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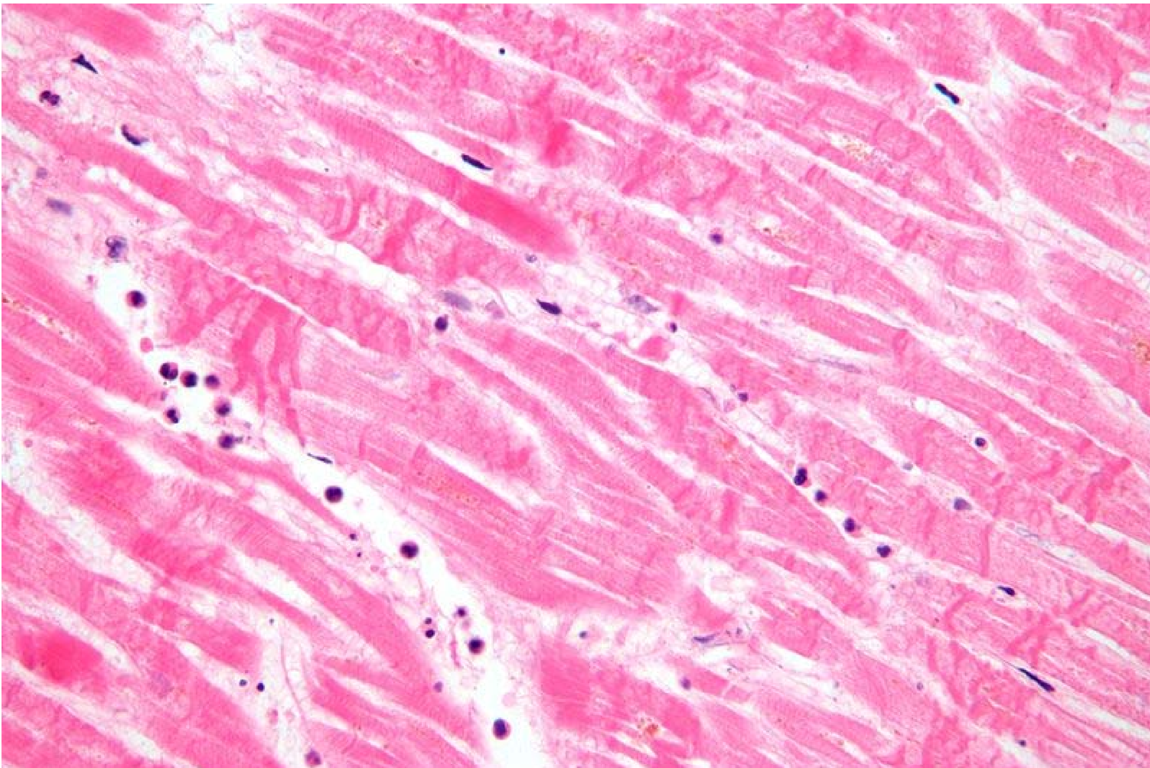
Chapter 1

Histopathology



Micrograph showing contraction band necrosis, a **histopathologic** finding of myocardial infarction (heart attack).

Histopathology (compound of three Greek words: *ἱστός* histos "tissue", *πάθος* pathos "disease-suffering", and -*λογία* -logia) refers to the microscopic examination of tissue in order to study the manifestations of disease. Specifically, in clinical medicine, histopathology refers to the examination of a biopsy or surgical specimen by a pathologist, after the specimen has been processed and histological sections have been placed onto glass slides. In contrast, cytopathology examines free cells or tissue fragments.

Collection of tissues

Histopathological examination of tissues starts with surgery, biopsy, or autopsy. The tissue is removed from the body or plant, and then placed in a fixative which stabilizes the tissues to prevent decay. The most common fixative is formalin (10% formaldehyde in water).

Preparation for histology

The tissue is then prepared for viewing under a microscope using either chemical fixation or frozen section.

Chemical fixation

In chemical fixation, the samples are transferred to a cassette, a container designed to allow reagents to freely act on the tissue inside. This cassette is immersed in multiple baths of progressively more concentrated ethanol, to dehydrate the tissue, followed by toluene or xylene, and finally extremely hot liquid (usually paraffin). During this 12 to 16 hour process, paraffin will replace the water in the tissue, turning soft, moist tissues into a sample miscible with paraffin, a type of wax. This process is known as **tissue processing**.

The processed tissue is then taken out of the cassette and set in a mold. Through this process of **embedding**, additional paraffin is added to create a paraffin block which is attached to the outside of the cassette.

The process of embedding then allows the sectioning of tissues into very thin (2 - 7 micrometer) sections using a microtome. The microtome slices the tissue ready for microscopic examination. The slices are thinner than the average cell, and are layered on a glass slide for staining.

Frozen section processing

The second method of histology processing is called frozen section processing. In this method, the tissue is frozen and sliced thinly using a microtome mounted in a below-freezing refrigeration device called the cryostat. The thin frozen sections are mounted on a glass slide, fixed immediately & briefly in liquid fixative, and stained using the similar staining techniques as traditional wax embedded sections. The advantages of this method is rapid processing time, less equipment requirement, and less need for ventilation in the laboratory. The disadvantage is the poor quality of the final slide. It is used in intra-operative pathology for determinations that might help in choosing the next step in surgery during that surgical session (for example, to preliminarily determine clearness of the resection margin of a tumor during surgery).

Staining of the Processed Histology Slides

This can be done to slides processed by the chemical fixation or frozen section slides. To see the tissue under a microscope, the sections are stained with one or more pigments. The aim of staining is to reveal cellular components; counterstains are used to provide contrast.

The most commonly used stain in histopathology is a combination of hematoxylin and eosin (often abbreviated H&E). Hematoxylin is used to stain nuclei **blue**, while eosin stains cytoplasm and the extracellular connective tissue **matrix pink**. There are hundreds of various other techniques which have been used to selectively stain cells. Other compounds used to color tissue sections include safranin, Oil Red O, congo red, silver salts and artificial dyes. **Histochemistry** refers to the science of using chemical reactions between laboratory chemicals and components within tissue. A commonly performed histochemical technique is the Perls Prussian Blue reaction, used to demonstrate iron deposits in diseases like Hemochromatosis.

Recently, antibodies have been used to stain particular proteins, lipids and carbohydrates. Called immunohistochemistry, this technique has greatly increased the ability to specifically identify categories of cells under a microscope. Other advanced techniques include **in situ hybridization** to identify specific DNA or RNA molecules. These antibody staining methods often require the use of frozen section histology. Digital cameras are increasingly used to capture histopathological images.

Interpretation

The histological slides are examined under a microscope by a pathologist, a medically qualified specialist. This medical diagnosis is formulated as a **pathology report** describing the histological findings and the opinion of the pathologist. In the case of cancer, this represents the **tissue diagnosis** required for most treatment protocols. In the removal of cancer, the pathologist will indicate whether the surgical margin is cleared, or is involved (residual cancer is left behind). This is done using either the bread loafing or CCPDMA method of processing.

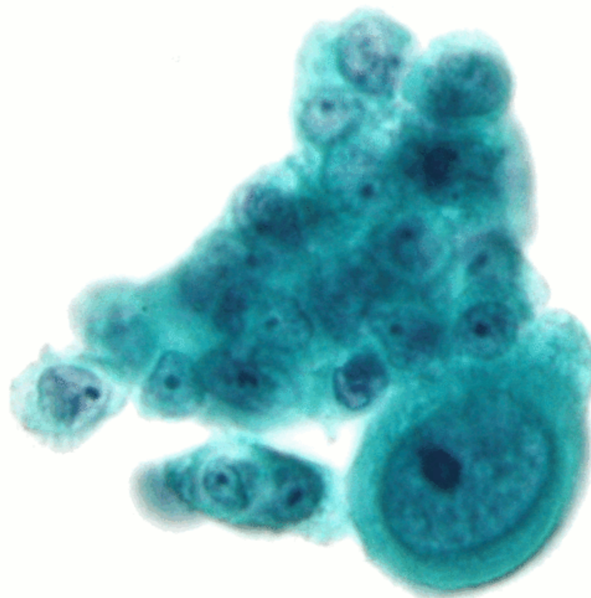
In myocardial infarction

After a myocardial infarction, no histopathology is seen the first ~30 minutes. The only possible sign the first 4 hours is waviness of fibers at border. Later, however, a coagulation necrosis is initiated, with edema and hemorrhage. After 12 hours, there can be seen karyopyknosis and hypereosinophilia of myocytes with contraction band necrosis in margins, as well as beginning of neutrophil infiltration. At 1 – 3 days there is continued coagulation necrosis with loss of nuclei and striations and an increased infiltration of neutrophils to interstitium. Until the end of the first week after infarction there is beginning of disintegration of dead muscle fibers, necrosis of neutrophils and beginning of macrophage removal of dead cells at border, which increases the succeeding days. After a week there is also beginning of granulation tissue formation at margins,

which matures during the following month, and gets increased collagen deposition and decreased cellularity until the myocardial scarring is fully mature at approximately 2 months after infarction.

Chapter 2

Cytopathology



A pair of micrographs of a cytopathology specimen showing a 3-dimensional cluster of cancerous cells (serous carcinoma)

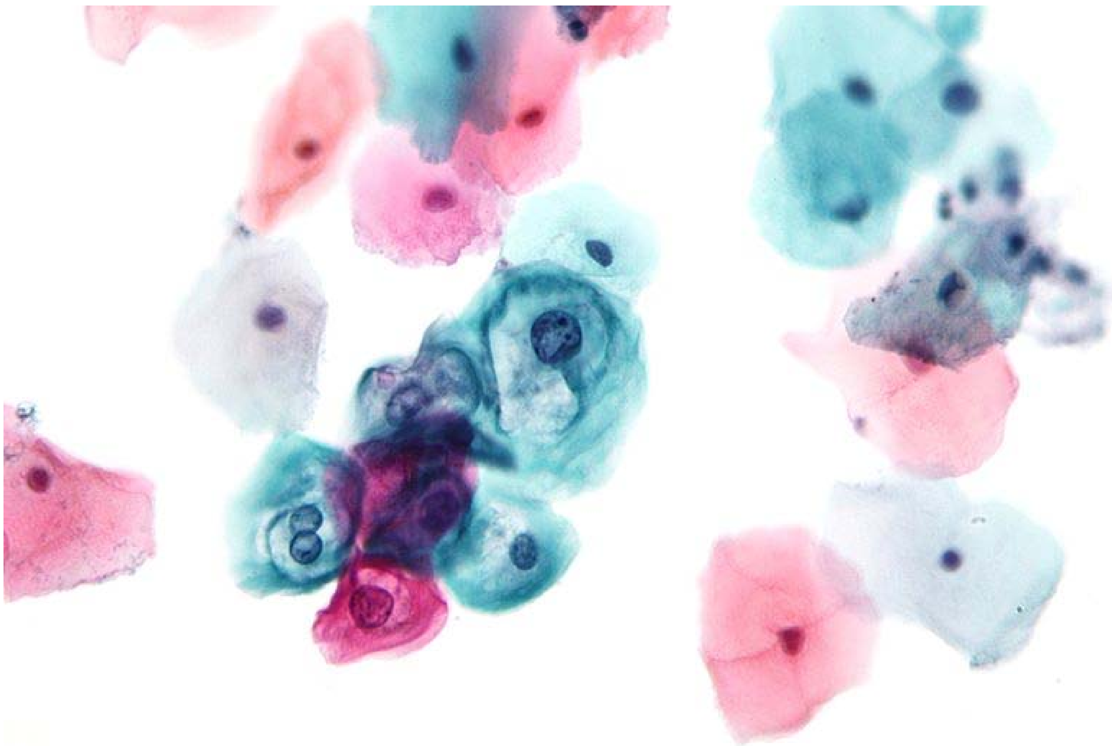
Cytopathology (from Greek κύτος, *kytos*, "a hollow"; πάθος, *pathos*, "fate, harm"; and -λογία, *-logia*) is a branch of pathology that studies and diagnoses diseases on the cellular level. The discipline was founded by Rudolf Virchow in 1858. A common application of cytopathology is the Pap smear, used as a screening tool, to detect precancerous cervical lesions and prevent cervical cancer. Cytopathogy is also commonly used to investigate thyroid lessions, diseases involving sterile body cavities (peritoneal, pleural, and cerebrospinal), and a wide range of other body sites. It is usually used to aid in the diagnosis of cancer, but also helps in the diagnosis of certain infectious diseases and other inflammatory conditions. Cytopathology is generally used on samples of free cells or tissue fragments, in contrast to histopathology, which studies whole tissues.

Cytopathologic tests are sometimes called **smear tests** because the samples may be smeared across a glass microscope slide for subsequent staining and microscopic examination. However, cytology samples may be prepared in other ways, including cytocentrifugation. Different types of smear tests may also be used for cancer diagnosis. In this sense, it is termed a *cytologic smear*.

Cytopathology is frequently, less precisely, called cytology, which means "the study of cells."

Cell collection

Two methods of collecting cells for cytopathologic analysis are:



A micrograph of an exfoliative cytopathology specimen (Pap test, Pap stain)

1. *Exfoliative Cytology* – In this method, cells are collected after they have been either spontaneously shed by the body ("spontaneous exfoliation") or manually scraped/brushed off of a surface in the body ("mechanical exfoliation"). An example of spontaneous exfoliation is when cells of the pleural cavity or peritoneal cavity are shed into the pleural or peritoneal fluid. This fluid can be collected via various methods for examination. Examples of mechanical exfoliation include Pap smears, where cells are scarped form the cervix with a cervical spatula, or bronchial brushings, where a bronchoscope is inserted into the trachea and used to evaluate a visible lesion by brushing cells from its surface and subjecting them to cytopathologic analysis.

2. *Fine Needle Aspiration Cytology* or *Needle aspiration biopsy* – A needle attached to a syringe is used to collect cells from lesions or masses in various body organs by microcoring, often with the application of negative pressure (suction) to increase yield. FNAC can be performed under palpation guidance (ie. the clinican can feel the lesion) on a mass in superficial regions like the neck, thyroid or breast; FNAC may also be assisted by ultrasound or CAT scan for sampling of deep-seated lesions within the body that cannot me localized via palpation. FNAC is is widely used in many countries, but success rate is dependent on the skill of the practitioner. If performed by a pathologist alone, or as team with pathologist-cytotechnologist, the success rate of proper diagnosis is superior than when performed by a non-pathologist. This may be due to the pathologist's ability to immediately evaluate specimens under a microscope and immediately repeat the procedure if sampling was inadequate.

Fine needles are 23 to 27 gauge. Because needles as small as 27 gauge can almost always yield diagnostic material, FNAC is often the least injurious way to obtain diagnostic tissue from a lesion. Sometime a syringe holder may be used to facilitate using one hand to perform the biopsy while the other hand is immobilizing the mass. Imaging equipment such as a CT scanner or ultrasound may be used to assist in locating the region to be biopsied.

Parameters

The nucleus of the cell is very important in evaluating the cellular sample. In cancerous cells, altered DNA activity can be seen as a physical change in the nuclear qualities. Since more DNA is unfolded and being expressed, the nucleus will be darker and less uniform, larger than in normal cells, and often show a bright red nucleolus.

While the cytologist's primary responsibility is to discern whether cancerous or precancerous pathology is present in the cellular sample analysed, other pathologies may be seen such as:

- microbial infections: parasitic, viral, and/or bacterial
- reactive changes
- immune reactions
- cell aging
- amyloidosis
- autoimmune diseases

Various normal functions of cell growth, metabolism, and division can fail or work in abnormal ways and lead to diseases.

Cytopathology is best used as one of three tools, the second and third being the physical exam and medical imaging. Cytology can be used to diagnose a condition and spare a patient from surgery to obtain a larger specimen. An example is thyroid FNA, many benign conditions can be diagnosed with a superficial biopsy and the patient can go back

to normal activities right away. If a malignant condition is diagnosed, the patient may be able to start radiation/chemotherapy, or may need to have surgery to remove and/or stage the cancer.

Some tumors may be difficult to biopsy, such as sarcomas. Other rare tumors my be dangerous to biopsy, such as pheochromocytoma. In general, a fine needle aspiration can be done anywhere it is safe to put a needle, including liver, lung, kidney, and superficial masses.

Many clinicians are not trained to perform fine needle aspiration biopsies properly, and then when they do not obtain diagnostic material, believe that cytology is not useful. Proper technique takes time to master. Cytotechnologists and cytopathologists can assist clinicians by going to procedures and assisting with collection techniques. A "quick read" is a peek under the microscope and can tell the clinician if they have obtained enough diagnostic material. Cytological specimens also need to be properly prepared so that the cells are not damaged.

Sometimes more information about the specimen is helpful. Immunohistochemical stains and molecular testing can be performed, especially if the sample is prepared using liquid based cytology. Often "reflex" testing is performed, such as HPV testing on an abnormal pap test or flow cytometry on a lymphoma specimen.

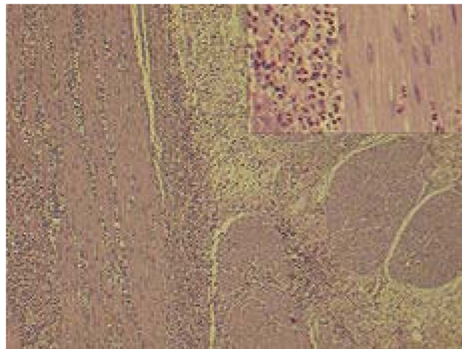
Body regions

Cytopathologic techniques are used in the examination of virtually all body organs and tissues:

- Gynaecologic cytology - concerning the female reproductive tract
- Urinary tract cytology - concerning the ureters, urinary bladder and urethra
- Effusion cytology - concerning fluids collections, especially within the peritoneum, pleura and pericardium
- Breast cytology - principally concerning the female breast
- Thyroid cytology - concerning the thyroid gland
- Lymph node cytology - concerning lymph nodes
- Respiratory cytology - concerning the lungs and airways
- Gastrointestinal cytology - concerning the alimentary tract
- Soft tissue, bone and skin cytology
- Kidney and adrenal cytology
- Liver and pancreas cytology
- Central nervous system cytology
- Eye cytology
- Salivary gland cytology

Chapter 3

Eosinophilic Gastroenteritis

Eosinophilic gastroenteritis	
 H&E Stain: Dense Eosinophilic infiltration of gastro-duodenal wall	
ICD-10	K52.8
ICD-9	558.3
DiseasesDB	32555
eMedicine	med/688

Eosinophilic gastroenteritis (EG) is a rare and heterogeneous condition characterized by patchy or diffuse eosinophilic infiltration of gastrointestinal (GI) tissue, first described by Kaijser in 1937 . Presentation may vary depending on location as well as depth and extent of bowel wall involvement and usually runs a chronic relapsing course. It can be classified into mucosal, muscular and serosal types based on the depth of involvement. Any part of the GI tract can be affected, and isolated biliary tract involvement has also been reported. The stomach is the organ most commonly affected, followed by the small intestine and the colon.

Pathophysiology

Peripheral blood eosinophilia and elevated serum IgE are usual but not universal. The damage to the gastrointestinal tract wall is caused by eosinophilic infiltration and degranulation.

As a part of host defense mechanism, eosinophil is normally present in gastrointestinal mucosa, though finding in deeper tissue is almost always pathologic . What triggers such dense infiltration in EG is not clear. It is possible that different pathogenetic mechanisms of disease is involved in several subgroups of patients. Food allergy and variable IgE response to food substances has been observed in some patients which implies role of hypersensitive response in pathogenesis. Many patients indeed have history of other atopic conditions like eczema, asthma etc.

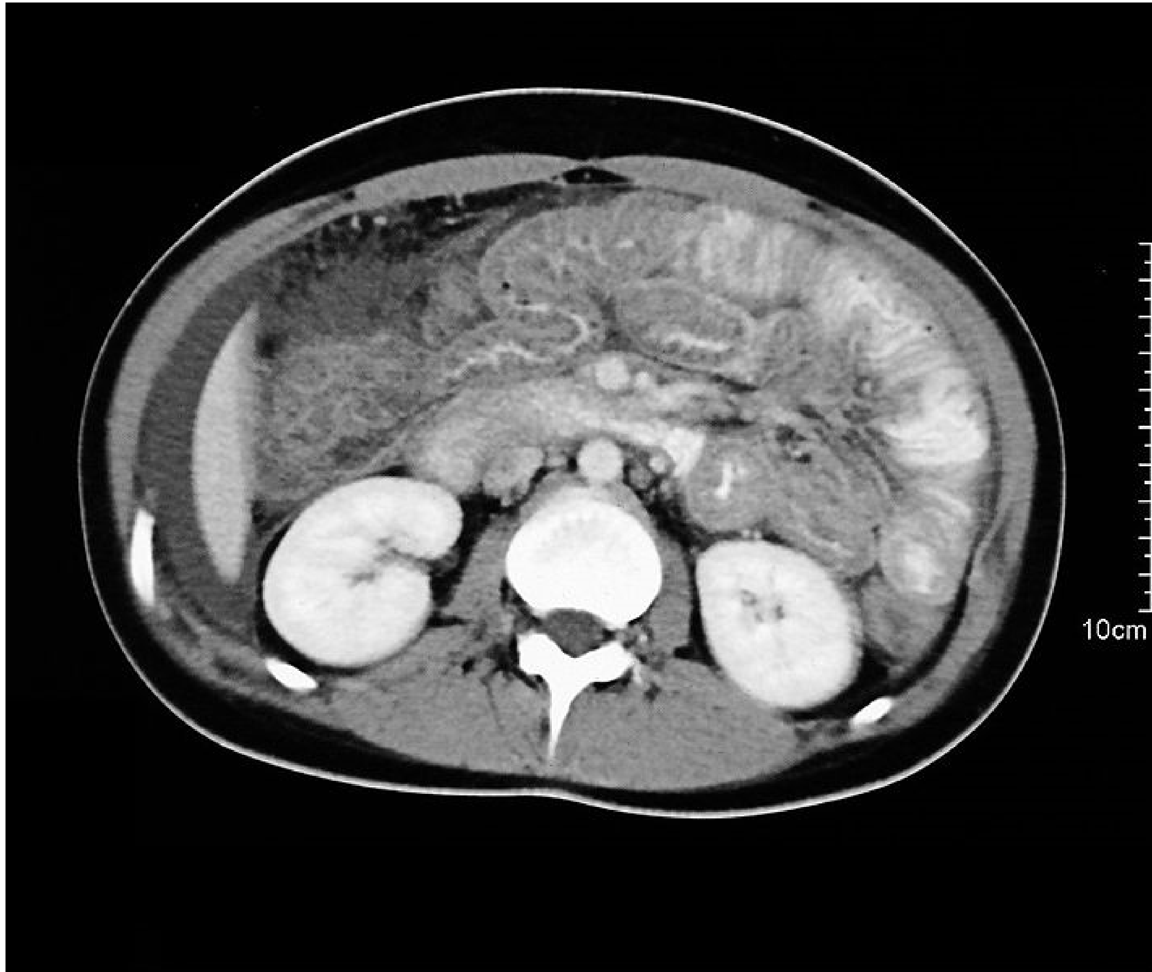
Eosinophil recruitment into inflammatory tissue is a complex process, regulated by a number of inflammatory cytokines. In EG cytokines IL-3, IL-5 and granulocyte macrophage colony stimulating factor (GM-CSF) may be behind the recruitement and activation. They have been observed immunohistochemically in diseased intestinal wall . In addition eotaxin has been shown to have an integral role in regulating the homing of eosinophils into the lamina propria of stomach and small intestine . In the allergic subtype of disease, it is thought that food allergens cross the intestinal mucosa and trigger an inflammatory response that includes mast cell degranulation and recruitment of eosinophils.

Symptoms and signs

EG typically presents with a combination of chronic nonspecific GI symptoms which include abdominal pain, nausea, vomiting, diarrhea, weight loss, and abdominal distension. Approximately 80% have symptoms for several years; a high degree of clinical suspicion is often required to establish the diagnosis, as the disease is extremely rare. Occasionally, the disease may manifest itself as an acute abdomen or bowel obstruction.

- **Mucosal EG** (25-100%)is the commonest variety, which presents with features of malabsorption and protein losing enteropathy. Failure to thrive and anaemia may also be present. Lower gastrointestinal bleeding may imply colonic involvement.
- **Muscular EG** (13-70%) present with obstruction of gastric outlet or small intestine; sometimes as an obstructing caecal mass or intussusception.
- **Subserosal EG** (4.5 % to 9 % in Japan and 13 % in the USA) presents with ascites which is usually exudative in nature, abundant peripheral eosinophilia, and has favourable responses to corticosteroids.
- Other documented features are Cholangitis, pancreatitis, eosinophilic splenitis, acute appendicitis and giant refractory duodenal ulcer.

Diagnosis



Spiral CT showing ascites and concentric thickening of colon and ileum in EG

Talley et al. suggested 3 diagnostic criteria which is still widely used:

1. the presence of gastrointestinal symptoms,
2. histological demonstration of eosinophilic infiltration in one or more areas of the gastrointestinal tract or presence of high eosinophil count in ascitic fluid (latter usually indicates subserosal variety),
3. no evidence of parasitic or extraintestinal disease.

Hypereosinophilia, the hallmark of allergic response, may be absent in up to 20% of patients, but hypoalbuminaemia and other abnormalities suggestive of malabsorption may be present.

CT scan may show nodular and irregular thickening of the folds in the distal stomach and proximal small bowel, but these findings can also be present in other conditions like Crohn's disease and lymphoma.

The endoscopic appearance in eosinophilic gastroenteritis is nonspecific; it includes erythematous, friable, nodular, and occasional ulcerative changes. Sometimes diffuse inflammation results in complete loss of villi, involvement of multiple layers, submucosal oedema and fibrosis.

Definitive diagnosis involves histological evidence of eosinophilic infiltration in biopsy slides. Microscopy reveals >20 eosinophils per high power field. Infiltration is often patchy, can be missed and laparoscopic full thickness biopsy may be required.

Radio isotope scan using technetium (^{99m}Tc) exametazime-labeled leukocyte SPECT may be useful in assessing the extent of disease and response to treatment but has little value in diagnosis, as the scan does not help differentiating EG from other causes of inflammation.

When eosinophilic gastroenteritis is observed in association with eosinophilic infiltration of other organ systems, the diagnosis of idiopathic hypereosinophilic syndrome should be considered.

Management

Corticosteroids are the mainstay of therapy with a 90% response rate in some studies. Appropriate duration of steroid treatment is unknown and relapse often necessitates long term treatment. Various steroid sparing agents e.g. sodium cromoglycate (a stabilizer of mast cell membranes), ketotifen (an antihistamine), and montelukast (a selective, competitive leukotriene receptor antagonist) have been proposed, centering around an allergic hypothesis, with mixed results. An elimination diet may be successful if a limited number of food allergies are identified.

Epidemiology

Epidemiology may differ between studies, as number of cases are small, with approximately 300 EG cases reported in published literature.

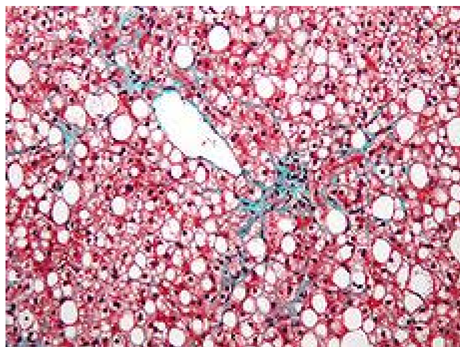
EG can present at any age and across all races, with a slightly higher incidence in males. Earlier studies showed higher incidence in the third to fifth decades of life.

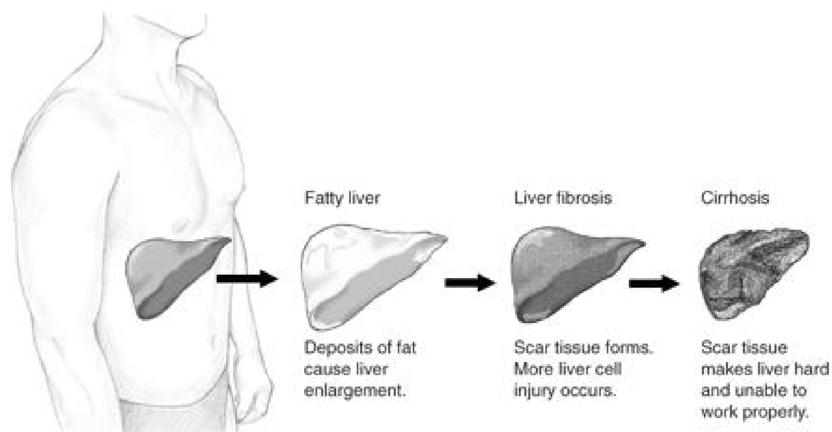
Other gastrointestinal conditions associated with allergy

- Eosinophilic esophagitis
- Eosinophilic ascites
- Coeliac disease
- Protein losing enteropathy from intolerance to cow's milk protein
- Infantile formula protein intolerance

Chapter 4

Fatty Liver

Fatty liver	
 Micrograph showing a **fatty liver** (macrovesicular steatosis), as seen in non-alcoholic fatty liver disease. Trichrome stain.	
ICD-10	K70., K76.0
ICD-9	571.0, 571.8
DiseasesDB	18844
eMedicine	med/775 article/170409
MeSH	*C06.552.241*



Different stages of liver damage

Fatty liver, also known as **fatty liver disease** (**FLD**), is a reversible condition where large vacuoles of triglyceride fat accumulate in liver cells via the process of steatosis (i.e. abnormal retention of lipids within a cell). Despite having multiple causes, fatty liver can be considered a single disease that occurs worldwide in those with excessive alcohol intake and those who are obese (with or without effects of insulin resistance). The condition is also associated with other diseases that influence fat metabolism. Morphologically it is difficult to distinguish alcoholic FLD from non alcoholic FLD and both show micro-vesicular and macrovesicular fatty changes at different stages.

Accumulation of fat may also be accompanied by a progressive inflammation of the liver (hepatitis), called steatohepatitis. By considering the contribution by alcohol, fatty liver may be termed alcoholic steatosis or non-alcoholic fatty liver disease (NAFLD), and the more severe forms as alcoholic steatohepatitis (part of alcoholic liver disease) and non-alcoholic steatohepatitis (NASH).

Causes

Fatty liver is commonly associated with alcohol or metabolic syndrome (diabetes, hypertension, obesity and dyslipidemia) but can also be due to any one of many causes:

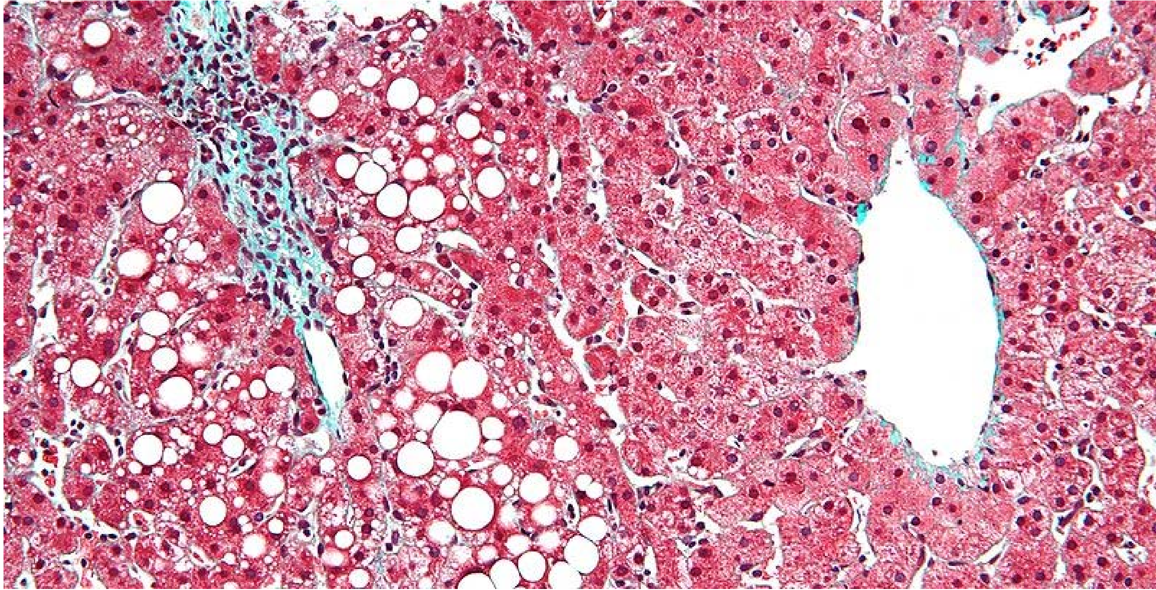
Metabolic
: Abetalipoproteinemia, glycogen storage diseases, Weber-Christian disease, acute fatty liver of pregnancy, lipodystrophy

Nutritional
: Malnutrition, total parenteral nutrition, severe weight loss, refeeding syndrome, jejuno-ileal bypass, gastric bypass, jejunal diverticulosis with bacterial overgrowth

Drugs and toxins
: Amiodarone, methotrexate, diltiazem, highly active antiretroviral therapy, glucocorticoids, tamoxifen, environmental hepatotoxins (e.g., phosphorus, mushroom poisoning)

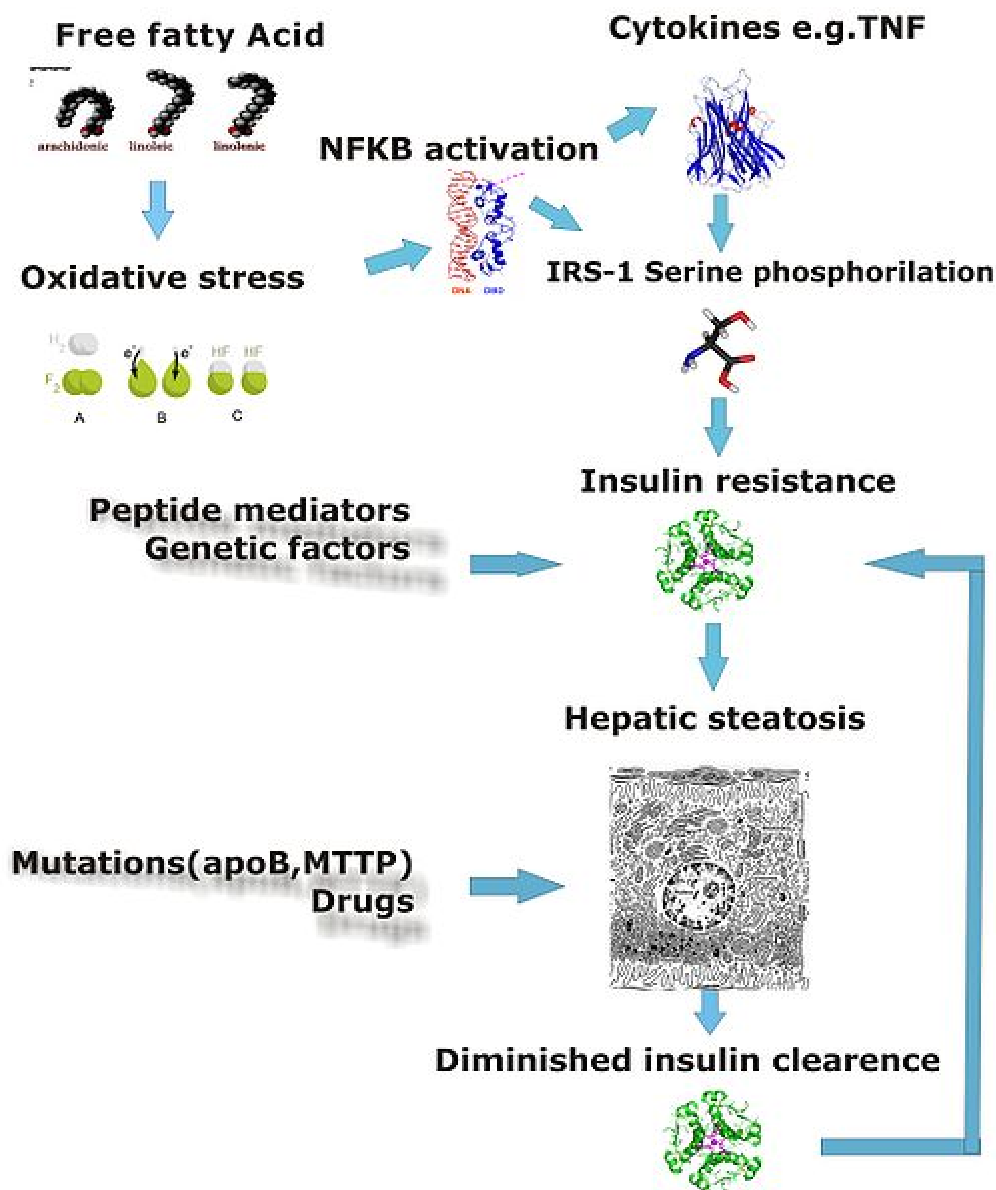
Other
: Inflammatory bowel disease, HIV, Hepatitis C especially genotype 3, and Alpha 1-antitrypsin deficiency

Pathology



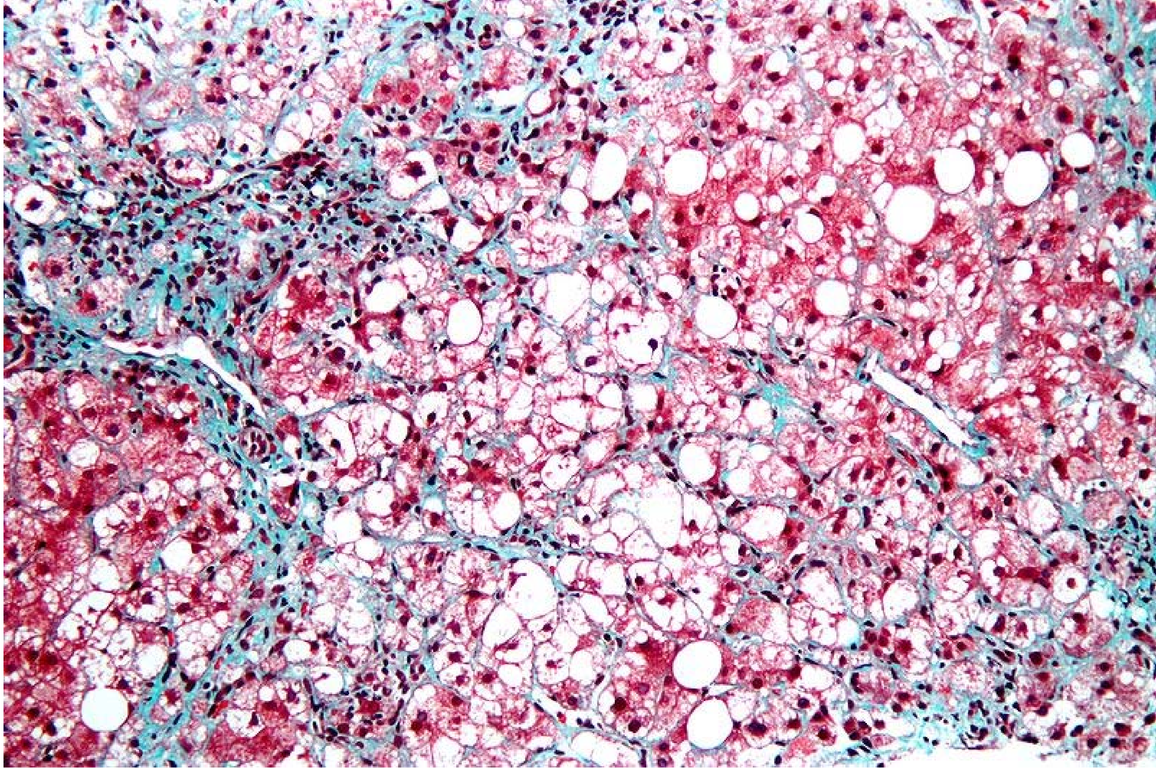
Micrograph of periportal hepatic steatosis, as may be seen due to steroid use. Trichrome stain.

Fatty change represents the intra-cytoplasmic accumulation of triglyceride (neutral fats). At the beginning, the hepatocytes present small fat vacuoles (liposomes) around the nucleus (microvesicular fatty change). In this stage liver cells are filled with multiple fat droplets that do not displace the centrally located nucleus. In the late stages, the size of the vacuoles increase pushing the nucleus to the periphery of the cell giving characteristic signet ring appearance (macrovesicular fatty change). These vesicles are well delineated and optically "empty" because fats dissolve during tissue processing. Large vacuoles may coalesce and produce fatty cysts which are irreversible lesions. Macrovesicular steatosis is the most common form and is typically associated with alcohol, diabetes, obesity and corticosteroids. Acute fatty liver of pregnancy and Reye's syndrome are examples of severe liver disease caused by microvesicular fatty change. The diagnosis of steatosis is made when fat in the liver exceeds 5–10% by weight.



Mechanism leading to hepatic steatosis

Defects in fat metabolism are responsible for pathogenesis of FLD which may be due to imbalance in energy consumption and its combustion resulting in lipid storage or can be a consequence of peripheral resistance to insulin, whereby the transport of fatty acids from adipose tissue to the liver is increased. Impairment or inhibition of receptor molecules (PPAR-α, PPAR-γ and SREBP1) that control the enzymes responsible for the oxidation and synthesis of fatty acids appears to contribute towards fat accumulation. In addition, alcoholism is known to damage mitochondria and other cellular structure further impairing cellular energy mechanism. On the other hand non alcoholic FLD may begin as excess of unmetabolised energy in liver cells. Hepatic steatosis is considered reversible and to some extent nonprogressive if there is cessation or removal of underlying cause.



Micrograph of inflamed fatty liver (steatohepatitis)

Severe fatty liver is sometimes accompanied by inflammation, a situation that is referred to as *steatohepatitis*. Progression to alcoholic steatohepatitis (ASH) or non-alcoholic steatohepatitis (NASH) depend on persistence or severity of inciting cause. Pathological lesions in both conditions are similar. However, the extent of inflammatory response varies widely and does not always correlate with degree of fat accumulation. Steatosis (retention of lipid) and onset of steatohepatitis may represent successive stages in FLD progression.

Liver with extensive inflammation and high degree of steatosis often progresses to more severe forms of the disease. Hepatocyte ballooning and hepatocyte necrosis of varying degree are often present at this stage. Liver cell death and inflammatory responses lead to the activation of stellate cells which play a pivotal role in hepatic fibrosis. The extent of fibrosis varies widely. Perisinusoidal fibrosis is most common, especially in adults, and predominates in zone 3 around the terminal hepatic veins.

The progression to cirrhosis may be influenced by the amount of fat and degree of steatohepatitis and by a variety of other sensitizing factors. In alcoholic FLD the transition to cirrhosis related to continued alcohol consumption is well documented but the process involved in non-alcoholic FLD is less clear.

Diagnosis

Most individuals are asymptomatic and are usually discovered incidentally because of abnormal liver function tests or hepatomegaly noted in unrelated medical condition. Elevated liver biochemistry is found in 50% of patients with simple steatosis. The serum ALT level usually is greater than the AST level in non-alcoholic variant and the opposite in alcoholic FLD (AST:ALT more than 2:1).

Imaging studies are often obtained during evaluation process. Ultrasonography reveals a "bright" liver with increased echogenicity. Medical imaging can aid in diagnosis of fatty liver; fatty livers have lower density than spleen on computed tomography (CT) and fat appears bright in T1-weighted magnetic resonance images (MRIs). No medical imagery, however, is able to distinguish simple steatosis from advanced NASH. Histological diagnosis by liver biopsy is sought when assessment of severity is indicated.

Treatment

The treatment of fatty liver depends on what is causing it, and generally, treating the underlying cause will reverse the process of steatosis if implemented at early stage.

Complication

Up to 10% of cirrhotic alcoholic FLD will develop hepatocellular carcinoma. Overall incidence of liver cancer in non-alcoholic FLD has not yet been quantified, but the association is well established.

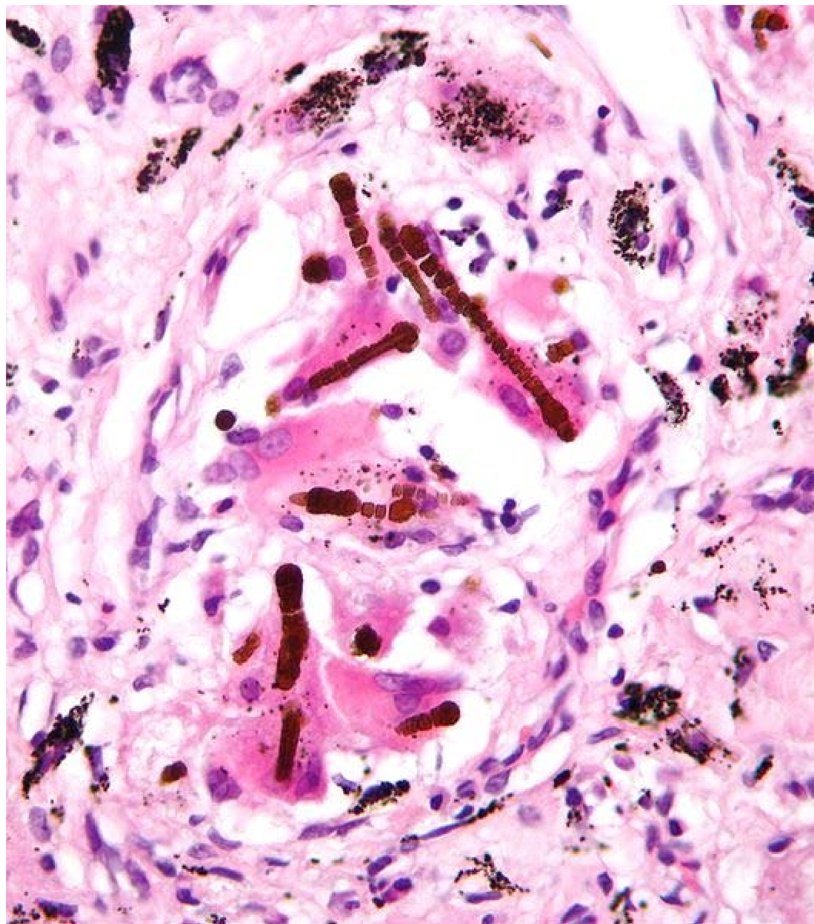
Epidemiology

The prevalence of FLD in the general population ranges from 10% to 24% in various countries. However, the condition is observed in up to 75% of obese people, 35% of whom will progress to non-alcoholic FLD, despite no evidence of excessive alcohol consumption. FLD is the most common cause of abnormal liver function test in the US. "Fatty livers occur in 33% of European-Americans, 45% of Hispanic-Americans, and 24% of African-Americans."

Chapter 5

Ferruginous Body, Fibrinoid Necrosis and Field Stain

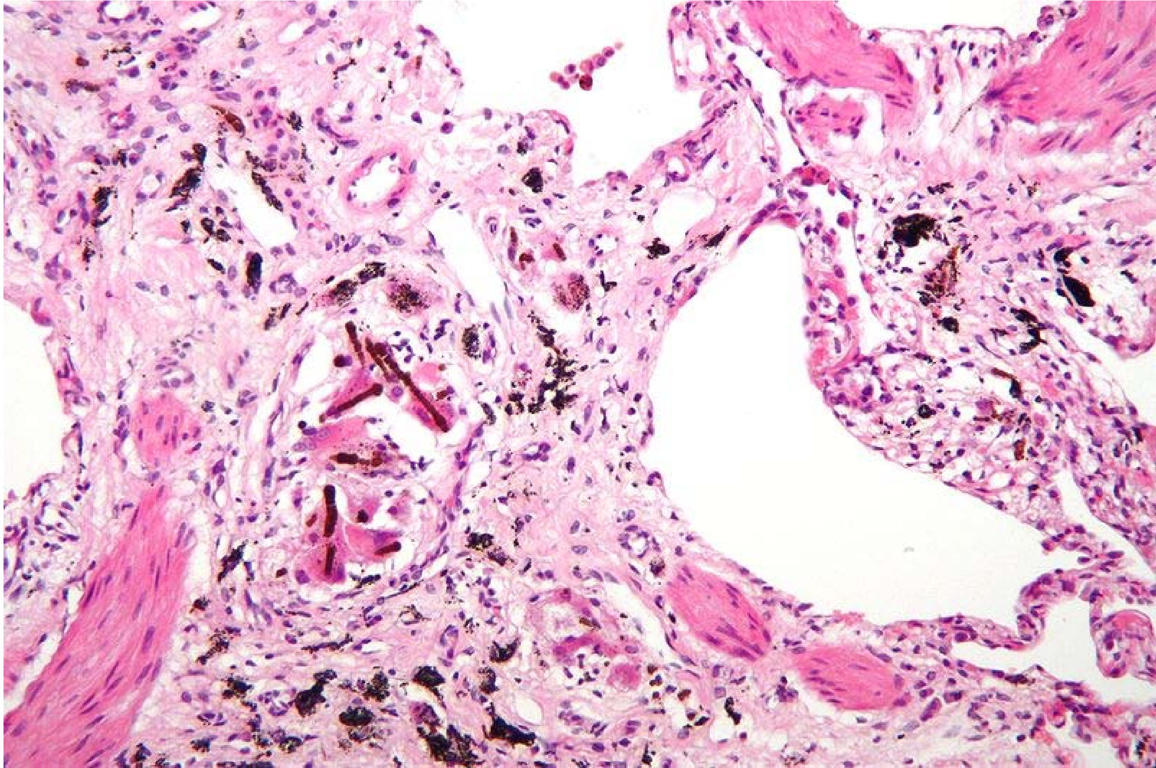
Ferruginous body



Ferruginous bodies. H&E stain.

