Human Histology

Manual of Practicals

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Acknowledgement

To all those who, with their work and effort, have contributed to the development and knowledge of Histology

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Sample Processing for Light and Transmission Electron Microscopy

1. OBTAINING THE SAMPLES

In Medicine, the samples use to come from patients from whom a portion of tissue is extracted, which is called a biopsy. They also come from material obtained by surgical treatment (surgical specimens) and, of course, from people who have died of some type of disease and whose family authorizes the autopsy to be performed (clinical autopsy). Many of the studies are carried out using extensions of exudates on slides, aspirated material or smears, which are generically known as cytology and/or extensions. The material must be obtained with great care, trying not to deform or damage the tissue: appropriate and sharp material must be used.

The origin of the samples largely determines the "quality" of the material. It is extremely important that as little time as possible elapses between obtaining the sample and its fixation in order to have the highest degree of resemblance to the "in vivo" state. In the following, we will refer to the study methods most commonly used in daily practice.

2. WHAT A HISTOLOGICAL SAMPLE IS

To be able to observe biological samples under a light or electron microscope, we must undergo a series of steps that aim:

a) To dehydrate the samples and embed them in a material that allows to made thin sections.

b) To rehydrate the sections again to dye/stain them since dyes are only soluble in water.

c) Once the samples have been stained, they must be dehydrated again so that we can use a medium that does not refract the light and allows us the best possible resolution.

3. SAMPLE PROCESSING FOR LIGHT MICROSCOPY

The histological specimen for light microscopy usually consists of a section about 5um-thick that is placed between two rectangular glass slides: one of the slides has a certain thickness and consistency (colloquially called as "holder") and the other is very thin ("coverslip"). Located between them, the stained sample is ready for observation under the light microscope. This is a routine preparation, which the student will use in these practicals.

All these resumed steps are known as "sample processing". Next, we will explain the goal of each step in detail, whose protocol is as follows:

1. Fixation: to make sure that the sample remains as similar as possible to the living state.
2. Dehydration: it replaces the water in the tissues with alcohol or acetone.
3. Clarification: this is the substitution of the dehydrating agent by another one, called "transitional" agent (formerly xylol and actually non-toxic substitutes for xylol), which in turn is miscible in the inclusion medium that provides the sample with the necessary hardness to be sectioned.
4. Inclusion/Embedding: the sample is embedded in a medium that provides it with sufficient hardness replacing the water that was initially in the cells and tissues. Paraffin is worldwide used: it is a wax and therefore not miscible in water. This substance has
5. Sectioning: histological sections of about 5um thickness are obtained and deposited on the slides, where they are left until they adhere to the glass.
6. Removal of the paraffin from sections: by immersion in the transition liquid (xylol, for example).
7. Substitution of xylol by 100% alcohol.
8. Substitution of alcohol by water: using decreasing concentrations.
9. Staining: the most commonly dyes (hematoxylin and eosin) are aqueous, so it is necessary to "undo" the steps.
10. Dehydration of the sections already stained (with increasing concentrations of alcohols).
11. Substitution of the alcohol by xylol.
12. Mounting: at this step, a viscous liquid is added on the histological section to cover it (this is the mounting medium, usually DPX) and then the coverslip is placed on top of it. The mounting medium is not soluble in water and has the virtue of allowing light to pass through it without producing distortions in the image. Moreover, mounting medium solidifies in about 24 hours, which allows histological preparations to be stored for many years.

The slide is now ready to be placed under the microscope.

As a resume, the first four steps are known as embedding, the fifth step is sectioning, numbers 6-9 correspond to "dehydration and staining" and the rest to "mounting". Any treatment of tissues that is necessary to impregnate them with a solid medium that facilitates the production of thin sections for microscopy has the generic name of inclusion (although inclusion really refers to the treatment necessary to impregnate the tissues with a solid medium that facilitates the production of sections for microscopy).

4. SAMPLE PROCESSING FOR TRANSMISSION ELECTRON MICROSCOPY

In order to be able to observe a sample through the transmission electron microscope, it must be included in a series of steps superimposable to those we have just seen for light microscopy. However, there is a huge difference with the size of the sample, which must be smaller (less than 1 mm^3).

The protocol can be summarized as follows:

1. Fixation: if possible, perfusion with 4% paraformaldehyde followed by 4% glutaraldehyde.
2. Washing with isoosmolar phosphate or cacodylate buffer (0.1M, pH 7.4) to remove the fixative.
3. Post-fixation: usually with osmium tetroxide.
4. Washing of the sample with isoosmolar buffer to remove the postfixative agent.
5. Dehydration with alcohols (or acetones) of increasing concentration.
6. During the dehydration process it is common to add heavy metal salts that increase the contrast when observing the sample under the microscope (such as uranyl acetate).
7. Replacement of the dehydrating agent by a so-called transition agent (usually propylene oxide) that in turn is miscible in the upcoming inclusion medium.
8. Inclusion of the sample: in the medium that provides the sample with the necessary hardness to be able to be sectioned. Usually epoxy type resins are used (araldite, epon, etc). Now, this substance replaces the water that initially was in the cells and tissues.
9. Sectioning: firstly, semi-thin sections (1um) are made to select the study area. Then, ultra-thin sections of about 60-80 nm thick are obtained, which are deposited on a grid of about 3mm that acts as a support, where they are left until they dry.
10. "Staining": this is not really staining, as we use heavy metals (lead citrate) that specifically impregnate the elements of the sample.
11. Washing of the sample with an isoosmolar buffer to remove the excess of lead citrate.

The grids, with the samples, are introduced into the electron microscope. The heavy metals that have been deposited on the sample prevent (more or less) electrons from passing through it.

5. GOAL OF THE DIFFERENT SAMPLE PROCESSING STEPS

5.1. **Fixation**

As above mentioned, fixation is the name given to procedures used to "suspend" cellular activities that prevent postmortem decomposition (autolysis).

The basic objectives of fixation are:

a) To prevent the phenomena of autolysis and bacterial attack.
b) To achieve that tissues and cells remain as similar as possible to the live state and that, ideally, small molecules are not lost.
c) To avoid changes in the appearance or volume of the material during subsequent processes.
d) To allow a clear staining of the structures.

Although the preservative effects of some substances, such as mercury and its salts, have been known since the time of Hippocrates, it was not until the middle of the nineteenth century that their research began to be systematically carried out. Thanks to industrial development and the increase in our knowledge of chemistry and physics, there has been an important advance in the knowledge of new substances and their mechanism of action. Fixing techniques can then be divided into physical and chemical.

5.1.1. *Physical techniques*

They are very accessible and allow us to avoid the possible artifacts that could be caused by the addition of different substances to the specimen to be studied. However, they do not preserve the fine morphology adequately. They are very useful in routine cytology work and in histochemical and cytochemical studies.

Within this group we will highlight:

a) Drying: it consists of leaving the specimen to be studied in the air, achieving the evaporation of water. It is practically useless in the study of tissues, since the autolysis processes have already begun before the specimen has completely dried out. It is very useful in cytology, since although the cells lose details of their fine structure, this disadvantage is offset by its ease and low cost.

b) Freezing: this technique is based on subjecting the specimen to be studied to temperatures below zero degrees. It is used as a routine method in intraoperative biopsies due to its speed. For histochemical studies, where it is necessary to preserve enzymatic activity, freezing is usually performed in liquid nitrogen at about 179º C below zero. The major drawback is the lower quality of cell structure and detail, as well as the risk of ice crystals forming and causing artifacts in the specimen. After freezing, the samples are directly sectioned (without any further intermediate step) and stained (see below).

5.1.2. *Chemical techniques*

These are based on the use of chemical substances capable of reacting with the cellular components and preventing their degradation. Although there are numerous fixatives, the most common have their effect by reacting with the proteins: they coagulate or precipitate the proteins. The most common method is to immerse the samples in the fixative liquid (immersion fixation), but on certain occasions (usually in research) the fixative solutions can be introduced through the vascular system of the experimental animal (perfusion fixation), which guarantees better fixation. There are numerous compounds which to a greater or lesser extent meet this requirement and can be summarized schematically in a few groups of substances:

a) Aldehydes: formaldehyde, glutaraldehyde, acrolein, etc. They establish bridges between protein molecules in a relatively short time. They are widely used; in fact, formaldehyde is the fixative par excellence in light microscopy, and glutaraldehyde is the fixative par excellence in electron microscopy. However, the preservation of the morphological structure is not complete and, in addition, they can prevent or hinder numerous cytochemical reactions.

b) Oxidizing agents such as osmium tetroxide, potassium permanganate, potassium dichromate, etc. This group of fixatives reacts with proteins and lipids, and their exact mechanism of action is not fully known. Osmium tetroxide is routinely used as a post-fixative after glutaraldehyde in conventional electron microscopy, both in transmission and scanning.

c) Protein denaturing agents like ethyl alcohol, methyl alcohol, acetic acid, etc. They preserve proteins acceptably, but carry away lipids during fixation. Their use is rather restricted but they are commonly used in the dehydration process following fixation.

d) Agents of unknown mechanism as picric acid, mercuric chloride. They have a very restricted usefulness.

e) Fixative mixtures: the most popular are Bouin's liquid (75 ml of saturated aqueous solution of picric acid plus 25 ml of 4% formalin plus 5 ml of glacial acetic acid) for light microscopy and Karnovsky's solution (2% formaldehyde and 1-2% glutaraldehyde) for electron microscopy.

5.2. Dehydration

The dehydration aims to replace all the existing water in the tissues by alcohols. It is tried to do it in the most delicate possible way, to avoid artifacts in the sample, the reason why increasing concentrations of alcohols are used.

5.3. Clearing

The purpose of this step is to use a liquid that is soluble in alcohols (or acetones) but also soluble in the inclusion medium (paraffin in optical microscopy and resins in transmission electron microscopy). The criteria for the choice of the clarifier include:

1. Speed to remove alcohol
2. Easiness to remove by the embedding agent
3. Flammability
4. Cost
5. Toxicity
6. Non-carcinogenic

In electron microscopy this step is sometimes "skipped" by using varying mixtures of alcohol and resin (3:1, 2:1, 1:1, 1:2, 1:3) before immersing the samples in pure resin, all at room temperature.

5.4. Inclusion

The most used substance as inclusion medium in optical microscopy is paraffin, which is maintained liquid (at 55°-60° C) so that it can be exchanged with the clarifying medium. After a certain time, the samples are removed from the paraffin bath and moved to a new (clean) paraffin, to make the blocks where the samples are placed to be sectioned. The set is at room temperature (or in cooled plates) so that the paraffin block can cool and then solidify. These blocks allow samples to be stored for decades.

In electron microscopy, epoxy resins are normally used, and the most popular being Araldit and Epon. These resins are actually supplied in bottles containing different liquid components (the polymer, the plasticizer, the hardener and the reaction accelerator, or catalyst). Once mixed, the lightening agent is replaced by the resin. The molds are then made and placed in an oven (at 60º C) for about 48 hours so the resin polymerizes.

Factors affecting the inclusion process include:

1. Agitation
2. Heat
3. Viscosity
4. Ultrasound
5. Vacuum

5.5. **Sectioning**

The block is placed on a device capable of making very thin, consecutive sections, which is called a microtome in the case of light microscopy and an ultra-microtome in electron microscopy.

The thickness of the sections that have been embedded in paraffin is routinely about 5um (4-6 um), being difficult to make them of lesser thickness. In special cases, thicker sections (of 20, 50 and even 100 um) are of interest in order to observe whole neurons with their branching in different planes, for example. Another option is to perform serial (consecutive) slices.

In the case of samples embedded in resin for electron microscopy, it is possible to obtain thinner sections, as the resin is much harder than paraffin. First, we made 1 um thick sections called semi-thin sections. These slices (which are observed with the optical microscope) are stained with toluidine blue and, in addition to being used for the morphological study (with higher quality than the samples from paraffin), they are used to select which part of the sample is of interest to study, proceeding to cut (carving) the already scarce sample. This carved sample is the one that is cut into ultra-thin sections of about 60-80 nm thick, which are the ones that will be stained with heavy metals and observed with the electron microscope.

After cutting the slices, these are transferred to the corresponding support. In the case of optical microscopy, rectangular crystals of 7.5x2.5 cm, with a thickness of 1mm, are used. In the case of the ultra-sections for electron microscopy, the slices (about half a dozen) are placed on a copper grid of 3 mm diameter, which can be previously covered with a thin film of a plastic material to provide a better support for the sections, as the grid is hollow or perforated, to allow the electrons to pass through it.

5.6. **Staining**

Most tissues are colorless, which makes it very difficult to observe under the microscope. For this reason, staining methods were introduced, which are carried out using mixtures of substances called dyes. Dyes are natural or artificial chemical compounds characterized by having one or more color-generating atomic groups (chromophores), as well as one or more groups with chemical affinity towards colorable substrates (auxochromes). In some cases, the auxochrome does not react di-

rectly with the colorable substrate but with an intermediate substance that has a double affinity: for the colorant and for the substrate, and is called mordant. A dye can color because its chromophores are capable of absorbing a certain portion or specific band of radiation within the visible region of the electromagnetic spectrum (wavelength between 400 and 750 nm). The term affinity measures the tendency of a dye to transfer from the staining solution to the tissue section.

Usually, routine histological sections are stained with two dyes: hematoxylin and eosin. Hematoxylin, which is found in a tree (in the medullary extract of the Palo de Campeche or *Hematoxylun campechianum*), is not intrinsically a dye, but shows an oxidation product: hematein (wine-red). Eosin is an artificial colorant derived from xanthene with diverse commercial types, staining with different shades of red and pink.

Both hematoxylin and eosin are so-called panoptic stains: those that stain generally, rather than specifically, all cellular components. The most popular of these stains is the hematoxylin-eosin double stain, being the most popular because of its ability to stain a huge number of different tissue structures, its simplicity and its applicability to different tissues from different sources. Hematoxylin is basic and therefore will bind to acidic structures (nucleic acids, for example). Eosin is an acidic dye and will bind to basic structures (basic proteins, for example). Those structures with an intermediate pH will stain with both dyes. Essentially, hematoxylin stains nuclei blue-blackish, with good intracellular detail, while eosin stains the cell cytoplasm and most connective tissue fibers with varying shades of pink, orange and red.

As we have already mentioned, in electron microscopy the samples are not stained but the tissue elements are impregnated with heavy metal salts, which deflect the electrons, as opposed to the non-impregnated areas that allow their passage. This results in a "black and white" image with different gray densities. The most commonly used heavy metals are lead citrate, uranyl acetate and potassium permanganate (the latter has a more limited use).

5.7. **Preparation and mounting**

In light microscopy, after staining the specimen, an extremely thin glass (coverslip, of 0.17 mm) is superimposed on the specimen to protect it. If a "cover slip" is used, a fluid substance whose refractive index is equal to that of the glass slides must be interposed between it and the "slide": this is the mounting medium. If this medium does not solidify spontaneously (water, glycerin, etc.) the mounting is temporary. If it hardens, it remains as a strong glue (DPX, Canada balsam, etc.) and the mounting is permanent.

6. PROCESSING OF SAMPLES FIXED BY FREEZING

As we have already commented, physical fixation does not excessively respect the structure, so the morphological quality will be inferior to that of samples fixed with chemical agents. However, this method is routinely used in hospitals because of its rapidity in providing an intraoperative diagnosis that will guide the course of surgery. The surgeon, when in doubt as to the nature of the pathology he/she is facing, removes a small portion of the tissue in question (takes an intraoperative biopsy) and sends it to the Pathology Department. While the surgeon waits, the pathologist issues a diagnosis that will determine the surgeon's attitude and the course of the operation. Despite all this requires maximum speed (sometimes 10-15 minutes), the quality of the sample suffers.

Cold fixation is also necessary in those situations in which chemical fixation deteriorates the molecules to be studied, as occurs in cytochemical studies, in which the aim could be revealing enzymatic reactions or antigenic determinants.

