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GUIDE TO THE NECROPSY OF THE MOUSE

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Preface

In the field of experimental pathology, as well as in that of human clinical pathology, the autopsy is of fundamental importance for the examination of morphological changes associated with pathology, or the states induced by the experimental treatments. Moreover, in particular fields of research, a complete autopsy represents an essential procedure in order not only to supply the researcher with an exact qualitative description of the pathological state, but also for giving precise quantitative estimates, in terms of frequency or incidence of diseases. It is finally superfluous to emphasize that the anatomo-pathological examination constitutes the most important aid, on a morphologic basis, for the study and the identification of pathogenetic mechanisms.

Indispensable to the attainment of such aims is the execution of a technically correct necropsy through the application of a series of systematic operations that are able to examine every part of the animal in the best possible way, without altering any particular characteristic. An improper application of such a rule can often be the cause of an incomplete or inexact appraisal of the normal and pathological characters of the organs and can make the pathologist reach an inaccurate diagnosis.

With a careful external and internal examination of the body, any particular characters of the organs can, in fact, be highlighted such as the position, the relationships with other organs, the volume, together with the shape, colour and consistency; this complete observation helps the pathologist to reach a conclusive judgment on the causes of death.

It is important to stress that, together with evidence found in the course of the autopsy, data on the history of the animal must be also taken into due consideration, as well as symptoms that precede a disease and laboratory examinations (microscopical, chemical and bacteriological) executed before or soon after death. Considered alone, these data collected during the life or after death of the animal cannot however be considered conclusive for an accurate appraisal of the cause of death. This will only be reached when all the above information is collected, analysed and synthesized in a conclusive appraisal that constitutes the epicrisis.

In all the laboratories where animal experiments are carried out, every investigator or expert technician must be trained to complete a careful post-mortem examination.

The scope of this handbook is to offer an essential guide on autopsy techniques for people who work with experimental animals and in particular with the mouse, which is used as an experimental model for the class of the Mammals, and is quickly extending to every field of biological research.

The author also hopes the present effort can provide useful information to help the diagnosis of the most frequent diseases of the mouse.

The first Italian edition was published by Boringhieri in 1972. The present edition follows somewhat the same plan as the first but is largely revised with new figures and photographs.

In the preparation of this edition the assistance of Vincenzo Di Majo (ENEA) was crucial and the author would like to record his indebtedness to him.

The author would also like to acknowledge the expert photographic expertise of the Audiovisual media group of the Department of Anatomy (University of Cambridge, UK) for the preparation of new colour photographs and image manipulation.

This work is the result of a collaboration between two Institutions, i.e. ENEA and University of Cambridge, carried out within the framework of the EULEP - Web Atlas of Pathology (www.eulep.org), a project funded by the European Commission (Contract number QLRT-1999-00320) .

Chapter 1

Introduction

1. General

In order to avoid the progression of post-mortem degeneration processes, the necropsy must be carried out as soon possible.

Autolysis begins as soon as cadaveric rigidity is over, is particularly rapid, especially in the mouse, and leads, in a short time, to the complete lysis of several organs of the animal, starting from the inner ones.

These degenerative and putrefactive phenomena manifest with variable speeds and intensities: the adrenals, the covering epithelia of the gut and the bone marrow are the first to be altered; then the liver, spleen and kidneys, while the heart and the skeletal muscles, the fibrous tissues, the skin and bones appear more resistant.

Figure1. Position of the mouse for autopsy

However, the variations of the environmental temperature can modify the time of appearance and the progressing of these phenomena. The putrefactive processes, in fact, are accelerated by few hours in a warm atmosphere, while a cold atmosphere can delay their initiation considerably. It is therefore necessary to store cadavers in a refrigerator (+ 2°C to +4°C) as soon as possible after death, in case the autopsy cannot be carried out immediately.

2. Materials

Appropriate equipment is necessary in order to carry out a careful necroscopic examination.

The mouse will have to be carefully laid down on an appropriate wooden surface, or on a smooth and square table of paraffin, a few centimetres thick, extended on the back, with the limbs spread and held firmly with pins fixed in the four paws (Fig. 1). The necessary instruments are scissors, pliers, scalpels of varied format and size, and small containers filled with fixative to collect tissue sections to be processed by histological techniques.

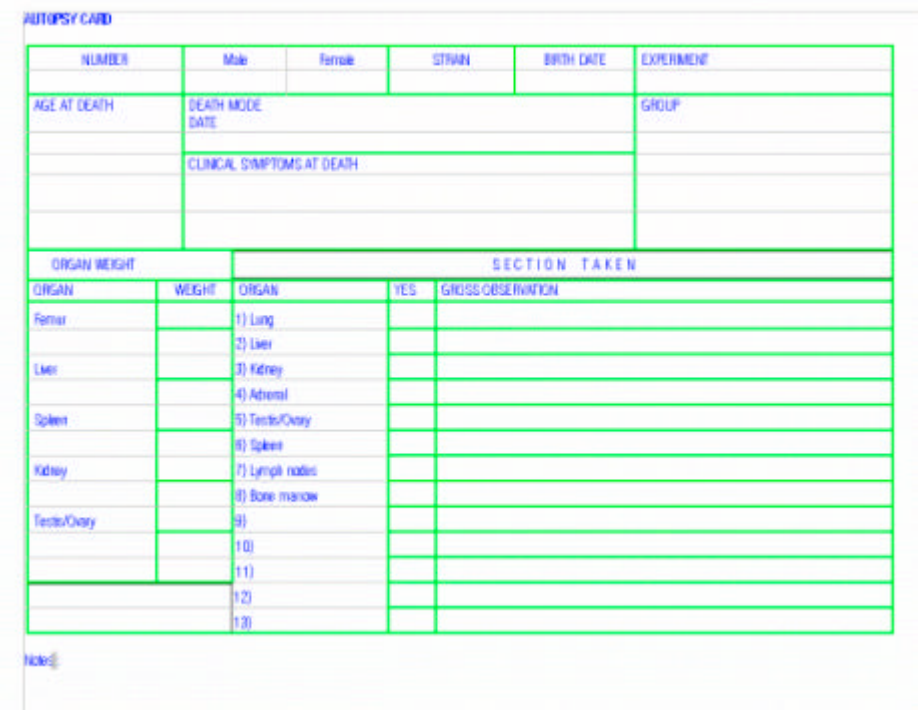
3. Description of the autopsy cards

During the autopsy, the investigator must transcribe, preferably on appropriate cards, all the observations made during the post-mortem examination. These will serve to obtain a general, indispensable objective picture, useful for the pathologist to express his final judgment on the causes of the death (epicrisis).

On the pathology card (Figs. 2 and 3) the information on the identification of the animal will be transcribed, and all the macroscopic observations made during the autopsy will be reported.

All these pieces of information are of great usefulness for the interpretation of the histological observations, particularly when the description of the macroscopic data can be decisive in establishing the diagnosis, or in determining the importance of one lesion as a cause of death.

It is important, during the compilation of the cards, to be as objective as possible in reporting what one is observing. The investigator must describe the detailed aspects of an organ or of a lesion without adding interpretation of what he observes and without attempting to make a pathological classification of the same lesion.


AUTOPSY CARD

NUMBER	Male	Female	STRAIN	BIRTH DATE	EXPERIMENT

AGE AT DEATH	DEATH MODE DATE	GROUP
	CLINICAL SYMPTOMS AT DEATH	

ORGAN WEIGHT		SECTION TAKEN		
ORGAN	WEIGHT	ORGAN	YES	GROSS OBSERVATION
Femur		1) Lung		
		2) Liver		
Liver		3) Kidney		
		4) Adrenal		
Spleen		5) Testis/Ovary		
		6) Spleen		
Kidney		7) Lymph nodes		
		8) Bone marrow		
Testis/Ovary		9)		
		10)		
		11)		
		12)		
		13)		

Notes:

Fig. 2 Mouse card reporting autopsy observations

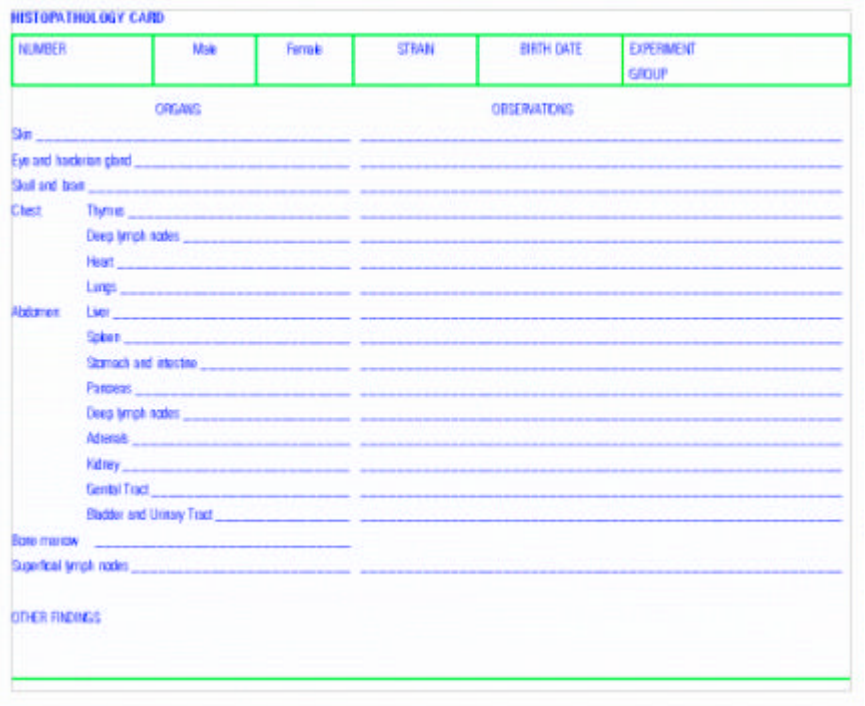


Fig. 3 Mouse card reporting histological diagnoses

In particular, a series of characteristics should be described like the position, the shape, the colour and the consistency of the organs, the aspect of the cut surfaces, the normal or pathological content of some hollow organs, like the bladder, pleura or intestine, about which it will be spoken below.

The better method of filling in the cards consists of dictating a complete description of the organs and the lesions observed to a person who assists.

The other face of the card should contain the histological description of the specimen of every organ taken.

4. Criteria for specimen collection and tissue fixation for histological examination

In general, a histological examination of some or all organs is necessary for a final diagnosis; in this respect, it is important to know how to deal with tissues taken during the autopsy.

First of all, it is not advisable to leave the organ specimens for even a short time in air; they should be fixed as soon as possible. The fixative can be 10% buffered formalin, 5% Zenker formolic acid, or Bouin 's fixative. The storage of the sections in the fixative should not exceed 24 or 48 hours.

It is rarely advisable to fix large entire organs, as they have been removed, but it is suggested to take only some sections, preferably those showing pathological characteristics. The instruments for this operation are a thin scalpel or a common razor blade. Moreover, it is convenient to deal each organ in a different way before the fixation. The heart will be divided in two halves with a median cut taken from the apex to the base, so that the fixative can be absorbed more quickly. A median cut along the greater axis will be executed for kidneys and, in some cases, also for the testes. These methods are essential in order to obtain good histological slides.

Chapter 2

External examination

The external examination is the first procedure to carry out on the animal's body.

1. General condition

The investigator will examine the animal's general condition: state of nutrition and development of the skeletal muscular masses, presence of skin alterations, fur, any superficial lesions.

An animal found in a bad nutritional condition suggests that a chronic disease might be the cause of death. If the body is in a good general state this is an indication of a sudden death, probably caused by an acute process of short duration.

2. Skin and cutaneous adnexa

From the examination of the skin, the presence of traumatic wounds will be obvious, as well as that of ulcers, acute or chronic infectious processes, some types of tumours of the skin, or of the mammary glands.

Oedematous phenomena can moreover be appreciated as they usually cause the skin to become swollen, smooth and glossy. Such phenomenon is called oedema when it is limited to some body areas, and anasarca when, instead, is generalized to the whole body. Phenomena of this type can be correlated with many causes, like post-infectious toxaemia or generalized leukaemic processes.

Another character to take into consideration during the external examination is the state of the fur. The fur appears rough, dry and hirsute in a mouse in bad shape, when a serious disease becomes chronic, or due to the presence of parasites. In these cases, fur has a powdery aspect; crusty and dry areas can also be present, frequently of grey-yellowy colour. In some strains, like the C57Bl, a similar aspect is frequently associated with the presence of parasites or fungi. Moreover, in this strain the depilated areas show particular characters, such as small specks, preferentially localized on the head or on the back. The phenomenon of depilation can also be present in animals aged or treated with high doses of X rays. In this last case, such phenomenon appears more prematurely.

In pigmented strains, variations of fur colour are also found after radiation exposure. In particular, it is observed that colour remains unchanged until the second month after the treatment, also after high doses, and then it begins to turn white in several body regions. The head is the first to become grey, then the depigmentation extends in the order to neck, to the back, and finally the entire fur turns to white. The depigmentation phenomenon is absent in unirradiated and aged mice. The depigmentation, in fact, is a phenomenon typical of the treatment with ionising radiation.

3. Natural orifices

It is advisable to report the anomalies and the lesions of the natural orifices, as the mouth, the nose and the anus to complete the external examination.

The lesions of the mouth are easily detectable by making a careful examination of the state of the tongue, of the mucosa of the fauces, of the lips, and of the teeth.

Anomalies of the tongue are rare and substantially consist of an increase or in a decrease in volume, usually associated with congenital alterations:

Interesting modifications can be observed sometimes on the labial and oral mucosa. Normally, the colour of this mucosa is rose, but one possible finding is a pale mucosa in case of erythroid anaemia or a bluish in passive hyperaemia. Moreover, in mice dead from infectious diseases, haemorrhages of the submucosa can be found as petechia; in some cases superficial erosions of the mucosa, ulcers, or vesicles, are also present.

Lesions of teeth are observable in almost all animals aged or treated with high doses of radiation, and consist of several degrees of loss, erosion or fracture, with particular regard to the incisors, which prevent the animal feeding adequately. It is therefore necessary, when a undernourished mouse is found, to carefully examine the state of its teeth, whose defects, if very serious, can be a cause of death.

The aspect of the mucosa of the nasal openings will have to be also reported, as well as the eventual presence of exudates or of haemorrhages. This last report, called epistaxis, may not only be connected to the action of a trauma, but also to ulcerative phenomena or rupture of the wall of a vessel.

