Exp Toxic Pathol 2004; **55**: 433–449
http://www.elsevier.de/etp

[1] Department of Information Technology and Databases, Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany
[2] Department of Toxicologic Pathology, Bayer HealthCare AG, Wuppertal, Germany
[3] Department of Product Safety, Regulations, Toxicology and Ecology, BASF AG, Ludwigshafen, Germany
[4] Drug Safety Evaluation Pathology, Aventis Pharma Deutschland GmbH, Hattersheim, Germany
[5] Schering-Plough Research Institute, Lafayette, NJ, USA
[6] Department of Toxicology/Pathology/Animal Care, Abbott GmbH Co. KG, Ludwigshafen, Germany
[7] Institute of Pathology and Toxicology, ALTANA Pharma AG, Hamburg, Germany
[8] Institute of Toxicology, Merck KGaA, Darmstadt, Germany

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

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

GERD MORAWIETZ[1], CHRISTINE RUEHL-FEHLERT[2], BIRGIT KITTEL[3], AXEL BUBE[4], KEVIN KEANE[5], SABINE HALM[6], ANKE HEUSER[7], and JÜRGEN HELLMANN[8]

With 48 figures

Received: March 25, 2004; Revised; May 17, 2004; Accepted: May 25, 2004

Address for correspondence: GERD MORAWIETZ, Department of Information Technology and Databases, Fraunhofer Institute of Toxicology and Experimental Medicine, Nikolai-Fuchs-Str. 1, 30625 Hannover, Germany; Fax: ++49 511 5350 155, E-mail: morawietz@item.fraunhofer.de

Key words: Trimming; RITA; NACAD; rat; mouse; standardization; guidelines; kidney; ureter; urinary bladder; brain; spinal cord; spinal nerve root; eye; optic nerve; Harderian gland; skeletal muscle; peripheral nerve; bone; cartilage; femur; joint; heart; aorta; thymus; spleen; bone marrow; sternum; lymph nodes.

*) 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 Corporate Toxicology, 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; Pfizer, Inc., Kalamazoo, MI, USA; Schering-Plough Research Institute, Lafayette, NJ, USA

Summary

This is the third 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 urinary, nervous, musculoskeletal, cardiovascular, and lymphoreticular 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 standardize tissue sampling and trimming for comparison 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 (CRISSMAN et al. 2004).

Brief introduction to the use of the individual organ guides

For each organ the following information is usually given (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 plane of section 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 placed in a cassette for processing. The sample size is determined by the size of the organ. 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, placing of organs in cassettes, etc.
6. Schematic **drawings** and/or **gross photographs** are given indicating the plane of section. Some of the gross photographs show the organ and trimming direction *in situ* for orientation purposes. However, it is recommended to remove the organ or tissue first. Trimming can then be performed 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.**

The following terms are used to describe 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.

Final technical remarks

Study types: Although carcinogenicity studies in rodents are the focus of this publication, the same trimming procedures are also recommended for short-term rodent studies, unless specific target tissues or organs require an adaptation. In general, it is advisable to follow one standard trimming procedure in the laboratory for all studies to avoid technical inconsistencies and to facilitate inter-study comparison.

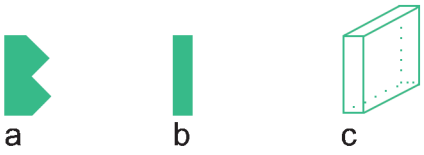


Fig. 1. Symbols used in the drawings and/or gross photographs to indicate the plane of section. **a:** cutting level parallel to the plane of the picture, **b:** cutting level perpendicular to the plane of the picture, **c:** cut level, 3-D.

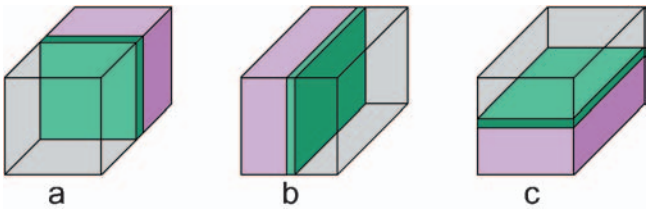


Fig. 2. Schematic presentation of the plane of section. **a:** transverse, **b:** longitudinal vertical, **c:** longitudinal horizontal.

Additional organs: Besides the organs addressed in these guides, special investigations or guidelines may require additional organs. Some can be found in the specimens described here, such as teeth in the transverse sections of the nasal cavity, brown adipose tissue at the renal hilus and heart base, white adipose tissue in the subcutis and the urethra of males in the prostate section. Others, however, may need adaptation of sampling procedures or collection of additional specimens.

Blocking: The following recommendations should be taken into account:

1. For reasons of economy it is desirable to have a small number of blocks per animal.
2. Unduly large numbers of specimens in one block can lead to a loss of quality. Besides the obvious limitation of specimen size, difficulties may arise when cutting tissues with different physical properties in the same block.
3. Small organs often benefit from being embedded by themselves which makes it easier to cut accurately the level of interest. Examples are the pituitary gland, the adrenal gland and the thyroid gland with parathyroid glands.
4. Organs with similar cutting properties are often combined in one block.
5. The pathologist benefits from being able to examine organs in functional groups, e.g. stomach together with intestine or a combination of the lymphoid organs in one block.

In order to reduce the number of slides, more than one block may be placed on a slide. Some laboratories see an advantage in collecting the adrenal glands, pituitary gland and thyroid gland with parathyroid glands on one slide which were processed in different blocks. An example of a blocking scheme reflecting the above mentioned considerations was given by KRINKE (2000).

Number of sections: The number of sections per specimen is usually one. If in some instances more than one section has to be taken, it should be borne in mind to evaluate the same amount of sections in all animals/groups to obtain comparable results.

Staining: The H&E stain is regarded as the standard in toxicological studies. Other histological stains and immunohistochemistry can be applied as a routine or on a case by case basis in addition to the H&E stained sections.

Fixation: In literature a volume ratio of tissue to fixative of 1:20 is often mentioned. However, much less fixative is sufficient, especially if a shaking device is used for freshly fixed tissues and/or fixative is replaced once. Tissues must be promptly and appropriately fixed by immersion. Adequate fixation time is necessary before tissue processing commences (CRISSMAN et al. 2004).

Internet: With kind permission of Elsevier Publishing Jena, an extended version of these guides for organ sampling and trimming will be available in the Internet under the URL www.item.fraunhofer.de/reni/trimming.

Acknowledgements: The authors would like to thank Mr. THEODOR LAFORME and Mrs. BRIGITTE POSCHMANN (Bayer HealthCare AG) for the gross preparation of organs and histological slides; Mrs. PETRA HARTMANN, Mrs MEIKE PETERSEN and Mrs. MARTINA NEUENHAUS (Bayer HealthCare AG) for scanning the histological slides, Dr. GERD-PETER FEHLERT for his help in image processing; Dr. WOLFGANG KAUFMANN (BASF AG) for his scientific advice regarding the nervous system, Dr. PAUL DESLEX (Pfizer Centre Recherche) for providing additional photographs and slides, Dr. THOMAS NOLTE (Boehringer Ingelheim Pharma GmbH & Co KG) and Dr. ANNE PROVENCHER BOLLIGER (Novartis Pharma AG) for help in establishing the bone marrow manuscript and Dr. MATTHEW JACOBSEN (Syngenta CTL) and Dr. RICHARD DOUGHTY (AstraZeneca) for their helpful input during the editing process.

References

BAHNEMANN R, JACOBS M, KARBE E, et al.: RITA – Registry of Industrial Toxicology Animal-data – Guides for organ sampling and trimming procedures in rats. Exp Toxic Pathol 1995; **47**: 247–266.

CRISSMAN JW, GOODMAN DG, HILDEBRANDT PK, et al: Best practice guideline: toxicologic histopathology. Toxicol Pathol 2004; **32**: 126–131.

KITTEL B, RUEHL-FEHLERT C, MORAWIETZ G, et al.: Revised guides for organ sampling and trimming in rats and mice – Part 2. Exp Toxic Pathol 2004; **55**: 413–432.

KRINKE GJ (ed): The laboratory rat. Academic Press, San Diego San Francisco New York, 2000.

RUEHL-FEHLERT C, KITTEL B, MORAWIETZ G, et al.: Revised guides for organ sampling and trimming in rats and mice – Part 1. Exp Toxic Pathol 2003; **55**: 91–106.

7 Urinary system

7.1 Kidney and Ureter

Localizations:	Kidney: both in the median, through the tip of papilla and renal pelvis Ureter: transverse section midway between kidneys and bladder. *Optional*: adjacent to the renal pelvis (not shown in the image)
Number of sections:	2 (1 per side)
Direction:	Kidney: one side longitudinal, other side transverse Ureter: longitudinal adjacent to kidney or transverse with adipose tissue
Remarks kidney:	Capsule should not be removed. Fixation can be improved by an incision at necropsy.