Processing consists of rapidly cooling the specimen (temperatures of –20° C for intraoperative biopsies and about –179° C for research work), then adding a substance that will allow the specimen to be placed on the microtome and cut into sections (5-6 um thick). The section is then stained with hematoxylin-eosin, dehydrated and mounted with DPX.

In electron microscopy, it is also possible to perform freezing inclusions. These cryo-techniques can basically maintaining the same principles, but are extremely delicate and difficult. It is necessary to consider the very high resolution of these devices and that the "tolerable/admissible" quality losses in optical microscopy are real alterations that nullify the possibility of interpretation in electron microscopy.

Some of the advantages of cold fixation are:

1. Extremely fast
2. Rapid arrest of cellular reactions
3. No diffusion of soluble elements
4. No introduction of foreign elements into the sample.
5. It is convenient that the samples are small so that the cold penetrates easily.
6. For electron microscopy, cryoprotective agents should be used to prevent the formation of ice crystals.

7. CYTOLOGICAL SMEARS

In this case, considering a liquid sample from a drop of fluid (blood, exudate, aspirate from any source, etc.) or by deposition in an cytology (cervix, vagina…), the cells are intended to be distributed homogeneously throughout the slide.

Once the sample has adhered to the slide, it is fixed and then stained. In the case of blood samples, panoptic stains such as May-Grünwald-Giemsa (commonly Giemsa) or Wright's are widely used. Vaginal smears are usually stained according to the Papanicolaou method.

8. PROBLEMS IN THE INTERPRETATION OF TISSUE SECTIONS: ARTIFACTS

The term artifact means artificial product or images, and refers to "a phenomena in the preparation that do not exist in the live tissue, which have been artificially created during the processing of the samples".

The most frequent are:

1. Tissue rupture. Consequence of inadequate procurement of the biological material (breaks, tears, compressions, etc.).
2. Defective fixation. If there is not a good fixation, the structural preservation will not be adequate and consequently the quality of the sections will not be satisfactory.

3. Retraction. It is the most frequent, and can be due to fixation with alcohol or other dehydrating agents (although sometimes these agents are necessary) or to a too fast dehydration. Shrinkage appears in the tissue as cracks or holes without any biological structure ("holes").

4. Damaged microtome blade. Imperfections or nicks in the microtome (or ultramicrotome) blade produce a defect in the cut that is observed as a straight line through the biological structures.

5. Variations in the thickness of the cut. They are usually the result of a bad adjustment of the microtome, of the blade (a screw not tightened properly) or of a bad fit of the block in its clamp.

6. Folds or wrinkles. These can be produced when cutting the sections or when placing them extended on the crystal (or on the grid).

7. Dye precipitaione. Sometimes colored crystals are observed on the histological section that correspond to dyes that have precipitated, or that have not been "sufficiently washed".

9. THREE-DIMENSIONAL INTERPRETATION OF THE SECTIONS

If we want to carry out a proper interpretation of the images when observing histological sections under the microscope and, we must bear in mind that what we see is a thin section, and therefore a two-dimensional view of a three-dimensional object.

Sections of the same object can show a completely different appearance. For instance, tube-shaped histological structures can have very different appearances. To determine the correct three-dimensional shape of an organ or large structure, serial slicing is often used, whereby consecutive slices of the entire organ or structure are made. A careful analysis of the relationships between slices in the whole series will give us an outline of the spatial configuration, and eventually build a model based on the series of slices: a three-dimensional reconstruction.

Methods of study of histological samples

1. INTRODUCTION

The human eye has a discrimination capacity (resolution) of 0.1 mm, which means that we cannot differentiate as separate two different points that are less than 0.1 mm (100 um) apart. To be able to "see" at a level beyond that of the naked eye, we must use instruments with higher resolution such as microscopes (hence the term microscopic structure), which requires us to prepare the samples to be studied. The light microscope has a resolution limit of about 0.2 um (because the wavelength of light is 0.4-0.7 um). Both transmission and scanning electron microscopes have a resolution limit of about 3 angstroms and about 3-20 nm, respectively, due to the limitations of the lens used to focus electrons onto the sample.

Histology is the science that deals with the study of the microscopic structure of live beings (*histo*: tissue, *logia*: knowledge) and, in our case, it is fundamentally limited to the human being. The knowledge of the microscopic structure of the human body has always aroused great interest. At first, the study was focused on the shape, size, peculiarities of the different cells, as well as on the organizations they constitute to form the different tissues and organs.

From a general point of view, we can consider that there are two main groups of methods applicable to the study of cells and tissues: i) those whose application does not harm the samples and are therefore called vital methods, and ii) those that kill the samples to study them, which are called non-vital methods. Both groups allow us to study both morphological and functional aspects, but, while the vital methods allow observation with practically no previous manipulation, the non-vital methods require a preparation that tends to "suspend" cellular activities without the cells and tissues undergoing important morphological transformations. We will describe the differences between both methods in the next section.

2. METHODS OF STUDY

2.1. **Vital methods**

Those that allow the study of cells or cell groupings (behavioral tests, explants, organotypic cultures, etc.) without killing the specimen. Vital methods avoid the possibility that fixation or

staining processes may remove or distort some cellular components. Obviously, the safest method is to observe the cells under the microscope while they are alive. This requires special optical systems designed to take advantage of the diffraction properties of the cells. When light passes through a live cell, the phase of the light wave is altered: light passing through a relatively thick or dense part of the cell, such as the nucleus, is retarded and its phase is correspondingly shifted relative to that of light that has passed through an adjacent thinner region of the cytoplasm. This wave interference effect is exploited by different microscopes.

2.2. **Non-vital methods**

These methods are the most used in daily practice and it is necessary to kill the cells. They are based on the paralysis of cellular activities at a given time to determine the structure or functional state of cells, tissues or organs. Before using these methods, it is necessary to fix and process the samples. This varies significantly depending on the final method of observation of the cells. Broadly speaking, we can consider that it is based on the rapid and non-artifacting arrest of cellular activities and its inclusion in substances not miscible in aqueous solutions, which allow us a perfect observation of the cytological details.

3. STUDY TECHNIQUES

In order to be able to observe visible beings beyond the resolution of our eye, we must resort to instruments that provide us with reliable images and provide us with as much information as possible. A number of different types of microscopes are used, which can be classified into two main groups according to the type of energy wave that strikes the preparation: optical or photonic microscopes, which use light of different wavelengths, and electron microscopes, which use electron beams.

The optical microscope has a system of lenses (condenser, objectives and eyepieces) through which the image of the objects is amplified and directed. The image quality provided depends on the resolution limit. Its value depends mainly on the wavelength of the light and the numerical aperture of the objective. In today's optical microscopes, the resolution limit is 0.2 um when using white light.

Electron microscopes use a source of electrons (filament) that, when subjected to a potential difference of about 60-80 kW, become incandescent and provide a beam of electrons, These are "channeled" through a narrow duct thanks to the action of several condensers (condenser lenses, objective, etc.), and pass through the sample. The sample has been previously "stained" with heavy metals, so that the electrons collide with the metals that are located on certain structures and are scattered giving a "negative image" on a phosphorescent screen. In transmission electron microscopes, the resolution limit is about 3 angstroms and in scanning electron microscopes it is about 3-20 nm.

3.1. **Bright-field microscopy**

The simplest method of observation is the direct transmission of light through the cell. This vital method is amazingly simple but, unfortunately, it does not allow us to visualize cellular details and we can only distinguish the contours of the cell membrane and the nucleus, with the rest of the cell constituting a grayish shadow.

3.2. Phase contrast microscopy

When light passes through a living cell, the phase of the light wave varies. The combination of both types of waves is used by this type of microscope to allow us the visualization of many details of the structure and appearance of cells without the need of killing the cell.

3.3. Darkfield microscopy

A simpler way to observe the details of an unstained living cell is to use the light scattered by the various cellular components. The darkfield microscope uses a beam of light directed from the side, so that only the scattered light penetrates the microscope lenses and, consequently, the cell appears as a luminous object on a black background.

3.4. Fluorescence microscopy

It is also possible to study in living cells their uptake of a substance added to the culture medium to which a fluorescent molecule has been conjugated, so that we can examine whether the particle has been taken up by the cells and what its destination is. This method can also be used with previously fixed cells, as in the case of the study of chromosome bands.

3.5. Conventional optical microscopy

It is based on the direct observation of structures through transmitted light microscopes that pass through the material under study. It is the most widely used method (practically all diagnoses in Pathology Departments use this method).

To be able to visualize it, it is necessary to resort to stainings. Although there are many types, we can include them in:

1. Panoptic stains. They are based on the non-specific staining of all cellular structures thanks to the combined use of acidic and basic dyes. Among the most used are Hematoxylin-Eosin staining (for histology and pathological anatomy), May-Grünwald-Giemsa staining and Wright's staining (the latter for blood and hematopoietic tissue). They are routinely used to study the shape, size, and morphological characteristics.
2. Specific stainings. The dyes used here have an affinity for some of the molecules present in the cells. They are used to demonstrate the existence or not of specific substances including lipids, carbohydrates, proteins, nucleic acids, pigments, and minerals, and those used to reveal and identify different microorganisms.

3.6. Enzymatic cytochemistry and immunocytochemistry

The purpose of enzymatic cytochemistry is to reveal the existence of certain enzymes inside the cells, which, moreover, can be quantified. It will therefore allow us to know the state of nu-

merous cellular metabolic processes. Among the most used cytochemical reactions are succinic dehydrogenase, lactate dehydrogenase, glucose 6-phosphate dehydrogenase, peroxidase, ATPase, and monoamine oxidase.

Immunocytochemistry allows us to identify specific substances that cannot be identified by specific staining. This technique is based on the detection of molecules by means of their corresponding antibodies. The first and most widely used method involves the binding of a fluorescent molecule to the antibody, called immunofluorescence. The limitation of immunofluorescence led to the development of immunocytochemistry, which is based on the replacement of the fluorescent molecule by an enzymatic marker (peroxidase) which is then revealed by cytochemical methods. Immunocytochemistry allows, in addition to a longer marker lifetime, the subsequent staining of the cells and comparison of the chromophore localization with the morphology.

3.7. Autoradiography

Autoradiography is defined as a method of localizing radioactive material on biological material. There are many methods for autoradiography, but the most common is to incubate cells or to inject tissues with a radioactively labeled metabolic precursor, such as thymidine (which is administered tritiated). Subsequently, the samples are spread on a well cleaned slide, on which a photographic emulsion is placed in intimate contact. This emulsion (consisting of a suspension of silver halide crystals on a gelatin support) is activated by the radiation of radioactive molecules, so that when the emulsion is photographically developed, the activated silver crystals are transformed into metallic silver. During the fixation process, the metallic silver remains, taking the form of small silver grains, while the unimpressed silver halide crystals are removed.

Autoradiography can be performed both on samples observable by light microscopy and by transmission electron microscopy. This technique allows us to reveal the rate of incorporation of molecules into cells, thanks to which we can, for example, know the rate of cell proliferation or the nature and distribution of diffusible substances whose fixation by conventional methods shows special problems. Since the appearance of fluorochromes, this technique has practically fallen into disuse, since its disadvantages include ts delicate nature, slowness and radiation problems.

3.8. Confocal microscopy

Basically, the confocal microscope is an optical microscope that uses a laser as a light source and an electronic system for image acquisition. Thanks to this, this instrument achieves a considerable increase in the resolution of images of extremely thin optical sections, eliminating the interference produced by the light coming from different optical fields (or focusing planes) of the entire thickness of the studied sample. In this way, focusing is achieved on a single plane, hence the term confocal. Thus, in confocal microscopy, structures are recognized in which the light emitted or reflected by a sample is concentrated in a single focal plane and superimposed on any light that does not come from that plane. This produces a scanning by planes that allows us to have an image in the three axes and, therefore, three-dimensional.

Thanks also to the fact that the images obtained are digital, it is possible to obtain high magnifications that were exceedingly difficult to achieve with optical microscopy.

3.9. **Transmission electron microscopy (TEM)**

Transmission electron microscopy allows us to visualize, with high resolution (3 angstroms), the cellular ultrastructure thanks to the ultrathin sections (60-80 nm) that are labelled with heavy metals.

The basic principle of specimen processing is the same as explained before, but with some differences: the fixation must extremely preserve the cellular structure, since any alteration of the same will be very conspicuous and will hinder the correct interpretation of the images obtained, and the inclusion medium must provide sufficient hardness to allow us to make very thin sections with the help of the yltramicrotome.

In addition to study morphology, we also can use special techniques for the demonstration of certain molecules, as well as cytochemistry and immunocytochemistry techniques, among others.

3.10. **Scanning electron microscopy (SEM)**

Unlike the previous one, scanning electron microscopy does not allow to observe sections of the samples, but to study their fragmented external surface, or directly the external surface. For this purpose, the samples are fixed and dehydrated in a similar way as for TEM. Once the dehydration (100% acetone) is finished, using the "critical point" technique, acetone is replaced by carbon dioxide and then sublimated, so that the piece is completely dried. The sample is then fractured, and its surface is sprinkled and coated with a very thin layer of gold molecules that allows the bombardment of the piece by electrons and the consequent formation of the image.

This method is extremely useful for viewing three-dimensional structures, to study the ability of cells to infiltrate and invade substrates, for assessing the spatial groupings of cells and their relationship to vascular systems, and to unravel cell locomotion.

3.11. **Other types of electron microscopy**

a) STEM electron microscopy: these are microscopes that are usually associated with a transmission electron microscope, greatly extending its advantages. They combine the possibilities of a scanning electron microscope with those of a transmission electron microscope.
On the one hand, an extremely fine scanning electron beam is used, which significantly increases the image resolution of small opaque samples. The samples can also be observed transparently, causing minimal damage to the sample and obtaining better contrast and the possibility of carrying out analytical determinations.
b) Scanning tunneling electron microscopy (STM): this is a type of scanning microscopy that involves the use of quantum mechanics. It does not use free particles (and therefore does not require any type of emission source or condenser lenses) but rather tracks the surface of the specimen, performing a delineation of the atomic topography of the surface. On the surface of solid bodies the electrons do not have a rigid position, but each electron behaves as a wave, and therefore its position is not well defined, it is blurred. This movement of the electrons is known as tunnel effect since it seems that the electrons dig tunnels beyond their position.

A very sharp tip is responsible for tracing the contours of the sample surface. If two solids are so close that their electronic distributions overlap, a current will flow between them if an electric potential is applied. The magnitude of the tunnel current depends very sensitively and exponentially on the spacing between the two solids (variations in spacing as small as one atomic diameter cause the magnitude of the current to change by three times its value). The current generated provides the signal that allows us to image the relative position of individual atoms on the surfaces of the sample under study.

Its application includes fields such as physics (study of superconductors), chemistry (composition of chemical reactions) and biology (direct non-destructive method of visualizing biological samples).

c) Atomic force scanning electron microscopy (AFM): this is a variant of the tunneling microscope. It produces images much closer to topography than the tunneling microscope and can also scan non-conductive surfaces. This microscope picks up the interatomic forces existing between the apex of a fine punch and the atoms of a sample, as the punch sweeps the sample.

d) Acoustic scanning electron microscopy (SAM): it uses ultrasonic imaging technology. Ultrasound waves dissipate rapidly in air, but in liquid media they can propagate and be used for imaging, but not for communication. Its ultrasonic waves have frequencies close to one gigahertz (one billion oscillations per second), whereas the human voice has a frequency of less than 20,000 hertz. For its operation, it uses a hemispherical acoustic lens to focus the acoustic emission from an ultrasonic transducer on a small region of the sample, through a binding medium such as water. Its resolution depends on the length of the acoustic wavelength in the specimen.

This type of microscopy allows us to study with extremely high contrast unstained cellular elements, so that changes in viscosity can be directly observed. It is especially useful for the study of live tissues specially to see the contraction of muscle fibers and cell motility processes.

e) Other techniques: the addition of other types of detectors (e.g. for EELS, X-rays, undispersed transmitted electrons, etc.) and the introduction of microprocessors have greatly expanded and facilitated the applications of current electron microscopy.