Moreover, equal attention should be paid to the examination of the anal opening. In the animal affected by diarrhoea, the anus is nearly always smeared by faeces and, in some cases, also by blood. A prolapsed intestine (rectal prolapse) can also be observed in the anal area. This lesion, noticeable also during the life of the animal, shows an intense dark-red colour with frequent ulcerative processes due to haemorrhagic stuffing of the walls of prolapsed intestine, and to bacterial invasion. Such lesions are rather frequent and are found in nearly all strains of mice.

Chapter 3

Internal examination

Following the external examination, the investigator can proceed to the dissection of the animal. In some cases the interest of the researcher is focussed on a specific area or organ; in this case the investigator would be tempted to limit his macroscopic examination to that particular region, without trying to have a general picture of all the organs of the animal. This type of limited survey is unadvisable. It is always better to carry out a complete necropsy of the mouse possibly with a fast and accurate method, which reveals the particular characteristics of all the organs and their evident lesions.

We will therefore describe a systematic method, designed to provide and accurate autopsy of a laboratory mouse.

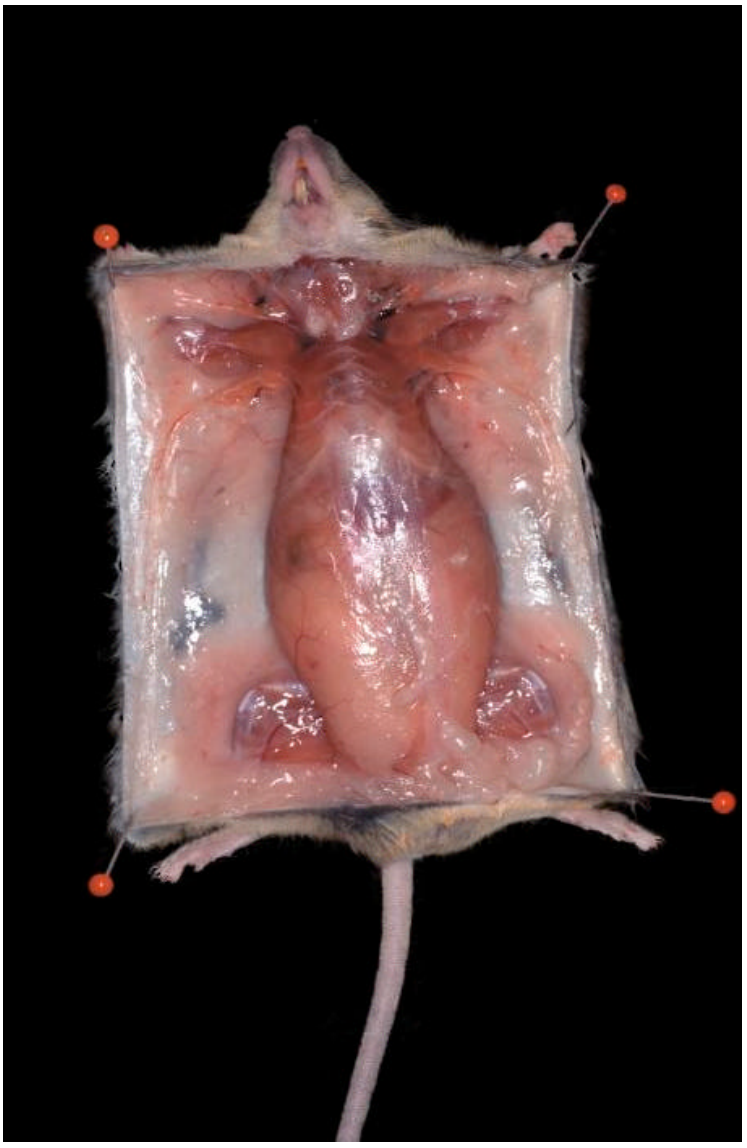


Fig. 4 Opening of the mouse skin: external examination of subcutaneous tissues, muscles, lymph nodes and mammary glands

Once the animal is fixed on the appropriate table in the position described previously, the investigator will make a small cut at the level of the pubis, such as to allow the entrance of one point of the scissors. Then, the investigator will perform a median longitudinal cut superiorly to the chin, having care to accurately separate the skin from of the underlying musculature.

The skin is then dissected and turned on one side and then on the other, so as to obtain an examination field as wide as possible (Fig. 4). The characteristics of the subcutaneous, superficial lymph nodes, mammary glands and of the skeletal muscles will then be apparent.

1. Subcutaneous

The presence of a glossy, gelatinous and clear liquid filled subcutaneous space confirms the diagnosis of oedema, also noticeable during the external examination, while a more or less extended collection of blood, with enlarged margins, indicates the presence of haematoma. Small, more or less numerous, haemorrhagic petechia can moreover be observed more frequently in mice affected by an infectious disease. Variations of the normal colour can also be found for the presence of particular pigments, as it happens in icterus.

2. Superficial and deep lymph nodes

Anatomical outline. In normal conditions, the lymph nodes of the mouse are easily detectable. They are numerous, of variable size in different strains of animals, greyish, and shaped as a small pea or bean. According to the localization, they can be classified as superficial lymph nodes, situated in the subcutaneous area and near the skeletal muscular masses, and as deep lymph nodes, situated inside the thoracic and abdominal cavity or close to the organs. Figure 5 shows a general picture of the morphological characteristics of every lymph node and its localization in the normal mouse.

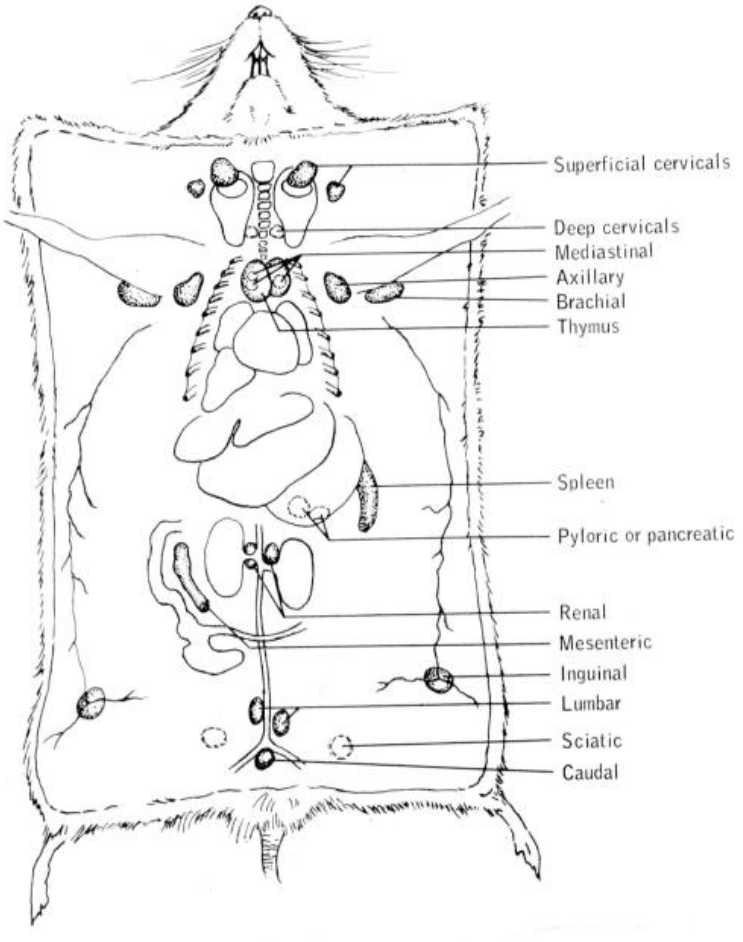


Fig. 5 Scheme reporting localization of the lymphatic system (from T. B. Dunn, 1954, courtesy of the Author)

All superficial lymph nodes are bilateral and can be classified as: cervical superficial lymph nodes, situated immediately above the submandibular salivary glands; axillary lymph nodes, present in the axillary fossa; brachial and retroscapular lymph nodes, in proximity to the angle of the scapula; inguinal lymph nodes situated closed to the bifurcation of the superficial epigastric vein.

The main deep lymph nodes are: the deep cervical lymph nodes, often difficult to localize, the more superficial ones are found in the cervical plane, hidden in the connective tissue that encircles the trachea;

mediastinum or thoracic lymph nodes situated on the posterior face of the two lobes of the intimately connected thymus; the pyloric or pancreatic lymph nodes near the margin of the pancreas; the renal lymph nodes situated between the median margin of kidneys, more often at level of the hilum and in correspondence of the abdominal aorta; the mesenteric lymph node, of lengthened shape, that lies between the mesentery membranes, close to the ascending portion of the colon; the lumbar and caudal lymph nodes localized in proximity to the bifurcation of the aorta.

Examination. Of the several lymph nodes, we will describe the particular characteristics as the shape, the volume, the consistency and the eventual relationships between them and the underlying plans.

The lymph nodes near the centre of an inflammatory process frequently show increase of volume and sometimes haemorrhagic characteristics.

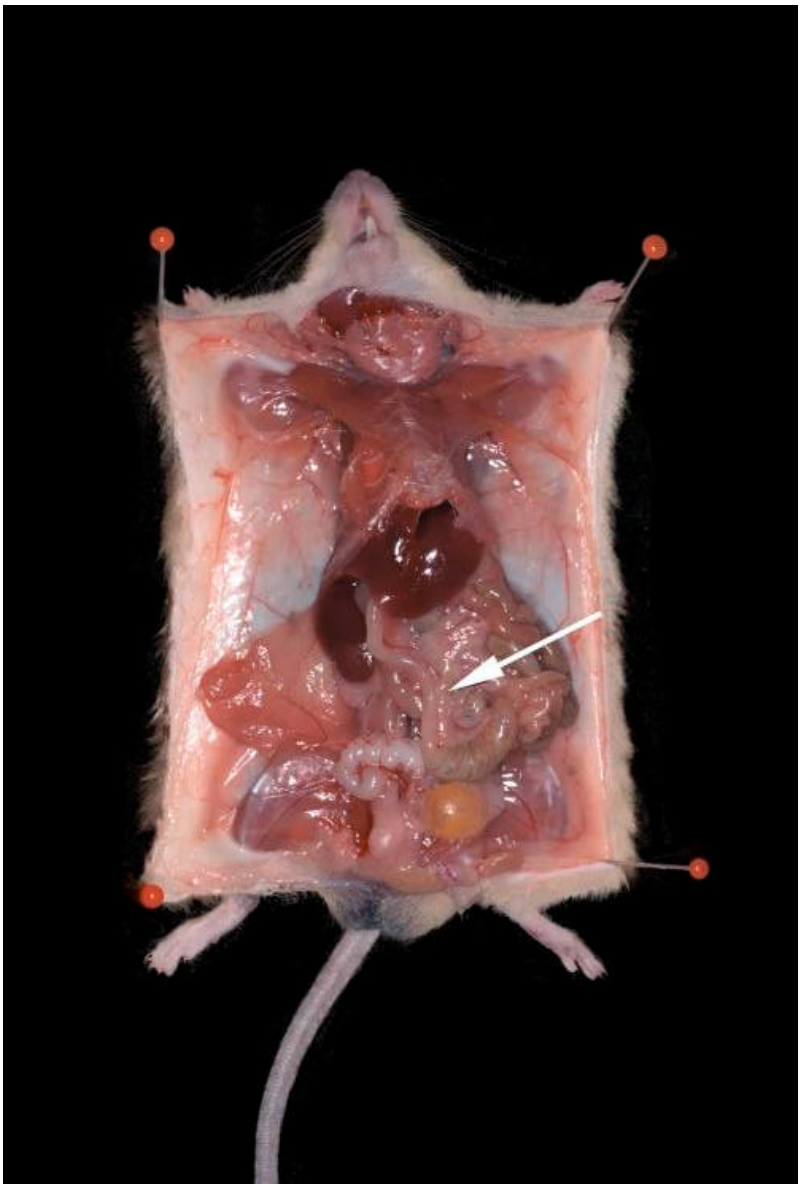


Fig. 6 Observation of the mesenteric lymph node

When, on the contrary, the whole lymphatic system of the animal is affected, this can be ascribed to a lymphoma. But also in these cases, the only character that can be observed macroscopically is enlargement which frequently is also of remarkable degree. Therefore, it will be more prudent to make a diagnosis of lymphoma, even if at this stage enough indicative, only when the necropsy is finished; alterations of the deep lymph nodes and of other organs, like the thymus, spleen and the liver, can be of help.

The investigator must also examine, with particular attention, the state of the mesenteric lymph node. This lymph node in the mouse is found intimately connected, by means of mesentery, to the ascending colon (Fig. 6), and is easily detectable when the small intestine is reflected on the left side. In some diseases, the

mesenteric lymph node can show remarkable variations in colour, consistency, and volume until becoming various times larger than the normal. This happens both in the lymphomas that in the mouse it is believed to originate just from the mesenteric lymph node, and in the so-called mesenteric syndrome described by Dunn (1954), in which you observe a marked proliferation of endothelial tissue, which forms numerous vessels overfilled by blood.

3. Mammary glands

In normal conditions, the mammary apparatus is formed by five pairs of glands (Fig. 7). Three pairs are situated in the thoracic and two in the abdominal region. This glandular tissue is formed by a system of lobules and excretory ducts. When it is completely developed, it is extended through nearly all the subcutaneous region, except in some areas of the back.