The transverse section of the kidney from the middle portion allows optimal examination of the renal pelvis, renal papilla and the junction with the ureter. The longitudinal section permits histological evaluation of a relatively large area of tissue which includes both renal poles. This is advantageous for the evaluation of any focal lesions. In addition, the regions of the renal pelvis close to the poles are of interest with respect to concretions and urothelial changes.

A slightly paramedian cut at trimming is helpful to get the full length of the renal papilla in section.

The ureter can be fixed on a cardboard together with a small amount of attached adipose tissue.

Related references

BACHMANN and KRIZ 1998, BANNASCH et al. 1974, COHEN et al. 2002, CORMAN and OWEN 1992, FRITH et al. 1998, KHAN and ALDEN 2002, LIEBELT 1998, SHORT and GOLDSTEIN 1992

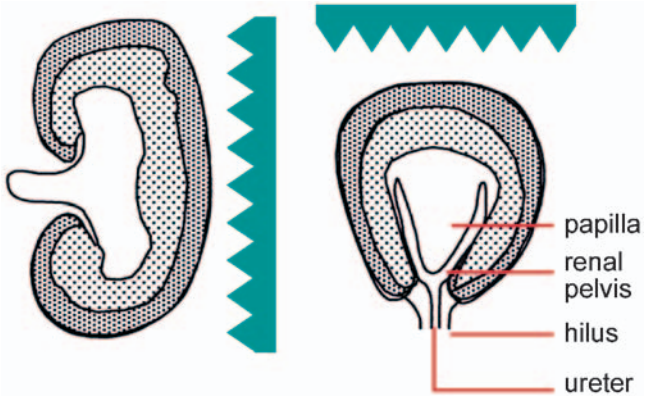


Fig. 7.1a. Kidney and ureter.

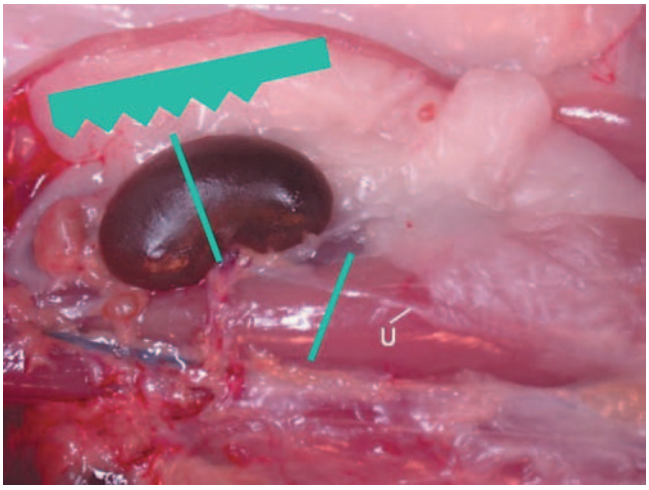


Fig. 7.1b. Kidney and ureter (U) *in situ*.

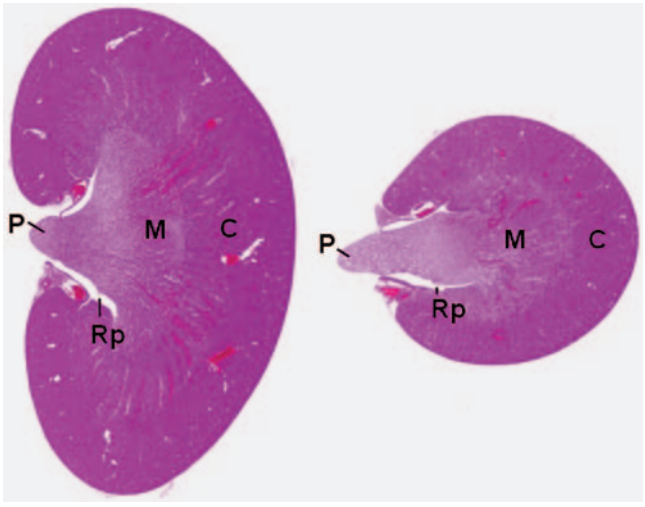


Fig. 7.1c. Kidneys (P: papilla, M: medulla, C: cortex, Rp: renal pelvis).

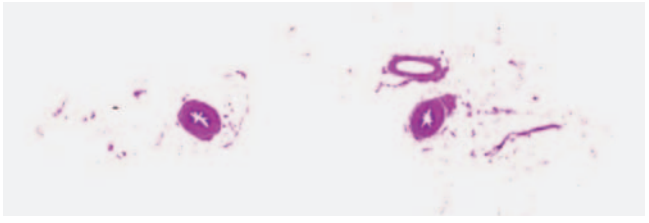


Fig. 7.1d. Ureters.

7 **Urinary system**

7.2 Urinary bladder

Number of sections:	1
Direction:	Longitudinal vertical
Remarks:	Instillation 0.2 ml rat, 0.05 ml mouse

For instillation, the needle is inserted via the urethra into the urinary bladder. For the mouse, fixative can be injected through the urinary bladder wall after ligation of the urethra. The organ is filled by injection of approximately 0.2 ml (rat) or 0.05 ml (mouse) of the fixative. Do not infuse if distended with urine. Ligation is performed with a ventral knot. In addition, it may be helpful to mark the ventral side of the bladder with a stick of silver nitrate. After removal of the bladder, fixation is continued in a suitable container. The bladder is cut vertically through the ventral knot to access the following regions:

1. Vertex and ventral body: The vertex is the area generally most prone to develop neoplasms; deposition of sediments and calculi occurs mainly in the ventral aspect, which can lead to urothelial alterations.
2. Dorsal part of the bladder.
3. Bladder neck with trigone: Whilst most of the urinary bladder in the embryo is of endodermal origin, the trigone is derived from the mesoderm of the Wolffian ducts.

Related references

Cohen at al. 2002, Frith 1980, Kunze 1992, Pauli et al. 1998

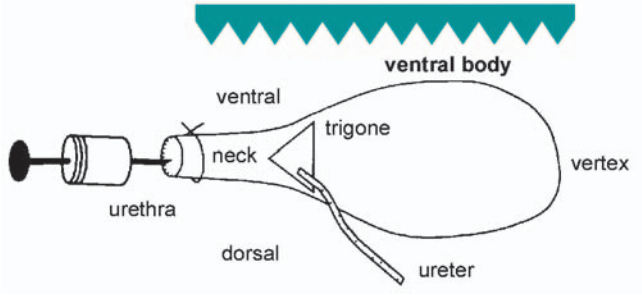


Fig. 7.2a. *In situ* localization and fixation of urinary bladder.

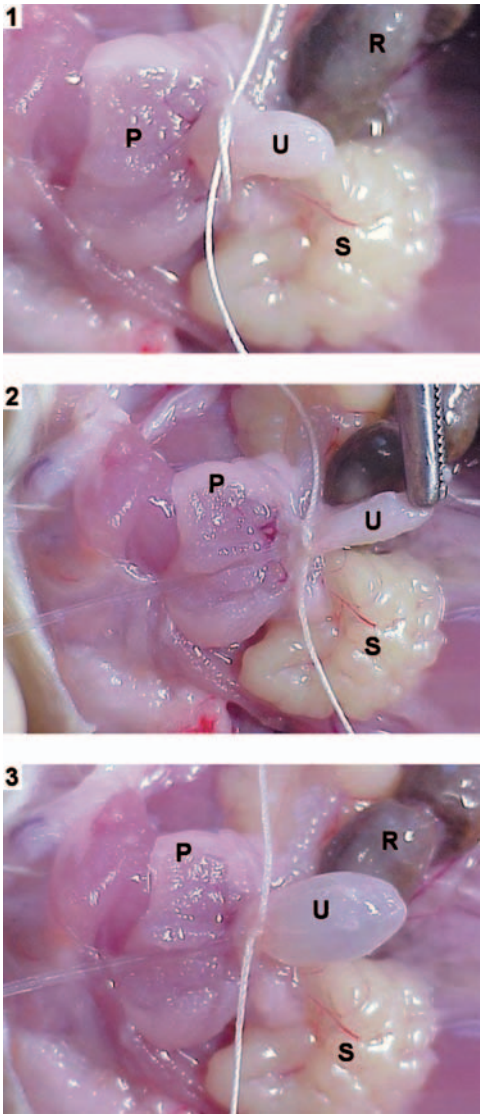


Fig. 7.2b. Instillation procedure: *in situ* localization, ventral aspect, after opening of the abdominal cavity. P: prostate, R: rectum, S: seminal vesicle, U: urinary bladder.

