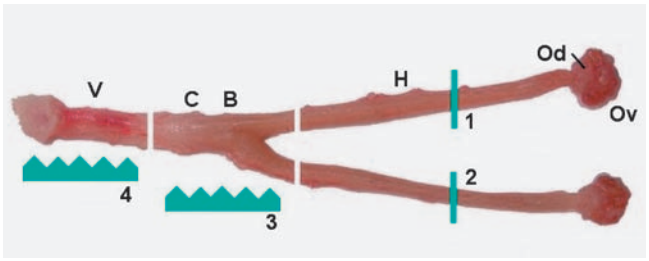

Fig. 5.2b. Uterus and vagina (V: vagina, C: cervix, B: body, H: uterine horn, Od: oviduct, Ov: ovary).

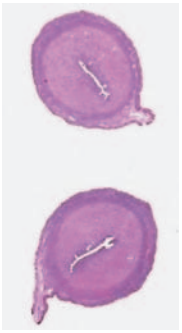

Fig. 5.2c. Uterine horn.

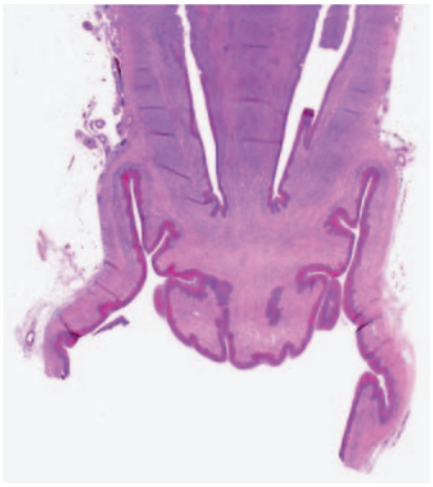

Fig. 5.2d. Uterine cervix.

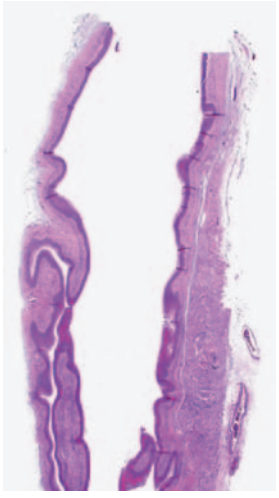

Fig. 5.2e. Vagina.

6 Endocrine system
6.1 Pituitary gland

Localization:	Sella turcica of sphenoid bone
Number of sections:	1
Direction:	Transverse, parallel to the caudodorsal surface of the gland.
Remarks:	To avoid destruction of the pituitary, fixation is recommended before removal from the skull and/or weighing. The organ is embedded *in toto* with its caudodorsal surface down, so that the section will include all three parts. *Optional:* trimmed *in situ*, closely caudal to the pituitary, caudal surface embedded down. Transverse section of decalcified skull.

The pituitary consists of three portions: pars distalis, pars intermedia, and pars nervosa. All three parts should be present in one histologic section with the largest possible area.

Relevant differences between rats and mice

In mice, *in situ* fixation may be particularly useful to avoid preparation artifacts and to obtain consistent planes of section (Mahler and Elwell, 1999).

Related references

Capen 1996a, Hebel and Stromberg 1986, Mahler and Elwell 1999, Osamura 1996, Satoh et al. 1997

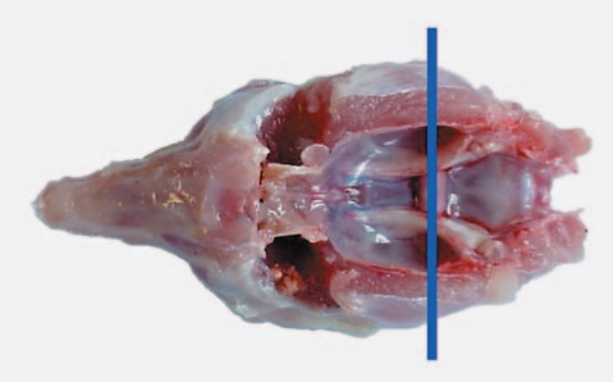

Fig. 6.1b. Skull, dorsal view. Level for optional in-situ trimming.