Likewise, the use of goniometers, laser optical diffraction methods, stereology and computerized image analysis techniques have also revolutionized purely morphological interpretation at the ultrastructural level.

3.12. **Cryofracture**

This technique consists of fracturing a frozen specimen by means of a blade at low temperature. To do so, the following steps are taken:

1. Stabilization of the object by abrupt freezing
2. Production of a fracture through the frozen preparation
3. Making a replica of the object by evaporation-condensation of a heavy metal and carbon

The advantages of this method lie in the purely physical nature of the specimen preparation, whereby the specimen remains unaltered and free of artifacts that come not only from

fixation, but also from those derived from postmortem processes and dehydration. The images obtained by this method allow the observation of un-sectioned organelles (nuclei, membranes, vacuoles, etc.), as well as fractured ones, in which case their internal structure can be appreciated.

The limitations of this method include the impossibility of to applying autoradiographic or immunocytochemical techniques, as well as the difficulty of interpretation of some structures, especially in the case of not being fractured.

Surface Epithelia

General objective of the practice

To know the basic shapes of surface epithelia. To identify some types of surface epithelia.

KIDNEY

Objectives to identify:

Simple flat/squamous epithelium

Simple cuboidal epithelium

We will focus on the renal corpuscles, which are only located in the cortex of the kidney. With the small objective (×4), we can recognize the renal corpuscles as rounded (spherical) structures located in the convex area of the histological section. Renal corpuscles consist of a dense area (glomerulus) surrounded by a clear edge, an empty space (urinary space), which is lined in its external boundary by a simple flat epithelium (Bowman's capsule).

With the use of higher magnifications (×10, ×40), we will be able to better perceive the epithelium. Between the corpuscles (×10) there are numerous tubes cut more or less perpendicularly. These structures, which are called convoluted tubules (proximal and distal), are lined (×40) by a simple cuboidal epithelium and leave a hole or lumen in their centre. Their nuclei are rounded and arranged in a central position.

ESOPHAGUS

Objectives to identify:

Non-keratinised stratified flat (or squamous) epithelium

The non-keratinised stratified flat epithelium can be easily found on the surface of the esophagus. Stratified squamous epithelium consists of a variable number of cell layers that exhibit maturation from a cuboidal basal layer to a flattened surface layer.

SKIN

Objectives to identify:

Keratinised stratified flat epithelium

The epithelium that lines the skin, called the epidermis, is a keratinised flat/squamous epithelium. A number of cell layers can be distinguished in the epithelium, related to their degree of maturation:

1. in the deepest strata, closer to the basement membrane, the nuclei are closer together because cells are smaller and, in addition, their cytoplasm is basophilic.
2. in the intermediate layers, the nuclei are more separated and their boundaries are well perceptible. Furthermore, shrinkage of cells during processing reveals the desmosomes, giving this layer a "prickly appearance", from which it derives its name, the prickle cell layer.
3. the most superficial living cells present keratohyalin granules (intracellular grains intensely basophilic). Later, the death of the cell and the disappearance of the nuclei forms the stratum corneum or corneal layer, which is nothing but keratin.

TRACHEA

Objectives to identify:

Ciliated pseudostratified epithelium

Goblet cells

Cilia

The cross section of the trachea constitutes a ring, in which a structure of intense blue color that is the cartilage is located in its wall. The outer part of the trachea consists of connective tissue, while its internal surface is lined by a pseudostratified columnar ciliated epithelium, also called as respiratory epithelium. This type of epithelium undergoes rapid post-mortem histolysis, and therefore we will discover large areas where we will not see anything but a thin layer of cells.

The epithelium is tall and the nuclei do not retain the arrangement in a single row but are disposed at different levels, thus creating the illusion of cellular stratification. Despite this apparent stratification, all cells contact with the basement membrane, hence it is called pseudostratified.

In this epithelium, two types of cells stand out (×10):

1. Ciliated cells: are tall, with eosinophilic cytoplasm and nuclei at different heights. They are characterized by having numerous cilia on their apical surface (×40).
2. Goblet cells: they are pale cells, scattered throughout the epithelium, and named for their resemblance to drinking goblets. Nuclei are basally located and the distended apical cytoplasm has a foamy appearance due to mucigen (mucus) granules.

URINARY BLADDER

Objectives to identify:

Transitional epithelium (or urothelium)

The urinary bladder is lined by an epithelium called transitional epithelium or urothelium, characterized by a stratified appearance (×4) presenting a variable number of layers, ranging from three to seven. Beneath the epithelium there are usually abundant blood vessels, sometimes giving the impression of being inside it. At medium (×10) and especially high magnification (×40), it is noted that surface cells (umbrella or dome cells) are large and rounded (projected towards the light) and may contain two nuclei.

KIDNEY

ESOPHAGUS

SKIN

TRACHEA

URINARY BLADDER

Glandular Epithelia

General objective of the practice

To acquire the ability to identify the glandular arrangement of epithelial elements. We will study a typical exocrine gland (the salivary gland) and the thyroid (an endocrine gland whose product of secretion is stored extracellularly). In the large intestine we will see a simple tubular gland and its relationship with the other layers of the organ. The liver has "cords" of cells that we will see interspersed between blood capillaries. We will also look at the breast in order to understand the structure of a glandular lobule: a morphofunctional unit.

SALIVARY GLAND

Objectives to identify:

Serous acinus

Mucous acinus

Mixed serous-mucous acinus

  Crescents of Giannuzzi (Serous demilune)

Intercalated duct

Striated duct

We observe an exocrine gland (×4), whose secretory unit is constituted by the acini (×10), named for the arrangement in clusters that secretory cells adopt. The serous acini show markedly stained cells (×10, ×40), while mucous acini, made up of mucous cells, have a clear cytoplasm (×10, ×40).

Acini that contain both serous and mucous cells are called mixed acini (×10). In these mixed acini, serous cells are located surrounding mucous cells, acquiring a crescent appearance: this image (which is actually an artifact that appears during sample processing) is the Gianuzzi's crescent

(×40). Small excretory ducts are located between the acini, formed by a row of cubic cells with eosinophilic cytoplasm called intercalated ducts (×10, ×40).

Acini are arranged in fairly well-defined groups called lobules. (×4). Within the lobule, the acini are embedded in a more or less loose connective tissue (×10). This connective tissue becomes denser when located between the different lobules (called interlobular). At this level we can find the larger-sized secretory ducts, the striated ducts (×40). These ducts consist of large columnar cells whose basal domain appears striated due to the presence of basal interdigitations (basal labyrinth).

LARGE INTESTINE

Objectives to identify:

Intestinal mucosa

Simple tubular gland

Absorptive cells (enterocytes)

Goblet cells (mucous cells)

The simple columnar epithelium that lines the intestinal mucosa is of the glandular type (×4), whose invaginations create tubular glands. The glands are lined by a simple columnar epithelium (×10) formed mainly by absorptive cells/enterocytes (with eosinophilic cytoplasm and basal nucleus) and by goblet cells that show a clear cytoplasm, with calyx shape, in which the apical plasmatic membrane is not clearly delimited (×40). We must note that glands are only a part of the intestinal mucosa.

BREAST GLAND

Objectives to identify:

Lobule

Epithelial component

Intralobular and terminal ducts: epithelium

Connective component

Intralobular stroma

Interlobular stroma

The mammary gland is constituted (×4) by a glandular epithelial element (parenchyma) embedded in a matrix of connective tissue (stroma). The epithelial element is arranged like a bunch/cluster (×10). These clusters (secretory in nature) form relatively large units (lobes) that are separated from each other (×4) by dense connective tissue (interlobular stroma). In the lobes, in turn, there are small secretory units (lobules) whose connective tissue is less dense than the previous one (intralobular stroma). Inside the lobule, we can see the epithelial component of the organ, which in resting breasts (not pregnant nor lactating) appears as small ductules (terminal ductules).

LIVER

Objectives to identify:

Parenchyma: hepatic cord (hepatocytes)

Capillary vascular network: sinusoids

The observation at small magnifications (×4) of a liver shows that it is formed by eosinophilic cords (cords of hepatocytes) that constitute the liver parenchyma. These cords are anastomosed with each other (×10), giving an irregular appearance, and flanked (×10) by very thin blood vessels called hepatic capillaries or hepatic sinusoids. This arrangement allows hepatocytes to exchange, with ease, a huge number of substances with blood.

THYROID

Objectives to identify:

Follicle

The histological section of the thyroid (×4) is very characteristic, since it is made up of rounded elements (follicles) consisting (×10) of a monolayer of cells, constituting the wall (follicular cells), and of an internal acellular zone containing a homogeneous material (colloid). The follicles are separated by a basement membrane from the adjacent connective tissue, which is richly vascularized (×40). Follicular cells synthesize thyroglobulin, which they store extracellularly (colloid) in the follicular lumen. Given the demand for thyroid hormones, portions of colloid are recaptured by follicular cells and, from thyroglobulin, form the active hormones that are released into the blood, at the blood capillaries located in the adjacent stroma.

SALIVARY GLAND

LARGE INTESTINE

BREAST GLAND

LIVER

THYROID

Connective and Adipose Tissue

General objective of the practice

To know and to acquire the ability to identify the main types of common connective tissue (loose, dense, reticular, mucous and elastic) and its main cell types. We will also study the adipose tissue, which is a variety of connective tissue with its own characteristics.

SKIN

Objectives to identify:

Epithelium: epidermis

Connective tissue: dermis and hypodermis

  Loose connective tissue (superficial or papillary dermis)

  Dense connective tissue (deep or reticular dermis)

  Reticular connective tissue

White adipose tissue

Adipocytes

The skin allows us to see not only abundant dense connective tissue, but also the reticular variety and adipose tissue. The first thing we must do is to orient ourselves in the preparation. To do this (×4), we will first look for an epithelial band: this is the epidermis, which corresponds to the variety of keratinized stratified flat/squamous epithelium (already studied in the practice of surface epithelium). Below the epidermis, we can find the dermis (dense connective tissue) and deeper, the hypodermis (with abundant adipose tissue).

The dermis is revealed to us (×4, ×10) as a connective tissue where the fibres of collagen prevail, giving the tissue a homogeneous and eosinophilic appearance (there are territorial variations

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that we will see when we study the skin). Collagen fibres are arranged in more or less sinuous bundles or fascicles, within which we can recognize fibroblasts, normally inactive. In the dermis, we will see large longitudinal or obliquely sectioned structures that are the hair follicles and, next to them, accumulations of clear cells that correspond to the sebaceous glands. In the deepest part of the dermis, we can also observe clear spaces with small cross-cut tubes with a spiral or swirling appearance: these are the sweat glands, which are immersed in a connective tissue rich in reticulin fibres: the connective reticular tissue.

The furthest part from the epidermis corresponds to the hypodermis (or cellular subcutaneous tissue) where white adipose tissue is abundant and whose appearance is that of a mesh or net. Note that when soft/light-stained, adipose tissue might pass unnoticed. At higher magnifications (×10, ×40), we identify its cell, the adipocyte, which appears as an empty and rounded space limited by fine margins corresponding to the plasma membrane together with few cytoplasmic organelles.

UMBILICAL CORD

Objectives to identify:

Mucous connective tissue

Mucous connective tissue is found exclusively in the umbilical cord and is so-called as "Wharton Jelly". At small magnification (×10), in a cross-sectional section of the umbilical cord, we can observe three vascular structures (one vein and two arteries), between which we find the mucous connective tissue. It is recognized (×10, ×40) as a basophilic granular matrix (rich in mucopolysaccharides) with few fibres and few cells.

AORTA

Objectives to identify:

Elastic connective tissue

Elastic fibres

Elastic connective tissue can be seen in the wall of the arteries, especially in elastic arteries like aorta, where elastic fibers are distributed over most of its wall. In routine stains (hematoxylin-eosin), elastic fibers appear pink (they are eosinophilic) and therefore very difficult to differentiate (×10) from smooth muscle cells that form the middle layer of the aorta, giving the whole wall a relatively homogeneous appearance. However, if we close the diaphragm slightly, we raise the light (moving the potentiometer to position 7-9) and observe with the 10x or 40x objectives, we will be able to notice the presence of fine wavy and shiny bands, with a parallel arrangement to each other.

SKIN

UMBILICAL CORD

AORTA

Cartilage, bone and bone development

General objective of the practice

To know and to acquire the ability to identify the main types of cartilage and bones, as well as the structural and cellular elements that compose them. It is also intended to study the epiphyseal growth plate and to correlate the physiological events that occur in it with its morphology.

TRACHEA

Objectives to identify:

Territorial and interterritorial matrix

Isogenic group

Chondral lacunae

Chondrocyte

Perichondrium

  Fibrous perichondrium

  Chondrogenic perichondrium: chondroblast

Hyaline cartilage is easily recognizable against the light due to its intense blue coloration (basophilia). Thus, in the trachea it is revealed as rings in the shape of the letter C. With the small lens (×4), we can see the peculiarity of this tissue, characterized by the homogeneity (hyaline texture) of its matrix, scattered with lighter spaces with cells (chondrocytes) inside (chondral lacunae) and usually grouped in a variable number (isogenic group). Isogenic groups receive this name because all of them come from the same cell.

Chondrocytes of the isogenic group produce matrix, so they separate from each other, this being mechanism responsible for the interstitial growth of the cartilage. Cells are located in a hole

or cell ("celda") called chondral lagoon. Artifact-retraction of chondrocytes makes these gaps appear empty, which are separated from each other by thin partitions.

With the objective of 10x, we can appreciate that the matrix where the cells of the isogenic groups are immersed (territorial matrix) is more basophilic than the matrix between isogenic groups (interterritorial matrix). This is due to the different composition of the matrices, as territorial matrix is more recent and richer in sulphated proteoglycans.

On the limits of each chondral zone, and always separating them from the surrounding connective tissue, we find the perichondrium. We can differentiate between two areas: the most external or peripheral (fibrous in appearance) and the innermost, which only differs from the underlying cartilage in the smallest size of the lacunae, where we find chondroblasts, a minor cell type and less differentiated than mature chondrocytes.

EPIGLOTTIS / LARYNX

Objectives to identify:

Territorial and interterritorial matrix

  Elastic fibres

Isogenic group

Chondral lacunae

Chondrocyte

Perichondrium

  Fibrous perichondrium

  Chondrogenic perichondrium: chondroblast

The elastic cartilage is quite similar to the hyaline one: isogenic groups, gaps and chondrocytes are superimposable to that seen in the hyaline cartilage. Perhaps isogenic groups show a less regular disposition. The real difference is found in its matrix, which is very rich in elastic fibers, giving a less homogeneous appearance in the sections stained with hematoxylin-eosin. By slightly closing the diaphragm, we can glimpse the elastic fibers due to their refringence. However, it is only with specific stains for elastic fibers like orcein when the elastic variety reveals its richness in elastic fibers. The perichondrium is similar to the already studied.

INTERVERTEBRAL DISC

Objectives to identify:

Territorial and interterritorial matrix

Isogenic group

Chondrocyte

Perichondrium

Despite fibrous cartilage shares the structure studied above, it presents some particular features that differentiate it even morphologically. On the one hand, isogenic groups are composed of few chondrocytes, which are scarce and housed in small lacunae. On the other hand, the territorial matrix can be distinguished by its greater basophilia when comparing to the interterritorial one, which is more fibrous in appearance and eosinophilic, with dense bundles of collagen fibers.

LAMINAR BONE

Objectives to identify:

Compact bone (cortical bone)

　Haversian canals

　Osteons

Trabecular bone (spongy bone; cancellous bone)

　Trabeculae

Bone elements

　Lamella

　Osteocyte lacunae

　Osteocyte

　Endosteum

　Periosteum

The laminar bone is the mature form of bone tissue, as immature bone has no lamellae. Therefore, the basic histological structure is laminar, but the arrangement of the lamellae/plates is different in the compact or in the trabecular varieties.