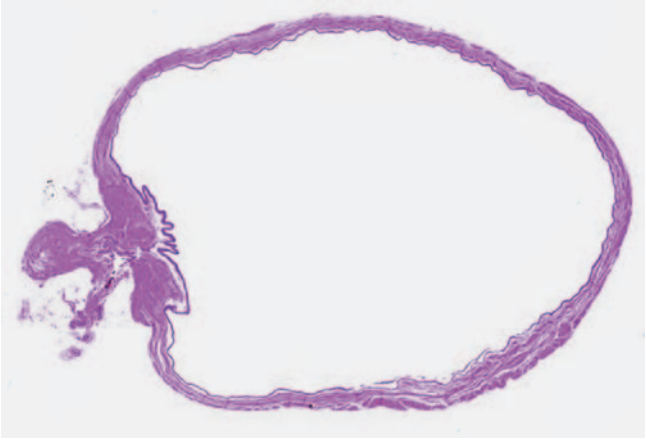


Fig. 7.2c. Urinary bladder after instillation.

8 Nervous system

8.1 Brain

Localizations:	1) Cerebrum at the optic chiasm 2) Cerebrum at the base of the posterior hypothalamus 3) Midcerebellum and medulla oblongata *Optional:* 4) Pons at the middle of its protrusion 5) cranial cervical cord
Number of sections:	3 (4)
Direction:	Transverse
Remarks:	Embedded with the anterior faces down. To achieve accurate brain weights, the spinal cord should be cut off at a consistent level.

Unless the scope of examinations in neurotoxicity studies is extended, it is advised to use the above mentioned brain sections for the morphological screening in all rodent studies concerned.

Transverse sections of the brain are required to assess whether the findings are distributed uni- or bilaterally and symmetrically or asymmetrically. The areas of the brain known to be susceptible to neurotoxicity, including oncogenicity, are: the cerebral and cerebellar cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, pons and medulla oblongata. Routinely, three transverse sections are obtained:

1. the first section at the level of optic chiasm including the basal ganglia, septum, cortex, anterior hypothalamus;
2. the second section at the level of hippocampus containing the cortex and brain stem at the transition of diencephalon to mesencephalon and
3. the third section containing the cerebellum and brain stem (medulla oblongata).

Location 4 is recommended for special investigations of the pons.

To achieve precise vertical cuts, brain matrix moulds can be used. Different sizes are available for different laboratory species. For rodents, the brain can be sliced at 1 mm intervals.

A sample of the cranial cervical cord can be removed together with the brain (location 5). Alternatively, it can be taken together with the cranial vertebral column to avoid artifacts (see 8.2: spinal cord).

Trimmed specimens should not be stored in alcohol for extended periods during routine processing to avoid artifactual vacuolation.

Related references

Dorman et al. 2002, Krinke 1989, Paxinos and Watson 1997, Schäppi and Krinke 1991, WHO 1986

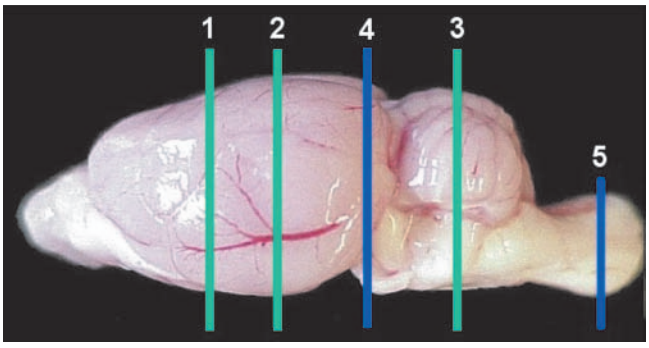


Fig. 8.1a. Brain, lateral view.

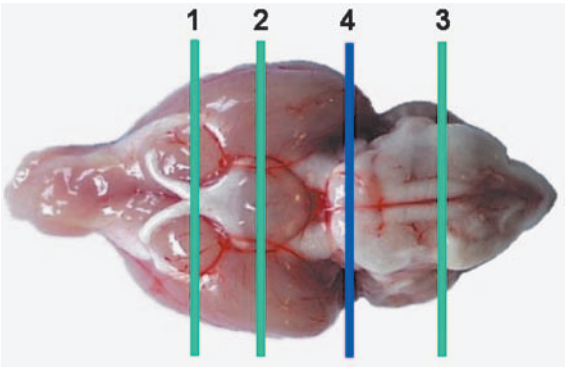


Fig. 8.1b. Brain, ventral view.

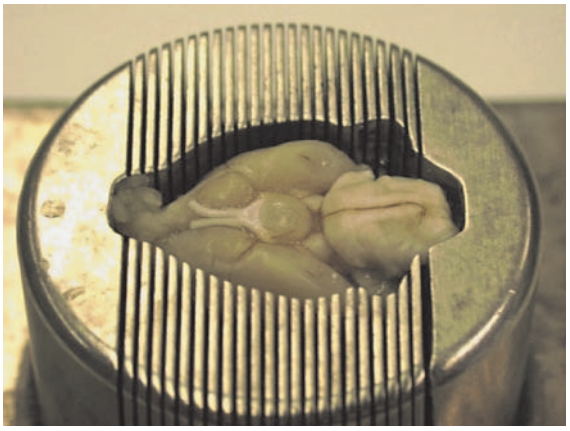


Fig. 8.1c. Coronal Brain Matrix System (supplier: A. Semrau GmbH und Co, 45549 Sprockhövel, Germany).

Abbreviations used in figures 8.1d to 8.1g:

Ac: anterior commissure
C: cerebellum
Cc: cerebral cortex
Cca: corpus callosum
H: hypothalamus
Hc: hippocampus
Mo: medulla oblongata
O: optic chiasm
P: pons
Sr: striatum
T: thalamus

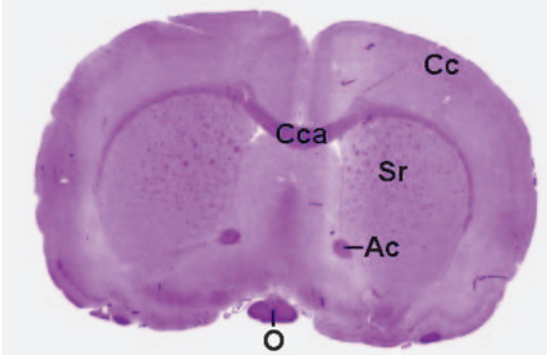


Fig. 8.1d. Brain, localization 1, cerebrum.

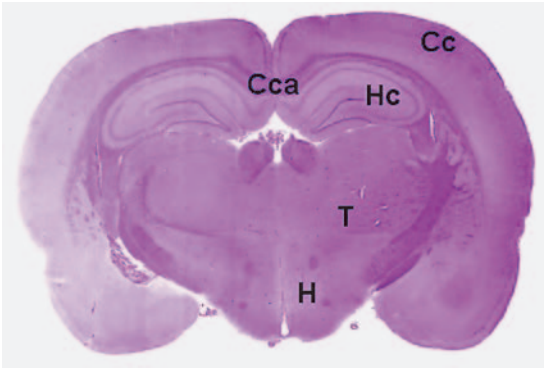


Fig. 8.1e. Brain, localization 2, cerebrum.

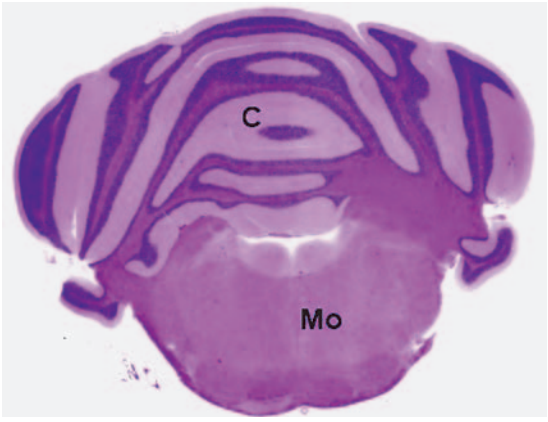


Fig. 8.1f. Brain, localization 3, cerebellum and medulla oblongata.

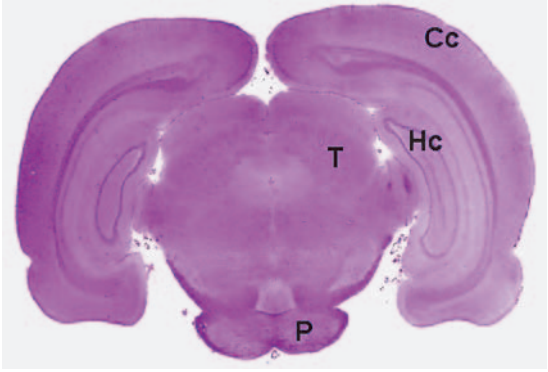


Fig. 8.1g. Brain, optional localization 4, cerebrum and pons.