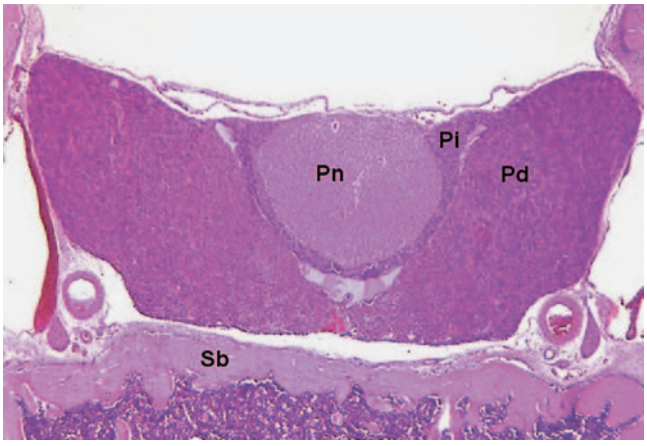

Fig. 6.1c. Pituitary gland *in situ* (Pn: pars nervosa, Pi: pars intermedia, Pd: pars distalis, Sb: sphenoid bone).

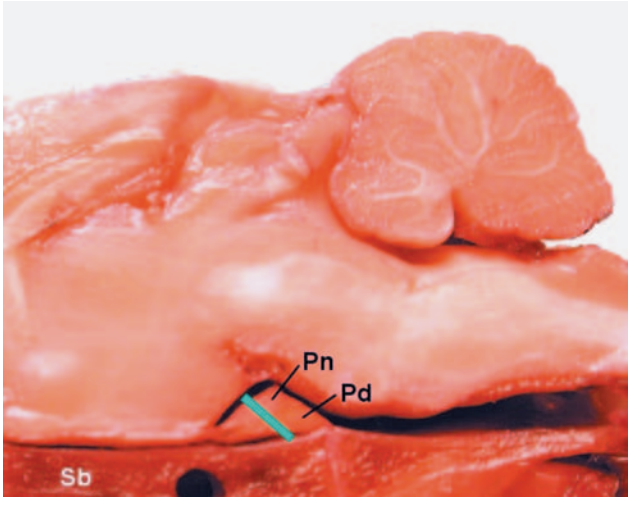

Fig. 6.1a. Pituitary gland, *in situ* localization, median aspect (Pn: pars nervosa, Pd: pars distalis, Sb: sphenoid bone).

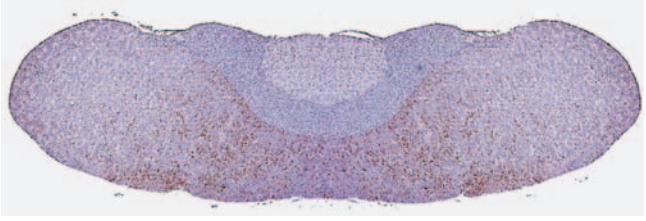

Fig. 6.1.d. Pituitary gland, male rat, immunohistochemical staining for prolactin: depending on the level of section, the positive cells are not evenly distributed.

6.2 Thyroid gland, Parathyroid gland, Trachea (oral study) and Esophagus

Localization:	In the area of the parathyroid gland
Number of sections:	1
Direction:	**If thyroid glands are not weighed:** Transverse section of trachea, esophagus, thyroid and parathyroid glands. *Optional:* longitudinal horizontal section of thyroid glands in conjunction with trachea. A separate transverse section of the esophagus is made.
	If thyroid glands are weighed: Longitudinal horizontal (largest cut surface), section of thyroid and parathyroid glands. Transverse section of trachea and esophagus.
Remarks:	Recuts are sometimes required to consistently include the parathyroid in the section. The number of focal lesions observed depends on the area of thyroid examined.

Relevant differences between rats and mice

The rat possesses only one pair of parathyroids. They are located on the anterior and lateral aspect of the thyroid lobes but may vary in position.

In the mouse, the position and the number of parathyroids is variable. Usually, there are two parathyroid glands located bilaterally just under the capsule near the dorsolateral border of each thyroid lobe. They are rarely found at the same level, sometimes one or both may be posterior to the thyroid; they may be deeply embedded in the thyroid tissue and there may be more than two.

Related references

Boorman and DeLellis 1983, Botts et al. 1991, Botts et al. 1994, Capen 1996b, Capen 1996c, Kittel et al. 1996a, Kittel et al. 1996b, Pozharisski 1990

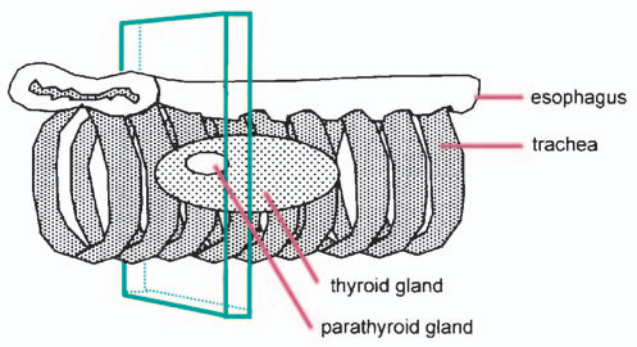

Fig. 6.2a. Transverse section.

