4

Pathology

Doctor 2017 | Medicine | JU

Sheet Slides

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Mechanism of Apoptosis

Recall from previous lecture, apoptosis has two distinct pathways:

1) Intrinsic pathway (Mitochondrial pathway): most common.

- it is related to activation of sensors inside the cytoplasm leading at the end to opening of the pores of mitochondrial wall involving Bax and Bak proteins.

2) Extrinsic pathway (Death receptor pathway)

- Involves binding between FAS receptor and FAS ligand (which is usually present on the effector cell). The receptor can either be FAS itself or type 1 TNF receptor.

There are two occasions in which death receptor pathway is used:

1) Death of targeted cells by the mediated Cytotoxic-T lymphocytes.

- Apoptosis mediated by Cytotoxic-t lymphocyte uses FAS receptor and FAS ligand interaction to cause death of these cells.
- when FAS ligand, present on the Cytotoxic-T lymphocyte (the effector cell), binds to FAS receptor on the target cell, it will trigger death by apoptosis.
- Target cells in this case are: Tumor cells or Virally infected cells.

2) Elimination of self-reactive lymphocytes:

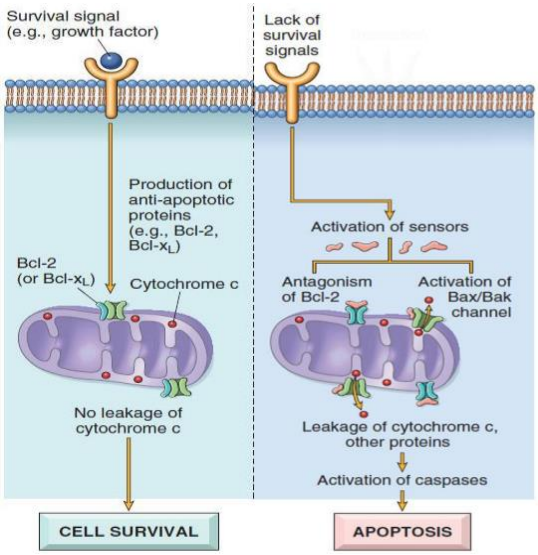
Lymphocytes capable of recognizing self-antigens. They are produced normally **but,** they must be killed by apoptosis in order not to develop autoimmune diseases.

- The binding between FAS ligand and FAS will activate the cytoplasmic domain of FAS receptor which is the death domain. when it is activated, it will activate caspase 8 unlike in mitochondrial pathway where caspase 9 is used.
- Then, caspase 8 will activate another series of caspases until we have activation of executioner caspases which will lead to the same mechanism of mitochondrial pathway; Formation of apoptotic bodies and detection of these bodies by phagocytes, phagocytosis of these bodies and getting rid of them.

Sometimes, Viruses use certain mechanisms to overcome this pathway. Example, some viruses produce a molecule called FLIP that normally acts as caspase antagonist; it will inhibit caspases, which is essential in apoptosis pathways. Therefore, apoptosis process will be inhibited, and the viral infection will progress.

Examples of Apoptosis

1) Growth Factor Deprivation.



When the cell is no longer receiving life signals such as growth factors or hormones that are needed to remain alive, the cell will activate the sensors in the cytoplasm and trigger the mitochondrial pathway.

Examples:

1) Hormone-sensitive cells deprived of hormones; like endometrium after menopause.

2) Unstimulated lymphocytes; lymphocytes aren't getting growth factor signals.

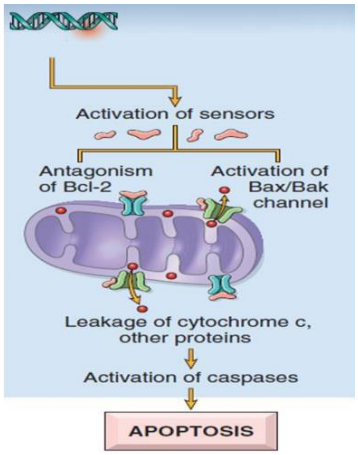
3) Neurons deprived of nerve growth factor.

Recall what have been discussed before:

- Normally we have a survival signal; growth factor stimulation of the cell. Therefore, the active proteins of Bcl2 family are Bcl2 and Bcl-xl antiapoptotic proteins which will maintain Bak and Bax in their inactive form and therefore, cytochrome c will **not** escape to the cytosol.
- However, Lack of survival signals causes the sensors to be activated which will lead to activation of Bak and Bax, their dimerization and insertion in mitochondrial wall leading to the escape of cytochrome c in order to activate caspase 9 and the mitochondrial pathway.

2) DNA Damage.