A **ferruginous body** (pl. ferruginous bodies), is a histopathologic finding in interstitial lung disease suggestive of significant asbestos exposure (asbestosis).

They appear as small brown nodules in the septum of the alveolus. Ferruginous bodies are typically indicative of asbestos inhalation (when the presence of asbestos is verified they are called "asbestos bodies"). In this case they are fibers of asbestos coated with an iron-rich material derived from proteins such as ferritin and hemosiderin. Ferruginous bodies are believed to be formed by macrophages that have phagocytized and attempted to digest the fibers.



Micrograph of asbestosis with prominent **ferruginous bodies**. H&E

Fibrinoid necrosis

Fibrinoid necrosis is a form of necrosis, or tissue death, in which there is accumulation of amorphous, basic, proteinaceous material in the tissue matrix with a staining pattern reminiscent of fibrin. It is associated with conditions such as immune vasculitis (e.g. Henoch-Schonlein purpura), malignant hypertension, or hyperacute transplant rejection.

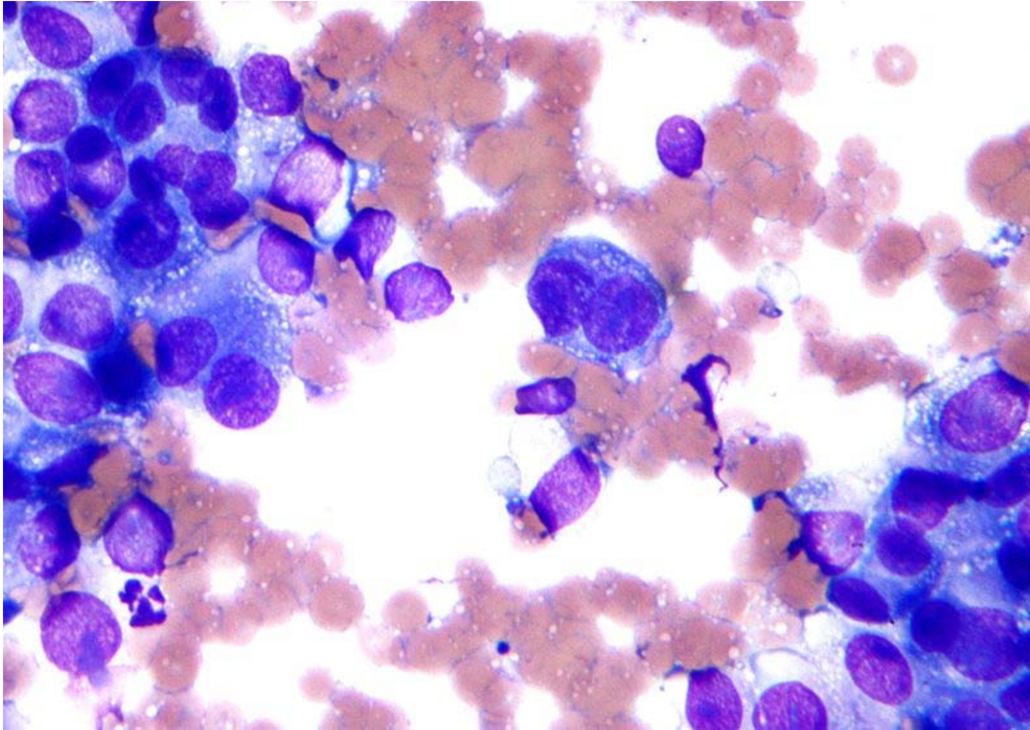
Fibrinoid material does usually contain fibrin and tends to be eosinophilic (staining red with the acidic dye eosin). However, in systemic lupus erythematosus the fibrinoid deposits may contain significant amounts of nuclear debris, including acidic DNA, and may be haematoxyphilic (staining purple or blue with the basic dye haematoxylin). Fibrinoid necrosis is distinguished from hyaline deposits, which are more homogeneous

and glassy, and caseous necrosis, which is associated with the tuberculosis. Fibrinoid is now a somewhat historic term, based on traditional haematoxylin and eosin staining. Its significance is different in different contexts, such as malignant hypertension and rheumatoid nodules. However, in context it remains a diagnostically useful term. The idea that autoimmune diseases such as systemic lupus and rheumatoid arthritis were based on a common lesion of 'fibrinoid necrosis of collagen' led to the popular term 'collagen vascular disease', which is now obsolete. It is cells that are dead, not collagen. The presence of fibrin indicates that nearby blood vessels have become highly permeable and often themselves destroyed but vasculitis is not necessarily present at sites of fibrinoid deposition.

Fibrinoid necrosis can be associated with immune-complex associated disease, as in the synovitis and nodules of rheumatoid arthritis and various forms of immune- based vasculitis and glomerular disease. It may be associated with deposition of immune complexes and complement components in vessel walls. In small vessel vasculitis fibrin plugs frequently occur in the vessel lumen, but the term fibrinoid is usually used to refer to material outside the lumen of a vessel.Fibrinoid necrosis also occurs in the walls of arterioles in malignant hypertension (blood pressure greater than 200/130 mmHg).

Fibrinoid necrosis involves the deposition of immune complexes in areas such as the glomerulus and capillaries. The immune complexes do not cause the damage, but activate the alternative complement pathway, specifically C5a, which attracts neutrophils. The neutrophils are then responsible for causing fibrinoid necrosis.

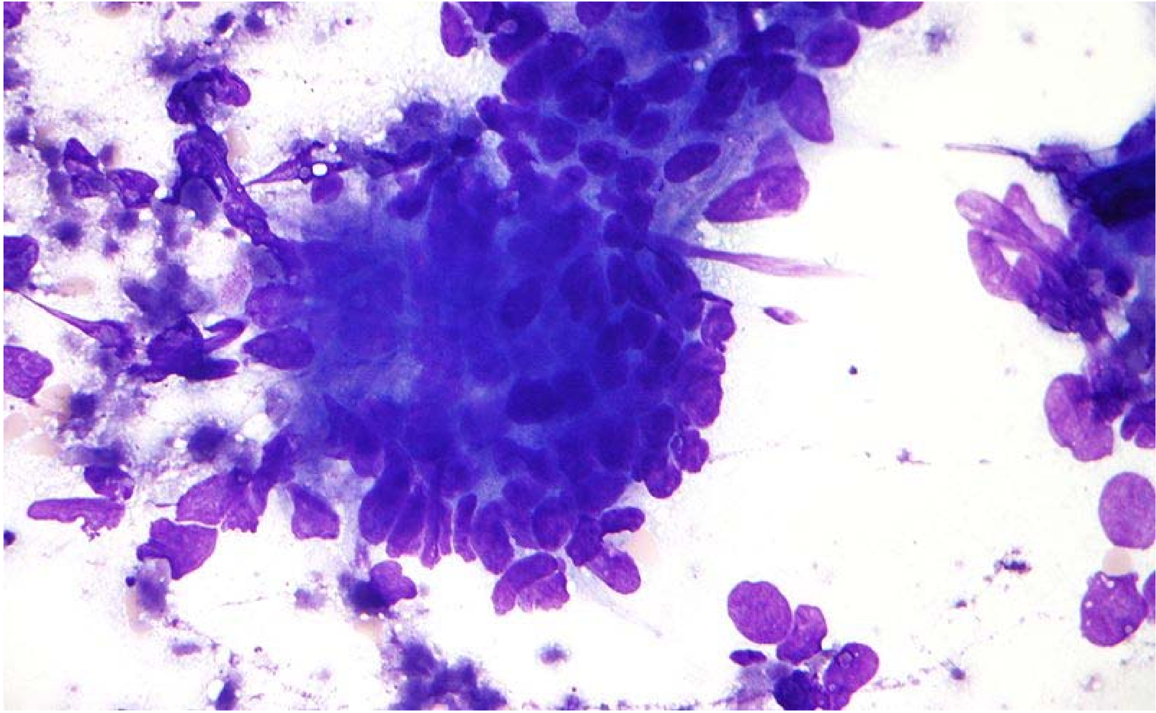
Field stain



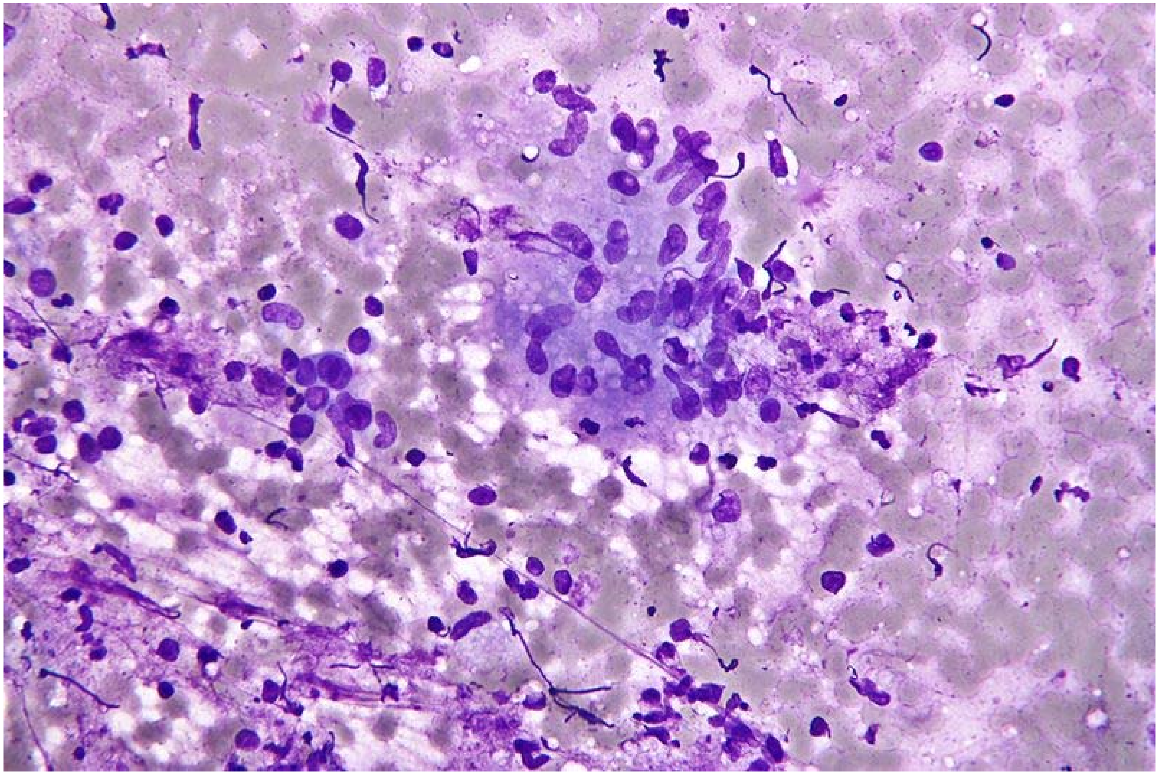
Micrograph of a Field stain showing malignant melanoma

Field stain is a histological method for staining of blood smears. It is used for staining thin blood films in order to discover malarial parasites. Field's stain is a version of a Romanowsky stain, used for rapid processing of the specimens.

Field's stain uses methylene blue and Azure 1 dissolved in phosphate buffer solution, and Eosin Y in buffer solution.



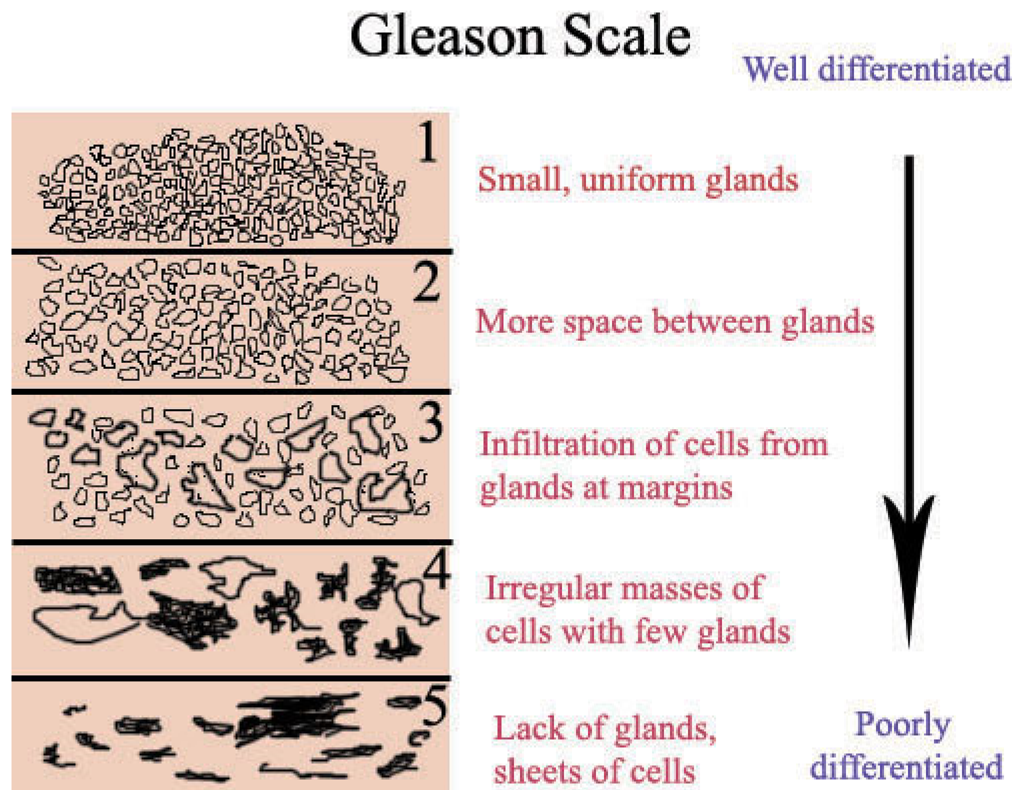
Colorectal adenocarcinoma. Field stain.



Granuloma. Field stain.

Chapter 6

Gleason Grading System



Gleason grade Lower grades are associated with small, closely packed glands. Cells spread out and lose glandular architecture as grade increases. Gleason score is calculated from grade as described in the text.

The **Gleason Grading system** is used to help evaluate the prognosis of men with prostate cancer. Together with other parameters, it is incorporated into a strategy of prostate cancer staging which predicts prognosis and helps guide therapy. A Gleason score is given to prostate cancer based upon its microscopic appearance. Cancers with a higher Gleason score are more aggressive and have a worse prognosis.

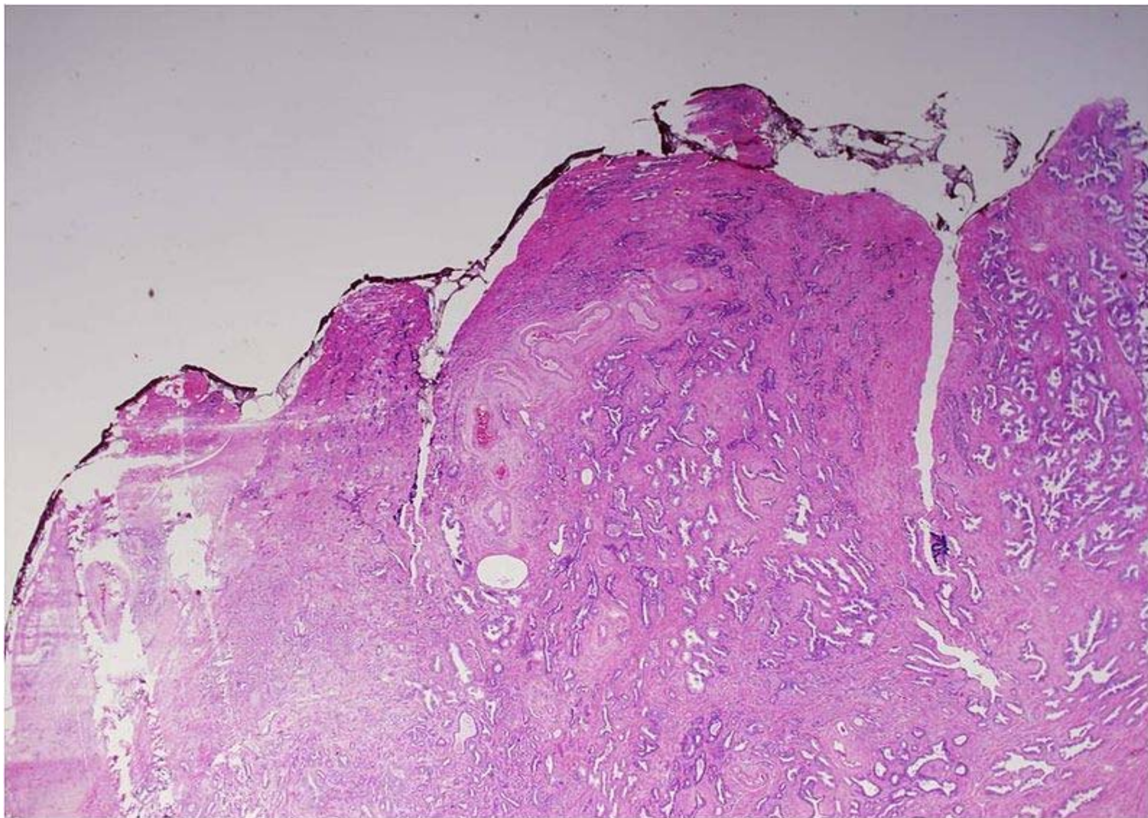
Process

Most often, a urologist or radiologist will remove a cylindrical sample (biopsy) of prostate tissue through the rectum, using hollow needles, and prepare microscope slides. After a prostate is removed in surgery, a pathologist will slice the prostate for a final examination.

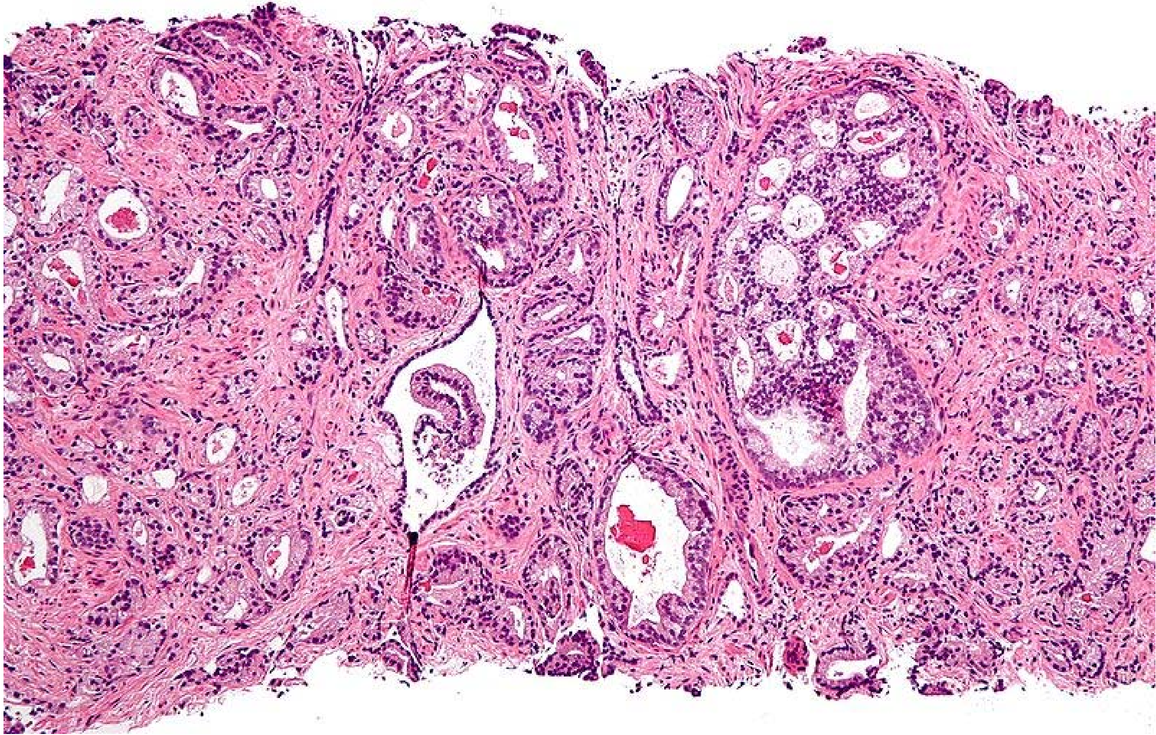
Grades and scores

The pathologist assigns a grade to the most common tumor pattern, and a second grade to the next most common tumor pattern. The two grades are added together to get a Gleason Score. For example, if the most common tumor pattern was grade 3, and the next most common tumor pattern was grade 4, the Gleason Score would be 3+4 = 7. The Gleason Grade is also known as the Gleason Pattern, and the Gleason Score is also known as the Gleason Sum. The Gleason Grade or Gleason Pattern ranges from 1 to 5, with 5 having the worst prognosis. The Gleason Score ranges from 2 to 10, with 10 having the worst prognosis. For Gleason Score 7, a Gleason 4+3 is a more aggressive cancer than a Gleason 3+4. Also, there is not really any difference between the aggressiveness of a Gleason Score 9 or 10 tumor.

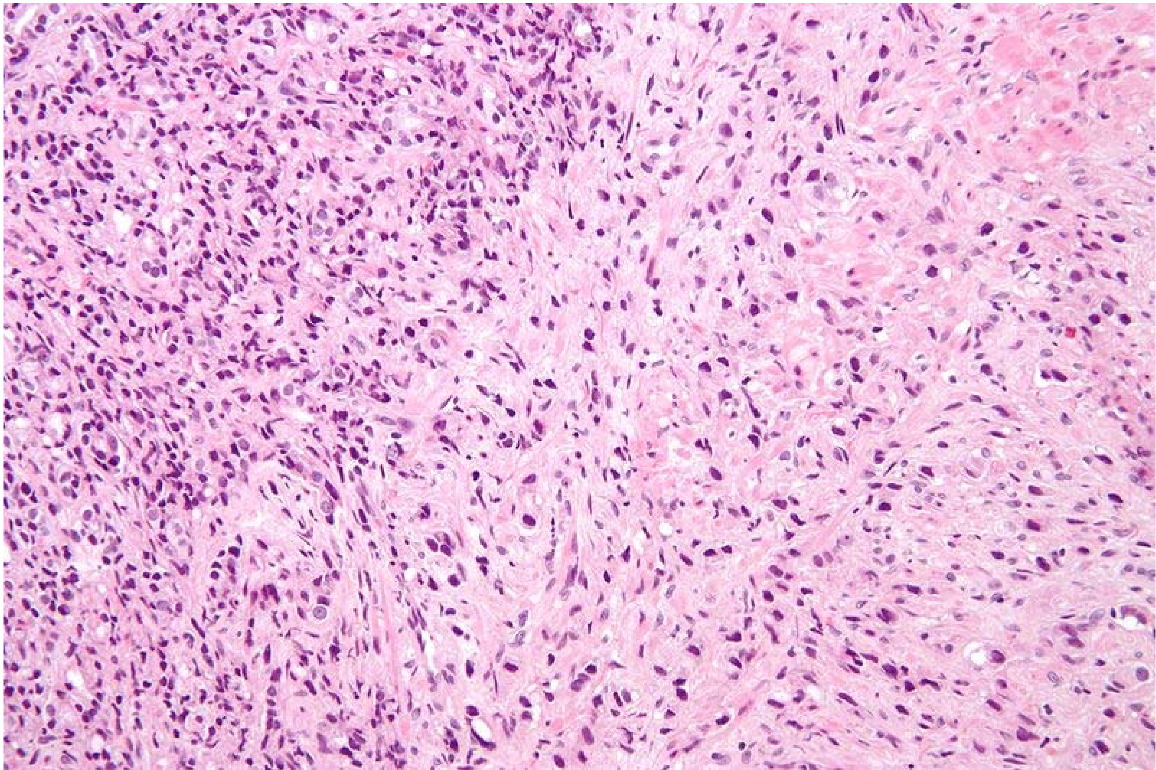
Patterns 1 through 5



Gleason Pattern 3. H&E stain.



Gleason pattern 4. H&E stain.



Gleason pattern 4 (left of image) and Gleason pattern 5 (right of image). H&E stain.

Gleason patterns are associated with the following features:

- Pattern 1 - The cancerous prostate closely resembles normal prostate tissue. The glands are small, well-formed, and closely packed.
- Pattern 2 - The tissue still has well-formed glands, but they are larger and have more tissue between them.
- Pattern 3 - The tissue still has recognizable glands, but the cells are darker. At high magnification, some of these cells have left the glands and are beginning to invade the surrounding tissue.
- Pattern 4 - The tissue has few recognizable glands. Many cells are invading the surrounding tissue
- Pattern 5 - The tissue does not have recognizable glands. There are often just sheets of cells throughout the surrounding tissue.

In the United Kingdom, prostate cancer of Gleason pattern 1 and 2 are almost never seen. Gleason pattern 3 is by far the most common.

Primary, secondary, and tertiary

A pathologist examines the biopsy specimen and attempts to give a score to the two patterns.

- First called the primary grade, represents the majority of tumor (has to be greater than 50% of the total pattern seen).
- Second - a secondary grade - relates to the minority of the tumor (has to be less than 50%, but at least 5%, of the pattern of the total cancer observed).

These scores are then added to obtain the final Gleason score.

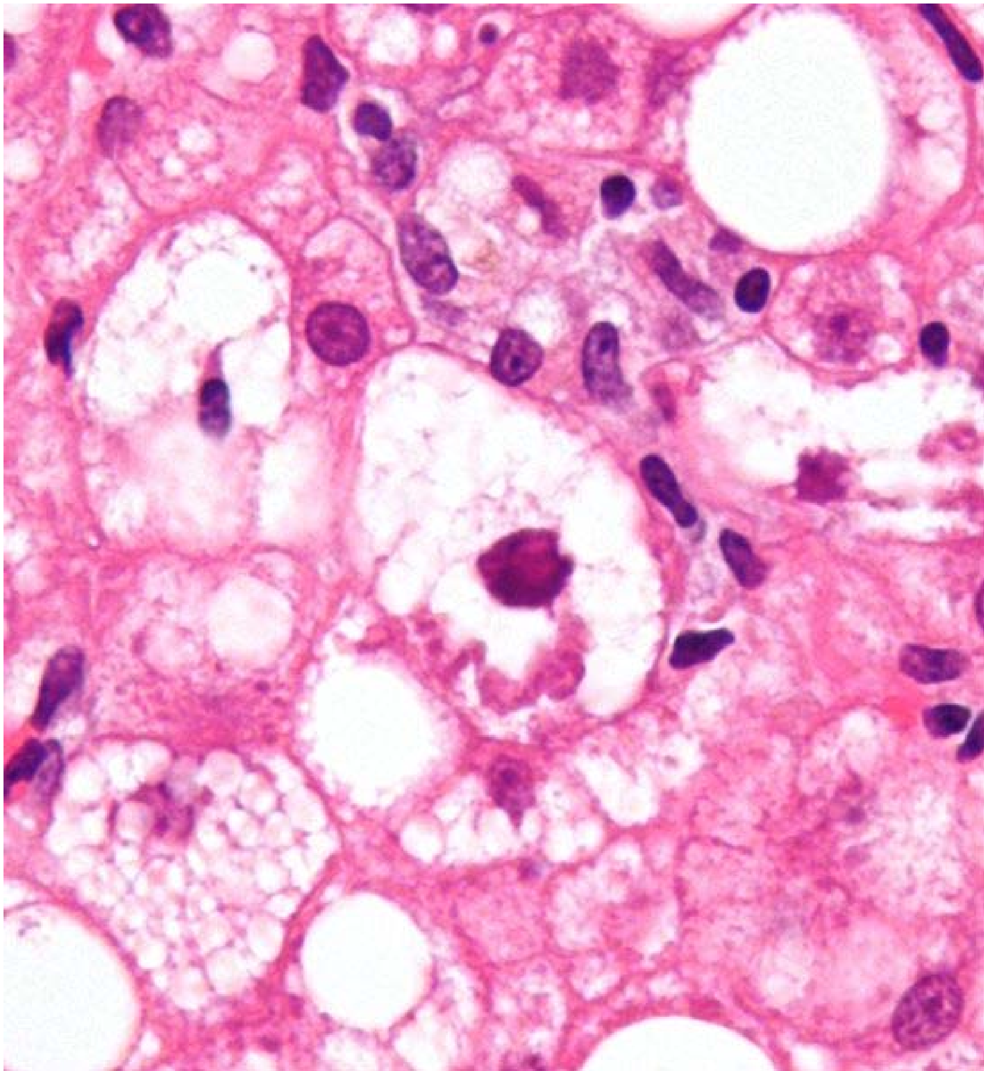
Increasingly, pathologists provide details of the "tertiary" component. This is where there is a small component of a third (generally more aggressive) pattern. So there could be a Gleason 3+4 with a tertiary component of pattern 5 - this would be considered to be more aggressive than a prostate cancer that was Gleason 3+4 with no tertiary pattern 5. Although it is debatable as to what the full extent the tertiary component has on the aggressiveness of a cancer.

History

The scoring system is named after Dr. Donald Gleason, a pathologist at the Minneapolis Veterans Affairs Hospital who developed it with other colleagues at that facility in the 1960s. It remains an important tool.

Chapter 7

Mallory body



Micrograph showing a **Mallory body** with the characteristic *twisted-rope* appearance (centre of image - within a ballooning hepatocyte). H&E stain.

In histopathology, a **Mallory body**, also known as **Drew Parlin bodies**, **Mallory-Denk body**, and **Mallory's hyaline**, is an inclusion found in the cytoplasm of liver cells.

Associated conditions

Mallory bodies are classically found in the livers of people suffering from alcoholic liver disease and were once thought to be specific for that.

They are most common in alcoholic hepatitis (prevalence of 65%) and alcoholic cirrhosis (prevalence of 51%).

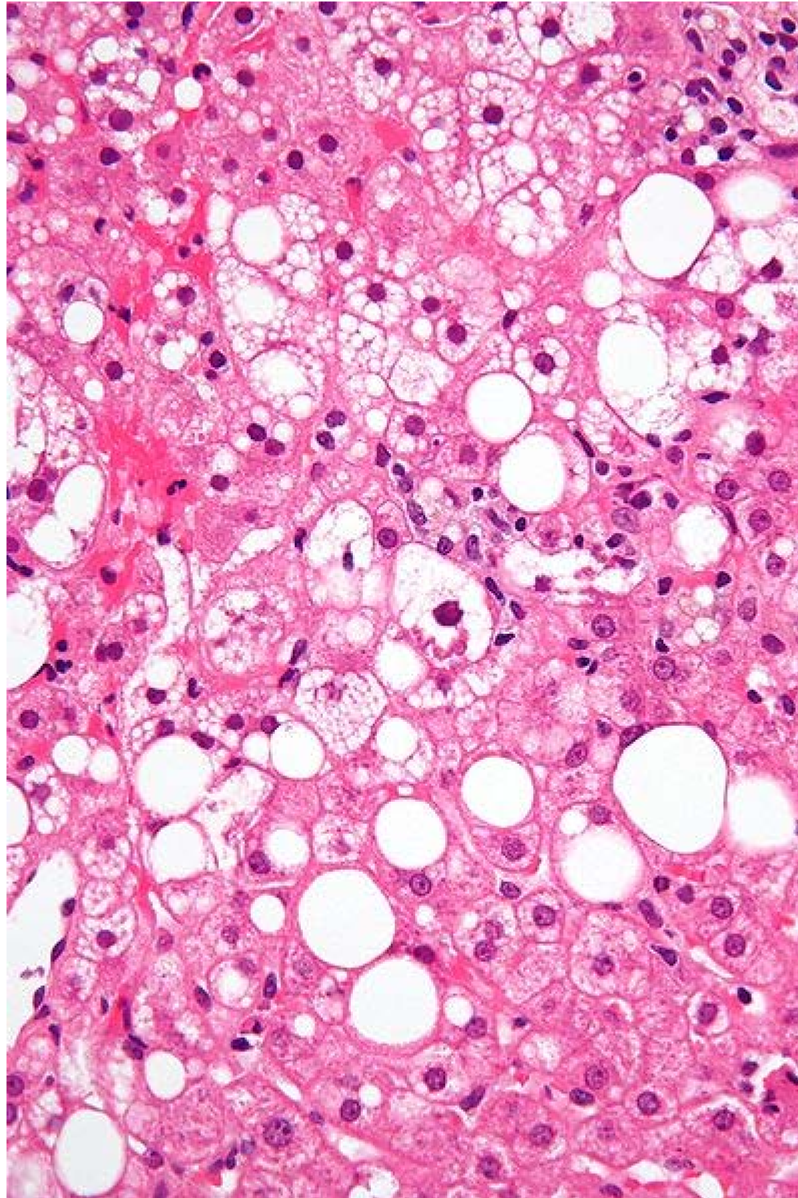
They are a recognized feature of Wilson's disease (25%), primary biliary cirrhosis (24%), non-alcoholic cirrhosis (24%), hepatocellular carcinoma (23%) and morbid obesity (8%), among other conditions.

Appearance

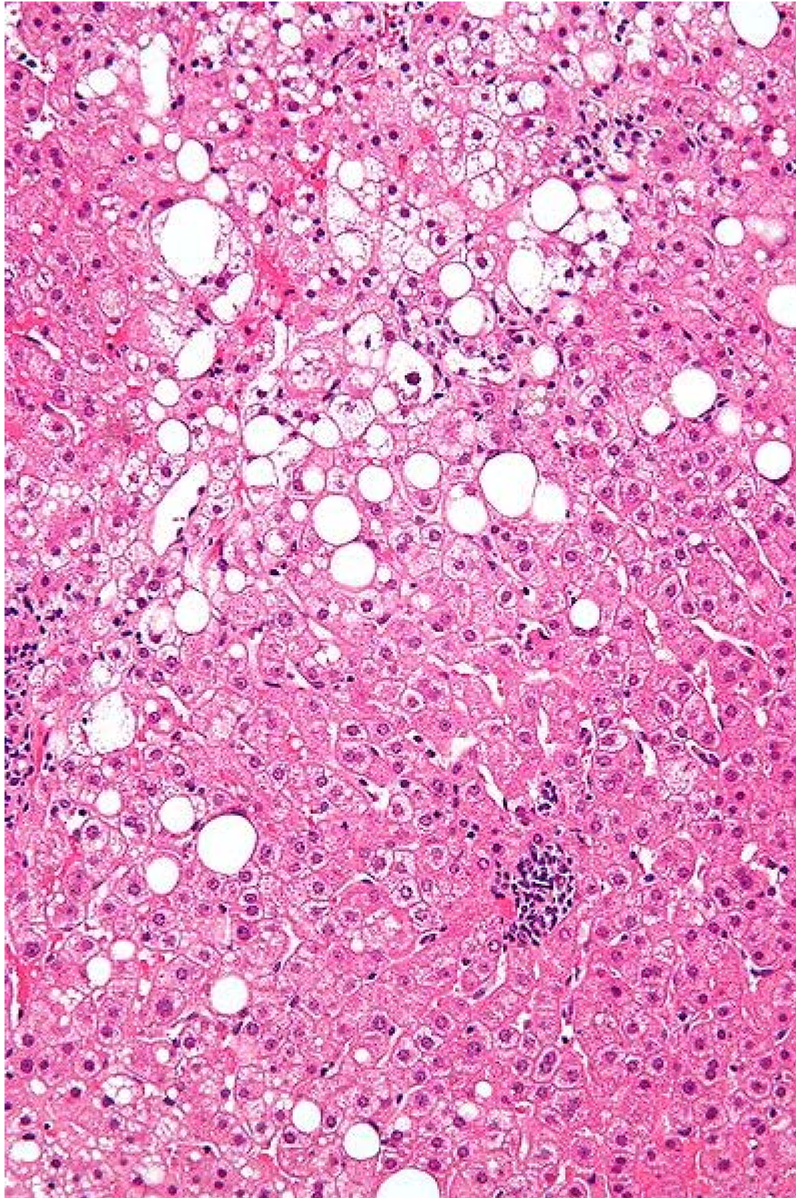
Mallory bodies are highly eosinophilic and thus appear pink on H&E stain. The bodies themselves are made up of intermediate keratin filament proteins that have been ubiquinated, or bound by other proteins such as heat shock proteins, or p62.

Eponym

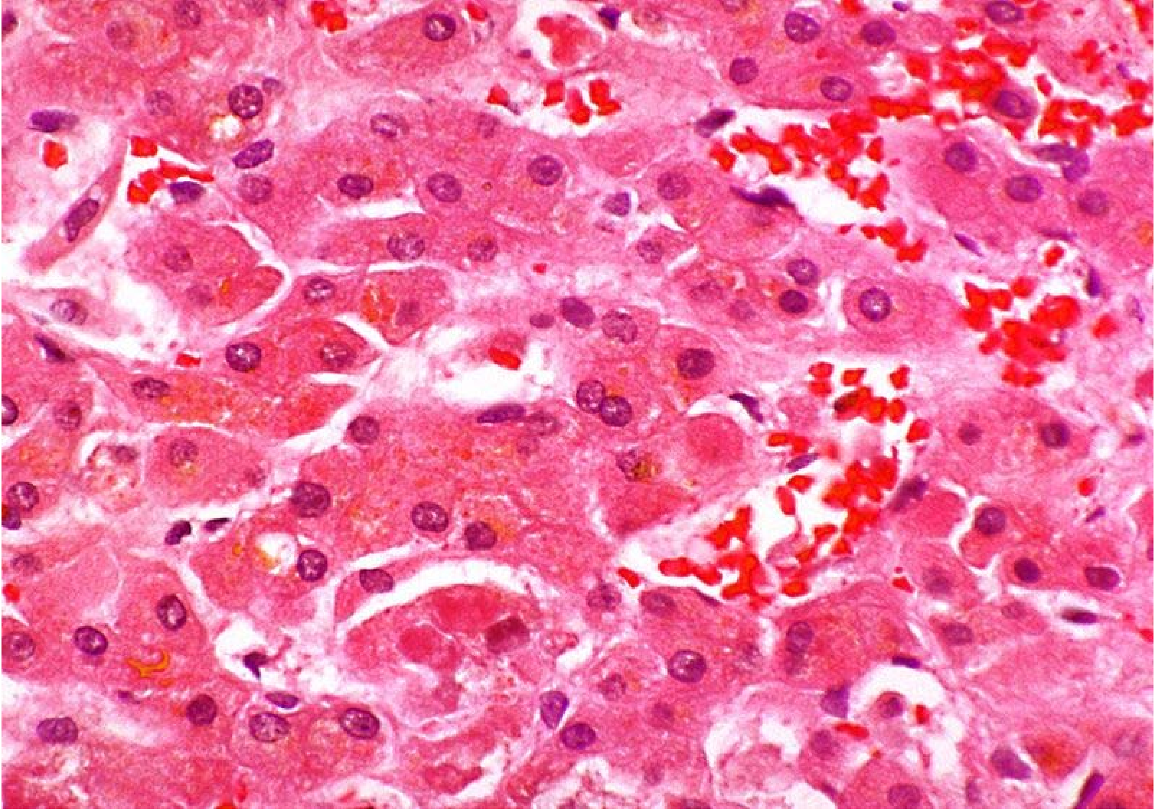
It is named for Frank Burr Mallory. It is was also described serendipitously at the same time by Dr. Drew Parlin.



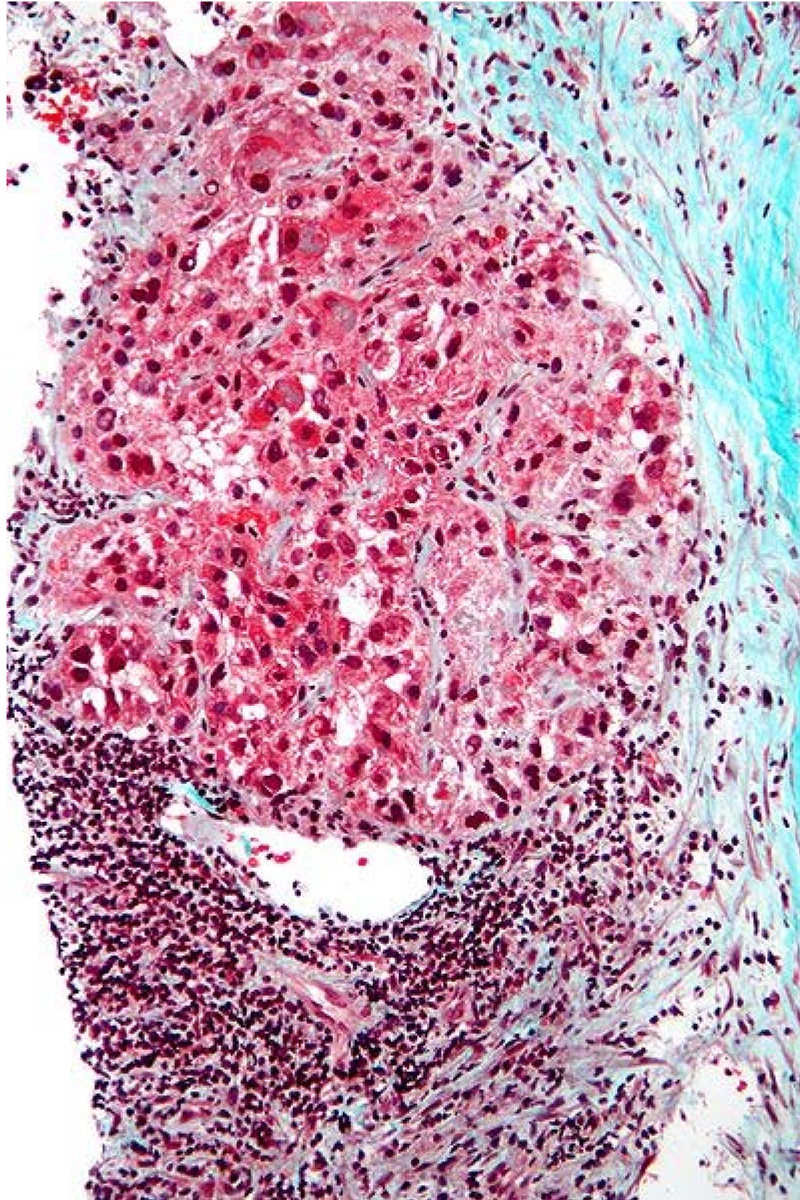
Micrograph showing a **Mallory body**. Original magnification



Micrograph showing a **Mallory body**. Original magnification.



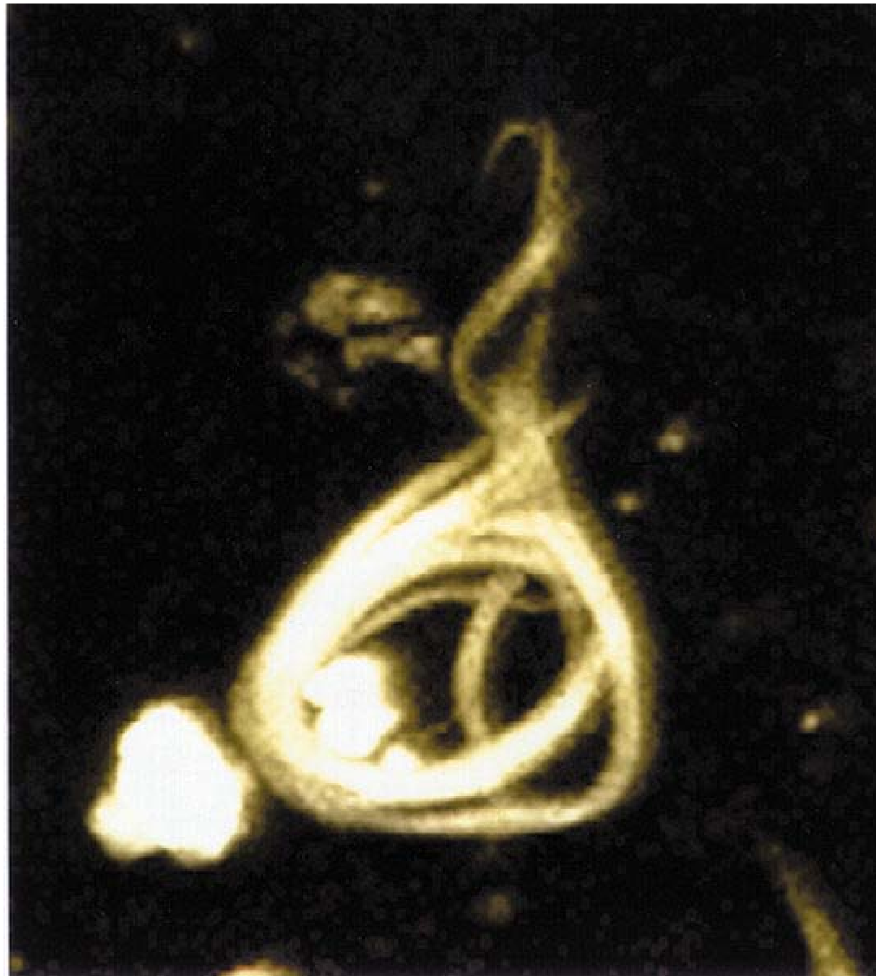
Liver micrograph showing abundant **Mallory bodies**, as seen in alcohol abuse



Mallory bodies in hepatocellular carcinoma. Trichrome stain.

Chapter 8

Neurofibrillary Tangle



Microscopy image of a neurofibrillary tangle, conformed by hyperphosphorylated tau protein

Neurofibrillary Tangles (NFTs) are aggregates of hyperphosphorylation tau that are most commonly known as a primary marker of Alzheimer's Disease. Their presence is also found in numerous other diseases known as Tauopathies. Little is known about their exact relationship to the different pathologies.

Formation

Neurofibrillary Tangles are formed by hyperphosphorylation of a microtubule-associated protein known as tau, causing it to aggregate, or group, in an insoluble form. (These aggregations of hyperphosphorylated tau protein are also referred to as PHF, or "paired helical filaments"). The precise mechanism of tangle formation is not completely understood, and it is still controversial whether tangles are a primary causative factor in disease or play a more peripheral role.

Cytoskeletal changes

Three different maturation states of NFT have been defined using anti-tau and anti-ubiquitin immunostaining. At stage 0 there are morphologically normal pyramidal cells showing diffuse or fine granular cytoplasmic staining with anti-tau. In other words cells are healthy with minimal tau presence; at stage 1 some delicate elongate inclusions are stained by tau antibodies (these are early tangles); stage 2 is represented by the classic NFT demonstration with anti-tau staining ; stage 3 is exemplified by ghost tangles (tangles outside of cells where the host neuron has died), which are characterized by a reduced anti-tau but marked anti-ubiquitin immunostaining.

