INTRODUCTION TO PATHOLOGY

AN OVERVIEW:

In this section, the student is introduced to the broad discipline of pathology. The basic information provided will be helpful in understanding or developing an appreciation for ways in which living tissues respond to injurious agents. The role of pathology in medicine, historical aspects of medicine, basic language of pathology, ways in which the body responds to injurious agents, means of detecting disease after death of the entire body, and causes of cell injury and/or disease, are discussed

GENERAL CONSIDERATIONS:

The term pathology is used when one refers to the **"scientific study of disease"** or the alterations that occur when abnormal influences (**bacteria, viruses, etc.**) affect cells, tissues, or body systems. More specifically, pathology may be defined as the **"scientific study of the molecular, cellular, tissue, or organ system response to injurious agents or adverse influences."**

For more than a hundred years, pathology has been one of the **"keystones"** of medicine. As an academic subject, it is taught as part of the medical curriculum to prepare the student for courses in clinical medicine and surgery. It serves as a **"bridge"** or **"link"** between the preclinical subjects (**anatomy, physiology, etc**.) and the courses in clinical medicine. Actually, pathology provides a logical means of relating the knowledge of normal structure and function (**anatomy and physiology**) to abnormal structure and function as encountered in a diseased individual. Thus, the factual background or knowledge needed for logical reasoning when solving real-life clinical problems is provided. It should be emphasized that pathology, as the scientific study of disease, follows the morbid process from its inception to its termination, and investigates the lesions produced. Therefore, a sound knowledge of pathology is the foundation of a solid understanding of disease as it occurs in the living patient.

Pathology has its roots deeply implanted in medical history. The earliest observers, from Celsus (**about 30 B.C. - A.D. 38**) to Morgagni in the 18th century, based their work upon the naked-eye appearances of diseased individuals and organs. Only as the technique of light microscopy improved was the Germanic School of Pathology, headed by Virchow (**1821-1905**), able to investigate changes at the cellular level. Today, technological advances are occurring so rapidly that no doctor can hope to have a working knowledge of all the methods available to study disease. Regardless, it is by utilizing all these complex techniques that problems are fully investigated. Thus, the student of medicine should have some understanding of the techniques which are available, and the types of problems which they might be able to solve. In this way, investigators conversant with many different disciplines are brought together to their mutual benefit. Techniques available in pathology include light microscopy, electron microscopy, microdissection, immunological techniques, ultracentrifugation, electrophoresis, chromatography, radioactive isotope technology, tissue culture, etc.

In summary, pathology is one of several mechanisms employed to solve those problems encountered in clinical situations. Thus, the student is required to make practical use of information accumulated in the General and Special Pathology courses. The compartmentalization and storage of knowledge for examination purposes is an exercise in futility. However, the utilization of accumulated knowledge in understanding clinical problems is an educational reality.

HISTORICAL ASPECTS OF MEDICINE/PATHOLOGY:

- -- The oldest civilized people (**Chinese, Indians, and Egyptians)** were under the impression that disease occurred when demons or evil spirits were displeased with an individual. The medicine men were concerned with appeasing these evil spirits.
- -- The Egyptians began to influence medicine around 4000 B.C. These people were adept in certain phases of medicine, especially skull surgery. In addition, embalming was an art employed by the Egyptians; many of these bodies **(mummies)** are still well preserved. There is no record of the Egyptians finding lesions or diseases during the embalming process.
- -- The **Mosaic Doctrine** (**1500 B.C.**) is the first recorded evidence of systematic meat inspection. These lays, as formulated by Moses and described in the books of Exodus and Leviticus, are similar to those we have today. Even though the Hebrews were advanced in food sanitation, they still accepted "divine displeasure" as the cause of disease.
- -- The Greek culture had a profound effect on the scientific approach to medicine. Greek physicians elucidated the principles of exact and careful clinical observations. However, they did not deal with the nature or the changes that occurred subsequent to disease.

HIPPOCRATES (460-375 B.C.),

AGreek physician, introduced the humoral theory of disease. He recognized four humors or fluids in the body:

- (1) blood which came from the heart;
- (2) phlegm which came from the brain;
- (3) yellow bile which came from the liver; and
- (4) black bile which came from the spleen.

Health was thought to be due to proper mixing of these four humors, while disease resulted from improper mixing. The humoral theory of disease was supported by three critical observations:

(1) diseases were often characterized by increased discharge of fluid (perspiration, fever, vomiting, diarrhea, catarrhal discharge, exudation, and transudation);

(2) blood was the vital tissue and the individual died if exsanquinated;

(3) the coagulation of blood was different in healthy and sick individuals.

Also, what was called phlegm is the same as fibrin. Those factors considered to be the effects of disease today were considered as causes by the humoral pathologists. The early humoral pathologists were not permitted to perform postmortem examinations on humans; thus, a confused concept of normal anatomy existed. Hippocrates is considered to be the **Father of Medicine.**

ARISTOTLE (384-323 B.C.),

A Greek philosopher, was the originator of modern anatomy and physiology. Also, he is considered to be the **Father of Zoology.** Aristotle dissected many animals, carried out experiments in physiology, and

studied the growth and development of animal life (human autopsy examinations were forbidden during this period).

CLAUDIUS GALEN (129-201 A.D.),

A Greek physician practicing in Rome, was a follower of the beliefs of Hippocrates. Humoral pathology was brought to its height and most extreme development by Galen. Also, he wrote numerous medical documents and held despotic authority over European medicine for thirteen centuries after his death. Also, Galen is remembered for his views on meat inspection. He insisted that animals used for human food should be inspected prior to slaughter.

CORNELIUS CELSUS (30 B.C.-38 A.D.)

Was not a physician, but a man of leisure with a variety of interests. A great deal of the history of the early humoral pathologists was recorded in his work. A great number of the conditions recognized today are described in the work of Celsus. His writings described and discussed the cardinal signs of inflammation (**redness, swelling, heat and pain**).

RENATUS VEGETIUS (450-500 A.D.),

A Roman veterinarian, is credited with being the first author to write a textbook devoted exclusively to veterinary medicine. He was among the first to urge people to disregard Divine Displeasure as the cause of disease and to base their treatment and concepts of disease on a thorough knowledge of anatomy, surgery and medicine. Vegetius is considered to be the **Father of Veterinary Medicine**.

- -- During the middle ages, medicine entered into an era of nonproductivity and few contributions were made. However, the Renaissance brought forth profound advances in medicine and pathology. The works of Galen were questioned and new investigations were made. During this period, Divine Displeasure as the cause of disease began to gradually disappear.

WILLIAM HARVEY (1578-1657)

Described the blood vascular system and the circulation of blood in 1628. His works have had a far reaching effect on medicine and pathology.

ANTONY VAN LEEUWENHOEK (1632-1723)

Was the first to show that the microscope had practical importance in the study of tissues and other small objects (**he is not credited with discovering the microscope**).

JEAN FERNEL (1497-1558),

A Frenchman, was one of the first to describe diseases according to organs or parts of the body. He generally divided his diseases into those affecting parts above the diaphragm, those involving parts below the diaphragm and external diseases. One of his books, entitled **Pathologiae Libri**, was the first medical work to be called a text of pathology.

GIOVANNI MORGAGNI (1682-1771),

An Italian, is recognized as one of the earliest pathologists and the originator of modern pathology. He was the first to correlate pathologic changes in the dead individual with clinical signs and symptoms shown by the individual during life.