8 Nervous system
8.2 Spinal Cord and Spinal nerve root

Localizations:	1) Cervical cord at upper cervical segment. *Optional:* cervical cord cut at the level of the first cervical nerve (see 8.1 brain) 2) Thoracic cord at mid-thoracic segment 3) Lumbar cord at 4th lumbar segment close to last rib
Number of sections:	3
Direction:	Transverse
Remarks:	In conjunction with vertebral body (decalcified). *Optional:* without bone to avoid decalcification. Embedded with anterior faces down.

Transverse sections of the spinal cord are required to assess whether the findings are distributed uni- or bilaterally, and symmetrically or asymmetrically. Sections should be obtained at the upper cervical, mid-thoracic and lumbar levels. The upper cervical area positioned at the transition of medulla oblongata to cervical spinal cord (level of the first cervical nerve) is important for the detection of distally accentuated neuropathy affecting the long spinal tracts. This region is known to produce the earliest and most prominent lesions in the dorsal cervical column. At necropsy, the spinal cord should be cut off directly posterior to the cerebellum and can be removed with the vertebral bone as with the thoracic and lumbar spinal cord.

In the lumbar spinal cord, the area of L4 segment should preferably be examined, as this segment provides the main contribution to the peripheral sciatic nerve. The L4 segment of the spinal cord is located at the junction of the thoracic and lumbar spine which is indicated by the last rib. If the lumbar specimen is taken more caudally, it will contain only the cauda equina.

It is advantageous to remove the spinal cord from the vertebrae before processing to avoid decalcification. However, processing *in situ* has the advantage to include dorsal root ganglia and nerve roots to assess radiculoneuropathy.

In neurotoxicity studies, sections from the cervical and lumbar swellings are recommended in current guidelines (OECD 424). The cervical swelling is located at the C3–C6 segments of the spinal cord. This section is not prioritized but can be taken as additional option in routine rodent studies.

To avoid artifactual vacuolation in the white matter, the specimens should not be stored in alcohol.

Related references

GELDERD and CHOPIN 1977, KRINKE 1989, KRINKE and FITZGERALD 1988, OECD 1997, SCHÄPPI and KRINKE 1991

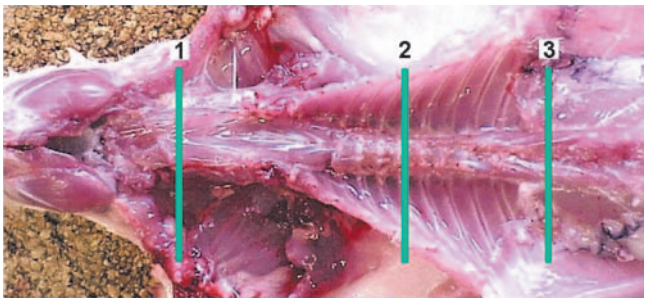


Fig. 8.2a. Spinal cord.

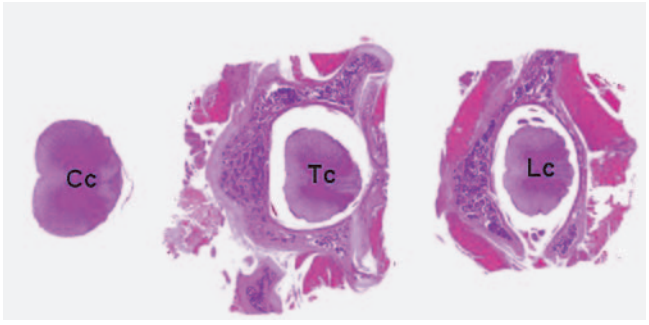


Fig. 8.2b. Spinal cord (Cc: cervical cord, Tc: thoracic cord, Lc: lumbar cord). Option without bone shown for the cervical cord.

8 Nervous system

8.3 Eye, Optic nerve and Harderian gland

Localization:	In the plane of the optic nerve
Number of sections:	2 (1 per side)
Direction:	Longitudinal vertical
Remarks:	Optic nerve included. *Optional:* together with Harderian gland. *Optional:* together with eye lids for better orientation. For long-term studies, formalin fixation is generally sufficient. For other study types, fixation in Davidson's fixative is recommended to avoid detachment of the retina.

Each eye can be removed from the socket together with the optic nerve and the Harderian gland (optional) after fixation of the head. These organs are embedded in such a way that the section plane includes the anterior pole of the eye, the lens, the optic nerve and the gland.

Alternatively, each eye with the optic nerve can be taken at necropsy followed by the removal of the Harderian gland; in this case, both samples are fixed and embedded separately.

As an option, the optic nerve specimen can be taken at the base of the brain.

Relevant differences between rats and mice

The rodent eye has no areas of increased visual acuity (fovea and macula), therefore orientation is not as critical as in the non-rodent eye. The Harderian gland lies intraorbitally and is cone-shaped in the rat and horseshoe-shaped in the mouse.

See also: 2.2 Salivary glands (extraorbital lacrimal gland)

Related references

LATENDRESSE et al. 2002, MOLON-NOBLOT and DUPRAT 1991, WHITELEY and PEIFFER 2002

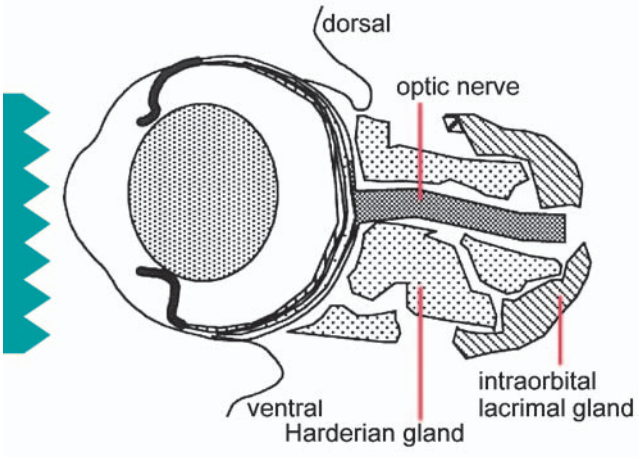


Fig. 8.3a. Eye, optic nerve and Harderian gland.

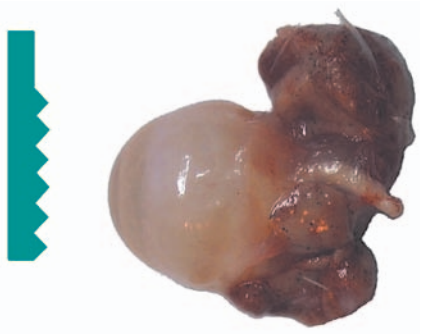


Fig. 8.3b. Eye.

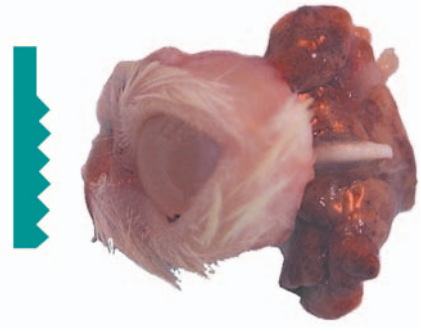


Fig. 8.3c. Eye and eye lid.

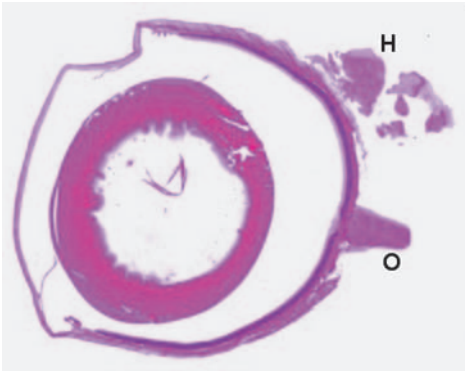


Fig. 8.3d. Eye, Davidson's fixative (O: optic nerve, H: Harderian gland).

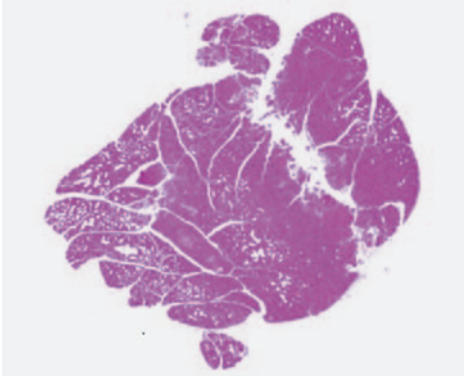


Fig. 8.3e. Harderian gland.