Note: Sentinel proteins were mentioned in the book



Recall from sheet 3: Exposure of cells to radiation (E.g: UV light) or chemotherapeutic agents, intracellular generation of ROS, and acquisition of mutations may all induce DNA damage, which if severe may trigger apoptotic death.

Mechanism:

- Damage to DNA is sensed/detected by intracellular sentinel proteins.
- These proteins will transmit signals that lead to accumulation of P53 protein.

- Any gene in the body will be translated to a protein. P53 gene is translated to p53 protein which acts as tumor suppressor; prevents tumor development.
- p53 first arrests the cell cycle (at the G1 phase) to allow the DNA to be repaired (by DNA repair genes) before it is replicated. if it is repaired the cell will continue proliferating.
- **However,** if the damage is too great to be repaired successfully, p53 triggers apoptosis, mainly by the activation of the mitochondrial pathway; stimulating BH3-only sensor proteins that ultimately activate Bax and Bak, proapoptotic members of the Bcl-2 family.
- When p53 is mutated or absent (NO APOPTOSIS, as it is in certain cancers), cells with damaged DNA (that would otherwise undergo apoptosis) survive. **Why?**
  - Due to inherited or sporadic mutation/absence in p53, the tumor suppressor effect will be lost and therefore any DNA damage will not be repaired, the cell will proliferate along with DNA damage. In such cells, the DNA damage may result in mutations or DNA rearrangements (e.g., translocations) that lead to malignancy/neoplastic transformation.

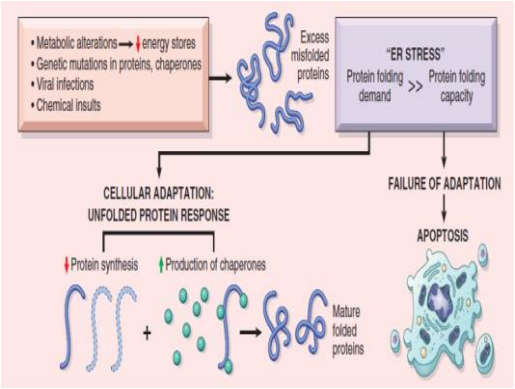
3) Accumulation of Misfolded Proteins: ER stress.

The accumulation of misfolded proteins in a cell can stress compensatory pathways in the ER and lead to cell death by apoptosis.

- In order for any protein to function it should be properly folded.
- During normal protein synthesis, **chaperones** in the ER control the proper folding of newly synthesized proteins.
- misfolded polypeptides are ubiquitinated and targeted for proteolysis.
- If a protein is not properly folded it is called a misfolded protein and therefore, it is not functional. If unfolded or misfolded proteins accumulate in the cytoplasm, they first induce a protective cellular response that is called the unfolded protein response.
- **unfolded protein response:** an adaptive mechanism/response that activates signaling pathways that increase the production of chaperones and decrease protein translation, thus reducing the levels of misfolded proteins in the cell.
- If this mechanism of adaptation fails due to accumulation of a large amount of misfolded protein that cannot be handled by the adaptive response, the signals that are generated result in activation of proapoptotic sensors of the BH3-only family as well as direct activation of caspases, leading to apoptosis usually by the mitochondrial (intrinsic) pathway.

- This is present in certain diseases in which the cell injury and cell death is mediated by endoplasmic reticulum stress. Cell death as a result of protein misfolding is recognized as a feature of a number of diseases, including the neurodegenerative disorders (mostly occur in brain) such as Alzheimer disease, Parkinson disease and Huntington disease (movement disorder).

Where do these Misfolded proteins come from?



1) Genetic abnormalities (Abnormality in the protein itself or in the chaperones).

2) Metabolic

Examples: decrease in nutrient supply (no ATP) or oxygen supply, cases of ischemia and hypoxia. Such cases will decrease protein folding due to abnormality in endoplasmic reticulum and ribosomes leading to accumulation of misfolded proteins.

3)Viral infections.

Like prion infections in the CNS cause accumulation of misfolded proteins and therefore death of virally infected cells by apoptosis.

4) Chemical insults.

Any chemical, toxic or chemotherapeutic agent that lead to accumulation of misfolded proteins.

- **Misfolded proteins either lead to a disease by**
  - Activation of apoptosis like neurodegenerative diseases and type 2 DB (Type 2 Diabetes Mellitus happens when the cells are not responding well to the hormone insulin [insulin resistance] which causes higher insulin levels and higher blood sugar levels).
  - Putting the cell at a state of deficiency of the protein since this misfolded protein is not functional.

    Example: Cystic Fibrosis which is caused by inherited mutations in a membrane transport protein that prevent its normal folding. This protein will produce in misfolded manner, the cell will have a state of deficiency of this protein and lead to unwanted symptoms.
  - ❖ Endoplasmic reticulum stress lead to a disease by triggering endoplasmic apoptosis or by having a state of deficiency of the affected protein.