MARIE-FRANCOIS XAVIER BICHAT (1771-1801),

A Frenchman, is credited with establishing the foundation for the study of histology, even though most of his work was done by physical and chemical methods **(he did not possess a microscope)**. Bichat presented a new concept of anatomy and showed that the body was composed of twenty-one (21) tissues (vascular, osseous, muscular, cartilaginous, etc.). He is considered to be the **Father of Histology.**

JACQUES LABRESSIE DE SOLLEYSEL (1617-1680)

Published the first complete veterinary classic of this period, entitled **Le Parfait Marechal**. In this publication, Solleysel pointed out the adverse situation created by allowing the veterinary art to fall so completely into the hand of the Farrier. This book marks the beginning of the end of the horseshoer's regimen and control of veterinary medicine.

CARL ROKITANSKY (1804-1878),

A German, is considered as the supreme descriptive pathologist of all time. He firmly established the structural basis of disease as well as necropsy technique. However, he explained practically all diseases on the basis of blood anomalies.

- -- The field of pathology was completely reformed by cellular teachings which originated in Germany. The inspiration for this development came from Johannes Mueller (**1801-1858**) who had many famous students (**Schwann, Henle, Virchow and Schleiden).**

RUDOLPH VIRCHOW (1821-1902)

Is known as the **Father of Cellular Pathology.**

He coined and explained many of the terms and concepts used today in pathology (**amyloidosis, fatty degeneration, etc**.). In addition, he started publication of "Virchow's Archives," a journal that has been in continuous publication since 1847. This is considered to be one of the most complete works of pathology in existence.

LOUIS PASTEUR,

Frenchman, was one of the originators of the field of bacteriology. He demonstrated the importance of infectious organisms (**bacterial**) in disease. Pasteur studied human and animal diseases (**pasteurellosis, anthrax, rabies, etc.**) and showed that individuals could be successfully immunized by vaccines prepared from organisms.

ROBERT KOCH (1843-1910),

A German bacteriologist, established the **Koch's Postulate**, a procedure employed for proving a specific microorganism as the cause of a disease. He was the first to use artificial solid media in the attainment of pure cultures.

EDWIN KLEBS (1834-1913),

A student of Virchow demonstrated the importance of bacteria in pathology.

JULIUS COHNHEIM (1839-1884)

A student of Virchow is credited with being the originator of modern experimental pathology. He revealed the vascular alterations that are the basis of the inflammatory response.

WILLIAM H. WELCH (1850-1934),

A student of Cohnheim, is credited with bringing pathology to the United States. He was Professor of Pathology at John Hopkins University in Baltimore, Maryland.

BASIC LANGUAGE OF PATHOLOGY:

In order for a subject or course to be meaningful, one should become familiar with the basic terminology applicable to that subject. Listed below are a few basic terms used repeatedly in pathology and/or medicine. The student should become familiar with these terms and their definitions.

DISEASE:

A disease may be defined as a **"state in which an individual exhibits an anatomical, physiological, or biochemical deviation from the normal.**" As generally used, the term "disease" is employed to describe a state in which there is sufficient departure from the normal for clinical signs or symptoms to be produced.

LESIONS:

The term lesion is generally used to refer to **"structural or morphological alterations associated with a diseased state in an individual."** It is the objective deviation from the normal.

Lesions may be recognized with the naked-eye (**gross lesions**), with the aid of a light microscope (**microscopic lesions**), or with the aid of the electron microscope (**ultrastructural lesions**). Biochemical or functional lesions are recognized as changes which result from disturbed function.

Pathognomonic Lesion: refers to a change which is specifically characteristic of a disease. When one sees a pathognomonic lesion, he knows that a particular disease is present.

HEALTH:

As generally used, the term **"health"** refers to the **"state in which an individual is living in complete harmony with his environment,"** it is a relative state. All body functions are performed normally even though lesions may be present in organs and/or tissues. It should be remembered that the transitional zone between health and disease is difficult to define.

ETIOLOGY:

The term **"etiology"** refers to a **"study of the cause of a disease."** An etiologic agent is the factor (**bacterium, virus, etc.**) responsible for lesions or a disease state.

Predisposing Causes of Diseases: refer to those factors which make an individual more susceptible to a disease (**damp weather, poor ventilation, etc.**)

Exciting Causes of Disease: refer to those factors which are directly responsible for a disease **(bacteria, viruses, hypoxia, chemical agents, etc**.).

CLINICAL SIGNS:

"Clinical signs" refer to any **"functional evidence of disease which can be determined objectively or by the observer"** (**lameness, salivation, increased respiratory efforts, etc.**). ***Remember, clinical signs are seen only in the living individual.***

The term clinical symptoms should be reserved for any **"functional evidence of disease that can be determined subjectively or by the patient" (feeling or abdominal discomfort, etc.)**.

PROGNOSIS:

The term **"prognosis"** refers to the probably outcome of a disease in a living individual. It is the clinician's estimate of the severity and possible result of a disease.

DIAGNOSIS:

The term **"diagnosis"** refers to the **"determination of the nature of a disease expressed in a concise manner."**

A morphologic or anatomic diagnosis is based on the location and nature of the lesion. Etiologic diagnosis is made on the basis of the cause. Definitive diagnosis is made on the basis of the specific disease entity involved**.** A clinical diagnosis is made on the basis of clinical signs observed in the living animal.

PATHOGENESIS:

The term **"pathogenesis"** refers to the **"progressive development (sequence of events) of a disease from the time it is initiated to its final conclusion in recovery or death."**

NECROPSY:

Common usage of the term **"necropsy"** in pathology refers to gross examination of the human remains by systematic dissection in order to evaluate any abnormal changes **(lesions)** that may be present.

However, a complete necropsy refers to all postmortem examinations including gross, microscopic, toxicologic, and microbiologic examinations. The term **"autopsy,"** used synonymously with necropsy, has been avoided by some pathologists because of the prefix **"auto"** meaning self, implying self-examination.

Biopsy refers to the removal and examination of tissue obtained from the living body.

Euthanasia refers to the intentional **"putting to death"** of an individual with an incurable or painful disease by employing humane means.

SOMATIC DEATH:

The term **"somatic death"** refers to death of the entire body; there is cessation of all body functions. The absence of heart beat, pulse, respiration or brain waves has been used to define somatic death.

Necrobiosis refers to death of cells at the end of their normal life-span within the living body **(epithelial cells of the skin, leukocytes, etc.).** Cell death occurs without harm to the individual because "normal functions" have been fulfilled.

Necrosis refers to the morphological changes caused by the progressive degradative action of enzymes on the lethally injured cell within the living body. After a cell dies, lysosomes rupture and their hydrolytic enzymes are released. The release and activation of these lysosomal enzymes are responsible for cell necrosis.

POSTMORTEM CHANGES:

"Postmortem changes" refer to cell death which accompanies or occurs after death of the entire body (**somatic death),** whereas antemortem changes refer to those alterations that occur in cells, tissues, organs, etc. prior to somatic death or in the living individual. It is important to differentiate postmortem changes from antemortem changes in order to interpret correctly those lesions encountered at necropsy.

Remember, postmortem changes develop only after the individual dies.

Postmortem Autolysis refers to self-digestion by enzymes that are present within or released into the cytoplasm of cells after death. It is due to total diffuse anoxia.

Postmortem Putrefaction refers to the decomposition of tissues by bacterial enzymes after death of the entire body.