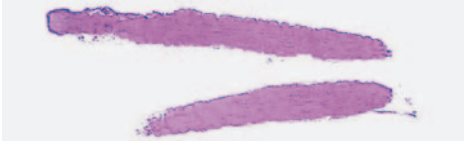


Fig. 8.3f. Optic nerve, taken from the brain near to the optic chiasm.

8 Nervous system

8.4 Skeletal muscle and peripheral nerve

Localizations:	Biceps femoris muscle
Sciatic nerve	
Number of sections:	3 (2)
Direction:	Skeletal muscle: longitudinal and/or transverse Sciatic nerve: longitudinal; optional: transverse
Remarks:	Muscle and nerve are sampled separately. *Optional:* skeletal muscle and sciatic nerve sampled attached to each other. Longitudinal and transverse sections in one cassette. If only one sample of muscle is processed, a transverse section is preferred.

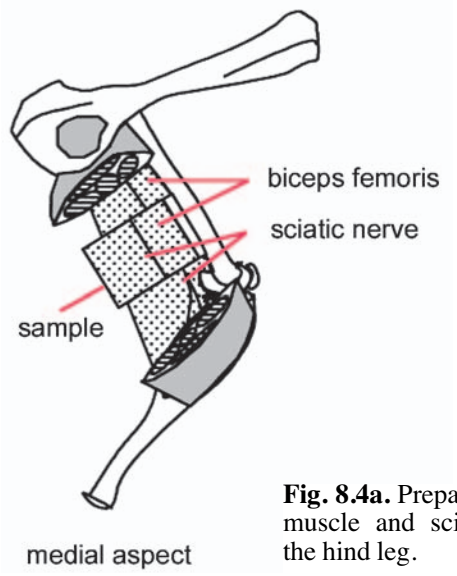


Fig. 8.4a. Preparation of skeletal muscle and sciatic nerve from the hind leg.

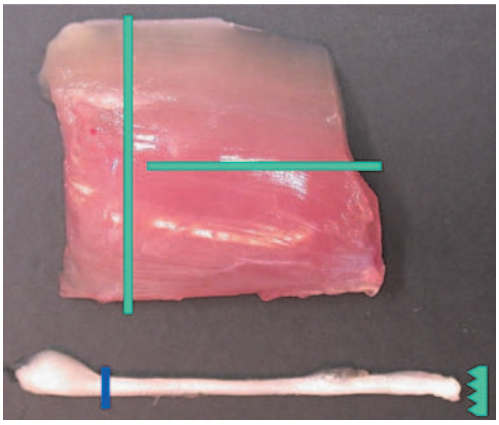


Fig. 8.4b. Skeletal muscle and sciatic nerve, transverse and longitudinal horizontal sections.

If skeletal muscle and sciatic nerve are sampled together, the gracilis, adductor, semimembranous and semitendinous muscles are removed from the medial aspect of the thigh to get access to the sciatic nerve running along the medial surface of the biceps femoris muscle. The sample is taken by proximal and distal transverse cuts. After fixation, transverse and longitudinal sections are prepared.

The sciatic nerve can be fixed on a card board, if not sampled together with muscle.

Related references

McGavin 1991, Popesko et al. 1992

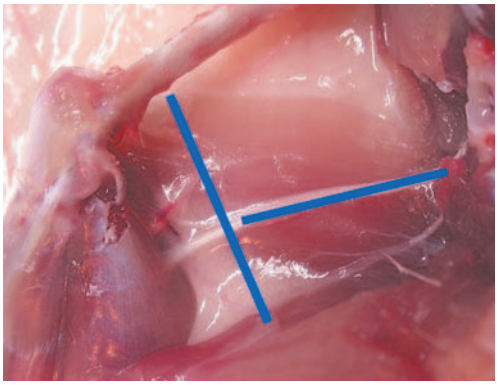


Fig. 8.4c. Muscle *in situ*, option.

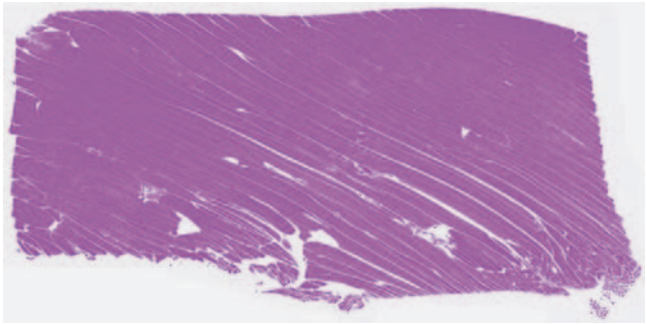


Fig. 8.4d. Skeletal muscle, longitudinal section.

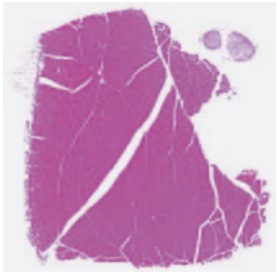


Fig. 8.4e. Skeletal muscle, transverse section.

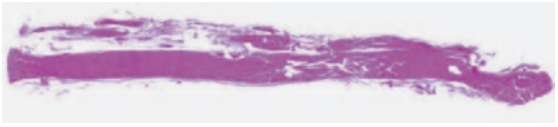


Fig. 8.4f. Sciatic nerve, longitudinal section.

9 Musculoskeletal system

9.1 Bone, Cartilage, Femur and Joint

Localization:	Knee joint with distal femur and proximal tibia
Number of sections:	1
Direction:	Longitudinal
Remarks:	Decalcified

For routine histological examination of bone (and bone marrow), the distal portion of one femur with the knee joint and proximal portion of the adjacent tibia are removed at necropsy, fixed and decalcified. A longitudinal section is then made through femur, knee joint and tibia, possibly including patella and/or menisci. Both long bones should be cut at similar length with inclusion of parts of their diaphyses. The section should be slightly lateral to the center of the joint to ensure that articular cartilage is present rather than ligaments. The use of large sections of bone with the joint has the advantage of maintaining anatomic integrity and allows separation of systemic pathologic conditions from reactive processes. With the proposed technique, the epiphyses, metaphyses, growth plates and articular cartilages of femur and tibia are also sectioned allowing assessment of growth, modeling, and remodeling parameters.

In old rats, the femur contains a high proportion of fat marrow.

Related references

WOODARD et al. 2002

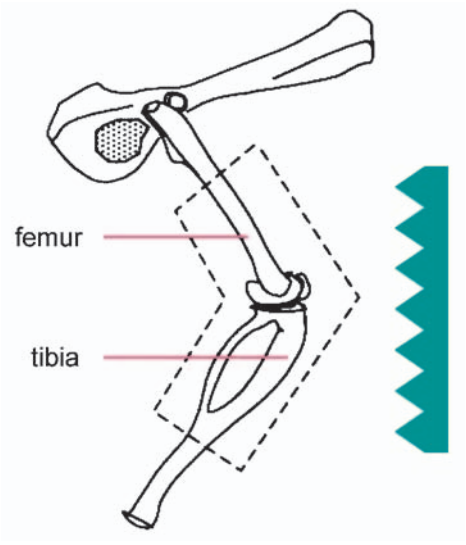


Fig. 9.1a. Femur and tibia.

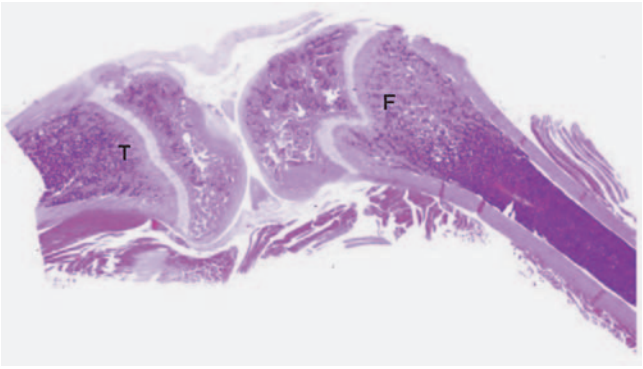


Fig. 9.1b. Femur (F) and tibia (T). Picture taken from a young rat.

10 Cardiovascular system
10.1 Heart

Localization:	Through ventricles and atria with auricles
Number of sections:	1
Direction:	Longitudinal
Remarks:	One half that contains the main vascular trunks

A longitudinal section through both ventricles should be made from the base to the apex of the heart. Do not open the heart at necropsy. The half with the main vessel trunks is blocked to get a section through the opened ventricles and atria with auricles as well as through base, septum, apex, papillary muscle and main vessels of the heart.