Causes

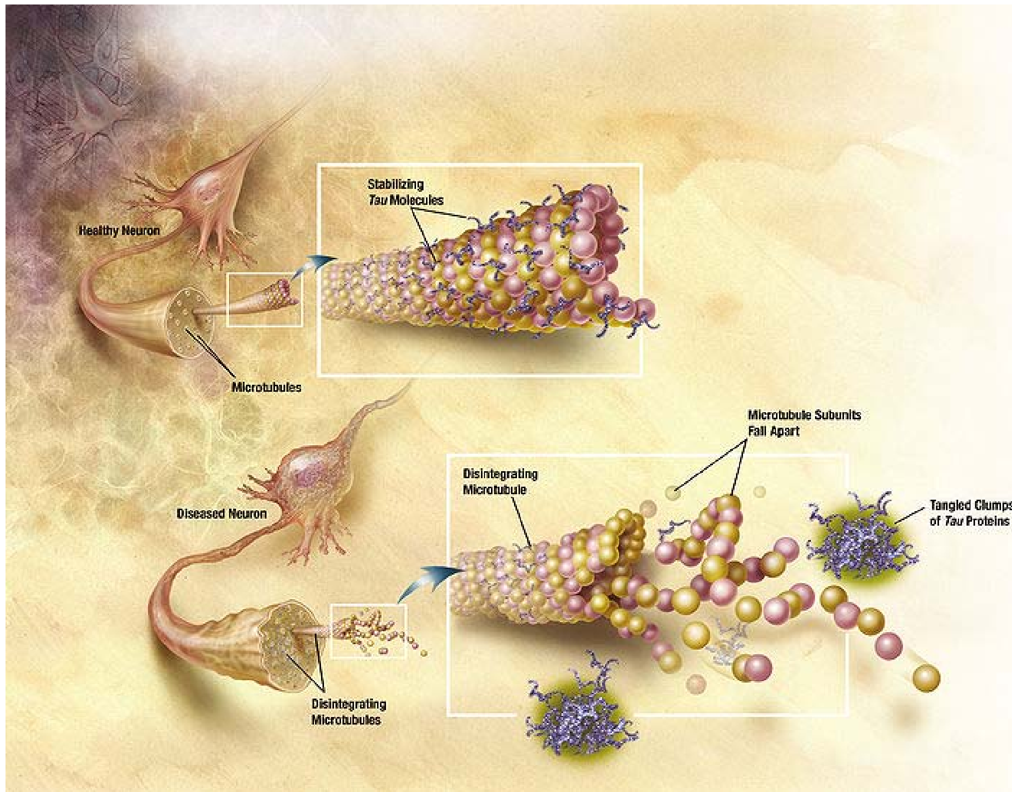


Diagram of how microtubules desintegrate with Alzheimer's disease

Mutated Tau

The traditional understanding is that tau binds to microtubules and assists with their formation and stabilization. However when tau is hyperphosphorylated, it is unable to bind and the microtubules become unstable and begin disintegrating. The unbound tau clumps together in formations called neurofibrillary tangles. More explicitly, intracellular lesions known as pretangles develop when tau is phosphorylated excessively and on improper amino acid residues. These lesions, over time, develop into filamentous neurofibrilary tangles (NFTs) which interfere with numerous intracellular functions. Seeking a reliable animal model for tau-related pathologies, researchers expressed the human mutant P301L tau gene in adult mice. This experiment resulted in the formation of neurofibrillary tangles and pretangle formations. The human mutant P301 tau gene is associated with frontotemporal dementia with parkinsonism, another tauopathy associated with NFTs. It was found that the degree of tau pathology was dependent on time and the level of gene expression. Groups receiving a combination of a promoter and enhancer in the vector saw increased tau expression, as early as 3 weeks after vector injection, which was measured using a Western blot.These groups also showed a greater pathology compared to those with less expression of the mutant tau. Additionally, NFTs were clearly detected by immunoelectron microscopy at 4 months but not at 2 months. However, at both 2 and 4 months, pretangle-like structures were observed suggesting the NFT formation is not complete by 4 months and will continue to progress with time.

Traumatic Brain Injury

Preliminary research indicates that iron deposits due to hemorrhaging, following traumatic brain injury (TBI), may increase tau pathology. While TBI does not routinely lead to accelerated NFT formation, further work may determine if other blood components or factors unrelated to hemorrhages are involved in this TBI-induced augmentation of tau pathology. NFTs are most commonly seen associated with repetitive mild TBI as opposed to one instance of severe traumatic brain injury. For example the clinical syndrome of dementia pugilistica, otherwise known as punch-drunk syndrome found in boxers, is highly associated with NFTs and neuropil threads.

Aluminum

The idea that there is a link between Aluminum exposure and the formation of neurofibrillary tangles has floated around the scientific community for sometime without having been definitively proven or disregarded. Recently a study examining the hippocampal CA1 cells from individuals with and with out Alzheimer’s disease showed a small portion of the pyramidal cells contain cytoplasmic pools within their somas containing early NFTs. These cytoplasmic pools are aggregates of an aluminum/hyperphosphorylated tau complex similar to mature NFTs. (Walton) While a connection between aluminum and NFTs and AD is maintained, there is evidence that Aluminum does not directly cause the formation of NFTs or AD.

Pathology

It has been shown that the degree of cognitive impairment in diseases such as AD is significantly correlated with the presence of neurofibrillary tangles.

Harmful or Protective?

There has been some suggestion that the formation of NFTs is not a causal relationship with disease. Rather that NFTs may be produced in response to a variety of conditions and may in fact be a compensatory response against oxidative stress and serves a protective function. Several points are made to argue the position that NFTs are perhaps protective instead of harmful. First there appears to be a dispute as to the impact of neurofibrillary tangles on neuronal viability because some neurons containing NFTs survive for decades. Furthermore, NFTs have been found in apparently healthy individuals, indicating that NFTs are not directly related to neural degeneration. It has been proposed that the formation of NFTs is part of a multifaced compensatory response where oxidative insult activates several kinases, which are then capable of phosphorylating tau. This then prompts the early formation of NFTs, which reduce oxidative damage and prolong the function of the neuron. While an intriguing theory, scientists have not come to a firm conclusion as to what role NFTs play in neurodegenerative diseases.

Neuron loss

Traditionally believed to play a major role in neuron loss, NFTs are an early event in pathologies such as Alzheimer's disease, and as more NFTs form, there is substantially more neuron loss. However, it has been shown that there is significant neuron loss before the formation of neurofibrillary tangles, and that NFTs account for only a small proportion (around 8.1%) of this neuron loss. Coupled with the longevity of neurons containing NFTs, it is likely that some other factor is primarily responsible for the bulk of neuron loss in these diseases, not the formation of neurofibrillary tangles.

NFT-predominant dementia vs. Classical Alzheimer's

It is currently unclear as to whether or not Neurofibrillary tangle-predominant dementia (NFTPD) (a.k.a. tangle-only dementia) is a variant of the traditional Alzheimer's disease, or a genetically distinct entity. Characterized by later onset and milder cognitive impairment, the distribution of NFT pathology is more closely related to that found in centenarians showing no or limited cognitive impairment. NFTs are generally limited to allocortical/limbic regions of the brain with limited progression to the neocortex but a greater density in the allocortical/hippocampal region. Plaques are generally absent.

Alzheimer disease with concomitant dementia with Lewy bodies (AD+DLB)

The degree of NFT involvement in AD is defined by Braak stages. Braak stages I and II are used when NFT involvent is confined mainly to the transentorhinal region of the brain. Stages III and IV indicated involvement of limbic regions such as the hippocampus, and V and VI when there's extensive neocortical involvement. This should not be confused with the degree of senile plaque involvement, which progresses differently.

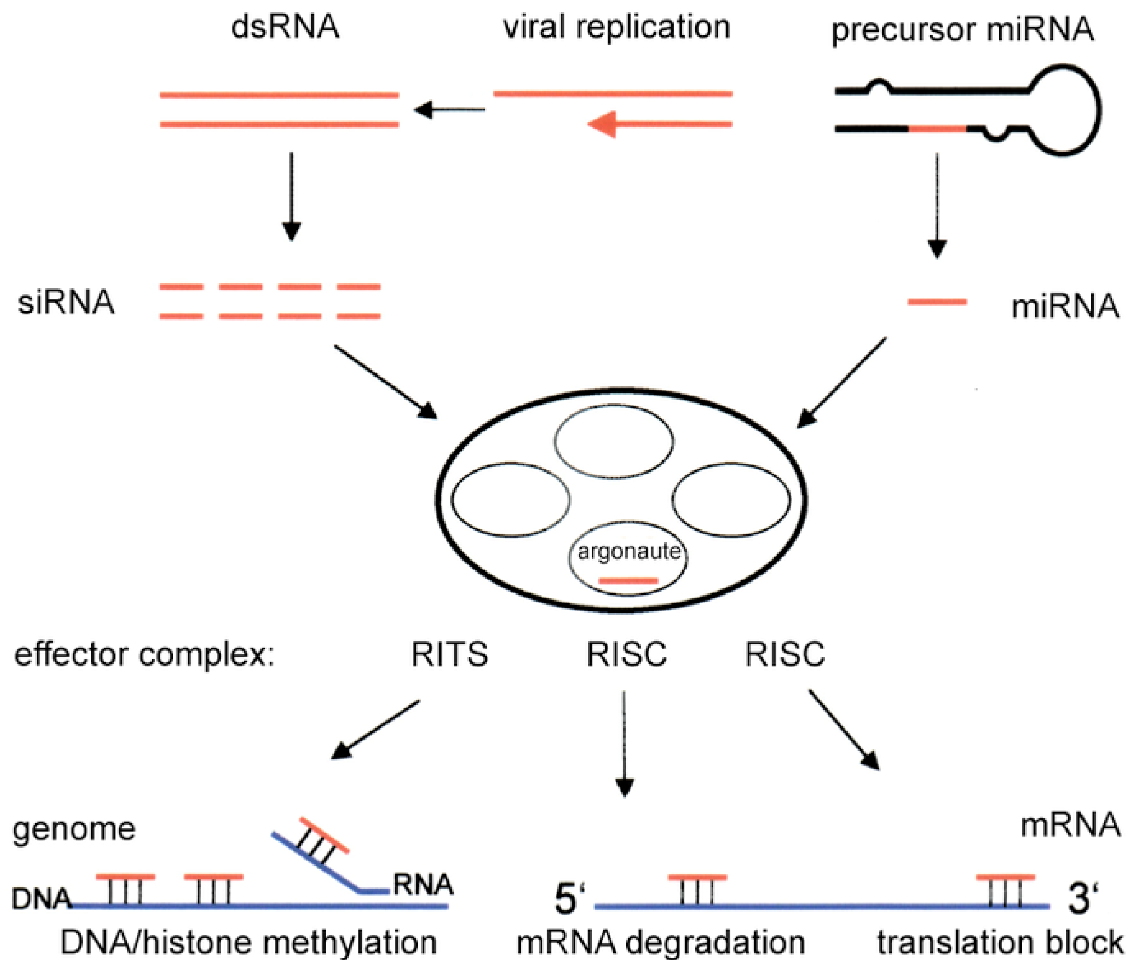
Neurofibrillary tangle and modified Braak scores were lower in AD+DLB, however, neocortical NFT scores show markedly different patterns between AD+DLB and Classical Alzheimers. In pure AD, NFT are predominately found at a high frequency: In AD+DLB, the distribution of NFT frequency was found to be bimodal: NFTs were either frequent or few to absent. Additionally, neocortical NFT frequency in the AD+DLB group tended to parallel the severity of other types of tau cytopathology.

Link to Aggression and Depression In Alzheimer's Patients

A recent study looked for correlation between the quantitative aspects of Alzheimer's disease (neuron loss, neuritic plaque and neurofibrillary tangle load) and aggression frequently found in Alzheimer's patients. It was found that only an increase in neurofibrillary tangle load was associated with severity of aggression and chronic aggression in Alzheimer's patients.While this study does indicate a correlation between NFT load and severity of aggression, it does not provide a causative argument.

Research has also indicated that patients with AD and comorbid depression show higher levels of neurofibrillary tangle formation than individuals with AD but no depression. Comorbid depression increased the odds for advanced neuropathologic disease stage even when controlling for age, gender, education and cognitive function.

Treatment



Overview of RNA interference

Statins

Statins have been shown to reduce the neurofibrillary tangle burden in mouse models, likely due to their anti-inflammatory capacities.

Cyclin-dependent kinase 5

Cyclin-dependent kinase 5 (CDK5) is a kinase that has been previously hypothesized to contribute to tau pathologies. RNA interference (RNAi) mediated silencing of the CDK5 gene has been proposed as a novel therapeutic strategy again tau pathology, such as neurofibrillary tangles. Knockdown of CDK5 has been shown to reduce the phosphorylation of tau in primary neuronal cultures and in mouse models. Furthermore, this silencing showed a dramatic reduction in the number of neurofibrillary tangles.

Lithium

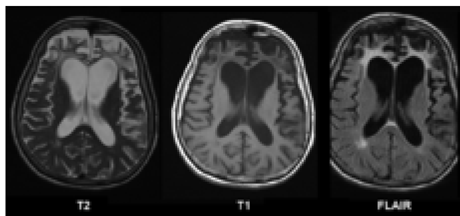
Lithium has been shown to decrease the phosphorylation of tau.Lithium treatment has been shown to reduce the density of neurofibrillary tangles in transgenic models in the hippocampus and spinal cord. Despite the decrease in density of NFTs, motor and memory deficits were not seen to improve following treatment. Additionally, no preventative effects have been see using lithium treatment.

Other conditions

- Progressive supranuclear palsy although with straight filament rather than PHF tau
- Dementia pugilistica (chronic traumatic encephalopathy)
- Frontotemporal dementia and parkinsonism linked to chromosome 17 however without detectable β-amyloid plaques.
- Lytico-Bodig disease (Parkinson-dementia complex of Guam)
- Ganglioglioma and gangliocytoma
- Meningioangiomatosis
- Subacute sclerosing panencephalitis
- As well as lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, and lipofuscinosis

Chapter 9

Pick's Disease

Pick's disease	
 Brain MRI in Pick's disease	
ICD-10	G31.0, F02.0
ICD-9	331.11
OMIM	172700
DiseasesDB	10034
eMedicine	neuro/311
MeSH	D020774

Pick's disease, also known as **Pick disease** and **PiD** (not to be confused with Pelvic Inflammatory Disease, also known as PID), is a rare neurodegenerative disease. While the term Pick's disease was once used to represent a class of clinical syndromes with symptoms attributable to frontal and temporal lobe dysfunction, it is now used (at least among professionals in the field) to mean a specific pathology that is just one of the causes of the clinical syndrome now known as frontotemporal lobar degeneration. Some people still use the term Pick's disease to mean the more general clinical syndrome of frontotemporal lobar degeneration, but this has previously led to confusion among both professionals and patients and so its use should be restricted to the specific pathological subtype described below.

Pick's disease (the pathology) causes progressive destruction of nerve cells in the brain and causes tau proteins in neurons to accumulate into silver-staining, spherical aggregations known as "**Pick bodies**" that are a defining characteristic of the disease.

History

Pick's disease is named after Arnold Pick, a professor of psychiatry from the University of Prague who first discovered and described the disease in 1892 by examining the brain tissue of several deceased patients with histories of dementia. As a result, the characteristic histological feature of this disease - a protein tangle that appears as a large body in neuronal tissue - is named a Pick body. In 1911, Alois Alzheimer also noted the complete absence of senile plaques and neurofilbrillary tangles as well as the presence of Pick Bodies and occasional ballooned neurons.

Symptoms

Pick's disease is one of the causes of the clinical syndrome of frontotemporal lobar degeneration which has three subtypes. Pick's disease pathology is associated more with the frontotemporal dementia and progressive nonfluent aphasia subtypes than the semantic dementia subtype.

Causes

While other pathologies causing frontotemporal lobar degeneration are associated with a genetic cause, there is no evidence in the modern literature that classical Pick's disease pathology can run in families or has a genetic cause.

Pathology and biochemistry

PiD was first recognized as a distinct disease separate from other neurodegenerative diseases because of the presence of large, dark-staining aggregates of proteins in neurological tissue as well as the aforementioned ballooned cells, which are known as Pick cells. Pick bodies are almost universally present in patients with PiD, but some new cases of atypical Pick's disease have come to light that lack noticeable Pick bodies. A variety of stains can aid in the visualization of Pick bodies and Pick cells, but immunohistochemical staining using anti-tau and anti-ubiquitin antibodies have proven the most efficient and specific. Hematoxylin and eosin staining allows visualization of another population of Pick cells, which are both tau and ubiquitin protein negative. Several different silver impregnation stains have been used, including the Bielschowsky, Bodian, and Gallyas methods. The latter two techniques are sensitive enough to allow PiD to be distinguished from Alzheimer's disease as the Bodian will bind preferentially to cells with PiD as compared to the Gallyas method, which preferentially binds to the cells with Alzheimer's.

Numerous different areas of the brain are affected by PiD, but the specific areas that are affected allow for differentiation between PiD and Alzheimer's disease. Pick bodies are almost always found in several different places in the brain, including the dentate gyrus, the pyramidial cells of the CA1 sector and subiculum of the hippocampus, and the neocortex as well as a plurality of other nuclei. Interestingly, it is the location within the different layers of the brain as well as the anatomical location that demonstrates some of

the unique features of PiD. A striking feature is that in the neocortex the Pick bodies are located in the II and IV layers of the cortex, which send neurons within the cortex and to thalamic synapses, respectively. While layers III and V have very few if any Pick bodies they show extreme neuronal loss that can, in some cases, be so severe as to leave a void in the brain altogether. Other regions that are involved include the caudate, which is severely affected, the dorsomedial region of the putamen, the globus pallidus, and locus cerulus. The hypothalamic lateral tuberal nucleus is also very severely affected. The cerebellar elements that are important in receiving input, including the mossy fibers as well as the monodendritic brush cells in the granule cell layer, and generating output signals, most notably the dentate nucleus, are stricken with lots of tau protein inclusions. Strangely, the substantia nigra is most often uninvolved or only mildly involved, but cases of extreme degeneration do exist.

PiD has several unique biochemical characteristics that allow for unique identification of Pick's disease as opposed to other pathological subtypes of frontotemporal lobar degeneration. The most striking of these is that this disease, which has tau protein tangles present in many affected neurons, contains only one or as many as two of the six different isoforms of the tau protein. All of these isoforms result from alternative splicing of the same gene. Pick bodies typically have the 3R isoform of tau proteins as not only the most abundant form but the only form of this protein, but a recent study has shown that a much greater number of different tau isoforms including 4R and mixed 3R/4R can be present in the Pick bodies. Not only do these tangles have the 3R tau protein predominately but they are also characteristically shaped with a round body and there is often an indentation in the area that faces the nucleus of the cell. The Pick bodies are also able to be labeled by N-terminal amyloid precursor protein segment, hyperphosphorylated tau, ubiquitin, Alz-50, neurofiliment proteins, clathrin, synaptophysin and neuronal surface glycoside (A2B5) specific stains. Moreover βII tubulin proteins are also suspected in playing a role in the formation of phosphor-tau aggregates that are seen in PiD as well as AD.

Differences from Alzheimer's disease

In Alzheimer's disease, all six isoforms of tau proteins are expressed. In addition, the presence of neurofibrillary tangles that are a hallmark of Alzheimer's can be stained with antibodies to basic fibroblast growth factor, amyloid P, and heparan sulfate glycosaminoglycan. Another difference is that in Pick's disease, a personality change occurs prior to any form of memory loss, unlike Alzheimer's, where memory loss typically presents first. This is used clinically to determine whether patient is suffering from Alzheimer's or Pick's.

Sample Presentation

(From biopsy "histological" verified, after death, case presentation)

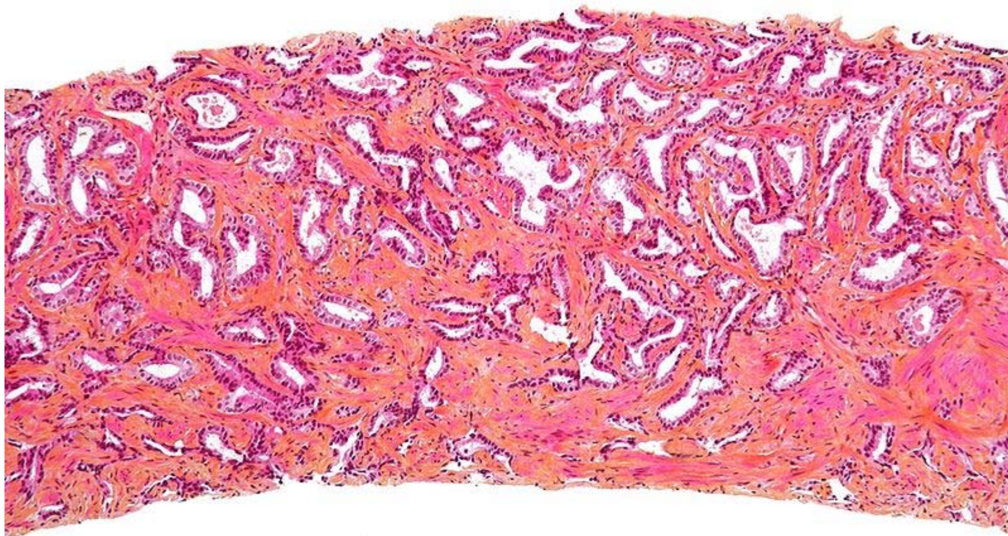
64 y/o retired professional male with progressive strange behavior according to his wife and children for several months without memory loss at start but mild problems with memory now (peoples names, and dates).

Additional signs/symptoms: Uncharacteristic impulsive behavior, easily distractible (again uncharacteristic for this former professional), lack of personal hygiene (stops bathing, brushing teeth, combing hair, etc without prompting from wife), appears unkempt/disheveled, uncharacteristically rude to friends and family, eats with fingers occasionally. No focal neurological signs/symptoms. Prominent grasp and snout reflex.

Chapter 10

Prostate Biopsy and Reed-Sternberg Cell

Prostate biopsy



Micrograph of a **prostate biopsy** showing prostate adenocarcinoma, acinar type, the most common type of prostate cancer. HPS stain.

Prostate biopsy is a procedure in which small samples are removed from a man's prostate gland to be tested for the presence of cancer. It is typically performed when the scores from a PSA blood test rise to a level that is associated with the possible presence of prostate cancer.

The procedure, usually an outpatient procedure, requires a local anesthetic, with fifty-five percent of men reporting discomfort during the biopsy. The most frequent complication of the procedure is bleeding in the urine for several days, some bleeding in the stool for several days, and blood in the ejaculate for several weeks afterwards.

The procedure may be performed transrectally, through the urethra or through the perineum. The most common procedure is transrectal, and may be done with tactile finger guidance, or, more commonly and precisely, with ultrasound guidance.

About a dozen samples are taken from the prostate gland through a thin needle - about six from each side. If the procedure is performed transrectally, antibiotics are prescribed to prevent infection. An enema may also be prescribed for the morning of the procedure. In both the transrectal and the transperineal procedure, the doctor inserts an ultrasound probe into the rectum to help guide the biopsy needles. A local anesthetic is then administered into the tissue around the prostate, similar to the local anesthetic administered for a dental procedure. A spring-loaded prostate tissue collection needle is then inserted into the prostate, through the rectum (or more rarely through the perineum), about a dozen times. It makes a clicking sound, and there may be considerable discomfort.

Prostate mapping

Prostate Mapping is a new biopsy method that uses a combination of multi-sequence MRI scans and template-guided multiple biopsies. It involves taking 30-50 biopsies through the skin that lies in front of the back passage instead of the rectum. The procedure is carried out under general anaesthetic. This method can determine with high accuracy the location and aggressiveness of cancer.

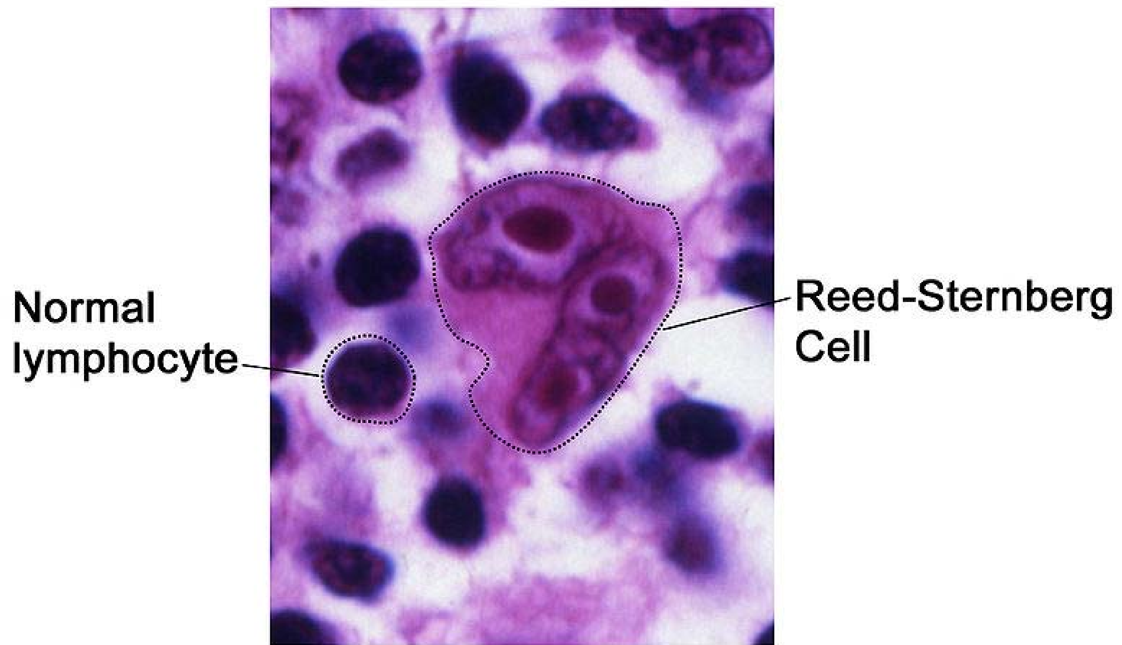
Gleason score

The tissue samples are then examined under a microscope to determine whether cancer cells are present, and to evaluate the microscopic features (or Gleason score) of any cancer found.

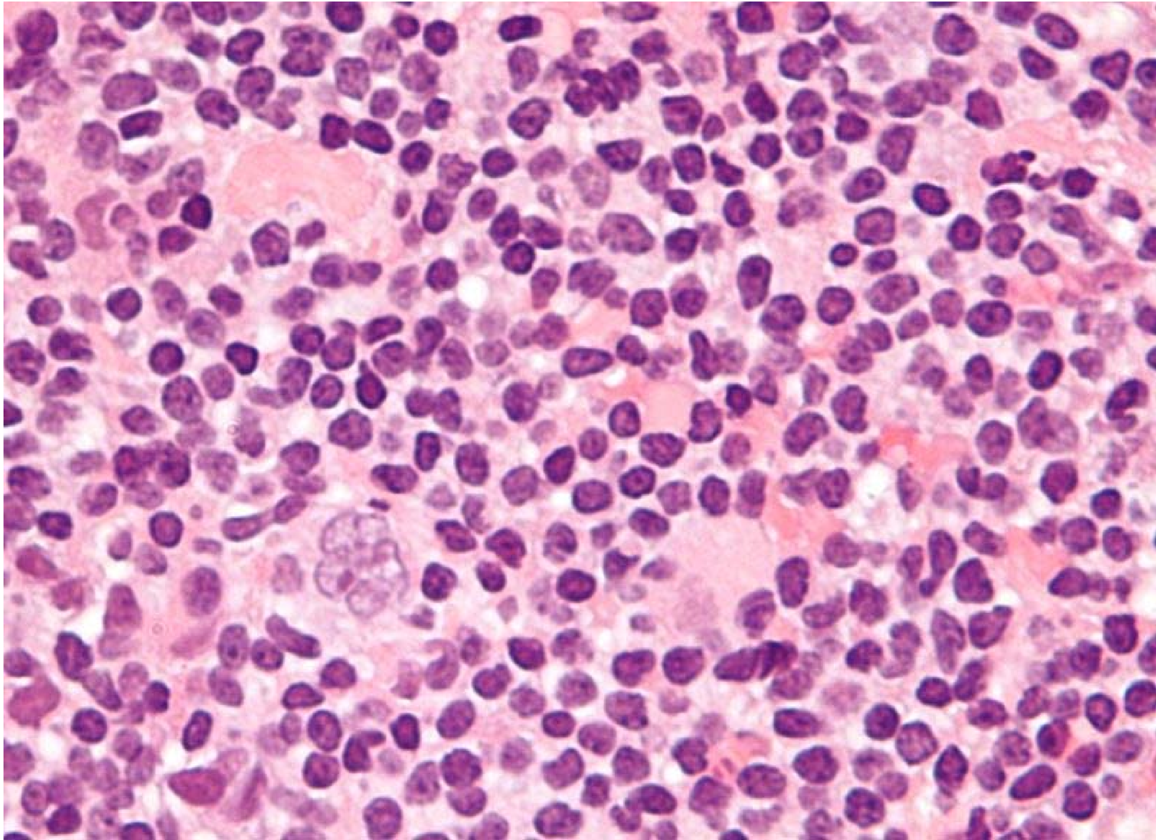
Tumor markers

Tissue samples can be stained for the presence of PSA and other tumor markers in order to determine the origin of maligant cells that have metastasized.

Reed-Sternberg cell



Micrograph showing a classic Reed-Sternberg cell



Micrograph showing a "popcorn cell", the Reed-Sternberg cell variant seen in nodular lymphocyte predominant Hodgkin lymphoma.H&E stain.

Reed-Sternberg cells (also known as **lacunar histiocytes** for certain types) are different giant cells found with light microscopy in biopsies from individuals with Hodgkin's lymphoma (aka Hodgkin's disease; a type of lymphoma) primarily due to EBV, and certain other disorders. They are usually derived from B lymphocytes.

They are named after Dorothy Reed Mendenhall (1874-1964) and Carl Sternberg (1872-1935), who provided the first definitive microscopic descriptions of Hodgkin's disease.

Reed-Sternberg cells are large and are either multinucleated or have a bilobed nucleus (thus resembling an "owl's eye" appearance) with prominent eosinophilic inclusion-like nucleoli. Reed-Sternberg cells are CD30 and CD15 positive, usually negative for CD20 and CD45. The presence of these cells is necessary in the diagnosis of Hodgkin's lymphoma - absence of Reed-Sternberg cells has very high negative predictive value. They can also be found in reactive lymphadenopathy (such as infectious mononucleosis, carbamazepine associated lymphadenopathy) and very rarely non-Hodgkin lymphomas.

A special type of Reed-Sternberg cells are *lacunar histiocytes*, whose cytoplasm retracts when fixed in formalin, so the nuclei give the appearance of cells that lie with empty spaces (called *lacunae*) between them. These are characteristic of the nodular sclerosis subtype of Hodgkin's lymphoma.

Chapter 11

Rheumatoid Nodule and Tauopathy

Rheumatoid nodule

Rheumatoid nodule

MeSH D012218

A **rheumatoid nodule** is a local swelling or tissue lump, usually rather firm to touch, like an unripe fruit, which occurs almost exclusively in association with rheumatoid arthritis. Very rarely rheumatoid nodules occur as 'rheumatoid nodulosis' in the absence of arthritis. They are usually subcutaneous especially over bony prominences such as the tip of the elbow or olecranon or over the finger knuckles. Less commonly they occur in the lining of the lung and other internal organs. The occurrence of nodules in the lung of miners exposed to silica dust was known as Caplan's syndrome. Nodules vary in size from that of a lentil or pea to that of a mandarin orange. Quite often they are associated with synovial pockets or bursae. About 5% of rheumatoid arthritis patients have such nodules within 2 years of disease onset, and the cumulative prevalence is about 25%. In the great majority of cases nodules are not painful or disabling in any way, being more of an unsightly nuisance, but in some cases they can be painful, especially if the overlying skin breaks down. Rarely, the nodules occur at diverse sites on body (e.g. upper eyelid, distal region of soles, vulva and internally in the gallbladder, lung, heart valves, larynx and spine).

Diagnosis

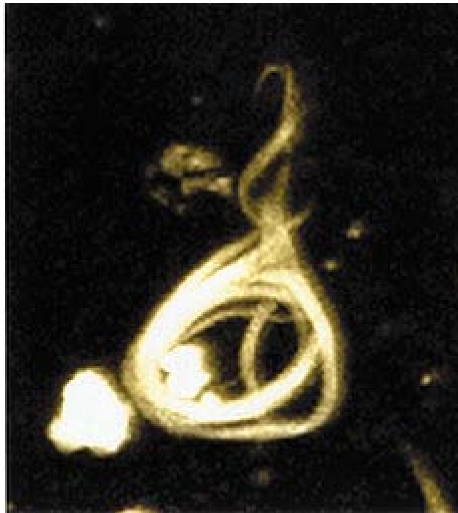
Histological examination of nodules shows that they consist of a shell of fibrous tissue surrounding a center of fibrinoid necrosis. Pea sized nodules have one centre. Larger nodules tend to be multilocular, with many separate shells or with connections between the necrotic centres. Individual necrotic centres may contain a cleft or several centres of necrosis may all open on to a large bursal pocket containing synovial fluid. The boundary between the necrotic centre and the outer fibrous shell is made up of the characteristic feature of the nodule, which is known as a cellular palisade. The palisade is a densely packed layer of macrophages and fibroblasts which tend to be arranged radially, like the seeds of a kiwi fruit or fig. Further out into the fibrous shell there is a zone that contains T lymphocytes and plasma cells in association with blood vessels. The overall

histological picture is essentially identical to that of rheumatoid synovitis with the main differences being that the palisade replaces the synovial intima (they may blend imperciptibly in bursae) and an almost total absence of B lymphocytes.

Treatment

Treatment of rheumatoid nodules is rarely a priority for people with rheumatoid arthritis. However, surgical removal is often successful, even if there is a tendency for nodules to regrow. Of the drug therapies commonly used in rheumatoid arthritis, methotrexate has the disadvantage of tending to make nodules worse. TNF inhibitors do not have a very reliable effect on nodules. B cell depletion with rituximab often leads to disappearance of nodules but this is not guaranteed.

Tauopathy

Tauopathy	
 Microscopy image of a neurofibrillary tangle, conformed by hyperphosphorylated tau protein	
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Tauopathies are a class of neurodegenerative diseases resulting from the pathological aggregation of tau protein in the human brain.

The best known of these illnesses is Alzheimer's disease (AD), where tau protein is deposited within neurons in the form of neurofibrillary tangles (NFTs). They were first described by the eponymous Alois Alzheimer in one of his patients suffering from the disorder. Tangles are formed by hyperphosphorylation of a microtubule-associated

protein known as tau, causing it to aggregate in an insoluble form. (These aggregations of hyperphosphorylated tau protein are also referred to as PHF, or "paired helical filaments"). The precise mechanism of tangle formation is not completely understood, and it is still controversial whether tangles are a primary causative factor in the disease or play a more peripheral role. AD is also classified as an amyloidosis because of the presence of senile plaques.

The degree of NFT involvement in AD is defined by **Braak stages.** Braak stages I and II are used when NFT involvent is confined mainly to the transentorhinal region of the brain, stages III and IV when there's also involvement of limbic regions such as the hippocampus, and V and VI when there's extensive neocortical involvement. This should not be confused with the degree of senile plaque involvement, which progresses differently.

Other conditions in which neurofibrillary tangles are commonly observed include:

- Progressive supranuclear palsy although with straight filament rather than PHF tau
- Dementia pugilistica (chronic traumatic encephalopathy)
- Frontotemporal dementia and parkinsonism linked to chromosome 17 however without detectable β-amyloid plaques.
- Lytico-Bodig disease (Parkinson-dementia complex of Guam)
- Tangle-predominant dementia, with NFTs similar to AD, but without plaques. Tends to appear in the very old.
- Ganglioglioma and gangliocytoma
- Meningioangiomatosis
- Subacute sclerosing panencephalitis
- As well as lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, and lipofuscinosis

In Pick's disease and corticobasal degeneration tau proteins are deposited in the form of inclusion bodies within swollen or "ballooned" neurons. Argyrophilic grain disease (AGD), another type of dementia, is marked by the presence of abundant argyrophilic grains and coiled bodies on microscopic examination of brain tissue. Some consider it to be a type of Alzheimer disease. It may co-exist with other tauopathies such as progressive supranuclear palsy and corticobasal degeneration.

Some other tauopathies include:

- Frontotemporal dementia
- Frontotemporal lobar degeneration

The non-Alzheimer's tauopathies are sometimes grouped together as "**Pick's complex**".

Chapter 12

Staining

A stained histologic specimen, sandwiched between a glass microscope slide and coverslip, mounted on the stage of a light microscope.

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), or organelles within individual cells.

In biochemistry it involves adding a class-specific (DNA, proteins, lipids, carbohydrates) dye to a substrate to qualify or quantify the presence of a specific compound. Staining and fluorescent tagging can serve similar purposes. Biological staining is also used to mark cells in flow cytometry, and to flag proteins or nucleic acids in gel electrophoresis.

Staining is not limited to biological materials, it can also be used to study the morphology of other materials for example the lamellar structures of semi-crystalline polymers or the domain structures of block copolymers.

In vivo *vs* In vitro

In vivo staining is the process of dyeing living tissues—*in vivo* means "in life" (compare with *in vitro* staining). By causing certain cells or structures to take on contrasting colour(s), their form (morphology) or position within a cell or tissue can be readily seen and studied. The usual purpose is to reveal cytological details that might otherwise not be apparent; however, staining can also reveal where certain chemicals or specific chemical reactions are taking place within cells or tissues.

In vitro staining involves colouring cells or structures that are no longer living. Certain stains are often combined to reveal more details and features than a single stain alone. Combined with specific protocols for fixation and sample preparation, scientists and physicians can use these standard techniques as consistent, repeatable diagnostic tools. A counterstain is stain that makes cells or structures more visible, when not completely visible with the principal stain.

- For example, crystal violet stains only Gram-positive bacteria in Gram staining. A safranin counterstain is applied which stains all cells, allowing the identification of Gram-negative bacteria as well.

Often these stains are called vital stains. They are introduced to the organism while the cells are still living. However, these stains are eventually toxic to the organism, some more so than others. To achieve desired effects, the stains are used in very dilute solutions ranging from 1:5000 to 1:500000 (Howey, 2000). Note that many stains may be used in both living and fixed cells.

In vitro methods

Preparation

The preparatory steps involved depend on the type of analysis planned; some or all of the following procedures may be required.

Fixation–which may itself consist of several steps–aims to preserve the shape of the cells or tissue involved as much as possible. Sometimes heat fixation is used to kill, adhere, and alter the specimen so it will accept stains. Most chemical fixatives (chemicals causing fixation) generate chemical bonds between proteins and other substances within

the sample, increasing their rigidity. Common fixatives include formaldehyde, ethanol, methanol, and/or picric acid. Pieces of tissue may be embedded in paraffin wax to increase their mechanical strength and stability and to make them easier to cut into thin slices.

Permeabilization involves treatment of cells with (usually) a mild surfactant. This treatment will dissolve the cell membranes, and allow larger dye molecules access to the cell's interior.

Mounting usually involves attaching the samples to a glass microscope slide for observation and analysis. In some cases, cells may be grown directly on a slide. For samples of loose cells (as with a blood smear or a pap smear) the sample can be directly applied to a slide. For larger pieces of tissue, thin sections (slices) are made using a microtome; these slices can then be mounted and inspected.

Staining proper

At its simplest, the actual staining process may involve immersing the sample (before or after fixation and mounting) in dye solution, followed by rinsing and observation. Many dyes, however, require the use of a mordant: a chemical compound which reacts with the stain to form an insoluble, coloured precipitate. When excess dye solution is washed away, the mordanted stain remains.

Most of the dyes commonly used in microscopy are available as **certified stains**. This means that samples of the manufacturer's batch have been tested by an independent body, the Biological Stain Commission and found to be meet or exceed certain standards of purity, dye content and performance in staining techniques. These standards are published in detail in the journal Biotechnic & Histochemistry Many dyes are inconsistent in composition from one supplier to another. The use of certified stains eliminates a source of unexpected results.

Negative staining

A simple staining method for bacteria which is usually successful even when the "positive staining" methods detailed below fail, is to employ a negative stain. This can be achieved simply by smearing the sample on to the slide, followed by an application of nigrosin (a black synthetic dye) or Indian ink (an aqueous suspension of carbon particles). After drying, the microorganisms may be viewed in bright field microscopy as lighter inclusions well-contrasted against the dark environment surrounding them. Note: negative staining is a mild technique which may not destroy the microorganisms therefore it is unsuitable for studying pathogens.

Specific techniques

Gram staining

Gram staining is used to determine gram status to classify bacteria broadly. It is based on the composition of their cell wall. Gram staining uses crystal violet to stain cell walls, iodine as a mordant, and a fuchsin or safranin counterstain to mark all bacteria. Gram status is important in medicine; the presence or absence of a cell wall will change the bacterium's susceptibility to some antibiotics.

Gram-positive bacteria stain dark blue or violet. Their cell wall is typically rich with peptidoglycan and lacks the secondary membrane and lipopolysaccharide layer found in Gram-negative bacteria.

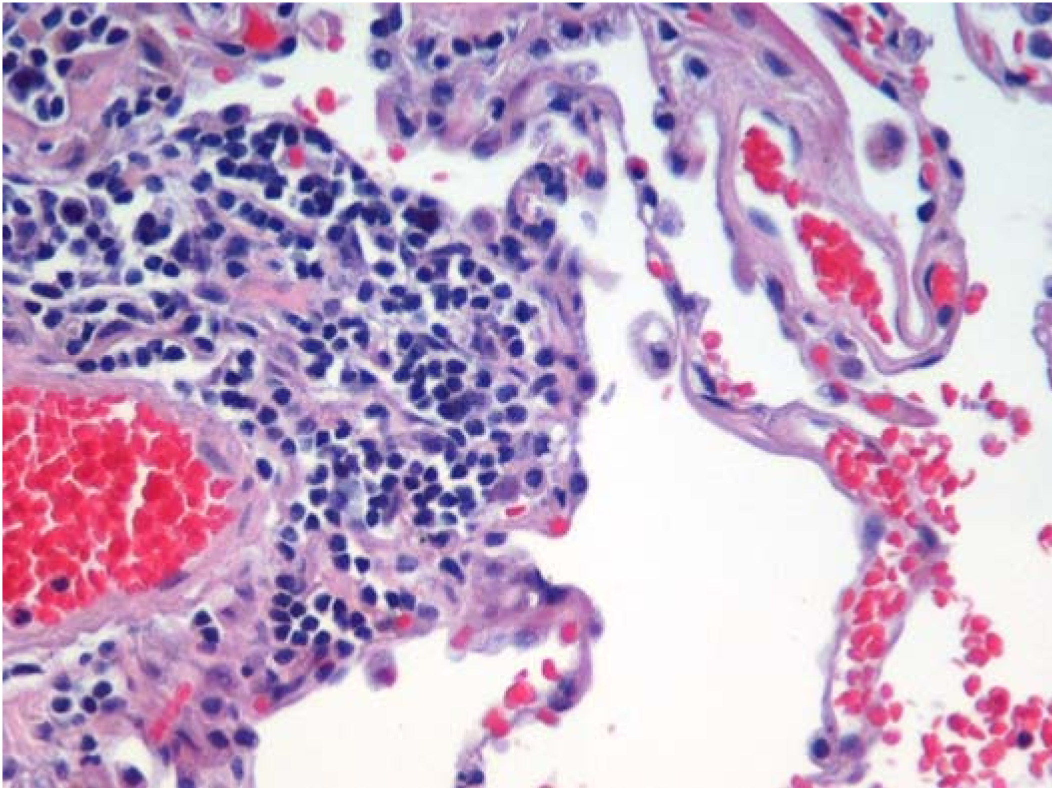
On most Gram-stained preparations, Gram-negative organisms will appear red or pink because they are counterstained. Due to presence of higher lipid content, after alcohol-treatment, the porosity of the cell wall increases, hence the CVI complex (Crystal violet - Iodine) can pass through. Thus, the primary stain is not retained. Also, in contrast to most Gram-positive bacteria, Gram-negative bacteria have only a few layers of peptidoglycan and a secondary cell membrane made primarily of lipopolysaccharide.

Ziehl-Neelsen stain

Ziehl-Neelsen staining is used to stain species of *Mycobacterium tuberculosis* that do not stain with the standard laboratory staining procedures like Gram staining.

The stains used are the red coloured Carbol fuchsin that stains the bacteria and a counter stain like Methylene blue or Malachite green.

Haematoxylin and eosin (H&E) staining



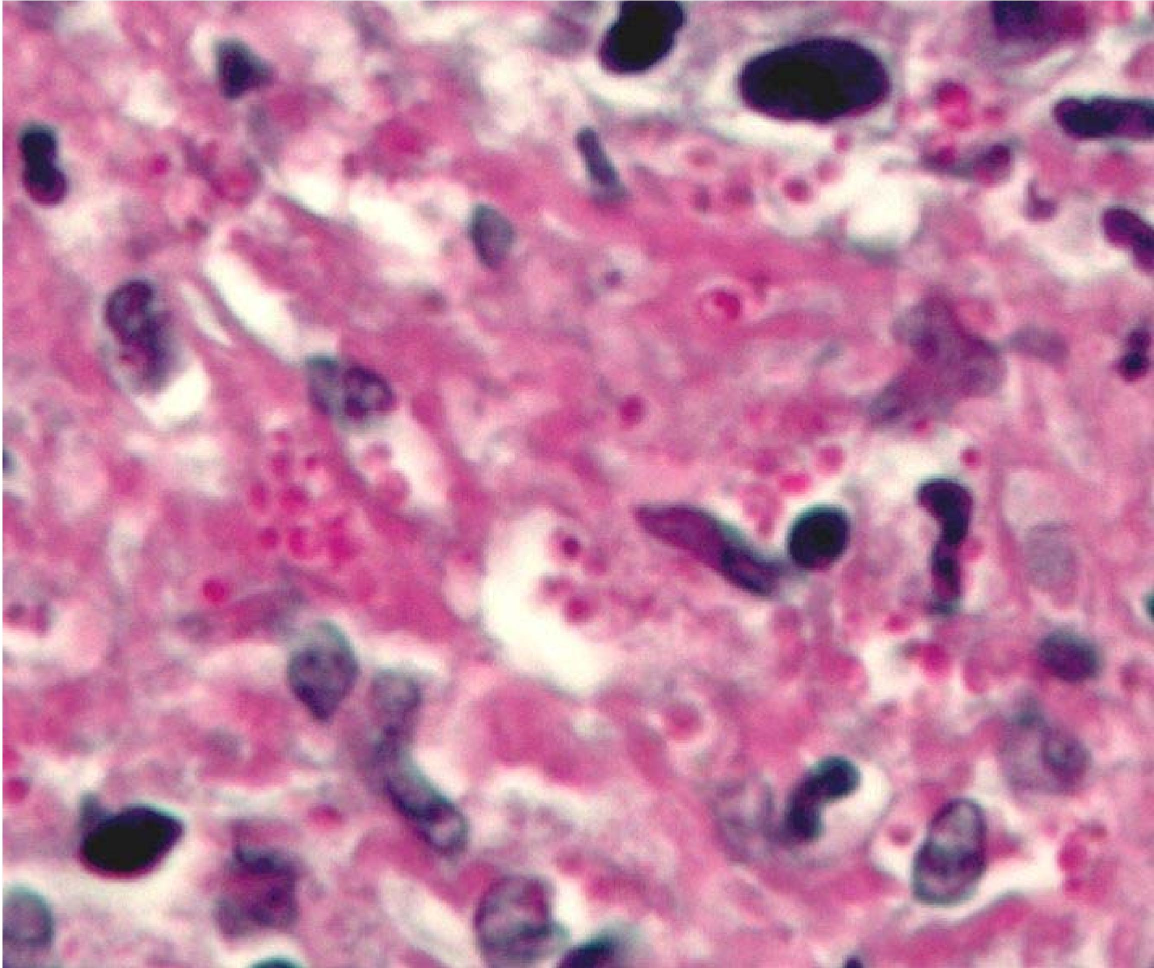
Microscopic view of a histologic specimen of human lung tissue stained with hematoxylin and eosin.

Haematoxylin and eosin staining protocol is used frequently in histology to examine thin sections of tissue. Haematoxylin stains cell nuclei blue, while eosin stains cytoplasm, connective tissue and other extracellular substances pink or red. Eosin is strongly absorbed by red blood cells, colouring them bright red. In a skilfully made H & E preparation the red blood cells are almost orange, and collagen and cytoplasm (especially muscle) acquire different shades of pink. When the staining is done by a machine, the subtle differences in eosinophilia are often lost.

Papanicolaou staining

Papanicolaou staining, or Pap staining, is a frequently used method for examining cell samples from various bodily secretions. It is frequently used to stain Pap smear specimens. It uses a combination of haematoxylin, Orange G, eosin Y, Light Green SF yellowish, and sometimes Bismarck Brown Y.

PAS staining



PAS diastase showing the fungus Histoplasma

Periodic acid-Schiff staining is used to mark carbohydrates (glycogen, glycoprotein, proteoglycans). It is used to distinguish different types of glycogen storage diseases.