4) Apoptosis of Self-Reactive Lymphocytes.

Mediated by **both** the Fas death receptor pathway and mitochondrial pathway.

If they don't go through apoptosis, the patient will develop autoimmune disease.

5) Cytotoxic T-Lymphocyte Mediated Apoptosis.

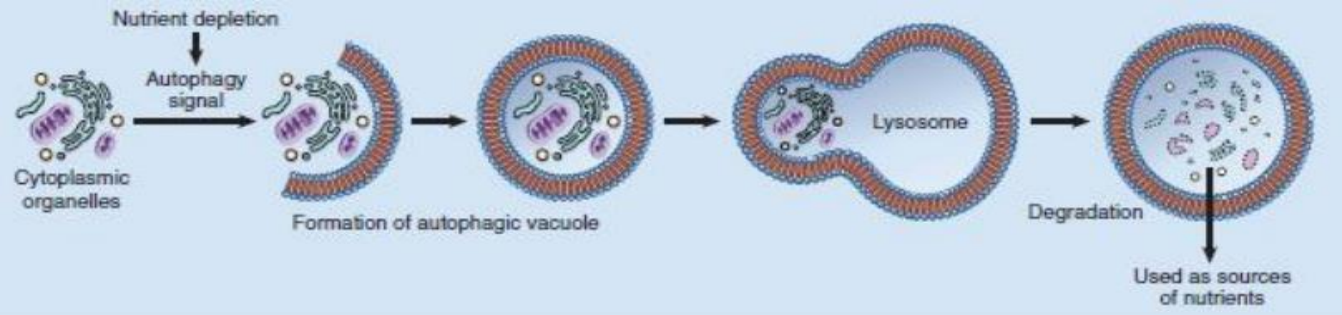
They mediate death of tumor cells and virally infected cells. **Mechanism:**

1) CTLs express FasL on their surface and may kill target cells by ligation of Fas receptors (Death receptor [extrinsic] pathway).

2) Production of an enzyme by the cytotoxic t-lymphocyte called **GRANZYME** which does a direct activation of the caspases, that can occur at the same time after the interaction FAS ligand and FAS. 16:10

Autophagy

Autophagy ("self-eating") refers to lysosomal digestion of the cell's own components.



Mechanism:

- In this process, intracellular organelles and portions of cytosol are first sequestered within an ER-derived autophagic vacuole, whose formation is initiated by cytosolic proteins that sense nutrient deprivation.
- The vacuole fuses with lysosomes to form a phagosome (autophagolysosome), in which lysosomal enzymes digest the cellular components.

Cytosolic proteins sense nutrient deprivation → Cytoplasmic organelles gather due to autophagy signal → A part of ER surrounds these organelles to form a vacuole → The vacuole fuses with a lysosome forming a phagosome → Lysosomal enzymes, produced by lysosome, will digest the contents of these vacuoles and the cell shrinks

Roles of Autophagy

1) A survival mechanism in times of nutrient deprivation.

The starved cell can live by eating its own contents and recycling these contents to provide nutrients and energy.

> **Recall from sheet 1: Atrophy's mechanisms:** decrease in protein synthesis, increase in protein degradation or by autophagy.

2) Atrophy (Adaptive).

In some circumstances, autophagy may be associated with atrophy of tissues due to several causes such as chronic ischemia, lack of hormonal or nutrient supply. Therefore, that may represent an adaptation that helps cells to survive lean times/stay alive by using its own substrates to limit energy consumption and also produce energy by digesting them. However, if the starved cell can no longer cope by devouring its contents, autophagy may eventually lead to apoptotic cell death.

3) Ischemia and myopathies.

Extensive autophagy is seen in ischemic injury and some types of myopathies.

4) With inflammatory bowel disease (unknown mechanism).

Polymorphisms in a gene involved in autophagy have been associated with inflammatory bowel disease, but the mechanistic link between autophagy and intestinal inflammation is not known.

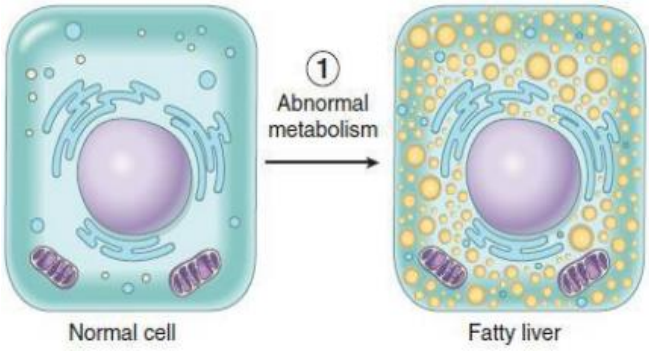
5) Role in cancer.