Rigor Mortis refers to stiffening of all muscles after death. It is related to a progressive decrease in oxygen, ATP, creatinine phosphate, and pH of muscles. Thus, muscle fibers shorten as they pass into rigor. Classically, rigor mortis begins in one (1) to six (6) hours after death and disappears 24 to 48 hours later.

Postmortem Clotting of Blood refers to the coagulation of blood in vessels and/or heart after somatic death. Postmortem clots may be dark red (**current jelly clots)** or the yellow color of plasma (**chicken fat clots)**. Such clots are smooth, shiny, uniform in texture, and unattached to the vessel or heart wall. On the other hand, antemortem clots or thrombi are friable, dull colored, roughened over the surfaces, and attached to the vessel wall.

BRANCHES OR PHASES OF PATHOLOGY:

There are many terms applied to different branches or phases of pathology. Among these are the following:

GENERAL PATHOLOGY:

Refers to the study of the basic alterations in tissues. These are changes which apply to most of the organs or tissues of the body and include such things as atrophy, necrosis and inflammation.

SYSTEMIC PATHOLOGY:

Refers to the study of the diseases of the organ systems of the body such as the respiratory system, digestive system and nervous system.

GROSS PATHOLOGY (macroscopic pathology, pathological anatomy,morbidanatomy):

Refers to the study of disease in which tissues and organs are examined with the unaided eye.

CELLULAR PATHOLOGY (microscopic pathology, histopathology):

Refers to the study of diseased tissues and organs with the aid of a microscope.

SURGICAL PATHOLOGY:

Refers to the study of tissues removed at the time of surgery.

CLINICAL PATHOLOGY:

Refers to the study of disease by examination of blood, urine, feces, skin scrapings, etc.

IMMUNOPATHOLOGY:

Refers to the study of diseases associated with abnormalities of the immune mechanisms of the body.

CHEMICAL PATHOLOGY:

Refers to the study of chemical changes in the fluids and tissues of the body as the result of disease. This branch of pathology is merely a portion of clinical pathology.

PHYSIOLOGICAL PATHOLOGY:

Refers to the study of the changes in the functions of organs or parts of the body as a result of disease.

GEOGRAPHICAL PATHOLOGY:

Refers to the study of the disease processes in population groups in different parts of the word. As an example, certain diseases of the heart and major blood vessels in man are much more prominent in the United States than in the Orient or other parts of the world.

SPECIMENS FOR HISTOPATHOLOGIC EXAMINATION:

If there is a need to examine lesions, etc. with the aid of the light microscope, tissue specimens are collected and placed in a 10% neutral-buffered solution of formalin which is the best general fixative available. It serves to

- (1) prevent autolytic changes;
- (2) preserve cellular constituents in as life-like a manner as possible;
- (3) protect by hardening the naturally soft tissues; etc.

The following guidelines should be adhered to when collecting tissue samples for histopathologic examination.

- --Collect samples immediately upon removal from the body or as soon after death as possible.
- --Specimens should be representative of the predominant gross alteration **(sample lesions near or at the junction of altered and unaltered tissue).**
- --Tissue sample should be approximately one-fourth inch thick (**to allow fixative to penetrate).**
- --Tissue samples should be immersed in at least 10 times their volume of 10% formalin

EXCITING CAUSES OF CELL INJURY/DISEASE:

Exciting causes refer to those factors or influences directly responsible for disease. Most of these adverse influences can be grouped into the following broad categories:

- (1) hypoxia,
- (2) physical injuries,

- (3) chemical injuries,
- (4) biological agents such as bacteria, viruses, fungi, etc.,
- (5) altered immune mechanisms,
- (6) genetic defects and
- (7) nutritional imbalances.

These causative factors are discussed briefly.

HYPOXIA :

Hypoxia refers to a lack of oxygen which is probably the most common cause of cell injury and disease. It may be the ultimate mechanism of damage initiated by a variety of physical, chemical and biological agents. Hypoxic injury to cells may be caused by:

- (1) loss of their blood supply (**e.g. ischemia subsequent to the presence of a thrombus in the lumen of a vessel**),
- (2) depletion of the oxygen-carrying capacity of the blood (**e.g. carbon monoxide poisoning in which monoxyhemoglobin replaces oxyhemoglobin and blocks the normal transport of oxygen),**
- (3) poisoning of the oxidative enzymes within cells (**e.g. cyanide poisoning in which cytochrome oxidase is inactivated).**

Ultimately, all of these derangements affect aerobic respiration.

PHYSICAL INJURIES:

Physical agents responsible for cell/tissue damage include trauma, pressure, obstructions of hollow organs, malpositions, thermal factors, changes in atmospheric pressure, light, electricity and radiation.

MECHANICAL TRAUMA:

Is usually an injury caused by sudden violent physical forces in which cells/tissues are torn or crushed. The types of trauma include:

- --Contusion **(bruise**) refers to an injury in which the covering skin is intact, but the underlying tissues are damaged.
- --Abrasion refers to an injury similar to a contusion, but one in which the skin is broken.
- --Incision refers to an injury produced by a sharp object, resulting in little tissue damage.
- --Laceration refers to an injury resulting from the tearing or tissues with a blunt object.
- --Perforation refers to a wound in which the point of entry of the mechanical force is small.
- --Rupture refers to an injury in which tissues are stretched until the fibers are disrupted (**rupture occurs in hollow organs or in the capsule of such organs as the kidneys, liver and spleen or in muscles and tendons**).
- --Fracture refers to a break in a hard substance such as bone or cartilage. A clean break, separating a bone into two parts, is called a simple fracture. If there are many parts, it is a comminuted fracture. If in addition to the break in the bone, there is an opening in the overlying skin, the lesion is a compound fracture. A fracture in which the periosteum remains intact and holds the ends of bone in place is a greenstick fracture.

- --Luxation (**dislocation)** refers to an injury of an articulation in which there is displacement of bone making up the articulation.

PRESSURE:

Results in mild or less violent injuries which usually take place over a prolonged period of time.

- --Decubitus ulcers or bed sores which occur over bony prominences of the body (**tuber coxae, zygomatic arch, etc.**) of an animal recumbent for several days is a classical example of pressure injury. Also, pressure injury occurs when casts or bandages are applied too tightly or when neoplasms, abscesses, etc. encroach upon parenchymal organs.

OBSTRUCTION OF HOLLOW ORGANS:

Hollow organs may be obstructed and subsequently injured by changes within the lumen **(rubber ball, etc.)**, pressure from outside the organ **(neoplasm, etc**.) or by changes within the wall of the organ **(abscesses, neoplasm, etc.**).

- --The term **"stenosis"** refers to any narrowing of the lumen of a hollow organ; whereas the term **"stricture"** is reserved for a form of stenosis caused by contraction of fibrous connective tissue (**scar tissue**) in the wall of an organ.

MALPOSITION:

Refers to the displacement of an organ or part that results in cell and/or tissue injury. The types of malpositions include the following:

- --Volvulus refers to the rotation of an organ or part around its mesenteric base of attachment. Loops of intestine are usually involved and the twist is usually more than 180 degrees.
- --Torsion refers to the rotation of an organ or part along its own long axis. The intestine, uterus, lung lobes, etc. may be involved.
- --Intussusception refers to the telescoping of one portion of a hollow organ into another portion **(e.g., a portion of the intestine is forced inside the segment just posterior to it).**
- --Prolapse is the appearance of an organ or portion of an organ at a natural or artificial body opening.
- --Hernia is the protrusion of an organ through a natural or an artificial body opening with the organ being covered with skin or the parietal layer of a serous membrane. It consists of three parts: the hernial ring, the hernial sac, and the contents.