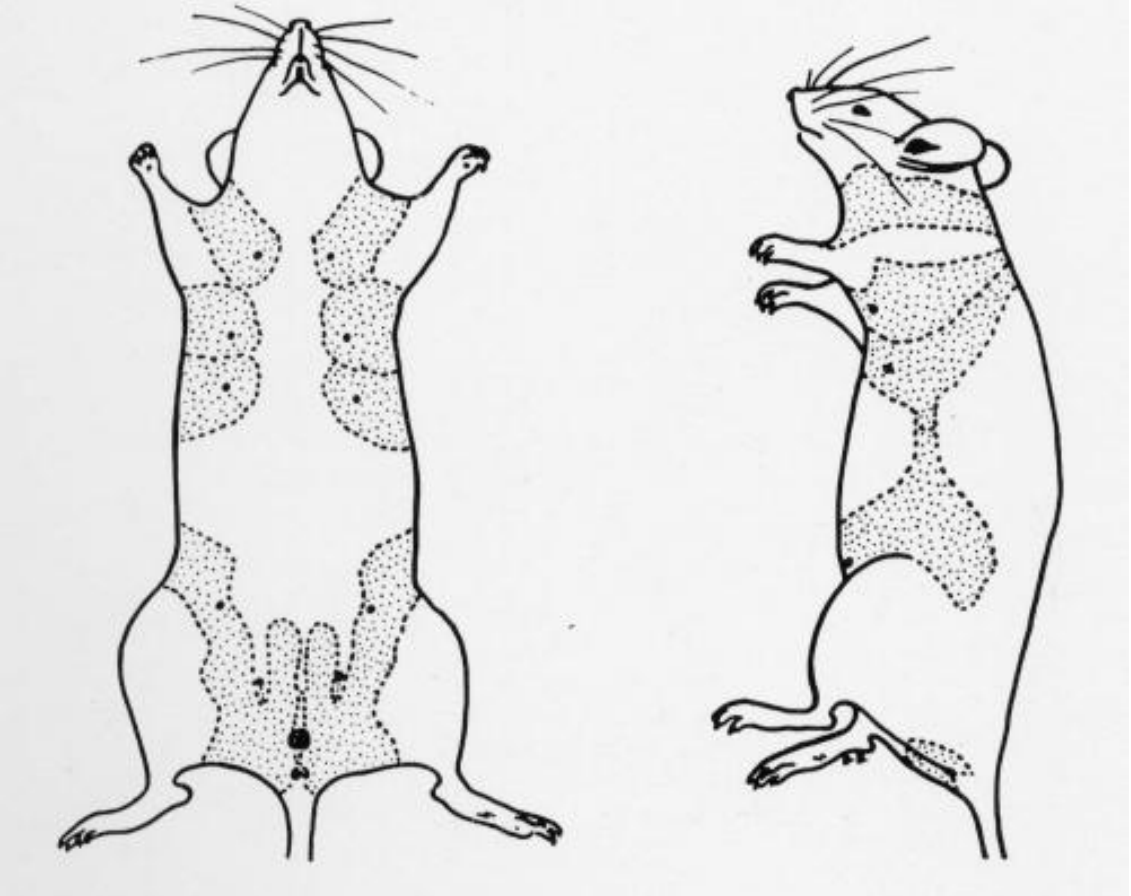


Fig. 7 Scheme reporting the localization of mammary glands (Murphy E.D., chapter 27 Characteristic Tumors, in E.L. Green Ed., "Biology of the Laboratory Mouse", reproduced by permission of McGraw-Hill, New York 1966).

The most frequent lesions of the mammary glands are the tumours. The benign or malignant tumours that originate from the duct epithelia are adenomas and adenocarcinoma. These tumours appear macroscopically as plates or nodules of various consistency, haemorrhagic, sometimes of a cystic aspect, of diameter variable from a few millimetres to some centimetres. Less frequent are tumours that have a connective tissue origin, like fibroma or fibrosarcoma, which show a wooden consistency, are markedly invasive, and often ulcerated at the skin surface.

In some strains of mice, in particular in many C3H lines, a spontaneous incidence as high as 100 percentage of these neoplasms is observed. In other strains, the increase of incidence of these neoplasms can be the consequence of chemical carcinogen, radiation or hormone treatment.

4. Skeleton and skeletal musculature

The various sections of the skeleton may be examined in a systematic way only when there are specific clinical-scientific interests. Otherwise, the inspection will be limited to those skeletal sections that appear as

the autopsy proceeds. A complete examination of the skeleton will be possible only by means of one good X-ray (Fig. 8).

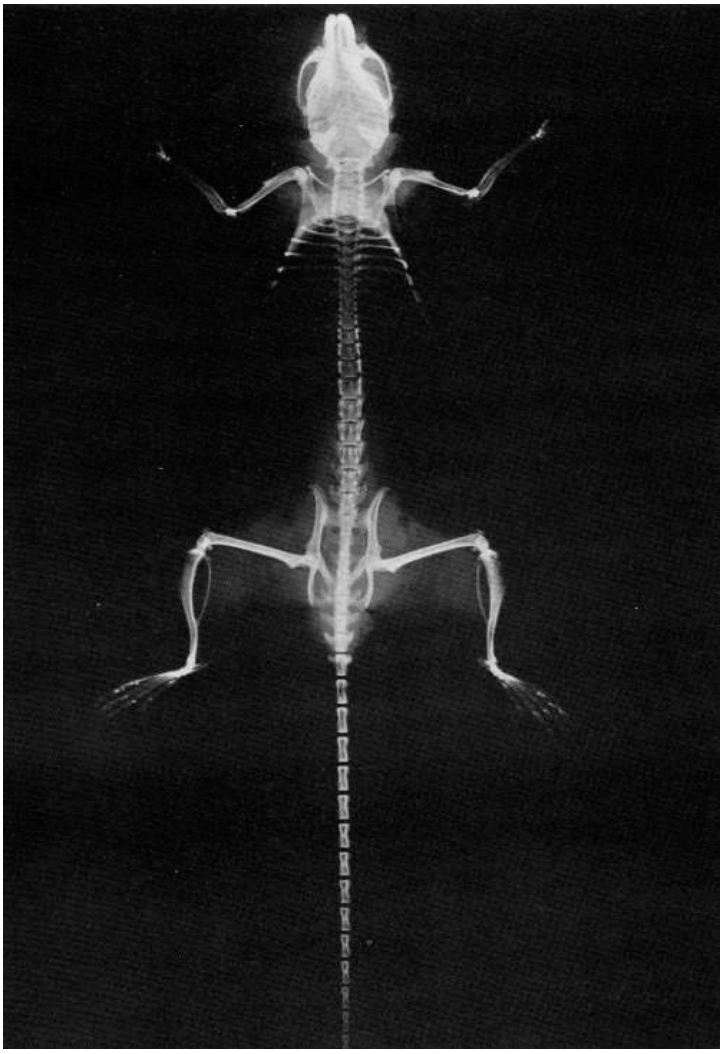


Fig. 8 Mouse X-ray picture

With this technique, it will be possible to detect, in a more detailed way, both the neoformations of the bone (tumours), in many cases macroscopically evident by their mass and for invasion of the adjacent soft parts, as well as thinning or atrophic lesions, particularly frequent in old animals. The state of the skeletal muscles must be then taken into consideration. A marked atrophy is a consequence of an infectious disease, often caused by viruses (dermatomyositis).

Once the observation of the superficial organs is over, the investigator will proceed with the examination of the inner cavities of the animal, starting from the abdomen, then the thorax, and, finally, the skull.

Chapter 4

Abdominal cavity

1. Opening and general examination

Once the abdominal cavity is opened, using a cut along the median axis of the muscular wall (Fig.9), the investigator should note phenomena which will be disturbed later in the progress of the autopsy. Therefore the position of the organs, the presence of any adhesions or liquids in the cavity, will have to be observed.

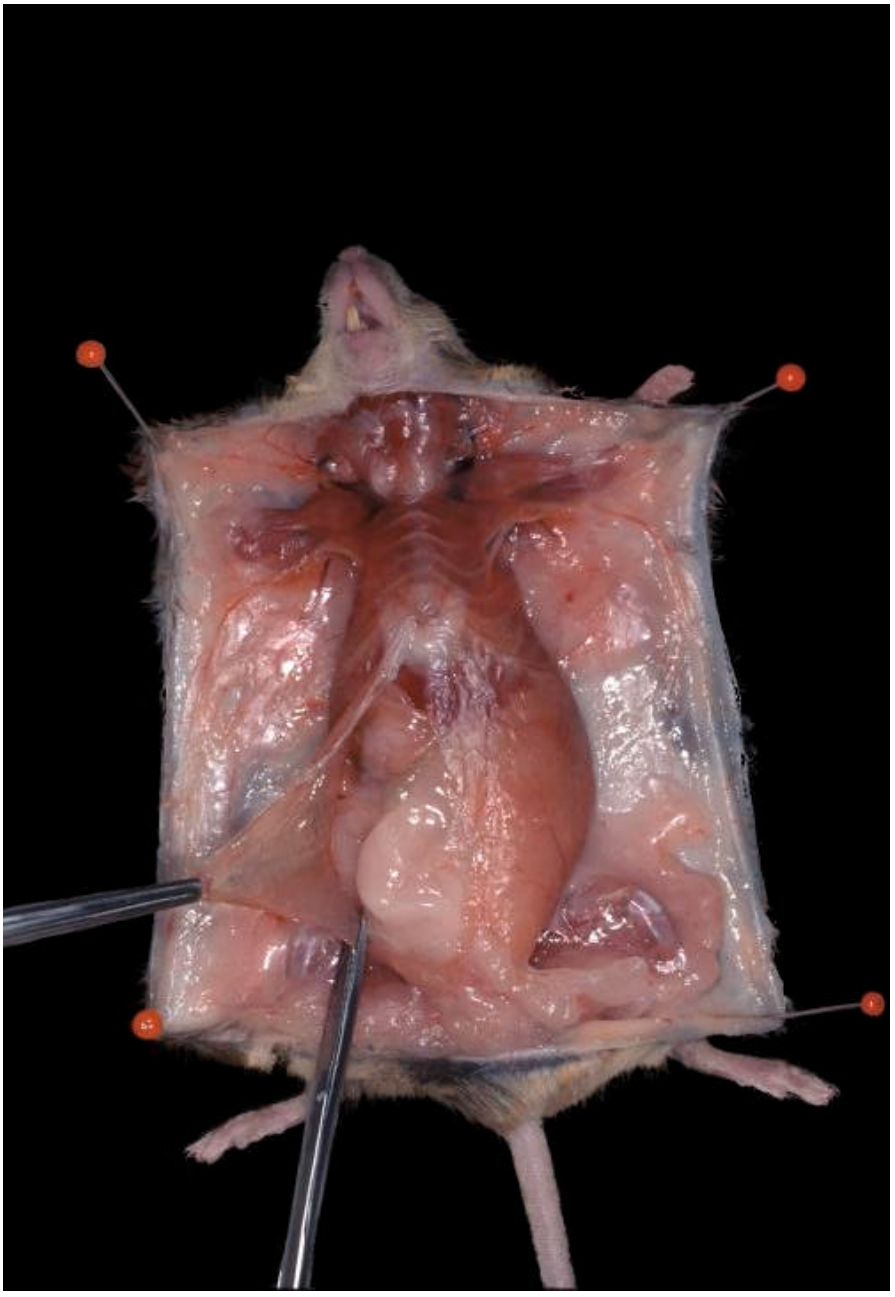


Fig. 9 Opening of the abdominal wall

Variations of organ position (ptosis or situs inversus), or absence of an organ, are congenital anomalies and very rare occurrence. Adhesions between several organs, due to inflammatory or neoplastic processes, as well as amounts of liquids, more or less abundant, are more frequently seen.

The abundant presence of liquids in the abdominal cavity is an indication of a pathological condition, and, in this case, it is important to carefully record this phenomenon, in order to achieve an accurate diagnosis.

An abundant, clear, lemon-coloured liquid (ascites) is as a result of portal hypertension, or of some severe lesions: renal (nephritis or nephrosclerosis), hepatic (cirrhosis) or cardiac lesion. These findings are also frequent in mice bearing generalized lymphoma or leukaemia, associated with the presence of abundant infiltration of neoplastic cells.

The presence of less or more abundant blood (hemoperitoneum) could be a consequence of abdominal blood vessel ruptures, or of inflammatory processes of the arteries (angitis, polyartheritis), trauma, or of spontaneous ruptures of aneurisms. In some cases, the hemoperitoneum can also be a consequence of a rupture of a vessel in a determined organ, affected by hepatic tumours or lymphoma/leukaemia.

The presence of blood in the abdominal cavity frequently produces an inflammatory reaction of the peritoneum, called peritonitis. Various types of peritonitis can be observed: the serum-fibrinous peritonitis or the purulent one, easily distinguishable on the basis of exudate peculiarities. The exudate will be serous with thin fibrinous filaments in the first case, and dense, creamy and yellowish in the second case.

In the abdominal cavity, mainly in the pelvic region, small masses of adipose necrotic tissue can also be found, attached by a connective peduncle to the abdominal wall. This adipose dark-coloured tissue can easily be confused with small glands or other structures.

Once the observation of the abdominal cavity is completed, the investigator can proceed to the extraction of the individual abdominal viscera and to their detailed examination.

2. Spleen

Anatomical outline. The spleen is an organ of predominantly lymph-erythropoietic function. The parenchyma of this organ contains: a) a tissue with erythropoietic function called red pulp, constituted by vessels and cords of various types of red cells (haemocytoblasts, erythrocytes, leukocytes, megakaryocytes); b) a lymphoid tissue called white pulp.

The spleen is situated in the left superior abdominal quadrant; it has a lengthened, oval, slightly curved shape.

The ventral face is smooth and convex; the dorsal one is slightly concave, oriented towards the stomach, to which it is connected by means of the gastrosplenic ligament. Moreover, the hilum, through which the splenic vessels enter the organ, lies on the dorsal face of the spleen.

In a young-adult mouse the spleen measures approximately 15 millimetres in length, 3 millimetres in width, 2 millimetres in thickness. Its average weight is approximately of 100 mg. Under normal conditions, the spleen has a friable and soft consistency, a smooth surface covered by a thin and transparent capsule, and a dark-red colour. Small variations in shape and volume, or the presence of rare aberrant accessories nodules of splenic tissue, can be rarely observed in animals of different strains and ages. In the spleen of the C57Bl mice it has been noticed, with a certain frequency, the presence of black spots, mainly localized on the postero-lateral and anterior part of the organ; these areas are found in the red pulp and are due to the presence of melanin in the cells.

Removal and examination. The spleen will be the first visceral organ of the abdominal cavity to be extracting using tweezers to hold and then cut the hilum together with the gastrosplenic ligament (Fig. 10).

The splenectomy is a simple operation since the organ is easily detached without abundant haemorrhage.

During this extraction the pancreas can also be easily removed due to its intimate connection with the spleen.

The anatomo-pathological examination of the spleen must take into consideration volume, consistency, colour, margins and any evident lesion.