The compact variety is typical of the cortical bones and, for this reason, we will see it in the periphery of the bones. At small magnifications, the Haversian canals stand out, clear and rounded spaces where the vessels and nerves run, centring the structural unit of the compact bone: the osteon (×10). The osteon is a structure characterized by the concentric arrangement of the lamellae, a fact that is revealed by slightly closing the diaphragm. Each lamella houses small cavities that are bone lacunae, where osteocytes lodge. If we move (×4) towards the inner-most regions of the bone (where we find the bone marrow), we can see how the compact bone is projected inwards as "cords" or trabeculae. These trabeculae appear as isolated "fragments" of bone, or as ramifications from the compact cortical zone. They correspond also to mature bone, and therefore, they have lamellae, although they do not have Haversian canals or osteons. Like compact bone, the cell that appears in the bone matrix is the osteocyte. The trabeculae are covered by the endosteum (×40).

At the margins of the bone and separating it from the surrounding connective tissue (×10), we find the periosteum. As in cartilage, two areas are differentiated: the outermost or peripheral (with its fibrous appearance) and the innermost one, which only differs from the underlying bone in the smaller size of lacunae, where osteoblasts (immature cells) are housed.

ENDOCONDRAL OSSIFICATION

Objectives to identify:

Epiphyseal plate

  Resting cartilage

  Zone of proliferating chondrocytes

  Zone of chondrocyte hypertrophy

  Zone of cell death

  Zone of vascular invasion

  Zone of mixed spicules

Chondral degeneration

Immature bone lamellae

Osteoblast

Osteoclast

Osteocyte

Osteoid

Periosteum

At the boundary between the epiphysis and the diaphysis we find the epiphyseal plate, characterized by the arrangement of chondrocytes in rows/columns parallel to the bone. In these rows, chondrocytes are arranged as a "pile of coins", in which a progressive increase in the size of the lacunae and a gradual swelling and degeneration of the chondrocytes as they approach to the diaphysis occur. These cytological changes are accompanied by the degeneration of the matrix that becomes more basophilic and ends up calcifying. These phenomena are expressive of chondral degeneration.

From here, the degenerated cartilage continues with immature bone trabeculae, in whose sinus are located the osteocytes, while the osteoblasts are outside. Osteoblasts are cells with great capacity of synthesis (basophilic cytoplasm) that are arranged in rows at the margins of the trabeculae, in the inner surface of the cortices, or between the cortex and the periosteum. Interspersed with osteoblasts, osteoclasts are found: this is a multinucleated cell, with eosinophilic staining and larger than the previous one, which belongs to the monocyte macrophage system. They are responsible for the erosion of trabeculae, being able to see them in small lagoons excavated by them in the free surface of trabeculae (Howship lagoons).

The trabeculae closest to the epiphyseal plate (metaphysis) present a very basophilic central matrix, which in many cases corresponds to the calcified chondral matrix (discussed above), surrounded by an eosinophilic matrix that has not been calcified yet (osteoid). It is at this level where osteoclasts and osteoblasts are most easily seen. The characteristics of the periosteum are similar to those seen in the previous preparation. However, as we have a very active growth process here, the layers, especially the internal or cellular, are more prominent, with visible osteoblasts.

TRACHEA

EPIGLOTTIS / LARYNX

INTERVERTEBRAL DISC
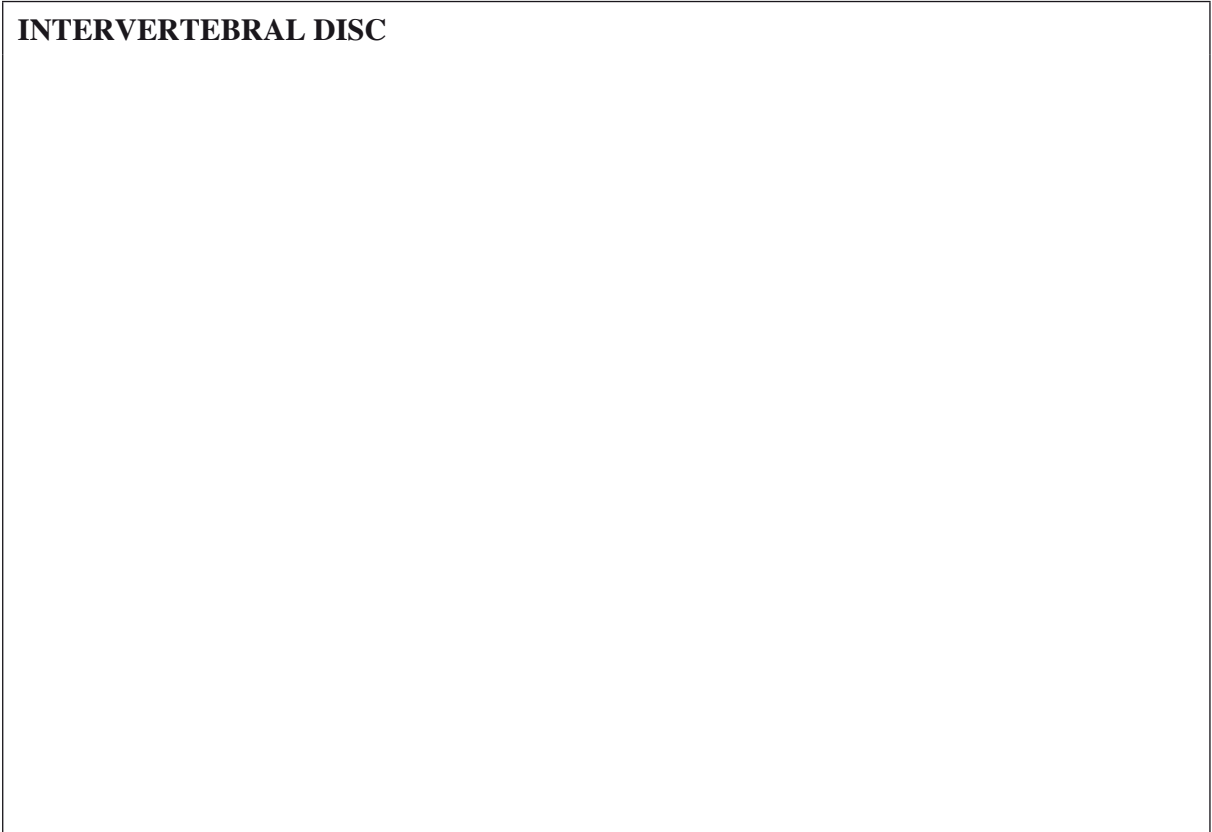
LAMINAR BONE

ENDOCONDRAL OSSIFICATION

Blood and Hematopoiesis

General objective of the practice

To know and to acquire the ability to identify the hematopoietic tissue and its main elements, both in smears as in histological sections. The observation of peripheral blood smears will allow us to recognize the cellular elements present in the blood. It is also intended that the student will be able to distinguish bone marrow smears from peripheral blood smears.

PERIPHERAL BLOOD

Objectives to identify:

Erythrocytes

Leukocytes

  Neutrophil and band cell

  Lymphocyte

Platelets

The study of a blood smear should be carried out in the so-called "observation area". In it, fundamentally, the following elements are (×40):

1. Erythrocytes: enucleated cells with eosinophilic staining.
2. Granulocytes: cells with a lobed or segmented nucleus in whose cytoplasm can be seen granules.
3. Lymphocytes: cells with a large nucleus/cytoplasm ratio, basophilic nucleus, hyperchromatic and scanty cytoplasm.
4. Platelets: they are small structures (smaller than 4 um) that correspond to cytoplasmic portions of megakaryocytes.

BONE MARROW EXTENSION

Objectives to identify:

Immature myelocytic forms (myelopoiesis)

Immature erythroid forms (erythropoiesis)

Megakaryocyte

In addition to the mature cells seen above, here we can find (×40):

1. Immature forms of the red series: they are cells with a dense, basophilic nucleus in a homogeneous cytoplasm
2. Immature forms of the white series: large cells with indented nuclei whose cytoplasm has numerous secretory granules
3. Megakaryocytes: huge cells with a multilobed nucleus. Easily visible with the objective of ×10 and even with the smallest one.

BONE MARROW BIOPSY

Objectives to identify:

Bone trabecula

Hematopoietic tissue

Megakaryocyte

Adipose tissue

In a bone marrow section, we can distinguish three main components:

1. Bone: made up of trabeculae of trabecular bone tissue that supports and houses the hematopoietic tissue.
2. Hematopoietic tissue: it constitutes the parenchyma itself. We distinguish basophilic cells arranged in nests (immature forms of the red series: erythroblastic series) together with diffusely arranged more eosinophilic cells (immature forms of the white series). Megakaryocytes are identified by their large size and multilobed nucleus.
3. Adipose tissue: adipocytes are arranged close to the bone trabeculae and within the hematopoietic tissue, easily recognizable by their morphology.

PERIPHERAL BLOOD

BONE MARROW EXTENSION

BONE MARROW BIOPSY

Muscle tissue

General objective of the practice

To know and to acquire the ability to identify the different varieties of muscle tissue. Likewise, it is intended that the student becomes familiar with the orientation of the cut in the section studied.

In transmission electron microscopy photography, we will focus on the identification of the sarcomere and its parts.

SKELETAL MUSCLE

Objectives to identify:

Skeletal striated muscle fiber (longitudinal section)

Skeletal striated muscle fiber (cross section)

The observation at small magnifications of a longitudinal section of skeletal muscle fibers reveals strongly eosinophilic bundles that present a striation perpendicular to its maximum axis (×10, ×40).

Each muscle fiber is well defined and has several nuclei that are arranged in the periphery (×10). In cross sections, the muscle fibers are rounded (×10), without striation and with marginalized nuclei (×10, ×40).

TRANSMISSION ELECTRON MICROSCOPY PHOTOGRAPHY

Objectives to identify:

Sarcomere

Z lines

A band, H band, M line and I Band

Mitochondria

In this TEM photography, a skeletal striated muscle fiber is observed. The ultrastructure offers an image characterized by the periodic striation existing in the cell cytoplasm. The striation is given by dark lines (Z lines) that delimit the sarcomere (muscular functional unit).

Inside the sarcomere, there is a darker central zone (more electrodense) that represents the A band. In the most central portion of the A band, there is a paler area which is the H band. In the middle portion of H band, in turn, M line is located. On each side of the A band and bounded by the Z lines, there are two paler-looking I semi-bands (low electro-density).

Cytoplasmic organelles are also observed in the muscle fiber, such as mitochondria and RER. Glycogen accumulations can also be observed.

SMOOTH MUSCLE

Objectives to identify:

Smooth muscle fascicle

General arrangement of the bundles of a smooth muscular organ

The uterus has a large muscular framework (myometrium) made up of smooth muscle fibers. With the objectives of ×4 and ×10, we can observe that the muscle fascicles cross over each other and have a less conspicuous staining than skeletal muscle fibers.

At higher magnifications (×40), we notice that each smooth muscle fiber has a single nucleus with rounded edges and central location.

CARDIAC MUSCLE

Objectives to identify:

Cardiac muscle fiber (longitudinal section)

Intercalated discs

Cardiac muscle fiber (cross section)

The heart is a muscular organ made up of striated muscle fibers called cardiac muscle fibers (longitudinal section), with different characteristics to the skeletal fiber. Each cardiac fiber has a single nucleus that is located in the central part of the fiber.

In this muscular variety, fibers are linked to each other by special structures called intercalated discs, which hint at higher magnification (×40). These discs allow the cardiac muscle fibers to form a kind of three-dimensional network. The cross section, as it occurs in the skeletal muscle fibers, does not allow us to see the striation.

SKELETAL MUSCLE

TRANSMISSION ELECTRON MICROSCOPY PHOTOGRAPHY

SMOOTH MUSCLE

CARDIAC MUSCLE

Nervous System

General objective of the practice

To identify the structure of a spinal ganglion and a peripheral nerve. In each of these structures we should be recognize their most significant elements. In the peripheral nerve preparation, it is also intended that the student recognizes the orientation of the histological section and in transmission electron microscopy photography be able to observe the structure of myelinated and unmyelinated nerves. To know and acquire the ability to recognize the spinal cord, brain and cerebellum. In each of these structures, we will recognize their principal structures as well as their main cell types.

SPINAL GANGLION (DORSAL ROOT GANGLION)

Objectives to identify:

Neurons

   Lipofuscin

Satellite cells

Nerve fibers

The observation at small magnifications of a spinal ganglion makes it possible to reveal two perfectly distinguishable constituents:

1. Neuronal cells: these cells stand out for presenting a large nucleus with a prominent nucleolus(×10). In the body of these cells, it is common to observe (×40) the existence of a yellowish pigment (lipofuscin) whose amount increases with age. Satellite cells can be seen around the neuronal bodies as rounded nuclei (remember a pearl necklace).
2. Nerve fibers: they represent the afferent and efferent myelinated neuronal fibers to the spinal ganglion. They are easily identified by the undulating image they present. Between the fibers are the nuclei of Schwann cells.

PERIPHERAL NERVE

Objectives to identify:

Nerve (longitudinal and transversal section)

Perineural sheaths: endoneurium, perineurium and epineurium

The longitudinal section of the peripheral nerves offers a characteristic undulating image (×4, ×10), which attracts attention thanks to the arrangement of the nuclei of Schwann cells (×10). The transversal section allows to observe the axons (×10, ×40) represented by a circular eosinophilic structure, surrounded by a clear, optically empty space, which represents the myelin that has dissolved with alcohols in the process of embedding the samples in paraffin. Outside of this clear halo, the nucleus of a Schwann cell is sometimes visible. (Correlate this image with transmission electron microscopy photography). The perineural sheaths are made up of bundles of connective tissue (×10).

TRANSMISSION ELECTRONIC MICROSCOPY PHOTOGRAPHY

Objectives to identify:

Myelinated nerve fiber

Schwann cell nucleus

Schwann cell cytoplasm

Myelin sheath

Axon

Unmyelinated nerve fiber

Basal membrane

Endoneural collagen

The myelin sheath, with a spiral constitution, stands out for its intense electrodensity. Inside the sheath we find the axon and outside the rest of the cytoplasm and the nucleus of the Schwann cell. The nerve fiber is separated from the surrounding connective tissue by a delicate basement membrane. Between different nerve fibers there are small collagen fibers (endoneural collagen) with different orientation.

Unmyelinated fibers are characterized because a single Schwann cell houses in its interior various axons. Outside of them, there is also a basement membrane.

SPINAL CORD

Objectives to identify:

Grey matter

   Ventral horn: motor neuron

   Grey commisure

   Dorsal horn

White matter

Central canal

The transversal sections of the spinal cord are represented (×4) by a section rounded with a small central hole that is the central canal. In the anterior part, we identify the anterior median fissure, and in the opposite side the posterior median sulcus.

The central canal is a tubular structure that runs through the center of the spinal cord. It is lined by a cuboidal epithelium formed by ependymocytes. In the spinal cord, the gray matter is arranged internally, surrounding the central canal, presenting an image reminiscent of the wings of a butterfly. The gray matter is represented by two anterior (ventral) horns and two posterior (dorsal) horns, symmetrical placed. The portion of grey matter around the central canal is named grey commisure, and it is continued without interruption with the horns. The white matter surrounds and encompasses gray matter. The widest areas of the gray matter (anterior horns) contain neuronal areas where motor neurons are located: they are cells of large size and pyramidal appearance with large nuclei and evident nucleolus.

BRAIN

Objectives to identify:

Grey matter (cerebral cortex)

   Molecular layer

   Pyramidal cells

White matter

   Oligodendrocytes

At small magnifications (×4, ×10), two zones are easily distinguished: an external one, rich in cells of different size and morphology: grey matter-cerebral cortex and another of internal disposition (white matter) where fibrillar structures are abundant and small cells with dense and rounded nuclei are recognized.