THERMAL INJURIES:

Extremes of temperature, such as freezing and burning, cause injury in several ways (**direct damage, vasconstriction, etc.).**

- --Burns refer to lesions produced directly by heat. Depending upon the extent of the injury, burns are classified as:
    - (1) first degree burns (**characterized by reddening of tissues),**

    - (2) second degree burns **(characterized by blister formation),**
    - (3) third degree burns (**characterized by death of cells and tissues or necrosis)** and
    - (4) fourth degree burns (**characterized by charring of tissues)**.
- --Heat retention (**heatstroke/sunstroke**) occurs when animals are unable to eliminate sufficient heat to maintain body temperature at a level compatible with life. Subsequently, cells and tissues are injured. Essential signs are high fever **(106 to 110 degrees F**), respiratory distress, discomfort, excitement, collapse, and sudden death.
- --Freezing of tissues occurs when the body is exposed to very low temperatures. When tissues freeze, there is damage to blood vessels, formation of thrombi and subsequent interference with circulation (**sloughing of tissues such as the tip of the ears or other extremities may occur).**

LIGHT:

Sunburn or overexposure to light is rare in animals due to skin pigmentation and protection by the hair coat. Photosensitization with damage to the skin occurs as the result of the action of sunlight on fluorescent pigments (**phylloerythrin, etc.**) in the skin.

ELECTRICITY:

Strong electrical currents from artificial sources or from lightning cause burns or result in somatic death. An animal produces a short circuit with his body between two conductors. The effects of electricity include burning of tissues, hemorrhages, **and/or** death due to interference with cardiac and respiratory functions.

RADIATION:

Injuries produced by ionizing radiation depend upon the type of radiation **(alpha, beta, gamma, etc.**) and the susceptibility of the cell or tissue exposed. Rapidly growing cells **(e.g., lymphoid cells**) are quite susceptible; whereas, more slow growing cells **(e.g., cells of bone) are more resistant**

CHEMICAL INJURIES:

The list of chemical that may produce cell/tissue injury defies compilation. Simple chemicals such as glucose or salt in hypertonic concentrations may cause cell injury by deranging the fluid and electrolyte homeostasis of cells; even oxygen in high concentrations is severely toxic. On the other hand, the levels of toxicity of certain substances are so high that they are known as poisons and trace amounts **(arsenic, cyanide, mercury salts**) may destroy a sufficient number of cells within minutes or hours to cause death. Under certain conditions, toxic materials may be formed within the body and subsequently cause cell damage. Severe burns, uremia, and gangrene may be associated with endogenous poisons.

BIOLOGICAL AGENTS:

Biological agents are important causes of cell injury and death. They include bacteria, viruses, rickettsiae, fungi and higher forms of parasites. The ways in which this heterogeneous group of biological agents cause injury are diverse and in many cases unknown*.*

Bacteria cause cell injury by means of various mechanisms. Certain bacteria, for example, elaborate exotoxins that inhibit oxidative processes and protein synthesis in cells. Other bacteria **(e.g., gram negative bacilli of the gastrointestinal tract)** cause cell injury by the release of endotoxin from their cell walls when the bacteria are killed. The endotoxin causes profound changes in blood vessels and in the

coagulation system. Also, bacteria can cause cell injury by the induction of immunologic responses to antigens contained within the microbiologic agent (**tuberculosis)**.

Viruses are infectious particles that contain only one nucleic acid, either DNA or RNA. They do not have ribosomes or other structures that synthesize protein, RNA or metabolic enzymes. Thus, viruses are intracellular parasites that survive within living cells where they subvert the metabolism of the host for their own survival. This results in decreased synthesis of all macromolecules vital to the host. Continued viral replication may therefore interfere with the host's own metabolism. The viral protein elaborated during viral replication may be directly toxic to infected cells. Also, viral RNA or DNA may become incorporated into the genome of the host cell and cause either cell death or cellular transformation and frequently increased cell proliferation. This last effect may lead to tumor formation, a point relevant to the viral causation of cancer.

Fungi are single cell, nucleated plant organisms which include a wide variety of pathogenic species as well as yeasts, molds, mushrooms, mildews and other similar organisms. Most fungi are omnipresent in the environment. Host resistance is therefore a dominant factor in disease. Opportunistic fungal infection results when animals are immunosuppressed, when their mechanisms of inflammation are inhibited and when stress is placed upon their systems over long periods.

Metazoan parasites generally cause disease by local destruction of cells of tissue, by their effects on blood circulation, by their effects as space-occupying lesions or by nutritive competition.

Rickettsial infections are caused by obligate intracellular organisms capable of multiplication only within certain cells of susceptible hosts. The organisms are smaller than bacteria and larger than viruses.

IMMUNOLOGIC REACTIONS:

Immunological reactions maybe life-saving or lethal. Some immune reactions may cause cell injury and death (**anaphylactic reaction to a foreign protein or drug, etc.)** , also, there is evidence that an immune reaction against **"self-antigens" (autoimmunity**) is the cause of certain diseases in human.

GENETIC DERANGEMENT:

Genetic defects are important causes of cellular injury. Genetic defects as causes of cell injury are of major interest to scientists and physicians today .The genetic injury may result in a defect as severe as the congenital malformations associated with Down syndrome, caused by a chromosomal abnormality, or as subtle as the decreased life of red blood cells caused by a single amino acid substitution in hemoglobin S in sickle cell anemia. The many inborn errors of metabolism arising from enzymatic abnormalities, usually an enzyme lack, are excellent examples of cell damage due to subtle alterations at the level of DNA. Variations in genetic makeup can also influence the susceptibility of cells to injury by chemicals and other environmental insults.

RESPONSE OF THE NORMAL CELL TO STRESS AND/OR INJURY:

The cellular adaptation responses **(atrophy, hypertrophy, hyperplasia, metaplasia)** allow the cell to survive by achieving an altered but steady state. The adapted cell is not injured, ill or dead. It may revert back to a normal state if the abnormal environmental influences are removed. On the other hand, the cell may become ill, or even die, if the intensity of the abnormal environmental influences is increased sufficiently.