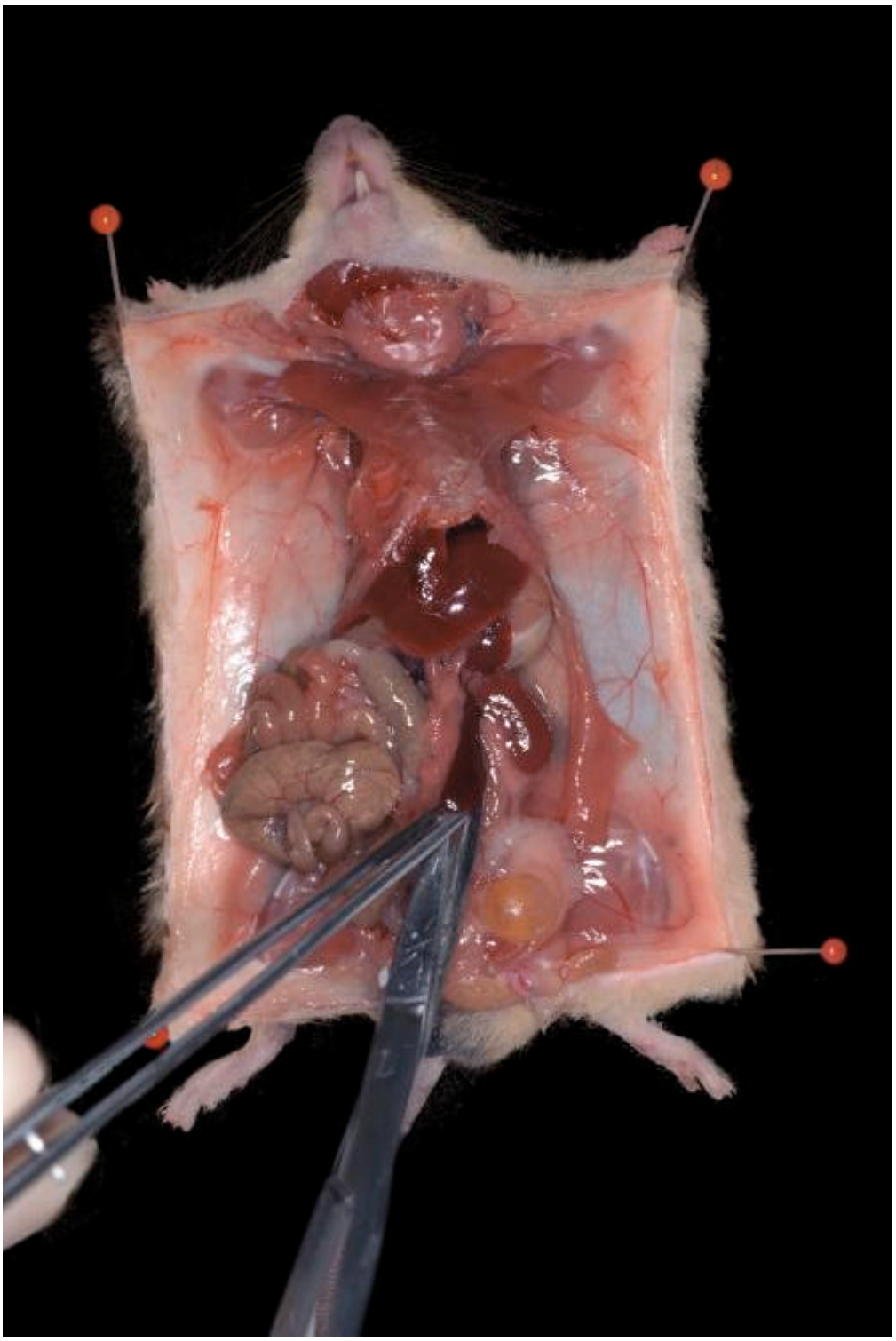


Fig. 10 Spleen extraction

There are lesions, like the lymphoma/leukaemia, in which the organ is enormously enlarged (splenomegaly) with a tense capsule, of a hard consistency, and of rose or greenish colour (chloroleukemia). In these cases, if a longitudinal cut of the organ is executed, the investigator will be able to find on the cut surface proliferation of red pulp (myeloid leukaemia), or presence of trabecular stripes, or many large lymphatic follicles easily visible on the red pulp (lymphoma). The presence of large haemorrhagic zones can be associated with the rupture of vessels.

An enlarged spleen of soft consistency and with an elastic capsule, through which a dark-red colour is visible, is an indication of infectious process at the moment of death. In this case, at the cut, the red pulp will appear hyperplastic, flowing, and easily removable with the scissors.

An enlargement is also seen in the so-called "spleen stasis" for the abundant presence of blood which pools in the organ. Among the causes that can produce this condition, it is worth remembering serious disturbances of the circulation caused by cardiopathies or pulmonary lesions.

Moreover the spleen can be affected by infarcts, which show some peculiar characteristics in relation to the period over which the lesion has been manifested. A strikingly triangular hemorrhagic area, with the base usually oriented toward the surface, suggests a recent infarct. On the contrary, a sclerotic cicatrix suggests that the lesion has taken place well before the death of the animal and, therefore, is not correlated with the cause of death.

Finally the presence of a circumscribed visible mass on the surface of the spleen, usually smooth, haemorrhagic and of dark-red colour is associated with vessel hyperplasia or neoplasia (angiomas).

3. Pancreas

The glandular pancreatic tissue in the mouse is completely enclosed in the mesenteric adipose tissue and does not appear as true compact organ as in the human being. Frequently, in the old animals, the pancreas is atrophic, because it is completely lacking in acini. The atrophy of the pancreas can also be the consequence of a chronic pancreatitis or nodular polyartheritis. In some cases, the pancreatic ducts can be found enlarged as cysts due to stenosis of the pancreatic duct, and its rupture may cause a disease such as fat necrosis (steatonecrosis).

Primary tumours are extremely rare in the mouse. Within these neoplasms, the benign types are represented by nodular hyperplasia, and by the so-called adenomatosis, very difficult to distinguish macroscopically. The malignant tumours, very often of epithelial origin (adenocarcinoma), are highly invasive, mainly in the mesentery and in the adjacent lymph nodes of the liver. Of course, all these tumours are detected more frequently after oncogenic drug treatment.

4. Digestive system

Anatomical outline. The oesophagus is a thin rectilinear tube passing through the pharynx to the stomach. For the two-thirds of its length, the oesophagus lies in the thoracic cavity, behind the trachea. Through the diaphragm, it enters into the abdominal cavity where it is linked to the stomach through the cardiac sphincter.

The stomach is a hollow organ with a bag shape that lies in the ventral part of the abdomen, located mainly in the superior left quadrant, and is partly covered by liver lobes.

The stomach is composed by two parts: the non-glandular and the glandular one delimited by a macroscopically visible edge. The non-glandular stomach includes the superior part of the organ (fundus), called forestomach, and is covered by squamous epithelium. The glandular stomach comprises the body of the organ and continues with the duodenum through the pyloric sphincter. The mucosa of the stomach is thick and with convoluted plica. Looking from the outside, the stomach shows a lesser and a greater curvature.

The intestine is approximately 40 centimetres long and comprises the small and the large intestine. The small intestine is divided in duodenum, jejunum, and jejunoileum. The duodenum starts from the stomach and has a horse shoe shape; it continues into the jejunum that represents the longest tract of the small intestine. The jejunoileum follows, and ends in the caecum. The large intestine comprises the caecum, which consists of a small bag located in the right inferior quadrant of the abdomen in the iliac fossa, and by the colon with its ascending portion (starting from the caecum), by a traverse short portion, and finally by the descending part. The rectum is the final portion of the intestine and it goes from the descending part of the colon to the anus.

Removal and examination. Once the external observation of the spleen and pancreas is completed, the investigator can proceed to the examination of the gut, including the stomach, and then to its extraction. To this aim, it is necessary to take into consideration the external aspect of the intestine and how it is arranged, in order to detect possible distension of the wall, usually caused by the presence of putrefactive gas, or colour variations, caused by enteritis, colitis, or for possible presence of blood.

At this is point, it is suggested that the investigator will first proceed to examination of the mesenteric lymph node.

With just one movement, the extraction of whole intestine and stomach can be achieved, starting with a single cut at the level of the rectum (Fig. 11).

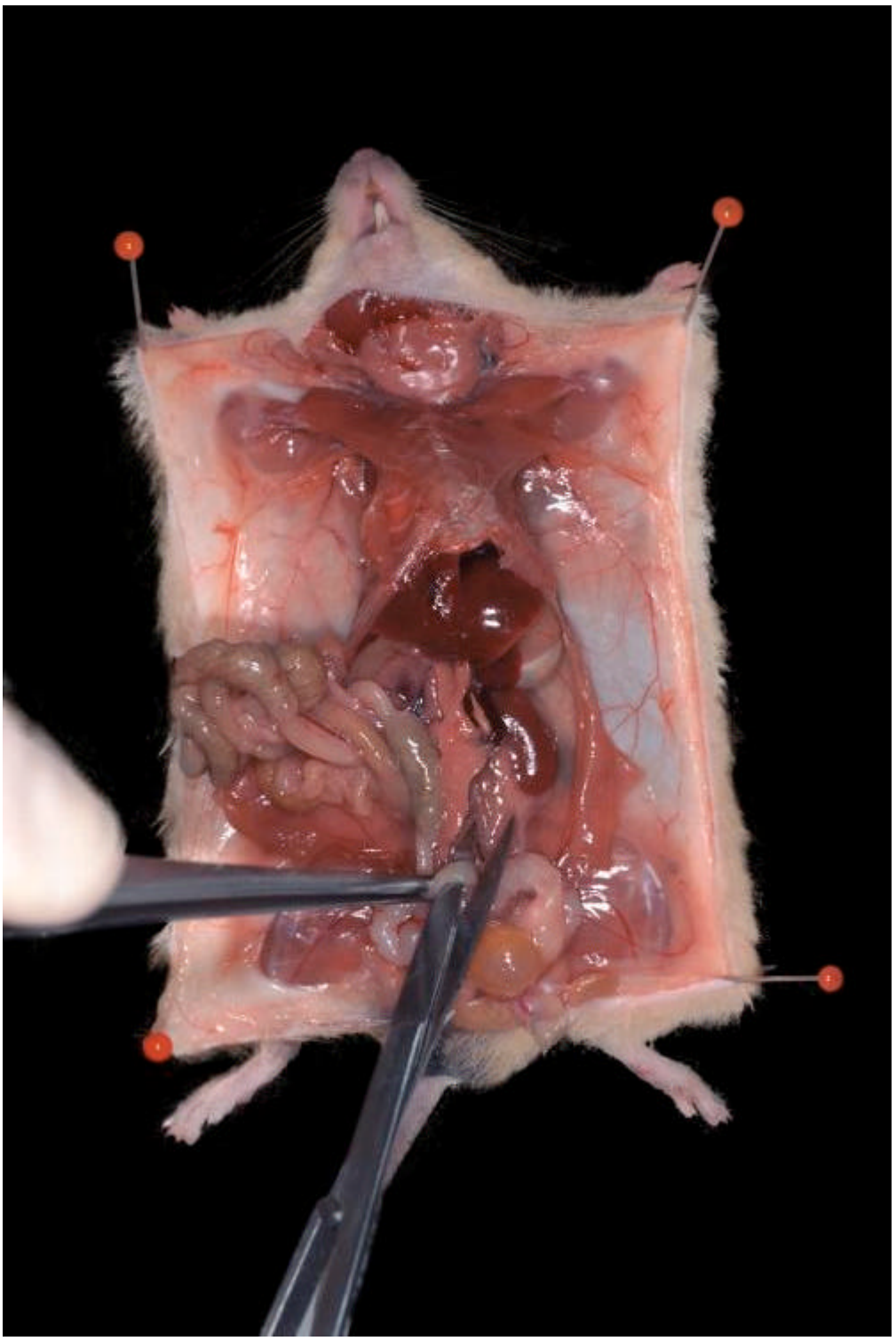


Fig. 11 Stomach and gut extraction

If the investigator holds this segment of intestine (the cut rectum) with forceps and lifts upwards and superiorly , the various intestinal segments can be gradually extracted proceeding in a caudal-cranial direction, cutting the insertion of the mesentery from the vertebral column up to the stomach. If you make another cut at the level of the oesophagus, closed to the cardiac sphincter, the procedure is completed so that the stomach and the intestine can be removed in one piece and are then ready for a detailed examination. Before proceeding to the luminal examination, however, it is advisable to carry out one technical procedure consisting in pulling out the full intestine, to achieve this you must proceed by cutting the intestine insertion of the mesentery (Fig. 12).

Opening of the stomach and the intestine. The opening of intestine and stomach can be made either during the autopsy, with fresh organs, or after a few hours of formalin fixation. To pass to the internal examination of

the gut lumen, the investigator must proceed with a single longitudinal cut up the intestine at the level of rectum, and from here continuing to the stomach, taking care that this cut be performed close to the mesentery insertion.

Fig. 12 Gut extracted and ready to be examined

The investigator is now able to investigate the contents of the intestine, wall thickness, colour, aspect of mucosa and its obvious lesions. In the glandular part of the stomach, particularly in DBA strain, it is possible to observe proliferative lesions of the mucosa, which can also be macroscopically diagnosed as a tumour. The mucosa of the large and small intestine appears reddish, hyperplastic and with fine haemorrhagic petechia or small ulcers in case of an inflammatory lesion (enteritis and colitis). Furthermore, in haemorrhagic gastritis there is an abundant presence of blood, which appears as a dark fluid.

The small intestine walls are normally scattered by small, white, protuberant nodules, called Peyer's patches, which, at the autopsy, can be erroneously diagnosed as small tumours or metastases. In generalized lymphoma, these patches, together with superficial and deep lymph nodes, can appear hardened in texture, and remarkably enlarged to become very big nodules of grey colour. Finally, in the advanced stage of this lesion, the Peyer 's patches can cover the entire intestine wall which will then appear uniformly thickened.

Digestive system tumours are frequent in the mouse and easy to diagnose. The epithelial malignant tumours (adenocarcinoma) are usually localized in the glandular part of the stomach and in the small and large intestine mucosa. They appear as large ulcerated masses with a distinct invasive character. More rarely the

investigator may observe large tumours in the forestomach and small tumours, or polyps, usually localized in the duodenum of the DBA strain.

5. Liver and biliary ducts

Anatomical outline. The liver is a large glandular organ which occupies a a large portion of the abdominal cavity of the mouse. With its superior convex surface, the liver adheres to the diaphragm, while its inferior concave surface is in contact with the stomach and the duodenum.