Masson's trichrome

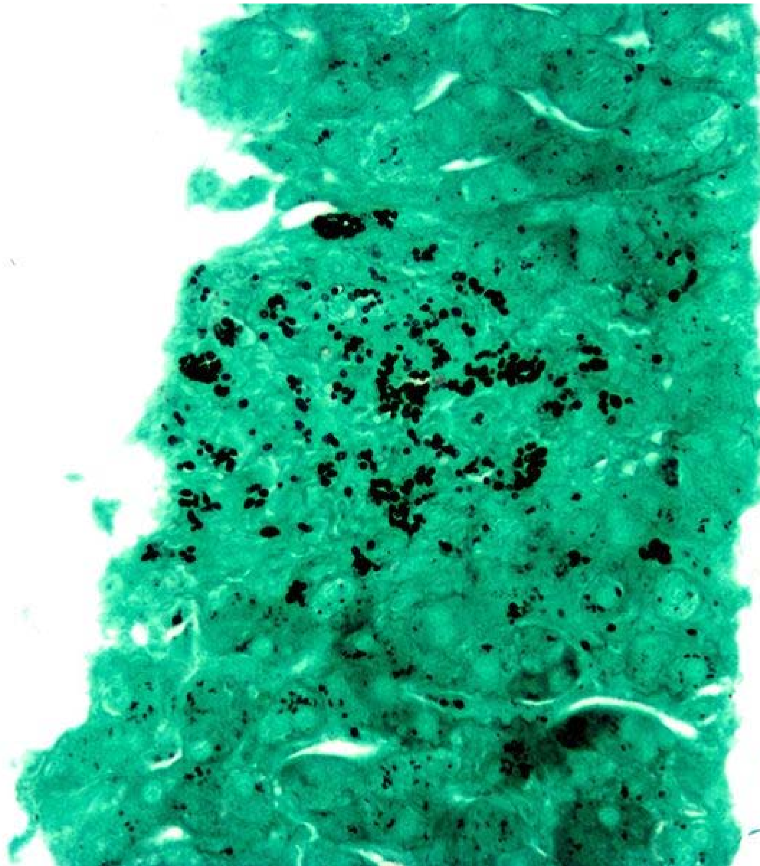
Masson's trichrome is (as the name implies) a three-colour staining protocol. The recipe has evolved from Masson's original technique for different specific applications, but all are well-suited to distinguish cells from surrounding connective tissue. Most recipes will produce red keratin and muscle fibers, blue or green staining of collagen and bone, light red or pink staining of cytoplasm, and black cell nuclei.

Romanowsky stains

The Romanowsky stains are all based on a combination of eosinate (chemically reduced eosin) and methylene blue (sometimes with its oxidation products azure A and azure B). Common variants include Wright's stain, Jenner's stain, Leishman stain and Giemsa stain.

All are used to examine blood or bone marrow samples. They are preferred over H&E for inspection of blood cells because different types of leukocytes (white blood cells) can be readily distinguished. All are also suited to examination of blood to detect blood-borne parasites like malaria.

Silver staining



Gömöri methenamine silver stain demonstrating histoplasma (black round balls)

Silver staining is the use of silver to stain histologic sections. This kind of staining is important especially to show proteins (for example type III collagen) and DNA. It is used to show both substances inside and outside cells. Silver staining is also used in temperature gradient gel electrophoresis.

Some cells are *argentaffin*. These reduce silver solution to metallic silver after formalin fixation. This method was discovered by Italian Camillo Golgi, by using a reaction

between silver nitrate and potassium dichromate, thus precipitating silver chromate in some cells. Other cells are *argyrophilic*. These reduce silver solution to metallic silver after being exposed to the stain that contains a reductant, for example hydroquinone or formalin.

Sudan staining

Sudan staining is the use of Sudan dyes to stain sudanophilic substances, usually lipids. Sudan III, Sudan IV, Oil Red O, and Sudan Black B are often used. Sudan staining is often used to determine the level of fecal fat to diagnose steatorrhea.

Conklin's staining

Special technique designed for staining true endospores with the use of malachite green dye, once stained, they do not decolourize.

Common biological stains

Different stains react or concentrate in different parts of a cell or tissue, and these properties are used to advantage to reveal specific parts or areas. Some of the most common biological stains are listed below. Unless otherwise marked, all of these dyes may be used with fixed cells and tissues; vital dyes (suitable for use with living organisms) are noted.

Acridine orange

Acridine orange (AO) is a nucleic acid selective fluorescent cationic dye useful for cell cycle determination. It is cell-permeable, and interacts with DNA and RNA by intercalation or electrostatic attractions. When bound to DNA, it is very similar spectrally to fluorescein.

Bismarck brown

Bismarck brown (also Bismarck brown Y or Manchester brown) imparts a yellow colour to acid mucins. Bismarck brown may be used with live cells.

Carmine

Carmine is an intensely red dye which may be used to stain glycogen, while Carmine alum is a nuclear stain. Carmine stains require the use of a mordant, usually aluminum.

Coomassie blue

Coomassie blue (also brilliant blue) nonspecifically stains proteins a strong blue colour. It is often used in gel electrophoresis.

Crystal violet

Crystal violet, when combined with a suitable mordant, stains cell walls purple. Crystal violet is an important component in Gram staining.

DAPI

DAPI is a fluorescent nuclear stain, excited by ultraviolet light and showing strong blue fluorescence when bound to DNA. DAPI binds with A=T rich repeats of chromosomes.DAPI also not visible with regular transmission microscopy. It may be used in living or fixed cells.

Eosin

Eosin is most often used as a counterstain to haematoxylin, imparting a pink or red colour to cytoplasmic material, cell membranes, and some extracellular structures. It also imparts a strong red colour to red blood cells. Eosin may also be used as a counterstain in some variants of Gram staining, and in many other protocols. There are actually two very closely related compounds commonly referred to as eosin. Most often used is eosin Y (also known as eosin Y ws or eosin yellowish); it has a very slightly yellowish cast. The other eosin compound is eosin B (eosin bluish or imperial red); it has a very faint bluish cast. The two dyes are interchangeable, and the use of one or the other is more a matter of preference and tradition.

Ethidium bromide

Ethidium bromide intercalates and stains DNA, providing a fluorescent red-orange stain. Although it will not stain healthy cells, it can be used to identify cells that are in the final stages of apoptosis - such cells have much more permeable membranes. Consequently, ethidium bromide is often used as a marker for apoptosis in cells populations and to locate bands of DNA in gel electrophoresis. The stain may also be used in conjunction with acridine orange (AO) in viable cell counting. This EB/AO combined stain causes live cells to fluoresce green whilst apoptotic cells retain the distinctive red-orange fluorescence.

Acid fuchsine

Acid fuchsine may be used to stain collagen, smooth muscle, or mitochondria. Acid fuchsine is used as the nuclear and cytoplasmic stain in Mallory's trichrome method. Acid fuchsine stains cytoplasm in some variants of Masson's trichrome. In Van Gieson's picro-fuchsine, acid fuchsine imparts its red colour to collagen fibres. Acid fuchsine is also a traditional stain for mitochondria (Altmann's method).

Haematoxylin

Haematoxylin (hematoxylin in North America) is a nuclear stain. Used with a mordant, haematoxylin stains nuclei blue-violet or brown. It is most often used with eosin in H&E (haematoxylin and eosin) staining—one of the most common procedures in histology.

Hoechst stains

Hoechst is a *bis*-benzimidazole derivative compound which binds to the *minor groove* of DNA. Often used in fluorescence microscopy for DNA staining, Hoechst stains appear yellow when dissolved in aqueous solutions and emit blue light under UV excitation. There are two major types of Hoechst: *Hoechst 33258* and *Hoechst 33342*. The two compounds are functionally similar, but with a little difference in structure. Hoechst 33258 contains a terminal hydroxyl group and is thus more soluble in aqueous solution, however this characteristics reduces its ability to penetrate the plasma membrane. Hoechst 33342 contains a ethyl substitution on the terminal hydroxyl group (i.e. an ethylether group) making it more hydrophobic for easier plasma membrane passage

Iodine

Iodine is used in chemistry as an indicator for starch. When starch is mixed with iodine in solution, an intensely dark blue colour develops, representing a starch/iodine complex. Starch is a substance common to most plant cells and so a weak iodine solution will stain starch present in the cells. Iodine is one component in the staining technique known as Gram staining, used in microbiology. Lugol's solution or Lugol's iodine (IKI) is a brown solution that turns black in the presence of starches and can be used as a cell stain, making the cell nuclei more visible. Iodine is also used as a mordant in Gram's staining, it enhances dye to enter through the pore present in the cell wall/membrane.

Malachite green

Malachite green (also known as diamond green B or victoria green B) can be used as a blue-green counterstain to safranin in the Gimenez staining technique for bacteria. It also can be used to directly stain spores.

Methyl green

Methyl green is used commonly with bright-field microscopes to dye the chromatin of cells so that they are more easily viewed.

Methylene blue

Methylene blue is used to stain animal cells, such as human cheek cells, to make their nuclei more observable. Also used to staining the blood film and used in cytology.

Neutral red

Neutral red (or toluylene red) stains Nissl substance red. It is usually used as a counterstain in combination with other dyes.

Nile blue

Nile blue (or Nile blue A) stains nuclei blue. It may be used with living cells.

Nile red

Nile red (also known as Nile blue oxazone) is formed by boiling Nile blue with sulfuric acid. This produces a mix of Nile red and Nile blue. Nile red is a lipophilic stain; it will accumulate in lipid globules inside cells, staining them red. Nile red can be used with living cells. It fluoresces strongly when partitioned into lipids, but practically not at all in aqueous solution.

Osmium tetroxide (formal name: osmium tetraoxide)

Osmium tetraoxide is used in optical microscopy to stain lipids. It dissolves in fats, and is reduced by organic materials to elemental osmium, an easily visible black substance.

Rhodamine

Rhodamine is a protein specific fluorescent stain commonly used in fluorescence microscopy.

Safranin

Safranin (or Safranin O) is a nuclear stain. It produces red nuclei, and is used primarily as a counterstain. Safranin may also be used to give a yellow colour to collagen.

Stainability of tissues

Positive affinity for a specific stain may be designated by the suffix *-philic*. For example, tissues that stain with an azure dye may be referred to as azurophilic. This may also be used for more generalized staining properties, such as acidophilic for tissues that stain by acidic stains (most notably eosin), basophilic when staining in basic dyes and *amphophilic* when staining with either acid or basic dyes. In contrast, Chromophobic tissues do not take up coloured dye readily.

Electron microscopy

As in light microscopy, stains can be used to enhance contrast in transmission electron microscopy. Electron-dense compounds of heavy metals are typically used.

Phosphotungstic acid

Phosphotungstic acid is a common negative stain for viruses, nerves, polysaccharides, and other biological tissue materials.

Osmium tetroxide

Osmium tetroxide is used in optical microscopy to stain lipids. It dissolves in fats, and is reduced by organic materials to elemental osmium, an easily visible black substance. Because it is a heavy metal that absorbs electrons, it is perhaps the most common stain used for morphology in biological electron microscopy. It is also used for the staining of various polymers for the study of their morphology by TEM. OsO_4 is very volatile and extremely toxic. It is a strong oxidizing agent as the osmium has an oxidation number of +8. It aggressively oxidizes many materials, leaving behind a deposit of non-volatile osmium in a lower oxidation state.

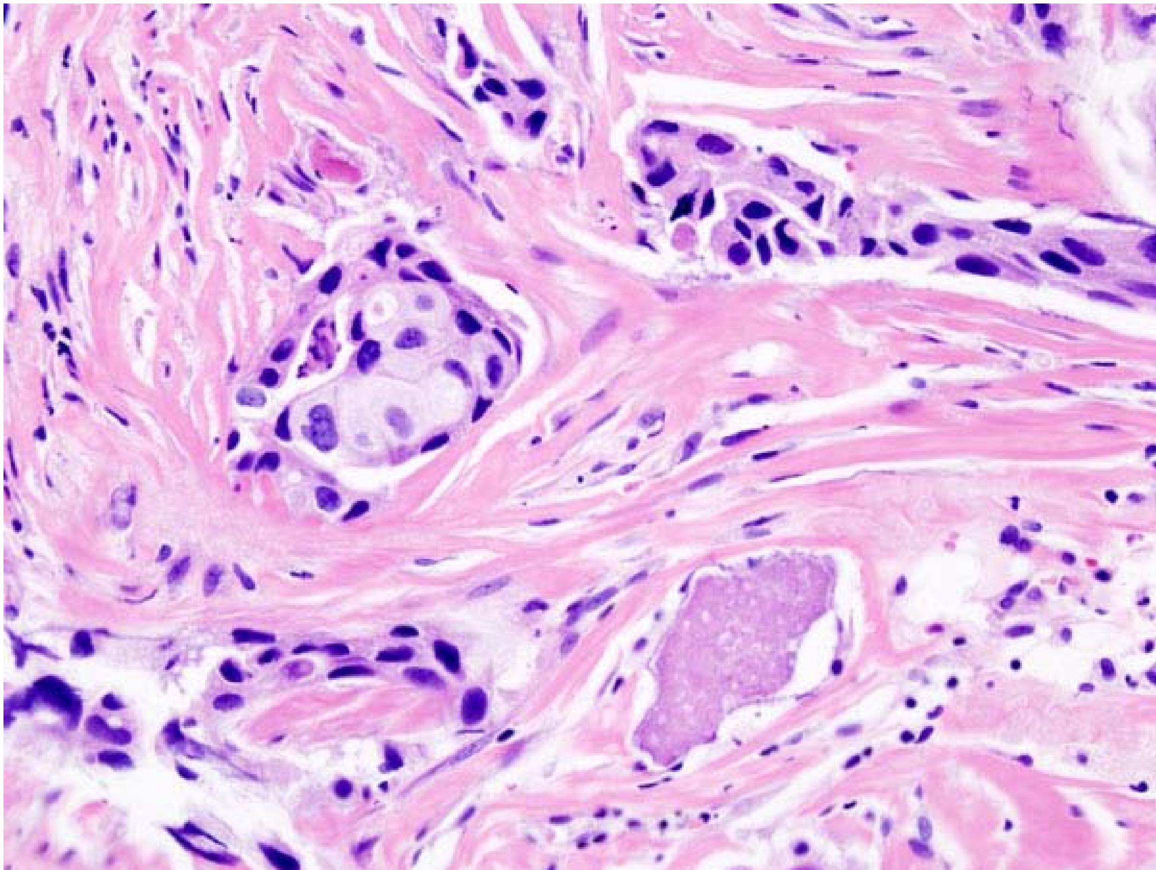
Ruthenium tetroxide

Ruthenium tetroxide is equally volatile and even more aggressive than osmium tetraoxide and able to stain even materials that resist the osmium stain, e.g. polyethylene.

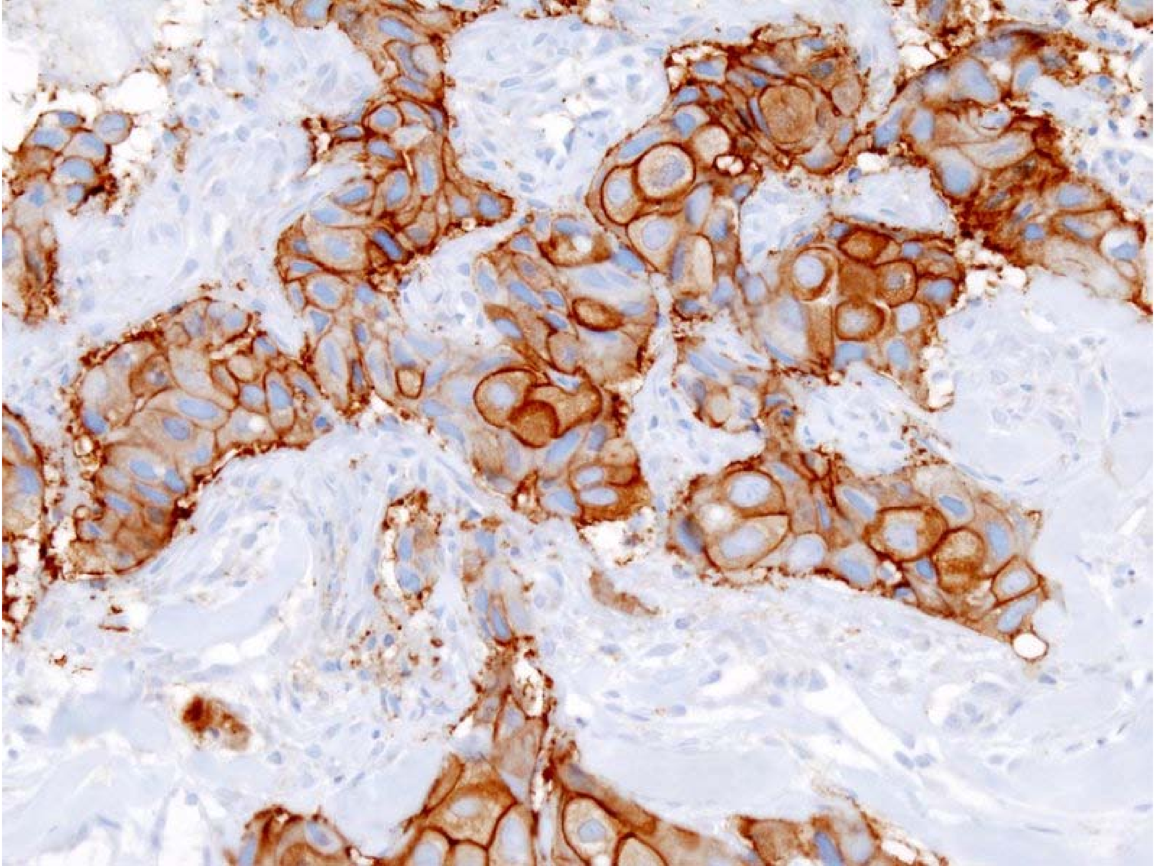
Other chemicals used in electron microscopy staining include: ammonium molybdate, cadmium iodide, carbohydrazide, ferric chloride, hexamine, indium trichloride, lanthanum nitrate, lead acetate, lead citrate, lead(II) nitrate, periodic acid, phosphomolybdic acid, potassium ferricyanide, potassium ferrocyanide, ruthenium red, silver nitrate, silver proteinate, sodium chloroaurate, thallium nitrate, thiosemicarbazide, uranyl acetate, uranyl nitrate, and vanadyl sulfate.

Chapter 13

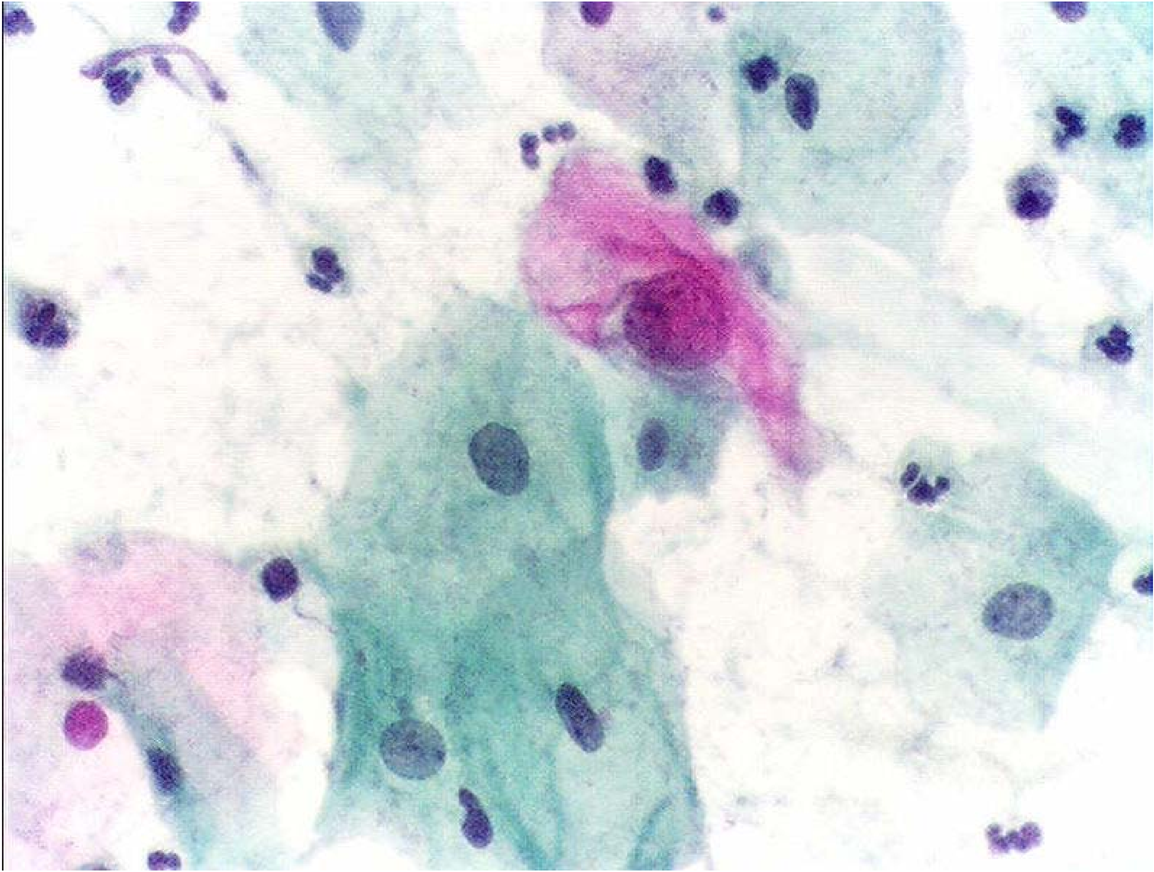
Anatomical Pathology



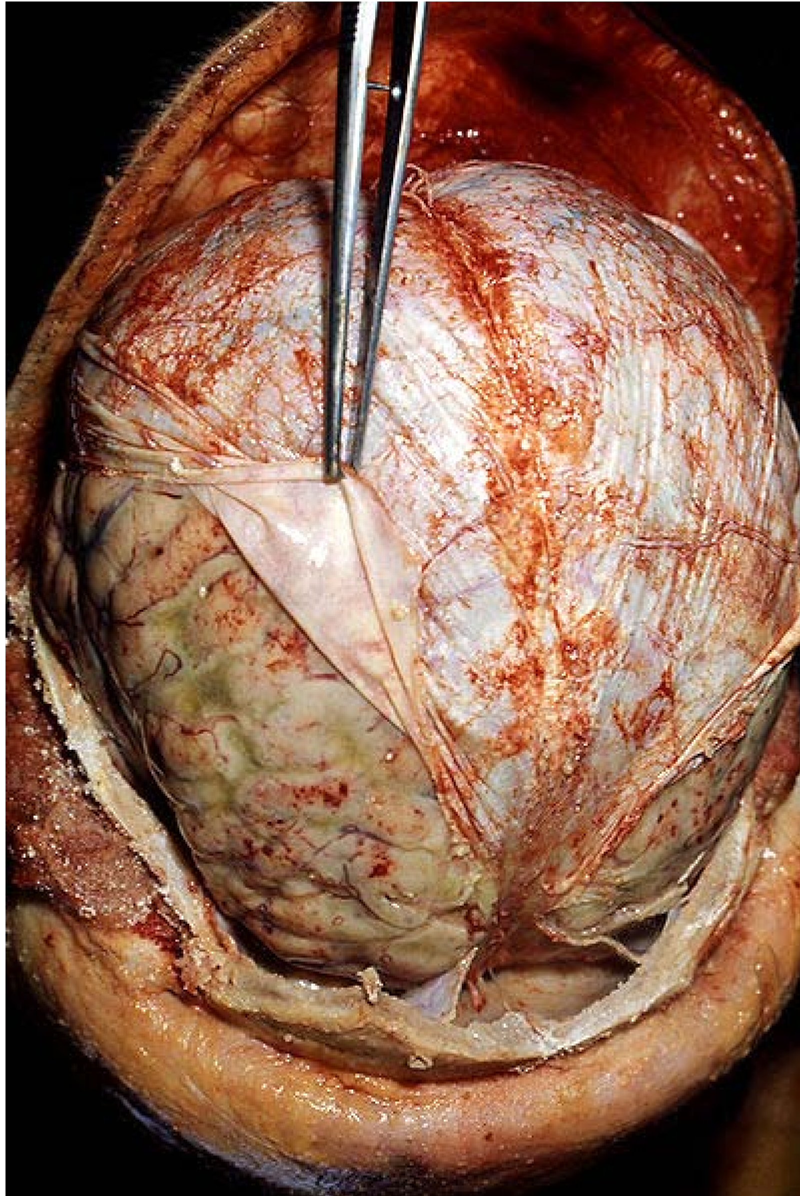
Histopathology: microscopic appearance of invasive ductal carcinoma of the breast. The slide is stained with Haematoxylin & Eosin.



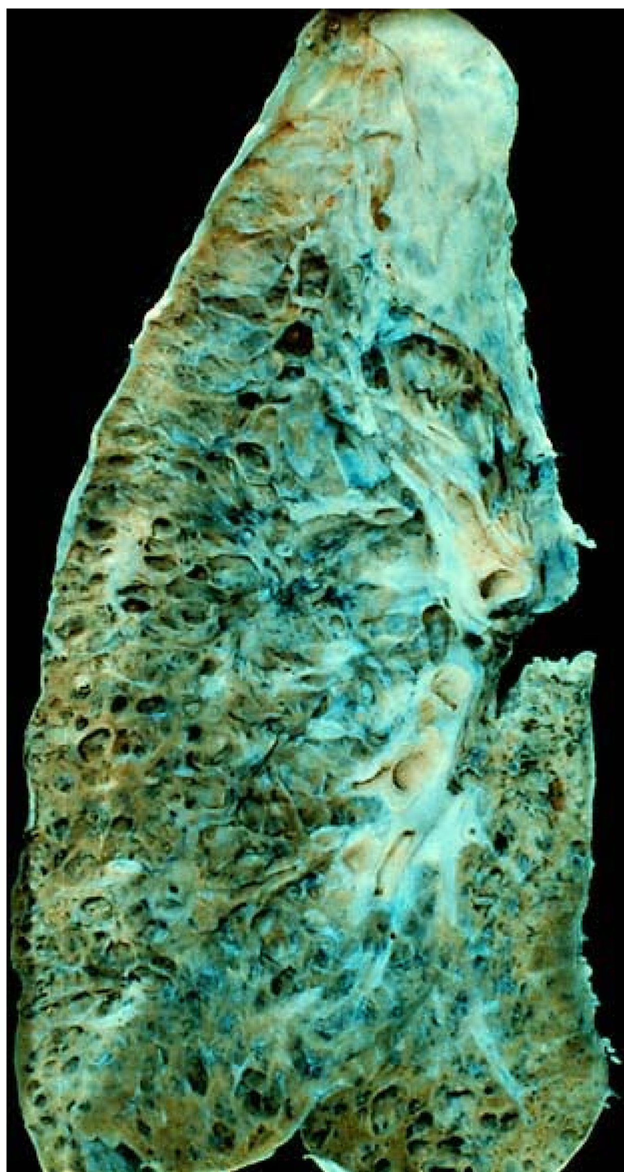
Histopathology: microscopic appearance of invasive ductal carcinoma of the breast. The slide is stained with an antibody (immunohistochemistry) against the ongene Her2neu. The dark brown reaction indicates that this tumor over-expresses this gene.



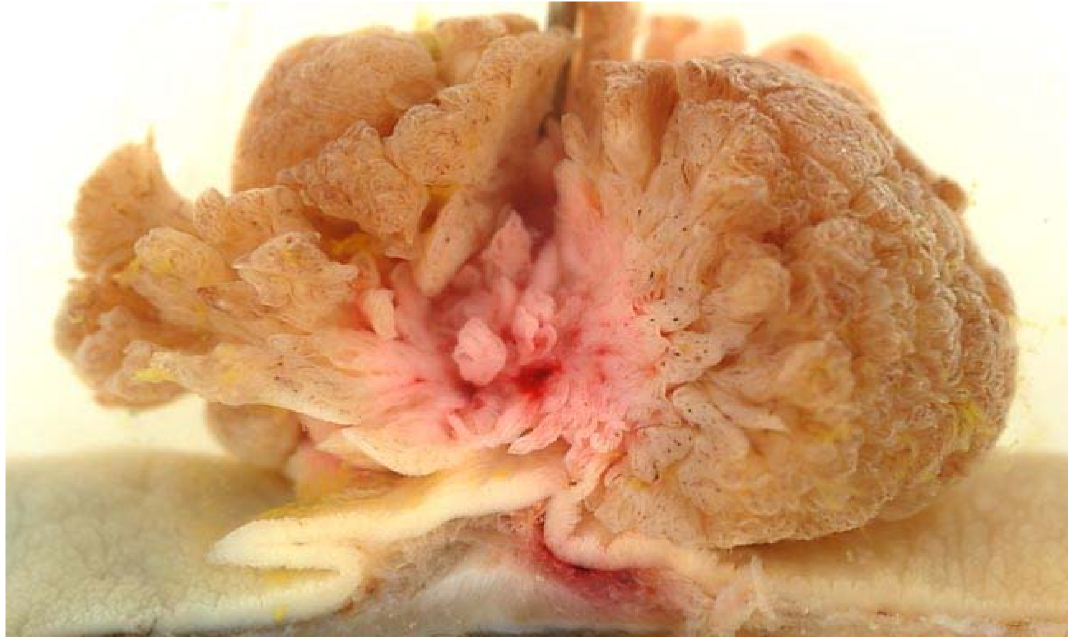
Cytopathology: microscopic appearance of a Pap test. The pink cell at the center with a large nucleus is abnormal, compatible with low grade dysplasia.



Autopsy: a brain surrounded by pus (the yellow-greyish coat around the brain, under the dura lifted by the forceps), the result of bacterial meningitis.



Gross examination: appearance of the cut surface of a lung showing the honeycomb pattern of end-stage pulmonary fibrosis.



Gross examination: appearance of a colorectal polyp (the cauliflower-shaped tumor) attached to the colon mucosa (the horizontal line at the bottom).

Anatomical pathology (*Commonwealth*) or **Anatomic pathology** (*U.S.*) is a medical specialty that is concerned with the diagnosis of disease based on the gross, microscopic, chemical, immunologic and molecular examination of organs, tissues, and whole bodies (autopsy).

Anatomical pathology is itself divided in subspecialties, the main ones being surgical pathology, cytopathology and forensic pathology. To be licensed to practice pathology, one has to complete medical school and secure a license to practice medicine. An approved residency program and certification (in the U.S., the American board of Pathology or the American Osteopathic Board of Pathology) is usually required to obtain employment or hospital privileges.

Anatomical pathology is one of two branches of pathology, the other being clinical pathology, the diagnosis of disease through the laboratory analysis of bodily fluids and/or tissues. Often, pathologists practice both anatomical and clinical pathology, a combination known as general pathology. The distinction between anatomic and clinical pathology is increasingly blurred by the introduction of technologies that require new expertise and the need to provide patients and referring physicians with integrated diagnostic reports. Similar specialties exist in veterinary pathology.

Skills and procedures

The procedures used in anatomic pathology include:

- Gross examination - the examination of diseased tissues with the naked eye. This is important especially for large tissue fragments, because the disease can often be visually identified. It is also at this step that the pathologist selects areas that will be processed for histopathology. The eye can sometimes be aided with a magnifying glass or a stereo microscope, especially when examining parasitic organisms.
- Histopathology - the microscopic examination of stained tissue sections using histological techniques. The standard stains are haematoxylin and eosin, but many others exist. The use of haematoxylin and eosin-stained slides to provide specific diagnoses based on morphology is considered to be the core skill of anatomic pathology. The science of staining tissues sections is called histochemistry.
- Immunohistochemistry - the use of antibodies to detect the presence, abundance, and localization of specific proteins. This technique is critical to distinguishing between disorders with similar morphology, as well as characterizing the molecular properties of certain cancers.
- In situ hybridization - Specific DNA and RNA molecules can be identified on sections using this technique. When the probe is labeled with fluorescent dye, the technique is called FISH.
- Cytopathology - the examination of loose cells spread and stained on glass slides using cytology techniques.
- Electron microscopy - the examination of tissue with an electron microscope, which allows much greater magnification, enabling the visualization of organelles within the cells. Its use has been largely supplanted by immunohistochemistry, but it is still in common use for certain tasks, including the diagnosis of kidney disease and the identification of immotile cilia syndrome among many others.
- Tissue cytogenetics - the visualization of chromosomes to identify genetics defects such as chromosomal translocation.
- Flow immunophenotyping - the determination of the immunophenotype of cells using flow cytometry techniques. It is very useful to diagnose the different types of leukemia and lymphoma.

Subspecialties

Surgical pathology

Surgical pathology is the most significant and time-consuming area of practice for most anatomical pathologists. Surgical pathology involves the gross and microscopic examination of surgical specimens, as well as biopsies submitted by non-surgeons such as general internists, medical subspecialists, dermatologists, and interventional radiologists. Surgical pathology increasingly requires technologies and skills traditionally associated with clinical pathology such as molecular diagnostics.

Renal pathology

Renal pathology is a sub-discipline of anatomical pathology concerned with the diagnosis of medical (non-tumor) kidney diseases, including both native kidneys and transplants.

Renal pathologists routinely employ techniques of immunofluorescence and electron microscopy, in addition to light microscopy, for kidney biopsy and nephrectomy specimens. The renal pathologist integrates the microscopic findings from these three techniques along with the clinical presentation of the patient and the patient's clinical laboratory results to arrive at a diagnosis.

Oral and maxillofacial pathology

In the United States, subspecialty-trained doctors of dentistry, rather than medical doctors, can be certified by a professional board to practice Oral and Maxillofacial Pathology.

Cytopathology

Cytopathology is a sub-discipline of anatomical pathology concerned with the microscopic examination of whole, individual cells obtained from smears or fine needle aspirates. Cytopathologists are trained to perform fine-needle aspirates of superficially located organs, masses, or cysts, and are often able to render an immediate diagnosis in the presence of the patient and consulting physician. In the case of screening tests such as the Papanicolaou smear, non-physician cytotechnologists are often employed to perform initial reviews, with only positive or uncertain cases examined by the pathologist. Cytopathology is a board-certifiable subspecialty in the U.S.

Molecular pathology

Molecular pathology is an emerging discipline within anatomical and clinical pathology which is focused on the use of nucleic acid-based techniques such as in-situ hybridization, reverse-transcriptase polymerase chain reaction, and nucleic acid microarrays for specialized studies of disease in tissues and cells. Molecular pathology shares some aspects of practice with both anatomic and clinical pathology, and is sometimes considered a "crossover" discipline.

Autopsy pathology

General anatomical pathologists are trained in performing autopsies, which are used to determine the disease factors contributing to a person's death. Autopsies are important in the ongoing medical education of clinicians, and in efforts to improve and verify the quality of medical care. Dieners are non-physicians who assist pathologists in the gross dissection portion of the autopsy. The recently established field of Pathologists Assistant has taken over the role of Diener and contributes a great deal to the pre-diagnosis of disease within the pathology laboratory. Autopsies represent less than 10% of the workload of typical pathologists in the United States. However, the autopsy is central to public perceptions of the field, in part due to portrayals of pathologists on television programs such as *Quincy, M.E.* and *Silent Witness*.

WORLD TECHNOLOGIES

Forensic pathology

Forensic pathologists receive specialized training in determining the cause of death and other legally relevant information from the bodies of persons who died suddenly with no known medical condition, those who die from non-natural causes, as well as those dying as a result of homicide, or other criminally suspicious deaths. A majority of the forensic pathologists cases are due to natural causes, often of a complex pathological nature. Often, additional tests such as toxicology, histology, and genetic testing will be used to help the pathologist determine the cause of death. Because of this, it is necessary for forensic pathologists to have a strong background in anatomical pathology before becoming forensic pathologists. Forensic pathologists will often testify in courts regarding their findings in cases of homicide and suspicious death. They also play a large role in public health, such as investigating deaths in the workplace, deaths in custody, as well as sudden and unexpected deaths in children. Forensic pathologists often have special areas of interest within their practice, such as sudden death due to cardiac pathology, deaths due to drugs, or Sudden Infant Death (SIDS), and various others.

Training and certification of Anatomical Pathologists

Australia

- **(Also New Zealand, Hong Kong, Singapore, Malaysia, and Saudi Arabia)**

Anatomical Pathology one of the specialty training programs offered by the Royal College of Pathologists of Australasia (RCPA). The RCPA. To qualify as a Fellow of the RCPA in Anatomical Pathology, the candidate must complete a recognised undergraduate or postgraduate medical qualification, then complete a minimum of 1 year of clinical medical experience (internship)as a prerequisite to selection as a training registrar. The training program is a minimum of 5 years, served in at least two laboratories, and candidates must pass a Basic Pathological Sciences examination (usually in first year), the Part 1 examination (not before 3rd year) and the Part 2 examination (not before 5th year). Fellows may then continue into subspecialty training.

Canada

Anatomical Pathology (AP) is one of the specialist certificates granted by the Royal College of Physicians and Surgeons of Canada. Other certificates related to pathology include general pathology (GP), forensic pathology, hematopathology, and neuropathology. Candidates for any of these must have completed four years of medical school and five years of residency training. After becoming certified in either AP or GP, it is common for pathologists to seek further fellowship training in a subspecialty of AP.

USA

Anatomic Pathology (AP) is one of the two primary certifications offered by the American Board of Pathology. The other is Clinical Pathology (CP). To be certified in

anatomic pathology, the trainee must complete four years of medical school followed by three years of residency training. Many US pathologists are certified in both AP and CP, which requires a total of four years of residency. After completing residency, many pathologists enroll in further years of fellowship training to gain expertise in a subspecialty of AP.

Anatomical pathology practice settings

- *Academic anatomical pathology* is practiced at university medical centers by pathologists who are also university faculty. As such, they often have diverse responsibilities that may include training pathology residents, teaching medical students, conducting basic, clinical, or translational research, and/or performing administrative duties, all in addition to the practice of diagnostic anatomical pathology. Pathologists in academic settings often sub-specialize in a particular area of anatomic pathology and may serve as consultants to other pathologists regarding cases in their specific area of expertise.

- *Group practice* is the most traditional private practice model. In this arrangement, a group of senior pathologists will control a partnership that employs junior pathologists and contracts independently with hospitals to provide diagnostic services, as well as attracting referral business from local clinicians who practice in the outpatient setting. The group often owns a laboratory for histology and ancillary testing of tissue, and may hold contracts to run hospital-owned labs. Many pathologists who practice in this setting are trained and certified in both anatomical pathology and clinical pathology, which allows them to supervise blood banks, clinical chemistry laboratories, and medical microbiology laboratories as well.

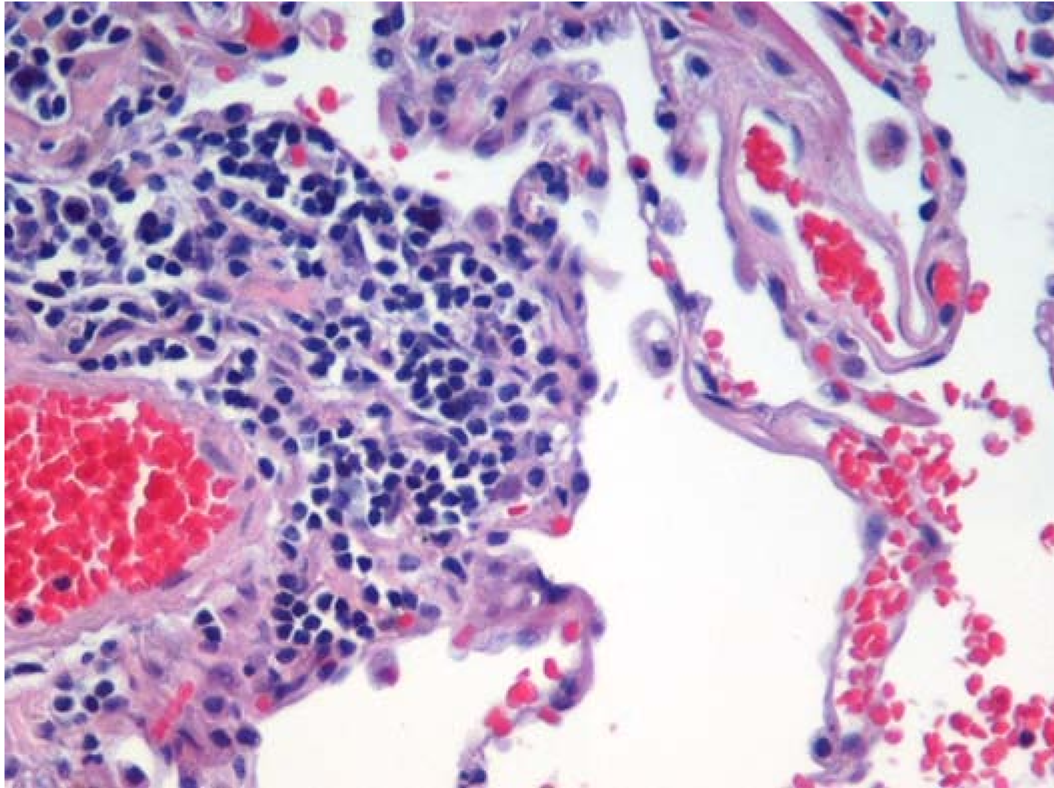
- Large *corporate providers of anatomical pathology services* have emerged in recent years, most notably *AmeriPath* in the United States. In this model, pathologists are employees, rather than independent partners. This model has been criticized for reducing physician independence, but defenders claim that the larger size of these practices allow for economies of scale and greater specialization, as well a sufficient volume to support more specialized testing methods.

- *Multispecialty groups*, composed of physicians from clinical specialties as well as radiology and pathology, are another practice model. In some case, these may be large groups controlled by an HMO or other large health care organization. In others, they are essentially clinician group practices that employ pathologists to provide diagnostic services for the group. These groups may own their own laboratories, or, in some cases may make controversial arrangements with "pod labs" that allow clinician groups to lease space, with the clinican groups receiving direct insurance payments for pathology services. Proposed changes to Medicare regulations may essentially eliminate these arrangements in the United States.

Chapter 14

Histology

A stained histologic specimen, sandwiched between a glass microscope slide and coverslip, mounted on the stage of a light microscope.



Microscopic view of a histologic specimen of human lung tissue stained with hematoxylin and eosin.

Histology (compound of the Greek words: *ἱστός* "tissue", and -*λογία* -logia) is the study of the microscopic anatomy of cells and tissues of plants and animals. It is performed by examining a thin slice (section) of tissue under a light microscope or electron microscope. The ability to visualize or differentially identify microscopic structures is frequently enhanced through the use of histological stains. Histology is an essential tool of biology and medicine.

Histopathology, the microscopic study of diseased tissue, is an important tool in anatomical pathology, since accurate diagnosis of cancer and other diseases usually requires histopathological examination of samples. Trained medical doctors, frequently board-certified as pathologists, are the personnel who perform histopathological examination and provide diagnostic information based on their observations.

The trained scientists who perform the preparation of histological sections are *histotechnicians,* histology technicians (HT), histology technologists (HTL), medical scientists, medical laboratory technicians, or biomedical scientists. Their field of study is called *histotechnology.*

Histology

Fixing

Chemical fixation with formaldehyde or other chemicals

Chemical fixatives are used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles (e.g., nucleus, endoplasmic reticulum, mitochondria). The most common fixative for light microscopy is 10% neutral buffered formalin (4% formaldehyde in phosphate buffered saline). For electron microscopy, the most commonly used fixative is glutaraldehyde, usually as a 2.5% solution in phosphate buffered saline. These fixatives preserve tissues or cells mainly by irreversibly cross-linking proteins. The main action of these aldehyde fixatives is to cross-link amino groups in proteins through the formation of CH_2 (methylene) linkage, in the case of formaldehyde, or by a C5H10 cross-links in the case of glutaraldehyde. This process, while preserving the structural integrity of the cells and tissue can damage the biological functionality of proteins, particularly enzymes, and can also denature them to a certain extent. This can be detrimental to certain histological techniques. Further fixatives are often used for electron microscopy such as osmium tetroxide or uranyl acetate

Formalin fixation leads to degradation of mRNA, miRNA and DNA in tissues. However, extraction, amplification and analysis of these nucleic acids from formalin-fixed, paraffin-embedded tissues is possible using appropriate protocols.

Frozen section fixation

Frozen section is a rapid way to fix and mount histology sections. It is used in surgical removal of tumors, and allow rapid determination of margin (that the tumor has been completely removed). It is done using a refrigeration device called a cryostat. The frozen tissue is sliced using a microtome, and the frozen slices are mounted on a glass slide and stained the same way as other methods. It is a necessary way to fix tissue for certain stain such as antibody linked immunofluorescence staining. It can also be used to determine if a tumour is malignant when it is found incidentally during surgery on a patient.

Processing - dehydration, clearing, and infiltration

The aim of Tissue Processing is to remove water from tissues and replace with a medium that solidifies to allow thin sections to be cut. Biological tissue must be supported in a hard matrix to allow sufficiently thin sections to be cut, typically 5 μm (micrometres; 1000 micrometres = 1 mm) thick for light microscopy and 80-100 nm (nanometre; 1,000,000 nanometres = 1 mm) thick for electron microscopy. For light microscopy, paraffin wax is most frequently used. Since it is immiscible with water, the main constituent of biological tissue, water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol to remove the water. This is followed by a hydrophobic clearing agent (such as

xylene) to remove the alcohol, and finally molten paraffin wax, the infiltration agent, which replaces the xylene. Paraffin wax does not provide a sufficiently hard matrix for cutting very thin sections for electron microscopy. Instead, resins are used. Epoxy resins are the most commonly employed embedding media, but acrylic resins are also used, particularly where immunohistochemistry is required. Thicker sections (0.35µm to 5µm) of resin-embedded tissue can also be cut for light microscopy. Again, the immiscibility of most epoxy and acrylic resins with water necessitates the use of dehydration, usually with ethanol.

Embedding

After the tissues have been dehydrated, cleared, and infiltrated with the embedding material, they are ready for external embedding. During this process the tissue samples are placed into molds along with liquid embedding material (such as agar, gelatine, or wax) which is then hardened. This is achieved by cooling in the case of paraffin wax and heating (curing) in the case of the epoxy resins. The acrylic resins are polymerised by heat, ultraviolet light, or chemical catalysts. The hardened blocks containing the tissue samples are then ready to be sectioned.

Because Formalin-fixed, paraffin-embedded (FFPE) tissues may be stored indefinitely at room temperature, and nucleic acids (both DNA and RNA) may be recovered from them decades after fixation, FFPE tissues are an important resource for historical studies in medicine.

Embedding can also be accomplished using frozen, non-fixed tissue in a water-based medium. Pre-frozen tissues are placed into molds with the liquid embedding material, usually a water-based glycol, OCT, TBS, Cryogel, or resin, which is then frozen to form hardened blocks.

Sectioning

Sectioning can be done in limited ways. Vertical sectioning perpendicular to the surface of the tissue is the usual method. Horizontal sectioning is often done in the evaluation of the hair follicles and pilosebaceous units. Tangential to horizontal sectioning is done in Mohs surgery and in methods of CCPDMA.

For light microscopy, a steel knife mounted in a microtome is used to cut 10-micrometer-thick tissue sections which are mounted on a glass microscope slide. For transmission electron microscopy, a diamond knife mounted in an ultramicrotome is used to cut 50-nanometer-thick tissue sections which are mounted on a 3-millimeter-diameter copper grid. Then the mounted sections are treated with the appropriate stain.

Frozen tissue embedded in a freezing medium is cut on a microtome in a cooled machine called a cryostat.

Staining

Biological tissue has little inherent contrast in either the light or electron microscope. Staining is employed to give both contrast to the tissue as well as highlighting particular features of interest. Where the underlying mechanistic chemistry of staining is understood, the term histochemistry is used. Hematoxylin and eosin (H&E stain) is the most commonly used light microscopical stain in histology and histopathology. Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink. Uranyl acetate and lead citrate are commonly used to impart contrast to tissue in the electron microscope.

Special staining: There are hundreds of various other techniques that have been used to selectively stain cells and cellular components. Other compounds used to color tissue sections include safranin, oil red o, Congo red, fast green FCF, silver salts, and numerous natural and artificial dyes that were usually originated from the development dyes for the textile industry.

Histochemistry refers to the science of using chemical reactions between laboratory chemicals and components within tissue. A commonly performed histochemical technique is the Perls Prussian blue reaction, used to demonstrate iron deposits in diseases like hemochromatosis.