CELLULAR ADAPTIVE RESPONSES TO INJURY
In general, cellular adaptation is a potentially reversible change in response to the environment.
Atrophy is a decrease in cell/organ size and functional ability. Causes of atrophy include decreased workload/disuse (immobilization); ischemia (atherosclerosis); lack of hormonal or neural stimulation, malnutrition, and aging.
Light microscopic examination shows small shrunken cells with lipofuscin granules. Electron microscopy shows decreased intracellular components and autophagosomes.
Hypertrophy is an increase in cell size and functional ability due to increased synthesis of intracellular components.
Causes of hypertrophy include:
• Increased mechanical demand can be physiologic (striated muscle of weight lifters) or pathologic (cardiac muscle in hypertension).
• Increased endocrine stimulation plays a role in puberty (growth hormone, androgens/estrogens, etc.), gravid uterus (estrogen), and lactating breast (prolactin and estrogen).
Hypertrophy is mediated by growth factors, cytokines, and other trophic stimuli and leads to increased expression of genes and increased protein synthesis.
Hypertrophy and hyperplasia often occur together.
Hyperplasia is an increase in the number of cells in a tissue or organ. Some cell types are unable to exhibit hyperplasia (e.g., nerve, cardiac, skeletal muscle cells).
• Physiologic causes of hyperplasia include compensatory mechanisms (e.g., after partial hepatectomy), hormonal stimulation (e.g., breast development at puberty), and antigenic stimulation (e.g., lymphoid hyperplasia).
• Pathologic causes of hyperplasia include endometrial hyperplasia and prostatic hyperplasia of aging.
Hyperplasia is mediated by growth factors, cytokines, and other trophic stimuli; increased expression of growth-promoting genes (proto-oncogenes); and increased DNA synthesis and cell division.
Metaplasia is a reversible change of one fully differentiated cell type to another, usually in response to irritation. It has been suggested that the replacement cell is better able to tolerate the environmental stresses. For example, bronchial epithelium undergoes squamous metaplasia in response to the chronic irritation of tobacco smoke.
The proposed mechanism is that the reserve cells (or stem cells) of the irritated tissue differentiate into a more protective cell type due to the influence of growth factors, cytokines, and matrix components.

In this section, reversible and irreversible cellular injuries are considered in detail.The biochemical, functional and structural cellular alterations discussed in the following sections are caused by a wide variety of injurious factors including hypoxia, bacteria, virsuses, trauma, chemicals, immunologic reactions, nutritional imbalances, genetic derangements, etc. However, hypoxia, chemicals and injury by infectious agents are encountered most commonly. In fact, hypoxic injury is considered to be a common pathway by which other injurious agents act.

Remember, those injurious agents responsible for cellular illness may also be responsible for cellular adaptation and/or cellular death (depending on the severity, duration, etc. of the injury).

It has not been possible to determine the exact biochemical site of action for most injurious agents even with the sophisticated methods of study available. However, the mitochondrium **(site of aerobic respiration involving oxidative phosphorylation and ATP production)**, cell membrane **(upon which the ionic and osmotic homeostasis of the cell and its organelles are dependent)**, endoplasmic reticulum **(site of synthesis of enzymatic and structural proteins)** and the nucleus **(site of the genetic apparatus)** are highly susceptible to the effects of injurious agents.

Remember, an injurious agent may affect or damage one biochemical function in one type of organelle first, but shortly thereafter, other functions and organelles are involved. In other words, regardless of the primary target that an injurious agent may damage within a cell, in time, all forms of injury will extend to involve the entire structure of the cell.

Although it has not been possible to determine the exact biochemical site of action for most injurious agents, it can be assumed that all forms of injury affect the cell initially by upsetting some important chemical reaction. Thus, a biochemical change is the first cellular alteration that develops following injury; such changes subsequently result in altered function (**i.e., the binding of mercury to cell membrane protein sulfhydryl groups in mercury chloride poisoning results in rapid increased cell membrane permeability and the movement of sodium and water into the cell).**

Remember, primary biochemical alterations are rarely detected. They are usually reflected by altered function. Functional changes are fairly advanced before structural changes of illness are detectable within a cell. Likewise, the structural manifestations of cell death (necrosis) only become evident some time after the cell has actually died. Thus, biochemical and functional changes always precede structural or morphologic alterations with a cell.

The time lag required to produce recognizable changes of cellular adaptation, cellular illness, or cellular death varies with the sophistication of the methods employed to detect these changes. Despite advanced methods of biochemical and morphologic investigation, the **"lines of demarcation"** between the normal, the adapted, the ill and the dead cell are still difficult to define. In other words, there are no clear **"hallmarks"** by which the severely stressed but normal cell can be distinguished from the cell that has been damaged to the point of illness. Likewise, there are no certain parameters by which the ill but still viable cell can be differentiated from one which has reached the **"point of no return"** and is doomed to die.

As the student embarks on a study of pathology, it should be remembered that the health of an individual has its origin in healthy cells and disease is due to dysfunction of a significant number of cells. The normal cell, the adapted cell, the ill cell and the dead cell are the primary concerns in pathology.

NOTE:Now that the student has gained an appreciation for certain aspects of "cellular response to injury," it is appropriate to consider the adapted, ill and dead cell in more detail.

Remember, these are hazily delimited states along a continuum of function and structure.

THE ADAPTED CELL "Cellular Adaptation"

GENERAL CONSIDERATIONS:

Cellular adaptation refers to those adjustments that a cell makes in response to alterations in the environment in which it must live. The adapted state is usually associated with altered functional demands placed upon the cell. However, those injurious agents responsible for cell illness and/or death may effectively alter the cell's environment resulting in an adaptive response. Insofar as a cell can adapt to its environment, it can escape injury. Actually, the adapted cell achieves an "altered but steady state" that permits it to survive.

Remember, the adapted cell is not injured, ill or dead.

REVERSIBLE CELLULAR INJURY (THE SICK OR ILL CELL)

GENERAL CONSIDERATIONS:

If the limits of adaptive capabilities are exceeded, the affected cell may become ill (**reversible cellular injury**) or even die **(irreversible cellular injury**). Actually, cellular adaptation, reversible cellular injury, and irreversible cellular injury are three states along a continuum of progressive encroachment on the cell's homeostasis. However, the manner in which a cell reacts to a specific injurious agent depends on varying factors, including the type, duration and severity of the injury (**i.e., a cell may die acutely without becoming ill or adapting to the adverse influence**).

Even though reversible cellular injury is one of the most common responses in disease, large gaps still exist in our knowledge and understanding of the process. Regardless, cells react to injurious agents in a limited number of ways. As mentioned earlier, damage to one biochemical or metabolic function may affect one type of organelle first, but eventually, other functions and organelles are involved. Therefore, whatever the primary target, in time, all forms of injury, if sustained, will extend to involve the entire cell.

Remember, the ill or sick cell is not a dead cell; it can revert back to a normal state or to an adapted state (spontaneously or after treatment with appropriate drugs, etc.).

ULTRASTRUCTURAL CHANGES IN THE SICK OR ILL CELL:

In the ill cell, prominent morphologic or functional changes are usually associated with the cell membrane, mitochondria, endoplasmic reticulum and lysosomes; nuclear changes are minimal. Significant ultrastructural alterations that occur in the ill or sick cell are summarized.

PLASMA MEMBRANE:

The plasma membrane is involved (**primarily or secondarily**) in virtually all forms of injury. Very early in the cell's response to injury, no ultrastructural alterations are observed; however, biochemical studies disclose increased cell membrane permeability which is reflected by increased intracellular accumulation of sodium, water and calcium ions, as well as by a leakage of potassium, enzymes and cofactors. Later in the cell's response to injury, distortion of microvilli, blebs, and vesicles occur in the membrane. Still later, breaks are seen in both the plasma membrane and the membranes enclosing the organelles.

MITOCHONDRIA:

The mitochondria are almost always altered when the cell is injured. Alterations occur rapidly after hypoxic injury but are delayed in many forms of chemical injury **(the following refers to hypoxic injury).** The earliest response to hypoxic injury is condensation of the mitochondrial matrical proteins **(associated with a loss of ATP**). However, this is quickly followed by swelling of mitochondria (**related to increased cell membrane permeability**). With progressive injury, the mitochondrial matrix becomes translucent.

Remember, flocculation of mitochondrial matrical proteins correlates with the onset of cell death; this is the earliest absolute sign of cell death.

