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17/MHS02/078

Cellular pathology assignment

Question

1) Write explicitly on 5 diagnostic techniques used in pathology, relevant illustration and examples required

2) Cellular adaptation precedes cell death, discuss. Diagrams essential.

Answers

A.

1. Flow cytometry
2. Enzyme histochemistry
3. Immunohistochemistry
4. Immunocystochemistry
5. Electron microscopy
6. Flow cystometry: Flow cytometry is used when there is a need to profile a large number of different cell types in a population. The cells are separated on the basis of differences in size and morphology. Additionally, fluorescently-tagged antibodies that target specific antigens on the cell surface can be used to identify and segregate various sub-populations.The basic steps include passing the cells through a narrow channel, such that each cell is illuminated by a laser one at a time. A series of sensors then detect the refracted or emitted light, and this data is integrated and compiled to generate information about the sample. 

A flow cytometer tube with suction straw

Flow cytometry helps to analyze several parameters of a cell simultaneously. Some of these parameters are described below:

* Functional analysis

This method can determine biological activity inside cells, such as the generation of reactive oxygen species, mitochondrial membrane changes during apoptosis, phagocytosis rates in labelled bacteria, native calcium content, and changing metal content in response response to drugs, etc.

* Determining cell viability

This method can also be used to assess cell viability after the addition of pathogenic organisms or drugs. Any breach in cell membrane integrity can be determined using dyes that can enter the punctured cell membrane. Fluorescent probes such as bis-oxonol can bind to proteins present on the cell membrane, allowing for the identification of various stages of necrosis.

* Measuring apoptosis and necrosis

Apoptosis or programmed cell death is accompanied by characteristic changes in cell shape, loss of structures, cell detachment, condensation of the cytoplasm, cell shrinkage, phagocytosis of cellular residues and changes in the nuclear envelope.

Some of the biochemical changes include proteolysis, DNA denaturation, cell dehydration, protein cross-linking, and a rise in the free calcium ions. These physical and biochemical changes can be detected using flow cytometry.

Oncosis is a necrotic event where the cell starts to swell rather than shrink. This leads to rupture of the plasma membrane and release of proteolytic enzymes that can also damage the surrounding tissues. These changes in the plasma membrane and cell shape can be assessed using flow cytometry.

* Cell cycle analysis

The amount of DNA present in the nucleus varies during each phase of the cell cycle. This variation in DNA content can be assessed using fluorescent dyes that bind to DNA or monoclonal antibodies, which can allow the detection of antigen expression.

Other factors including the content of cell pigments such as chlorophyll, DNA copy number variation, intracellular antigens, enzymatic activity, oxidative bursts, glutathione, and cell adherence can similarly be measured using this method.

How do flow cytometers work?

* Fluidics: During flow cytometry, a sheath fluid hydrodynamically focuses the cell suspension through a small nozzle such that only one cell passes the laser light at a time. A detector is placed in front of the laser beam such that it can capture the forward scattered light from the cells, while several detectors are also placed to the sides to measure the amount and intensity of light scattered in each direction.

Forward scatter refers to the light refracted by a cell that is traveling in the same direction as it was traveling originally. The proportion of light that is forward scattered is correlated with the cell size, where larger-sized particles produce more forward scattered light.

Side scatter refers to the refracted light that is orthogonal to the direction of the light path. Side scattered light provides information about the granularity, where the highly granular cells produce more side-scattered light compared to cells with low granularity.

For example, while performing flow cytometry of blood samples, large and granular granulocytes show a high forward and side scatter; monocytes that are large but not granular show a high forward but lower side scatter. Thus, based on the proportion of light that is forward and side scattered, different types of populations can be separated.

* Separation based on fluorescence emission

Apart from forward and side scatter, different types of cells can also be separated based on the light emitted by fluorescent molecules. This fluorescence may be due to naturally fluorescing materials inside a cell, or fluorescence-tagged antibodies. For example, fluorochrome is used to stain a protein of interest so that incident laser light of the appropriate wavelength allows the cells containing this protein to be detected.