The liver has four large lobes which join themselves in the dorsal region around the hilum. It can be distinguished a median lobe, two lateral (one right and one left), and one caudal lobe, subdivided into dorsal and ventral half. A thin transparent capsule, called the Glisson capsule, covers the organ. At the macroscopic examination, the cut surface shows a granular aspect due to a typical lobular structure. In fact, the lobules constitute the most important entity of the liver and they are composed by several cords of epithelial cells, arranged in a radial pattern around a vessel.

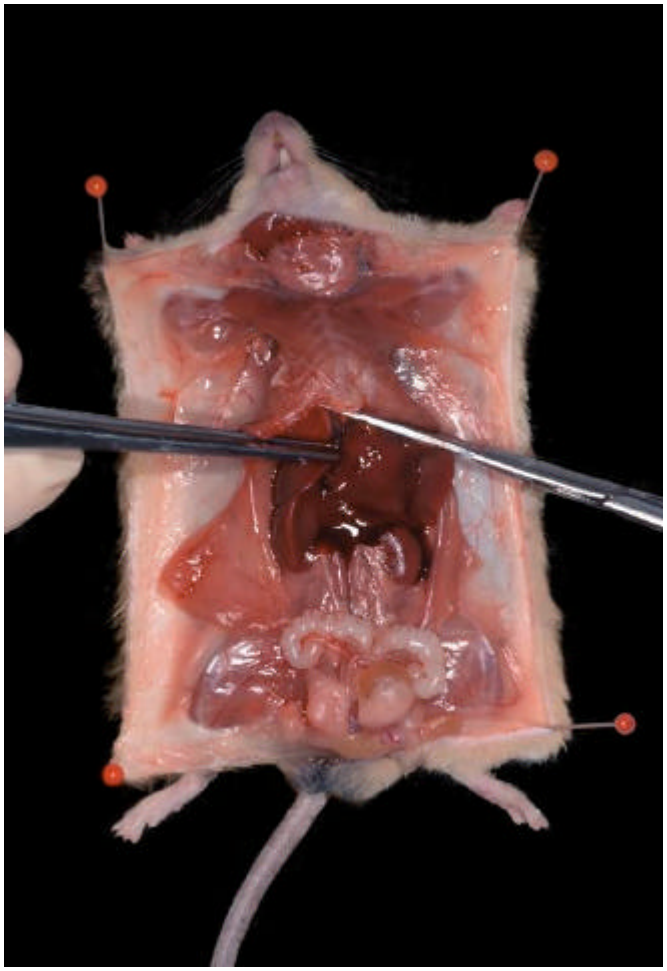


Fig. 13 Liver extraction

The normal colour of the liver is dark-reddish. The liver is hard, but friable when one exerts pressure on the parenchyma. The weight of the liver is of approximately 1.34 g in a mouse of three months of age.

The cholecyst, also called gall bladder, is visible on the inferior surface of the organ where it appears like a small a bag of a few millimetres in diameter. The bile, produced by the liver, reaches the gall bladder via the hepatic duct, where it is concentrated; the bile reaches the intestine through the cystic and the common bile ducts.

Removal and examination. In order to remove the liver, the investigator cuts, with scissors, the falciform and coronary ligaments that keep the organ intimately connected with the diaphragm, taking care not to cause lesions (Fig. 13).Once the liver is isolated, it will be removed, together with the inferior vena cava and the hepatic vessels. The investigator will then pass to the external examination of the organ, taking care to describe the key characteristics such as volume, consistency, colour, and general aspects of the cut surface.

In some lesions, variation of the volume of the organ is often associated with modification of the liver margins: in the case of organ atrophy, such as acute atrophy of the liver and cirrhosis, the margins are thin and sharp; while they become large and round in all the lesions associated with hepatomegaly, such as hepatostasis, fatty degeneration, and inflammation.

Of course, an increase in liver volume is also observed in neoplasia, and it must be remembered that hepatomas are among the most frequent tumours observed in the mouse, particularly in the C3H strain. These tumours consist of globular masses of variable size, from a few millimetres to some centimetres, and are usually localized in one of the greater lobes of the liver; they are usually well circumscribed and of soft consistency; their colour is similar to that of normal liver tissue.

A liver enlargement can also be observed in the case of generalized leukaemia/lymphoma accompanied with an increase in firmness, and variation of the colour from rose to dark-greenish. In some cases, the presence of irregular nodules on the organ surface is observed.

If the investigator proceeds to make a longitudinal cut along the greater lobes, he will then be able to observe more carefully the structure and the consistency of the organ. Possible variations of the colour and aspect of the lobe can be seen.

In many types of lymphoma, the pathologist might observe a massive infiltration of neoplastic cells associated with a pronounced proliferation of the connective tissue, with the appearance of small islands or large stripes, which usually alter the normal architecture of the lobes.

In fatty degeneration, some yellowish areas, surrounded by dark-red parenchyma, are observed at the cut surface. On the contrary, in the condition of "stasis", the normal lobular structure seems preserved, even if a marked congestion is present, due to an abundant blood content in the lobes.

Once the liver observation is completed, the organ is turned with its inferior face towards the investigator to examine the gall bladder. The external characteristics of this organ should be recorded, such as shape, size, and, soon after having produced one small cut, the content of the organ and the state of the covering mucosa.

The volume of the gall bladder is increased by bile retention, mainly caused by the presence of stones or as a consequence of the pressure of abdominal tumour masses. Other conditions may cause a volume decrease; such as chronic inflammatory diseases, ductal obstruction, and tumours.

The gall bladder liquid content, when present in sufficient amount, should be drawn up into a pipette and examined. Usually, the liquid is more or less clear and of a brown colour. Very rarely, the investigator will note the presence of stones; these are small, with smooth irregular surfaces, of different colours from dark-brown to grey, due to their different contents. Finally, the mucosa should be examined. In normal conditions, it has a smooth aspect and shows an irregular, reddish surface due to inflammatory diseases only in few cases.

6. Urinary apparatus

Once the abdominal cavity is freed of the organs previously removed, the investigator will be able to more easily examine the urinary apparatus.

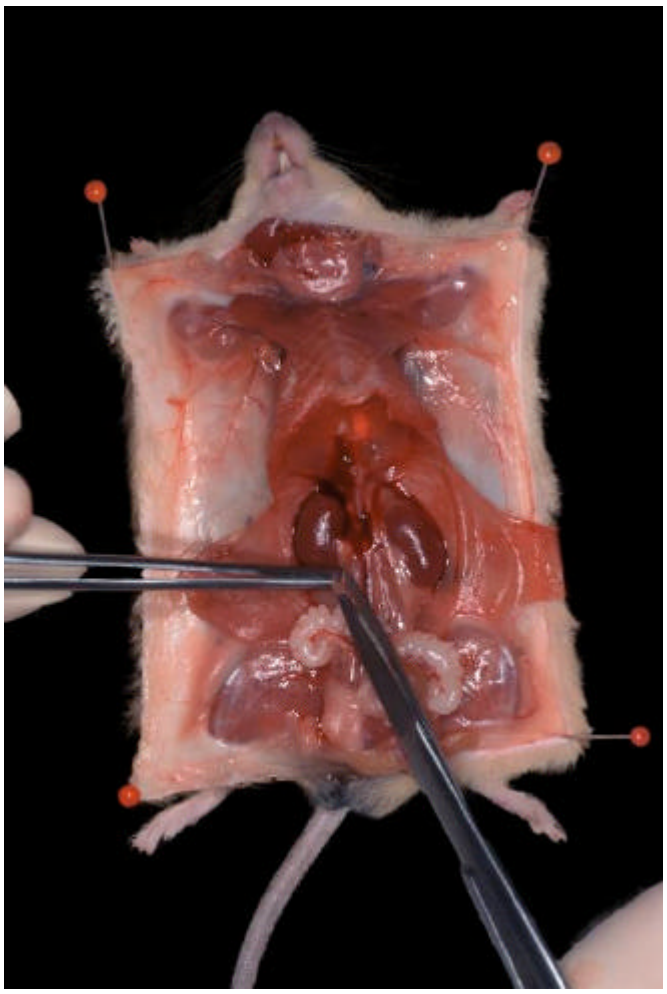


Fig. 14 Kidney and ureter extraction

Anatomical outline. The urinary apparatus consists of kidneys, ureters, bladder and urethra. The kidneys are paired organs, located on the dorsal (posterior) wall of the abdomen, beside the vertebral column. They have a bean shape. In the concave medial margin of the organ, you can appreciate the presence of the hilum of the kidney, from which the main vessels and nerves exit. The ureter also originates from this same area, called the renal pelvis.

The variation in dimensions or position of these organs might be easily appreciated by comparing the two kidneys. In fact, the right is usually larger and heavier that the left one, and it is also positioned more cranially. The weight of the right kidney is approximately 210 milligrams, and that of the left is 200 milligrams. The kidneys show a hard compact consistency, and a reddish colour that sometimes turns to yellow.

The ureters are two thin small ducts that connect the kidneys to the bladder. Their main function is to allow the urine, produced by kidneys, to be collected in the bladder. The ureters end up separately in the posterior part of the bladder, close to the neck.

The bladder appears as a small oval bag, covered by a thin greyish wall, and lies in the anteromedial area of the abdominal cavity. In its inferior part, this bag shrinks and continues in the neck and then in the urethra.

The urethra consists in one medial small duct that goes from the neck of the bladder to the external opening of the penis in the male mouse, and in the fossa clitoridis in females. This tract represents the last part of the urinary system, through which the urine is eliminated.

Just for topographical reasons, the adrenal glands are described in this chapter, dealing with the urinary system, because these glands lie on the superior pole of the kidneys and are intimately joined to them by fibrous and fat tissue. In normal conditions, it would be very difficult to isolate and extract these organs for their small size, that do not usually exceeds the head of a pin.

Removal and examination of kidneys, adrenal glands and ureters.
An useful technique for extracting the kidneys and ureters altogether consists in grasping the inferior part of the ureter with forceps near to its opening (Fig. 14) and lifting upwards.

The ureter is then detached from its attachments to the dorsal body wall up to the renal pelvis; here the main vessels are cut so that the investigator might proceed to the removal; in this way the adrenal glands will be also removed.

Nephrosclerosis and glomerulosclerosis are the most common lesions of the mouse kidney, macroscopically detectable mainly in aged animals. In these cases, the organ will be reduced in volume, pale, and with a shrunken surface. In the terminal stage, the lesion may also cause an increased hardness of the whole organ, which is easily detected when the longitudinal cut is subsequently made ; sometimes small cysts, filled with urine, can also be seen.

Hydronephrosis and pyonephrosis, on the contrary, may cause an enlargement of the kidney. In such a case, the investigator should make a careful examination of the ureters because some disease, such as inflammation, presence of stones or of tumours, can be closely correlated with this enlargement.

Of course, the enlargement of the kidney is also detectable in presence of tumours; rare in the mouse. The tumours of the kidney generally consist in a whitish nodule present just in one of the two organs. Finally, in leukaemia/lymphoma, a diffuse or nodular whitish tissue is detected, with the same characteristics observed in other organs when similarly affected (spleen, liver).

At macroscopic examination, the adrenal glands show some morphological differences correlated with the sex of the animal. In the females, the adrenals appear of an opaque pale colour, because of the high lipid content, while, in the male, they are often rose-coloured and translucent. The volume of the adrenals can also be greatly increased by the presence of a large nodular formation or tumour of variable dimensions and of dark colour.

Removal and examination of the bladder. The technique for the extraction of the bladder is identical in both sexes and is executed by grasping the highest point of the bladder with forceps and making a small cut with the scissors, as a keyhole, on the front wall. The liquid present in the cavity should be sucked up through the opening with a small pipette.

This operation will become necessary in case of vesical urinary retention, due to paresis or blocked outflow, due to the abundant amount of stagnant urine. In this case, the bladder walls are so distended that removal of the contents is very difficult. Therefore, after having emptied the organ of its contents, the investigator makes a cut at level of the neck, possibly in the lowest part.

With this technique, the prostate gland, which is very difficult to detect macroscopically, will then be removed.

Diseases that alter the shape and the volume of this organ (prostatic hypertrophy, tumours) are observed very rarely in the mouse; in these cases only, the investigator will be able to describe its macroscopic characteristics.

The female and male genital apparatuses will be examined separately, having specific configurations in each sex and different relationships with the surrounding organs.

7. Female genital apparatus

Anatomical outline. The genital apparatus of the female mouse includes the uterus, the ovaries, the oviducts and the vagina.

The uterus is formed by a tubular median part (body of the uterus) and two lateral formations (lateral horns). The caudal part of the uterus body is called the neck.

The uterus is found in the inferior part of the abdominal cavity and adheres to the posterior wall of the abdomen. In particular, the body of the uterus is placed anteriorly to the urinary bladder and is covered by a serosa adherent to the posterior wall of the abdomen. In normal conditions the colour of the organ is greyish.

The ovaries lie close to the inferior pole of the kidneys and are tied to the posterior wall of the abdomen by means of the mesovarium. They are small bilateral spherical organs with smooth surfaces. During the period

of the sexual maturity, the ovaries assume a nodular aspect due to the presence of follicles and corpora lutea.

Beyond their role as endocrine glands, the ovaries have the fundamental function of production of the oocytes that, once expelled, pass into the uterus through the oviducts, and there are fertilized.

The oviducts are two tight and small tubes, wrapped around themselves as a ball; they connect the ovaries to the horns of the uterus.

Finally, the vagina is a short channel that begins from the neck of the uterus and ends externally in front of the anal opening. In the abdominal cavity, the vagina lies in front of the rectum and behind the urethra.