ENDOPLASMIC RETICULUM:

The endoplasmic reticulum responds very early when the cell is injured; it swells due to an intake of water. This swelling is followed by detachment of ribosomes and desegregation of polysomes resulting in decreased synthesis of protein. Subsequently, progressive fragmentation of the endoplasmic reticulum occurs.

Remember, swelling of the endoplasmic reticulum is usually the first manifestation of cell injury.

LIGHT MICROSCOPIC CHANGES IN THE SICK OR ILL CELL

As viewed with the light microscope, the alterations that develop within a sick cell are found principally in the cytoplasm. The nucleus is unaffected except for some clumping of chromatin against the nuclear membrane. Two patterns of reversible cellular injury can be recognized, **"cellular swelling"** and **"fatty change."** Cellular swelling **(also referred to as cloudy swelling)** occurs whenever the cell is unable to maintain ionic and fluid homeostasis. It reflects an excessive accumulation of water in the cell's cytoplasm. Fatty change refers to an abnormal accumulation of fat within parenchymal cells. It occurs principally in those cells involved in and dependent on fat metabolism. Fat accumulates in the cell's cytoplasm because the sick cell is unable to utilize or export fat that it normally receives. The term hydropic change is commonly used to refer to advanced stages of cellular swelling. Cellular swelling is an early manifestation of cellular illness whereas fatty change represents a more severely ill cell. However, cellular swelling and fatty change are reversible; affected cells may revert to a normal or adapted state **(with treatment, etc.)** or they may progress to the point of cellular death.

CELLULAR SWELLING:

Cellular swelling refers to an increase in cell size due to increased intracellular accumulation of water. It is the earliest or first manifestation viewed with the light microscope in almost all sick or ill cells. In the early stages, water tends to accumulate in the endoplasmic reticulum; later, all cytoplasmic structures are involved. As viewed with the light microscope, the involved cell is swollen and its cytoplasm has a cloudy, indistinct, ground-glass appearance. If an extensive amount of water accumulates, small clear vacuoles

appear in the cytoplasm. This pattern is oftentimes referred to as hydropic change. As viewed with the naked eye, the involved organ may appear enlarged and pale (**cellular swelling is difficult to appreciate on gross inspection**). The ultrastructural alterations are the same as those described for the sick or ill cell.

Remember, cellular swelling or hydropic change occurs when an injurious agent causes disruption of controls for ionic and osmotic homeostasis at the cell membrane level, resulting in increased permeability. The so-called sodium-potassium pump is deranged and sodium concentration tends to equalize on both sides of the cell membrane; water passively follows sodium into the cell resulting in cellular swelling. Significant functional defects may not develop in involved cells. Cellular swelling is an indicator of mild injury and it is of primary importance as a possible incidence to more severe cellular injury (fatty change or even cell death).

"**Cellular swelling is a difficult morphologic change to appreciate with the light microscope since early postmortem autolysis results in a similar appearance or pattern. Actually, cellular swelling is more readily recognized ultrastructurally and paradoxically, on gross examination of the whole organ than under light microscopy."**

FATTY CHANGE:

"Fatty change" refers to an abnormal accumulation of fat within parenchymal cells. It occurs most frequently in those cells involved in, or dependent on, fat metabolism **(cells of the liver, heart and kidneys).** Fat accumulates in the cytoplasm of a **"sick"** or **"ill"** cell because such a cell is unable to metabolize, utilize and/or export the normal levels of lipids it receives on a daily basis. Fatty change may be preceded by **"cellular swelling"** and is, therefore, a morphologic expression of reversible cellular injury.

"In addition to its accumulation in the sick or ill cell, fat may accumulate within cells which are initially normal. The normal cell may synthesize and store excessive quantities of fat when presented with excess substrate. This occurs primarily in hepatic cells of obese individuals. In other organs and tissues (heart, pancreas, etc.) the fat accumulates within adipose cells in connective tissue stroma when presented with excess substrate."

As viewed with the light microscope **(utilizing routine hematoxylin and eosin stain)** fat appear as small or large empty vacuoles within the cell's cytoplasm. Grossly, the involved organ is enlarged, friable, pale and the surfaces bulge when incised. Ultrastructurally, fatty change begins with the development of minute liposomes or membrane-bound inclusions which are closely applied and probably derived from endoplasmic reticulum.

In the liver, fatty change is manifested first by the appearance of small vacuoles in the cytoplasm near the nucleus. As the process progresses, the vacuoles coalesce and the larger vacuoles displace the nuclei to the periphery **(hepatic cells may resemble adult adipose cells).** In severe cases, the liver will float in water **(fat lowers the surface tension)**. In the kidneys, fatty change is most prominent in the proximal convoluted tubules and the ascending limb of Henle's loop; however, all renal epithelial cells may be involved. Under light microscopy, the cytoplasmic fat droplets are small and indistinct. They are usually over-looked until demonstrated with special stain. In the heart, fat appears as very fine droplets within the cytoplasm of myocytes. These fat droplets are not easily detected unless a suitable special stain is employed. Grossly, the fat within myocytes cannot be judged accurately.

"Remember, fat appears as clear cytoplasmic vacuoles within parenchymal cells when routine hematoxylin and eosin stain is used in preparing microscopic slides. Likewise, intracytoplasmic accumulations of water and glycogen appear as clear vacuoles when hematoxylin and eosin is employed. Therefore, it becomes necessary to resort to special stains to distinguish between these three types of clear vacuoles. To identify cytoplasmic fat, it is necessary to prepare frozen tissue sections on fresh or formalin fixed tissues in order to preserve the lipid content of the vacuoles. These sections are then stained with a suitable fat stain (Oil-Red-O, Sudan II, Sudan IV, etc.). To identify glocygen, Best Carmine stain is commonly employed. When neither fat nor glycogen can be demonstrated within a clear vacuole, it can be assumed that water nor fluid with a low protein content is present (water does not stain)."

STROMAL FATTY INFILTRATION:

Stromal fatty infiltration refers to the deposition of lipids in the cytoplasm of adipose tissue cells found among interstitial connective tissue cells throughout the body. If the amount of stromal fat is excessive, **"obesity"** is used as a descriptive term. This is not considered to be cellular injury.

NOTE: At this time, the student is familiar with the alterations that develop in "sick cells" or in reversible cellular injury. Remember, sick cells may recover spontaneously or they may recover if the injurious agent is removed via the treatment with drugs, etc. Unfortunately, however, cell death may ensue and under such circumstances, lesions and/or disease may be reflected in the body as a whole.

IRREVERSIBLE CELLULAR INJURY (The Dead Cell)

GENERAL CONSIDERATIONS:

Cellular death is oftentimes an unfortunate extension of cell injury. A dead cell cannot revert to a normal, adapted or ill state; it is doomed to be degraded. ***Remember,*** a cell may or may not exhibit cellular swelling, fatty change, etc., prior to death (**depending on severity of injury).** The point at which cell death occurs is still largely undetermined. Neither biochemical nor morphological studies can predict with certainty which sick cells have passed the "point of no return" and are doomed to die. Ultrastructurally, flocculation of the mitochondrial matrical proteins correlates with the onset of cell death. This is considered to be the earliest absolute sign of cell death. Utilizing the light microscope, a cell can be recognized as dead only after it has undergone a sequence of changes referred to as necrosis. There is a time lapse of 6 to 12 hours between the onset of cell death and the non-controversial recognition that the cell is dead by light microscopy. On the other hand, necrosis may be apparent to the naked eye after 3 to 4 hours, or much sooner (**tissues become abnormally opaque and pale)**.