As a cell passes through the laser beam, a pulse of photon emission is created. This pulse is detected by the photomultiplier tube and converted to a voltage pulse, which can be interpreted by the flow cytometer. The higher the intensity of fluorescence, the higher the voltage pulse.

Data interpretation

Each cell that passes through the laser light is detected as a separate event. Also, different types of detected light: forward-scatter, side-scatter, and specific wavelengths of fluorescence emission, is assigned a distinct channel. The data for each of these events is plotted independently and can be represented by two methods: histograms and dot-plots.

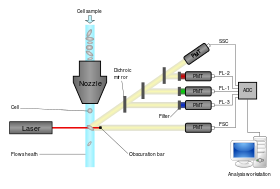
Histograms compare a single parameter, where intensity is plotted on one axis and the number of events is plotted on a separate axis. Dot-plots can compare more than one parameter simultaneously, where each event is displayed as a single point and the intensity values of two or three channels are represented on the various axis.

In this scenario, events that have similar intensities cluster together in the dot plot. While dot-plots can compare multiple parameters together, histograms are easier to read and understand. In many cases, dot-plots and histograms are not mutually exclusive, and in many flow cytometry experiments both types of graphs are plotted to represent and assess multi-parametric data.

Gating in flow cytometry

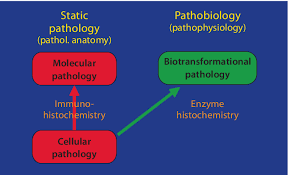
Gating is a method used in flow cytometry where more information is preferentially collected about a certain sub-population of cells. This adds further resolution to flow cytometry and can be used to analyze multiple parameters.

In a gated flow cytometry experiment, data is collected about one or more channels from the dot-plot. Then, a gate box is drawn and a subpopulation of cells are selected for more analysis. This subpopulation is highlighted in other plots that display information from alternate channels. This method helps to provide greater flexibility and single-cell resolution for each channel.



Schematic diagram of a flow cytometer, from sheath focusing to data acquisition.

1. Enzyme histochemistry: Enzyme histochemistry serves as a link between biochemistry and morphology. It is based on metabolization of a substrate provided to a tissue enzyme in its orthotopic localization. Visualization is accomplished with an insoluble dye product. It is a sensitive dynamic technique that mirrors even early metabolic imbalance of a pathological tissue lesion, combined with the advantage of histotopographic enzyme localization. With the advent of immunohistochemistry and DNA-oriented molecular pathology techniques, the potential of enzyme histochemistry currently tends to be underrecognized. This review aims to draw attention to the broad range of applications of this simple, rapid and inexpensive method. Alkaline phosphatase represents tissue barrier functions in brain capillaries, duodenal enterocyte and proximal kidney tubule brush borders. Decrease in enzyme histochemical alkaline phosphatase activity indicates serious functional impairment. Enzyme histochemical increase in lysosomal acid phosphatase activity is an early marker of ischemic tissue lesions. Over the last four decades, acetylcholinesterase enzyme histochemistry has proven to be the gold standard for the diagnosis of Hirschsprung disease and is one of the most commonly applied enzyme histochemical methods today. Chloroacetate esterase and tartrate-resistant phosphatase are both resistant to formalin fixation, EDTA decalcification and paraffin embedding. Early enzyme histochemical insight into development of a pathologic tissue lesion and evaluation of function and vitality of tissue enhance our understanding of the pathophysiology of diseases. In this process, enzyme histochemistry constitutes a valuable complement to conventional histology, immunohistochemistry and molecular pathology for both diagnostic and experimental pathology.



**Enzyme histochemistry**

General Principles of Enzyme Histochemical Techniques

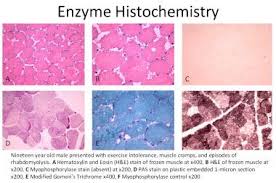
Enzyme histochemistry combines the biochemical analysis of enzyme activity with information on its topographical localization. In order to briefly acquaint the reader unfamiliar with these techniques, we describe two of the most important enzyme histochemical techniques in detail.