Histology samples have often been examined by radioactive techniques. In historadiography, a slide (sometimes stained histochemically) is X-rayed. More commonly, autoradiography is used to visualize the locations to which a radioactive substance has been transported within the body, such as cells in S phase (undergoing DNA replication) which incorporate tritiated thymidine, or sites to which radiolabeled nucleic acid probes bind in in situ hybridization. For autoradiography on a microscopic level, the slide is typically dipped into liquid nuclear tract emulsion, which dries to form the exposure film. Individual silver grains in the film are visualized with dark field microscopy.

Recently, antibodies have been used to specifically visualize proteins, carbohydrates, and lipids. This process is called immunohistochemistry, or when the stain is a fluorescent molecule, immunofluorescence. This technique has greatly increased the ability to identify categories of cells under a microscope. Other advanced techniques, such as nonradioactive *in situ* hybridization, can be combined with immunochemistry to identify specific DNA or RNA molecules with fluorescent probes or tags that can be used for immunofluorescence and enzyme-linked fluorescence amplification (especially alkaline phosphatase and tyramide signal amplification). Fluorescence microscopy and confocal microscopy are used to detect fluorescent signals with good intracellular detail. Digital cameras are increasingly used to capture histological and histopathological image.

Common laboratory stains

Stain	Common use	Nucleus	Cytoplasm	Red blood cell (RBC)	Collagen fibers	Specifically stains
Haematoxylin	General staining when paired with eosin (i.e. H&E)	Blue	N/A	N/A	N/A	Nucleic acids—blue ER (endoplasmic reticulum)—blue
Eosin	General staining when paired with haematoxylin (i.e. H&E)	N/A	Pink	Orange/red	Pink	Elastic fibers—pink Collagen fibers—pink Reticular fibers—pink
Toluidine blue	General staining	Blue	Blue	Blue	Blue	Mast cells granules—purple
Masson's trichrome stain	Connective tissue	Black	Red/pink	Red	Blue/green	Cartilage—blue/green Muscle fibers—red
Mallory's trichrome stain	Connective tissue	Red	Pale red	Orange	Deep blue	Keratin—orange Cartilage—blue Bone matrix—deep blue Muscle fibers—red
Weigert's elastic stain	Elastic fibers	Blue/black	N/A	N/A	N/A	Elastic fibers—blue/black
Heidenhain's AZAN trichrome stain	Distinguishing cells from extracellular components	Red/purple	Pink	Red	Blue	Muscle fibers—red Cartilage—blue Bone matrix—blue
Silver stain	Reticular fibers, nerve fibers, fungi	N/A	N/A	N/A	N/A	Reticular fibers—brown/black Nerve fibers—brown/black
Wright's stain	Blood cells	Bluish/purple	Bluish/gray	Red/pink	N/A	Neutrophil

						granules—purple/pink Eosinophil granules—bright red/orange Basophil granules—deep purple/violet Platelet granules—red/purple
Orcein stain	Elastic fibres	Deep blue [or crazy red]	N/A	Bright red	Pink	Elastic fibres—dark brown Mast cells granules—purple Smooth muscle—light blue
Periodic acid-Schiff stain (PAS)	Basement membrane, localizing carbohydrates	Blue	N/A	N/A	Pink	Glycogen and other carbohydrates—magenta

The Nissl method and Golgi's method are useful in identifying neurons.

Alternative techniques

Alternative techniques include cryosection. The tissue is frozen using a cryostat, and cut. Tissue staining methods are similar to those of wax sections. Plastic embedding is commonly used in the preparation of material for electron microscopy. Tissues are embedded in epoxy resin. Very thin sections (less than 0.1 micrometer) are cut using diamond or glass knives. The sections are stained with electron dense stains (uranium and lead) so that they can possibly be seen with the electron microscope.

History

In the 19th century, histology was an academic discipline in its own right. The 1906 Nobel Prize in Physiology or Medicine was awarded to histologists Camillo Golgi and Santiago Ramon y Cajal. They had dueling interpretations of the neural structure of the brain based in differing interpretations of the same images. Cajal won the prize for his correct theory and Golgi for the staining technique he invented to make it possible.

Histological classification of animal tissues

There are four basic types of tissues: muscle tissue, nervous tissue, connective tissue, and epithelial tissue. All tissue types are subtypes of these four basic tissue types (for example, blood cells are classified as connective tissue, since they generally originate inside bone marrow).

- Epithelium: the lining of glands, bowel, skin, and some organs like the liver, lung, and kidney
- Endothelium: the lining of blood and lymphatic vessels
- Mesenchyme: the cells filling the spaces between the organs, including fat, muscle, bone, cartilage, and tendon cells
- Blood cells: the red and white blood cells, including those found in lymph nodes and spleen
- Neurons: any of the conducting cells of the nervous system
- Germ cells: reproductive cells (spermatozoa in men, oocytes in women)
- Placenta: an organ characteristic of true mammals during pregnancy, joining mother and offspring, providing endocrine secretion and selective exchange of soluble, but not particulate, blood-borne substances through an apposition of uterine and trophoblastic vascularised parts

Note that tissues from plants, fungi, and microorganisms can also be examined histologically. Their structure is very different from animal tissues.

Related sciences

- Cell biology is the study of living cells, their DNA and RNA and the proteins they express.
- Anatomy is the study of organs visible by the naked eye.
- Morphology studies entire organisms.

Artifacts

Artifacts are structures or features in tissue that interfere with normal histological examination. These are not always present in normal tissue and can come from outside sources. Artifacts interfere with histology by changing the tissues appearance and hiding structures. These can be divided into two categories:

Pre-histology

These are features and structures that have being introduced prior to the collection of the tissues. A common example of these include: ink from tattoos and freckles (melanin) in skin samples.

Post-histology

Artifacts can result from tissue processing. Processing commonly leads to changes like shrinkage, washing out of particular cellular components, color changes in different tissues types and alterations of the structures in the tissue. Because these are caused in a laboratory the majority of post histology artifacts can be avoided or removed after being discovered. A common example is mercury pigment left behind after using Zenker's fixative to fix a section.

Chapter 15

Fixation

In the fields of histology, pathology, and cell biology, **fixation** is a chemical process by which biological tissues are preserved from decay, either through **autolysis** or **putrefaction**. Fixation terminates any ongoing biochemical reactions, and may also increase the mechanical strength or stability of the treated tissues.

Purpose of fixation

Fixation preserves a sample of biological material (tissue or cells) as close to its natural state as possible in the process of preparing tissue for examination. To achieve this, several conditions usually must be met.

First, a fixative usually acts to disable intrinsic biomolecules – particularly proteolytic enzymes—which otherwise digests or damages the sample.

Second, a fixative typically protects a sample from extrinsic damage. Fixatives are toxic to most common microorganisms (bacteria in particular) that might exist in a tissue sample or which might otherwise colonise the fixed tissue. In addition, many fixatives chemically alter the fixed material to make it less palatable (either indigestible or toxic) to opportunistic microorganisms.

Finally, fixatives often alter the cells or tissues on a molecular level to increase their mechanical strength or stability. This increased strength and rigidity can help preserve the morphology (shape and structure) of the sample as it is processed for further analysis.

Even the most careful fixation does alter the sample and introduce artifacts that can interfere with interpretation of cellular ultrastructure. A prominent example is the bacterial *mesosome*, which was thought to be an organelle in gram-positive bacteria in the 1970s, but was later shown by new techniques developed for electron microscopy to be simply an artifact of chemical fixation. Standardization of fixation and other tissue processing procedures takes this introduction of artifacts into account, by establishing what procedures introduce which kinds of artifacts. Researchers who know what types of artifacts to expect with each tissue type and processing technique can accurately interpret sections with artifacts, or choose techniques that minimize artifacts in areas of interest.

Fixation process

Fixation is usually the first stage in a multistep process to prepare a sample of biological material for microscopy or other analysis. Therefore, the choice of fixative and fixation protocol may depend on the additional processing steps and final analyses that are planned. For example, immunohistochemistry uses antibodies that bind to a specific protein target. Prolonged fixation can chemically mask these targets and prevent antibody binding. In these cases, a 'quick fix' method using cold formalin for around 24 hours is typically used.

Types of fixation

There are generally three types of fixation process:

Heat fixation: After a smear has dried at room temperature, the slide is gripped by tongs or a clothespin and passed through the flame of a Bunsen burner several times to heat-kill and adhere the organism to the slide. Routinely used with bacteria and archaea. Heat fixation generally preserves overall morphology but not internal structures. Heat fixation cannot be used in the capsular stain method as heat fixation will shrink or destroy the capsule (glycocalyx) and cannot be seen in stains.

Perfusion: Fixation via bloodflow. The fixative is injected into the heart with the injection volume matching cardiac output. The fixative spreads through the entire body, and the tissue doesn't die until it is fixed. This has the advantage of preserving perfect morphology, but the disadvantages that the subject dies and the cost is high (because of the volume of fixative needed for larger organisms)

Immersion: The sample of tissue is immersed in fixative of volume at a minimum of 20 times greater than the volume of the tissue to be fixed. The fixative must diffuse through the tissue to fix, so tissue size and density, as well as type of fixative must be considered. Using a larger sample means it takes longer for the fixative to reach the deeper tissue.

Chemical Fixation

In this process, structures are preserved in a state (both chemically and structurally) as close to living tissue as possible. This requires a chemical fixative that can **stabilise** the proteins, nucleic acids and mucosubstances of the tissue by making them **insoluble**.

Types of Chemical Fixatives

Crosslinking fixatives - Aldehydes

Crosslinking fixatives act by creating covalent chemical bonds between proteins in tissue. This anchors soluble proteins to the cytoskeleton, and lends additional rigidity to the tissue.

By far the most commonly used fixative in histology is formaldehyde. It is usually used as a 10% Neutral Buffered Formalin (NBF), that is aprox. 3.7% formaldehyde in phosphate buffered saline. Because formaldehyde is a gas at room temperature, formalin-formaldehyde gas dissolved in water (~37% w/v)-is used when making the former fixative. Paraformaldehyde is a polymerised form of formaldehyde, usually obtained as a fine white powder, which depolymerises back to formalin when heated. Formaldehyde fixes tissue by cross-linking the proteins, primarily the residues of the basic amino acid lysine. Its effects are reversible by excess water and it avoids formalin pigmentation. Other benefits include: Long term storage and good tissue penetration. It is particularly good for immunohistochemistry techniques. Also the formaldehyde vapour can be used as a fixatives for cell smears.

Another popular aldehyde for fixation is glutaraldehyde. It operates in a similar way to formaldehyde by causing deformation of the alpha-helix structures in proteins. However glutaraldehyde is a larger molecule, and so its rate of diffusion across membranes is slower than formaldehyde. Consequently glutaraldehyde fixation on thicker tissue samples may be hampered, but this problem can be overcome by reducing the size of the tissue sample. One of the advantages of glutaraldehyde fixation is that it may offer a more rigid or tightly linked fixed product—its greater length and two aldehyde groups allow it to 'bridge' and link more distant pairs of protein molecules. It causes rapid and irreversible changes, fixes quickly, is well suited for electron microscopy, fixes well at 4°C, and gives best overall cytoplasmic and nuclear detail. However it is not ideal for immunohistochemistry staining.

Some fixation protocols call for a combination of formaldehyde and glutaraldehyde so that their respective strengths complement one another.

These crosslinking fixatives–especially formaldehyde–tend to preserve the secondary structure of proteins and may protect significant amounts of tertiary structure as well.

Precipitating fixatives - Alcohols

Precipitating (or *denaturing*) fixatives act by reducing the solubility of protein molecules and (often) by disrupting the hydrophobic interactions that give many proteins their tertiary structure. The precipitation and aggregation of proteins is a very different process from the crosslinking that occurs with the aldehyde fixatives.

The most common precipitating fixatives are ethanol and methanol. They are commonly used to fix frozen sections and smears. Acetone is also used and has been shown to produce better histological preservation than frozen sections when employed in the Acetone Methylbenzoate Xylene (AMEX) technique.

The protein denaturants - methanol, ethanol and acetone - are rarely used alone for fixing blocks unless studying nucleic acids.

Acetic acid is a denaturant that is sometimes used in combination with the other precipitating fixatives. The alcohols, by themselves, are known to cause considerable shrinkage and hardening of tissue during fixation while acetic acid alone is associated with tissue swelling; combining the two may result in better preservation of tissue morphology.

Oxidising agents

The oxidising fixatives can react with various side chains of proteins and other biomolecules, allowing formation of crosslinks that stabilize tissue structure. However they cause extensive denaturation despite preserving fine cell structure and are used mainly as secondary fixatives.

Osmium tetroxide is often used as a secondary fixative when samples are prepared for electron microscopy. (It is not used for light microscopy as it penetrates thick sections of tissue very poorly.)

Potassium dichromate, chromic acid, and potassium permanganate all find use in certain specific histological preparations.

Mercurials

Mercurials such as B-5 and Zenker's have an unknown mechanism that increases staining brightness and give excellent nuclear detail. Despite being fast, mercurials penetrate poorly and produce tissue shrinkage. Their best application is for fixation of hematopoietic and reticuloendothelial tissues. Also note that since they contain mercury care must be taken with disposal.

Picrates

Picrates penetrate tissue well to react with histones and basic proteins to form crystalline picrates with amino acids and precipitate all proteins. It is a good fixative for connective tissue, preserves glycogen well, and extracts lipids to give superior results to formaldehyde in immunostaining of biogenic and polypeptide hormones However, it causes a loss of basophilia unless the specimen is thoroughly washed following fixation.

HOPE Fixative

Hepes-glutamic acid buffer-mediated organic solvent protection effect (HOPE) gives formalin-like morphology, excellent preservation of protein antigens for immunohistochemistry and enzyme histochemistry, good RNA and DNA yields and absence of crosslinking proteins.

Frozen Sections

Small pieces of tissue (5X5X3mm) are placed in a cryoprotective embedding medium - OCT, TBS or Cryogel - then snap frozen in isopentane cooled by liquid nitrogen. Tissue is then sectioned in a freezing microtome or cryostat. Sections are then fixed in one of the following fixatives: Absolute acetone for 10–15 minutes, 95% ethanol for 10–15 minutes or Absolute acetone 10minutes followed by 95% ethanol 10minutes

Advantages

- Give better preservation of antigenicity
- Minimal exposure to fixative
- Not exposed to the organic solvents

Disadvantages

- Lack morphological detail
- Present a potential biohazard

Target and Chemical Fixative Do's and Don'ts

Target	Fixative of Choice	Fixative to Avoid
Proteins	Neutral Buffered Formalin, Parafomaldehyde	Osmium Tetroxide
Enzymes	Frozen Sections	Chemical Fixatives
Lipids	Frozen Sections*, Glutaraldehyde/Osmium Tetroxide	Alcoholic fixatives, Neutral Buffered Formalin
Nucleic Acids	Alcoholic fixatives, HOPE	Aldehyde fixatives
Mucopolysaccharides	Frozen Sections	Chemical fixatives
Biogenic Amines	Bouin's~, Neutral Buffered Formalin	
Glycogen	Alcoholic based fixatives	Osmium Tetroxide

- Frozen Sections preserve RNA and Lipids despite poor morphology. Compare to Paraffin sections, synonymous to Chemical Fixatives in the table, which destroy RNA and affect some antigens BUT give good morphology.

~ A picrate

Factors Affecting Fixation

pH

Should be kept in the physiological range, between pH 4-9. The pH for the ultrastructure preservation should be buffered between 7.2 to 7.4

Osmolarity

Hypertonic solutions give rise to cell shrinkage.

Hypotonic solutions result in cell swelling and poor fixation.

Size of the Specimen

1-4mm Thickness

Volume of the Fixative

At least 15-20 times greater than tissue volume

Temperature

Increasing the temperature increases speed of fixation. However, care is required to avoid cooking the specimen.

Duration

As a general rule 1hr per 1mm

Time from Removal to Fixation

Fixation is a chemical process, and time must be allowed for the process to complete. Although "over fixation" can be detrimental, under-fixation has recently been appreciated as a significant problem and may be responsible for inappropriate results for some assays.

Chapter 16

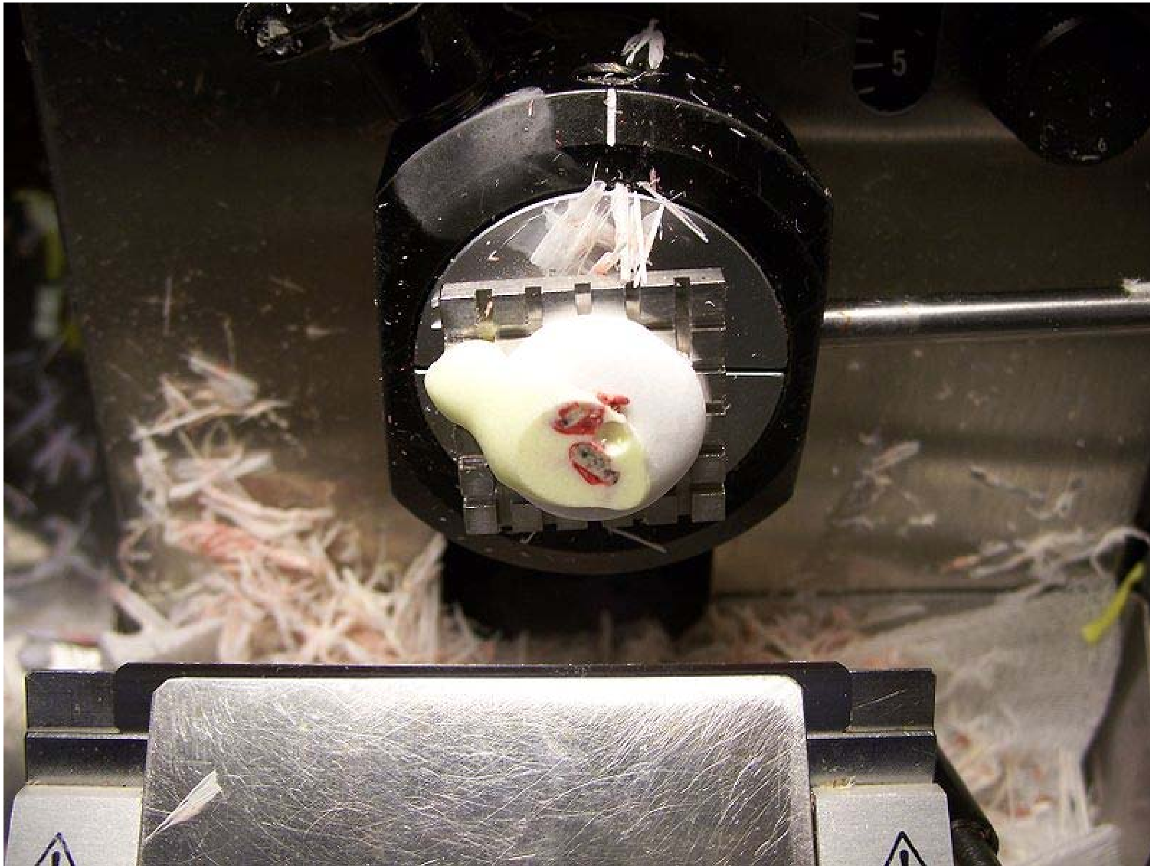
Frozen Section Procedure

The **frozen section procedure** is a pathological laboratory procedure to perform rapid microscopic analysis of a specimen. It is used most often in oncological surgery. The technical name for this procedure is **cryosection**.

The quality of the slides produced by frozen section is of lower quality than formalin fixed, wax embedded tissue processing. While diagnosis can be rendered in many cases, fixed tissue processing is preferred in many conditions for more accurate diagnosis.

The **intraoperative consultation** is the name given to the whole intervention by the pathologist, which includes not only frozen section but also gross evaluation of the specimen, examination of cytology preparations taken on the specimen (e.g. touch imprints), and aliquoting of the specimen for special studies (e.g. molecular pathology techniques, flow cytometry). The report given by the pathologist is usually limited to a "benign" or "malignant" diagnosis, and communicated to the surgeon operating via intercom. When operating on a previously confirmed malignancy, the main purpose of the pathologist is to inform the surgeon if the surgical margin is clear of residual cancer, or if residual cancer is present at the surgical margin. The method of processing is usually done with the bread loafing technique. But margin controlled surgery (CCPDMA) can be performed using a variety of tissue cutting and mounting methods, including mohs surgery.

Procedure



Tissue embedded within OCT, mounted on a chuck in a cryostat and ready for section production

The key instrument for cryosection is the cryostat (more correctly: cryotome), which is essentially a microtome inside a freezer. The microtome can be compared to a very accurate "deli" slicer, capable of slicing sections as thin as 1 micrometre. The usual histology slice is cut at 5 to 10 micrometres. The surgical specimen is placed on a metal tissue disc which is then secured in a chuck and frozen rapidly to about −20 to -30 °C. The specimen is embedded in a gel like medium consisting of poly ethylene glycol and poly vinyl alcohol; this compound is known by many names and when frozen has the same density as frozen tissue. At this temperature, most tissues become rock-hard. Usually a lower temperature is required for fat or lipid rich tissue. Each tissue has a preferred temperature for processing. Subsequently it is cut frozen with the microtome portion of the cryostat, the section is picked up on a glass slide and stained (usually with hematoxylin and eosin, the H&E stain). The preparation of the sample is much more rapid than with traditional histology technique (around 10 minutes vs 16 hours). However, the technical quality of the sections is much lower. The entire laboratory can occupy a space less than 9-square-foot (0.84 m^2), and minimal ventilation is required compared to a standard wax embedded specimen laboratory.

Uses

The principal use of the frozen section procedure is the examination of tissue while surgery is taking place. This may be for various reasons:

- In the performance of Mohs surgery - a simple method for 100% margin control of a surgical specimen.
- If a tumor appears to have metastasized, a sample of the suspected metastasis is sent for cryosection to confirm its identity. This will help the surgeon decide whether there is any point in continuing the operation. Usually, aggressive surgery is performed only if there is a chance to cure the patient. If the tumor has metastasized, surgery is usually not curative, and the surgeon will choose a more conservative surgery, or no resection at all.
- If a tumor has been resected but it is unclear whether the surgical margin is free of tumor, an intraoperative consultation is requested to assess the need to make a further resection for clear margins.
- In a sentinel node procedure, a sentinel node containing tumor tissue prompts a further lymph node dissection, while a benign node will avoid such a procedure.
- If surgery is explorative, rapid examination of a lesion might help identify the possible cause of a patient's symptoms. It is important to note, however, that the pathologist is very limited by the poor technical quality of the frozen sections. A final diagnosis is rarely offered intraoperatively.
- Rarely, cryosections are used to detect the presence of substances lost in the traditional histology technique, for example lipids. They can also be used to detect some antigens masked by formalin.
- The cryostat is available in a small portable device weighing less than 80 lb (36 kg), to a large stationary device 500 lb (230 kg) or more. The entire histologic laboratory can be carried in one portable box, making frozen section histology a possible tool in primitive medicine.

History

The frozen section procedure as practiced today in medical laboratories is based on the description by Dr Louis B. Wilson in 1905. Wilson developed the technique from earlier reports at the request of Dr William Mayo, surgeon and one of the founders of the Mayo Clinic. Earlier reports by Dr Thomas S. Cullen at Johns Hopkins Hospital in Baltimore also involved frozen section, but only after formalin fixation, and pathologist Dr William Welch, also at Hopkins, experimented with Cullen's procedure but without clinical consequences. Hence, Wilson is generally credited with truly pioneering the procedure (Gal & Cagle, 2005).

Chapter 17

Epithelium

Epithelium is one of the four basic types of animal tissue, along with connective tissue, muscle tissue and nervous tissue. Epithelial tissues line the cavities and surfaces of structures throughout the body, and also form many glands. Functions of epithelial cells include secretion, selective absorption, protection, transcellular transport and detection of sensation.

Epidermis is a special type of epithelium forming the outer layer of skin.

Epithelial tissue lies on top of connective tissue, from which it is separated by a basement membrane. It is composed of tightly clustered cells connected by tight junctions and desmosomes. Epithelial tissue is avascular, so it must receive nourishment via diffusion of substances from the underlying connective tissue, through the basement membrane.

General structure

The cells in epithelium are very densely packed together, leaving very little intercellular space. The cells form continuous sheets which are attached to each other at many locations by tight junctions and desmosomes.

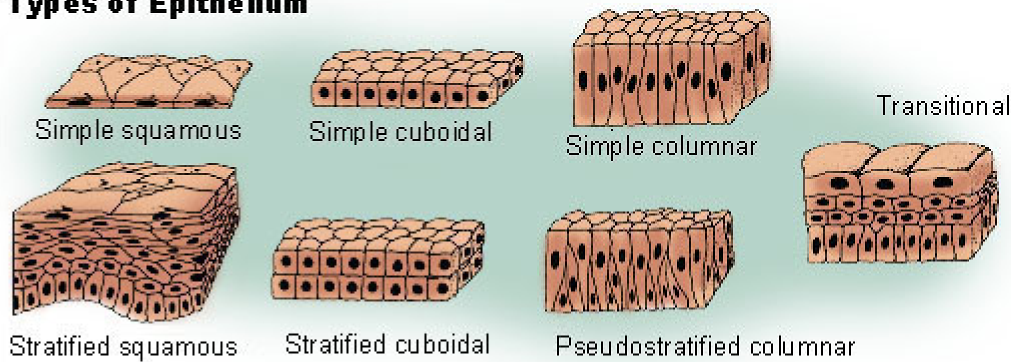
Basement membrane

All epithelial cells rest on a basement membrane, which acts as a scaffolding on which epithelium can grow and regenerate after injuries. Epithelial tissue is innervated, but avascular. Thus epithelial tissue must be nourished by substances diffusing from the blood vessels in the underlying tissue. The basement membrane acts as a selectively permeable membrane that determines which substances will be able to enter the epithelium.

Cell junctions

Cell junctions are especially abundant in epithelial tissues. They consist of protein complexes and provide contact between neighbouring cells, between a cell and the extracellular matrix, or they build up the paracellular barrier of epithelia and control the paracellular transport.

Classification of epithelial tissue



Types of epithelium

Epithelial tissues are generally classified by the morphology of their cells, and the number of layers they are composed of. Epithelial tissue that is only one cell thick is known as simple epithelium. If it is two or more cells thick, it is known as stratified epithelium. However, when taller simple epithelial cells are viewed in cross section with several nuclei appearing at different heights, they can be confused with stratified epithelia. This kind of epithelium is therefore described as "pseudostratified" epithelium.

Simple epithelium

Simple epithelium is one cell thick, that is, every cell is in direct contact with the underlying basement membrane. It is generally found where absorption and filtration occur. The thinness of the epithelial barrier facilitates these processes.

Simple epithelial tissues are generally classified by the shape of their cells. The four major classes of simple epithelium are: (1) simple squamous; (2) simple cuboidal; (3) simple columnar; (4) pseudostratified.

Type	Description
Squamous	Squamous cells have the appearance of thin, flat plates. They fit closely together in tissues; providing a smooth, low-friction surface over which fluids can move easily. The shape of the nucleus usually corresponds to the cell form and helps to identify the type of epithelium. Squamous cells tend to have horizontally flattened, elliptical (oval or shaped like an egg) nuclei because of the thin flattened form of the cell. Classically, squamous epithelia are found lining surfaces utilizing simple passive diffusion such as the alveolar epithelium in the lungs. Specialized squamous epithelia also form the lining of cavities such as the blood vessels (endothelium) and pericardium (mesothelium) and the major cavities found within the body.
Cuboidal	As their name implies, cuboidal cells are roughly cuboidal in shape,

	appearing square in cross section. Each cell has a spherical nucleus in the centre. Cuboidal epithelium is commonly found in secretive or absorptive tissue: for example the (secretive) exocrine gland the pancreas and the (absorptive) lining of the kidney tubules as well as in the ducts of the glands. They also constitute the germinal epithelium that covers the female ovary.
Columnar	Columnar epithelial cells are elongated and column-shaped. Their nuclei are elongated and are usually located near the base of the cells. Columnar epithelium forms the lining of the stomach and intestines. Some columnar cells are specialized for sensory reception such as in the nose, ears and the taste buds of the tongue. Goblet cells (unicellular glands) are found between the columnar epithelial cells of the duodenum. They secrete mucus, which acts as a lubricant.
Pseudostratified	These are simple columnar epithelial cells whose nuclei appear at different heights, giving the misleading (hence "pseudo") impression that the epithelium is stratified when the cells are viewed in cross section. Pseudostratified epithelium can also possess fine hair-like extensions of their apical (luminal) membrane called cilia. In this case, the epithelium is described as "ciliated" pseudostratified epithelium. Cilia are capable of energy dependent pulsatile beating in a certain direction through interaction of cytoskeletal microtubules and connecting structural proteins and enzymes. The wafting effect produced causes mucus secreted locally by the goblet cells (to lubricate and to trap pathogens and particles) to flow in that direction (typically out of the body). Ciliated epithelium is found in the airways (nose, bronchi), but is also found in the uterus and Fallopian tubes of females, where the cilia propel the ovum to the uterus.

Stratified epithelium

Stratified epithelium differs from simple epithelium in that it is multilayered. It is therefore found where body linings have to withstand mechanical or chemical insult such that layers can be abraded and lost without exposing subepithelial layers. Cells flatten as the layers become more apical, though in their most basal layers the cells can be squamous, cuboidal or columnar.

Stratified epithelial tissue also differs from simple epithelial tissue in that stratified epithelial tissues do not contain junctional complexes, and have their cells bound together only by desmosomes.

Stratified epithelia (of columnar, cuboidal or squamous type) can have the following specializations:

Specialization	Description
Keratinized	In this case, the most apical layers (exterior) of cells are dead and lose

	their nucleus and cytoplasm, instead contain a tough, resistant protein called keratin. This specialization makes the epithelium waterproof, so is found in the mammalian skin. The lining of the esophagus is an example of a non-keratinized or "moist" stratified epithelium.
Transitional	Transitional epithelium, like pseudostratified epithelium, is almost a class of its own; it is found in tissues that stretch and it can appear to be stratified cuboidal when the tissue is not stretched or stratified squamous when the organ is distended and the tissue stretches. It is sometimes called the urothelium since it is almost exclusively found in the bladder, ureters and urethra.

Functions

The primary functions of epithelial tissues are: (1) to protect the tissues that lie beneath it from radiation, dessication, toxins, and physical trauma; (2) the regulation and exchange of chemicals between the underlying tissues and a body cavity; and/or (3) the secretion of biological enzymes from epithelial tissue known as glandular epithelium.

Secretory epithelia

As stated above, secretion is one major function of epithelial cells. Glands are formed from the invagination / infolding of epithelial cells and subsequent growth in the underlying connective tissue. There are two major classifications of glands: endocrine glands and exocrine glands. Endocrine glands are glands that secrete their product directly onto a surface rather than through a duct. This group contains the glands of the Endocrine system.

Sensing the extracellular environment

"Some epithelial cells are ciliated, and they commonly exist as a sheet of polarised cells forming a tube or tubule with cilia projecting into the lumen." Primary cilia on epithelial cells provide chemosensation, thermosensation and mechanosensation of the extracellular environment by playing "a sensory role mediating specific signalling cues, including soluble factors in the external cell environment, a secretory role in which a soluble protein is released to have an effect downstream of the fluid flow, and mediation of fluid flow if the cilia are motile."

Emybryological development

In general, there are epithelial tissues deriving from all of the embryological germ layers:

- from ectoderm (e.g., the epidermis);
- from endoderm (e.g., the lining of the gastrointestinal tract);
- from mesoderm (e.g., the inner linings of body cavities).

However, it is important to note that pathologists do not consider endothelium and mesothelium (both derived from mesoderm) to be true epithelium. This is because such tissues present very different pathology. For that reason, pathologists label cancers in endothelium and mesothelium sarcomas, whereas true epithelial cancers are called carcinomas. Also, the filaments that support these mesoderm-derived tissues are very distinct. Outside of the field of pathology, it is, in general, accepted that the epithelium arises from all three germ layers.

Growing in culture

When growing epithelium in culture, one can determine whether or not a particular cell is epithelial by examining its morphological characteristics. Epithelial cells tend to cluster together, and have a "characteristic tight pavementlike appearance". But this is not always the case, such as when the cells are derived from a tumor. In these cases, it is often necessary to use certain biochemical markers to make a positive identification. The intermediate filament proteins in the cytokeratin group are almost exclusively found in epithelial cells, and so are often used for this purpose.

Location

Epithelium lines both the outside (skin) and the inside cavities and lumen of bodies. The outermost layer of our skin is composed of dead stratified squamous, keratinized epithelial cells.

Tissues that line the inside of the mouth, the esophagus and part of the rectum are composed of nonkeratinized stratified squamous epithelium. Other surfaces that separate body cavities from the outside environment are lined by simple squamous, columnar, or pseudostratified epithelial cells. Other epithelial cells line the insides of the lungs, the gastrointestinal tract, the reproductive and urinary tracts, and make up the exocrine and endocrine glands. The outer surface of the cornea is covered with fast-growing, easily-regenerated epithelial cells. Endothelium (the inner lining of blood vessels, the heart, and lymphatic vessels) is a specialized form of epithelium. Another type, mesothelium, forms the walls of the pericardium, pleurae, and peritoneum.

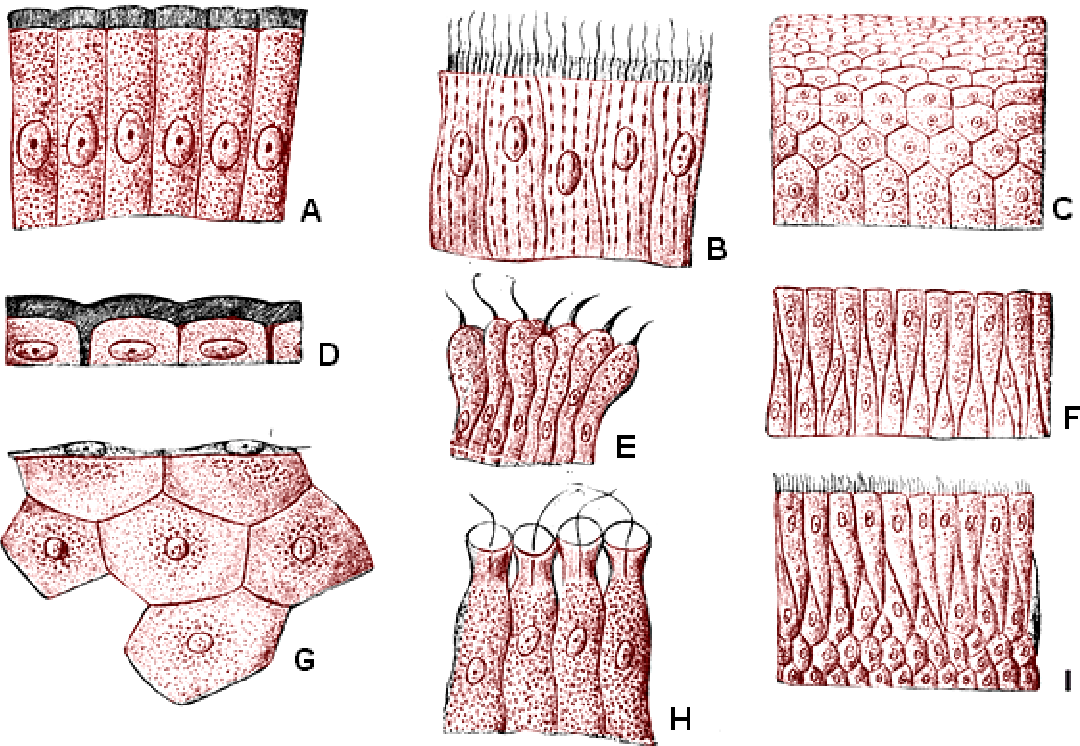
System	Tissue	Epithelium	Subtype
circulatory	blood vessels	Simple squamous	endothelium
digestive	ducts of submandibular glands	Stratified columnar	-
digestive	attached gingiva	Stratified squamous, keratinized	-
digestive	dorsum of tongue	Stratified squamous, keratinized	-
digestive	hard palate	Stratified squamous, keratinized	-

digestive	oesophagus	Stratified squamous, non-keratinized	-
digestive	stomach	Simple columnar, non-ciliated	-
digestive	small intestine	Simple columnar, non-ciliated	intestinal epithelium
digestive	large intestine	Simple columnar, non-ciliated	intestinal epithelium
digestive	rectum	Simple columnar, non-ciliated	-
digestive	anus	Stratified squamous, non-keratinized superior to Hilton's white line Stratified squamous, keratinized inferior to Hilton's white line	-
digestive	gallbladder	Simple columnar, non-ciliated	-
endocrine	thyroid follicles	Simple cuboidal	-
nervous	ependyma	Simple cuboidal	-
lymphatic	lymph vessel	Simple squamous	endothelium
integumentary	skin - dead superficial layer	Stratified squamous, keratinized	-
integumentary	sweat gland ducts	Stratified cuboidal	-
integumentary	mesothelium of body cavities	Simple squamous	mesothelium
reproductive - female	ovaries	Simple cuboidal	germinal epithelium (female)
reproductive - female	Fallopian tubes	Simple columnar, ciliated	-
reproductive - female	endometrium (uterus)	Simple columnar, ciliated	-
reproductive - female	cervix (endocervix)	Simple columnar	-
reproductive - female	cervix (ectocervix)	Stratified squamous, non-keratinized	-
reproductive - female	vagina	Stratified squamous, non-keratinized	-
reproductive - female	labia majora	Stratified squamous, keratinized	-
reproductive - male	tubuli recti	Simple cuboidal	germinal epithelium (male)
reproductive -	rete testis	Simple cuboidal	-

male			
reproductive - male	ductuli efferentes	Pseudostratified columnar	-
reproductive - male	epididymis	Pseudostratified columnar, with stereocilia	-
reproductive - male	vas deferens	Pseudostratified columnar	-
reproductive - male	ejaculatory duct	Simple columnar	-
reproductive - male (gland)	bulbourethral glands	Simple columnar	-
reproductive - male (gland)	seminal vesicle	Pseudostratified columnar	-
respiratory	oropharynx	Stratified squamous, non-keratinized	-
respiratory	larynx	Pseudostratified columnar, ciliated	respiratory epithelium
respiratory	larynx - True vocal cords	Stratified squamous, non-keratinized	-
respiratory	trachea	Pseudostratified columnar, ciliated	respiratory epithelium
respiratory	respiratory bronchioles	Simple cuboidal	-
sensory	cornea	Stratified squamous, non-keratinized	corneal epithelium
sensory	nose	Pseudostratified columnar	olfactory epithelium
urinary	kidney - proximal convoluted tubule	Simple cuboidal, with microvilli	-
urinary	kidney - ascending thin limb	Simple squamous	-
urinary	kidney - distal convoluted tubule	Simple cuboidal, without microvilli	-
urinary	kidney - collecting duct	Simple cuboidal	-
urinary	renal pelvis	Transitional	urothelium
urinary	ureter	Transitional	urothelium
urinary	urinary bladder	Transitional	urothelium
urinary	prostatic urethra	Transitional	urothelium
urinary	membranous urethra	Pseudostratified columnar, non-ciliated	-

urinary	penile urethra	Pseudostratified columnar, non-ciliated	-
urinary	external urethral orifice	Stratified squamous	-

Additional images



Chapter 18

Endothelium and Mesothelium

Endothelium

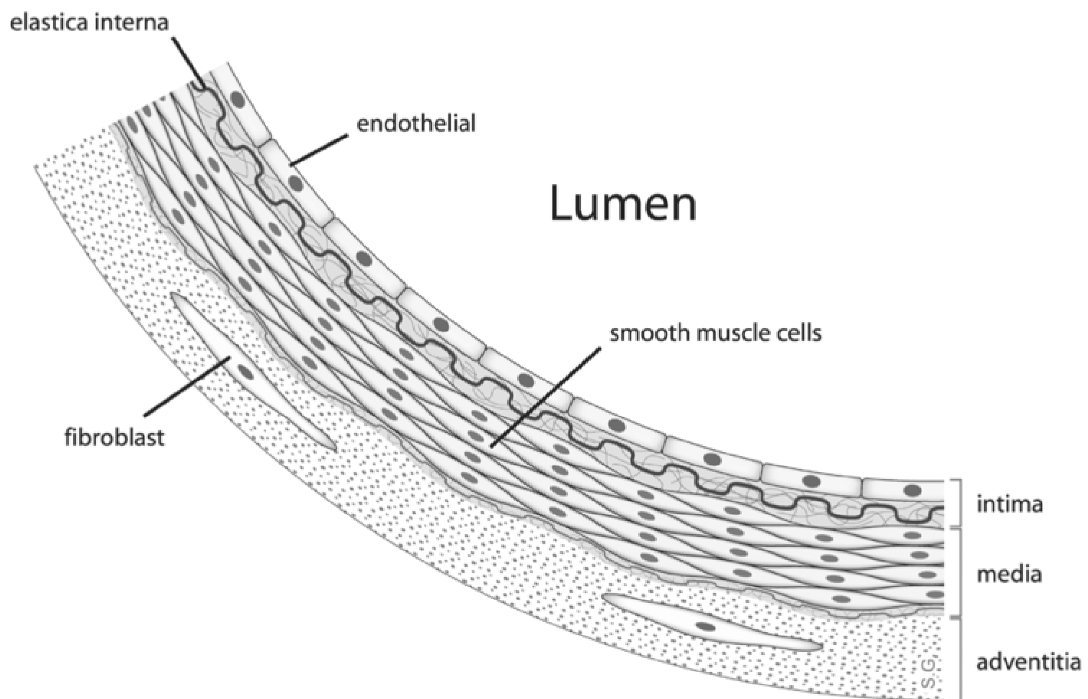
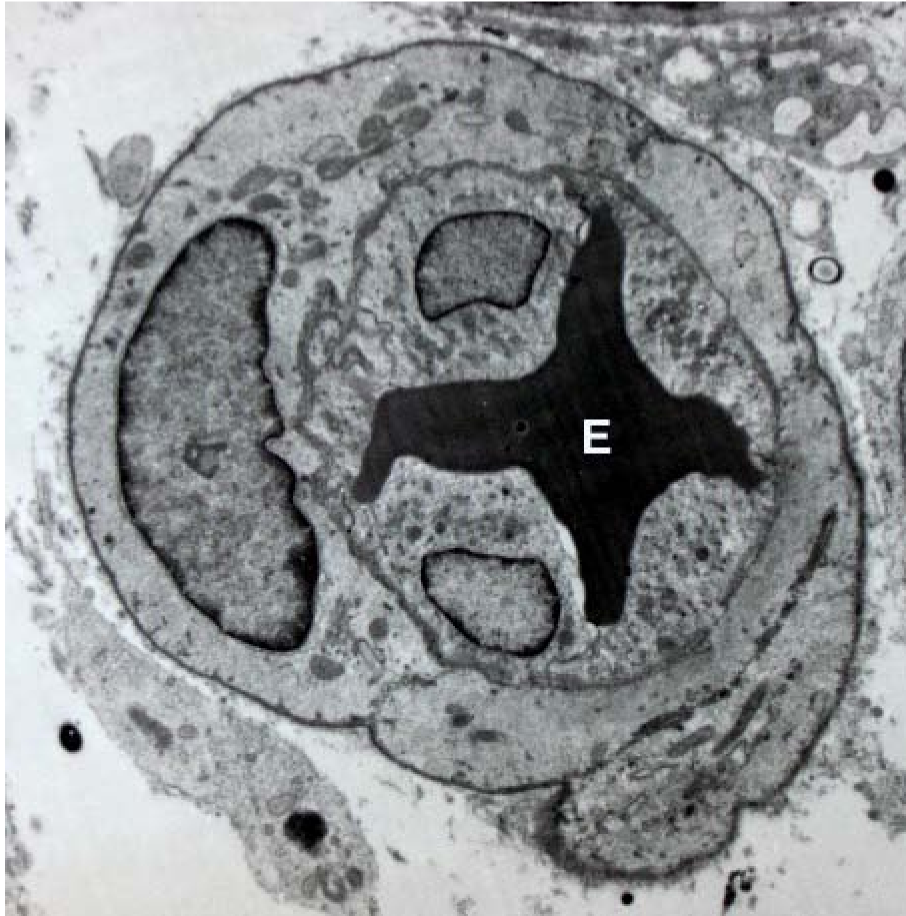


Diagram showing the location of endothelial cells



Endothelial cells, which form the tunica intima, encircle an erythrocyte (E).

The **endothelium** is the thin layer of cells that lines the interior surface of blood vessels, forming an interface between circulating blood in the lumen and the rest of the vessel wall. These cells are called **endothelial cells**. Endothelial cells line the entire circulatory system, from the heart to the smallest capillary. These cells reduce turbulence of the flow of blood, allowing the fluid to be pumped farther.

Endothelial tissue is a specialized type of epithelium tissue (one of the four types of biological tissue in animals). More specifically, it is simple squamous epithelium.

The endothelium normally provides a non-thrombogenic surface because it contains heparan sulfate which acts as a cofactor for activating antithrombin III, a protease that cleaves several factors in the coagulation cascade.

Terminology

The foundational model of anatomy makes a distinction between endothelial cells and epithelial cells on the basis of which tissues they develop from and states that the presence of vimentin rather than keratin filaments separate these from epithelial cells.

Endothelium of the interior surfaces of the heart chambers are called endocardium. Both blood and lymphatic capillaries are composed of a single layer of endothelial cells called a monolayer.

Function

Endothelial cells are involved in many aspects of vascular biology, including:

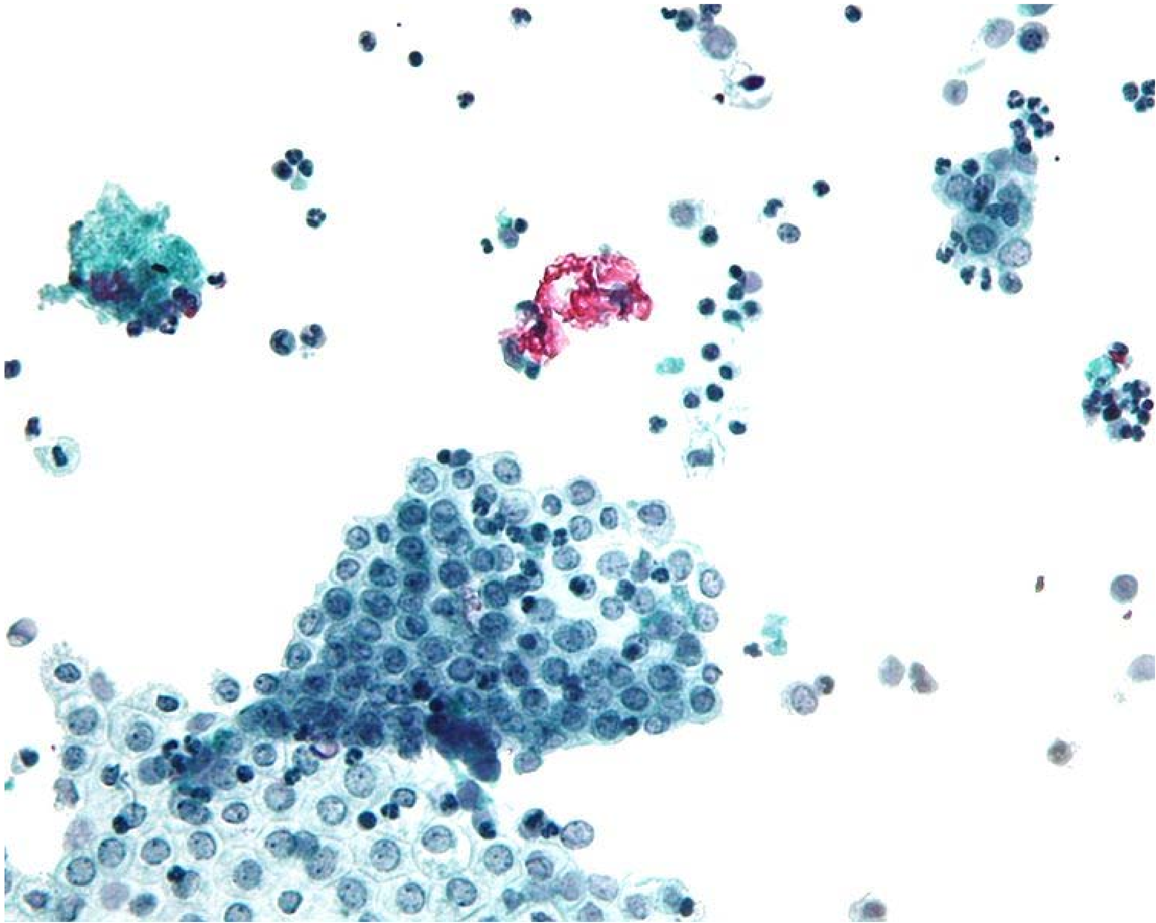
- Atherosclerosis
- Barrier function - the endothelium acts as a selective barrier between the vessel lumen and surrounding tissue, controlling the passage of materials and the transit of white blood cells into and out of the bloodstream. Excessive or prolonged increases in permeability of the endothelial monolayer, as in cases of chronic inflammation, may lead to tissue oedema/swelling.
- Blood clotting (thrombosis & fibrinolysis)
- Inflammation
- Formation of new blood vessels (angiogenesis)
- Vasoconstriction and vasodilation, and hence the control of blood pressure

In some organs, there are highly differentiated endothelial cells to perform specialized 'filtering' functions. Examples of such unique endothelial structures include the renal glomerulus and the blood-brain barrier.