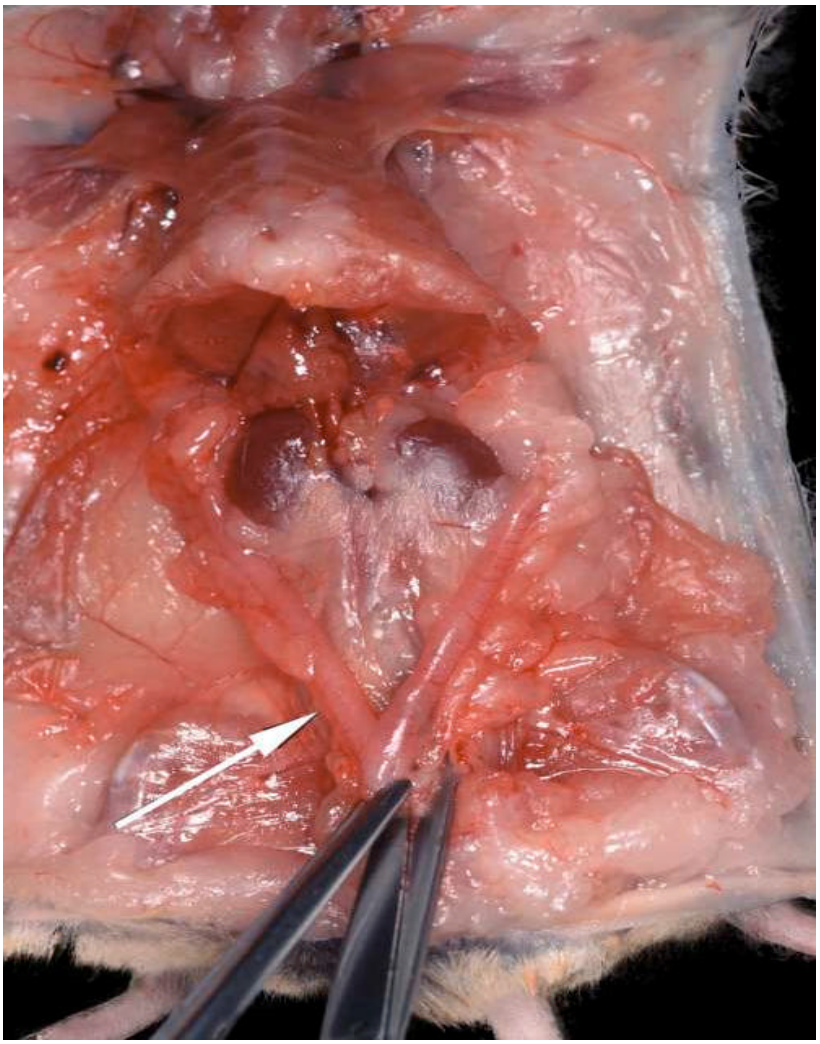


Fig. 15 Uterus Removal

Removal and examination. The Female genital apparatus is excised by making a cut on the median body of the uterus close to the neck (Fig. 15). From here, proceeding upwards, the mesometrium ligaments, that fix the organ to the posterior wall of the abdomen, are cut up to the level of the ovaries at the back. The investigator will then delicately cut those ligaments by which the ovaries are attached to the inferior poles of the kidneys, as well as from the mesovarium ligaments, with which the ovaries are joined to the posterior wall of the abdomen. Isolated organs are extracted and examined for shape, volume, consistency and obvious lesions.

Variations of uterine shape are very rare and are associated usually with congenital malformations. More frequently, the enlargement of this organ is associated with the presence of neoplasia (fibrosarcoma), or to the collection of pathological liquids (hydrometra, pyometra). In these latter cases the uterus will be markedly enlarged.

The investigator will make some cuts on the surface of the organ to investigate the consistency, the thickness of the walls and the character of the contents. Moreover, enlargements are found in case of leukaemia/lymphoma or cystic hyperplasia of the endometrium.

The ovaries are then examined for their volume and surfaces. Tumours (luteoma, tubular-adenoma), cysts, leukaemia/lymphoma are some of the lesions that more frequently affect this organ and induce variations of shape or volume.

8. Male genital apparatus

Anatomical outline. In the male mouse, the genital apparatus includes the testes, receptacula seminis, the epididymis, the penis and the preputial glands.

The testes are two organs of oval shape with a diameter of a few millimetres, situated at the side of the bladder and inside the scrotum. They are covered by a smooth and transparent membrane (tunica albuginea). Under normal conditions, they are greyish-white, of soft elastic consistency. The average weight is 103 mg for the right and 101 mg for the left testis.

The testes consist of numerous tubules covered with various cell layers and their main function is the production of mature sexual cells or spermatozoa. Moreover the testes produce male sexual hormones or androgens.

The spermatozoa produced from the testes pass into the urethra through the excretory ducts (epididymis and the deferent ducts).

The receptacula seminis are two bilateral structures that are found at the bladder sides and are in communication with the urethra. They are formed from numerous small secretion filled cavities. They have a curved flattened shape and a clear-grey colour. Owing to their vesicular conformation these formations have a soft consistency.

In the mouse, the preputial glands are included in the connective tissue, they are leaf shaped, and lie above the penis. They are of a dark-grey colour and a soft consistency.

Removal and examination. The investigator first of all must check the position of the organ, because, when the abdominal cavity is opened, the testes are often found outside the scrotum, leaning to the posterior wall of the abdomen and at the sides of the bladder; therefore, by grasping them delicately, they may be removed, cutting the excretory ducts close to their outlet in the membranous urethra (Fig. 16).

The investigator will then record the shape, the volume, the consistency, the presence of tumour masses and the characters of the epididymis. If a median longitudinal cut of the testes is made, he will also appreciate the colour, cut surface consistency, presence of inflammatory zones or of neoplasms.

A rupture of a blood vessel that accompanies the excretory ducts or the same testis usually causes the enlargement of the organ. In this cases the testis appears markedly enlarged with wide areas of blood infiltration, the remaining parenchyma is reduced to a small necrotic portion.

The receptacula seminis are often expanded and filled of serous liquid. This happens when an obstacle is present in the ejaculatory duct. In other cases, they are enlarged and filled of a yellowish liquid (pus), as it happens in the purulent inflammation. These lesions are of great diagnostic importance in the mouse, because they can be often the cause of peritonitis.

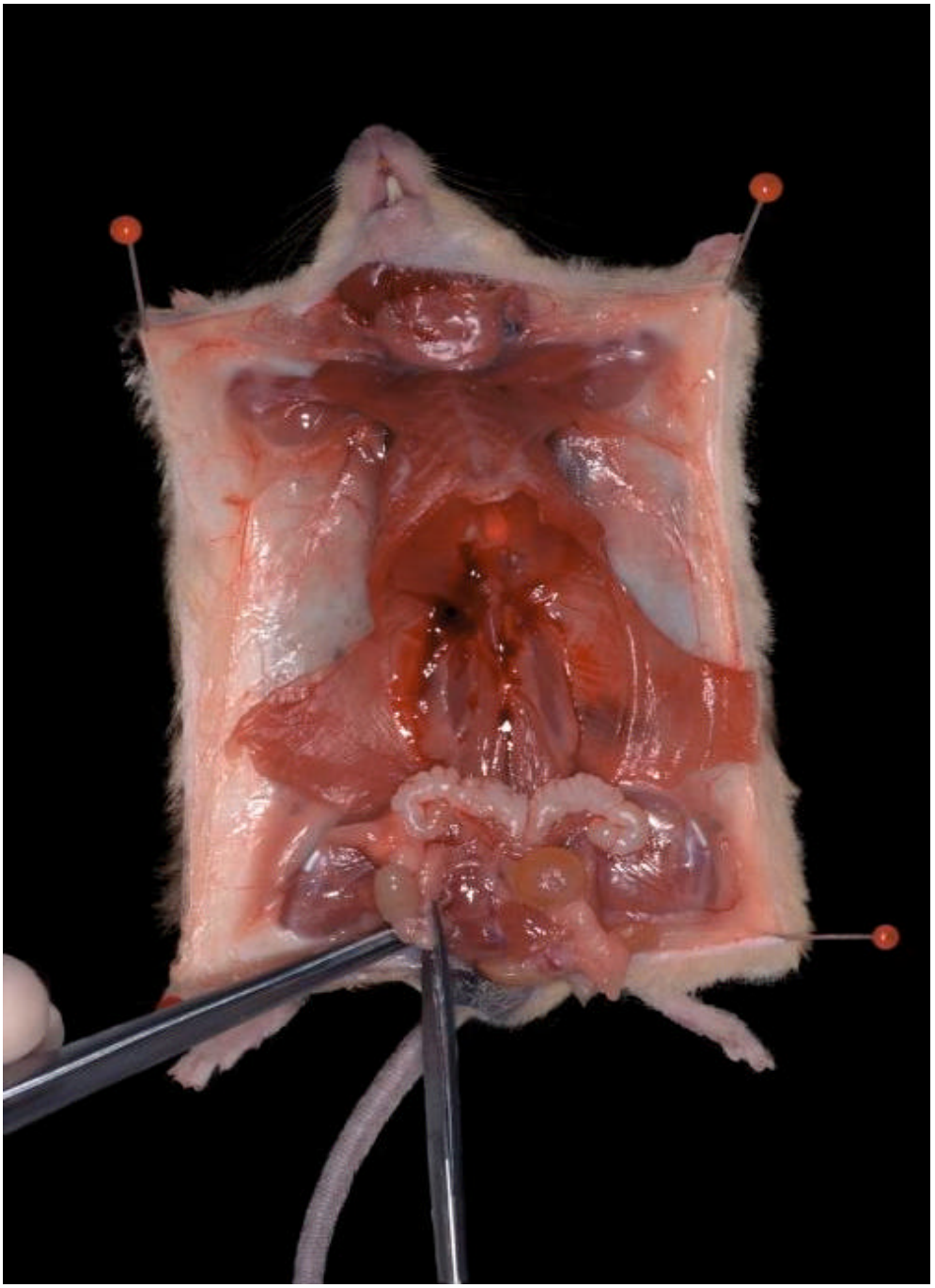


Fig. 16 Testis extraction

In order to complete the examination of the male genital apparatus, the investigator will then examine the penis and the preputial glands. The abscesses of these glands represent a rather common lesion in old animals and they can sometimes be detectable on the correspondent ulcerated skin.

Chapter 5

Thoracic cavity

1. Opening and general examination

The investigator will now proceed to open the thoracic cavity starting from the last rib by cutting the costal cartilages at their point of union with the bone. Continue cutting, proceeding up both sides, until reaching the articulation of the sternum with the ribs (Fig. 17); the inferior part of the sternum is raised with the clamp and, then, its insertions with the front margins of the diaphragm are cut. Keeping the sternal plate raised, the insertions of its inner face with the parietal pleura and the pericardium are finally separated.

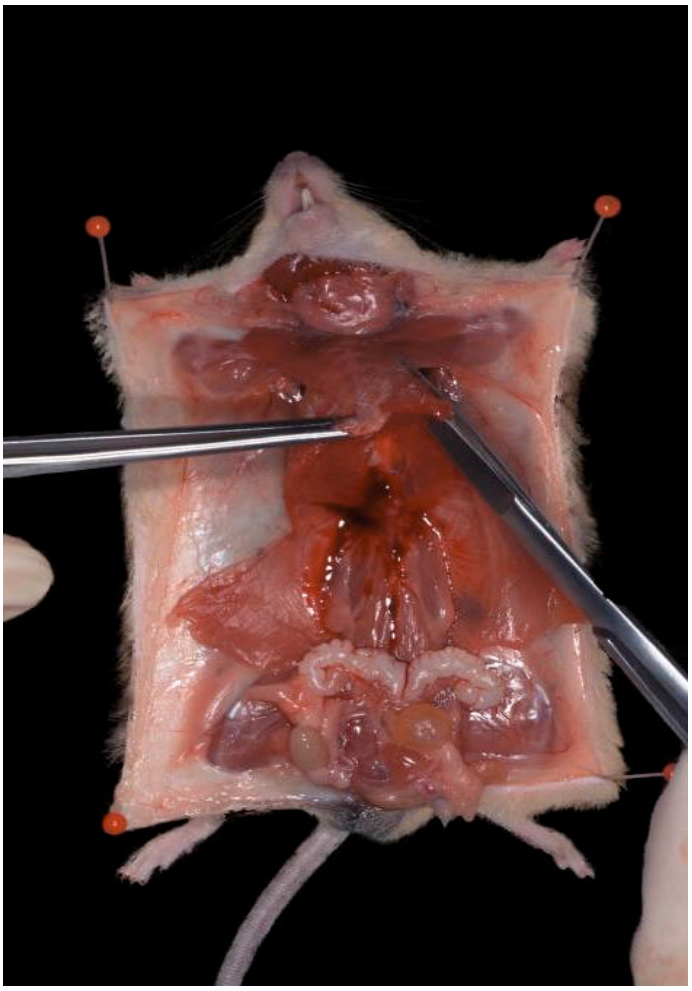


Fig. 17 Opening of the thoracic cavity

Once the thoracic cavity is opened, the investigator will be able to pass to the removal of the viscera.

Also in this case, as it was indicated for the abdominal cavity, it is necessary to pay attention to some characteristics that can be modified as a consequence of the extraction techniques. In this respect, the investigator must consider the position of the organs (situs inversus, congenital anomalies), the presence of adhesions (as a result of pleuritis or pericarditis) or hydro- and haemothorax.

2. Thymus

Anatomical outline. The thymus in the mouse consists of two lobes, and lies on the median line of the vertebral column, close to the base of the heart.

The right lobe is longer than the left, and has an oval shape flattened in its antero-posterior diameter. The left lobe is wider and shorter and has a triangular shape. A smooth thin capsule covers the two lobes. The lobes have a parenchymatous consistency and white colour. In normal conditions, the thymus of an adult male mouse of three months of age has an average weight of 30 mg.

Removal and examination. The thymus is the first organ of the thoracic cavity to be extracted. One of the two lobes in its inferior part is raised with a clamp and the ligaments between its inferior face and the pericardium are unglued with the scissors.

In normal conditions, the organ shows variations of dimensions in relation to the age of the animal. In fact, in the young mouse (Fig. 18), the thymus is well developed, while in the adult or in the old age is atrophic and difficult to detect macroscopically.