In a dehydrogenase reaction, enzyme substrates like sodium succinate or sodium L -lactate are oxidized and a stoichiometric color indicator tetranitrotetrazolium chloride blue (TNBT) is reduced to black or blue formazan. The formazan immediately binds to local protein and permits the precise localization of the enzyme dehydrogenase in a particular tissue compartment. The enzyme histochemical reaction follows the stoichiometric principles of biochemistry. Whereas biochemistry is applied to tissue homogenates or extracts, expressing enzyme activity in turnover rates, enzyme histochemistry indicates the locus of an enzyme in the tissue section. A second group of enzyme reactions use diazonium salt instead of tetrazolium chloride as color indicator. Enzymes stained with this kind of reaction are mainly esterases and phosphatases. The ester group or phosphate group of a naphthyl salt is split off by the enzyme reaction and the naphthyl rest couples to a diazo-salt and stains the esterase- or phosphatase-containing compartment like in a formazan color reaction]. Almost all enzyme histochemical investigations are performed with frozen tissue, because most enzymes are inactivated by formalin fixation. In fact, formalin fixation is even employed to stop enzyme histochemical reactions like lactic dehydrogenase or succinic dehydrogenase reactions]. With the requirement of frozen tissue, transportation and storage of biopsies or surgical specimens become an issue and lead to frequently asked questions in daily routine practice. It is recommended to freeze specimens on dry ice (CO 2 ) at –80°C or in isopentane stored in a –25°C freezer. Liquid nitrogen carries the disadvantage of cracking freezing artifact in large tissue specimens]. After freezing as described, the tissue can be stored without loss of enzymatic activity for longer periods at –25°C in a small Eppendorf tube (biopsies) or plastic bag (surgical resections). Similarly, an important technical point to stress is the minimal section thickness. It is recommendable to cut cryostat sections at 15 m in order to overcome the minimal enzyme activity threshold for the enzymatic starting reaction . It is important to be aware that a 15- m-thick cryostat section loses 70% of its thickness by thawing, spreading and drying, resulting in a final thickness of 4 m, whereas an originally 4- m-thick native cryostat section has a final thickness of 1.2 m, so that in thin sections the enzyme reaction does not reliably develop because the enzyme activity may drop below the enzymatic starting reaction .

Enzyme Histochemistry versus Biochemical Analysis

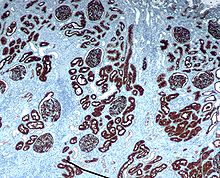
Biochemistry employs tissue extracts or tissue homogenates. Analysis of such tissue specimens reveals a general increase or decrease in enzyme activity measured in turnover rates. In contrast, enzyme histochemistry localizes enzyme activity to cells or tissue compartments but does not provide the exact quantitative data of biochemical analysis. The capability to localize enzyme histochemical activity on a cellular level justifies the Greek prefix ‘-topo’ in the term ‘enzyme histotopochemistry’, distinguishing enzyme histochemistry from biochemistry. Overall, there is a good correlation between histochemically judged enzyme activity per volume (area ! thickness) and biochemically determined activity per milligram tissue . The strength of biochemistry lies in the potential for quantification: biochemistry permits exact quantification of enzymatic turnover rates, whereas enzyme histochemistry has the disadvantage of a lack of reliable quantification, so that only semiquantitative assessment is achieved if microspectrophotometry is applied. However, the elective staining of a particular tissue structure in enzyme histochemistry permits precise morphometric size measurement by means of optic electronic morphometry equipment, e.g. the ASBA3 system. The level of monochromic light absorption permits semiautomatic calculation, e.g. of the extension of an ischemic brain infarct. Another example of morphometric analysis in conjunction with enzyme histochemistry is the determination of nerve cell size, nerve cell number and nerve cell distance of the myenteric plexus stained with lactic dehydrogenase enzyme histochemistry. Biochemistry incurs the disadvantage of tissue dilution effect by tissue surrounding the structure of interest. Chromatography, electrophoresis or density gradient centrifugation is applied to compensate for this disadvantage. Compared to enzyme histotopochemistry, the biochemical procedure is therefore time-consuming but nevertheless falls short to localize the altered structure, which is more easily demonstrated by enzyme histotopochemical means.