The cerebral cortex is also called gray matter since its richness in cellular somas give it a grayish color on fresh observation. On the contrary, white matter is named because being rich in myelin and having lower cell density it offers a whitish coloration.

In the cerebral cortex (×4), and immediately below the pia mater, there is a zone poor in nuclei: molecular layer (×10). Below the molecular layer, we find the rest of the cortical layers, with abundant somas. These include (×10, ×40) some that have a pyramidal morphology: pyramidal cells. Cells with small nuclei are abundant in the white matter, which correspond to a variety of glia, the oligodendroglia, whose cells are called oligodendrocytes (×10, ×40). The core image of this cell is very typical, with a clear halo around its round nucleus (×40), and artifactual product of the histological processing of nervous tissue.

CEREBELLUM

Objectives to identify:

Grey matter (cerebellar cortex)

   Molecular layer

   Purkinje cell layer

   Granular layer

White matter

   Oligodendrocyte

As in the brain, in the cerebellum, a gray matter (cerebellar cortex) and a white substance are distinguished. From the outside in, the cortex is constituted by three layers (×4):

1. Molecular layer: with morphological characteristics similar to that observed in the cortex cerebral, although comparatively thicker.
2. Purkinje cell layer: a discontinuous monolayer made up of large pyramidal cells (×4, ×10), with eosinophilic staining and prominent nucleolus (×40), which emits long and profusely branched dendrites into the molecular layer.
3. Granular layer: made up of numerous, round and very densely stained nuclei that at low magnifications look like basophilic grains.
   Internally to the layer of grains we find the white matter of the cerebellum, with similar characteristics to that observed in the brain and with the presence of oligodendrocytes.

SPINAL GANGLION (DORSAL ROOT GANGLION)

PERIPHERAL NERVE

TRANSMISSION ELECTRON MICROSCOPY PHOTOGRAPHY

SPINAL CORD

BRAIN

CEREBELLUM

Special sense organs

General objective of the practice

To know the structure of the eyeball and its parts. Special attention will be paid to the cornea and the retina and their respectively layers. In the ear, to understand the bony and membranous labyrinths. In the vestibule be able to recognize the sensory end organs. To describe the components of the cochlea including the organ of Corti. To recognize the taste buds.

THE EYE

Objectives to identify:

Fibrous Tunic

    Sclera

    Cornea

        Corneal epithelium

        Bowman's membrane

        Stroma

        Descemet's membrane

        Corneal endothelium

Vascular Tunic

    Choroid

    Ciliary body

        Ciliary process

        Ciliary muscle

        Ciliary body epithelium

Iris

   Stroma

   Sphincter muscle

   Pigment layer (posterior epithelium)

Nervous Tunic

   Ciliary body epithelium

   Pigment layer of Iris (posterior epithelium)

   Retina

      Pigment epithelium

      Photoreceptor layer (layer of cones and rods)

      Outer limiting layer

      Outer nuclear layer (nuclei of cones and rods)

      Outer plexiform layer

      Inner nuclear layer

      Inner plexiform layer

      Ganglion cell layer

      Optic nerve fibres (fibres of the optic nerve)

      Inner limiting layer

   Lens

      Lens capsule

      Lens epithelium (anterior epithelium)

      Lens fibers

The simple direct observation (without a microscope) of a histological section provides the most useful guidance on the arrangement of the different components studied in this practice.

The outer layer of the eye is the Fibrous Tunic. The sclera (×4) is the outer layer of dense connective tissue that surrounds the eyeball, except in its anterior pole, where the cornea is arranged. The cornea is made up of five layers: two epithelia that sit on their respective basal cells and a stroma in the central area. So, from outside to inside, we find (×10) the following layers:

1. A non-keratinized stratified flat epithelium resting (×40)
2. On a thick basement membrane (Bowman's membrane)
3. More deeply, a dense, avascular connective tissue is arranged, in which the fibers of the extracellular matrix adopt a very regular arrangement

4. Then, we find other basement membrane (Descemet's membrane), thinner than the previous one.
5. Finally, a thin epithelium (posterior corneal epithelium) called corneal endothelium.

The middle layer of the eye is the Vascular Tunic. In the posterior area of the eyeball (where there is no cornea), we find the choroid, which has numerous blood vessels. In the anterior zone, the choroid is continued with the ciliary body and the Iris. The ciliary body is constituted by the ciliary processes, the ciliary muscle and the ciliary body epithelium (the latter, architecturally, would be included in the nervous tunic). The iris has a stromal body within which the dilator and constrictor muscles of the pupil are arranged. Its anterior face does not have an epithelial layer but melanocytes, (×10, ×40), and its posterior face has a pigmentary layer (which is the epithelium of the pupil and, architecturally, would be included in the nervous tunic).

The inner layer of the eye is the Vascular Tunic, and is constituted from back to front by the retina, the ciliary body epithelium and the pigmentary layer of the iris. The retina: separated from the choroid by a basement membrane (Bruch's membrane or vitrea) has ten layers (×10, ×40):

1. The outermost (close to the choroid) is made up of a monolayer of cells very pigmented (*pigmented epithelium*).
2. Immediately below is the *layer of rods and cones* (which is composed of the photosensitive processes of the *photoreceptors*)
3. Then, the *outer limiting membrane*
4. Below, a group of nuclei, the *outer nuclear layer*, that correspond to the nuclei of cones and rods
5. The presence of cytoplasmic processes gives this layer an appearance frayed and its name of *outer plexiform layer*
6. Then, a nuclear cluster, the *inner nuclear layer*, where they are arranged bipolar, horizontal, amacrine and Müller cells (the latter are glial cells)
7. Followed by another *inner plexiform layer*
8. Few nuclei constitute the *layer of ganglion cells*, whose fibers constitute
9. the fibers of the optic nerve (*optic nerve fibers*)
10. Finally, the entire retina is delimited by the *inner limiting layer*

The lens (×4) is an eosinophilic body that, together with its ligament, delimits two spaces: one anterior (which contains the aqueous humor) and another posterior (vitreous humor). The anterior face of the lens presents (×10) the anterior epithelium of the lens, which is characterized by orienting its thick basement membrane outwards (×40), as consequence of its embryologic development. Between the cornea and the lens is the iris, which divides the anterior space of the eye in an anterior and a posterior chamber. In the anterior chamber, the aqueous humor formed in the ciliary process (posterior chamber) is reabsorbed.

THE EAR

Objectives to identify:

Temporal bone

Fibers of cochlear or acoustic nerve

Vestibule: utricle & saccule (maculae) and crista ampullaris at the ampullae of the semicircular canals

Cochlea

　Modiolus

　Spiral ganglion

　Scala media, scala vestibuli and scala tympani

　Membranes separating the scalae: basilar and vestibular (Reissner's) membranes

　Stria vascularis

　The organ of Corti

Before working with this preparation, please be aware that the cochlea is a coiled structure and so is always sectioned repeatedly and often oddly.

The inner ear is composed by compartments, one contained within the other: the bony and the membranous labyrinths. The former consists of interconnecting cavities and ducts full of perilymph, creating 3 communicated spaces located within the temporal bone: i) three semicircular canals (superior, horizontal and posterior) ii) two sacs that form the vestibule; and iii) the cochlea or snail. The membranous labyrinth is formed by the three semicircular (membranous) canals, which open to the utricle, with their ampullary ridges/cristae; ii) the utricle and the saccule; and iii) the cochlear duct. The *scala media* of the cochlear duct represents the central chamber of the cochlea, is filled with endolymph and contains the organ of Corti. It runs in parallel with the *scala vestibui* (upper) and the *scala tympani* (lower), tubular spaces filled with perilymph that communicate at the apex or helicotrema.

TONGUE: TASTE BUDS

Objectives to identify:

Circumvallate papilla

Taste bud

As detailed before in the gastrointestinal tract, the section of the tongue shows a peripheral mucosa and inner bundles of skeletal muscle. The squamous epithelium of the mucosa is elevated on the dorsal surface of the tongue into papillae. In humans, there are three types of papillae: filiform, fungiform and circumvallate.

Despite taste buds can appear in other parts of the tongue, in humans they are mainly located in these circumvallate papillae, appearing as depressions along their lateral walls. Taste buds are the chemoreceptors for the sense of taste and they resemble "barrels" that open at the surface of the papilla by means of a taste pore. The three types of cells within the taste bud are sensory, supporting and basal, but they are difficult to identify with routine staining.

EYE

EAR

TASTE BUDS

Circulatory System

General objective of the practice

To acquire the skills and competencies to identify the main elements of the cardiovascular system. The circulatory system consists of the heart and vessels, the latter being arteries, capillaries or veins. They all have basically the same structure consisting of three layers: intima, media and adventitia (called endocardium, myocardium and epicardium in the heart).

HEART

Objectives to identify:

Atrial wall

Ventricular wall

Atrioventricular valve

Endocardium

   Endothelium

   Subendothelial connective tissue

Myocardium (cardiac muscle)

Epicardium (visceral layer of serous pericardium)

Coronary branch

With the small objective (×4), the three layers of the heart wall are easily observed:

1. The endocardium (intimal layer), made up of the endothelial lining that rests on a scarce layer of connective tissue (it continues without interruption with the endothelium of vessels entering and leaving the heart).

2. The myocardium (middle layer) represents almost the entire thickness of the heart. It is made up of cardiac striated muscle cells (cardiac muscle fibers) that form the atria and ventricles. It shows a variable thickness: greater in the ventricles than in the atria.
3. The outer layer of the heart is the visceral pericardium. It consists of a coating mesothelial tissue that rests on a layer of loose connective tissue that is reflected and forms the parietal pericardium. Between both pericardial leaves they leave a virtual space called pericardial cavity.

The myocardium is made up of cardiac muscle fibers, already studied. In the atrioventricular limit (×4) there is a projection of fibrous tissue lined by endocardium which is the atrioventricular valve. At the atrioventricular junction, you can see (×4, ×10) muscular arteries that correspond to the coronary arteries.

ELASTIC ARTERY

Objectives to identify:

Tunica intima

Tunica media

Tunica adventitia

The arterial wall, like other blood vessels (except capillaries and venules), is constituted (×4, ×10) by the three layers above mentioned (intima, media and adventitia). The aorta is an elastic artery, named for its richness in elastic fibers. The intima is constituted (×10, ×40) by an endothelial layer that rests between loose connective tissue. The intimate layer has, in its innermost zone, a band of elastic fibers (internal limiting lamina) that is continued with the layer, with which sometimes is confused.

The most characteristic element is the richness in elastic fibers, which almost occupy the entire thickness of its wall (×10), being easily observable in the middle layer. As we already commented on the practice of connective tissue, elastic fibers are eosinophilic and are confused with the rest of the wall. To highlight them (×10, ×40), we will close the diagram slightly and see them as fine, wavy and shiny fibers, parallel to each other. The outer layer or adventitia is made up of loose connective tissue (×10) with small vessels inside.

MUSCULAR ARTERY

Objectives to identify:

Muscular artery

The muscular arteries are recognized in their cross section by presenting a circular appearance with a rounded light (×4). The intimate layer has, in its innermost zone, a band of elastic fibers (internal limiting lamina) with a wavy appearance due to the postmortem contraction of the vessel: its refractive character is appreciated when closing the. The middle layer

is made up of abundant parallel muscle fibers (×10), limited by the internal and external elastic laminae. The loose connective tissue of the adventitia continues with the perivascular connective tissue.

ARTERY – ARTERIOLE – VENULE – VEIN – CAPILLARY

Objectives to identify:

Arteriole - Venule - Capillary

Arterioles are included in the micocirculation (less than 100 μm thickness). In the transversal section (the most frequent), the arteriole is recognized by presenting (×10) a thick wall of muscle fibers (3-4 layers) and a rounded lumen, small in diameter compared to the one of the muscular wall. The middle layer mainly consists of smooth muscle layers of circular orientation. The elastic sheets that we saw in the arteries are not recognized (×40). The venule, similar to the vein although smaller, presents (×10) a wide light compared to its wall thickness, which is very thin, and in which few smooth muscle fibers can be observed (×40). The capillary (×10) is recognized as a circular ring made up (×40) by the thin cytoplasm of one or two endothelial cells that may occasionally show their nuclei in the preparation. Inside, one or two red blood cells are usually observed, occluding practically all the vascular lumen.

HEART

ELASTIC ARTERY

MUSCULAR ARTERY

ARTERY-ARTERIOLE-VENULE-VEIN-CAPILLARY

Immune System

General objective of the practice

Be able to identify the main lymphoid organs and their most representative structures, thus becoming familiar with their main morphological features.

THYMUS

Objectives to identify:

Lobule

Capsule and septa

Cortex

   Lymphocytes

Medulla

   Corpuscle of Hassall

The thymus is an organ covered by a thin capsule that is organized (×4) in two main lobes. The capsule projects inwards partially dividing the lobes into incomplete lobules that converge with each other (×4). The outermost area of the thymic lobule (thymic cortex) is darker (×4), as it is made up of a high density of mature lymphocytes (×10). On the contrary, the central area of the lobule (thymic medulla) is clearer, as it contains more dispersed larger and immature lymphocytes.

In the thymus, in addition to T lymphocytes, there are also thymic epithelial cells, that at the level of the medulla have an eosinophilic cytoplasm and can be seen in different degrees of degeneration, sometimes becoming spindle-shaped and even pearly structures: corpuscles of Hassall.

LYMPH NODES

Objectives to identify:

Capsule and connective trabeculae

Subcapsular sinus

Cortex

   Nodular (external) region: lymphoid follicles

   Paracortical (inner) region

Medulla

   Medullary cords

   Medullary sinuses

Hilum

The lymph node is an oval or reniform structure made up of a parenchyma of lymphoid tissue surrounded by a fibrous capsule that emits septa (or trabeculae) towards the interior, which is incompletely divided. The peripheral area of the parenchyma is called the cortex and the internal area the medulla. It has a double circulation:

1. blood circulation: blood vessels enter and exit through the hilum.
2. lymphatic circulation: lymphocytes penetrate the convex zone of the capsule (afferent lymphatic vessels), cross it and exit through the hilum (efferent lymphatic vessels).

Once the afferent lymphatic vessels pass through the capsule, the lymph is distributed subcapsularly by the subcapsular (or marginal) sinus consisting of a space lined by endothelial cells. From this sinus, the lymph traverses the lymph node through the cortical sinuses, from where it goes to the medullary sinuses to finally leave the lymph node.

In the cortex, we can observed two main zones: the nodular (external) region, where the lymphoid follicles (rich in B lymphocytes) are placed, and the paracortical (inner) region (rich in T lymphocytes), where "postcapillary venules" with a high endothelium are located (where lymphocyte homing takes place).

Throughout its journey through the sinuses, many lymphoid cells leave the sinuses crossing to the parenchyma, while other cells cross from the parenchyma to the sinuses. In the external cortex, there are the lymphoid follicles (×4), which consist of more or less spherical accumulations of lymphocytes. Some of the follicles have (×10) an outer crown of mature lymphocytes (with dense chromatin and little cytoplasm) surrounding a lighter area (germinal center) made up of more immature lymphocytes (larger size, less dense nucleus and more cytoplasm). These follicles are called secondary lymphoid follicles.

In the medulla we find medullary cords, which contain mainly macrophages, plasma cells and their precursors, and mature lymphocytes. Between the medullary cords we find the sinuses or channels (medullary sinuses) (×10) that drain the lymph to hilum. These sinuses are the result of the confluence of the numerous sinuses that pass through the ganglion.

SPLEEN

Objectives to identify:

Capsule and connective trabeculae

White pulp

White pulp follicles (corpuscles of Malpighi)

Central arteriole

Periarteriolar lymphoid sheath (PALS)

Red pulp

Cords of Billrroth

Splenic sinuses

The spleen, like the preceding organs, is surrounded (×4) by a fibrous capsule that emits trabeculae inwards. Fresh observation of the spleen shows a reddish background (red pulp) on which small whitish areas (white pulp) stand out. The white pulp constitutes the splenic parenchyma and is composed (×4, ×10) by lymphoid accumulations creating:

1. Nodules (corpuscles of Malpighi): oval structures that are pierced by an arteriole called central arteriole (despite not passing through the center).
2. Lymphoid sheaths around arterioles, also called as periarteriolar lymphoid sheaths or PALS, cylindrical cuff of lymphoid tissue around the central arteriole.