Pathology

Endothelial dysfunction, or the loss of proper endothelial function, is a hallmark for vascular diseases, and is often regarded as a key early event in the development of atherosclerosis. Impaired endothelial function is often seen in patients with coronary artery disease, diabetes mellitus, hypertension, hypercholesterolemia, as well as in smokers. Endothelial dysfunction has also been shown to be predictive of future adverse cardiovascular events. One of the main mechanisms of endothelial dysfunction is the diminishing of nitric oxide, often due to high levels of asymmetric dimethylarginine, which interfere with the normal L-arginine-stimulated nitric oxide synthesis. The most prevailing mechanism of endothelial dysfunction is an increase in reactive oxygen species, which can impair nitric oxide production and activity via several mechanisms. The signalling protein ERK5 is essential for maintaining normal endothelial cell function.

Mesothelium

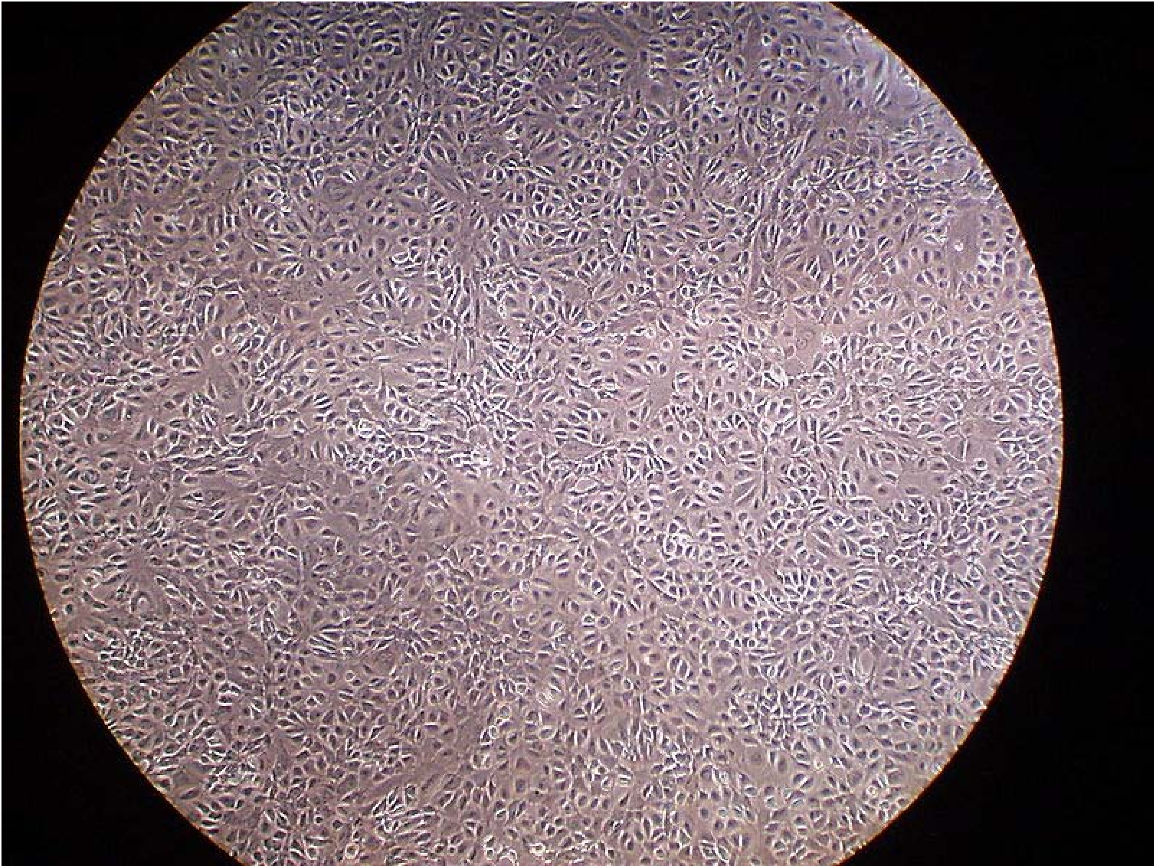


Micrograph of benign mesothelial cells. Peritoneal wash. Pap stain

The **mesothelium** is a membrane that forms the lining of several body cavities: the pleura (thoracic cavity), peritoneum (abdominal cavity including the mesentery) and pericardium (heart sac). Mesothelial tissue also surrounds the male internal reproductive organs (the tunica vaginalis testis) and covers the internal reproductive organs of women (the tunica serosa uteri). Mesothelium that covers the internal organs is called visceral mesothelium, while the layer that covers the body walls is called the parietal mesothelium.

Origin

Mesothelium derives from the embryonic mesoderm cell layer, that lines the coelom (body cavity) in the embryo. It develops into the layer of cells that covers and protects most of the internal organs of the body.



A layer of mesothelial cells grown in cell culture, featuring the typical "cobblestone" appearance

Structure

The mesothelium forms a monolayer of flattened squamous-like epithelial cells resting on a thin basement membrane supported by Dense irregular connective tissue. Cuboidal mesothelial cells may be found at areas of injury, the milky spots of the omentum, and the peritoneal side of the diaphragm overlaying the lymphatic lacunae. The luminal surface is covered with microvilli. The proteins and serosal fluid trapped by the microvilli provide a frictionless surface for internal organs to slide past one another.

Function

The mesothelium is composed of an extensive monolayer of specialized cells (mesothelial cells) that line the body's serous cavities and internal organs. The main purpose of these cells is to produce a lubricating fluid that is released between layers, providing a slippery, non-adhesive and protective surface to facilitate intracoelomic movement.

The mesothelium is also implicated in the transport and movement of fluid and particulate matter across the serosal cavities, leukocyte migration in response to

inflammatory mediators, synthesis of pro-inflammatory cytokines, growth factors and extracellular matrix proteins to aid in serosal repair, and the release of factors to promote the disposition and clearance of fibrin (such as plasminogen). It is an antigen presenting cell. Furthermore, the secretion of glycosaminoglycans and lubricants may protect the body against infection and tumor dissemination.

Role in disease

- **Mesothelioma:** (cancer of the mesothelium) is a disease in which cells of the mesothelium become abnormal and divide without control or order. They can invade and damage nearby tissues and organs. Cancer cells can also metastasize (spread) from their original site to other parts of the body. Most cases of mesothelioma begin in the pleura or peritoneum. More than 90% of mesothelioma cases are linked to asbestos exposure.

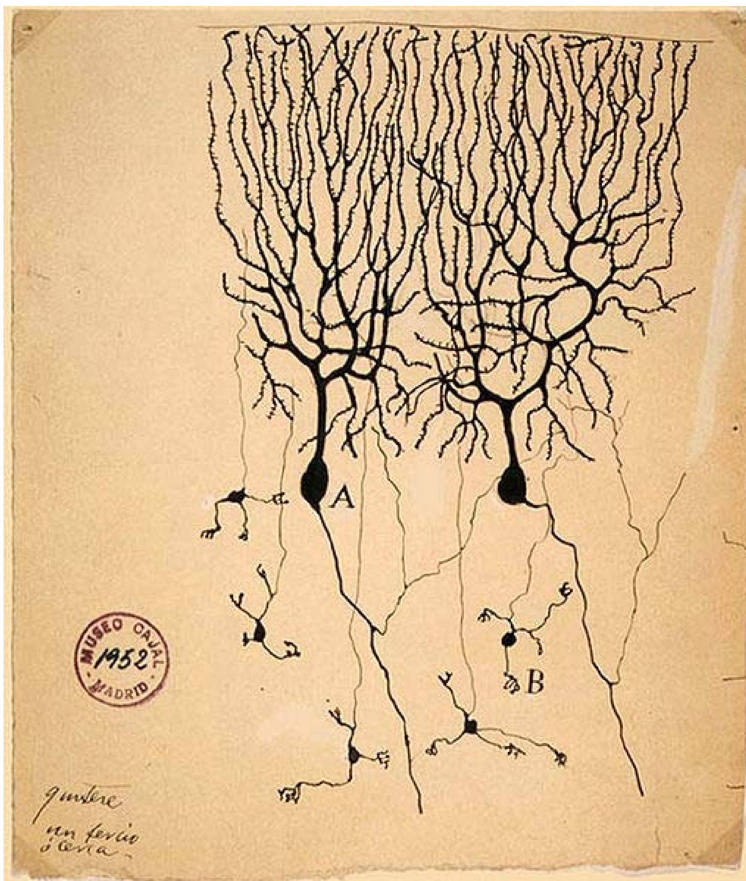
- **Intra-abdominal adhesions:** Normally, the mesothelium secretes plasminogen, which removes fibrin deposits. During surgical procedures, the mesothelium may be damaged. Its fibrinolytic capacity becomes insufficient and fibrin accumulates, causing fibrous adhesions between opposing surfaces. These adhesions cause intestinal obstruction and female infertility if it occurs in the abdomen, and may impair cardiac and lung function in the thorax.

- **Ultrafiltration failure:** The peritoneal mesothelium is implicated in the long-term development of ultrafiltration failure in peritoneal dialysis patients. The presence of supra-physiological glucose concentrations, acidity, and glucose degradation products in peritoneal dialysis fluids contribute to the fibrosis of the peritoneal mesothelium, either by epithelial-mesenchymal transition or increased proliferation of existing fibroblasts. A fibrosed peritoneum results in the increased passage of solutes across the peritoneum and ultrafiltration failure.

Chapter 19

Neuron

Neuron: Nerve Cell



Drawing by Santiago Ramón y Cajal of neurons in the pigeon cerebellum. (A) Denotes Purkinje cells, an example of a multipolar neuron. (B) Denotes granule cells which are also multipolar.

NeuroLex ID	*sao1417703748*

A **neuron** is an electrically excitable cell that processes and transmits information by electrical and chemical signaling. Chemical signaling occurs via synapses, specialized connections with other cells. Neurons connect to each other to form networks. Neurons are the core components of the nervous system, which includes the brain, spinal cord, and peripheral ganglia. A number of specialized types of neurons exist: sensory neurons respond to touch, sound, light and numerous other stimuli affecting cells of the sensory organs that then send signals to the spinal cord and brain. Motor neurons receive signals from the brain and spinal cord, cause muscle contractions, and affect glands. Interneurons connect neurons to other neurons within the same region of the brain or spinal cord.

A typical neuron possesses a cell body (often called the soma), dendrites, and an axon. Dendrites are filaments that arise from the cell body, often extending for hundreds of micrometres and branching multiple times, giving rise to a complex "dendritic tree". An axon is a special cellular filament that arises from the cell body at a site called the axon hillock and travels for a distance, as far as 1 m in humans or even more in other species. The cell body of a neuron frequently gives rise to multiple dendrites, but never to more than one axon, although the axon may branch hundreds of times before it terminates. At the majority of synapses, signals are sent from the axon of one neuron to a dendrite of another. There are, however, many exceptions to these rules: neurons that lack dendrites, neurons that have no axon, synapses that connect an axon to another axon or a dendrite to another dendrite, etc.

All neurons are electrically excitable, maintaining voltage gradients across their membranes by means of metabolically driven ion pumps, which combine with ion channels embedded in the membrane to generate intracellular-versus-extracellular concentration differences of ions such as sodium, potassium, chloride, and calcium. Changes in the cross-membrane voltage can alter the function of voltage-dependent ion channels. If the voltage changes by a large enough amount, an all-or-none electrochemical pulse called an action potential is generated, which travels rapidly along the cell's axon, and activates synaptic connections with other cells when it arrives.

Neurons of the adult brain do not generally undergo cell division, and usually cannot be replaced after being lost, although there are a few known exceptions. In most cases they are generated by special types of stem cells, although astrocytes (a type of glial cell) have been observed to turn into neurons as they are sometimes pluripotent.

Overview

A neuron is a special type of cell that is found in the bodies of most animals (all members of the group Eumetazoa, to be precise—this excludes only sponges and a few other very simple animals). The features that define a neuron are electrical excitability and the presence of synapses, which are complex membrane junctions used to transmit signals to other cells. The body's neurons, plus the glial cells that give them structural and metabolic support, together constitute the nervous system. In vertebrates, the majority of neurons belong to the central nervous system, but some reside in peripheral ganglia, and many sensory neurons are situated in sensory organs such as the retina and cochlea.

Although neurons are very diverse and there are exceptions to nearly every rule, it is convenient to begin with a schematic description of the structure and function of a "typical" neuron. A typical neuron is divided into three parts: the soma or cell body, dendrites, and axon. The soma is usually compact; the axon and dendrites are filaments that extrude from it. Dendrites typically branch profusely, getting thinner with each branching, and extending their farthest branches a few hundred micrometres from the soma. The axon leaves the soma at a swelling called the axon hillock, and can extend for great distances, giving rise to hundreds of branches. Unlike dendrites, an axon usually maintains the same diameter as it extends. The soma may give rise to numerous dendrites, but never to more than one axon. Synaptic signals from other neurons are received by the soma and dendrites; signals to other neurons are transmitted by the axon. A typical synapse, then, is a contact between the axon of one neuron and a dendrite or soma of another. Synaptic signals may be excitatory or inhibitory. If the net excitation received by a neuron over a short period of time is large enough, the neuron generates a brief pulse called an action potential, which originates at the soma and propagates rapidly along the axon, activating synapses onto other neurons as it goes.

Many neurons fit the foregoing schema in every respect, but there are also exceptions to most parts of it. There are no neurons that lack a soma, but there are neurons that lack dendrites, and others that lack an axon. Furthermore, in addition to the typical axodendritic and axosomatic synapses, there are axoaxonic (axon-to-axon) and dendrodendritic (dendrite-to-dendrite) synapses.

The key to neural function is the synaptic signalling process, which is partly electrical and partly chemical. The electrical aspect depends on properties of the neuron's membrane. Like all animal cells, every neuron is surrounded by a plasma membrane, a bilayer of lipid molecules with many types of protein structures embedded in it. A lipid bilayer is a powerful electrical insulator, but in neurons, many of the protein structures embedded in the membrane are electrically active. These include ion channels that permit electrically charged ions to flow across the membrane, and ion pumps that actively transport ions from one side of the membrane to the other. Most ion channels are permeable only to specific types of ions. Some ion channels are voltage gated, meaning that they can be switched between open and closed states by altering the voltage difference across the membrane. Others are chemically gated, meaning that they can be switched between open and closed states by interactions with chemicals that diffuse through the extracellular fluid. The interactions between ion channels and ion pumps produce a voltage difference across the membrane, typically a bit less than 1/10 of a volt at baseline. This voltage has two functions: first, it provides a power source for an assortment of voltage-dependent protein machinery that is embedded in the membrane; second, it provides a basis for electrical signal transmission between different parts of the membrane.

Neurons communicate by chemical and electrical synapses in a process known as synaptic transmission. The fundamental process that triggers synaptic transmission is the action potential, a propagating electrical signal that is generated by exploiting the

electrically excitable membrane of the neuron. This is also known as a wave of depolarization.

Anatomy and histology

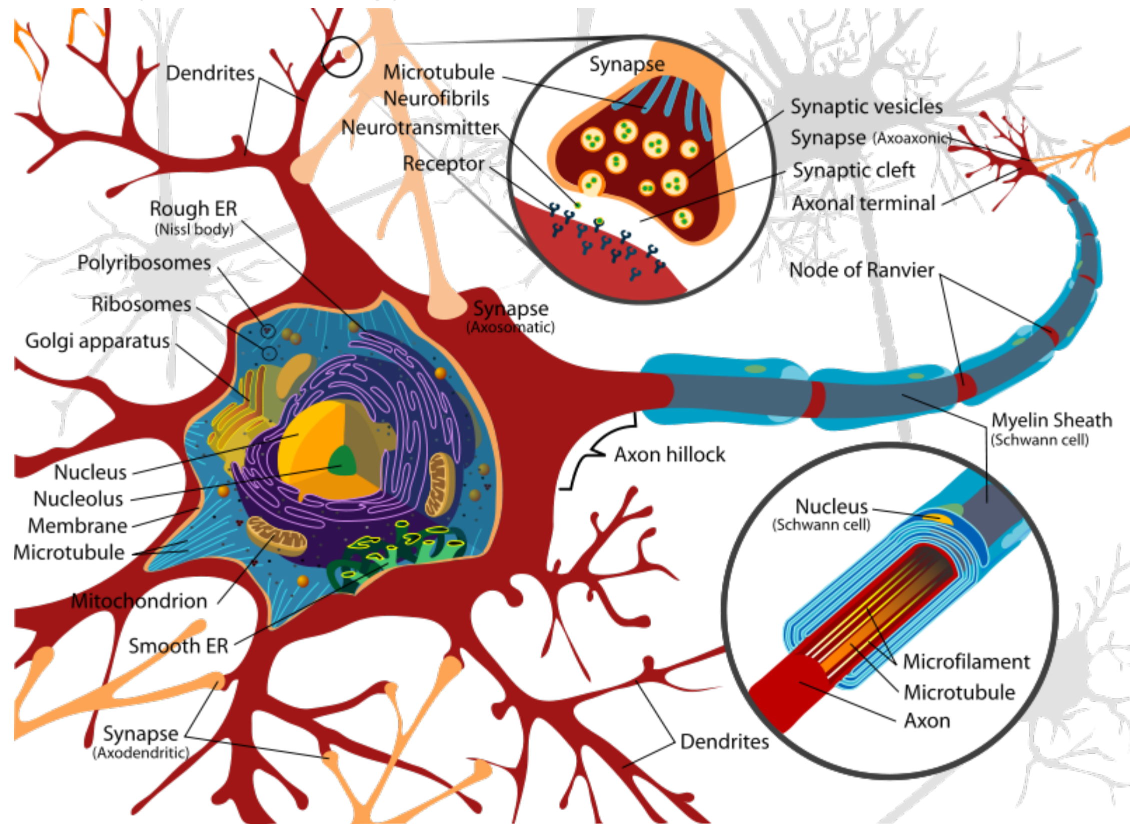


Diagram of a typical myelinated vertebrate motoneuron

Neurons are highly specialized for the processing and transmission of cellular signals. Given the diversity of functions performed by neurons in different parts of the nervous system, there is, as expected, a wide variety in the shape, size, and electrochemical properties of neurons. For instance, the soma of a neuron can vary from 4 to 100 micrometers in diameter.

- The soma is the central part of the neuron. It contains the nucleus of the cell, and therefore is where most protein synthesis occurs. The nucleus ranges from 3 to 18 micrometers in diameter.

- The dendrites of a neuron are cellular extensions with many branches, and metaphorically this overall shape and structure is referred to as a dendritic tree. This is where the majority of input to the neuron occurs.

- The axon is a finer, cable-like projection which can extend tens, hundreds, or even tens of thousands of times the diameter of the soma in length. The axon carries nerve signals away from the soma (and also carries some types of information

back to it). Many neurons have only one axon, but this axon may—and usually will—undergo extensive branching, enabling communication with many target cells. The part of the axon where it emerges from the soma is called the axon hillock. Besides being an anatomical structure, the axon hillock is also the part of the neuron that has the greatest density of voltage-dependent sodium channels. This makes it the most easily-excited part of the neuron and the spike initiation zone for the axon: in electrophysiological terms it has the most negative action potential threshold. While the axon and axon hillock are generally involved in information outflow, this region can also receive input from other neurons.

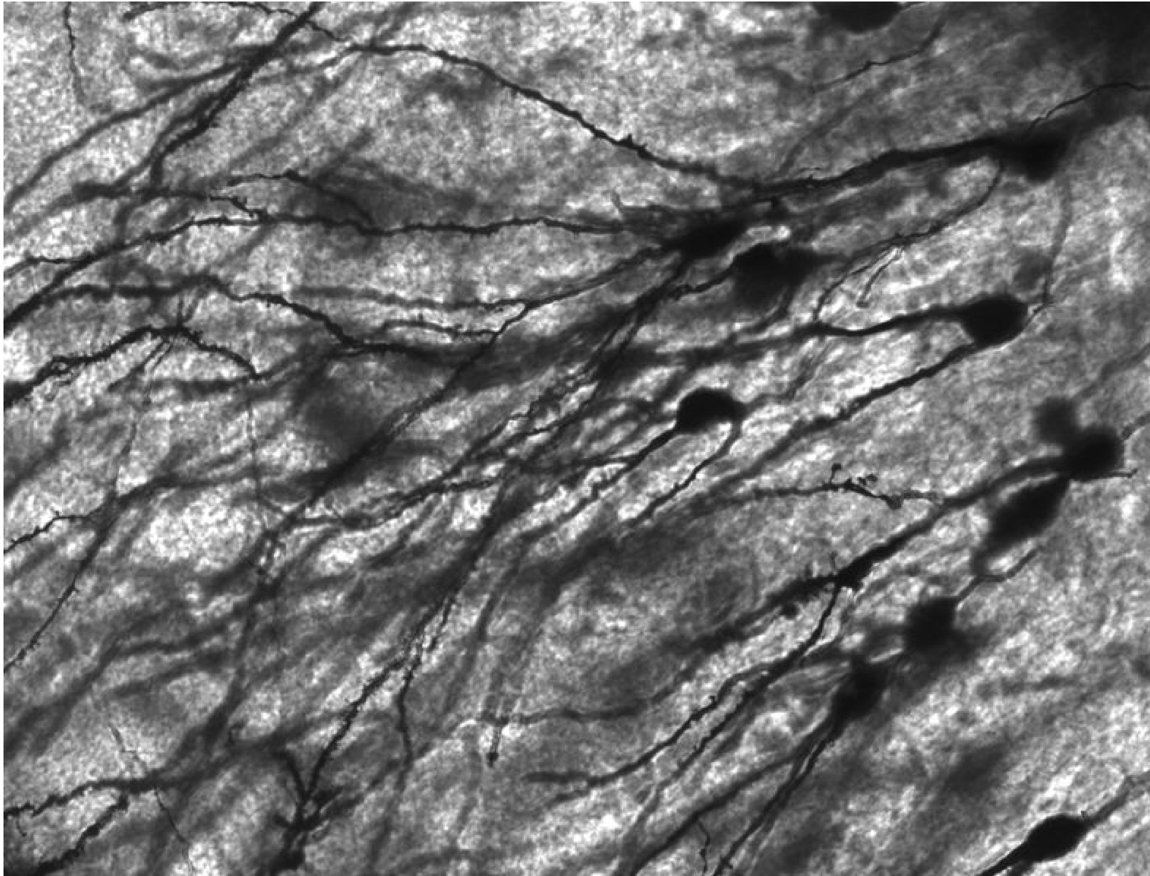
- The axon terminal contains synapses, specialized structures where neurotransmitter chemicals are released in order to communicate with target neurons.

Although the canonical view of the neuron attributes dedicated functions to its various anatomical components, dendrites and axons often act in ways contrary to their so-called main function.

Axons and dendrites in the central nervous system are typically only about one micrometer thick, while some in the peripheral nervous system are much thicker. The soma is usually about 10–25 micrometers in diameter and often is not much larger than the cell nucleus it contains. The longest axon of a human motoneuron can be over a meter long, reaching from the base of the spine to the toes. Sensory neurons have axons that run from the toes to the dorsal columns, over 1.5 meters in adults. Giraffes have single axons several meters in length running along the entire length of their necks. Much of what is known about axonal function comes from studying the squid giant axon, an ideal experimental preparation because of its relatively immense size (0.5–1 millimeters thick, several centimeters long).

Fully differentiated neurons are permanently amitotic; however, recent research shows that additional neurons throughout the brain can originate from neural stem cells found throughout the brain but in particularly high concentrations in the subventricular zone and subgranular zone through the process of neurogenesis.

Histology and internal structure



Golgi-stained neurons in human hippocampal tissue

Nerve cell bodies stained with basophilic dyes show numerous microscopic clumps of Nissl substance (named after German psychiatrist and neuropathologist Franz Nissl, 1860–1919), which consists of rough endoplasmic reticulum and associated ribosomal RNA. The prominence of the Nissl substance can be explained by the fact that nerve cells are metabolically very active, and hence are involved in large amounts of protein synthesis.

The cell body of a neuron is supported by a complex meshwork of structural proteins called neurofilaments, which are assembled into larger neurofibrils. Some neurons also contain pigment granules, such as neuromelanin (a brownish-black pigment, byproduct of synthesis of catecholamines) and lipofuscin (yellowish-brown pigment that accumulates with age).

There are different internal structural characteristics between axons and dendrites. Typical axons almost never contain ribosomes, except some in the initial segment. Dendrites contain granular endoplasmic reticulum or ribosomes, with diminishing amounts with distance from the cell body.

Classes

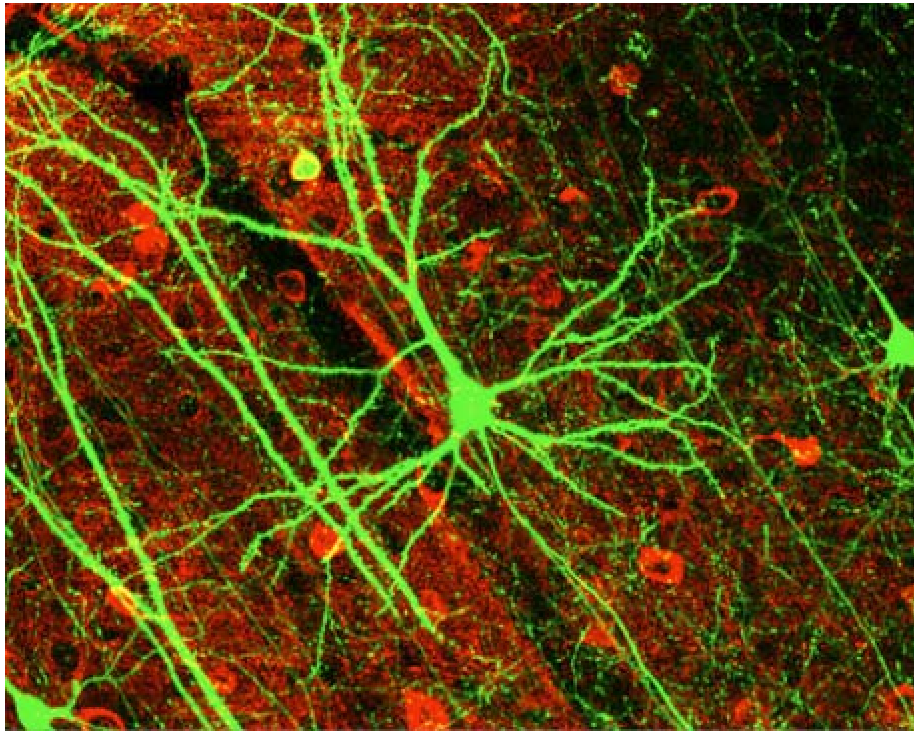
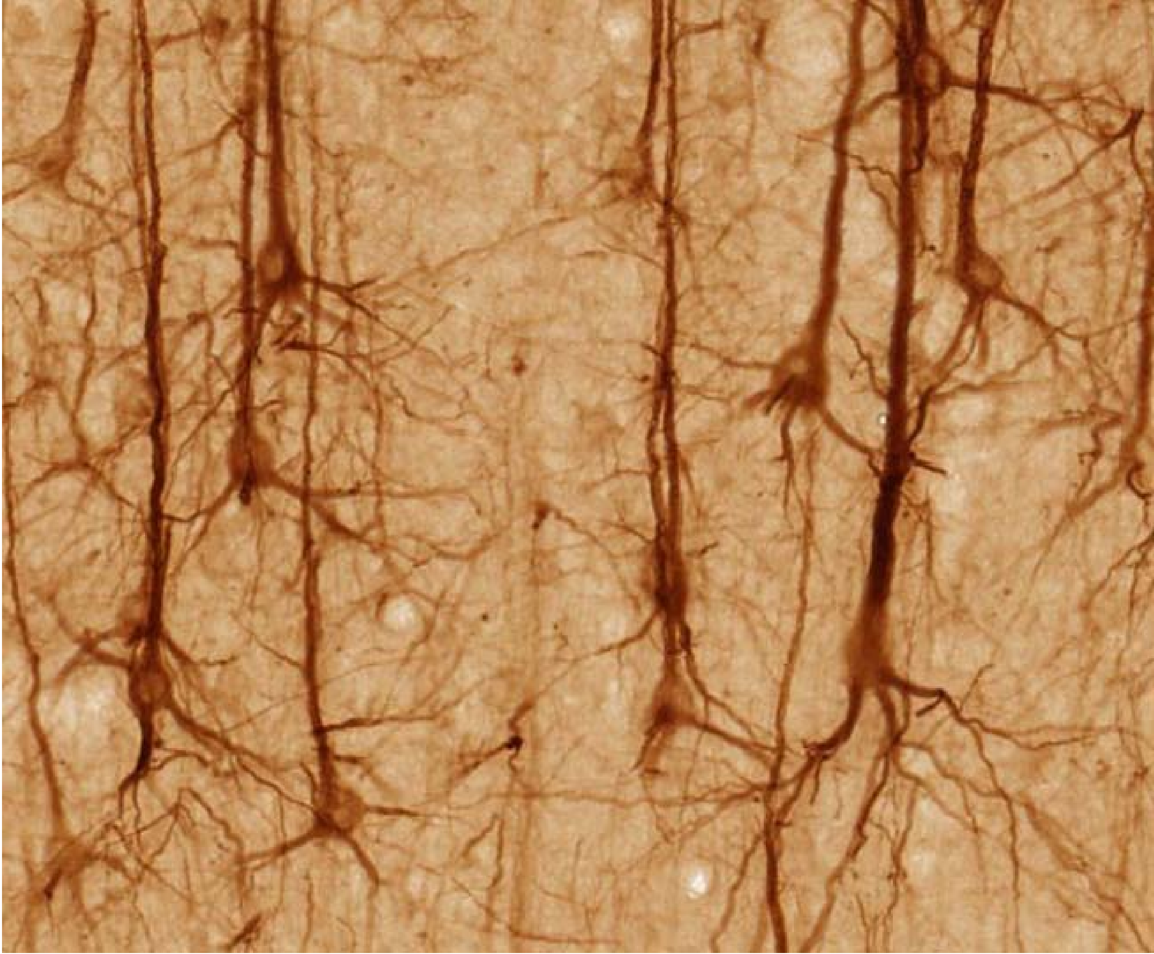


Image of pyramidal neurons in mouse cerebral cortex expressing green fluorescent protein. The red staining indicates GABAergic interneurons.



SMI32-stained pyramidal neurons in cerebral cortex

Neurons exist in a number of different shapes and sizes and can be classified by their morphology and function. The anatomist Camillo Golgi grouped neurons into two types; type I with long axons used to move signals over long distances and type II with short axons, which can often be confused with dendrites. Type I cells can be further divided by where the cell body or soma is located. The basic morphology of type I neurons, represented by spinal motor neurons, consists of a cell body called the soma and a long thin axon which is covered by the myelin sheath. Around the cell body is a branching dendritic tree that receives signals from other neurons. The end of the axon has branching terminals (axon terminal) that release neurotransmitters into a gap called the synaptic cleft between the terminals and the dendrites of the next neuron.

Structural classification

Polarity

Most neurons can be anatomically characterized as:

- Unipolar or pseudounipolar: dendrite and axon emerging from same process.
- Bipolar: axon and single dendrite on opposite ends of the soma.
- Multipolar: more than two dendrites:
    - Golgi I: neurons with long-projecting axonal processes; examples are pyramidal cells, Purkinje cells, and anterior horn cells.
    - Golgi II: neurons whose axonal process projects locally; the best example is the granule cell.

Other

Furthermore, some unique neuronal types can be identified according to their location in the nervous system and distinct shape. Some examples are:

- Basket cells, interneurons that form a dense plexus of terminals around the soma of target cells, found in the cortex and cerebellum.
- Betz cells, large motor neurons.
- Medium spiny neurons, most neurons in the corpus striatum.
- Purkinje cells, huge neurons in the cerebellum, a type of Golgi I multipolar neuron.
- Pyramidal cells, neurons with triangular soma, a type of Golgi I.
- Renshaw cells, neurons with both ends linked to alpha motor neurons.
- Granule cells, a type of Golgi II neuron.
- Anterior horn cells, motoneurons located in the spinal cord.

Functional classification

Direction

- Afferent neurons convey information from tissues and organs into the central nervous system and are sometimes also called sensory neurons.
- Efferent neurons transmit signals from the central nervous system to the effector cells and are sometimes called motor neurons.
- Interneurons connect neurons within specific regions of the central nervous system.

Afferent and efferent can also refer generally to neurons which, respectively, bring information to or send information from the brain region.

Action on other neurons

A neuron affects other neurons by releasing a neurotransmitter that binds to chemical receptors. The effect upon the target neuron is determined not by the source neuron or by the neurotransmitter, but by the type of receptor that is activated. A neurotransmitter can be thought of as a key, and a receptor as a lock: the same type of key can here be used to open many different types of locks. Receptors can be classified broadly as *excitatory* (causing an increase in firing rate), *inhibitory* (causing a decrease in firing rate), or *modulatory* (causing long-lasting effects not directly related to firing rate).

In fact, however, the two most common neurotransmitters in the brain, glutamate and GABA, have actions that are largely consistent. Glutamate acts on several different types of receptors, but most of them have effects that are excitatory. Similarly GABA acts on several different types of receptors, but all of them have effects (in adult animals, at least) that are inhibitory. Because of this consistency, it is common for neuroscientists to simplify the terminology by referring to cells that release glutamate as "excitatory neurons," and cells that release GABA as "inhibitory neurons." Since well over 90% of the neurons in the brain release either glutamate or GABA, these labels encompass the great majority of neurons. There are also other types of neurons that have consistent effects on their targets, for example "excitatory" motor neurons in the spinal cord that release acetylcholine, and "inhibitory" spinal neurons that release glycine.

The distinction between excitatory and inhibitory neurotransmitters is not absolute, however. Rather, it depends on the class of chemical receptors present on the target neuron. In principle, a single neuron, releasing a single neurotransmitter, can have excitatory effects on some targets, inhibitory effects on others, and modulatory effects on others still. For example, photoreceptor cells in the retina constantly release the neurotransmitter glutamate in the absence of light. So-called OFF bipolar cells are, like most neurons, excited by the released glutamate. However, neighboring target neurons called ON bipolar cells are instead *inhibited* by glutamate, because they lack the typical ionotropic glutamate receptors and instead express a class of inhibitory metabotropic glutamate receptors. When light is present, the photoreceptors cease releasing glutamate, which relieves the ON bipolar cells from inhibition, activating them; this simultaneously removes the excitation from the OFF bipolar cells, silencing them.

Discharge patterns

Neurons can be classified according to their electrophysiological characteristics:

- **Tonic or regular spiking**. Some neurons are typically constantly (or tonically) active. Example: interneurons in neurostriatum.
- **Phasic or bursting**. Neurons that fire in bursts are called phasic.
- **Fast spiking**. Some neurons are notable for their high firing rates, for example some types of cortical inhibitory interneurons, cells in globus pallidus, retinal ganglion cells.

Classification by neurotransmitter production

Neurons differ in the type of neurotransmitter they manufacture. Some examples are:

- Cholinergic neurons—acetylcholine. Acetylcholine is released from presynaptic neurons into the synaptic cleft. It acts as a ligand for both ligand-gated ion channels and metabotropic (GPCRs) muscarinic receptors. Nicotinic receptors, are pentameric ligand-gated ion channels composed of alpha and beta subunits that bind nicotine. Ligand binding opens the channel causing influx of Na^+ depolarization and increases the probability of presynaptic neurotransmitter release.

- GABAergic neurons—gamma aminobutyric acid. GABA is one of two neuroinhibitors in the CNS, the other being Glycine. GABA has a homologous function to ACh, gating anion channels that allow Cl- ions to enter the post synaptic neuron. Cl- causes hyperpolarization within the neuron, decreasing the probability of an action potential firing as the voltage becomes more negative (recall that for an action potential to fire, a positive voltage threshold must be reached).

- Glutamatergic neurons—glutamate. Glutamate is one of two primary excitatory amino acids, the other being Aspartate. Glutamate receptors are one of four categories, three of which are ligand-gated ion channels and one of which is a G-protein coupled receptor (often referred to as GPCR).

  1. AMPA and Kainate receptors both function as cation channels permeable to Na^+ cation channels mediating fast excitatory synaptic transmission
  2. NMDA receptors are another cation channel that is more permeable to Ca^{2+}. The function of NMDA receptors is dependant on Glycine receptor binding as a co-agonist within the channel pore. NMDA receptors will not function without both ligands present.
  3. Metabotropic receptors, GPCRs modulate synaptic transmission and postsynaptic excitability.

  Glutamate can cause excitotoxicity when blood flow to the brain is interrupted, resulting in brain damage. When blood flow is suppressed, glutamate is released from presynaptic neurons causing NMDA and AMPA receptor activation moreso than would normally be the case outside of stress conditions, leading to elevated Ca^{2+} and Na^+ entering the post synaptic neuron and cell damage.

- Dopaminergic neurons—dopamine. Dopamine is a neurotransmitter that acts on D1 type (D1 and D5) Gs coupled receptors which increase cAMP and PKA or D2 type (D2,D3 and D4)receptors which activate Gi-coupled receptors that decrease cAMP and PKA. Dopamine is connected to mood and behavior, and modulates both pre and post synaptic neurotransmission. Loss of dopamine neurons in the substantia nigra has been linked to Parkinson's disease.

- Serotonergic neurons—serotonin. Serotonin,(5-Hydroxytryptamine, 5-HT), can act as excitatory or inhibitory. Of the four 5-HT receptor classes, 3 are GPCR and 1 is ligand gated cation channel. Serotonin is synthesized from tryptophan by tryptophan hydroxylase, and then further by aromatic acid decarboxylase. A lack of 5-HT at postsynaptic neurons has been linked to depression. Drugs that block the presynaptic serotonin transporter are used for treatment, such as Prozac and Zoloft.

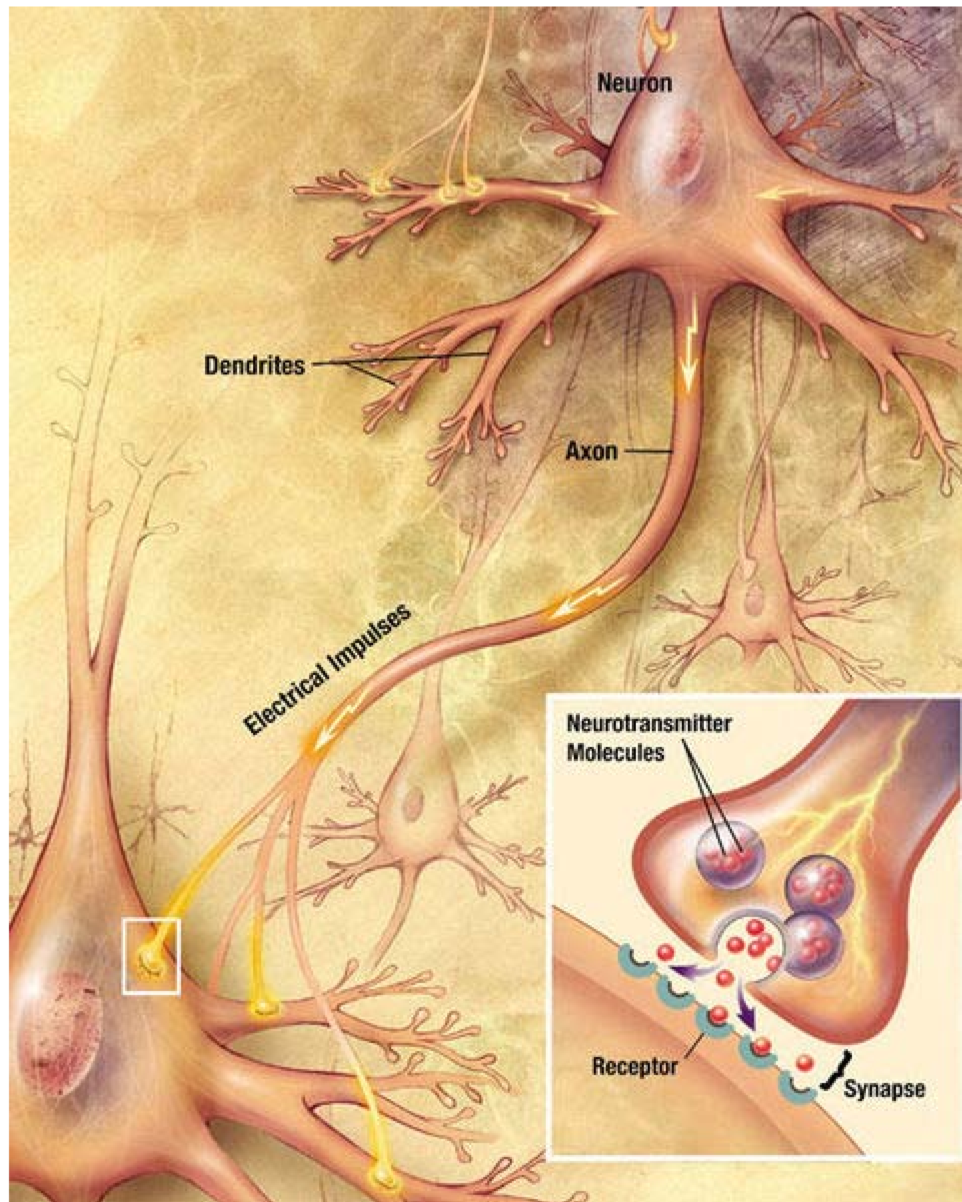
Connectivity

Neurons communicate with one another via synapses, where the axon terminal or *en passant* boutons (terminals located along the length of the axon) of one cell impinges upon another neuron's dendrite, soma or, less commonly, axon. Neurons such as Purkinje cells in the cerebellum can have over 1000 dendritic branches, making connections with tens of thousands of other cells; other neurons, such as the magnocellular neurons of the supraoptic nucleus, have only one or two dendrites, each of which receives thousands of synapses. Synapses can be excitatory or inhibitory and will either increase or decrease activity in the target neuron. Some neurons also communicate via electrical synapses, which are direct, electrically-conductive junctions between cells.

In a chemical synapse, the process of synaptic transmission is as follows: when an action potential reaches the axon terminal, it opens voltage-gated calcium channels, allowing calcium ions to enter the terminal. Calcium causes synaptic vesicles filled with neurotransmitter molecules to fuse with the membrane, releasing their contents into the synaptic cleft. The neurotransmitters diffuse across the synaptic cleft and activate receptors on the postsynaptic neuron.

The human brain has a huge number of synapses. Each of the 10^{11} (one hundred billion) neurons has on average 7,000 synaptic connections to other neurons. It has been estimated that the brain of a three-year-old child has about 10^{15} synapses (1 quadrillion). This number declines with age, stabilizing by adulthood. Estimates vary for an adult, ranging from 10^{14} to 5×10^{14} synapses (100 to 500 trillion).

Mechanisms for propagating action potentials



A signal propagating down an axon to the cell body and dendrites of the next cell

In 1937, John Zachary Young suggested that the squid giant axon could be used to study neuronal electrical properties. Being larger than but similar in nature to human neurons, squid cells were easier to study. By inserting electrodes into the giant squid axons, accurate measurements were made of the membrane potential.

The cell membrane of the axon and soma contain voltage-gated ion channels which allow the neuron to generate and propagate an electrical signal (an action potential). These signals are generated and propagated by charge-carrying ions including sodium (Na^+), potassium (K^+), chloride (Cl^-), and calcium (Ca^{2+}).

There are several stimuli that can activate a neuron leading to electrical activity, including pressure, stretch, chemical transmitters, and changes of the electric potential across the cell membrane. Stimuli cause specific ion-channels within the cell membrane to open, leading to a flow of ions through the cell membrane, changing the membrane potential.

Thin neurons and axons require less metabolic expense to produce and carry action potentials, but thicker axons convey impulses more rapidly. To minimize metabolic expense while maintaining rapid conduction, many neurons have insulating sheaths of myelin around their axons. The sheaths are formed by glial cells: oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system. The sheath enables action potentials to travel faster than in unmyelinated axons of the same diameter, whilst using less energy. The myelin sheath in peripheral nerves normally runs along the axon in sections about 1 mm long, punctuated by unsheathed nodes of Ranvier which contain a high density of voltage-gated ion channels. Multiple sclerosis is a neurological disorder that results from demyelination of axons in the central nervous system.

Some neurons do not generate action potentials, but instead generate a graded electrical signal, which in turn causes graded neurotransmitter release. Such nonspiking neurons tend to be sensory neurons or interneurons, because they cannot carry signals long distances.

Neural coding

Neural coding is concerned with how sensory and other information is represented in the brain by neurons. The main goal of studying neural coding is to characterize the relationship between the stimulus and the individual or ensemble neuronal responses, and the relationships amongst the electrical activities of the neurons within the ensemble. It is thought that neurons can encode both digital and analog information.

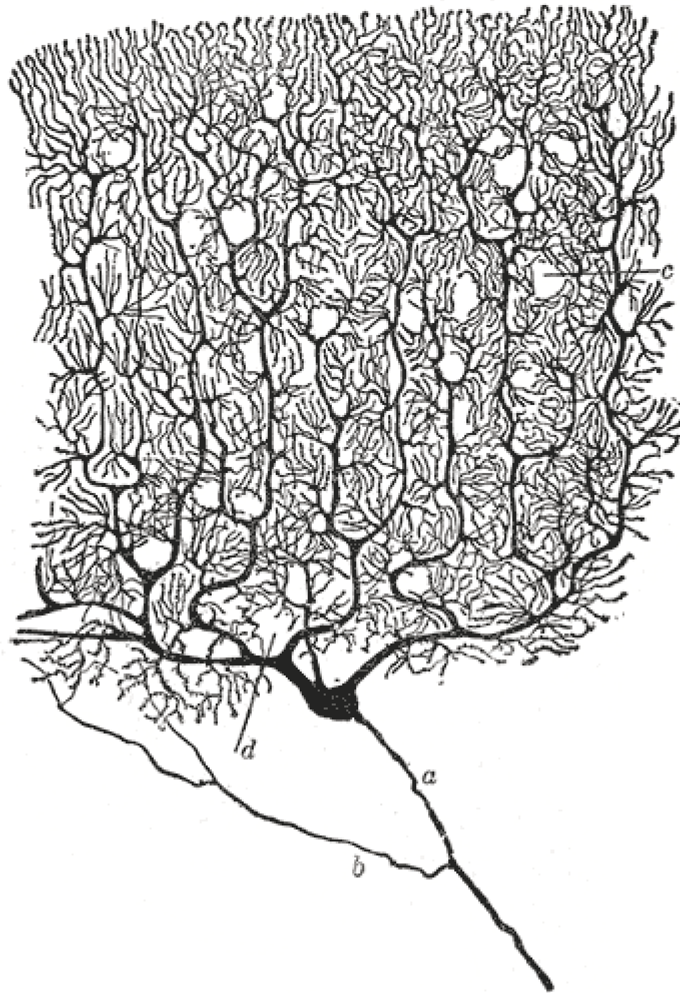
All-or-none principle

The conduction of nerve impulses is an example of an all-or-none response. In other words, if a neuron responds at all, then it must respond completely. Greater intensity of stimulation does not produce a stronger signal but can produce *more* impulses per second. There are different types of receptor response to stimulus, slowly adapting or tonic receptors respond to steady stimulus and produce a steady rate of firing. These tonic receptors most often respond to increased intensity of stimulus by increasing their firing frequency, usually as a power function of stimulus plotted against impulses per second. This can be likened to an intrinsic property of light where to get greater intensity of a specific frequency (color) there have to be more photons, as the photons can't become "stronger" for a specific frequency.