Intracellular Accumulations

- Under some circumstances, cells may accumulate abnormal amounts of various substances, which may be harmless or may cause varying degrees of injury.
- The substance may be located in the cytoplasm, within organelles (typically lysosomes), or in the nucleus, and it may be synthesized by the affected cells or it may be produced elsewhere.
  - Origin of this material can be endogenous (inside the cells) or exogenous (from outside of the cells).

Intracellular accumulations occur in these 4 instances:

1) Inadequate removal of a normal substance (fatty change in the liver)

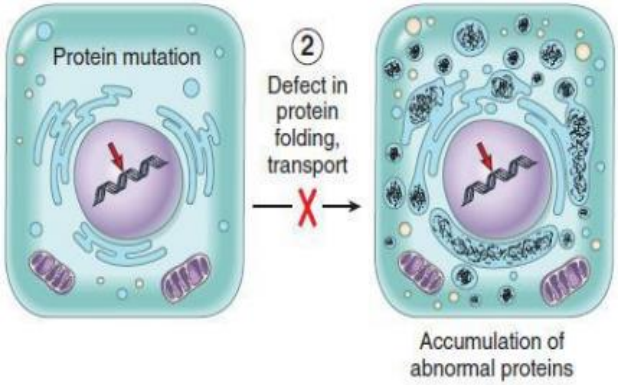


Such substances are normally present in the cell, **but** the cell needs to get rid of them. Deficiency in their removal or inadequate removal will lead to their accumulation.

Example: Accumulation of fat:

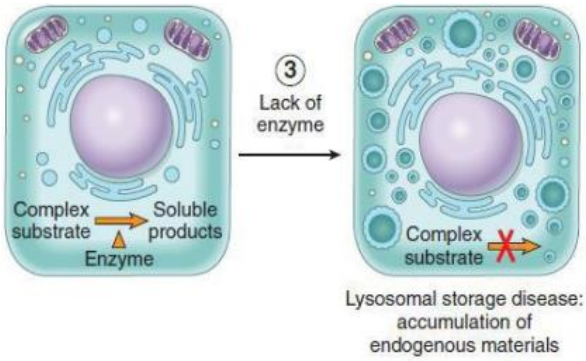
Fat is normally present in the cell. In cases Like CCl4 toxicity in liver cells which decreases protein synthesis, transport of fat from hepatocytes to outside the cell is deficient and fat will accumulate in the cell.

2) Accumulation of an abnormal endogenous substance (α1-antitrypsin)



The material is endogenous, produced in the cell but it is not normally present (unlike fat which is normally present) due to deficiency of certain enzymes it will accumulate, like alpha 1 antitrypsin deficiency.

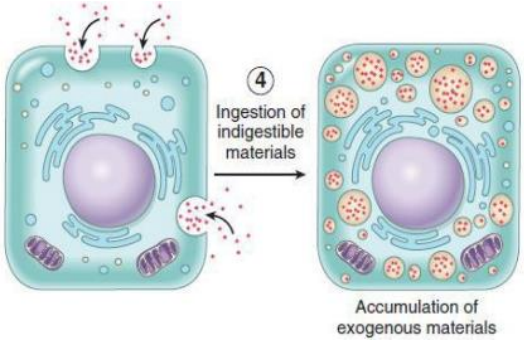
3) Failure to degrade a metabolite due to inherited enzyme deficiencies (storage diseases).



deficiency in a metabolite enzymatic digestion like in lysosomal/glycogen storage diseases.

Example: Glycogen is metabolized by certain enzymes. If they are deficient, the cell will start to accumulate glycogen in excessive amounts.

4) Deposition and accumulation of an abnormal exogenous substance (carbon and selica)

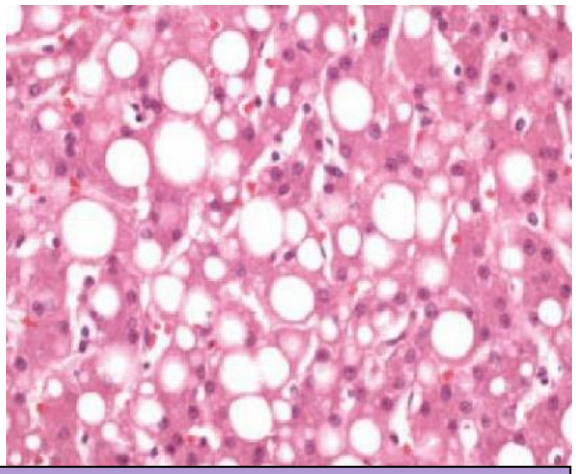


Deposition can be an exogenous material from outside the cell like carbon or silicon.