The red pulp is constituted (×10) by cellular cords (cords of Billrroth) between which vascular sinuses (splenic sinuses) filled with blood are arranged (×40). The cords are formed by a cyto-reticulum, in such a way that, instead of solid, they are practically hollow and filled with blood cells (mainly erythrocytes). The arteries that enter the spleen end:

1. directly into the sinusoids and then cross into the venous system (closed circulation)
2. in the cords, from where they cross into the sinusoids and then into the veins (open circulation)

Due to the presence of blood in both the cords and sinusoids, it is very difficult to individualize them, so the red pulp looks like a shapeless structure.

THYMUS

LYMPH NODES

SPLEEN

Endocrine System

General objective of the practice

To know and to acquire the ability to identify the hypophysis (pituitary gland) and its different parts (adenohypophysis, pars intermedia and neurohypophysis). We will look at the main cell types and the fibrillary aspect of the neurohypophysis. We will also study the thyroid gland, already seen in the practice of glandular epithelium due to the peculiarity of its follicles. In addition, we will study the parathyroid gland and the adrenal gland. In the latter we will focus on the cortex with its peculiar layers and then on the medulla.

HYPOPHYSIS (PITUITARY GLAND)

Objectives to identify:

Adenohypophysis (Pars Distalis/Anterior Pituitary)

   Chromophil cell: acidophil and basophil

   Chromophobe cells

Pars intermedia: Rathke cleft cysts

Neurohypophysis (Pars Nervosa/Posterior Pituitary)

   Nerve fibers

   Pituicytes

   Herring bodies

Against the light, we can get a precise idea of the different parts of the pituitary gland. The more stained and cellular portion corresponds to the adenohypophysis, while the paler, somewhat basophilic and less cellular, to the neurohypophysis. Between them, it is usually possible to observe small cysts of the intermediate part, the Rathke cleft cysts. With the objective ×4, we will observe again these structures to make a macro-microscopic correlation.

The observation at higher magnification (×10, ×40) of the adenohypophysis stained with hematoxylin-eosin will allow us to classify the epithelial cells into two groups, one whose cells have a high appetite for dyes (chromophilic cells) and the other with a low affinity (chromophobe cells). Within the chromophilic cells, we find cells with affinity for acidic dyes (acidophilic) or for basic dyes (basophilic). Chromophobic cells give an image of pale cytoplasm enhanced by the scarcity of granules in their cytoplasm.

The neurohypophysis is characterized by a fibrillar appearance (×10), which corresponds to the axons of neurons located in the supraoptic (vasopressin) and paraventricular (oxytocin) nuclei of the hypothalamus. Between the axons it is possible to see (×40) cell nuclei corresponding to pituicytes, which are glial cells. Between the adenohypophysis and the neurohypophysis is the Pars intermedia, with epithelial characteristics (×10), characterized by cystic spaces (Rathke's cysts) with a homogeneous eosinophilic content, which are vestiges of the lumen of Rathke's clefts.

THYROID

Objectives to identify:

Follicle

Follicular cell

Parafollicular cell (C cells; clear cells)

The thyroid is an exocrine gland that stores its content in the follicles, so that it is not in contact with the blood. Note that this storage is done extracellularly, so its contents (the colloid) is not recognized as its own, but as foreign, by the organism. The follicle is a more or less spherical structure of different sizes, surrounded by a basement membrane and lined internally by follicular cells (×10) that form a simple, cylindrical or cubic epithelium depending on the size of the follicle and its activity.

The follicular cells take up precursors (mainly amino acids and iodine) from the capillaries located on the other side of the basement membrane through their basal pole. They product of synthesis, thyroglobulin, is functionally inactive, and is dumped and stored in the follicle lumen forming the colloid. When there is a demand for thyroid hormones, the follicular cells capture by pinocytosis portions of thyroglobulin, whose vesicles fuse with lysosomes (phagolysosomes), resulting in thyroxine (T3) and tetrathyroxine (T4), which are functionally active hormones that are released through the basal pole into the stromal capillaries.

In the wall of the follicles, there is another type of cell called parafollicular cells or C cells (×10) because they secrete calcitonin. These cells are arranged singly or in small groups, scattered among the follicular cells. They are included within the basal lamina of the follicles, but without their apical borders in contact with the follicular lumen. They are characterized by their reticular appearance and low staining (×40), which also gives them the name of clear cells.

PARATHYROID

Objectives to identify:

Chief cells

Oxyphil cells

At low magnification, the tissue is basically constituted by glandular parenchyma arranged in anastomosed cords. As an endocrine gland, it presents a rich network of blood capillaries arranged between the cords, where the cells release their secretion.

At higher magnification (×10), we distinguish two cell types:

1. Chief cells: they have a spherical central nucleus and a clear cytoplasm. They are responsible for parathyroid hormone (PTH) synthesis.
2. Oxyphilic cells: they are characterized by a homogeneous eosinophilic cytoplasm (due to their richness in mitochondria). They are thought to be derived from the chief cells.

ADRENAL GLAND

Objectives to identify:

Cortex

   Zona glomerulosa, fasciculata and reticularis

Medulla

Medullar cells

In the adrenal (suprarenal) gland, two zones are distinguished (×4): one peripherally located with large cells with eosinophilic cytoplasm (cortex) and one centrally located (medulla).

In the cortex, the cells are organized in three layers (×4, ×10):

1. A layer located immediately below the capsule: zona glomerulosa, where the cells are grouped in small, rounded clusters reminiscent of renal glomeruli. These cells secrete aldosterone.
2. A middle layer where the cells are organized in fascicles perpendicular to the capsule: zone fasciculata. Its cells secrete corticosteroids.
3. An innermost layer, called the zone reticularis, where the cells are arranged irregularly. Its cells secrete sexual hormones.

Between the cells of the adrenal gland, regardless of their arrangement, a developed capillary plexus appears, due to the endocrine condition of this gland.

The medulla is derived from the neural crest and is considered a chromaffin paraganglia. Its cells (medullary cells ×10) are basophilic, show an irregular arrangement and are immersed in a highly vascularized stroma. They synthesize adrenaline and noradrenaline. As a paraganglia, it is possible to observe neuronal somas.

HYPOPHYSIS (PITUITARY GLAND)

THYROID

PARATHYROID

ADRENAL GLAND

Respiratory System

General objective of the practice

To know and to be able to identify the different segments of the respiratory tract. In the larynx, we will stop at the vocal cords observing their different constitution. In the trachea, we will focus on its different layers. In the lung, we will stop at the airways, showing the differences between bronchi and bronchioles, and at the terminal airways, the alveoli and the pleura. We will also pay attention to the presence of mucosa-associated lymphoid tissue (MALT).

LARYNX

Objectives to identify:

Epiglottis

Elastic cartilage

False Cord

   Respiratory epithelium (ciliated columnar)

   Seromucous glands

Ventricle

True Cord

   Stratified squamous epithelium (non-keratinized)

   Vocal muscle

   Reinke´s space

In a longitudinal section of the larynx, we can easily recognize the epiglottis, together with the elastic cartilage around which there are seromucous glands. The epithelial lining of the larynx presents areas of respiratory epithelium (pseudostratified cylindrical ciliated) and of stratified flat epithelium.

TRACHEA

Objectives to identify:

Mucosa: epitelium and lamina propria

Submucosa: seromucous glands

Hyaline cartilage: perichondrium

Smooth muscle

The cross section shows us a ring in which, at first glance, what most attracts our attention is the cartilage due to its intense basophilic staining. We will need to focus towards the interior part of the sample to find the respiratory epithelium.

Below the epithelium it is located the lamina propria (constituting the mucosa together with the epithelium), made up of loose connective. The lamina propria is continuous with the submucosa, in which we will find seromucous glands and fascicles of smooth muscle. These elements are more abundant in the posterior part of the trachea, where the cartilage ring is absent. Next, it is the tracheal cartilage (of hyaline variety) and more externally the adventitia, which is a zone of connective tissue continuous with the structures surrounding the trachea.

LUNG

Objectives to identify:

Intrapulmonary bronchi

Hyaline cartilage

Intrapulmonary blood vessels (arteries and veins)

Mucosa: epithelium

Smooth muscle

Bronchiole (mucosa, epithelium and smooth muscle)

Alveolar ducts

Alveoli

Capillaries

Pleura

At small magnifications (×4), we are able to recognize the structure of the lung: it this is an aerial structure, loose, with the appearance of a net or a mesh. In it, there are thickened areas where we can normally locate the largest bronchi.

The intrapulmonary bronchi (×10) are a section of the airway with a folded mucosa (with a respiratory epithelium resting on a lamina propria), below which there is a submucosa with

smooth muscle fibers, seromucous glands and lymphoid tissue (the latter can extend to the lamina propria). Surrounding the bronchus along its entire perimeter, we can observe fragments of hyaline cartilage arranged as incomplete irregular plates. Arteries, veins and lymphatics accompany each bronchus.

Of smaller size (less than 1 millimeter in diameter), we can also recognize the bronchioles. They are similar to the intrapulmonary ones, except for small changes in the cytology of their epithelium (Clara cells begin to appear, which will gradually replace goblet and ciliated cells). The main difference when comparing to bronchi is the absence of submucosal glands or cartilage.

The bronchi branch out into smaller segments until they finally end in the alveolar sacs, which are blind sacs made up of two or more sets of alveoli. Each alveolus is an air space that has a thin wall that contains fine capillaries and constitutes the respiratory barrier.

LARYNX

TRACHEA

LUNG

Gastrointestinal System: Digestive Tract

General objective of the practice

To identify the main early portions of the digestive tract, such as the tongue, the esophagus, the stomach, the small intestine, the large intestine and the appendix.

We will cover the general structure of the gastrointestinal tract and we will delve into the mucosa.

TONGUE

Objectives to identify:

Lingual mucosa: papillae

Skeletal muscle

The section of a tongue reveals a peripheral zone (the mucosa) surrounding a large central axis of skeletal muscle. The mucosa is formed by a flat stratified epithelium, scarcely keratinized, supported by a dense connective tissue (lamina propria). In the dorsal region of the tongue, we can recognize how the epithelium forms the taste papillae, and within these are arranged the taste buds.

The most striking feature of the tongue is its muscular axis, which constitutes almost the entire thickness of the tongue. In addition, the muscle bundles are characteristically oriented in all directions, crisscrossing each other, which explains the great lingual mobility. Between the muscle bundles there is a highly vascularized connective tissue rich in nerve fibers with clusters of seromucous glands.

ESOPHAGUS

Objectives to identify:

Mucosa

   Epithelium (non-keratinized stratified squamous)

   Lamina propria

   Muscularis mucosae

Submucosa

Muscularis externa (muscularis propia): smooth and skeletal muscle

Myenteric plexus (Auerbach´s plexus)

In the esophagus, we can see the general structure of the digestive tract, which from the inside out consists of: mucosa, submucosa, muscularis propria and serosa (or adventitia). The mucosa comprises, in turn, three constituents: epithelium, lamina propria and muscularis mucosae. This organization, which already begins in the pharynx, is found in the esophagus, stomach, small intestine, appendix and large intestine. The major differences between the various segments are found at the mucosal level.

The mucosa of the esophagus is constituted by a non-keratinized stratified flat epithelium that sits on connective tissue (lamina propria) and by a band of smooth muscle fibers that constitute the muscularis mucosae.

Underneath there is a wide band of connective tissue (submucosa) rich in vascular structures, where the submucosal plexus or Meissner's plexus is located. Next we find the muscularis externa (muscularis propia). Under 10x magnification, intertwined muscle bundles are visible, some with more vivid staining and in which the nuclei of the muscle fibers are arranged in the periphery (striated muscle) and other paler bundles with a central nucleus (smooth muscle). The relative proportion of these fibers will depend on the segment of the esophagus from which the sample is taken. The upper third is dominated by skeletal bundles, the lower third by smooth muscle bundles, and the middle third shows both types. The smooth muscle bundles are arranged in two layers: one internal and circular and the other external and longitudinal.

On the outside it is the adventitia, made up of connective tissue with blood vessels and nerve endings

STOMACH

Objectives to identify:

Mucosa

    Gastric gland (surface mucous, mucous neck, parietal and chief cells)

    Lamina propia

    Muscularis mucosae

Submucosa

Muscularis externa (muscularis propia)

Myenteric plexus (Auerbach´s plexus)

Serosa/Adventitia

Under 4x magnification, a glandular-type mucosa is observed, which comprises more than half of the thickness of the gastric wall. The glands are subdivided into superficial portion, neck and body. The superficial portion has mucous cells (×10); the neck portion has mucous, reserve and parietal cells, and the body portion has parietal cells in the uppermost and chief cells in the vicinity of the glandular fundi.

The parietal (ClH-secreting) cells are large, rounded and eosinophilic (×40), whereas the chief (pepsin-secreting) cells are prismatic and basophilic (×40). In the lower portions of the glands there are also enteroendocrine cells (producing gastrin, somatostatine, etc.) which are not distinguishable in routine hematoxylin-eosin stained sections. Surrounding and enveloping the epithelial structures appears a thin layer of loose connective tissue (lamina propria). The innermost portion of the mucosa is formed by smooth muscle fibers that are arranged forming the muscularis mucosae.

Next, we find the submucosa (×10), and immediately below (×4, ×10) there are two smooth muscle layers of different orientation: an internal circular and an external longitudinal (the internal one is usually reinforced by a more internal oblique layer). Between the muscular layers there are parasympathetic ganglia and nerve fibers (×10), which constitute the myenteric or Auerbach's plexus. Externally, the stomach presents a thin band of loose connective tissue that constitutes the adventitial layer.

SMALL INTESTINE

Objectives to identify:

Overview of the mucosa

Plicae circulares, valvulae conniventes or folds of Kerckring

Villi

Crypts of Lieberkün

Absorptive cells, goblets cells and Paneth cells

Lamina propia and muscularis mucosae

Submucosa

Muscularis externa (muscularis propia)

The intestinal wall has four layers: mucosa, submucosa, muscularis and serosa. The small intestine presents three systems to increase the absorption surface:

1. Plicae circulares (valvulae conniventes or folds of Kerckring). It is a fold whose axis is the submucosa. It is approximately 10mm high, 4mm thick and 5-6cm long. They are exclusive of the small intestine (absent in the first part of the duodenum).
2. Intestinal villi. Villi are finger-like projections whose axis is the lamina propria. They measure between 0.5 and 1mm in height and their number ranges between 10 and 40 per mm^2 of intestine. They are exclusive of the small intestine.
3. Microvilli. They are a differentiation of the apical cell-membrane of the absorptive cells (enterocytes). They are finger-like extensions of 1um in length and about 0.5um in width with an axis of 40 actin filaments anchored in the terminal network. An absorptive cell has thousands of microvilli and their presence increases the absorptive surface area about 25 times.

The direct observation of the preparation, without the aid of a microscope, allows us to identify the plicae circulares or connivent valves: these are numerous folds that introduce into the intestinal lumen and whose axis is constituted by the submucosa.

Both in these valves and in the rest of the mucosa (×10) there are folds or projections of the mucosa called the intestinal villi: they are smaller and with an axis of lamina propria. The intestinal villi are lined by a simple columnar epithelium (×40) with goblet cells and absorptive (enterocytes, with microvilli at the apical pole) cells.

Between the intestinal villi (×10), there are arranged the intestinal glands or crypts of Lieberkühn, which are inserted in the thickness of the lamina propria. In its deepest portion, and almost exclusively in the duodenum, there are cells with a granular and intensely eosinophilic cytoplasm, called Paneth cells (×40).