"Necrosis may be defined as the morphological changes caused by the degradative action of lysosomal enzymes on the lethally injured cell. It is the sum of the morphological changes following cell death within the living body."

After a cell dies, lysosomes rupture and their hydrolytic enzymes are released into the cell. The release and activation of these lysosomal enzymes are largely responsible for cell necrosis.

Remember, necrotic cells are dead cells, but dead cells are not necessarily necrotic.

ULTRASTRUCTURAL CHANGES IN DEAD/NECROTIC CELLS:

Immediately after cell death, the ultrastructural changes are similar to and cannot be distinguished from those of reversible cellular injury (**sick cell)**. As mentioned previously, flocculation of mitochondrial matrical proteins correlates with the onset of cell death as seen ultrastructurally. Later, lysosomes rupture and disappear as recognizable structures. Cellular components are progressively degraded and the dead cell may become replaced by myelin figures (**masses composed of phospholipids**). These phospholoipd myelin figures are either digested by phagocytic cells or degraded further into fatty acids.

LIGHT MICROSCOPIC AND GROSS CHANGES IN DEAD/NECROTIC CELLS:

As stated previously, cells can be recognized as dead with the light microscope only when the morphologic changes of necrosis develop. Nuclear changes are the **"hallmark"** of cellular necrosis **(cell death)**. The nuclear changes appear in the form of one of three patterns (**pyknosis, karyorrhexis and karyolysis).** Pyknosis refers to a nucleus that progressively shrinks and becomes transformed into a small, dense, wrinkled mass of tightly packed chromatin. Karyorrhexis refers to a nucleus that breaks or fragments into many clumps or pieces. Karyolysis refers to a nucleus in which there is progressive dissolution of the chromatin and eventual disappearance of the nucleus. The cytoplasm of a necrotic cell becomes transformed into an acidophilic, granular opaque mass. After a cell dies and undergoes the early changes of cell death, immediate dissolution may not occur. Instead, one of three distinctive patterns may ensue, depending on the balance between progressive proteolysis, coagulation of proteins and calcification. Thus, the necrotic cell may undergo coagulative necrosis, liquefactive necrosis or, in special circumstances, caseous, gangrenous or fat necrosis.

COAGULATIVE NECROSIS:

Coagulative necrosis refers to an area of necrosis in which the gross and microscopic architecture of the tissue and some of the cells are preserved. Presumably, the cell's structural and enzymatic proteins are denatured and rendered insoluble soon after cell death; thus, autolysis or self-digestion is hindered. As a result, the dead cells remain in a **"state of coagulation"** (**cooked appearance)**, at least for a few days. Eventually, the coagulated cells become liquified slowly through heterolysis and/or phagocytized by phagocytes (**neutrophils and macrophages)**. Microscopically, tissue structures and cellular outlines are recognizable; nuclei are pyknotic or absent; the cytoplasm is strongly acidophilic and opaque. (**e.g., an entire renal tubule may undergo coagulative necrosis but still be recognized as a "tubule" due to preservation of its cylindrical shape and the outlines of the tubular epithelial cells.**) Grossly, necrotic tissue is grey to white **(unless filled with blood**), firm, dense, and often depressed compared to surrounding normal tissue. Coagulative necrosis is most commonly the result of:

- 1) sudden severe ischemia **(infarcts),**
- (2) certain acute acting toxins (**mercury chloride, etc.)**,
- (3) toxin produced by certain bacteria and
- (4) mild burns.

The term **"Zenker's necrosis"** refers to coagulation of proteins of sarcoplasm. The condition occurs only in striated muscle. Microscopically, individual fibers are swollen, homogeneous and hyaline in texture.

The sarcoplasm is usually eosinophilic, the myofibrils are indistinct and the nuclei are pyknotic. Grossly, involved muscle fibers are pale, rather shiny, and swollen. (**The term "hyaline degeneration" has been used to refer to this muscle change**.)

LIQUEFACTIVE NECROSIS:

Liquefactive necrosis refers to an area of necrosis which disintegrates very rapidly into a liquid mass, resulting in a loss of cellular and architectural outlines. The very rapid liquefaction is due to autolysis (**release of enzymes from the cell's own lysosomes)** and to heterolysis **(lysosomal enzymes from invading neutrophils**). In liquefactive necrosis, the dead cells are digested, creating a defect which is filled usually by invading neutrophils. A tissue defect of this nature frequently occurs in nervous tissue soon after death due to the high content of lipid and small amounts of coagulable protein. Thus, there are two principal situations in which liquefactive necrosis occurs - abscesses found in any body site and in the central nervous system. Pyogenic bacteria (**staphylococci, streptococci, etc.**) are usually the cause of abscesses. Microscopically, the necrotic area may appear as empty spaces with frayed and irregular edges (**commonly observed in the central nervous system)**; or it may be represented by a dehydrated residue of neutrophils, tissue debris and fibrin.

Remember, the fluid in liquified areas is removed in the process of tissue dehydration prior to sectioning. Grossly, the necrotic area is represented by a cavity containing clear fluid or by an abscess (pus).

"Remember, all necrotic tissue tends to disappear ultimately by a slow process of liquefaction. However, liquefactive necrosis refers to an area of necrosis that disintegrates very rapidly into a liquid mass."

CASEOUS NECROSIS:

Caseous necrosis refers to a distinctive pattern of necrosis which is a combination of coagulative and liquefactive necrosis. The gross and microscopic architecture of the cells/tissue is lost, but the necrotic tissue is not completely liquified. Caseous necrosis is associated with diseases in which granulomatous lesions occur **(tuberculosis, mycotic infections, etc.)**. The caseous material usually remains in place for prolonged periods of time and is prone to undergo calcification. Liquefaction and disappearance seldom occur. Microscopically, the necrotic cells are not totally liquified nor are their outlines preserved, creating a distinctive amorphous granular debris. The necrotic material is usually enclosed by a connective tissue capsule. Grossly, the necrotic tissue is soft to firm, dry, friable, grayish-white to yellow, and resembles **"milk curds"** or cottage cheese. The term **"caseous**" is derived from the gross appearance of the necrotic tissue **(white and cheesy)**.

FAT NECROSIS:

Fat necrosis is a distinctive type involving adipose tissue. It occurs in the body cavities (**especially the abdomen**) and beneath the skin.

Enzymatic fat necrosis occurs subsequent to pancreatic damage and the release of activated pancreatic enzymes into the abdominal cavity. The activated lipases split the triglyceride esters of adipose tissue into fatty acids and glycerin. The fatty acids combine with metallic ions **(calcium, potassium sodium, etc.**) to form soap within what was once a fat cell. Microscopically, the fat within adipose tissue cells is replaced by a soap which is solid, opaque and nearly homogeneous. The necrotic fat cell takes a bluish to pinkish tinge, depending on the presence of sodium or potassium, respectively. It is purple if calcium is deposited. Cholesterol clefts are often present. Remember, the soap formed within necrotic fat cells is not dissolved out **(as is fat)** by fat solvents used in sectioning techniques. Grossly, necrotic fat is opaque,

whitish, firm, chalky and somewhat granular. Enzymatic fat necrosis is not a specific form of necrosis. The cellular changes are essentially liquefactive.