Applications of Enzyme Histochemistry as a Tool in Diagnostic Pathology and Pathobiology Enzyme histochemistry can be used in pathohistological routine diagnosis as it has simple technical requirements. Enzyme histotopochemical demonstration of acetylcholinesterase activity in frozen colon mucosal biopsies has proven a reliable tool and is the current gold standard in the diagnosis of Hirschsprung disease today. In particular, in the diagnosis of ultrashort Hirschsprung disease and aganglionosis limited to the internal sphincter, the so-called sphincter achalasia, the acetylcholinesterase reaction is the choice technique. Another attractive application of enzyme histochemistry is the verification of a peracute myocardial infarction during autopsy. This can be performed macroscopically by a succinic dehydrogenase reaction in a Petri dish with succinate (13 g in 100 ml phosphate buffer at pH 7.4) and neotetrazolium chloride (100 mg dissolved in phosphate buffer). The reduced succinic dehydrogenase activity in the ischemic area of the myocardial infarction can quickly and reliably be recognized macroscopically. The enzyme alkaline phosphatase conveys a range of important information. On the one hand, alkaline phosphatase is eliminated by bile secretion. An increase or accumulation of alkaline phosphatase in bile capillaries of the liver indicates a disturbed mechanism of secretion.Toxic lesions of the liver, infectious hepatitis or seriously congested liver show similar pictures. These observations demonstrate that a simple enzyme histochemical reaction for alkaline phosphatase indicates a functional disturbance of the liver in biopsy or autoptic liver tissue. In addition to the transport function of bile capillaries for alkaline phosphatase, this enzyme represents a barrier function and is observed in structures with directed transport function. Alkaline phosphatase of brain capillaries represents the bloodbrain barrier. The alkaline phosphatase of the brush border of the proximal renal tubules is responsible for absorption of metabolites from the primary urine and is quite sensitive to toxic or ischemic injury. Of particular diagnostic value is alkaline phosphatase in the brush border of the duodenal mucosa in the diagnosis of celiac disease. Alkaline phosphatase activity is high in young adults and low in postweaning children . This enzyme is a fairly sensitive parameter in the diagnosis and therapy of celiac disease.

  
Genetics of glycogen storage disease type V

1. Immunohistochemistry: Immunohistochemistry (IHC) is the most common application of immunostaining. It involves the process of selectively identifying antigens (proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. IHC takes its name from the roots "immuno", in reference to antibodies used in the procedure, and "histo", meaning tissue (compare to immunocytochemistry). Albert Coons conceptualized and first implemented the procedure in 1941. Visualising an antibody-antigen interaction can be accomplished in a number of ways, mainly either of the following:

Chromogenic immunohistochemistry (CIH), wherein an antibody is conjugated to an enzyme, such as peroxidase (the combination being termed immunoperoxidase), that can catalyse a colour-producing reaction. Immunofluorescence, where the antibody is tagged to a fluorophore, such as fluorescein or rhodamine. Immunohistochemical staining is widely used in the diagnosis of abnormal cells such as those found in cancerous tumors. Specific molecular markers are characteristic of particular cellular events such as proliferation or cell death (apoptosis). Immunohistochemistry is also widely used in basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue.



Chromogenic immunohistochemistry of a normal kidney targeting the protein CD10.