The rest of the layers towards the external aspect are similar to the stomach, except for the last layer where we can find an adventitia or a serosa. The serosa is formed by a thin layer of loose connective tissue covered by the peritoneal mesothelium.

LARGE INTESTINE

Objectives to identify:

Intestinal gland (crypt)

Absorptive cells and goblets cells

Lamina propria and muscularis mucosae

Submucosa

Muscularis externa (muscularis propia)

Myenteric plexus (Auerbach´s plexus)

As already mentioned, the large intestine lacks villi. It consists of simple tubular glands (×4) lined by absorptive cells and numerous goblet cells, morphologically similar to those seen in the small intestine (×40). There are also numerous enteroendocrine cells (not visible in routine staining).

In the lamina propria, numerous lymphocytes can be seen (×10, ×40), sometimes organized in lymphoid follicles, some of which have a germinating center (part of the MALT: mucosa-associated lymphoid tissue).

It is common to see some areas of the mucosa sectioned transversely, so that the glands are also transversely sectioned. This causes to see (×4, ×10) the glandular lumen centering the epithelium, so that the mucosal cells resemble the petals of a flower and give the whole a "daisy" appearance, being referred to as a "daisy field image".

The muscular layers offer no histological peculiarities and between them, there is a prominent myenteric plexus (×10, ×40). Outside, the adventitia or serosa.

APPENDIX (VERMIFORM APPENDIX)

Objectives to identify:

Mucosa: epithelium, lamina propia and muscularis mucosae

Lymphoid follicles

The structure of the appendix is similar to that of the large intestine, although its glands are more scarce and smaller. In the epithelium, there are numerous enteroendocrine cells. In the lamina propria, there is abundant lymphoid tissue (forming follicles) and the muscularis mucosae is very thin. The lymphoid tissue of the lamina propria also extends into the submucosa, even with interruption of the muscularis mucosae.

The muscular layer also consists of two layers, although the outer longitudinal one is thinner. Between the muscular layers, the myoenteric plexus is easily observable (×10, ×40). The external surface of the appendix has a serosa (×40).

TONGUE

ESOPHAGUS

STOMACH

SMALL INTESTINE

LARGE INTESTINE

APPENDIX (VERMIFORM APPENDIX)

Gastrointestinal System: Associated Organs

General objective of the practice

To know and to acquire the ability to identify the main types of glands associated with the digestive tract: salivary glands, liver and pancreas. In each of them, we must be able to identify the main constituent elements, both structural and cytological.

SALIVARY GLAND

Objectives to identify:

Acinus: serous, mucous, and mixed serous-mucous (Gianuzzi's halfmoon)

Mucous and serous cells

Myoepithelial cells

Striated duct

Interlobular duct

Against the light, the histological section reveals a lobular-like organization typical of exocrine glands. This configuration is also evident under the microscope, using the smallest objective. Later (×10), we will distinguish how the lobes are organized into lobules, the secretory unit is the acinus.

Some acini are made up of serous cells (serous acini), others are made up of mucous cells (mucous acini), and finally, other acini have both mucous and serous cells (mixed acini), the latter located eccentrically (×10, ×40). In the case of mixed acini, the serous cells are actually intercalated between the mucous ones, but due to an artifact produced during the preparation of the samples, they appear located on the outer part: this characteristic image receives the name of Gianuzzi's crescent or half-moon). Outside the serous or mucosal cells and inside the basement membrane, sometimes we will distinguish the nuclei of myoepithelial cells (better seen in mucosal-type acini).

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Between the acini, we can observe some eosinophilic ducts (×10), lined by a simple cuboidal epithelium, whose cells present a developed basal labyrinth, thus giving a striated appearance to the subnuclear area of the cells: this is the striated duct (×40). Between the glandular lobules, there are larger excretory ducts that are the interlobular ducts (×10), later showing a gradual stratification.

LIVER

Objectives to identify:

Overview: hepatic lobules

Classic liver lobule

Portal area and portal triad (artery, vein and bile duct)

Central vein

Hepatocytes

Sinusoids

At small magnifications (×4, ×10), we can distinguish how the liver is composed by eosinophilic cords of hepatocytes and sinusoidal capillaries. If we focus on detail, we will see zones of connective tissue between the hepatocytes and the sinusoids with triangular shape: these areas are the portal spaces or Kiernan spaces. The portal spaces are more or less located at the vertices of a hexagon, which is the structural unit of the liver and is called the classic lobule. In the center of the lobule, it is the central vein (or terminal hepatic). Thus, the lobules are made up of irregular and anastomosed cords of intimately attached hepatocytes, between which, the hepatic sinusoids are arranged (×10). The cords of cells are the hepatic trabeculae made up of hepatocytes (×40).

Inside the portal spaces (×10), we notice a vein (portal vein), an artery (portal artery) and ducts lined by cuboidal cells (bile ducts). These three elements constitute the portal triad. The portal spaces are connected to each other by the periportal spaces.

Thus, the blood that enters the liver reaches the portal spaces (through branches of the hepatic artery or of the portal vein) and from there italso reaches the periportal spaces, then flowing towards the central vein circulating through the sinusoids. In the liver, the functional unit is the acinus and its criterion is the degree of oxygenation of the hepatocytes, with a gradient from the periportal areas to the central areas of the lobule.

PANCREAS

Objectives to identify:

Overview: exocrine and endocrine pancreas

Serous acinus

Interlobular duct

Islet of Langerhans

At small magnifications (×10), we observe an exocrine gland made up of lobules, whose secretory units are the serous acini, between which there are excretory ducts of different sizes. We have already seen serous acini previously, and they do not have major particularities, except for the existence of intercalated ducts, which are hardly visible in routine preparations.

At medium magnification, we can see how some rounded and light-stained structures irregularly are located between the serous acini: they are the islets of Langerhans, which constitute the endocrine pancreas. With the 40x magnification objective, we see that islets are composed of a group of cells perfectly separated from the adjacent exocrine pancreas. However, we cannot distinguish the different cell types that constitute the islets of Langerhans.

SALIVARY GLAND

LIVER

PANCREAS

Urinary System

General objective of the practice

To know and to acquire the ability to identify the kidney, with its cortex and medulla. In the cortex, we will focus our attention on the glomeruli, with their different elements. We will also study the renal tubules. In the transmission electron microscopy image, we will deepen in the study of the glomerulus and the filtration barrier. Finally, we will focus on the urinary bladder.

KIDNEY

Objectives to identify:

Overview: capsule, cortex and medulla

Corpuscle vs glomerulus

Bowman´s capsule (parietal layer, visceral layer and urinary space)

Glomerulus: capillaries, mesangial cells and podocytes

Proximal tubule

Distal tubule

The direct inspection of the histological section (without the aid of lenses), allows differentiating two different regions: one, of peripheral location and granular aspect, which is the renal cortex, and another internal of radial aspect, which is the renal medulla.

The kidneys are enclosed by a band of dense connective tissue (×10), which is the renal capsule. The granular aspect of the renal cortex is due to the renal corpuscles, which are rounded structures (renal glomerulus) surrounded almost entirely by a clear space (the urinary space). The glomeruli are a vascular ball/tangle formed by about 10-20 capillary loops supported by the me-

sangium and covered by the visceral layer of Bowman's capsule. The glomerulus is connected to the renal vascular system (vascular pole) through a vascular tuft where an afferent arteriole and an efferent arteriole (glomerular arterioles) are arranged These arterioles are difficult to see in all the glomeruli, because we are observing a histological section that, evidently, cannot cut the vascular poles of all the glomeruli.

Capillaries are interposed between both arterioles forming a high-pressure portal system. The glomerular ultrafiltrate "falls" into the urinary or Bowman's space from where, through the urinary pole of the renal corpuscle, it passes to the proximal tubule (the first portion of the renal tubular system). The external part of the urinary space is delimited by the parietal layer of Bowman's capsule.

In the cortex, and at low magnification, we also observe numerous cross-sectional tubules located between the glomeruli. At 10x magnification we noticed that, although all tubules are lined by a simple cubic epithelium, some present an epithelium of cells with numerous microvilli that seem to occupy the lumen (proximal tubules) while others (distal tubules) have fewer and less developed microvilli and offer a sharper apical border.

In the medulla of the kidney, with the objective of 4x magnification, we confirmed its radial aspect, with numerous tubules of longitudinal section leaving from the cortex. In the medulla there are no glomeruli.In addition to the proximal and distal convoluted tubules, we also see thin-walled tubular portions (loops of Henle) and collecting tubules and ducts. These are lined (×10) by a simple cubic epithelium.

KIDNEY. TRANSMISSION ELECTRONIC MICROSCOPY PHOTOGRAPHY

Objectives to identify:

Glomerular filtration barrier

    Endothelial cells (highly fenestrated)

    Basement membrane

    Podocytes

In this photograph, the capillaries with fenestrated endothelium, surrounded by a thick basement membrane, stand out. The basement membrane has a more electrodense central zone (lamina densa) flanked by radiolucent bands (inner and outer rare lamina). On the opposite side of the basement membrane where the blood capillaries are located, the feet of the podocytes (pedicels) are observed. These feet constitute the tertiary ramifications of the podocytes (glomerular visceral layer). Between the different pedicels there are free zones (the filtration slit) with a thin diaphragm between them (filtration slit membrane). These three elements, the endothelium, the basement membrane and the pedicels constitute the glomerular filtration barrier.
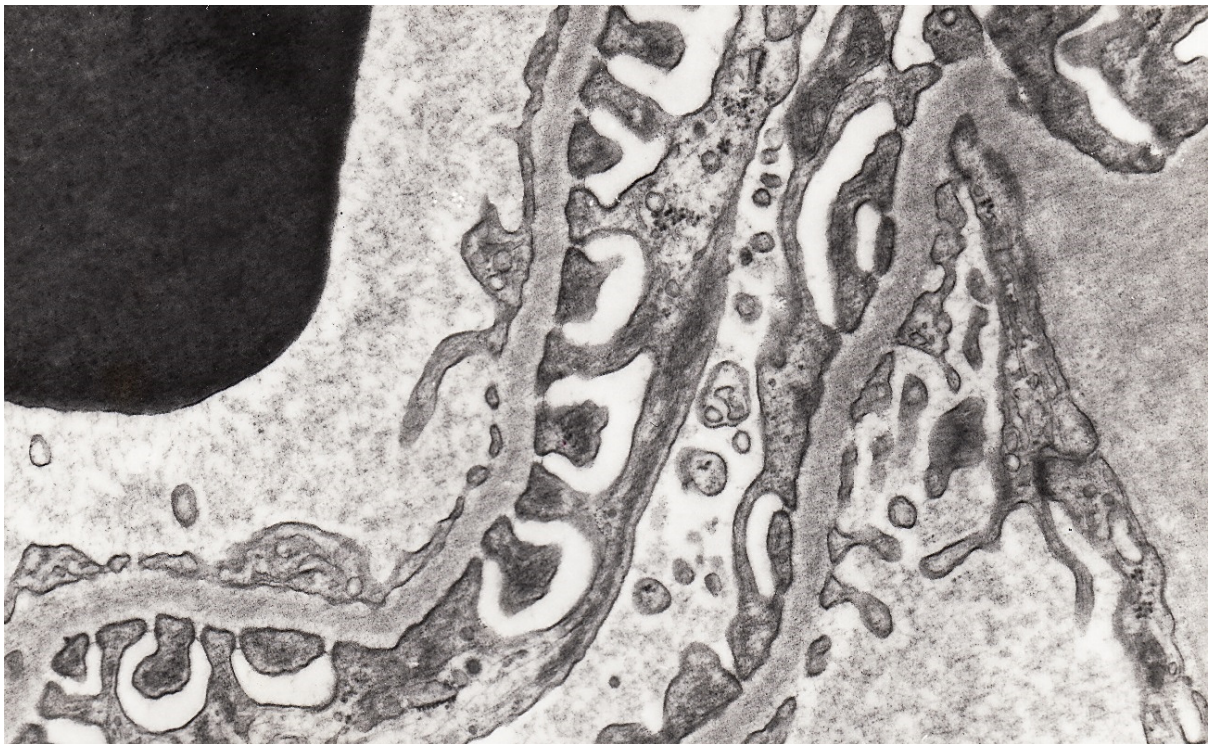
URINARY BLADDER/URETER

Objectives to identify:

Mucosa

   Transitional epithelium

   Lamina propria

Smooth muscle

Serosa

   With the small objective, we observe the general organization of these structures, which basically consist of a mucosa and muscular layers in different orientations. The mucosa shows a transitional epithelium resting on a cellular lamina propria. With 10x and 40x magnification, we will focus on the transitional or urinary epithelium, already explained in the practice of surface epithelial tissue. The muscular layers are made up of smooth muscle fibres (×10, ×40).

KIDNEY

KIDNEY. TRANSMISSION ELECTRONIC MICROSCOPY PHOTOGRAPHY

URINARY BLADDER/URETER

Male Reproductive System

General objective of the practice

To know and to acquire the ability to identify the testicle, both prepubertal and adult. In the adult, we will focus on the seminiferous epithelium and its cytology. The prostate will also be studied in this practice: we must be able to recognize it through its characteristic glands and its fibromuscular stroma.

ADULT TESTE

Objectives to identify:

Seminiferous tubules

Spermatogonia

Spermatozoa

Sertoli cells

Leydig cells

Epididymis

At small magnifications, we can see how permeable the seminiferous tubes of the adult are, presenting a wide lumen. At both 10x and 40x magnification, we observe that the seminiferous epithelium shows cells with a large, hyperchromatic nucleus located next to the basement membrane: they are the spermatogonia. At mid-height level, we find the spermatocytes. Primary spermatocytes are more basally arranged and have a large nucleus, whereas secondary ones are smaller and appear closer to the lumen of the tube. Next we find the spermatids, and in the apical portion of the epithelium and in the lumen, the spermatozoa. It must be taken into account that, unlike what is usually seen in any epithelium, the maturation of the seminiferous epithelium cannot be seen in a single section of tube, as it occurs in a process known as sperm wave, in such a way that we will only see some cellular associations.

In the middle of the epithelium and arranged perpendicular to the wall of the tube, there are cells with a clear and notched nucleus: they are Sertoli cells (representing 10%). Germ cells in different maturational stages are arranged around them. Between the seminiferous tubules (×10) there is a connective stroma rich in vessels, where large, polyhedral and eosinophilic cells, which are Leydig cells (×40), are seen alone or in groups (×40).

The epididymis is located next to the testicle, being also surrounded by a capsule of connective tissue. Inside it, there is a network of tubes lined by a pseudostratified cylindrical epithelium with abundant stereocilia.

PREPUBERAL TESTE (INFANT)

Objectives to identify:

Tunica albuginea

Seminiferous tubules

Epididymis

With the smallest objective, we see a dense capsule of fibrous connective tissue that is the albuginea, surrounding the testicular parenchyma. The testicular parenchyma is made up of numerous tubules (seminiferous tubes) grouped into lobules and immersed in loose connective tissue (×10). The tubes are immature and not yet fully permeabilized (×10). The tubes are internally lined by an immature germinal epithelium that practically obliterates the tubular lumen. The epithelium is made up of small, inactive cells of two types: Sertoli (most) and spermatogonia. Arranged on one side of the preparation, it is possible to observe the epididymis, as a set of regular tubules larger than the seminiferous ones.

PROSTATE

Objectives to identify:

Glands

*Corpora amylacea*

Fibromuscular stroma

Prostatic urethra

The prostate, like the rest of the exocrine glands, is made up of a parenchyma and a stroma. The parenchyma is formed by tubuloalveolar glands (prostatic glands) with a more or less wide lumen, arranged concentrically around the urethra. In the lumen, we can find concentric eosinophilic amyloid concretions: corpora amylacea, whose number increases with age. The prostatic stroma is composed of dense connective tissue and smooth muscle fibers (fibromuscular stroma). Inside the prostate, and depending on the level of the section, we can see portions of the prostatic urethra, lined by transitional epithelium.