Example: inhaling carbon from air pollutant or smoke will lead to its deposition in cells of lungs, lymphatics and chest.

Selected examples of each are described as follows:

Fatty change: Steatosis



Section in the Liver: Large droplet of colorless fat pushing nucleus to the periphery

- It happens in tissues that metabolize fat.
- Fatty change, also called **steatosis** refers to any accumulation of triglycerides within parenchymal cells.
- It is most often seen in the liver, since this is the major organ involved in fat metabolism, but may also occur in heart, skeletal muscle, kidney, and other organs.
- **Causes:** Toxins (like CCl4 toxicity), protein malnutrition, diabetes mellitus (DM), obesity, or anoxia.
  - Protein malnutrition: Lipids need proteins to be transported since they are insoluble. If there is a protein deficiency, lipids will accumulate in the cell.
  - Anoxia: complete cut of oxygen supply to the tissue which leads to its injury. (Hypoxia: decrease in oxygen supply, whereas anoxia means no oxygen).
- Alcohol abuse and DM+obesity are the most common causes of fatty liver.
  - Most common cause in western countries: Alcohol abuse.
  - Most common cause in our countries: DM+obesity.

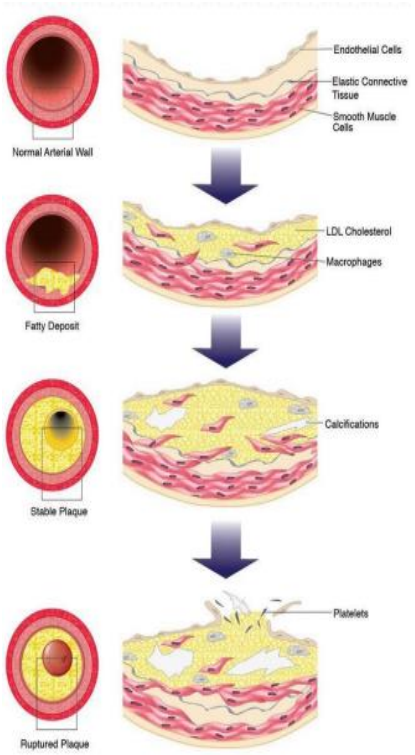
❖ Fat is a normal endogenous material, there is a problem in its transport and metabolism, so it will accumulate in the cell.

Cholesterol and Cholesteryl Esters

Cellular cholesterol metabolism is tightly regulated to ensure normal generation of cell membranes (in which cholesterol is a key component) without significant intracellular accumulation.

However, phagocytic cells may become overloaded with lipid (triglycerides, cholesterol, and cholesteryl esters) in several different pathologic processes, mostly characterized

by increased intake or decreased catabolism of lipids. Of these, atherosclerosis is the most important.



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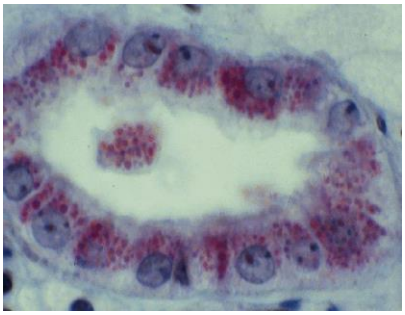
- **Atherosclerosis:** Deposition of cholesterol, cholesteryl esters in the walls of the blood vessels.
  - ➢ When cholesterol, cholesteryl esters, triglycerides levels are high, they enter the cytoplasm of microphages after being phagocytosed by them to deposit in the walls of the blood vessels.
  - ➢ It starts as a small deposition (atheroma) then it increases so that it starts narrowing the lumen of the blood vessels and sometimes superimpose thrombus.
  - If occurred in brain, can cause stroke.
  - If occurred in heart, can cause infraction.
  - If occurred in lumbar limbs, can cause ischemia.

Proteins

Morphologically visible protein accumulations are much less common than lipid accumulations; they may occur when excesses are presented to the cells or if the cells synthesize excessive amounts. (Either excess external or internal synthesis)

Examples:

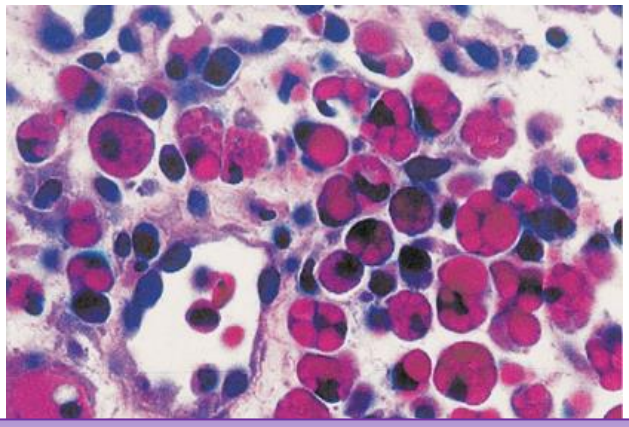
1) Proximal renal tubules in nephrotic syndrome (external)