Diagnostic IHC markers

IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. It is also an effective way to examine the tissues. This has made it a widely used technique in the neurosciences, enabling researchers to examine protein expression within specific brain structures. Its major disadvantage is that, unlike immunoblotting techniques where staining is checked against a molecular weight ladder, it is impossible to show in IHC that the staining corresponds with the protein of interest. For this reason, primary antibodies must be well-validated in a Western Blot or similar procedure. The technique is even more widely used in diagnostic surgical pathology for immunophenotyping tumors (e.g. immunostaining for e-cadherin to differentiate between DCIS (ductal carcinoma in situ: stains positive) and LCIS (lobular carcinoma in situ: does not stain positive). More recently, Immunohistochemical techniques have been useful in differential diagnoses of multiple forms of salivary gland, head, and neck carcinomas. The diversity of IHC markers used in diagnostic surgical pathology is substantial. Many clinical laboratories in tertiary hospitals will have menus of over 200 antibodies used as diagnostic, prognostic and predictive biomarkers. Examples of some commonly used markers include:

* BrdU: used to identify replicating cells. Used to identify tumors as well as in neuroscience research.
* Cytokeratins: used for identification of carcinomas but may also be expressed in some sarcomas.
* CD15 and CD30 : used for Hodgkin's disease
* Alpha fetoprotein: for yolk sac tumors and hepatocellular carcinoma
* CD117 (KIT): for gastrointestinal stromal tumors (GIST) and mast cell tumors
* CD10 (CALLA): for renal cell carcinoma and acute lymphoblastic leukemia
* Prostate specific antigen (PSA): for prostate cancer
* estrogens and progesterone receptor (ER & PR) staining are used both diagnostically (breast and gyn tumors) as well as prognostic in breast cancer and predictive of response to therapy (estrogen receptor)
* Identification of B-cell lymphomas using CD20
* Identification of T-cell lymphomas using CD3  
  Sample preparation

Preparation of the sample is critical to maintain cell morphology, tissue architecture and the antigenicity of target epitopes. This requires proper tissue collection, fixation and sectioning. A solution of paraformaldehyde is often used to fix tissue, but other methods may be used.

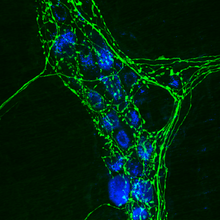
* Preparing tissue slices

The tissue may then be sliced or used whole, dependent upon the purpose of the experiment or the tissue itself. Before sectioning, the tissue sample may be embedded in a medium, like paraffin wax or cryomedia. Sections can be sliced on a variety of instruments, most commonly a microtome, cryostat, or vibratome. Specimens are typically sliced at a range of 3 µm-5 μm.[citation needed] The slices are then mounted on slides, dehydrated using alcohol washes of increasing concentrations (e.g., 50%, 75%, 90%, 95%, 100%), and cleared using a detergent like xylene before being imaged under a microscope. Depending on the method of fixation and tissue preservation, the sample may require additional steps to make the epitopes available for antibody binding, including deparaffinization and antigen retrieval. For formalin-fixed paraffin-embedded tissues, antigen-retrieval is often necessary, and involves pre-treating the sections with heat or protease. These steps may make the difference between the target antigens staining or not staining.

* Reducing non-specific immuno-staining

Depending on the tissue type and the method of antigen detection, endogenous biotin or enzymes may need to be blocked or quenched, respectively, prior to antibody staining. Although antibodies show preferential avidity for specific epitopes, they may partially or weakly bind to sites on nonspecific proteins (also called reactive sites) that are similar to the cognate binding sites on the target antigen. A great amount of non-specific binding causes high background staining which will mask the detection of the target antigen. To reduce background staining in IHC, ICC and other immunostaining methods, samples are incubated with a buffer that blocks the reactive sites to which the primary or secondary antibodies may otherwise bind. Common blocking buffers include normal serum, non-fat dry milk, BSA, or gelatin. Commercial blocking buffers with proprietary formulations are available for greater efficiency. Methods to eliminate background staining include dilution of the primary or secondary antibodies, changing the time or temperature of incubation, and using a different detection system or different primary antibody. Quality control should as a minimum include a tissue known to express the antigen as a positive control and negative controls of tissue known not to express the antigen, as well as the test tissue probed in the same way with omission of the primary antibody (or better, absorption of the primary antibody).