ADULT TESTE

PREPUBERAL TESTE (INFANT)

PROSTATE
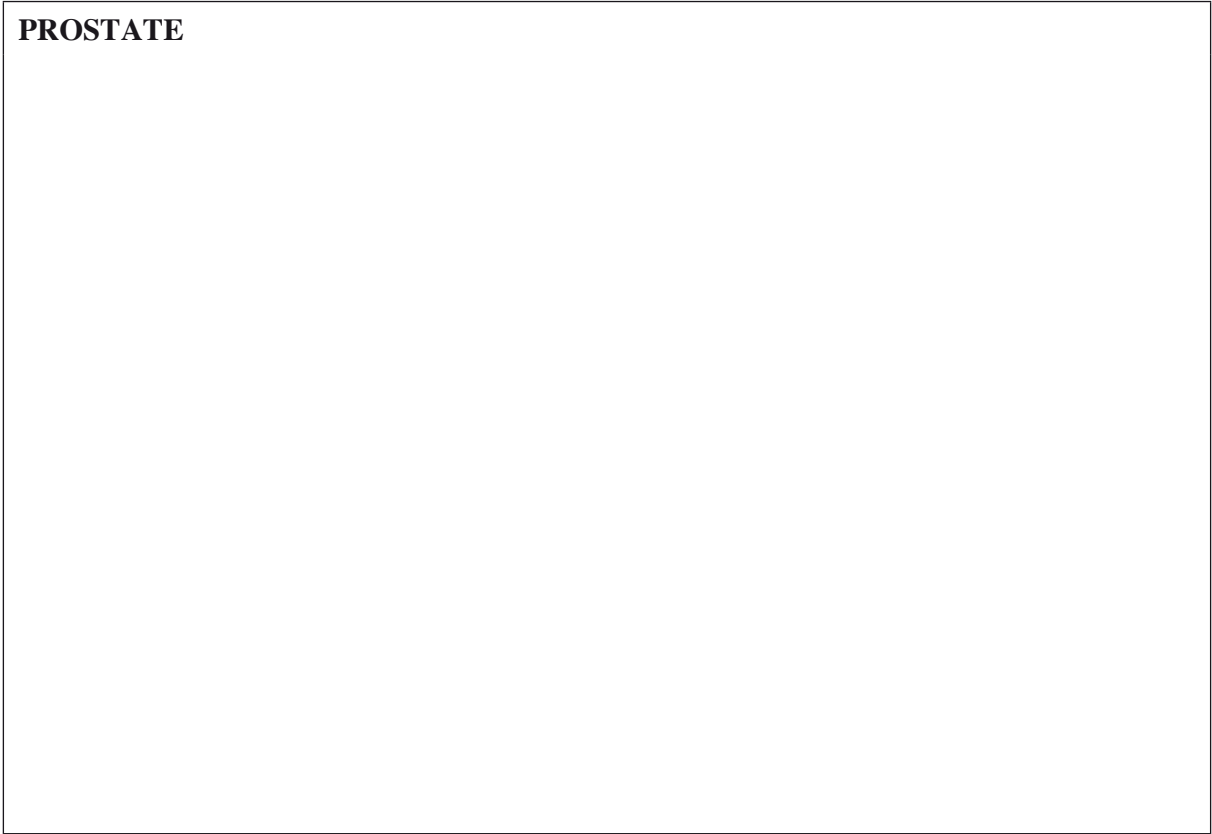
Female Reproductive System

General objective of the practice

To recognize and to acquire the ability to identify the main parts of an ovary and a fallopian tube. In the case of the ovary, we will have to keep in mind the variations that can be observed depending on the age of the woman. We will also study the study the corpus luteum.

To know and to acquire the ability to identify the constituent elements of the uterus, both the body and the cervix. In addition, we will study the placenta, where we will look at the chorionic villi and the disposition of the blood vessels with respect to the trophoblast.

OVARY

Objectives to identify:

Overview: cortex, medulla and hilum

Tunica albuginea

Germinal epithelium

Cortex: follicles and stromal cells

  Primordial follicles

  Primary follicles

  Secondary follicles Graafian follicles

Corpus albicans

Corpus luteum: lutein cells

Medulla

Hilum: blood vessels

At low magnification, two well differentiated zones can be seen in the ovary: a central one with abundant vascular structures —the ovarian medulla—, and another region surrounding it, the

ovarian cortex, which is made up of a special connective tissue called ovarian stroma in which the follicles are arranged. The ovary is lined externally by a simple cubic epithelium called germinal epithelium (×10, ×40). Beneath the germinal epithelium, there is a condensation of the cortical stroma constituting the tunica albuginea (×10).

In the cortical stroma, we observe rounded structures of various sizes (×10), which are the ovarian follicles:

1. The smallest ones consist of a layer of flattened cells, which surround an oocyte: these are the primordial follicles. Surrounding each follicle, there is a basement membrane that separates them from the surrounding stroma.
2. When the follicles are activated (follicular maturation), the flattened follicular cells become taller and proliferate (granulosa cells) and the primary follicle is then called the primary follicle. Between the oocyte and the follicular cells there is a thick glycoprotein layer called the pellucid membrane.
3. Subsequently, fluid appears between the granulosa cells, forming a cavity or antrum, and the follicle is then called secondary (or antral) follicle. The area of lipid-laden follicular cells is called the granulosa layer. A corona of granulosa cells (corona radiata) is attached around the oocyte. The stromal cells surrounding the follicle become active, forming the theca (inner and outer).
4. The follicle continues to develop until ovulation expels the oocyte. Once this happens, the follicle remains in the ovary, thus constituting the corpus luteum (or "yellow body"), in which the follicular cell layers have proliferated to form a macroscopically visible wall whose cells (now called as lutein cells) are completely filled with lipids (×10). The inner portion of the corpus luteum (where the antral cavity and the oocyte used to be) is now occupied by connective tissue and areas of hemorrhage (×4, ×10).

The large follicles that do not reach the end of their development and the corpus luteum undergo progressive involution and fibrosis that end up with irregular structures of spongy/brainy aspect, formed by dense connective tissue (×10): the corpus albicans. They represent, therefore, the final stage of fibrosis and scarring of the atretic ovarian follicles and corpus luteum.

The central area of the ovary, which is called the medulla, continues with the area of the ovary through which the vessels that were seen in the medulla penetrate, which is called the hilum (×4).

FALLOPIAN TUBE

Objectives to identify:

Mucosa: folds

   Epithelium

   Lamina propria

Smooth muscle layers

Serosa/Adventitia

An overview (×4) of the tube reveals a tubular structure with smooth muscular layers as a framework. In the internal portion of the tube, we observe a mucosa forming numerous arborescent folds towards the lumen.

The folds are constituted (×4) by an axis of lamina propria, on which it rests a simple cylindrical epithelium. The muscular layers, two in number, present different spatial orientation, being circular the internal and longitudinal the external. Externally, the tube is lined by an adventitia or a serosa.

UTERINE BODY

Objectives to identify:

Myometrium: smooth muscle fibers

Endometrium: stratum basalis and stratum functionalis

Endometrial glands

   Proliferative phase

   Secretory phase

Stroma (spiral arteries)

The uterus (×4) is a more or less pyriform organ consisting of a large muscular layer (myometrium) with smooth muscle fibers crisscrossing in all directions and a mucosa (endometrium) arranged internally.

The endometrium (×4), consists of glands and stroma. The glands (×10) run from the proximity of the myometrium, the basal portion of the gland, where they are straight, to the lumen of the uterus where they acquire a more tortuous morphology. The appearance of the glands depends on the functional stage of the endometrium.

During the first phase of the endometrial cycle, the glands are rectilinear and their epithelium shows frequent mitoses (proliferative endometrium). After ovulation, the glands stop mitosis, begin to show signs of secretion and become increasingly flexuous and with broader lumen (secretory endometrium). The epithelium (×40) is pseudostratified in the proliferative phase and simple cylindrical in the secretory phase.

The stroma is composed of specialized connective tissue that, like the glands, responds to hormonal stimuli. By day 23 of the endometrial cycle, spiral arteries can be seen (×10) in the stroma, which can be recognized by the close proximity of several sections of the same arteriole (due to their spiral configuration).

CERVIX

Objectives to identify:

Overview: ectocervix and endocervix

Ectocervical mucosa: stratified squamous epithelium

Endocervical mucosa

Glands: simple columnar epithelium with mucous-secreting cells

Stroma

The cervix (×4) presents a framework of dense connective tissue (cervical stroma) lined in the portion facing the vagina by a non-keratinized stratified flat epithelium (ectocervical mucosa) (×10), and by a glandular-type epithelium (endocervical mucosa) in its inner portion, the one facing the endocervical canal.

The endocervical mucosa (×4) consists of an arborescent zone formed by clefts and tubules lined by a simple ciliated epithelium of mucous cells (×10, ×40) that invaginate into the stroma, thus resembling glands by their appearance in histological sections. The glands.

PLACENTA

Objectives to identify:

Amnion

Chorionic plate (fetal side)

Villi

Syncytiotrophoblast

Cytotrophoblast

Maternal plate/Basal plate (maternal side)

Decidual cells

The placenta is the organ generated to allow the exchange of gases and substances between the fetal and maternal blood circulations during pregnancy. The placenta is attached on one side to the uterine wall and, on the opposite side, it faces the amniotic cavity. Its size makes it difficult to observe both ends in the same histological section.

The amnion appears as a thin homogeneous connective band, on which rests a simple cubic epithelium (×10, ×40). The connective tissue of the amnion continues with that of the chorionic plate, rich in vessels, which are the branches of the umbilical vein and arteries (×4, ×10).

From the chorionic plate, and focusing towards the interior of the placenta, we will discover the chorionic villi, thicker at first and thinning as they divide (×10). At higher magnification, we will stop on a villus and observe that it is constituted by a central axis of connective tissue in which abundant blood vessels are located.

Surrounding the villi (×40), the trophoblastic cells appear, either individualized (cytotrophoblast, ligther) or forming cell syncytia (syncytiotrophoblast), the latter easily recognizable by consisting of clusters of basophilic rounded nuclei, distributed in a more or less regular manner.

The clear spaces between the villi form the intervillous space, where maternal blood is visible (×10). With the objective of 4x magnification, and looking towards the edge opposite to that of the amnion, we can study the maternal side of the placenta, the basal plate. Below this basal plate, which is made up of the decidual cells, endometrial glands can sometimes be seen.

OVARY

FALLOPIAN TUBE

UTERINE BODY

CERVIX

PLACENTA

Integumentary System and Mammary Gland

General objective of the practice

To know and to acquire the ability to identify the skin and its appendages/adnexa. We will study the epidermis (with its layers and cells), the dermis (papillary and reticular) and the hypodermis. The hair, sebaceous glands, hair erector muscle and eccrine sweat glands will also be the subject of our attention. The mammary gland is an apocrine gland of great importance. We will focus on the mammary lobule (the functional unit) with its epithelial component: terminal ductules, intralobulillar ductules and its peculiar stroma (intralobulillar stroma). We will also deal with the interlobular portion.

SKIN

Objectives to identify:

Overview: epidermis, dermis and hypodermis

Epidermis: keratinized stratified flat epithelium

  Stratum basale

  Stratum spinosum

  Stratum granulosum

  Stratum corneum (keratine)

  Cells: keratinocytes and melanocytes

Dermis: papillary (superficial) and reticular (deep)

  Fibroblasts and collagen fibers

  Blood vessels

Pilosebaceous unit

   Hair follicle and arrector pili muscle

   Sebaceous glands

Ecrine sweat gland (immersed in reticular connective tissue)

Hypodermis: adipocytes

The epidermis consists of a keratinized stratified flat epithelium (×4, ×10), which relies on a connective tissue called the dermis. Deeper down, we find a less fibrous tissue with abundant adipocytes: the hypodermis or subcutaneous cellular tissue.

The epidermis is a keratinized stratified flat epithelium in which several strata or layers can be distinguished. From the inside out they are:

1. Stratum basale: where the basal cells are arranged
2. Stratum spinosum (or Malphigi's): so called because it presents numerous desmosomes that when the cells retract resemble spikes/thorns
3. Stratum granulosum: due to its richness in keratohyalin granules
4. Stratum corneum: in which no nuclei can be distinguished and consists of keratin.

The cells that mature in these strata are the keratinocytes (keratin producers).

In the basal layer, another type of cell also stands out, the melanocytes, easily identifiable because they present a clear perinuclear halo. Their secretion product, melanin, is found in the adjacent keratinocytes as a yellowish-brown pigment.

The epidermis invaginates into the more superficial dermis forming prolongations (ridges), between which the dermis is arranged forming papillae (papillary dermis). The ridges are called interpapillary ridges. This superficial or papillary dermis is less dense and more cellular than the deep dermis, showing numerous capillaries and sensory endings (Meissner and Pacini corpuscles).

The deeper dermis (deep dermis or reticular dermis) is made up of dense connective tissue with larger vessels; within it there are the cutaneous appendages. The dermis continues in depth, without having a precise limit (×4), with the hypodermis, rich in adipose tissue.

In the dermis there are prominent epithelial structures (×4) that continue with the epidermis, forming sheaths inside which there is a material similar to keratin: these are the hair follicles. Their wider lower portion is called the bulb, and is penetrated at its base by connective tissue (connective papilla). Associated with the follicles (×4), there are ovoid glands made up (×10) of clear cells with a round central nucleus and foamy cytoplasm: sebaceous glands. Also, in relation to the hair follicles, there are (×10) small bundles of smooth muscle: arrector pili muscle. These structures constitute the pilosebaceous unit.

The eccrine sweat glands, located in the deep dermis, are not associated with the hair follicle and drain directly to the outer surface of the skin. Their secretory component is spiral, so when cut the sample, we see them as several tubes very close to each other immersed in reticular connective tissue (×40).

MAMMARY GLAND (BREAST)

Objectives to identify:

Lobule

   Glandular component: terminal ducts (epithelium and myoethelial cells)

   Stromal component: intralobular stroma

Interlobular stroma

Ducts: intralobular and interlobular

Adipocytes

The breast is a modified apocrine sweat gland that develops more in women and is subject to hormonal changes. In the resting breast, there are glandular clusters (×4): mammary lobules. Within the lobules, the glandular epithelial component is arranged as small blind ducts (like a cul-de-sac), which are called terminal ducts. These terminal ducts lead to small ducts, the intralobulillar ducts, that will become interlobular ducts when they leave the lobule. During gestation and lactation, the terminal ducts dilate and form alveolar structures (the place where secretion will occur). The ductus are lined by a low cylindrical epithelium surrounded on the outside by myoepithelial cells (recognizable by their clear halo, and difficult to perceive their limits/boundaries). Outside both cell types there is the basement membrane, which separates them from the intralobulillar stoma.

The intralobular stroma is more loose and more cellular (lymphocytes, plasma cells) than the interlobular stroma. It also has numerous blood capillaries and responds to hormonal stimuli. The interlobular ducts are larger and immersed in the interlobular stroma, which also contains a variable number of adipocytes.

NIPPLE

Objectives to identify:

Skin of the nipple

   Epidermis: stratified keratinized flat epithelium with abundant melanin

   Sebaceous glands

Lactiferous ducts

The nipple is lined by a stratified keratinized flat epithelium with numerous small invaginations. The highly pigmented epithelium tends to form broad interpapillary ridges that often merge with each other, thus giving a reticular or lacy appearance (×10). Beneath the epithelium, there are numerous sebaceous glands not associated with hair structures. Around 15 to 20 lactiferous ducts lead to the nipple. As explained before, the interlobular ducts lead to ducts between the lobes, and these finally to the lactiferous ducts (before this last portion there is a dilatation called the lactiferous sinus). The stroma consists of dense connective tissue with numerous nerve endings and smooth muscle bundles.

SKIN

MAMMARY GLAND (BREAST)

NIPPLE