Related references
Hebel and Stromberg 1986, Piper 1981, Van Vleet et al. 2002

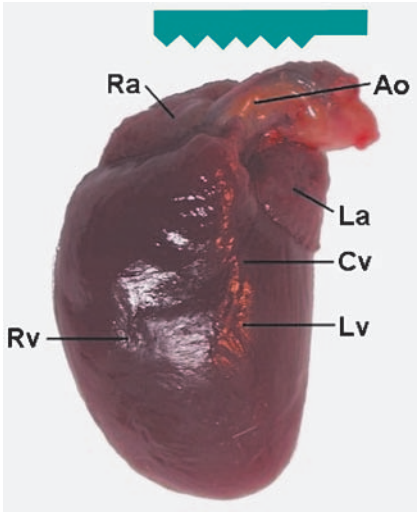


Fig. 10.1a. Heart (Ao: aorta, Cv: conoventricular vein, La: left auricle, Lv: left ventricle, Ra: right auricle, Rv: right ventricle).

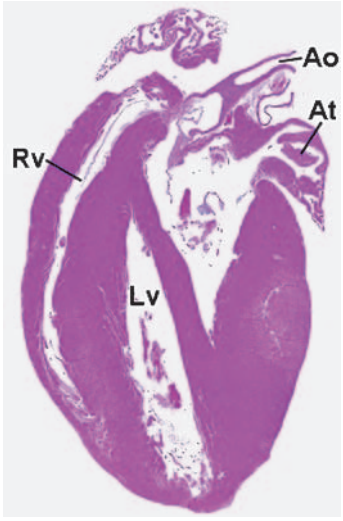


Fig. 10.1b. Heart (Ao: aorta, At: atrium, Lv: left ventricle, Rv: right ventricle).

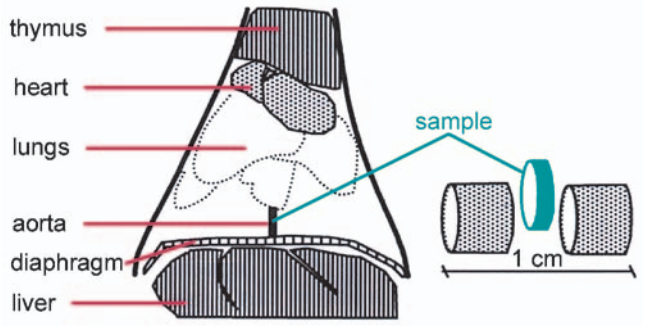
10 Cardiovascular system
10.2 Aorta

Localization:	Thoracic region
Number of sections:	1
Direction:	Transverse

The section should be taken from the middle of the last 1 cm caudal segment of the thoracic aorta. This region is closely attached to the thoracic vertebrae and can easily be removed during necropsy.

Induced lesions of the vascular system are rare in rats except for some pharmaceutical compounds such as vasodilators. Most of the induced changes are observed in smaller arteries and arterioles of various organs but not in thick-walled major blood vessels such as the aorta.

Related references
Van Vleet et al. 2002



↑

Fig. 10.2a. Aorta, ventral view.

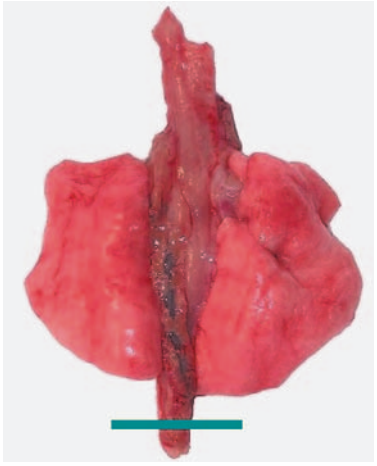


Fig. 10.2b. Aorta, dorsal view.

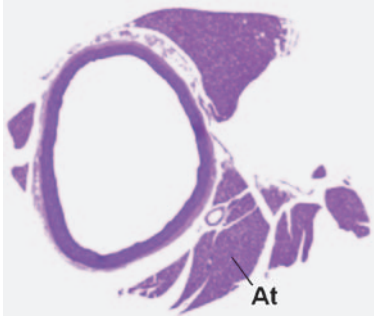


Fig. 10.2c. Aorta and adjacent brown adipose tissue (At).

11 Lymphoreticular system

11.1 Thymus

Localization:	Along the length of one lobe *Option*: whole organ
Number of sections:	1
Direction:	Longitudinal
Remarks:	Largest area Thymic region in old animals

The whole thymus is fixed and trimmed along the length of one lobe. This gives a standardized longitudinal section showing all anatomical structures of this organ.

In immuno-toxicological assays, it is advisable to embed the whole organ, ventral aspect down, and to cut both lobes. In case of thymic atrophy or involution, the whole organ/thymic region should be embedded.

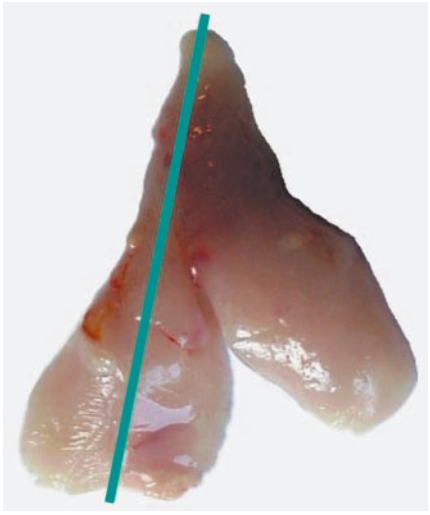


Fig. 11.1a. Thymus, young rat.

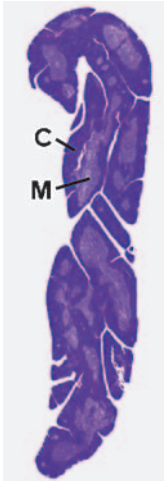


Fig. 11.1b. Thymus (C: cortex, M: medulla).

Related references

Djikstra and Sminia 1990, Kuper et al. 2000, Kuper et al. 2002, Schuurman et al. 1994, U.S. Food and Drug Administration 2002

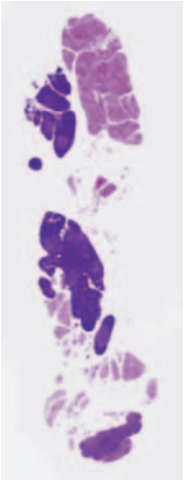


Fig. 11.1c. Atrophic thymus.

11 Lymphoreticular system
11.2 Spleen

Localization:	At largest extension *Option*: whole organ
Number of sections:	1
Direction:	Transverse *Option*: longitudinal horizontal (not shown in the image)

A transverse section is made at the largest extension of the organ, showing red and white pulp. This plane of section guarantees the presence of all relevant anatomical structures and hallmarks of the white pulp, e.g. PALS (periarteriolar lymphatic sheath), marginal zone and follicles.

Related references
DJIKSTRA and VEERMAN 1990, KUPER et al. 2000, KUPER et al. 2002, SCHUURMAN et al. 1994, U.S. Food and Drug Administration 2002

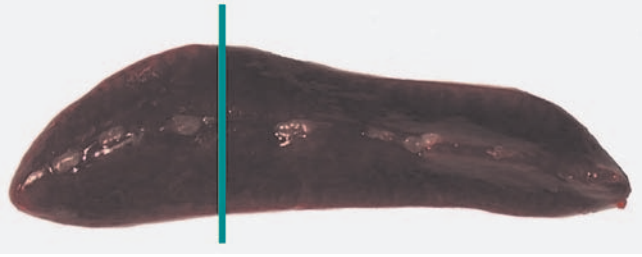


Fig. 11.2a. Spleen.

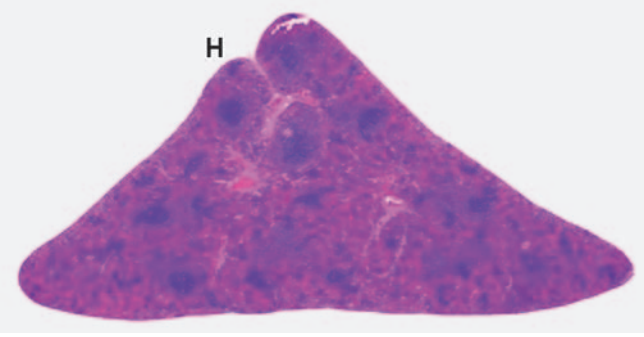


Fig. 11.2b. Spleen (H: hilus).