- In the kidney, for example, trace amounts of albumin filtered through the glomerulus are normally reabsorbed by pinocytosis in the proximal convoluted tubules.
- However, in disorders with heavy protein leakage from blood to urine across the glomerular filter (e.g., **Nephrotic syndrome:** the kidney filters out proteins to go out along with urine), much more of the protein is reabsorbed, and vesicles containing this protein accumulate, giving the histologic appearance of pink, hyaline cytoplasmic droplets. The process is reversible: if the proteinuria abates, the protein droplets are metabolized and disappear.

2) Russell bodies in plasma cells. (internal)

- Plasma cells produces antibodies; immunoglobulins, which are proteins.
- In some disease situations, these plasma cells produce excessive amounts of these immunoglobulins which leads to marked accumulation in the cytoplasm forming rounded, eosinophilic (pink) droplets called **Russell bodies.**



Intracellular accumulation of immunoglobulins (Russell bodies) inside plasma cells

3) Alcoholic hyaline in liver.

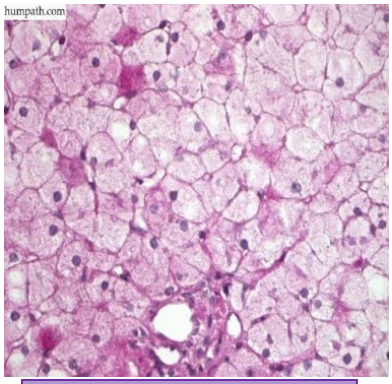
4) Neurofibrillary tangles in neurons.

These are also internal examples, but the doctor said to focus on the first two examples.

Glycogen

Glycogen is the stored form of glucose.

Excessive intracellular deposits/accumulations of glycogen are associated with abnormalities in the metabolism of either glucose or glycogen. Examples:



Section of the liver, hepatocytes are filled with glycogen. PAS stain is used to detect presence of glycogen.

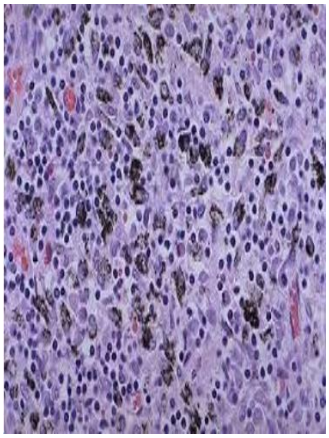
1) In poorly controlled diabetes mellitus, the prime example of abnormal glucose metabolism, glycogen accumulates in renal tubular epithelium, cardiac myocytes, and β cells of the islets of Langerhans (pancreas), and there is no way to control the metabolism since we have insulin deficiency or insulin resistance.

2) Glycogen storage diseases. Glycogen also accumulates within cells in a group of related genetic disorders collectively referred to as glycogen storage diseases, or glycogenosis, where we have inherited deficiencies of the enzymes.

Pigments Deposition or Accumulation

Pigments are colored substances that can be seen by naked eye or under the microscope. They are either exogenous, coming from outside the body, such as carbon, or are endogenous, synthesized within the body itself, such as lipofuscin, melanin, and certain derivatives of hemoglobin. Examples:

1) Carbon Deposition



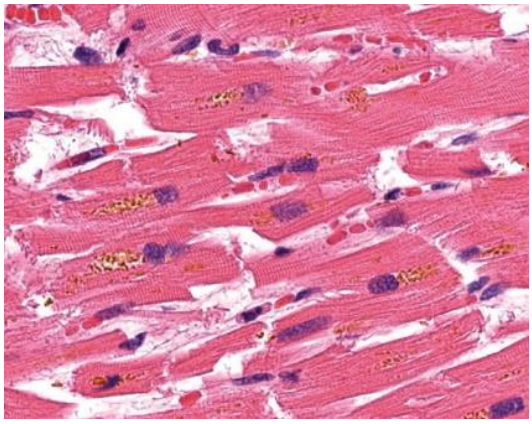
Carbon is the most common exogenous pigment.

It is deposited in the lung due to coal dust, ubiquitous air pollutant of urban life or smoking.

Carbon is indigestible material. When inhaled, it is phagocytosed by alveolar macrophages and transported through lymphatic channels to the regional tracheobronchial lymph nodes. Aggregates of the pigment blacken the draining lymph nodes and pulmonary parenchyma.