1. Immunocystochemistry: Immunocytochemistry (ICC) is a common laboratory technique that is used to anatomically visualize the localization of a specific protein or antigen in cells by use of a specific primary antibody that binds to it. The primary antibody allows visualization of the protein under a fluorescence microscope when it is bound by a secondary antibody that has a conjugated fluorophore. ICC allows researchers to evaluate whether or not cells in a particular sample express the antigen in question. In cases where an immunopositive signal is found, ICC also allows researchers to determine which sub-cellular compartments are expressing the antigen. Immunocytochemistry differs from immunohistochemistry in that the former is performed on samples of intact cells that have had most, if not all, of their surrounding extracellular matrix removed. This includes individual cells that have been isolated from a block of solid tissue, cells grown within a culture, cells deposited from suspension, or cells taken from a smear.



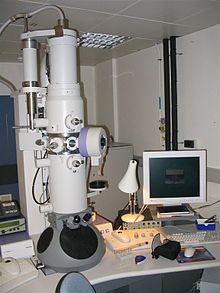
Immunocytochemistry labels individual proteins within cells, such as TH (green) in the axons of sympathetic autonomic neurons.

There are many ways to prepare cell samples for immunocytochemical analysis. Each method has its own strengths and unique characteristics so the right method can be chosen for the desired sample and outcome. Cells to be stained can be attached to a solid support to allow easy handling in subsequent procedures. This can be achieved by several methods: adherent cells may be grown on microscope slides, coverslips, or an optically suitable plastic support. Suspension cells can be centrifuged onto glass slides (cytospin), bound to solid support using chemical linkers, or in some cases handled in suspension. Concentrated cellular suspensions that exist in a low-viscosity medium make good candidates for smear preparations. Dilute cell suspensions existing in a dilute medium are best suited for the preparation of cytospins through cytocentrifugation. Cell suspensions that exist in a high-viscosity medium, are best suited to be tested as swab preparations. The constant among these preparations is that the whole cell is present on the slide surface. For any intercellular reaction to take place, immunoglobulin must first traverse the cell membrane that is intact in these preparations. Reactions taking place in the nucleus can be more difficult, and the extracellular fluids can create unique obstacles in the performance of immunocytochemistry. In this situation, permeabilizing cells using detergent (Triton X-100 or Tween-20) or choosing organic fixatives (acetone, methanol, or ethanol) becomes necessary. Antibodies are an important tool for demonstrating both the presence and the subcellular localization of an antigen. Cell staining is a very versatile technique and, if the antigen is highly localized, can detect as few as a thousand antigen molecules in a cell. In some circumstances, cell staining may also be used to determine the approximate concentration of an antigen, especially by an image analyzer.

Methods

There are many methods to obtain immunological detection on tissues, including those tied directly to primary antibodies or antisera. A direct method involves the use of a detectable tag (e.g., fluorescent molecule, gold particles, etc., ) directly to the antibody that is then allowed to bind to the antigen (e.g., protein) in a cell. Alternatively, there are many indirect methods. In one such method, the antigen is bound by a primary antibody which is then amplified by use of a secondary antibody which binds to the primary antibody. Next, a tertiary reagent containing an enzymatic moiety is applied and binds to the secondary antibody. When the quaternary reagent, or substrate, is applied, the enzymatic end of the tertiary reagent converts the substrate into a pigment reaction product, which produces a color (many colors are possible; brown, black, red, etc.,) in the same location that the original primary antibody recognized that antigen of interest. Some examples of substrates used (also known as chromogens) are AEC (3-Amino-9-EthylCarbazole), or DAB (3,3'-Diaminobenzidine). Use of one of these reagents after exposure to the necessary enzyme (e.g., horseradish peroxidase conjugated to an antibody reagent) produces a positive immunoreaction product. Immunocytochemical visualization of specific antigens of interest can be used when a less specific stain like H&E (Hematoxylin and Eosin) cannot be used for a diagnosis to be made or to provide additional predictive information regarding treatment (in some cancers, for example). Alternatively the secondary antibody may be covalently linked to a fluorophore (FITC and Rhodamine are the most common) which is detected in a fluorescence or confocal microscope. The location of fluorescence will vary according to the target molecule, external for membrane proteins, and internal for cytoplasmic proteins. In this way immunofluorescence is a powerful technique when combined with confocal microscopy for studying the location of proteins and dynamic processes (exocytosis, endocytosis, etc.).