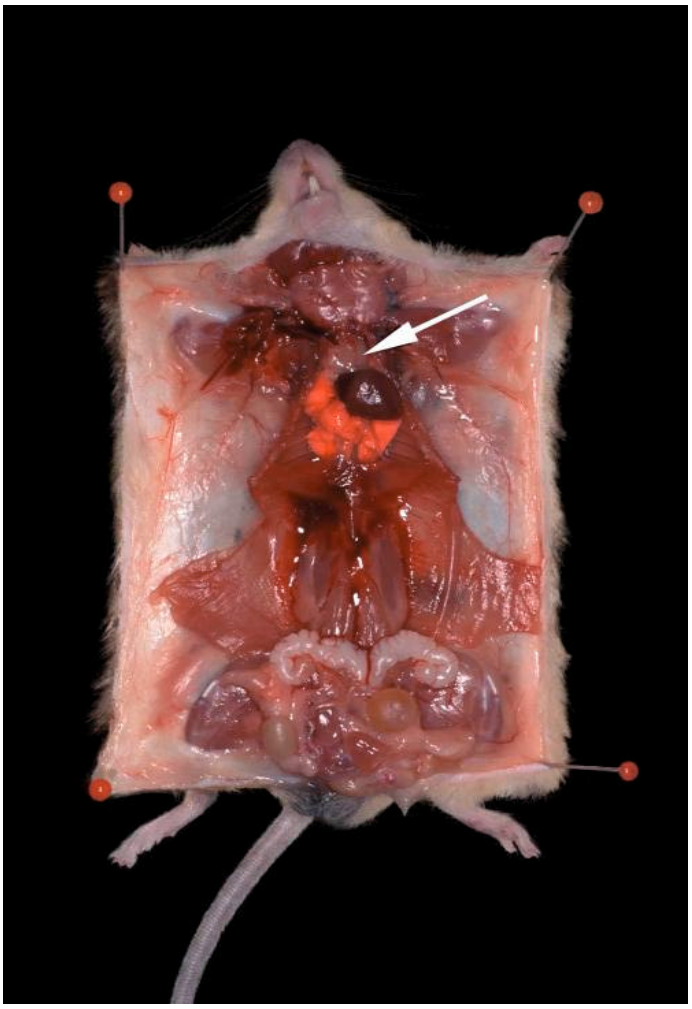


Fig. 18 Thymus observation

Size variations of thymus are found in some diseases, with particular regard to localized lymphatic lymphoma (thymic lymphoma). In these cases, the thymus is enormously enlarged, increased in hardness, of whitish colour; sometimes, it occupies a great part of thoracic cavity. In this case, the cause of the death is the compression that the thymic mass exercises on the respiratory movements.

Sometimes, the investigator will also observe the presence of clear liquid in the thoracic cavity, and the enlargement of the lymph nodes of the thoracic chain.

3. Lungs and heart

Anatomical outline. The lungs are two large organs of the thoracic cavity and are composed of lobes. The right lung of the mouse, like that of all other small rodents, is larger than the left one. This is due to the fact that the heart occupies, with its diameter, the greater part of the left thoracic cavity. While the right lung is composed of four lobes (the superior, the mean, the post-caval, and the middle), the left lung shows a single lobe.

In normal conditions, the colour of the lung depends on the amount of air and blood present, usually it is pale rose-coloured. The lungs are elastic and have a spongy consistency.

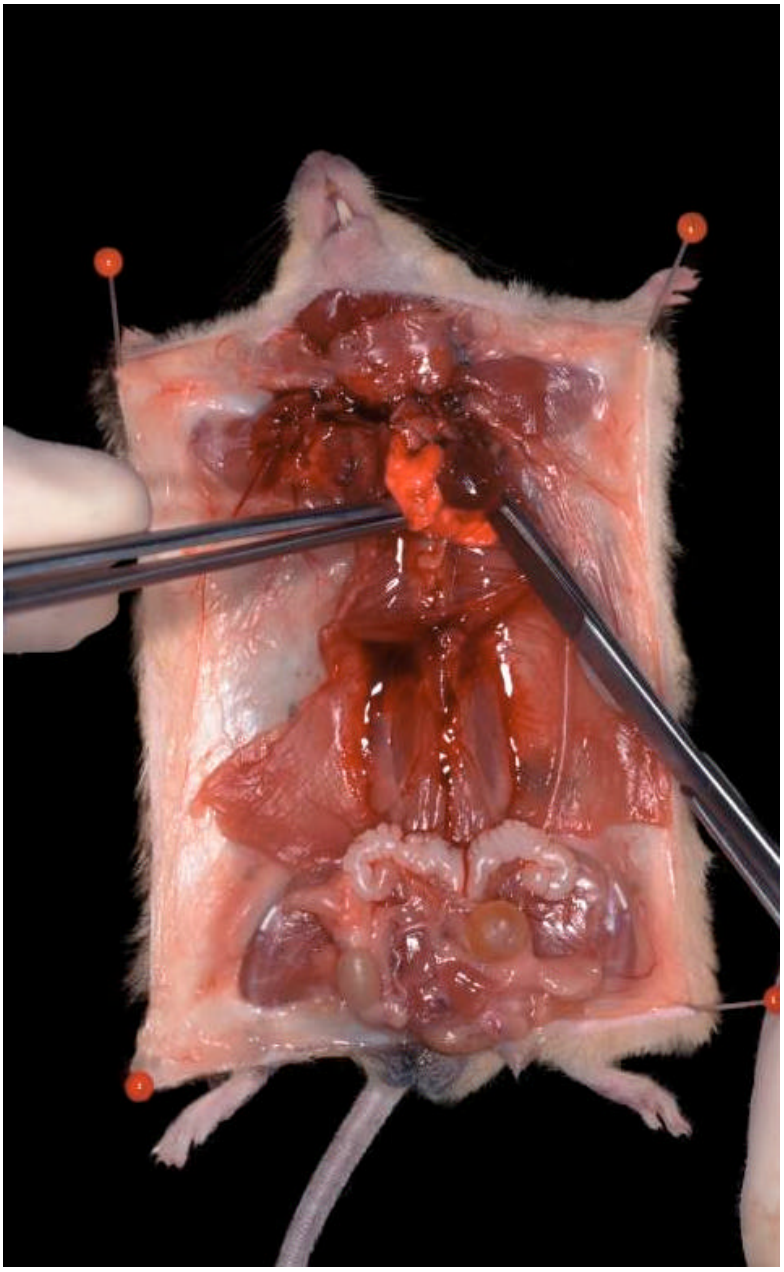


Fig. 19 Lungs and heart observation

A thin and transparent serosa membrane, called visceral pleura, covers the lungs. At the level of the median wall, in proximity of the hilum of the lung, the pleura is reflected and covers the inner wall of the thoracic cavity and then becomes the parietal pleura. A thin space is present between these two membranes (pleural cavity).

The heart has a pyramidal triangular shape, with its greater axis oriented obliquely to the left. It is divided, by the septum, in one left and one right half with no communication between them. An atrium and a ventricle form each half of the heart. The blood from the atrium goes into the ipsilateral ventricle and then into the circulation, either pulmonary on the right hand side or systemic on the left. The atrioventricular canals are supplied with a valve that allows the blood to flow from the atrium to the ventricle, but not vice versa. The tricuspid valve lies between the right atrium and the ventricle; the bicuspid valve is found between the left atrium and the ventricle. Through the vena cava, the non-oxygenated blood arrives in the right atrium and then passes in the ipsilateral ventricle. Through the pulmonary artery, the blood reaches the lungs where it is oxygenated, and it returns to the left atrium, through the pulmonary veins; then it goes into the ipsilateral ventricle and finally it is distributed to all regions of the body through the aorta.

In the mouse, the length of the apical-basal diameter of the heart is of approximately 9 millimetres, while its transverse diameter, at the level of the ventricles, is approximately 5-6 millimetres.

A serosal membrane called the pericardium covers the heart; the heart is of reddish colour and weighs around 130mg.

Removal of lungs and heart. The removal of all the thoracic organs (heart, lungs, trachea, and main vessels) must be simultaneous. First, the oesophagus and main vessels (vena cava and thoracic aorta) will be cut with scissors at the level of the diaphragm (Fig. 19).

Thereafter, pulling anteriorly, the attachment of the aorta to the vertebral column is removed and then, proceeding anteriorly towards the neck, the attachments of the trachea and the oesophagus. Making another cross-sectional cut in the proximal part of the neck, possibly over the larynges and the thyroid, the investigator can complete the extraction. This simple technique allows the isolation of all the thoracic organs without causing any lesion or changes in their mutual relationships.

Alternatively, the investigator may proceed to the separation of the heart from the lungs, leaving trachea, bronchi and thyroid in situ.

Examination of the lungs. During the external examination of the lungs, some particular characters will have to be annotated accurately (shape, volume, consistency and colour) and their obvious variation.

Partial reduction of lung volume may be observed in atelectasis due to a compression of adjacent pulmonary masses or of other masses close to the mediastinum, or by obstruction of the bronchi. In these cases, the organ is diminished in volume and more compact and of dark-red colour.

Some diseases, like oedema, emphysema, infections (bronchopneumonia), or neoplasia may, on the contrary, determine an enlargement of the organs.

The oedematous lung appears translucent, enlarged, and of pale colour, due to abundant liquid. At a longitudinal cut, performed along the greater axis of each lobe, these characters may be better appreciated; when you press the organ surface, you might appreciate the loss of discrete amount of a clear liquid. A similar picture is present in the case of emphysema, where the lungs appear equally pale and abnormally expanded. In this case cutting the lobes is accompanied by a thin crackle, caused from the abundant air coming out from the alveolar cavities.

A diffuse or circumscribed enlargement of one lobe may be an indication for a diagnosis of pneumonia. In this case, the lung shows peculiar characters such as an intense dark-reddish colour and hardness similar to that of liver parenchyma (hepatization). Moreover, the cut surfaces appear finely granular and, under pressure, a loss of haemorrhagic fluid is detected.

The presence of some hard whitish nodules easily allows the investigator to make a diagnosis of lung tumours (adenoma or metastases).

Removal of the heart. For an accurate examination of the organ, the investigator must isolate the heart from the lungs, delicately cutting the main vessels using scissors.

The external examination of the heart consists of a description of the external characteristics together with possible variations, using the same methodology followed for the other organs already described. However, due to the small size of the heart and consequent handling difficulty, the investigator, in the majority of cases, should limit his task to make a simple external examination of the organ, without opening the inner cavities (atria and ventricles) in order to avoid damage to the endocardium and the valves; these tissues will be better examined histologically.

At the external examination, other heart variations such as colour, volume etc., may be also detected.

Enlargement of the heart will be observed in the case of cardiac hypertrophy, connected with severe blood flow disturbances, lesions of the coronary vessels, hypertension, and in chronic degenerative kidney disease.

A reduction in size is very rarely observed and, when present, is due to a chronic disease of the myocardium or to age.

Different lesions may cause myocardium colour variations: the investigator will observe a pale-rose myocardium in the case of severe anaemia, or dark-coloured in hypertrophy or in acute infectious diseases.

The entire aspect of the heart will then be altered by the presence of infarcts, tumours (very rare), and large thrombi, often found in the atrium. In the last case, the investigator will observe a marked expansion of the atrium with irregular sclerotic areas, and sometimes massive haemorrhage in the pericardium and pleura, as a consequence of the rupture of the thin atrium wall.

4. Thyroid

Anatomical outline. The thyroid consists of two small long lobes with an oval shape adherent to the lateral and dorsal surfaces of the trachea. They are easily visible if the investigator removes the salivary glands reflecting them laterally, together with the relative sternum muscles.

Each lobe is approximately 2-3 millimetres long and 1 millimetre wide. Under normal conditions, the thyroid is translucent due to the presence of colloid, of yellow colour, and has a soft consistency. Aberrant thyroid follicles can be very rarely found in the adipose tissue, near the base of the heart.

Examination. The investigator will then observe the shape and the volume of the gland. Enlargement of the thyroid is usually observed in the presence of circumscribed or diffuse tumourous nodules.

Spontaneous tumours are very rare in the mouse, although their incidence increases if the animal is treated with particular drugs or radioactive ^{131}I. Among non-neoplastic lesions which cause enlargements of the gland, follicular epithelial hyperplasia associated with a diminished colloid content in the follicles must be considered. Sometimes, the presence of an atrophic thyroid, associated with fibrous processes, could be also present in old mice.

Chapter 6

Cranial cavity

1. Opening and general observation.

In order to complete the autopsy, the investigator must proceed to the opening of the cranium for examination of the brain. The techniques used are numerous, but we would like to suggest one that, in our opinion, appears the simplest for a small rodent.

Keeping the head firmly with the aid of large forceps, the skin must be cut with a median-longitudinal cut from the nape to the snout (Fig. 20) reflecting then the two edges of the skin from one side to the other and then pulling them toward outside so that the investigator might better observe the entire skull. Applying this technique, the eyes together with the Harderian glands, which lie in the orbital cavity behind eye-ball (Fig. 21), will be displayed. Finally, these organs could be examined before the investigator proceeds to opening the skull.