Another name for carbon pigment deposition in the lungs is **Anthracosis.**

2) Lipofuscin pigment: endogenous.

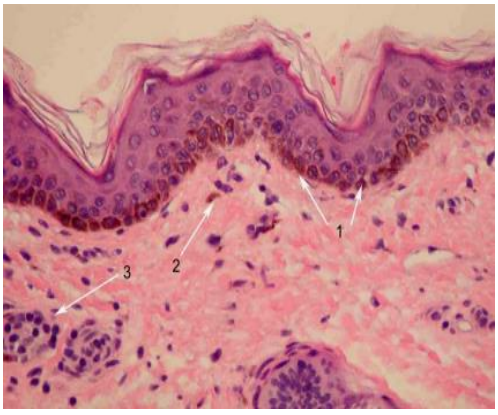


- Also called "wear-and-tear pigment," is an insoluble brownish-yellow granular intracellular material that accumulates/deposits in a variety of tissues (particularly the heart, liver, and brain) with aging or atrophy.
- Lipofuscin represents complexes of lipid and protein that are produced by the free radical–catalyzed peroxidation of polyunsaturated lipids of subcellular membranes (membrane of organelles) or the cell membrane. These degraded membrane components (lipids and proteins) will deposit in the cell and give a yellow-brown appearance.

➢ The brown pigment, when present in large amounts (When the tissue is highly atrophic with large amounts of deposition of this material), imparts an appearance to the whole tissue (mostly happens with aging) that is called **Brown atrophy**.

❖ Clinically insignificant; It is not injurious to the cell but is a marker/indication of a past free radical injury or aging. The significance comes from the atrophied cell.

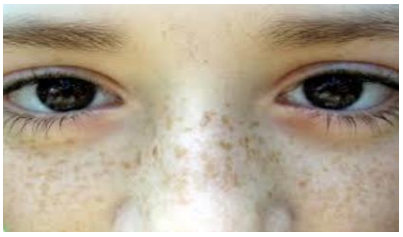
3) Melanin



Melanin is an endogenous, brown-black pigment that is synthesized by melanocytes located in the epidermis and acts as a screen/protection against harmful UV radiation.

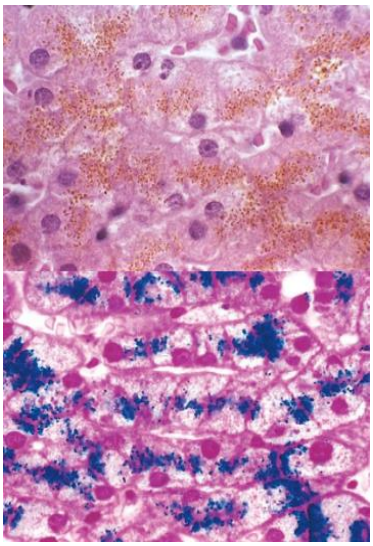
➢ Light skin people more vulnerable to UV light damage than dark skin people who have more melanin.

Although melanocytes are the only source of melanin, adjacent basal keratinocytes and dermal microphages in the skin can accumulate the pigment (e.g., in **Freckles**, a melanin pigment, which happens due to exposure to sun light) When?



- When melanin is produced excessively in the melanocytes, it will diffuse to adjacent keratinocytes in the skin and give the skin this brown-black coloration. 33:35

4) Hemosiderin or Iron pigments



- Comes from heme which has iron in its structure that makes this hemosiderin pigment. Hemosiderin is a hemoglobin-derived granular pigment that is golden yellow to brown in H&E stain (similar to lipofuscin) and accumulates in tissues when there is a local or systemic excess of iron.
- Iron is normally stored within cells in association with the protein apoferritin, forming **ferritin micelles**. Hemosiderin pigment represents large aggregates of these ferritin micelles, readily visualized by light and electron microscopy.
- Although hemosiderin accumulation is usually pathologic, it can also be physiologic.

Physiologic: Rapid turnover of RBCs; small amounts of this pigment are normal in the mononuclear phagocytes of the bone marrow, spleen, and liver (reticular epithelial system), where aging red cells are normally degraded. (this is normal physiologic, it does not indicate a disease process).

Pathologic: Examples:

a) Localized accumulation due to trauma such as in bruises.

The color changes from red to blue (deoxygenated blood) to brownish then fades to yellow color. This brown discoloration is due to deposition of iron. Damaged RBCs will be destroyed by microphages, leading at the end to deposition of iron; local pathologic deposition from hemorrhage.

b) Hemosiderosis: systemic pathologic deposition of hemosiderin: the pigment is diffused all over the body if we have extensive accumulations of iron which is seen in hereditary hemochromatosis (iron overload), hemolytic anemias or repeated blood transfusion like in Thalassemia.