11 Lymphoreticular system
11.3 Bone marrow (Sternum)

Localization:	Sternum
Number of sections:	1
Direction:	Longitudinal horizontal
Sample size:	2–3 sternebrae
Remarks:	Decalcified

The bone marrow is generally examined concurrently with the bone tissue. From this decalcified section it is possible to evaluate the cellularity, number of megakaryocytes and the stromal compartment. Examination of a bone marrow smear of a core sample from the femur may be useful for evaluation of iron content and more precise cytology.

Related references
KUPER et al. 2000, MACKENZIE and EUSTIS 1990, VALLI et al. 1990, VALLI et al. 2002, WICKRAMASINGHE 1992

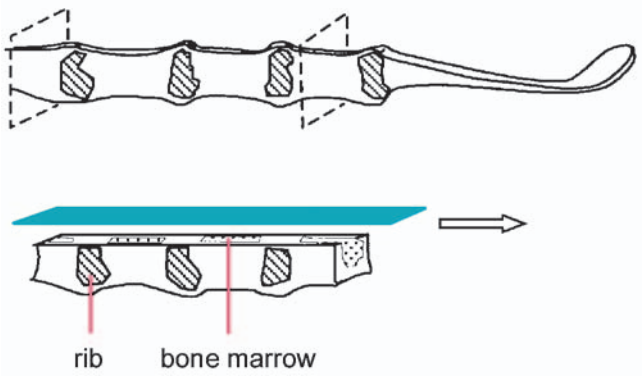


Fig. 11.3a. Sternum with bone marrow.

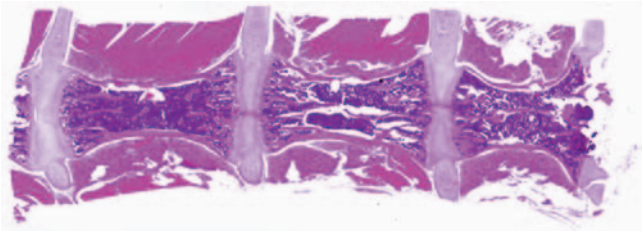


Fig. 11.3b. Sternum with bone marrow.

11 Lymphoreticular system
11.4 Bone marrow smear

Localization:	Core sample from the femoral diaphysis
Number of smears:	1
Remarks:	Slides should only be stored after fixation in methanol. Staining with the Pappenheim, May-Gruenwald-Giemsa or Wright method.

Various techniques have been described. For each method training is necessary to obtain satisfactory smears. Femur diaphysis is recommended as localization because no bone trabecula are intermingled with the hematopoietic tissue.

The proximal and distal epiphyses are cut off with scissors. For removal of the bone marrow, air is blown from one end into the marrow cavity and the marrow cast is collected onto a glass slide. The smear is prepared conventionally with a cover glass. A smear of adequate quality contains grossly visible particles. Marrow smears should be prepared as fresh as possible to avoid blood clotting.

For collection of the bone marrow, aspiration with a pipette containing anticoagulated serum or a small paint brush or a cotton bud from the longitudinally opened femur can also be used.

Related references
VALLI et al. 1990, VALLI et al. 2002

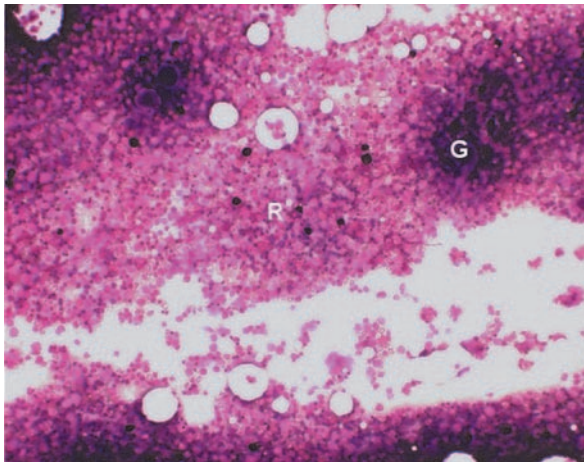


Fig. 11.4a. Low magnification of a rat bone marrow smear, stained with May-Gruenwald-Giemsa, showing grossly visible particles (G) and regions suitable for evaluation (R).

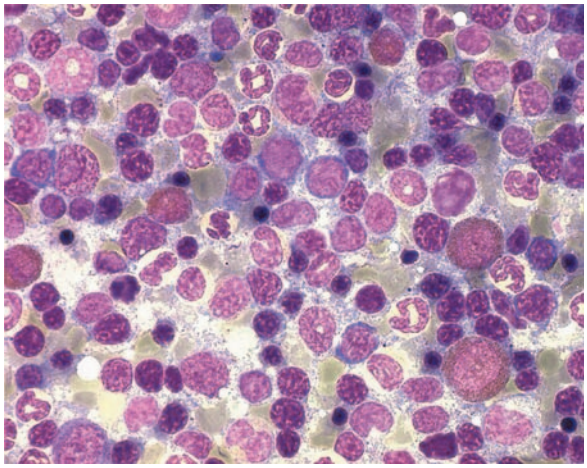


Fig. 11.4b. High power view of a rat bone marrow smear stained with May-Gruenwald-Giemsa.

11 Lymphoreticular system
11.5 Lymph nodes

Localization:	Mesenteric lymph node *Optional:* mandibular lymph node, axillary lymph node, popliteal lymph node, auricular lymph node, inguino-femoral lymph node *Inhalation study:* lung associated lymph nodes
Number of sections:	1 (per lymph node)
Direction:	Longitudinal (largest cut surface)
Sample size:	Whole organ
Remarks:	In case of parenteral application, one lymph node draining the application site and another distant from it should be chosen

The peripheral lymph nodes that are most often examined are the mandibular, axillary and/or popliteal lymph nodes. The lymph nodes are often embedded untrimmed as whole organ due to their small size. It is important that a section is taken through the middle of the longitudinal axis of the lymph node in order to be able to examine all major areas, such as cortex, paracortex and medulla.

Related references

KUPER et al. 2000, KUPER et al. 2002, SCHUURMAN et al. 1994, U.S. Food and Drug Administration 2002, WARD 1990

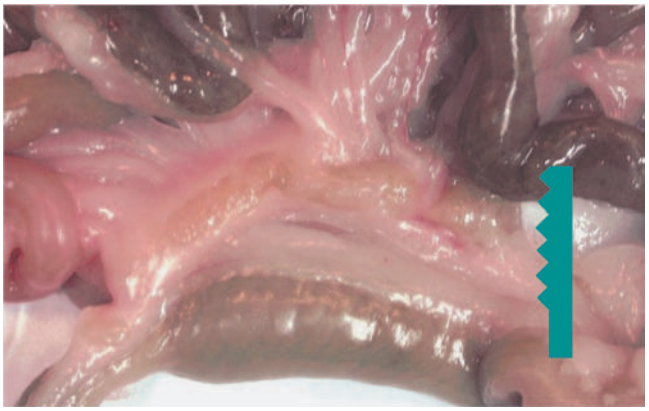


Fig. 11.5a. Mesenteric lymph node.

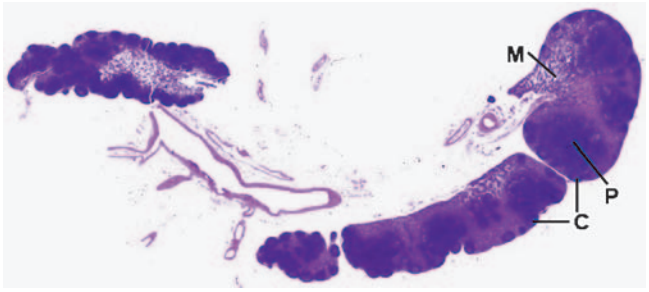


Fig. 11.5b. Mesenteric lymph node (C: cortex, M: medulla, P: paracortex).

References for organ guides

BACHMANN S, KRIZ W: Anatomy, cytology, ultrastructure nephron and collecting duct structure in the kidney, rat. In: JONES TC, HARD GC, MOHR U (eds) Monographs on pathology of laboratory animals. Urinary system, 2nd edition. Springer, Berlin Heidelberg New York Tokyo 1998, pp 3–36.

BANNASCH P, SCHACHT U, STORCH E: Morphogenese und Mikromorphologie epithelialer Nierentumoren bei Nitrosomorpholin-vergifteten Ratten. I. Induktion und Histologie der Tumoren. Z Krebsforsch 1974; **81**: 311–331.

COHEN SM, WANIBUCHI H, FUKUSHIMA S: Lower urinary tract. In: HASCHEK WM, ROUSSEAUX CG, WALLIG MA (eds) Handbook of toxicologic pathology, 2nd edition, Vol 2. Academic Press, San Diego New York Boston, 2002; pp 337–362.