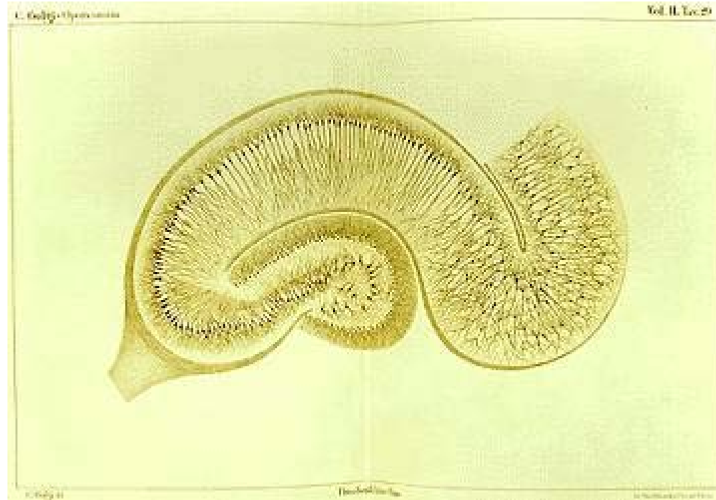
There are a number of other receptor types that are called quickly-adapting or phasic receptors, where firing decreases or stops with steady stimulus; examples include: skin when touched by an object causes the neurons to fire, but if the object maintains even

pressure against the skin, the neurons stop firing. The neurons of the skin and muscles that are responsive to pressure and vibration have filtering accessory structures that aid their function. The pacinian corpuscle is one such structure; it has concentric layers like an onion which form around the axon terminal. When pressure is applied and the corpuscle is deformed, mechanical stimulus is transferred to the axon, which fires. If the pressure is steady, there is no more stimulus; thus, typically these neurons respond with a transient depolarization during the initial deformation and again when the pressure is removed, which causes the corpuscle to change shape again. Other types of adaptation are important in extending the function of a number of other neurons.

History



Drawing of a Purkinje cell in the cerebellum cortex done by Santiago Ramón y Cajal, demonstrating the ability of Golgi's staining method to reveal fine detail



Drawing by Camillo Golgi of a hippocampus stained with the silver nitrate method

The term *neuron* was coined by the German anatomist Heinrich Wilhelm Waldeyer. The neuron's place as the primary functional unit of the nervous system was first recognized in the early 20th century through the work of the Spanish anatomist Santiago Ramón y Cajal. Cajal proposed that neurons were discrete cells that communicated with each other via specialized junctions, or spaces, between cells. This became known as the neuron doctrine, one of the central tenets of modern neuroscience. To observe the structure of individual neurons, Cajal improved a silver staining process known as Golgi's method, which had been developed by his rival, Camillo Golgi. Cajal's improvement, which involved a technique he called "double impregnation", is still in use. The silver impregnation stains are an extremely useful method for neuroanatomical investigations because, for reasons unknown, it stains a very small percentage of cells in a tissue, so one is able to see the complete micro structure of individual neurons without much overlap from other cells in the densely packed brain.

The neuron doctrine

The neuron doctrine is the now fundamental idea that neurons are the basic structural and functional units of the nervous system. The theory was put forward by Santiago Ramón y Cajal in the late 19th century. It held that neurons are discrete cells (not connected in a meshwork), acting as metabolically distinct units.

Later discoveries yielded a few refinements to the simplest form of the doctrine. For example, glial cells, which are not considered neurons, play an essential role in information processing. Also, electrical synapses are more common than previously thought, meaning that there are direct, cytoplasmic connections between neurons. In fact, there are examples of neurons forming even tighter coupling: the squid giant axon arises from the fusion of multiple axons.

Cajal also postulated the Law of Dynamic Polarization, which states that a neuron receives signals at its dendrites and cell body and transmits them, as action potentials,

along the axon in one direction: away from the cell body. The Law of Dynamic Polarization has important exceptions; dendrites can serve as synaptic output sites of neurons and axons can receive synaptic inputs.

Neurons in the brain

The number of neurons in the brain varies dramatically from species to species. One estimate puts the human brain at about 100 billion (10^{11}) neurons and 100 trillion (10^{14}) synapses. Another estimate is 86 billion neurons of which 16.3 billion are in the cerebral cortex and 69 billion in the cerebellum. By contrast, the nematode worm *Caenorhabditis elegans* has just 302 neurons making it an ideal experimental subject as scientists have been able to map all of the organism's neurons. The fruit fly *Drosophila melanogaster*, a common subject in biology experiments, has around 100,000 neurons and exhibits many complex behaviors. Many properties of neurons, from the type of neurotransmitters used to ion channel composition, are maintained across species, allowing scientists to study processes occurring in more complex organisms in much simpler experimental systems.

Neurological disorders

Charcot-Marie-Tooth disease (CMT), also known as Hereditary Motor and Sensory Neuropathy (HMSN), Hereditary Sensorimotor Neuropathy (HMSN), or Peroneal Muscular Atrophy, is a heterogeneous inherited disorder of nerves (neuropathy) that is characterized by loss of muscle tissue and touch sensation, predominantly in the feet and legs but also in the hands and arms in the advanced stages of disease. Presently incurable, this disease is one of the most common inherited neurological disorders, with 37 in 100,000 affected.

Alzheimer's disease (AD), also known simply as Alzheimer's, is a neurodegenerative disease characterized by progressive cognitive deterioration together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. The most striking early symptom is loss of short-term memory (amnesia), which usually manifests as minor forgetfulness that becomes steadily more pronounced with illness progression, with relative preservation of older memories. As the disorder progresses, cognitive (intellectual) impairment extends to the domains of language (aphasia), skilled movements (apraxia), recognition (agnosia), and functions such as decision-making and planning get impaired.

Parkinson's disease (also known as Parkinson disease or PD) is a degenerative disorder of the central nervous system that often impairs the sufferer's motor skills and speech. Parkinson's disease belongs to a group of conditions called movement disorders. It is characterized by muscle rigidity, tremor, a slowing of physical movement (bradykinesia), and in extreme cases, a loss of physical movement (akinesia). The primary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of dopamine, which is produced in the dopaminergic neurons of the brain. Secondary symptoms may include high level cognitive dysfunction and subtle language problems. PD is both chronic and progressive.

Myasthenia Gravis is a neuromuscular disease leading to fluctuating muscle weakness and fatigability. Weakness is typically caused by circulating antibodies that block acetylcholine receptors at the post-synaptic neuromuscular junction, inhibiting the stimulative effect of the neurotransmitter acetylcholine. Myasthenia is treated with immunosuppressants, cholinesterase inhibitors and, in selected cases, thymectomy.

Demyelination

Demyelination is the act of demyelinating, or the loss of the myelin sheath insulating the nerves. When myelin degrades, conduction of signals along the nerve can be impaired or lost, and the nerve eventually withers. This leads to certain neurodegenerative disorders like multiple sclerosis, chronic inflammatory demyelinating polyneuropathy.

Axonal degeneration

Although most injury responses include a calcium influx signaling to promote resealing of severed parts, axonal injuries initially lead to acute axonal degeneration (AAD), which is rapid separation of the proximal and distal ends within 30 minutes of injury. Degeneration follows with swelling of the axolemma, and eventually leads to bead like formation. Granular disintegration of the axonal cytoskeleton and inner organelles occurs after axolemma degradation. Early changes include accumulation of mitochondria in the paranodal regions at the site of injury. Endoplasmic reticulum degrades and mitochondria swell up and eventually disintegrate. The disintegration is dependent on Ubiquitin and Calpain proteases (caused by influx of calcium ion), suggesting that axonal degeneration is an active process. Thus the axon undergoes complete fragmentation. The process takes about roughly 24 hrs in the PNS, and longer in the CNS. The signaling pathways leading to axolemma degeneration are currently unknown.

Nerve regeneration

It has been demonstrated that neurogenesis can sometimes occur in the adult vertebrate brain, and it is often possible for peripheral axons to regrow if they are severed. The latter can take a long time: after a nerve injury to the human arm, for example, it may take months for feeling to return to the hands and fingers.

Chapter 20

Germ Cell

In biology, **germ cells** are the cells that give rise to the gametes of organisms that reproduce sexually. In many animals, the germ cells originate near the gut and migrate to the developing gonads. There, they undergo cell division of two types, mitosis and meiosis, followed by cellular differentiation into mature gametes, either eggs or sperm. Unlike animals, plants do not have a germ line set aside in early development. Instead, germ cells can come from somatic cells in the adult floral meristem.

Introduction

Multicellular eukaryotes are made of two fundamental cell types. Germ cells produce gametes and are the only cells that can undergo meiosis as well as mitosis. These cells are sometimes said to be immortal because they are the link between generations. Somatic cells are all the other cells that form the building blocks of the body and they only divide by mitosis. The lineage of germ cells is called germ line. Germ cell specification begins during cleavage in many animals or in the epiblast during gastrulation in birds and mammals. After transport, involving passive movements and active migration, germ cells arrive at the developing gonads. In humans, sexual differentiation starts approximately 6 weeks after conception. The end-products of the germ cell cycle are the egg or sperm.

Under special conditions *in vitro* germ cells can acquire properties similar to those of embryonic stem cells (ES). The underlying mechanism of that change is still unknown. These changed cells are then called embryonic germ cells (EG). Both EG and ES are pluripotent. Recent studies have demonstrated that it is possible to give rise to primordial germ cells from ES.

Specification

There are two mechanisms to establish the germ cell lineage in the embryo. The first way is called preformistic and involves that the cells destined to become germ cells inherit the specific germ cell determinants present in the germ plasm (specific area of the cytoplasm) of the egg (ovum). The unfertilized egg of most animals is asymmetrical: different regions of cytoplasm contain different amounts mRNA and proteins. By this germ cells obtained by the first divisions of the fertilized egg are characterized by specific molecules of a particular region of the egg cytoplasm. The second way is found in birds and mammals, where germ cells are not specified by such determinants but by signals

controlled by zygotic genes. In mammals, a few cells of the early embryo are induced by signals of neighboring cells to become primordial germ cells. Mammalian eggs are somewhat symmetrical and after the first divisions of the fertilized egg, the produced cells are all totipotent. This means that they can differentiate in any cell type in the body and thus germ cells.

Migration

Primordial germ cells, germ cells that still have to reach the gonads, also known as PGCs, precursor germ cells or gonocytes, divide repeatedly on their migratory route through the gut and into the developing gonads.

Invertebrates

In the model organism *Drosophila*, pole cells passively move from the posterior end of the embryo to the posterior midgut because of the infolding of the blastoderm. Then they actively move through the gut into the mesoderm. Endodermal cells differentiate and together with Wunen proteins they induce the migration through the gut. Wunen proteins are chemorepellants that lead the germ cells away from the endoderm and into the mesoderm. After splitting into two populations, the germ cells continue migrating laterally and in parallel until they reach the gonads. Columbus proteins, chemoattractants, stimulate the migration in the gonadal mesoderm.

Vertebrates

In the *Xenopus* egg, the germ cell determinants are found in the most vegetal blastomeres. These presumptive PGCs are brought to the endoderm of the blastocoel by gastrulation. They are determined as germ cells when gastrulation is completed. Migration from the hindgut along the gut and across the dorsal mesentery then takes place. The germ cells split into two populations and move to the paired gonadal ridges. Migration starts with 3-4 cells that undergo three rounds of cell division so that about 30 PGCs arrive at the gonads. On the migratory path of the PGCs, the orientation of underlying cells and their secreted molecules such as fibronectin play an important role.

Mammals have a migratory path comparable to that in *Xenopus*. Migration begins with 50 gonocytes and about 5,000 PGCs arrive at the gonads. Proliferation occurs also during migration and lasts for 3-4 weeks in humans.

PGCs come from the epiblast and migrate subsequently into the mesoderm, the endoderm and the posterior of the yolk sac. Migration then takes place from the hindgut along the gut and across the dorsal mesentery to reach the gonads (4.5 weeks in human beings). Fibronectin maps here also a polarized network together with other molecules. The somatic cells on the path of germ cells provide them attractive, repulsive, and survival signals. But germ cells also send signals to each other.

In reptiles and birds, germ cells use another path. PGCs come from the epiblast and move to the hypoblast to form the germinal crescent (anterior extraembryonic structure). The gonocytes then squeeze into blood vessels and use the circulatory system for transport. They squeeze out of the vessels when they are at height of the gonadal ridges. Cell adhesion on the endothelium of the blood vessels and molecules such as chemoattractants are probably involved in helping PGCs migrate.

*Sex determining region of Y (*Sry*) gene*

The sex of a mammalian individual is determined by the *Sry* gene on the Y chromosome. It induces the somatic cells of the gonadal ridge to develop into a testis. *Sry* is expressed in a small group of somatic cells of the developing gonad and influence these cells to become Sertoli cells (supporting cells in testis). Sertoli cells are responsible for sexual development along a male pathway in many ways. One of these ways involves stimulation of the arriving primordial cells to differentiate into sperm. In the absence of the *Sry* gene, primordial germ cells differentiate into eggs. Removing genital ridges before they started to develop into testes or ovaries results in the development of a female, independent of the carried sex chromosome.

Gametogenesis

Gametogenesis, the development of diploid germ cells into either haploid eggs or sperm, (respectively oogenesis and spermatogenesis) is different for each species but the general stages are similar. Oogenesis and spermatogenesis have many features in common, they both involve:

- Meiosis
- Extensive morphological differentiation
- Incapacity of surviving for very long if fertilization does not occur

Despite their homologies they also have major differences:

- Spermatogenesis has equivalent meiotic divisions resulting in four equivalent spermatids while oogenic meiosis is asymmetrical: only one egg is formed together with three polar bodies.
- Different timing of maturation: oogenic meiosis is interrupted at one or more stages (for a long time) while spermatogenic meiosis is rapid and uninterrupted.

Oogenesis

After migration primordial germ cells will become oogonia in the forming gonad (ovary). The oogonia proliferate extensively by mitotic divisions, up to 5-7 million cells in humans. But then many of these oogonia die and about 50,000 remain. These cells differentiate into primary oocytes. In week 11-12 *post coitus* the first meiotic division begins (before birth for most mammals) and remains arrested in prophase I from a few days to many years depending on the species. It is in this period or in some cases at the

beginning of sexual maturity that the primary oocytes secrete proteins to form a coat called zona pellucida and they also produce cortical granules containing enzymes and proteins needed for fertilization. Meiosis stands by because of the follicular granulosa cells that send inhibitory signals through gap junctions and the zona pellucida. Sexual maturation is the beginning of periodic ovulation. Ovulation is the regular release of one oocyte from the ovary into the reproductive tract and is preceded by follicular growth. A few follicle cells are stimulated to grow but only one oocyte is ovulated. A primordial follicle consists of an epithelial layer of follicular granulosa cells enclosing an oocyte. The pituitary gland secrete follicle-stimulating hormones (FSHs) that stimulate follicular growth and oocyte maturation. The thecal cells around each follicle secrete estrogen. This hormone stimulates the production of FSH receptors on the follicular granulosa cells and has at the same time a negative feedback on FSH secretion. This results in a competition between the follicles and only the follicle with the most FSH receptors survives and is ovulated. Meiotic division I goes on in the ovulated oocyte stimulated by luteinizing hormones (LHs) produced by the pituitary gland. FSH and LH block the gap junctions between follicle cells and the oocyte therefore inhibiting communication between them. Most follicular granulosa cells stay around the oocyte and so form the cumulus layer. Large non-mammalian oocytes accumulate egg yolk, glycogen, lipids, ribosomes, and the mRNA needed for protein synthesis during early embryonic growth. These intensive RNA biosynthese are mirrored in the structure of the chromosomes, which decondense and form lateral loops giving them a lampbrush appearance. Oocyte maturation is the following phase of oocyte development. It occurs at sexual maturity when hormones stimulate the oocyte to complete meiotic division I. The meiotic division I produces 2 cells differing in size: a small polar body and a large secondary oocyte. The secondary oocyte undergoes meiotic division II and that results in the formation of a second small polar body and a large mature egg, both being haploid cells. The polar bodies degenerate. Oocyte maturation stands by at metaphase II in most vertebrates. During ovulation, the arrested secondary oocyte leaves the ovary and matures rapidly into an egg ready for fertilization. Fertilization will cause the egg to complete meiosis II. In human females there is proliferation of the oogonia in the fetus, meiosis starts then before birth and stands by at meiotic division I up to 50 years, ovulation begins at puberty.

Egg growth

A 10 - 20 µm large somatic cell generally needs 24 hours to double its mass for mitosis. By this way it would take a very long time for that cell to reach the size of a mammalian egg with a diameter of 100 µm (some insects have eggs of about 1,000 µm or greater). Eggs have therefore special mechanisms to grow to their large size. One of these mechanisms is to have extra copies of genes: meiotic division I is paused so that the oocyte grows while it contains two diploid chromosome sets. Some species produce many extra copies of genes, such as amphibians, which may have up to 1 or 2 million copies. A complementary mechanism is partly dependent on syntheses of other cells. In amphibians, birds, and insects, yolk is made by the liver (or its equivalent) and secreted into the blood. Neighboring accessory cells in the ovary can also provide nutritive help of two types. In some invertebrates some oogonia become nurse cells. These cells are connected by cytoplasmic bridges with oocytes. The nurse cells of insects provide

oocytes macromolecules such as proteins and mRNA. Follicular granulosa cells are the second type of accessory cells in the ovary in both invertebrates and vertebrates. They form a layer around the oocyte and nourish them with small molecules, no macromolecules, but eventually their smaller precursor molecules, by gap junctions.

Spermatogenesis

Mammalian spermatogenesis is representative for most animals. In human males, spermatogenesis begins at puberty in seminiferous tubules in the testes and go on continuously. Spermatogonia are immature germ cells. They proliferate continuously by mitotic divisions around the outer edge of the seminiferous tubules, next to the basal lamina. Some of these cells stop proliferation and differentiate into primary spermatocytes. After they proceed through the first meiotic division, two secondary spermatocytes are produced. The two secondary spermatocytes undergo the second meiotic division to form four haploid spermatids. These spermatids differentiate morphologically into sperm by nuclear condensation, ejection of the cytoplasm and formation of the acrosome and flagellum.

The developing male germ cells do not complete cytokinesis during spermatogenesis. Consequently cytoplasmic bridges assure connection between the clones of differentiating daughter cells to form a syncytium. In this way the haploid cells are supplied with all the products of a complete diploid genome. Sperm that carry a Y chromosome, for example, is supplied with essential molecules that are encoded by genes on the X chromosome.

Diseases

Germ cell tumor is a rare cancer that can affect people at all ages. 2.4 children out of 1 million suffer the disease, and it counts for 4% of all cancers in children and adolescents younger than 20 years old.

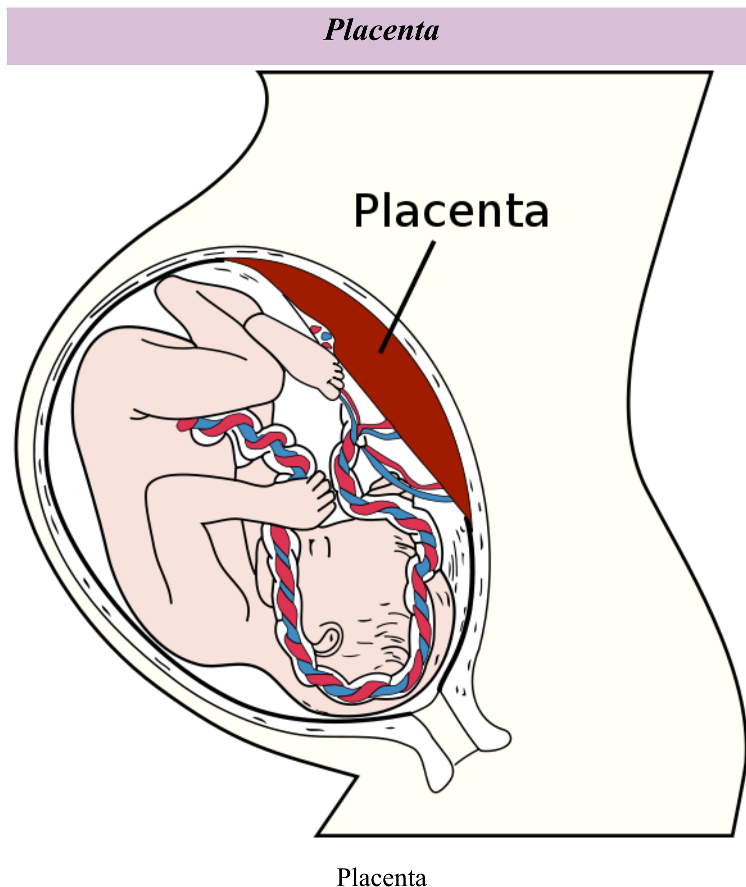
Germ cell tumors are generally located in the gonads but can also appear in the abdomen, pelvis, mediastinum, or brain. Germ cells migrating to the gonads may not reach that intended destination and a tumor can grow wherever they end up, but the exact cause is still unknown. These tumors can be benign or malignant.

Induced differentiation from stem cells

Culture of human embryonic stem cells in mitotically inactivated porcine ovarian fibroblasts (POF) causes differentiation into germ cells, as evidenced by gene expression analysis.

Chapter 21

Placenta

Placenta	
 Placenta	
Precursor	decidua basalis, chorion frondosum

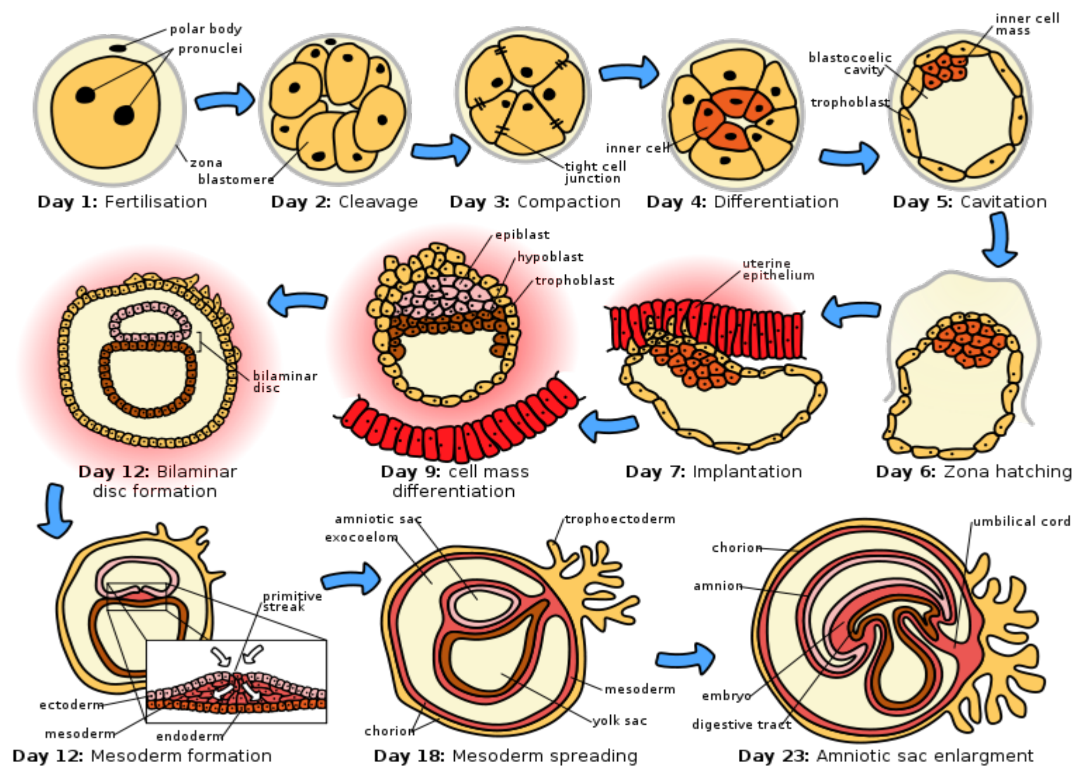
The **placenta** is an organ that connects the developing fetus to the uterine wall to allow nutrient uptake, waste elimination, and gas exchange via the mother's blood supply. Placentas are a defining characteristic of eutherian or "placental" mammals, but are also found in some snakes and lizards with varying levels of development up to mammalian levels. The word *placenta* comes from the Latin for *cake*, from Greek *plakóenta/plakoúnta*, accusative of *plakóeis/plakoús – πλακόεις, πλακούς*, "flat, slab-like", in reference to its round, flat appearance in humans. Protherial (egg-laying) and metatherial (marsupial) mammals produce a choriovitelline placenta that, while

connected to the uterine wall, provides nutrients mainly derived from the egg sac. The placenta develops from the same sperm and egg cells that form the fetus, and functions as a fetomaternal organ with two components, the fetal part (Chorion frondosum), and the maternal part (Decidua basalis).

Structure

In humans, the placenta averages 22 cm (9 inch) in length and 2–2.5 cm (0.8–1 inch) in thickness (greatest thickness at the center and become thinner peripherally). It typically weighs approximately 500 grams (1 lb). It has a dark reddish-blue or maroon color. It connects to the fetus by an umbilical cord of approximately 55–60 cm (22–24 inch) in length that contains two arteries and one vein. The umbilical cord inserts into the chorionic plate (has an eccentric attachment). Vessels branch out over the surface of the placenta and further divide to form a network covered by a thin layer of cells. This results in the formation of villous tree structures. On the maternal side, these villous tree structures are grouped into lobules called cotyledons. In humans the placenta usually has a disc shape, but size varies vastly between different mammalian species.

Development



The initial stages of human embryogenesis

The placenta begins to develop upon implantation of the blastocyst into the maternal endometrium. The outer layer of the blastocyst becomes the trophoblast which forms the

outer layer of the placenta. This outer layer is divided into two further layers: the underlying cytotrophoblast layer and the overlying syncytiotrophoblast layer. The syncytiotrophoblast is a multinucleate continuous cell layer which covers the surface of the placenta. It forms as a result of differentiation and fusion of the underlying cytotrophoblast cells, a process which continues throughout placental development. The syncytiotrophoblast (otherwise known as syncytium), thereby contributes to the barrier function of the placenta.

The placenta grows throughout pregnancy. Development of the maternal blood supply to the placenta is suggested to be complete by the end of the first trimester of pregnancy (approximately 12–13 weeks).

Placental circulation

Maternal placental circulation

In preparation for implantation, the uterine endometrium undergoes 'decidualisation'. Spiral arteries in decidua are remodelled so that they become less convoluted and their diameter is increased. This increases maternal blood flow to the placenta and also decreases resistance so that blood flow is increased. The relatively high pressure as the maternal blood enters the intervillous space through these spiral arteries bathes the villi in blood. An exchange of gases takes place. As the pressure decreases, the deoxygenated blood flows back through the endometrial veins.

Maternal blood flow is approx 600–700 ml/min at term.

Fetoplacental circulation

Deoxygenated fetal blood passes through umbilical arteries to the placenta. At the junction of umbilical cord and placenta, the umbilical arteries branch radially to form chorionic arteries. Chorionic arteries, in turn, branch into cotyledon arteries. In the villi, these vessels eventually branch to form an extensive arteriocapillary venous system, bringing the fetal blood extremely close to the maternal blood; but no intermingling of fetal and maternal blood occurs ("placental barrier").

Endothelin and prostanoids cause vasoconstriction in placental arteries, while nitric oxide vasodilation. On the other hand, there is no neural vascular regulation, and catecholamines have only little effect.

Functions

Nutrition and immunity

The perfusion of the intervillous spaces of the placenta with maternal blood allows the transfer of nutrients and oxygen from the mother to the fetus and the transfer of waste products and carbon dioxide back from the fetus to the mother. Nutrient transfer to the

fetus is both actively and passively mediated by proteins called nutrient transporters that are expressed within placental cells.

Adverse pregnancy situations, such as those involving maternal diabetes or obesity, can increase or decrease levels of nutrient transporters in the placenta resulting in overgrowth or restricted growth of the fetus.

IgG antibodies can pass through the human placenta, thereby providing protection to the fetus in uterus.

Endocrine function

In humans, aside from serving as the conduit for oxygen and nutrients for fetus, placenta secretes hormone (secreted by syncytial layer/syncytiotrophoblast of chorionic villi)that is important during pregnancy.

Hormones:

Human Chorionic Gonadotropin (hCG). The first placental hormone produced is hCG, which can be found in maternal blood and urine as early as the first missed menstrual period (shortly after implantation has occurred) through about the 100th day of pregnancy. This is the hormone analyzed by pregnancy test; a false-negative result from a pregnancy test may be obtained before or after this period. Women's blood serum will be completely negative for hCG by one to two weeks after birth. hCG testing is proof that all placental tissue is delivered. hCG is only present during pregnancy because it is secreted by the placenta, which of course is present only during pregnancy. hCG also ensures that the corpus luteum continue to secrete progesterone and estrogen. Progesterone is very important during pregnancy because when its secretion decreases, endometrial lining will slough off and pregnancy will be lost. hCG suppresses the maternal immunologic response so that placenta is not rejected.

Human Placental Lactogen (hPL [Human Chorionic Somatomammotropin]). This hormone is lactogenic and growth-promoting properties. It promotes mammary gland growth in preparation for lactation in the mother. It also regulates maternal glucose, protein, fat levels so that this is always available to the fetus.

Estrogen. It is referred to as the "hormone of woman" because it influences the female appearance. It contributes to the woman's mammary gland development in preparation for lactation and stimulates uterine growth to accommodate growing fetus.

Progesterone. This is referred to as the "hormone of mothers" because it is necessary to maintain endometrial lining of the uterus during pregnancy. This hormone prevents preterm labor by reducing myometrial contraction. This hormone is high during pregnancy.

Cloaking from immune system of mother

The placenta and fetus may be regarded as a foreign allograft inside the mother, and thus must evade from attack by the mother's immune system.

For this purpose, the placenta uses several mechanisms:

- It secretes Neurokinin B containing phosphocholine molecules. This is the same mechanism used by parasitic nematodes to avoid detection by the immune system of their host.
- Also, there is presence of small lymphocytic suppressor cells in the fetus that inhibit maternal cytotoxic T cells by inhibiting the response to interleukin 2.

However, the placental barrier is not the sole means to evade the immune system, as foreign fetal cells also persist in the maternal circulation, on the other side of the placental barrier.

Other functions

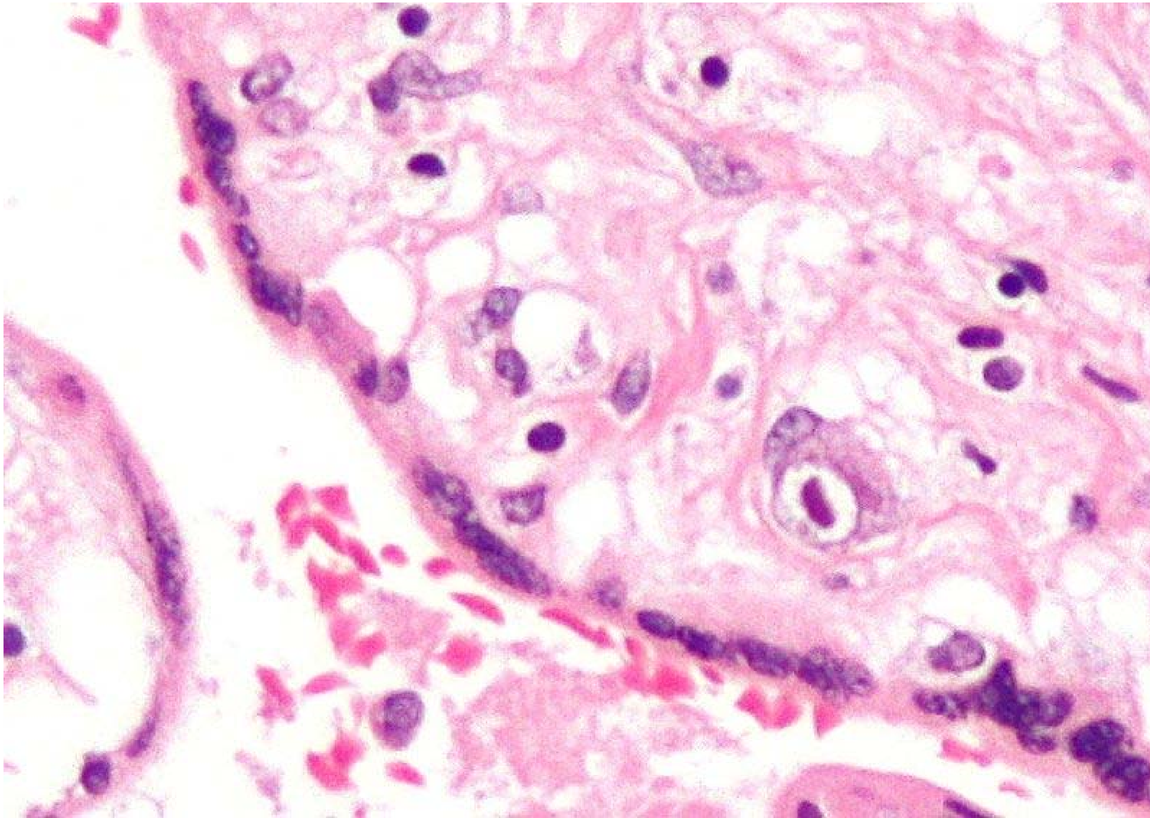
The placenta also provides a reservoir of blood for the fetus, delivering blood to it in case of hypotension and vice versa, comparable to a capacitor.

Birth

Placental expulsion begins as a physiological separation from the wall of the uterus. The period from just after the fetus is expelled until just after the placenta is expelled is called the *third stage of labor*. The placenta is usually expelled within 15–30 minutes of the baby being born.

Placental expulsion can be managed actively, for example by giving oxytocin via intramuscular injection followed by cord traction to assist in delivering the placenta. Alternatively, it can be managed expectantly, allowing the placenta to be expelled without medical assistance. A Cochrane database study suggests that blood loss and the risk of postpartum bleeding will be reduced in women offered active management of the third stage of labour.

Pathology



Micrograph of a cytomegalovirus (CMV) infection of the placenta (CMV placentitis). The characteristic large nucleus of a CMV infected cell is seen off-centre at the bottom-right of the image. H&E stain.

Numerous pathologies can affect the placenta.

When the placenta implants too deeply:

- Placenta accreta
- Placenta praevia
- Placental abruption/abruptio placentae

Infections involving the placenta:

- Placentitis, such as the TORCH infections.
- Chorioamnionitis.

Cultural practices and beliefs

The placenta often plays an important role in various human cultures, with many societies conducting rituals regarding its disposal. In the Western world, the placenta is most often incinerated.

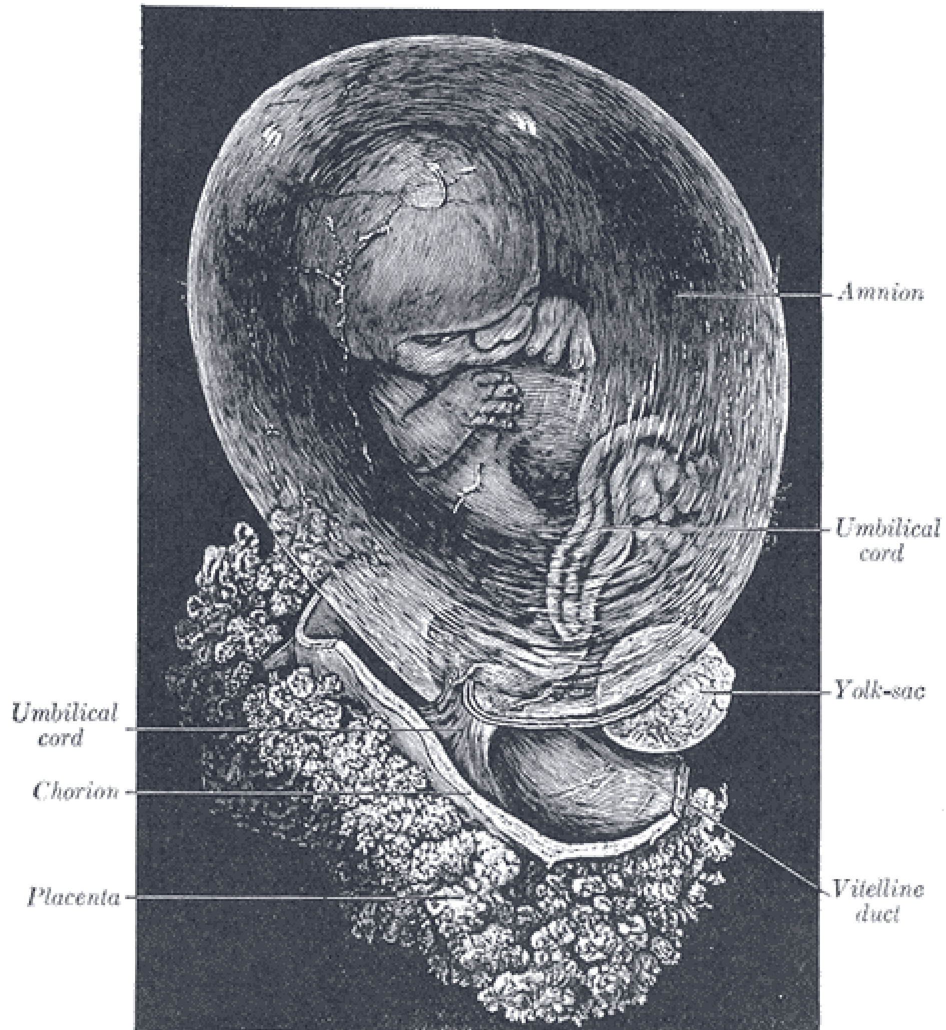
Some cultures bury the placenta for various reasons. The Māori of New Zealand traditionally bury the placenta from a newborn child to emphasize the relationship between humans and the earth. Similarly, the Navajo bury the placenta and umbilical cord at a specially chosen site, particularly if the baby dies during birth. In Cambodia and Costa Rica, burial of the placenta is believed to protect and ensure the health of the baby and the mother. If a mother dies in childbirth, the Aymara of Bolivia bury the placenta in a secret place so that the mother's spirit will not return to claim her baby's life.

The placenta is believed by some communities to have power over the lives of the baby or its parents. The Kwakiutl of British Columbia bury girls' placentas to give the girl skill in digging clams, and expose boys' placentas to ravens to encourage future prophetic visions. In Turkey, the proper disposal of the placenta and umbilical cord is believed to promote devoutness in the child later in life. In Ukraine, Transylvania, and Japan, interaction with a disposed placenta is thought to influence the parents' future fertility.

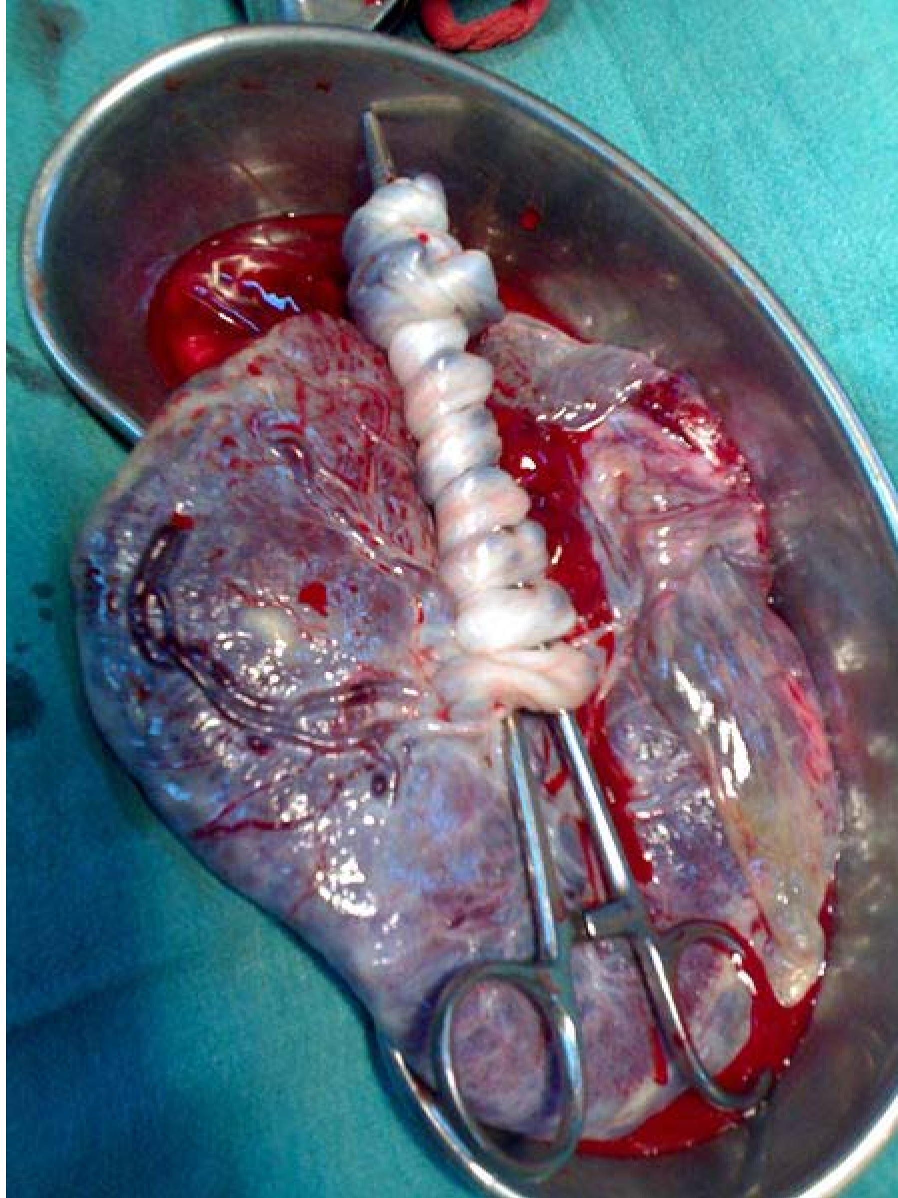
Several cultures believe the placenta to be or have been alive, often a relative of the baby. Nepalese think of the placenta as a friend of the baby's; Malaysian Orang Asli regard it as the baby's older sibling. The Ibo of Nigeria consider the placenta the deceased twin of the baby, and conduct full funeral rites for it. Native Hawaiians believe that the placenta is a part of the baby, and traditionally plant it with a tree which can then grow alongside the child.

In some cultures, the placenta is eaten, a practice known as placentophagy. In some eastern cultures, such as China and Hong Kong, the placenta is thought to be healthful and is used in medicine and various health products.

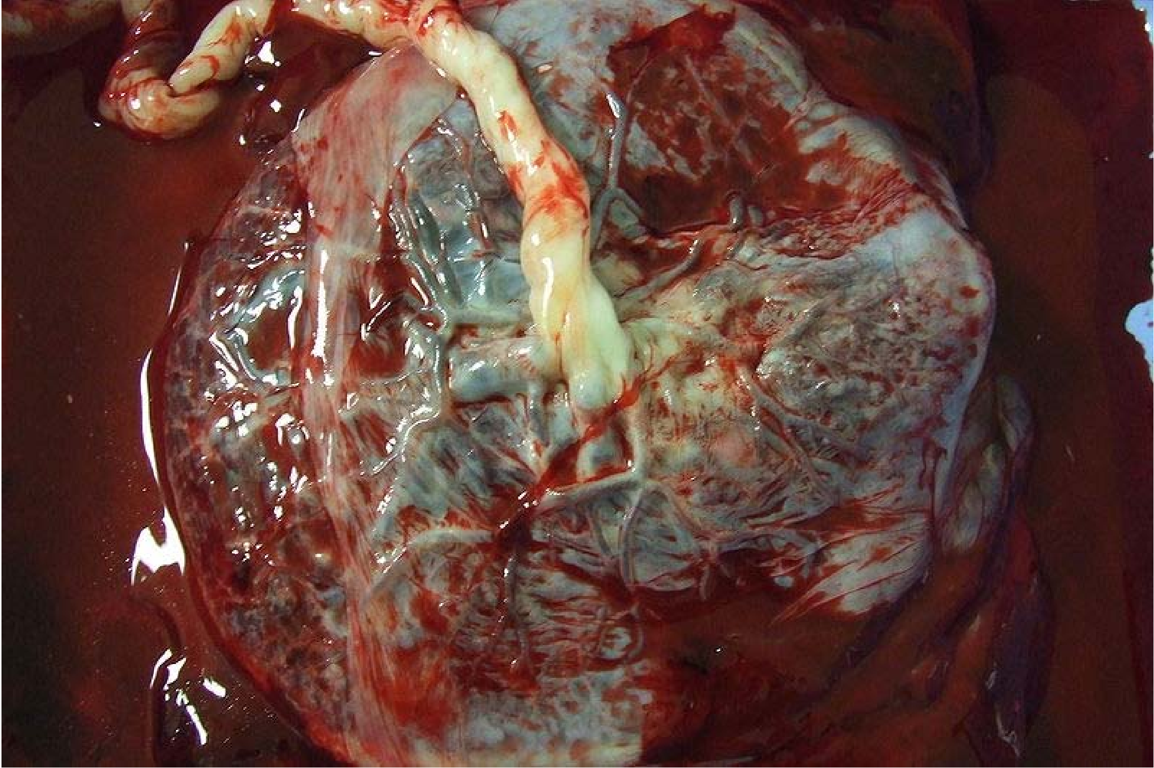
Additional images



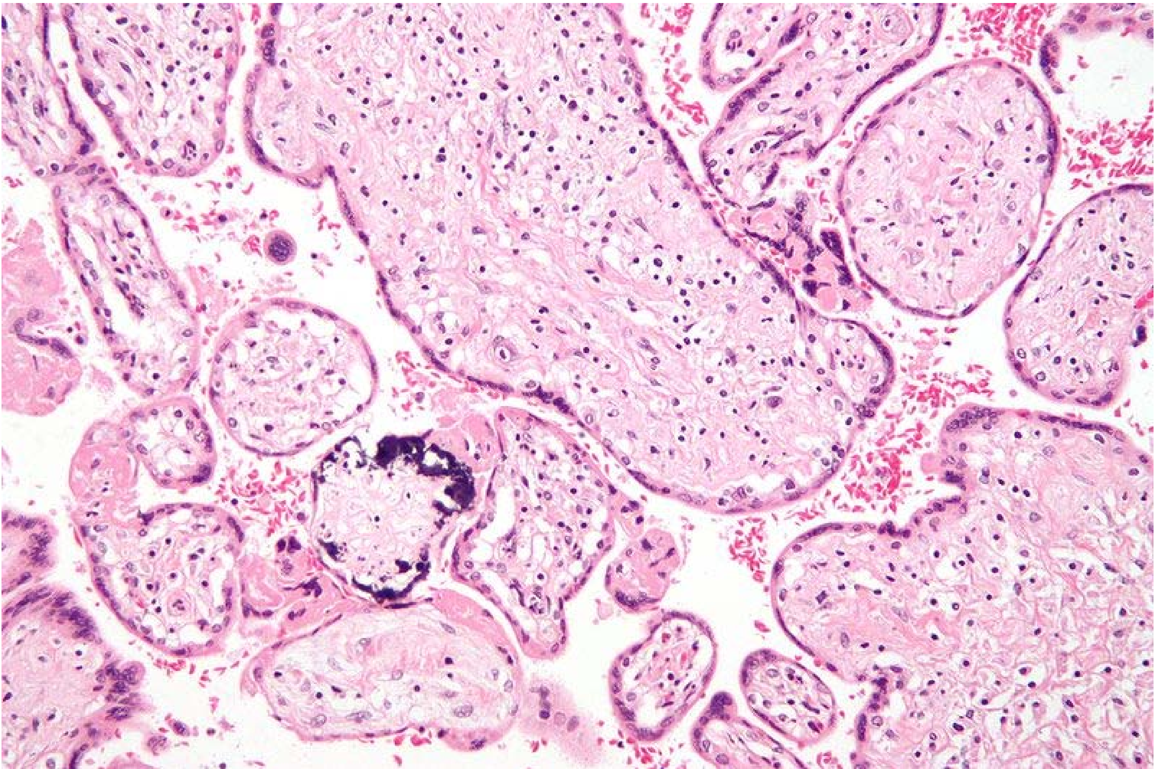
Fetus of about 8 weeks, enclosed in the amnion. Magnified a little over two diameters.