1. Electron microscopy: Electron microscopy (EM) is a technique for obtaining high resolution images of biological and non-biological specimens. It is used in biomedical research to investigate the detailed structure of tissues, cells, organelles and macromolecular complexes. The high resolution of EM images results from the use of electrons (which have very short wavelengths) as the source of illuminating radiation. Electron microscopy is used in conjunction with a variety of ancillary techniques (e.g. thin sectioning, immuno-labeling, negative staining) to answer specific questions. EM images provide key information on the structural basis of cell function and of cell disease. There are two main types of electron microscope – the transmission EM (TEM) and the scanning EM (SEM). The transmission electron microscope is used to view thin specimens (tissue sections, molecules, etc) through which electrons can pass generating a projection image. The TEM is analogous in many ways to the conventional (compound) light microscope. TEM is used, among other things, to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture). Conventional scanning electron microscopy depends on the emission of secondary electrons from the surface of a specimen. Because of its great depth of focus, a scanning electron microscope is the EM analog of a stereo light microscope. It provides detailed images of the surfaces of cells and whole organisms that are not possible by TEM. It can also be used for particle counting and size determination, and for process control. It is termed a scanning electron microscope because the image is formed by scanning a focused electron beam onto the surface of the specimen in a raster pattern. The interaction of the primary electron beam with the atoms near the surface causes the emission of particles at each point in the raster (e.g., low energy secondary electrons, high energy back scatter electrons, X-rays and even photons). These can be collected with a variety of detectors, and their relative number translated to brightness at each equivalent point on a cathode ray tube. Because the size of the raster at the specimen is much smaller than the viewing screen of the CRT, the final picture is a magnified image of the specimen. Appropriately equipped SEMs (with secondary, backscatter and X-ray detectors) can be used to study the topography and atomic composition of specimens, and also, for example, the surface distribution of immuno-labels.

A modern transmission electron microscope Scanning electron microscope

B. Cell injury is classified as reversible if the injured cell can regain homeostasis and return to a morphologically (and functionally) normal state. Acute cell swelling is the classic morphologic change in reversible injury; however, it is also the typical early change of irreversible cell injury. Irrespective of the nature of the initial injury, hypoxia is often the ultimate cause of acute cell swelling because it results in adenosine triphosphate depletion. The hypoxic cell then swells because of loss of volume control when membrane adenosine triphosphatase ionic pumps fail. Acute cell swelling is also a response to direct cell membrane damage from lipid peroxidation (by reactive oxygen species), binding of certain toxins, damage to ion channels, or insertion of transmembrane pore-forming complexes. Because acute cell swelling is a common early response to both reversible and irreversible injury, it is well to think of this morphologic change as a marker of potentially reversible cell injury. Cells, depending on their reparative or regenerative capacities, may recover from potentially irreversible cell injury; however, if the injury is severe or sustained, acute cell swelling becomes the initial step in the process of cell death. If the injury is not so severe as to be lethal, then the cell may not succumb but (again depending on the nature of the injury and of the cell) is unlikely to recover completely or to return to its “normal” structural and functional state. Major mechanisms of acute cell swelling, which is a result of cell injury, are

(1) hypoxia, (including ischemia) and

(2) membrane injury caused by lipid peroxidation or the formation of lytic pores through insertion of a MAC via the complement pathway or by bacterial cytolysins.

The cellular response to injury depends on

(1) the type of cell injured and its susceptibility and/or resistance to hypoxia and direct membrane injury and

(2) the nature, severity, and duration of the injury. As examples, neurons, cardiac myocytes, endothelium, and epithelium of the proximal tubule of the kidney are cells that are extremely susceptible to hypoxia, whereas fibroblasts, adipocytes, and other mesenchymal structural cells are less susceptible.