CORMAN BJ, OWEN RA: Normal development, growth, and aging of the kidney. In: MOHR U, DUNGWORTH DL, CAPEN CC (eds) Pathobiology of the aging rat. Vol I. ILSI Press, Washington 1992, pp 195–209.

DJIKSTRA CD, SMINIA T: Thymus. Structure and function. Normal anatomy, histology, immunohistology, ultrastructure, rat. In: JONES TC, WARD JM, MOHR U, et al. (eds) Monographs on pathology of laboratory animals. Hemopoietic system. Springer, Berlin Heidelberg New York Tokyo, 1990; pp 249–256.

DIJKSTRA CD, VEERMAN AJP: Spleen. Structure and function. Normal anatomy, histology, ultrastructure, rat. In: JONES TC, WARD JM, MOHR U, et al. (eds) Monographs on pathology of laboratory animals. Hemopoietic system. Springer, Berlin Heidelberg New York Tokyo, 1990; pp 185–193.

DORMAN DC, BRENNEMAN KA, BOLON B: Nervous system. In: HASCHEK WM, ROUSSEAUX CG, WALLIG MA (eds) Handbook of toxicologic pathology, 2nd edition, Vol 2. Academic Press, San Diego New York Boston, 2002; pp 509–537.

FRITH CH: Biologic and morphologic characteristics of urinary bladder neoplasms induced in BALB/c female mice with 2-acetylaminofluorene. J Environ Pathol Toxicol 1980; **3**: 103–119.

FRITH CH, TOWNSEND JW, AYRES PH: Histology, ultrastructure, urinary tract, mouse. In: JONES TC, HARD GC, MOHR U (eds) Monographs on pathology of laboratory animals. Urinary system, 2nd edition. Springer, Berlin Heidelberg New York Tokyo 1998, pp 319–322.

GELDERD JB, CHOPIN SF: The vertebral level of origin of spinal nerves in the rat. Anat Rec 1977; **188**: 45–48.

HEBEL R, STROMBERG MW: Anatomy and embryology of the laboratory rat. BioMed, Wörthsee, 1986; pp 97–116.

KHAN KNM, ALDEN CL: Kidney. In: HASCHEK WM, ROUSSEAUX CG, WALLIG MA (eds) Handbook of toxicologic pathology, 2nd edition, Vol 2. Academic Press, San Diego New York Boston, 2002; pp 255-336.

KRINKE GJ: Neuropathologic screening in rodent and other species. J Am Coll Toxicol 1989; **8**: 141–146.

KRINKE GJ, FITZGERALD R: The pattern of pyridoxine-induced lesion: difference between the high and the low toxic level. Toxicology 1988; **49**: 171–178.

KUNZE E: Nonneoplastic and neoplastic lesions of the urinary bladder, ureter, and renal pelvis. In: MOHR U, DUNGWORTH DL, CAPEN CC (eds) Pathobiology of the aging rat. Vol I. ILSI Press, Washington 1992; pp 259–284.

KUPER CF, DE HEER E, VAN LOVEREN H, et al.: Immune system. In: HASCHEK WM, ROUSSEAUX CG, WALLIG MA (eds) Handbook of toxicologic pathology, 2nd edition, Vol 2. Academic Press, San Diego New York Boston, 2002; pp 585–646.

KUPER CF, HARLEMAN JH, RICHTER-REICHELM HB, et al.: Histopathologic approaches to detect changes indicative of immunotoxicity. Toxicol Pathol 2000; **28**: 454–466.

LATENDRESSE JR, WARBRITTION AR, JONASSEN H, et al.: Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. Toxicol Pathol 2002; **30**: 524–533.

LIEBELT AG: Unique features of anatomy, histology and ultrastructure, kidney, mouse. In: JONES TC, HARD GC, MOHR U (eds) Monographs on pathology of laboratory animals. Urinary system, 2nd edition. Springer, Berlin Heidelberg New York Tokyo 1998, pp 37–57.

MACKENZIE WF, EUSTIS SL: Bone marrow. In: BOORMAN GA, EUSTIS SL, ELWELL MR, et al. (eds) Pathology of the Fischer rat. Reference and atlas. Academic Press, San Diego New York London, 1990; pp 421–488.

MCGAVIN MD: Procedures for morphological studies of skeletal muscle, rat, mouse, and hamster. In: JONES TC, MOHR U, HUNT RD (eds) Monographs on pathology of laboratory animals. Cardiovascular and musculoskeletal systems. Springer, Berlin, Heidelberg New York Tokyo, 1991; pp 101–108.

MOLON-NOBLOT SM, DUPRAT P: Anatomy of the ocular surfaces, cornea and conjunctiva, rat and mouse. In: JONES TC, MOHR U, HUNT RD (eds) Monographs on pathology of laboratory animals. Eye and ear. Springer, Berlin Heidelberg New York Tokyo, 1991; pp 3–16.

OECD: Test guideline 424. Neurotoxicity study in rodents, 1997.

PAULI BU, GRUBER AD, WEINSTEIN RS: Transitional epithelium, bladder, rat. In: JONES TC, HARD GC, MOHR U (eds) Monographs on pathology of laboratory animals. Urinary system, 2nd edition. Springer, Berlin Heidelberg New York Tokyo, 1998; pp 319–322.

PAXINOS G, WATSON C: The rat brain in stereotaxic coordinates. 3rd edition. Academic Press, San Diego London, 1997.

PIPER RC: Morphological evaluation of the heart in toxicologic studies. In: BALAZC T (ed) Cardiac toxicology, Vol. 3, CRC Press, Boca Raton, 1981; pp 111–136.

POPESKO P, RAJTOVA V, HORAK J: A colour atlas of the anatomy of small laboratory animals. Vol. 2. Rat, mouse, golden hamster. Wolfe Publishing, Bratislava, 1992; p 89.

SCHÄPPI U, KRINKE G: Die Erfassung toxischer Wirkungen am Nervensystem. In: HESS R (ed) Arzneimittel-Toxikologie. Georg Thieme Verlag, Stuttgart New York, 1991; pp 238–258.

SHORT BG, GOLDSTEIN RS: Nonneoplastic lesions in the kidney. In: MOHR U, DUNGWORTH DL, CAPEN CC (eds) Pathobiology of the aging rat. Vol I. ILSI Press, Washington 1992, pp 212–225.

SCHUURMAN HJ, KUPER CF, VOS JG: Histopathology of the immune system as a tool to assess immunotoxicity. Toxicology 1994; **86**: 187–212.

U.S. Food and Drug Administration: Guidance for industry. Immunotoxicology evaluation of investigational new drugs, 2002.

VALLI VE, MCGRATH JP, CHU I: Hematopoietic system. In: HASCHEK WM, ROUSSEAUX CG, WALLIG MA (eds) Handbook of toxicologic pathology, 2nd edition, Vol 2. Academic Press, San Diego New York Boston, 2002; pp 647–679.

VALLI VE, VILENEUVE DC, REED B, et al.: Evaluation of blood and bone marrow, rat. In: JONES TC, WARD JM, MOHR U, et al. (eds) Monographs on pathology of laboratory animals. Hemopoietic system. Springer, Berlin Heidelberg New York Tokyo, 1990; pp 9–26.

VAN VLEET JF, FERRANS VJ, HERMAN E: Cardiovascular and skeletal muscle system. In: HASCHEK WM, ROUSSEAUX CG, WALLIG MA (eds) Handbook of toxicologic pathology, 2nd edition, Vol 2. Academic Press, San Diego New York Boston, 2002; pp 363–455.

WARD JM: Classification of reactive lesions of lymph nodes. In: JONES TC, WARD JM, MOHR U, et al. (eds) Monographs on pathology of laboratory animals. Hemopoietic system. Springer, Berlin Heidelberg New York Tokyo, 1990; pp 155–161.

WHITELEY HE, PEIFFER RL: The eye. In: HASCHEK WM, ROUSSEAUX CG, WALLIG MA (eds) Handbook of toxicologic pathology, 2nd edition, Vol 2. Academic Press, San Diego New York Boston, 2002; pp 539–584.

WICKRAMASINGHE SN: Bone marrow. In: STERNBERG SS (ed) Histology for pathologists. Raven Press, New York, 1992; pp 1–31.

WOODARD JC, BURKHARDT JE, LEE W: Bones and joints. In: HASCHEK WM, ROUSSEAUX CG, WALLIG MA (eds) Handbook of toxicologic pathology, 2nd edition, Vol 2. Academic Press, San Diego New York Boston, 2002; pp 457–508.

WHO: Principles and methods for the assessment of neurotoxicity associated with exposure to chemicals. In: Environmental health criteria 60. World Health Organization, Geneva; 1986.