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**DEPARTMENT: NURSING SCIENCE**

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**QUESTION**  
1. Write explicitly on 5 diagnostic techniques use in pathology, relevant illustrations and examples required. 2. Cellular Adaptation precedes cell death, discuss. Diagrams essential.

**ANSWERS**

1. ♦Staining

♦Immunohistochemistry

♦Electron microscopy

♦Flow cytometry

♦Molecular pathology/Cytogenetics

**SPECIAL STAINS**

Pathologists use the chemical properties of components of the tissues being studied in their choice of different stains. The stain(s) are applied to the thin sections on glass slides to allow the pathologist to see the cells under the microscope. The most widely used stain is haematoxylin and eosin. This stain is a combination of a basic stain (haematoxylin) and an acidic stain (eosin), which react with acidic and basic cell components in the tissue on the slide to give purple and pink colors to the tissues. Other stains available highlight fats, different tissue fibres, different types of mucus, microorganisms, protein etc.

**IMMUNOHISTOCHEMISTRY**

A major change in histopathology in recent times has been the development of immunohistochemistry. Where special stains are a relatively crude and, in most cases, relatively non-specific way of staining tissue components, immunohistochemical stains are by comparison far more specific in what they stain.

This technique involves attaching a dye to an antibody that will only bind to a certain protein type on or within a cell. Antibodies are like keys that can only open a certain lock (cell protein or antigen). Hundreds of antibodies are available which allow labeling of hundreds or even thousands of different protein types. Where a special stain may allow the pathologist to identify, for example, a cell as being cancerous, immunohistochemistry can identify which organ in the body that cancerous cell came from and how aggressively it may behave.

The dyes that attach to the labelling antibody can be also altered, including using different coloured dyes or even fluorescent dyes that are easier to see on microscopy. Some laboratories can use fluorescence-labeled antibodies to allow for computerized slide analysis, reducing the time taken to examine large numbers of slides and identifying which slides need to be reviewed by a pathologist and which are within the normal range.

**ELECTRON MICROSOCY**

The usual microscopes used by pathologists are not powerful enough to see the smallest parts that make up a cell. This is not usually a problem, but some diseases can only be diagnosed at this sub-cellular level. Examples include types of kidney disease: aggressive cancers which lose their normal proteins, making immunohistochemistry less useful in their identification.

In these cases a very powerful type of microscope is used called the electron microscope. This utilises beams of electrons rather than visible light to magnify the cells in a tissue sample. It can magnify up to 2 million times, whereas the maximum power of a conventional light microscope is only 1 to 2 thousand times.

**FLOW CYTOMETRY**

This technique is used most commonly as an adjunct in the diagnosis of cancers of the blood cells (leukemia). Cells are suspended in a liquid and passed through a laser beam (single wave length light beam). A detector measures how the beam is scattered and if fluorescent light is emitted from excited particles on the cells. This is interpreted by a computer as a number of cells/ particles/ proteins (whatever substance is being examined for) and is shown on a graph. This can be used to give the quantities and relative proportions of different types of cells in the blood and identify any abnormal cells (e.g. Leukaemia).

**MOLECULAR PATHOLOGY AND CYTOGENETICS**

With the explosion of information about cell DNA (the genetic coding material) and genes that has resulted since the completion of the Human Genome Project, increasing numbers of genes are being recognized that, if faulty, may be involved in the development of disease including cancers. This is shaping up to change the way that disease is thought of, diagnosed and treated.

Molecular pathology is an umbrella term for the analysis of the genetic material (chromosomes and their DNA) of cells, and is becoming an increasingly widely requested component of the pathology workup of a submitted tissue. One of the subdivisions of molecular pathology is cytogenetics, which is the analysis of chromosomes (the form in which DNA is found in the cell nucleus). The two most commonly used techniques in molecular pathology and cytogenetics are florescence in situ hybridization (FISH) and direct sequencing of DNA.

FISH is a technique used to stain chromosomes to reveal areas where genes may have been deleted, duplicated or broken. Fluorescent labels are attached to specific DNA sequences (parts of specific genes) which allow faulty genes to be seen when examining the cells under a special type of microscope.

Direct sequencing of cell DNA is a way of looking at individual genes or groups of genes, to detect and characterize which mutation is present in a particular patient’s tumour.

As an example of the usefulness of cytogenetics one can look at breast cancer. Anatomical pathology can give a diagnosis of what type of breast cancer a patient may have, how far it has spread, whether or not it is likely to be an aggressive tumour and whether it will respond to hormone and targeted therapies. Cytogenetics can add to this information by identifying whether the patient has a faulty gene(s) which predisposed them to the development of breast cancer. If present, this would mean that they have an increased chance of developing cancer in the opposite breast and of developing other specific cancer types (e.g. ovarian cancer). It also has implications for the patient’s direct relatives and offspring. Did they inherit the faulty gene(s) and what are the chances that they will develop cancer in the future? By direct sequencing of the faulty gene, the close relatives of the patient can be screened for the mutation, after appropriate consent, allowing for preventative steps to be taken to minimize their chances of developing a similar cancer in the future.  There are also treatments being developed which will target the products of specific gene mutations in a patient.

2. **CELLULAR ADAPTATION PRECEEDS CELL DEATH**

Injury to tissues and organs begins at the cellular level. Rudolf Virchow (1821-1902), known as the father of cellular pathology, based his study of diseased cells on the observation of structural alterations (morphologic lesions). However, Virchow also realized that biochemical changes in the cell, which preceded the appearance of lesions, more completely explained the functional disturbances in diseased cells and, in some cases, were the only detectable changes. Thus the pathologist must always correlate lesions with their biochemical bases and remember that a cell can be damaged functionally (biochemically) yet have no apparent morphologic alterations.

Simplistically, cell injury disrupts cellular homeostasis. Cells are injured by numerous and diverse causes (etiologic agents) from intrinsic and extrinsic sources; however, all of these causes, and they number in the thousands, activate one or more of four final common biochemical mechanisms leading to cell injury . These fundamental underlying biochemical mechanisms of cell injury are (1) ATP depletion, (2) permeabilization of cell membranes, (3) disruption of biochemical pathways, and (4) damage to DNA.

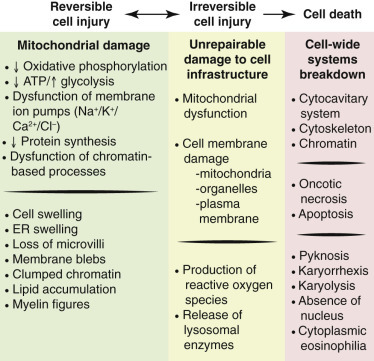
**MORPHOLOGY OF ACUTE CELL SWELLING**

Acute cell swelling increases the volume and weight of parenchymal organs and imparts pallor to them. It is important to distinguish hydropic degeneration from more positive adaptations, such as hypertrophy or hyperplasia, which, if extensive, also increase the size of an organ. Liver and kidney (especially the renal cortex) are two organs in which the lesions of acute cell swelling can be striking. An affected liver weighs more than normal, appears pale and swollen with rounded edges, and has an accentuated lobular pattern. In the CNS the cell swelling of cytotoxic edema has little effect on the color of neuroparenchyma but does increase the weight and volume of the affected tissue. Even a slight increase in volume of the brain has catastrophic consequences because there is little space in the cranium to accommodate swelling.

**IRREVERSIBLE CELL INJURY AND CELL DEATH**

Major mechanisms of acute cell swelling, 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.



**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.