The response to injury can be degenerative, adaptive, or completely reversible with restoration of normal structure and function for the affected cell; however, with more severe or persistent injury, acute cell swelling can progress to irreversible cell injury and cell death. The cellular alterations that differentiate reversible cell injury from irreversible cell injury have been and are being studied extensively.

So what is cell Death?

The death of cells is an essential “value-added” part of embryonic development and maturation of the fetus and of homeostasis within populations of adult somatic cells. In these physiologic examples of cell death, cells that are no longer needed are removed during development or remodeling of tissues. However, cell death is also a point-of-no-return response to severe injury, and it is this pathologic form of cell death that is the topic of this section. Cell death typically assumes one of two morphologic forms : necrosis or apoptosis. The term necrosis has evolved to mean death by swelling of the cell (oncosis) with eventual rupture of cell membranes. Necrotic cell death typically involves groups or zones of cells and elicits an inflammatory reaction because of the release of cell contents into the ECM. Apoptosis, in contrast, is directed by cellular signaling cascades and typically affects individual cells. Apoptosis is a process of condensation and shrinkage of the cell and its organelles with eventual fragmentation of the cell. Importantly, apoptotic cell fragments remain membrane bound; thus no cellular components that could induce inflammation are released. Autophagy is a third possible mechanism of cell death, but it is more commonly a means of cell survival. Whereas apoptosis has long been recognized as a regulated or programmed process, not only responsible for physiologic removal of surplus cells but also occurring as a reaction to certain injuries, necrosis was once considered an entirely accidental and random response to injury. However, with the discovery that inhibition of apoptosis could shift cells from apoptotic death to a regulated process of oncotic death, the idea arose that necrosis could, at least in certain situations, be regulated by cellular signaling pathways.

Adaptations to Chronic Cell Injury

In the case of repetitive or continuous injury that is not inherently or immediately lethal, cells of many different types can survive, even without complete recovery, by adapting. Depending on the cell type—not all cells are capable of all possible responses—cellular adaptations to chronic injury include the following:

1. Hypertrophy, an increase in cell size by virtue of an increase in number and size of organelles

2. Hyperplasia, an increase in cell number that only those cells capable of mitosis can undergo

3. Metaplasia, a change from one differentiated cell type to another of the same germ layer (e.g., from ciliated epithelium to stratified squamous epithelium in the respiratory tract)

4. Dysplasia, abnormal differentiation with features of cellular atypia

5. Atrophy, a decrease in cell size by virtue of a decrease in number and size of organelles

6. Intracellular accumulations of endogenous or exogenous substances

Certain adaptations (e.g., myocardial hypertrophy) can increase the functional capacity of cells or tissues, at least temporarily, but more often cellular adaptations to chronic injury serve as means of protection (for example, keratinized stratified squamous epithelium offers more protection to underlying tissue than does pseudostratified ciliated epithelium) or survival (an alternative to cell death) and result in altered or diminished function of cells or tissues. Dysplasia is an adaptation without apparent advantages to the host. Indeed, dysplasia can be a precursor to malignant neoplasia (cancer). Injured cells may also accumulate materials including fat, cholesterol, protein, glycogen, or pigment. When cells are irreversibly injured and dying, specific nuclear changes may be visible, including pyknosis, karyorrhexis, and karyolysis. In summary, cell adaptation is the ability of cells to respond to various types of stimuli and adverse environmental changes.

These adaptation include hypertrophy (enlargement of individual cells), hyperplasia (increase in cell number), atrophy (reduction in size and cell number), metaplasia (transformation from one type of epithelium to another) and dysplasia (disordered growth of cells)

Tissues adapt differently depending on the replicative characteristics of the cells that make up the tissue for example labile tissues such as the skin can rapidly replicate and therefore can also regenerate after injury, where as permanent tissue such as neural and cardiac tissue cannot regenerate after injury ( cell injury). If cells are not able to adapt to the adverse environmental changes cell death occurs physiologically in the form of apoptosis or pathologically in the form of necrosis. **Cell death occurs as a result of inability of cells to adapt to injury.**