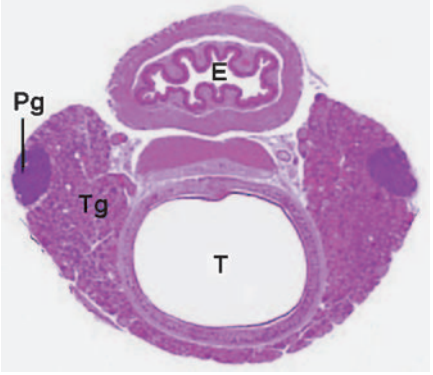

Fig. 6.2b. Transverse section (T: trachea, Tg: thyroid gland, Pg: parathyroid gland, E: esophagus).

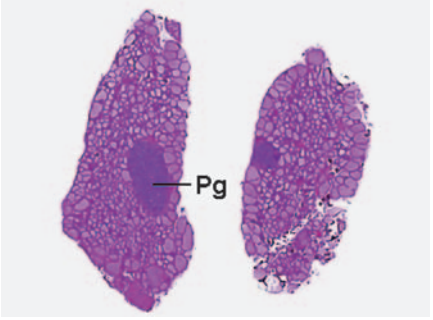

Fig. 6.2c. Thyroid gland, longitudinal section, with parathyroid gland (Pg).

6.3 Adrenal gland

Localization:	Through cortex and medulla
Number of sections:	2 (1 per side)
Direction:	Longitudinal (largest cut surface)
Remarks:	Embedded *in toto*; careful microtome sectioning until recommended cutting level is obtained.

Median sections of the adrenal glands (one per side) are required in order to demonstrate a representative part of both cortical and medullary tissues.

Relevant differences between rats and mice

The mouse adrenal differs from that of the rat by the absence of a zona reticularis in the inner cortex and an additional "X-zone" at the junction between cortex and medulla in females which regresses with age (Nyska and Maronpot, 1999). The adrenal gland in male mice is very small. Therefore, careful cutting is required to obtain medullary tissue in the section.

Related references

Frith 1996, Hebel and Stromberg 1986, Nyska and Maronpot 1999, Paget and Thomson 1979

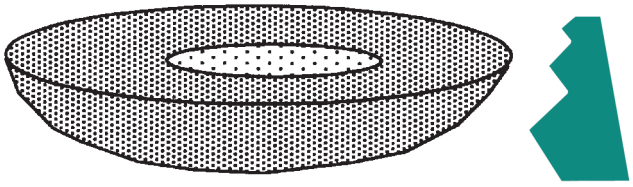

Fig. 6.3a. Adrenal gland.

Fig. 6.3b. Adrenal glands.

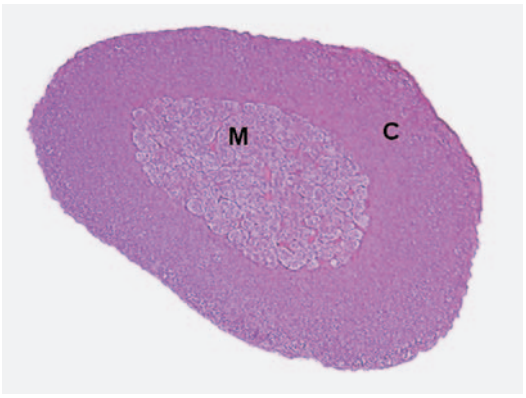

Fig. 6.3c. Adrenal gland, male mouse (C: cortex, M: medulla).

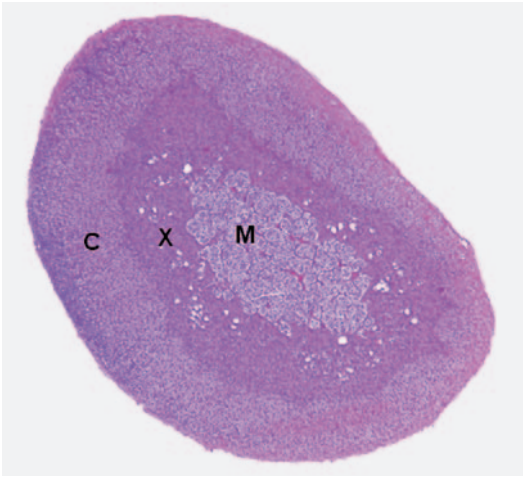

Fig. 6.3d. Adrenal gland, female mouse, age about 3 months (C: cortex, M: medulla, X: X-zone).

References

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