- If the deposition is little it will not have any clinical significance.

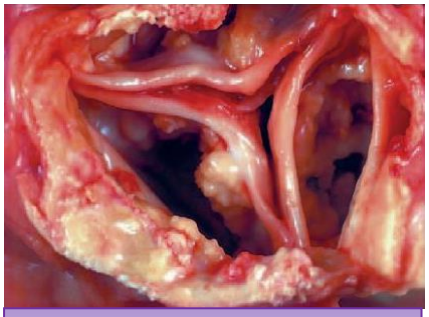
- If deposition of iron is huge the patient will develop organ disfunction.
- So, it depends on the amount of deposition to know whether it gives clinical significance or not.

- **Prussian blue stain:** a special stain that is used as a marker for iron deposition, the iron can be unambiguously identified by the Prussian blue histochemical reaction. This stain is specific, if u use it at lipofuscin pigment it will **not** give a blue color.

Pathologic Calcification

Pathologic calcification, a common process in a wide variety of disease states, is a result of an abnormal deposition of calcium salts, together with smaller amounts of iron, magnesium, and other minerals. It can occur in two ways:

1) Dystrophic Calcification



- Initiation → propagation
- Extracellular/Intracellular
- Calcium phosphate crystals

In this form, calcium metabolism is normal, but it deposits in injured or dead tissue, such as areas of necrosis of any type.

- Just like what we said in fat necrosis, that fatty acids will attract the calcium to do saponification and appear as chalky white material.
- Not necessarily caused by hypercalcemia. However, it can be exacerbated by hypercalcemia.
- May be a cause of organ dysfunction; if deposited in large amounts.

Examples:

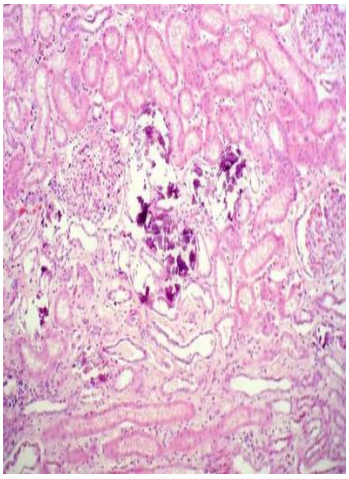
1) Incidental finding indicating insignificant past cell injury, like abscess that was healed. If we took a biopsy of it, we can see the calcification. It has no clinical significance, but it is an indicator for a past injury.

2) Necrosis of any type (e.g. atheromas, aging or damaged heart valves [aortic stenosis], tuberculosis).

  - It is virtually ubiquitous in the arterial lesions of advanced atherosclerosis.
  - Calcification can develop in aging or damaged heart valves, resulting in severely compromised valve motion. Dystrophic calcification of the aortic valves is an important cause of aortic stenosis in elderly persons.
- Seen as whitish coloration by naked eye (chalky material).

- Under microscope, it will have a purple color by H&E stain.

2) Metastatic Calcification



- Metastasis in medicine means spread of a cancer. **But,** it is not necessarily associated with cancer in metastatic calcification.
- Metastatic calcification is almost always associated with abnormal Ca2+ metabolism (hypercalcemia). Deposition occurs in normal tissues (no necrosis).
- In VESSELS, LUNG, KIDNEY.

The major causes of hypercalcemia are

1) Increased secretion of parathyroid hormone, due to either primary parathyroid tumors or production of parathyroid hormone–related protein by other malignant tumors, causing high levels of calcium and its accumulation.

2) Destruction of bone (which causes the elaboration of calcium from the bone to the blood and therefore building up, depositing anywhere) due to the effects of accelerated turnover (e.g., Paget disease), immobilization, or tumors (increased bone catabolism associated with **M**ultiple **M**yeloma, leukemia, or diffuse skeletal metastasis).

3) Vitamin D–related disorders including vitamin D intoxication and sarcoidosis (an autoimmune disease, in which macrophages activate a vitamin D precursor).

4) Renal failure, in which phosphate retention leads to secondary hyperparathyroidism.

In renal failure, patients will have hypocalcemia. Due to that, secondary hyperparathyroidism occurs which leads to hypercalcemia; calcium deposits everywhere mostly in blood vessel walls, lungs and kidney.

- If deposited material is small, no clinical significance.
- However, if there is severe calcification of calcium, it will cause organelle disfunction, like if the whole kidney is calcified, Nephrocalcinosis occurs and causes renal failure.

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END OF CHAPTER

NOTE: Cell aging will be discussed with neoplasia lectures