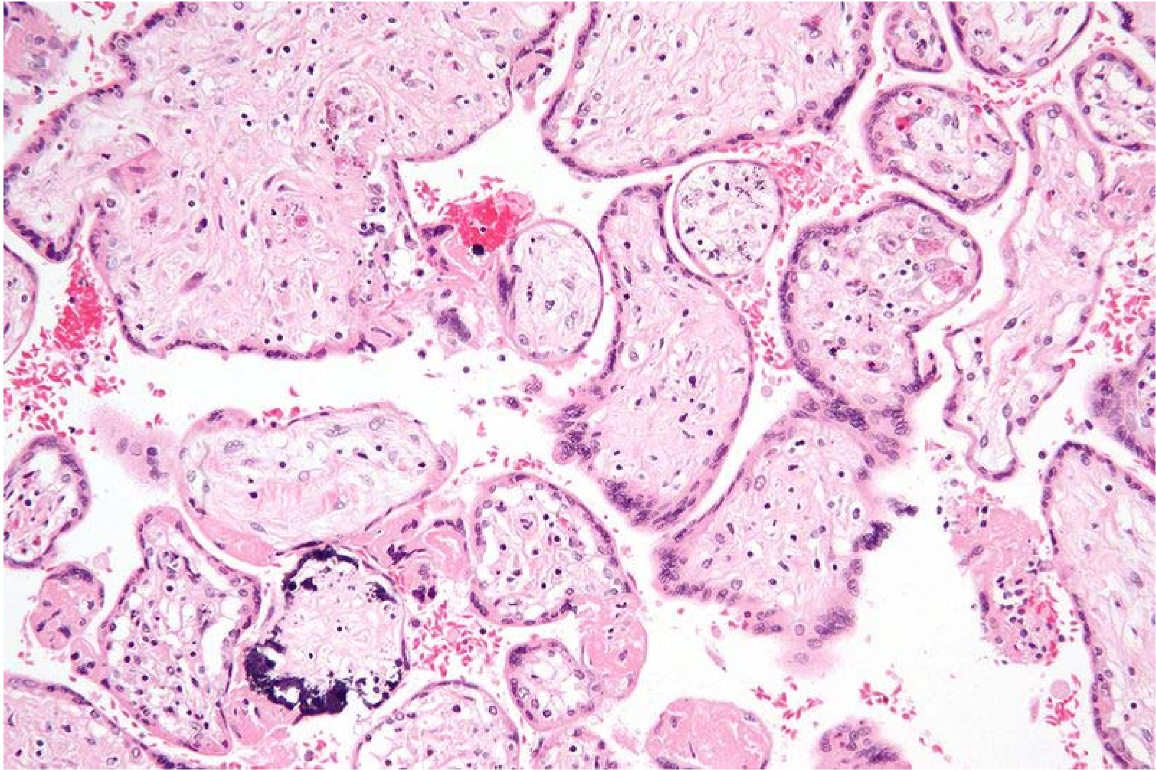
Picture of freshly delivered placenta and umbilical cord wrapped around Kelly clamps



Fresh human placenta



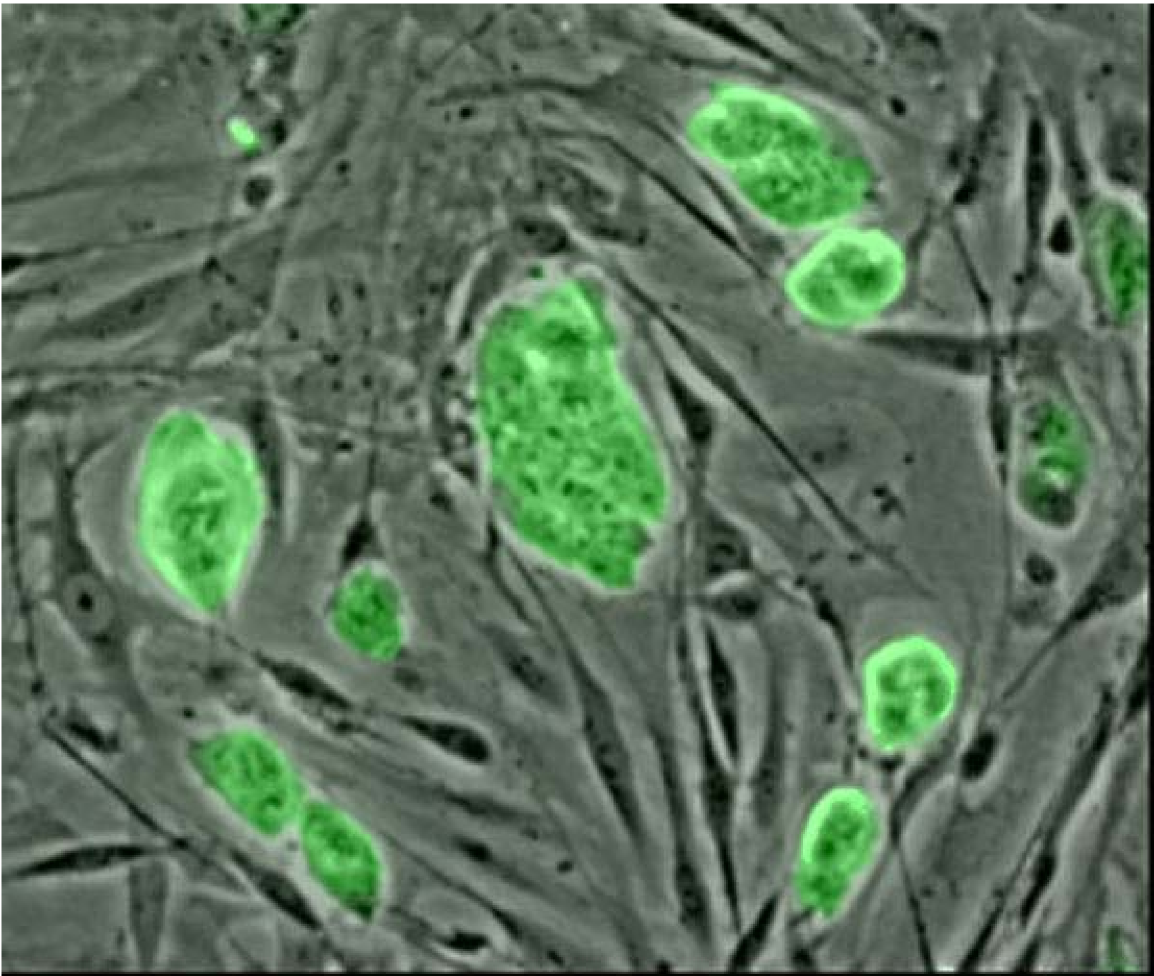
Micrograph of a placental infection (CMV placentitis)



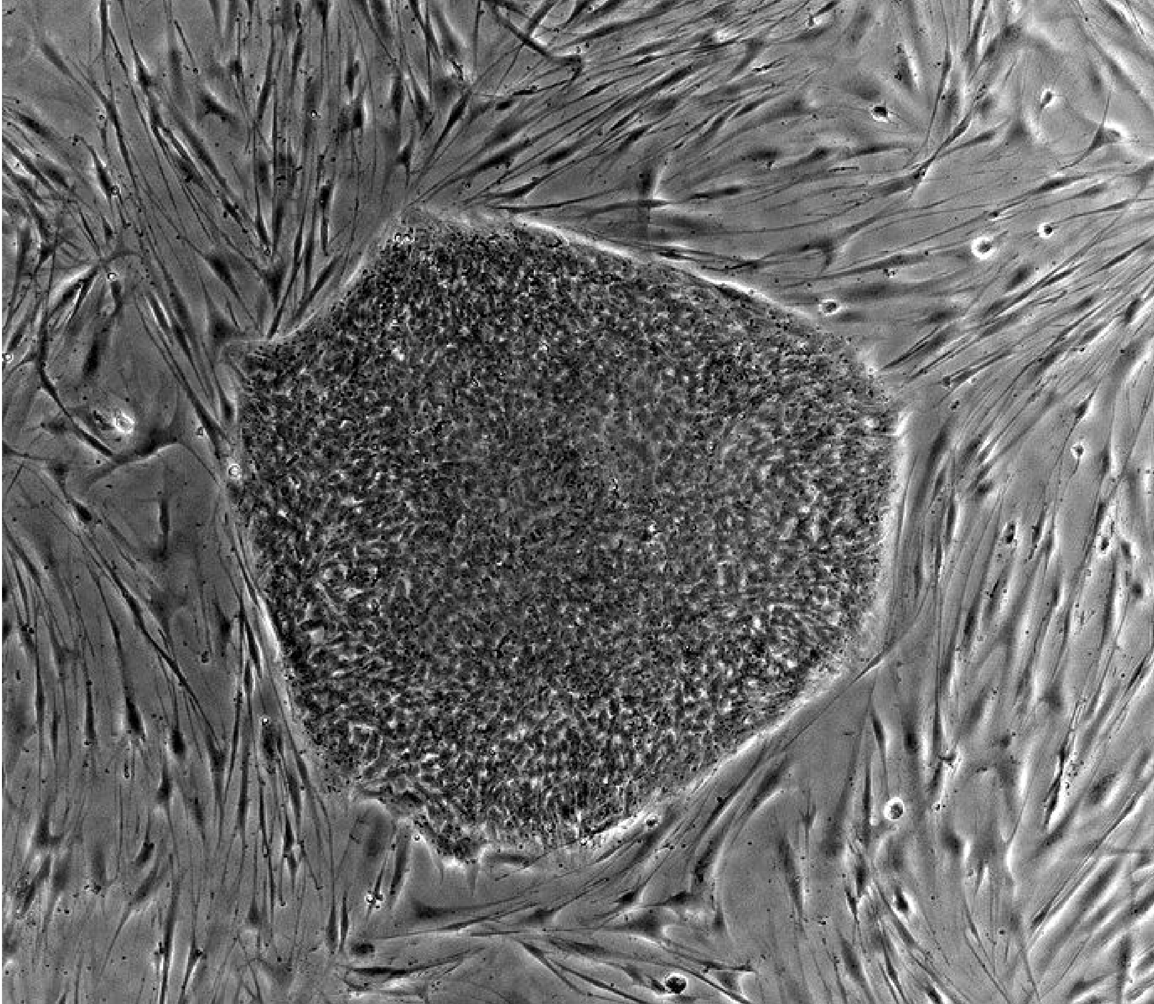
Micrograph of CMV placentitis

Chapter 22

Stem Cell



Mouse embryonic stem cells with fluorescent marker



Human embryonic stem cell colony on mouse embryonic fibroblast feeder layer

Stem cells are cells found in all multicellular organisms. They are characterized by the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. Research in the stem cell field grew out of findings by Ernest A. McCulloch and James E. Till at the University of Toronto in the 1960s.

The two broad types of mammalian stem cells are: embryonic stem cells that are isolated from the inner cell mass of blastocysts, and adult stem cells that are found in different tissues. In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenished in adult tissues. In a developing embryo, stem cells can differentiate into all of the specialized cells, but also maintain the normal turnover of regenerative organs, such as blood, skin, or intestinal tissues.

Stem cells can now be grown and transformed into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells from a variety of sources, including umbilical cord blood and bone marrow, are routinely used in medical therapies. Embryonic cell

lines and autologous embryonic stem cells generated through therapeutic cloning have also been proposed as promising candidates for future therapies.

Properties

The classical definition of a stem cell requires that it possess two properties:

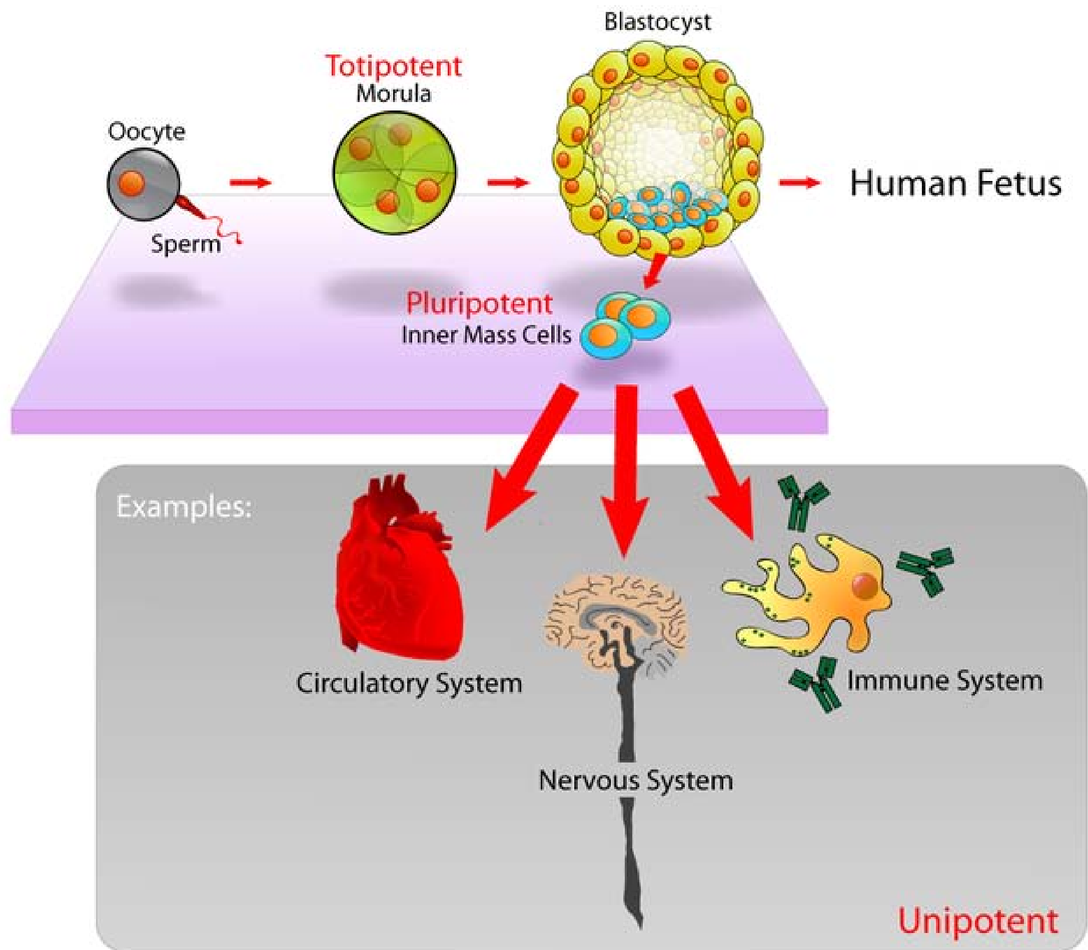
- *Self-renewal* - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state.
- *Potency* - the capacity to differentiate into specialized cell types. In the strictest sense, this requires stem cells to be either totipotent or pluripotent - to be able to give rise to any mature cell type, although multipotent or unipotent progenitor cells are sometimes referred to as stem cells.

Self-renewal

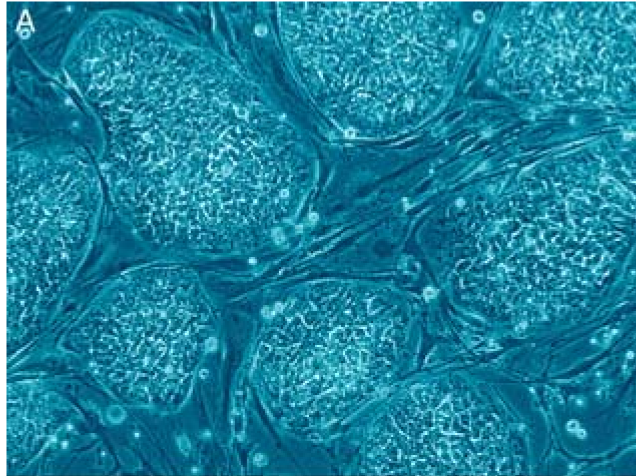
Two mechanisms exist to ensure that the stem cell population is maintained:

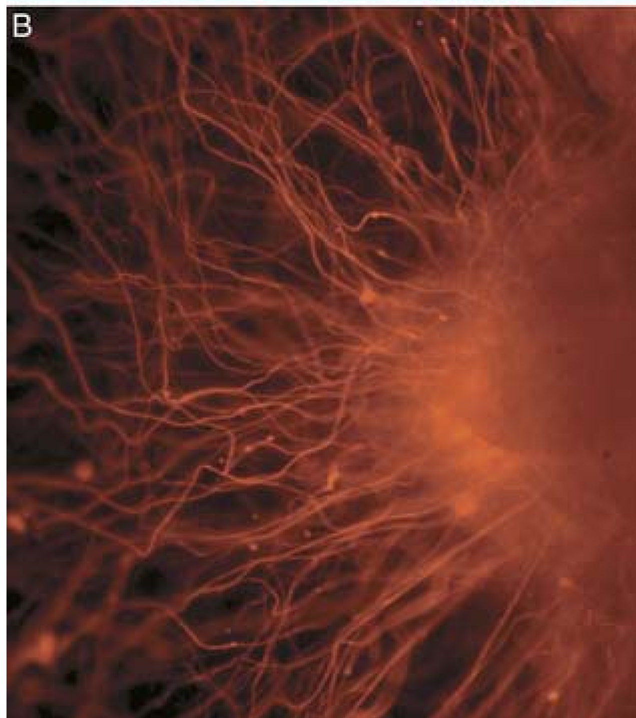
1. Obligatory asymmetric replication - a stem cell divides into one father cell that is identical to the original stem cell, and another daughter cell that is differentiated
2. Stochastic differentiation - when one stem cell develops into two differentiated daughter cells, another stem cell undergoes mitosis and produces two stem cells identical to the original.

Potency definitions



Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta.





Human embryonic stem cells
A: Cell colonies that are not yet differentiated.
B: Nerve cell

Potency specifies the differentiation potential (the potential to differentiate into different cell types) of the stem cell.

- Totipotent (a.k.a omnipotent) stem cells can differentiate into embryonic and extraembryonic cell types. Such cells can construct a complete, viable, organism. These cells are produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.
- Pluripotent stem cells are the descendants of totipotent cells and can differentiate into nearly all cells, i.e. cells derived from any of the three germ layers.

- Multipotent stem cells can differentiate into a number of cells, but only those of a closely related family of cells.
- Oligopotent stem cells can differentiate into only a few cells, such as lymphoid or myeloid stem cells.
- Unipotent cells can produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

Identification

The practical definition of a stem cell is the functional definition - a cell that has the potential to regenerate tissue over a lifetime. For example, the gold standard test for a bone marrow or hematopoietic stem cell (HSC) is the ability to transplant one cell and save an individual without HSCs. In this case, a stem cell must be able to produce new blood cells and immune cells over a long term, demonstrating potency. It should also be possible to isolate stem cells from the transplanted individual, which can themselves be transplanted into another individual without HSCs, demonstrating that the stem cell was able to self-renew.

Properties of stem cells can be illustrated *in vitro*, using methods such as clonogenic assays, where single cells are characterized by their ability to differentiate and self-renew. As well, stem cells can be isolated based on a distinctive set of cell surface markers. However, *in vitro* culture conditions can alter the behavior of cells, making it unclear whether the cells will behave in a similar manner *in vivo*. Considerable debate exists whether some proposed adult cell populations are truly stem cells.

Embryonic

Embryonic stem cell lines (ES cell lines) are cultures of cells derived from the epiblast tissue of the inner cell mass (ICM) of a blastocyst or earlier morula stage embryos. A blastocyst is an early stage embryo—approximately four to five days old in humans and consisting of 50–150 cells. ES cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. In other words, they can develop into each of the more than 200 cell types of the adult body when given sufficient and necessary stimulation for a specific cell type. They do not contribute to the extra-embryonic membranes or the placenta.

Nearly all research to date has taken place using mouse embryonic stem cells (mES) or human embryonic stem cells (hES). Both have the essential stem cell characteristics, yet they require very different environments in order to maintain an undifferentiated state. Mouse ES cells are grown on a layer of gelatin and require the presence of Leukemia Inhibitory Factor (LIF). Human ES cells are grown on a feeder layer of mouse embryonic fibroblasts (MEFs) and require the presence of basic Fibroblast Growth Factor (bFGF or FGF-2). Without optimal culture conditions or genetic manipulation, embryonic stem cells will rapidly differentiate.

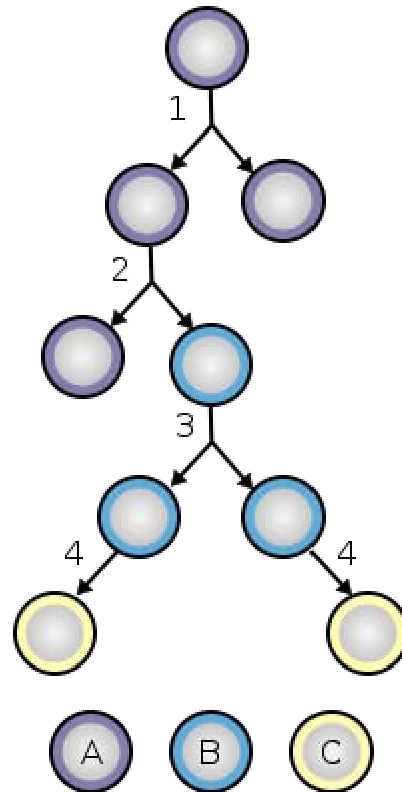
A human embryonic stem cell is also defined by the presence of several transcription factors and cell surface proteins. The transcription factors Oct-4, Nanog, and Sox2 form the core regulatory network that ensures the suppression of genes that lead to differentiation and the maintenance of pluripotency. The cell surface antigens most commonly used to identify hES cells are the glycolipids SSEA3 and SSEA4 and the keratan sulfate antigens Tra-1-60 and Tra-1-81. The molecular definition of a stem cell includes many more proteins and continues to be a topic of research.

After nearly ten years of research, there are no approved treatments using embryonic stem cells. The first human trial was approved by the US Food & Drug Administration in January 2009. However, as of August 2010, the first human trial had not yet been initiated. The first human medical trial for embryonic stem cells started in Atlanta on October 13, 2010 for spinal injury victims. ES cells, being pluripotent cells, require specific signals for correct differentiation - if injected directly into another body, ES cells will differentiate into many different types of cells, causing a teratoma. Differentiating ES cells into usable cells while avoiding transplant rejection are just a few of the hurdles that embryonic stem cell researchers still face. Many nations currently have moratoria on either ES cell research or the production of new ES cell lines. Because of their combined abilities of unlimited expansion and pluripotency, embryonic stem cells remain a theoretically potential source for regenerative medicine and tissue replacement after injury or disease.

Fetal

Fetal stem cells are primitive cell types found in the organs of fetuses.

Adult



Stem cell division and differentiation. A - stem cell; B - progenitor cell; C - differentiated cell; 1 - symmetric stem cell division; 2 - asymmetric stem cell division; 3 - progenitor division; 4 - terminal differentiation

Also known as somatic (from Greek Σωματικός, "of the body") stem cells and germline (giving rise to gametes) stem cells, they can be found in children, as well as adults.

Pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including umbilical cord blood. A great deal of adult stem cell research has focused on clarifying their capacity to divide or self-renew indefinitely and their differentiation potential. In mice, pluripotent stem cells are directly generated from adult fibroblast cultures. Unfortunately, many mice don't live long with stem cell organs.

Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, dental pulp stem cell, etc.).

Adult stem cell treatments have been successfully used for many years to treat leukemia and related bone/blood cancers through bone marrow transplants. Adult stem cells are also used in veterinary medicine to treat tendon and ligament injuries in horses.

The use of adult stem cells in research and therapy is not as controversial as embryonic stem cells, because the production of adult stem cells does not require the destruction of an embryo. Additionally, because in some instances adult stem cells can be obtained from the intended recipient, (an autograft) the risk of rejection is essentially non-existent in these situations. Consequently, more US government funding is being provided for adult stem cell research.

An extremely rich source for adult mesenchymal stem cells is the developing tooth bud of the mandibular third molar. While considered multipotent they may prove to be pluripotent. The stem cells eventually form enamel (ectodrm), dentin,periodontal ligament, blood vessels, dental pulp, nervous tissues, including a minimum of 29 different unique end organs. Because of extreme ease in collection at 8–10 years of age before calcification and minimal to no morbidity will probably constitute a major source for personal banking, research and multiple therapies. These stem cells have been shown capable of producing hepatocytes.

Amniotic

Multipotent stem cells are also found in amniotic fluid. These stem cells are very active, expand extensively without feeders and are not tumorigenic. Amniotic stem cells are multipotent and can differentiate in cells of adipogenic, osteogenic, myogenic, endothelial, hepatic and also neuronal lines. All over the world, universities and research institutes are studying amniotic fluid to discover all the qualities of amniotic stem cells, and scientists such as Anthony Atala and Giuseppe Simoni have discovered important results.

From an ethical point of view, stem cells from amniotic fluid can solve a lot of problems, because it's possible to catch amniotic stem cells without destroying embryos. For example, the Vatican newspaper "Osservatore Romano" called amniotic stem cell "the future of medicine".

It's possible to collect amniotic stem cells for donors or for autologuous use: the first US amniotic stem cells bank opened in 2009 in Medford, MA, by Biocell Center Corporation and collaborates with various hospitals and universities all over the world.

Induced pluripotent

These are not adult stem cells, but rather reprogrammed cells (e.g. epithelial cells) given pluripotent capabilities. Using genetic reprogramming with protein transcription factors, pluripotent stem cells equivalent to embryonic stem cells have been derived from human adult skin tissue. Shinya Yamanaka and his colleagues at Kyoto University used the transcription factors Oct3/4, Sox2, c-Myc, and Klf4 in their experiments on cells from human faces. Junying Yu, James Thomson, and their colleagues at the University of Wisconsin–Madison used a different set of factors, Oct4, Sox2, Nanog and Lin28, and carried out their experiments using cells from human foreskin.

As a result of the success of these experiments, Ian Wilmut, who helped create the first cloned animal Dolly the Sheep, has announced that he will abandon nuclear transfer as an avenue of research.

Frozen blood samples can be used as a source of induced pluripotent stem cells, opening a new avenue for obtaining the valued cells.

Lineage

To ensure self-renewal, stem cells undergo two types of cell division. Symmetric division gives rise to two identical daughter cells both endowed with stem cell properties. Asymmetric division, on the other hand, produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell. It is possible that the molecular distinction between symmetric and asymmetric divisions lies in differential segregation of cell membrane proteins (such as receptors) between the daughter cells.

An alternative theory is that stem cells remain undifferentiated due to environmental cues in their particular niche. Stem cells differentiate when they leave that niche or no longer receive those signals. Studies in *Drosophila* germarium have identified the signals dpp and adherens junctions that prevent germarium stem cells from differentiating.

The signals that lead to reprogramming of cells to an embryonic-like state are also being investigated. These signal pathways include several transcription factors including the oncogene c-Myc. Initial studies indicate that transformation of mice cells with a combination of these anti-differentiation signals can reverse differentiation and may allow adult cells to become pluripotent. However, the need to transform these cells with an oncogene may prevent the use of this approach in therapy.

Challenging the terminal nature of cellular differentiation and the integrity of lineage commitment, it was recently determined that the somatic expression of combined transcription factors can directly induce other defined somatic cell fates; researchers identified three neural-lineage-specific transcription factors that could directly convert mouse fibroblasts (skin cells) into fully functional neurons. This "induced neurons" (iN) cell research inspires the researchers to induce other cell types implies that *all* cells are totipotent: with the proper tools, all cells may form all kinds of tissue.

Treatments

Medical researchers believe that stem cell therapy has the potential to dramatically change the treatment of human disease. A number of adult stem cell therapies already exist, particularly bone marrow transplants that are used to treat leukemia. In the future, medical researchers anticipate being able to use technologies derived from stem cell research to treat a wider variety of diseases including cancer, Parkinson's disease, spinal cord injuries, Amyotrophic lateral sclerosis, multiple sclerosis, and muscle damage, amongst a number of other impairments and conditions. However, there still exists a

great deal of social and scientific uncertainty surrounding stem cell research, which could possibly be overcome through public debate and future research, and further education of the public.

One concern of treatment is the possible risk that transplanted stem cells could form tumors and have the possibility of becoming cancerous if cell division continues uncontrollably.

Stem cells, however, are already studied extensively. While some scientists are hesitant to associate the therapeutic potential of stem cells as the first goal of the research, they find the investigation of stem cells as a goal worthy in itself.

Contrarily, supporters of embryonic stem cell research argue that such research should be pursued because the resultant treatments could have significant medical potential. It is also noted that excess embryos created for in vitro fertilization could be donated with consent and used for the research.

The recent development of iPS cells has been called a bypass of the legal controversy. Laws limiting the destruction of human embryos have been credited for being the reason for development of iPS cells, but they are less efficient and reliable than natural stem cells. Various methods are being developed to bypass this problem by removing mutation.

Research patents

The patents covering a lot of work on human embryonic stem cells are owned by the Wisconsin Alumni Research Foundation (WARF). WARF does not charge academics to study human stem cells but does charge commercial users. WARF sold Geron Corp. exclusive rights to work on human stem cells but later sued Geron Corp. to recover some of the previously sold rights. The two sides agreed that Geron Corp. would keep the rights to only three cell types. In 2001, WARF came under public pressure to widen access to human stem-cell technology.

These patents are now in doubt as a request for reviewing the US Patent and Trademark Office has been filed by non-profit patent-watchdogs The Foundation for Taxpayer & Consumer Rights, and the Public Patent Foundation as well as molecular biologist Jeanne Loring of the Burnham Institute. According to them, two of the patents granted to WARF are invalid because they cover a technique published in 1993 for which a patent had already been granted to an Australian researcher. Another part of the challenge states that these techniques, developed by James A. Thomson, are rendered obvious by a 1990 paper and two textbooks.

The outcome of this legal challenge is particularly relevant to the Geron Corp. as it can only license patents that are upheld.

Key research events

- 1908 - The term "stem cell" was proposed for scientific use by the Russian histologist Alexander Maksimov (1874–1928) at congress of hematologic society in Berlin. It postulated existence of haematopoietic stem cells.
- 1960s - Joseph Altman and Gopal Das present scientific evidence of adult neurogenesis, ongoing stem cell activity in the brain; like André Gernez, their reports contradict Cajal's "no new neurons" dogma and are largely ignored.
- 1963 - McCulloch and Till illustrate the presence of self-renewing cells in mouse bone marrow.
- 1968 - Bone marrow transplant between two siblings successfully treats SCID.
- 1978 - Haematopoietic stem cells are discovered in human cord blood.
- 1981 - Mouse embryonic stem cells are derived from the inner cell mass by scientists Martin Evans, Matthew Kaufman, and Gail R. Martin. Gail Martin is attributed for coining the term "Embryonic Stem Cell".
- 1992 - Neural stem cells are cultured *in vitro* as neurospheres.
- 1997 - Leukemia is shown to originate from a haematopoietic stem cell, the first direct evidence for cancer stem cells.
- 1998 - James Thomson and coworkers derive the first human embryonic stem cell line at the University of Wisconsin–Madison.
- 1998 - John Gearhart (Johns Hopkins University) extracted germ cells from fetal gonadal tissue (primordial germ cells) before developing pluripotent stem cell lines from the original extract.
- 2000s - Several reports of adult stem cell plasticity are published.
- 2001 - Scientists at Advanced Cell Technology clone first early (four- to six-cell stage) human embryos for the purpose of generating embryonic stem cells.
- 2003 - Dr. Songtao Shi of NIH discovers new source of adult stem cells in children's primary teeth.
- 2004–2005 - Korean researcher Hwang Woo-Suk claims to have created several human embryonic stem cell lines from unfertilised human oocytes. The lines were later shown to be fabricated.
- 2005 - Researchers at Kingston University in England claim to have discovered a third category of stem cell, dubbed cord-blood-derived embryonic-like stem cells (CBEs), derived from umbilical cord blood. The group claims these cells are able to differentiate into more types of tissue than adult stem cells.
- 2005 - Researchers at UC Irvine's Reeve-Irvine Research Center are able to partially restore the ability of mice with paralyzed spines to walk through the injection of human neural stem cells.
- August 2006 - Mouse Induced pluripotent stem cells: the journal *Cell* publishes Kazutoshi Takahashi and Shinya Yamanaka.
- October 2006 - Scientists at Newcastle University in England create the first ever artificial liver cells using umbilical cord blood stem cells.
- January 2007 - Scientists at Wake Forest University led by Dr. Anthony Atala and Harvard University report discovery of a new type of stem cell in amniotic fluid. This may potentially provide an alternative to embryonic stem cells for use in research and therapy.

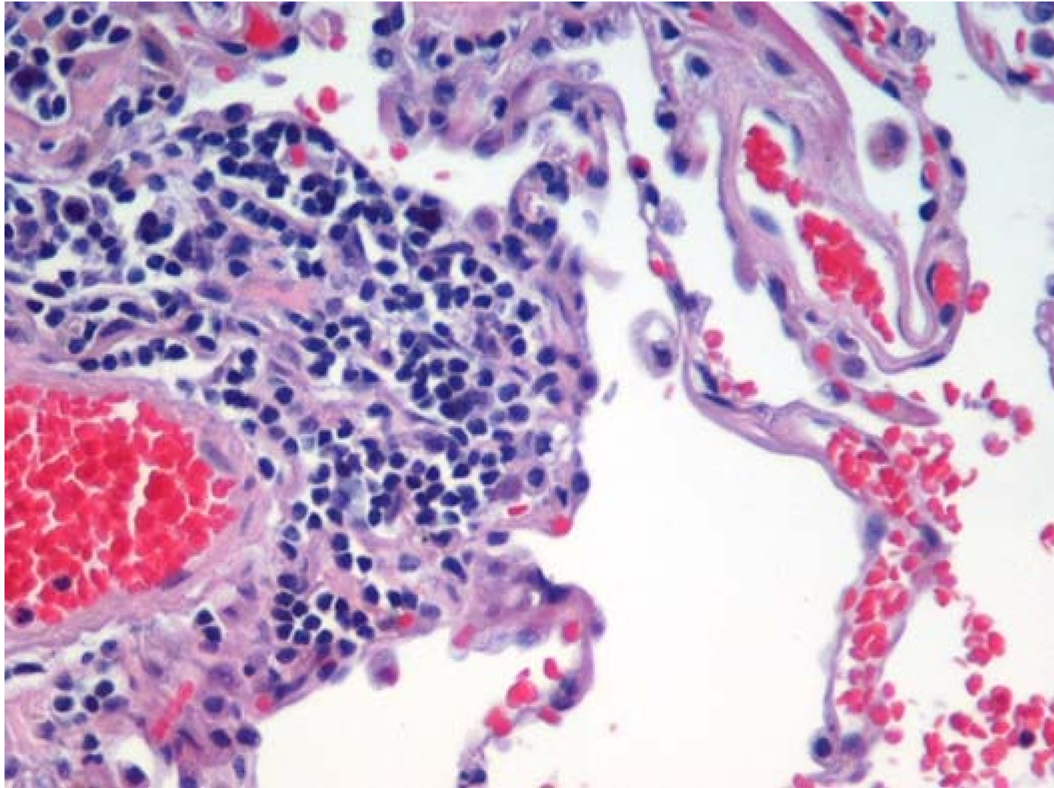
- June 2007 - Research reported by three different groups shows that normal skin cells can be reprogrammed to an embryonic state in mice. In the same month, scientist Shoukhrat Mitalipov reports the first successful creation of a primate stem cell line through somatic cell nuclear transfer
- October 2007 - Mario Capecchi, Martin Evans, and Oliver Smithies win the 2007 Nobel Prize for Physiology or Medicine for their work on embryonic stem cells from mice using gene targeting strategies producing genetically engineered mice (known as knockout mice) for gene research.
- November 2007 - Human induced pluripotent stem cells: Two similar papers released by their respective journals prior to formal publication: in *Cell* by Kazutoshi Takahashi and Shinya Yamanaka, "Induction of pluripotent stem cells from adult human fibroblasts by defined factors", and in *Science* by Junying Yu, et al., from the research group of James Thomson, "Induced pluripotent stem cell lines derived from human somatic cells": pluripotent stem cells generated from mature human fibroblasts. It is possible now to produce a stem cell from almost any other human cell instead of using embryos as needed previously, albeit the risk of tumorigenesis due to c-myc and retroviral gene transfer remains to be determined.
- January 2008 - Robert Lanza and colleagues at Advanced Cell Technology and UCSF create the first human embryonic stem cells without destruction of the embryo
- January 2008 - Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts
- February 2008 - Generation of pluripotent stem cells from adult mouse liver and stomach: these iPS cells seem to be more similar to embryonic stem cells than the previously developed iPS cells and not tumorigenic, moreover genes that are required for iPS cells do not need to be inserted into specific sites, which encourages the development of non-viral reprogramming techniques.
- March 2008-The first published study of successful cartilage regeneration in the human knee using autologous adult mesenchymal stem cells is published by clinicians from Regenerative Sciences
- October 2008 - Sabine Conrad and colleagues at Tübingen, Germany generate pluripotent stem cells from spermatogonial cells of adult human testis by culturing the cells in vitro under leukemia inhibitory factor (LIF) supplementation.
- 30 October 2008 - Embryonic-like stem cells from a single human hair.
- 1 March 2009 - Andras Nagy, Keisuke Kaji, *et al.* discover a way to produce embryonic-like stem cells from normal adult cells by using a novel "wrapping" procedure to deliver specific genes to adult cells to reprogram them into stem cells without the risks of using a virus to make the change. The use of electroporation is said to allow for the temporary insertion of genes into the cell.
- 28 May 2009 Kim *et al.* announced that they had devised a way to manipulate skin cells to create patient specific "induced pluripotent stem cells" (iPS), claiming it to be the 'ultimate stem cell solution'.
- 11 October 2010 First trial of embryonic stem cells in humans.

- 25 October 2010 - Ishikawa et *al.* write in the Journal of Experimental Medicine that research shows that transplanted cells which contain their new host's nuclear DNA could still be rejected by the invidual's immune system due to foreign mitochondrial DNA. Tissues made from a person's stem cells could therefore be rejected, because mitochondrial genomes tend to accumulate mutations.

Chapter 23

Automated Tissue Image Analysis

A stained histologic specimen, sandwiched between a glass microscope slide and coverslip, mounted on the stage of a light microscope.



Microscopic view of a histologic specimen of human lung tissue stained with hematoxylin and eosin.

Automated tissue image analysis is a process by which computer-controlled automatic test equipment to evaluate tissue samples, using computations to derive quantitative measurements from an image to avoid subjective errors.

In a typical application, automated tissue image analysis could be used to measure the aggregate activity of cancer cells in a biopsy of a cancerous tumor taken from a patient. In breast cancer patients, for example, automated tissue image analysis may be used to test for high levels of proteins known to be present in more aggressive forms of breast cancers.

Applications

Automated tissue imaging analysis can significantly reduce uncertainty in characterizing tumors compared to evaluations done by histologists, or improve the prediction rate of recurrence of some cancers. As it is a digital system, suitable for networking, it also facilitates cooperative efforts between distant sites. Systems for automatically analyzing tissue samples also reduce costs and save time.

High-performance CCD cameras are used for acquiring the digital images. Coupled with advanced widefield microscopes and various algorithms for image restoration, this

approach can provide better results than confocal techniques at comparable speeds and lower costs.

Processes

The United States Food and Drug Administration classifies these systems as medical devices, under the general instrumentation category of automatic test equipment.

ATIS have seven basic processes (sample preparation, image acquisition, image analysis, results reporting, data storage, network communication, and self-system diagnostics) and realization of these functions highly accurate hardware and well-integrated, complex, and expensive software.

Preparation

Specimen preparation is critical for evaluating the tumor in the automated system. In the first part of the preparation process the biopsied tissue is cut to an appropriate size (typically 4 mm), fixed in buffered formalin, dehydrated in ethanol-xylene, embedded in paraffin, thin sectioned typically to 4 um slices, then moutned onto at least two barcoded slides (a control and a test). Next the paraffin is removed from the tissue, the tissue is rehydrated, then stained. Any inconsistency in these procedures from case to case may result in uncertainties in the outcome of the analysis. These potential and irreducible inconsistencies in analysis results motivated the development of Automated Tissue Image Systems.

Acquisition

Digital micrographs are acquired of the stained specimen on the glass slide. The images are taken by a set of charge-coupled devices (CCD).

Analysis

Image analysis involves complex computer algorithms which identify and characterize cellular color, shape, and quantity of the tissue sample using image pattern recognition technology based on vector quantization. Vector representations of objects in the image, as opposed to bitmap representations, have superior zoom-in ability. Once the sample image has been acquired and resident in the computer's random access memory as a large array of 0's and 1's, a programmer knowledgeable in cellular architecture can develop deterministic algorithms applied to the entire memory space to detect cell patterns from previously defined cellular structures and formations known to be significant.

The aggregate algorithm outcome is a set of measurements that is far superior to any human sensitivity to intensity or luminance and color hue, while at the same time improving test consistency from eyeball to eyeball.

Reporting

The systems have the capability of presenting the resulting data in text and graphically, including on high definition monitors, to the system user. Computer printers, as relatively low image resolution devices, are used mostly to present final pathology reports that could include text and graphics.

Storage

Storage of the acquired data (graphical digital slide files and text data) involves saving system information in a data storage device system having at least convenient retrieval, and file management capabilities.

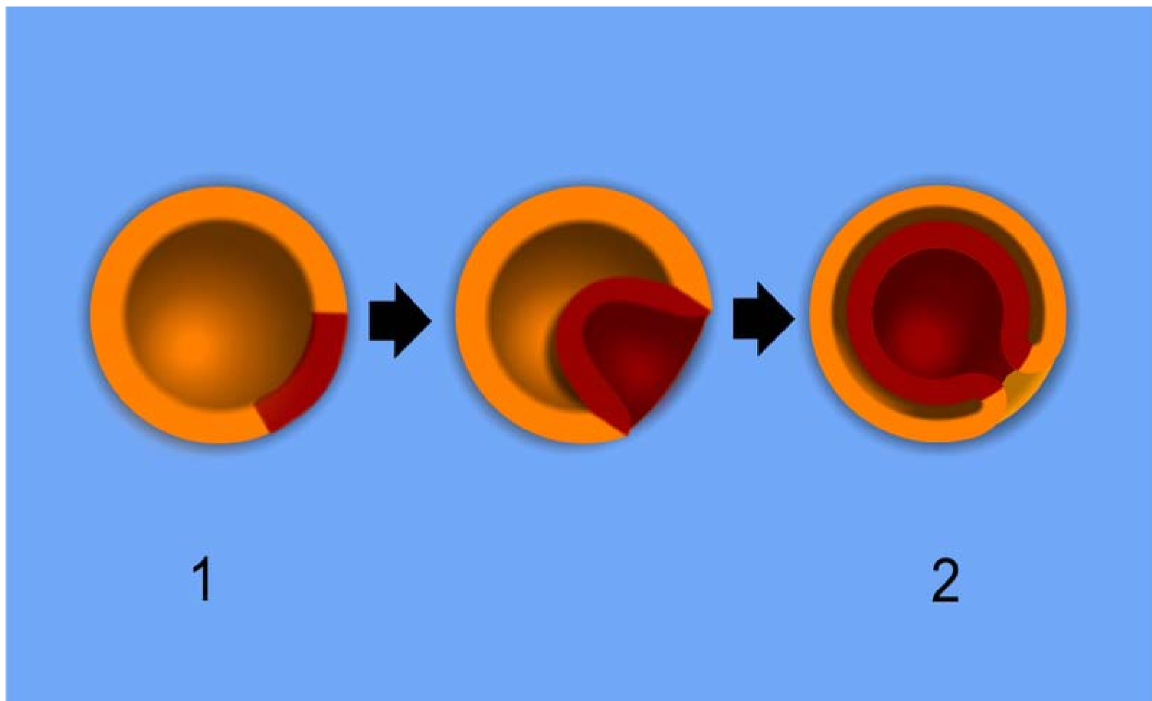
Medical imaging industry standards includes the Picture Archiving and Communication Systems (PACS), of European origin, which are image and information management solutions in computer networks that allow hospitals and clinics to acquire, distribute and archive medical images and diagnostic reports across the enterprise. Another standard of European origin is the Data and Picture Archiving and Communication System (DPACS). Although medical images can be stored in various formats, a common format has been Digital Imaging and Communications in Medicine (DICOM).

Chapter 24

Histogenesis

Histogenesis is the formation of different tissues from undifferentiated cells. These cells are constituents of three primary germ layers, the endoderm, mesoderm, and ectoderm. The science of the microscopic structures of the tissues formed within histogenesis is termed histology.

Germ layers



Gastrulation of a diploblast: The formation of germ layers from a (1) blastula to a (2) gastrula. Some of the ectoderm cells (orange) move inward forming the endoderm (red).

A **germ layer** is a collection of cells, formed during animal and mammalian embryogenesis. Germ layers are typically pronounced within vertebrate organisms; however, animals or mammals more complex than sponges (eumetazoans and agnotozoans) produce two or three primary tissue layers. Animals with radial symmetry, such as cnidarians, produce two layers, called the ectoderm and endoderm. Therefore, they are diploblastic. Animals with bilateral symmetry produce a third layer in-between

called mesoderm, making them triploblastic. Germ layers will eventually give rise to all of an animal's or mammal's tissues and organs through a process called organogenesis.

Endoderm

The **endoderm** is one of the germ layers formed during animal embryogenesis. Cells migrating inward along the archenteron form the inner layer of the gastrula, which develops into the endoderm. Initially, the endoderm consists of flattened cells, which subsequently become columnar.

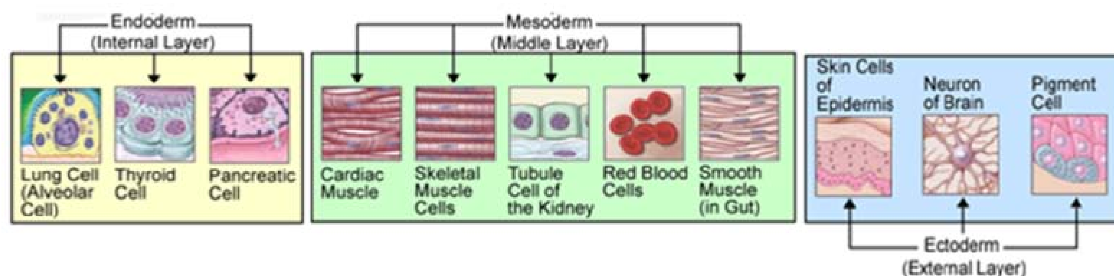
Mesoderm

The **mesoderm** germ layer forms in the embryos of animals and mammals more complex than cnidarians, making them triploblastic. During gastrulation, some of the cells migrating inward to form the endoderm form an additional layer between the endoderm and the ectoderm. This key innovation evolved hundreds of millions of years ago and led to the evolution of nearly all large, complex animals. The formation of a mesoderm led to the formation of a coelom. Organs formed inside a coelom can freely move, grow, and develop independently of the body wall while fluid cushions and protects them from shocks.

Ectoderm

The **ectoderm** is the start of a tissue that covers the body surfaces. It emerges first and forms from the outermost of the germ layers.

Production



The endoderm produces tissue within the lungs, thyroid, and pancreas. The mesoderm aids in the production of cardiac muscle, skeletal muscle, smooth muscle, tissues within the kidneys, and red blood cells. The ectoderm produces tissues within the epidermis and aids in the formation of neurons within the brain, and melanocytes.

The proceeding graph represents the products produced by the three germ layers.

Germ Layer	Category	Product
Endoderm	General	Gastrointestinal tract
Endodern	General	Respiratory tract
Endoderm	General	Endocrine glands and organs (liver and pancreas)
Mesoderm	General	Bones
Mesoderm	General	Most of the Circulatory system
Mesoderm	General	Connective tissues of the gut and integuments
Mesoderm	General	Excretory Tract
Mesoderm	General	Mesenchyme
Mesoderm	General	Mesothelium
Mesoderm	General	Muscles
Mesoderm	General	Peritoneum
Mesoderm	General	Reproductive System
Mesoderm	General	Urinary System
Mesoderm	Vertebrate	Chordamesoderm
Mesoderm	Vertebrate	Paraxial mesoderm
Mesoderm	Vertebrate	Intermediate mesoderm
Mesoderm	Vertebrate	Lateral plate mesoderm
Ectoderm	General	Nervous system
Ectoderm	General	Outer part of integument
Ectoderm	Vertebrate	Skin (along with glands, hair, nails)
Ectoderm	Vertebrate	Epithelium of the mouth and nasal cavity
Ectoderm	Vertebrate	Lens and cornea of the eye
Ectoderm	Vertebrate	Melanocytes
Ectoderm	Vertebrate	Peripheral nervous system
Ectoderm	Vertebrate	Facial cartilage
Ectoderm	Vertebrate	Dentin (in teeth)
Ectoderm	Vertebrate	Brain (rhombencephalon, mesencephalon and prosencephalon)
Ectoderm	Vertebrate	Spinal cord and motor neurons
Ectoderm	Vertebrate	Retina
Ectoderm	Vertebrate	Posterior pituitary