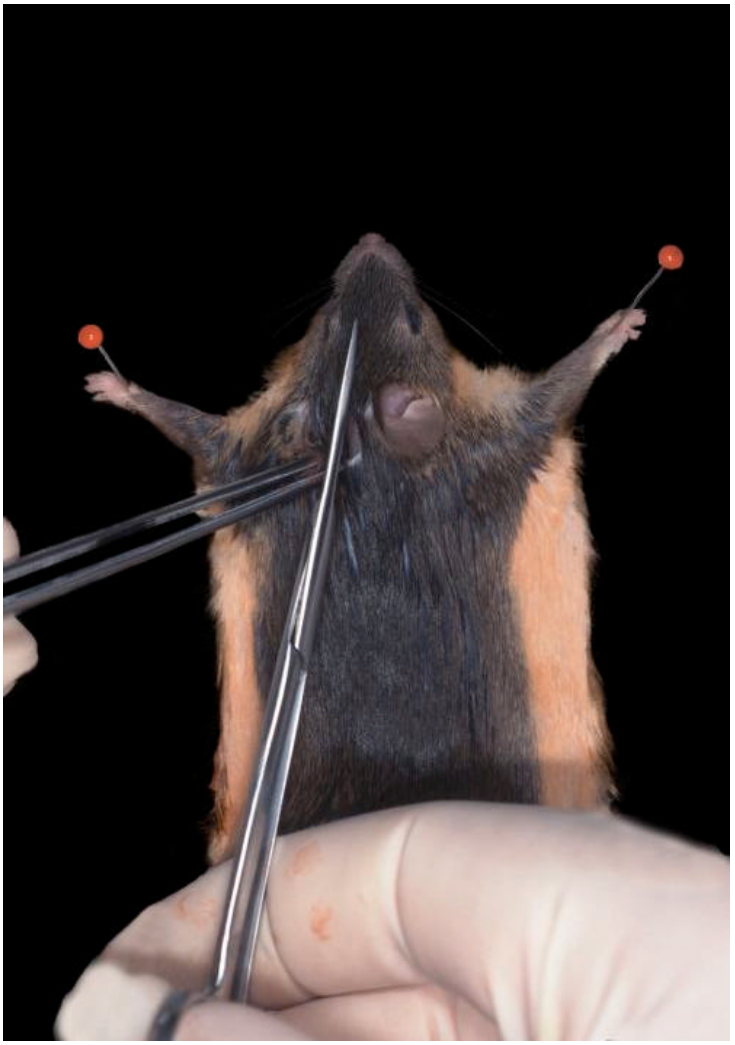


Fig. 20 Skin opening for skull observation

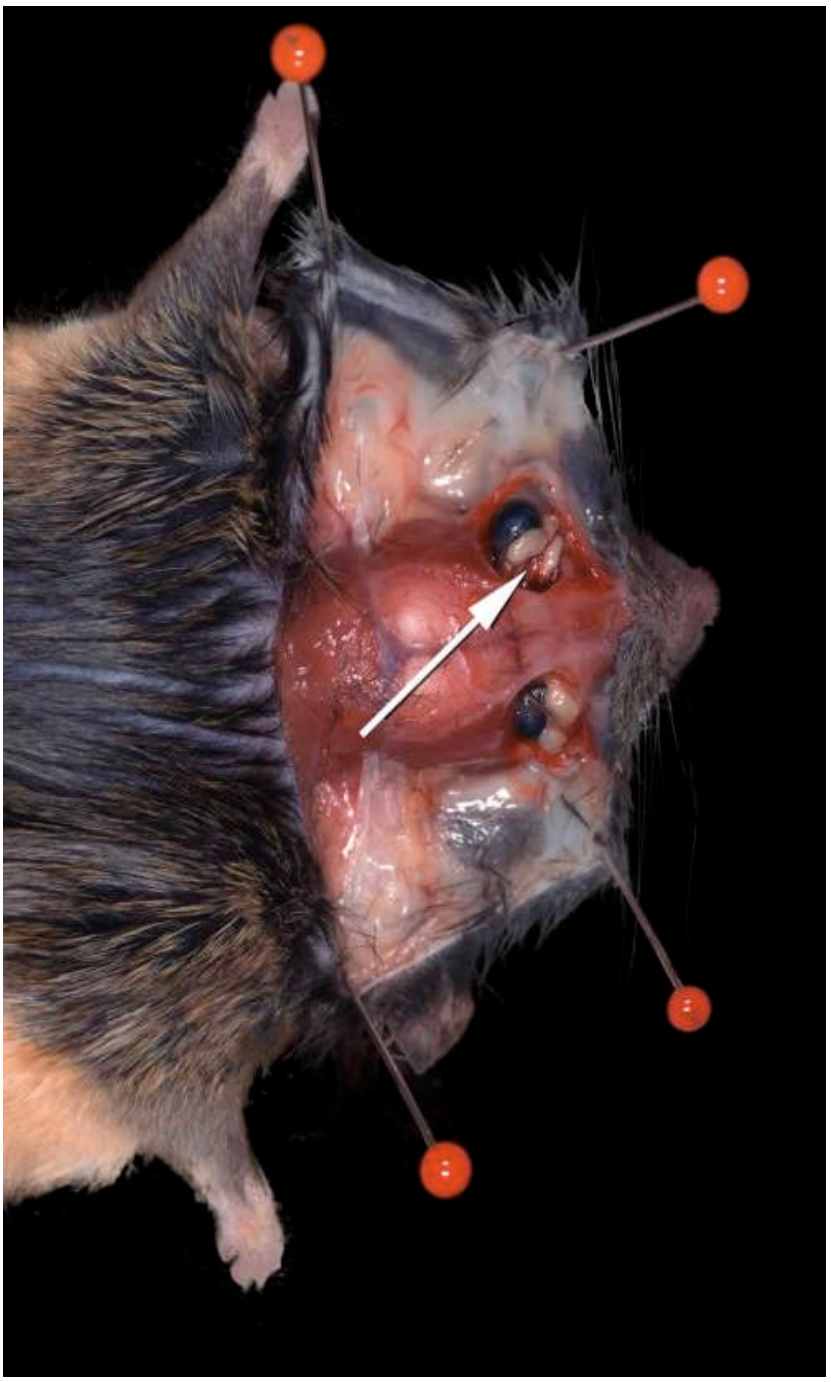


Fig. 21 Observation of eyes and Harderian glands

2. Eye

Anatomical outline. The eye, located in the orbital cavity, has a nearly spherical shape and is connected at the back to the distal part of the optical nerve.

A transparent, uncoloured and non-vascularised membrane, called the cornea, covers the front half of the eye. The sclera, which covers the posterior part of the eye, is, of course, not visible until the organ is removed: it is opaque, white, and bright. The sensitive portion of the eye consists of an inner membrane, called the retina; a nervous matrix that includes the final part of the optical nerve.

The iris is located behind the cornea and consists in a circular disk with a central hole called the pupil.

The lens lies behind the iris and inside the eyeball. It has a nearly spherical shape, and is transparent, uncoloured, of an elastic consistency and occupies almost the entire ocular globe. Due to protein denaturation, the lens might show different degrees of opacity (cataracts).

The cataract is a lesion that might spontaneously appear in an old mouse or as a possible late effect after treatment with radiation. Associated with this lesion, atrophy of the iris is sometimes present. In this case, the investigator will observe a dark radial strips around the pupil together with the presence of spots of pigments in the frontal part of the eye. In the advanced stage of this lesion, the iris can be completely absent or with the presence of some pieces left and located at the pupil edges. Whitish irregular plates might be observed in the central zone of the cornea, mainly in old DBA mice.

3. Harderian gland

Anatomical outline. The Harderian gland (or retro-orbital gland) is located in the orbital cavity behind the eye. It is U-shaped and embraces the ocular globe at the back. Under normal conditions, the gland has a grey colour.

Fig. 22 Opening of the skull

Examination. The benign and malignant tumours of this gland are frequent and they cause, in the majority of cases, the protrusion of the eye from the eyelids. When these masses are very large, they encircle the ocular globe so that they can be easily diagnosed macroscopically. Finally, ulceration of the cornea and conjunctiva is associated to this lesion.

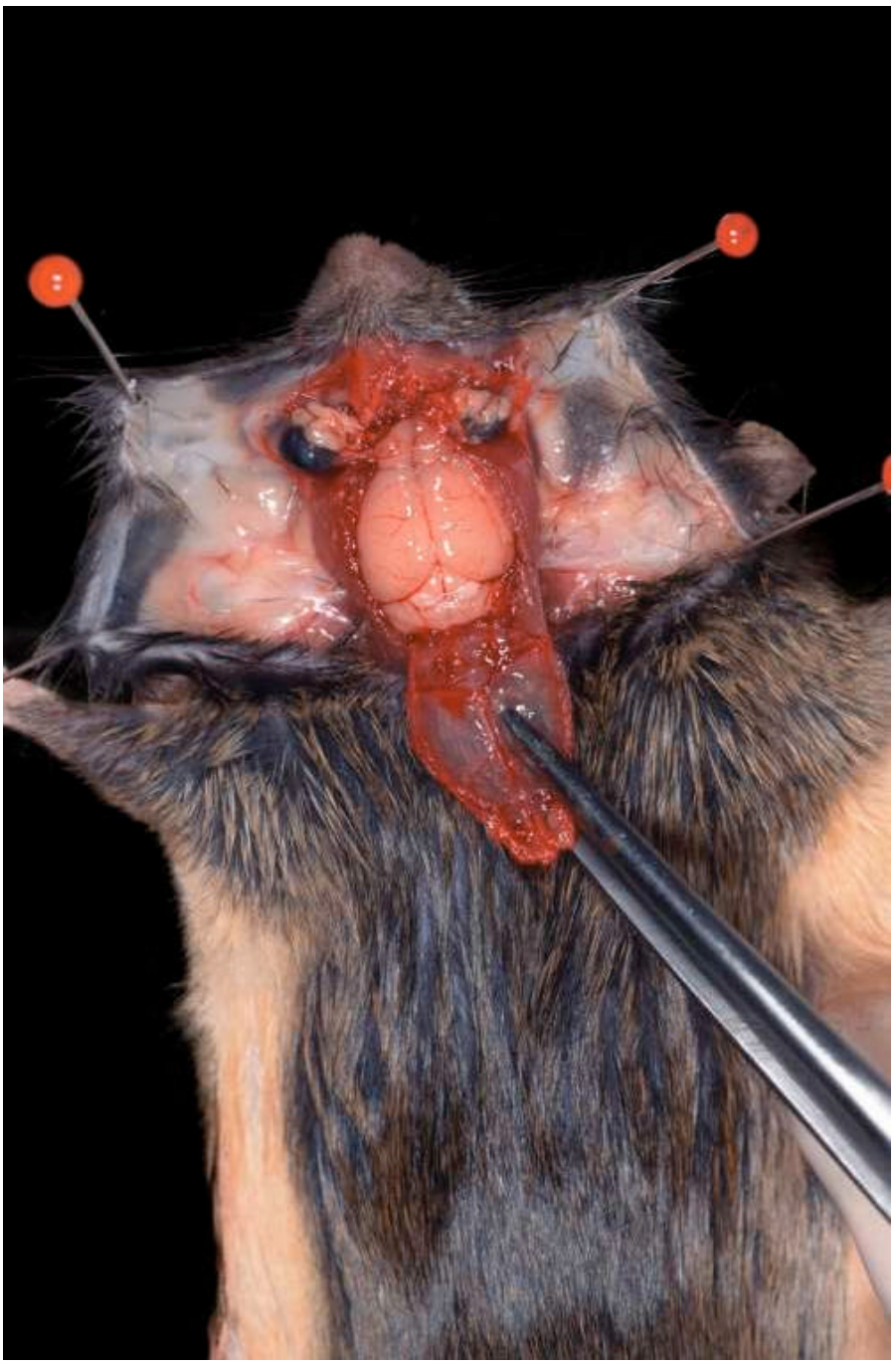


Fig. 23 Brain extraction

4. Brain

Anatomical outline. The brain represents the main part of the central nervous system (CNS) and it is contained in the cranial cavity. The brain has an ovoidal shape with the longest axis oriented in the skull's caudal direction. Three connective membranes, called meninges, cover the brain. The outer one is the dura mater; it is fibrous, strong and is in direct contact with the inner face of the skull; under this membrane, a thin, transparent, invisible membrane, called the arachnoid, lies. Under this one, the investigator can see the pia mater, highly vascularised and intimately adherent to the surface of the brain.

The investigator will then pass to examine the dorsal surface of the brain which consists of two large hemispheres smooth, of grey colour, both separated by one deep longitudinal channel. The olfactory bulb is located in front of the hemisphere, while the cerebellum is localized at the back. The cerebellum is composed of two small grey hemispheres and one median lobe (vermis) placed between the two hemispheres; it is covered by thin convolutions.

On the inferior surface of the brain, the investigator will observe the medulla oblongata that is in direct communication with the spinal cord.

The pons, composed by a white nervous substance, is located in the cranial part of the spinal cord. The optical chiasma, into which the optical nerves end, is visible more ahead.

In the young-adult mouse the average brain weight is approximately 450 milligrams.

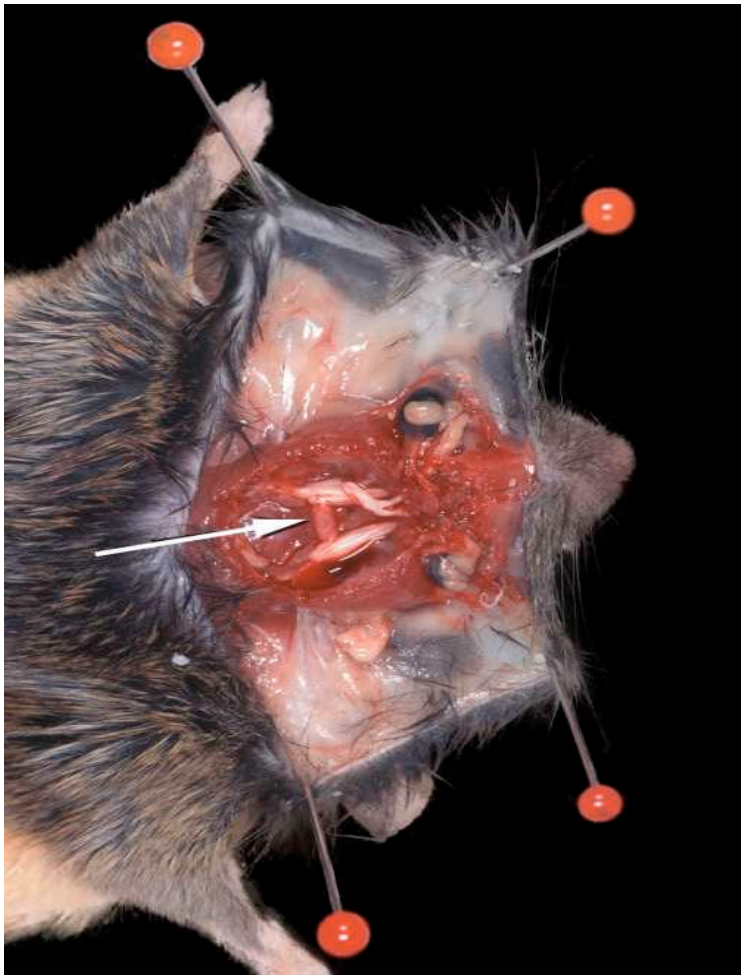


Fig. 24 Observation of the hypophysis after brain removal.

Removal and Examination. The opening of the cranial cavity is carried out using scissors that must be used only for this procedure.

The investigator will make a crosscut at the level of the nasal septum, which divides the two orbital cavities. From this point, paying attention that the scissors do not penetrate the nasal cavity too deeply, the investigator proceeds to cut the occipital and parietal bones (Fig. 22). Afterwards, the skull is removed and the brain and meninges can be seen (Fig. 23).

Diseases of the meninges consist mainly of diffuse haemorrhage for rupture of meningeal vessels. In this case, the presence of circumscribed regions of collected blood, usually in correspondence with the affected lobe, can be seen.

When the skull is completely removed, it is possible also to observe partial or total brain enlargement as in the case of diffuse oedema or for tumours.

In order to complete the examination for other possible diseases, it is necessary to proceed to the removal of the encephalon. The technique consists in raising the brain by gently introducing forceps under the frontal lobe. The nerves and the vessels of the brain base are then cut and, proceeding towards the brain base, the bulb is separated from the spinal cord with a single cut. The encephalon is then removed in toto, so that the cranial base can now be examined with particular regard to the hypophysis (Fig. 24). The investigator will

then observe the volume and the shape of this gland that, in normal conditions, is an oblate spheroid with its greater axis perpendicular to the cranial base.

With these manual operations, the investigator's job is completed and it is now the turn of the pathologist, who will carry out another phase of investigation and study for an exact microscopic diagnosis through histological examination.

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