Traumatic fat necrosis occurs primarily in subcutaneous adipose tissue. It is associated with mechanical trauma and pressure. However, the exact etiologic mechanism has not been clearly elucidated. Apparently, there is local damage to fat cells due to trauma with the release of fatty acids.

GANGRENOUS NECROSIS (GANGRENE):

Gangrenous necrosis or gangrene refers to an area of necrosis (**usually coagulative**) which is invaded by saprophytic and/or putrefactive bacteria.

"Initially, the tissues undergo coagulative necrosis; subsequently, the coagulated tissues are invaded by saprophytic and/or putrefactive bacteria which attract neutrophils to the area; the liquefactive action of the bacteria and the lysosomal enzymes released by the invading neutrophils modify the coagulated tissue."

Thus, gangrenous necrosis does not represent a distinctive pattern. It is, in reality, a combination of coagulative and liquefactive necrosis. If the coagulative pattern is dominant, the process is called dry gangrene. However, if the liquefactive pattern is more pronounced, it is designated as wet gangrene. Dry gangrene occurs primarily in the extremities **(limbs, ears, etc.**), whereas, wet gangrene occurs chiefly in visceral organs. Microscopically, the changes described for coagulative and/or liquefactive necrosis along with bacteria are observed. Grossly, in dry gangrene, the affected tissue is cool, dry, pale, shriveled and leather-like. There is a sharp line of demarcation between normal and gangrenous tissue. In moist gangrene, affected tissue is swollen, soft, pulpy, foul smelling and usually dark or black in color.

Gas gangrene is the term commonly used when necrotic tissue is invaded by bacteria that produce large amounts of gas from constituents of the dead tissue. Several species of genus Clostridium are capable of producing gas gangrene. These anaerobic, spore-forming bacteria can live in dead as well as in living tissue. ***For example,*** Clostridium species continue to multiply in it, producing large amounts of gas.

NOTE: Necrosis of cells/tissues is one of the most common and important lesions observed in disease. If a sufficient number of cells die in a given organ or tissue, the individual as a whole may exhibit clinical signs of illness, or somatic death may ensue. Death of cells (necrosis) in one organ may lead to secondary effects in other organs and tissues (e.g., death and necrosis of heart muscle cells may lead to a weakened heart which is unable to pump blood at its normal rate; subsequently, blood accumulates in many organs and tissues; liver necrosis occurs, and edematous fluid collects in body cavities, etc.).

CALCIUM SALT DEPOSITION IN NECROTIC TISSUE:

The term dystrophic calcification is used when calcium salts are deposited in dead or dying tissues. This condition occurs in the presence of normal serum levels of calcium and in the absence of derangement in calcium metabolism. It is not related to calcium content of the blood which normally is around 10 mg/100 ml. Dystrophic calcification may be especially prominent in necrotic tissue that persists in the body for long periods of time. It occurs in areas of coagulative, liquefactive, caseous and fat necrosis. The precise pathogenesis is poorly understood and may involve several different pathways.

"The term metastatic calcification is used to refer to the deposition of calcium salts in living tissues. It occurs subsequent to some derangement in calcium metabolism that results in hypercalcemia."

Grossly, calcium salts appear as fine, white granules or clumps, which give a gritty feeling when incised. Microscopically, calcium salts have a basophilic, amorphorous appearance with H and E stain. However, it can be confirmed with special staining procedures such as Von Kossa and Alzarin-Red-S techniques.

INTRACELLULAR ENZYMES RELEASED BY DEAD OR DYING CELLS:

Injured or dead cells tend to leak intracellular enzymes across their abnormally permeable plasma membrane. The enzymes diffuse into the intercellular fluid and subsequently into the bloodstream. In the bloodstream, these enzymes can be assayed by relatively simple laboratory techniques. Thus, elevated blood levels of enzymes released from dead cells is an important diagnostic aid for the recognition of dead cells/tissues within the living body. Elevated blood levels of such intracellular enzymes as glutamic oxaloacetic acid transminase, lactic dehydrogenase and creatine phosphokinase suggest the presence of severely damaged or dead cells (**necrosis**) within the body (**the diagnostic importance of the enzymes is discussed in your clinical chemistry course).**

THE OUTCOME OF NECROTIC TISSUE:

Overview: What do the outcomes mean to the host in terms of continued health?

Necrotic tissue tends to incite an inflammatory reaction in surrounding viable tissue since it acts as an irritant. Therefore, invading leukocytes surround the necrotic tissue and assist in its liquefaction. Eventually, the liquified tissue is removed via the bloodstream and/or lymphatic system. This is the usual outcome of necrotic tissue when the number of dead cells are few, and/or when the central nervous system is involved. Large necrotic masses of tissue are liquified and removed very slowly. In addition to liquefaction and removal by the bloodstream and/or lymphatic system, necrotic tissue may be handled by the body in the following ways.

- 1.Liquefaction by Autolysis and Heterolysis with the Formation of a Cyst-like Accumulation of Fluid: This occurs when fluid accumulates faster than it is drained away by the blood and lymph streams.
- 2.Liquefaction by Autolysis and Heterolysis with the Formation of Abscesses: This occurs when pyogenic bacteria are present in the necrotic tissue. Pus is formed.
- 3.Encapsulation without Liquefaction: This may occur when there is very little moisture in a part and the inflammatory reaction is not intense enough to assist in the liquefaction of the necrotic mass. Thus, within a few days, there is a proliferation of fibrous connective tissue around the necrotic tissue. Eventually, encapsulation occurs. Encapsulated caseous or coagulative necrotic tissue may persist in the body for a long time with little or not harm to the host.
- 4.Desquamation or Sloughing of Necrotic Tissue: This refers to the separation of necrotic tissue from viable tissue on an external or internal body surface **(skin, intestine, etc.).** The term **"desquamate"** is used when thin layers of necrotic cells in the epithelial layer separate from the underlying viable tissue **(the defect that remains is referred to as an erosion).** The term "sloughing" is used when larger masses of

necrotic cells **(extends beyond the surface epithelum)** are separated from the underlying viable tissue (**the defect that remains is referred to as an ulcer)**.

- 5.Regeneration of Replacement by Connective Tissue of Cells Lost via Necrosis: This occurs as a terminal stage. After necrotic tissue is removed, the damaged organ or part is restored as nearly as possible to its previous normal condition. The term regeneration refers to the process whereby lost cells are replaced by others of the same kind (**some cells regenerate readily whereas others do not)**. In replacement by connective tissue or scar tissue formation, the lost cells are replaced by fibrous connective tissue.

NOTE: At this point, the student has been exposed to the ways in which a normal cell responds when it encounters stress or an injurious agent. Cellular adaptation, cellular illness and cellular death are the common responses to injury. Remember, the adapted cell achieves an "altered but steady state." Its health is preserved, but its structure is altered and its functions are performed at a different level than that of a normal cell. The reversibly injured or ill cell is actually damaged but not killed by the injurious agent. It may revert to an adapted or normal state. The irreversibly injured or dead cell is doomed to be completely degraded. In the next segment, an attempt is made to solidify aspects of cellular injury by discussing possible mechanisms of reversible and irreversible cellular injury.

MECHANISMS OF REVERSIBLE AND IRREVERSIBLE CELLULAR INJURY

GENERAL CONSIDERATIONS:

The student is reminded that immensely complex problems are encountered in attempts to determine the precise molecular or biochemical event or events that initiate cellular injury or cellular death. It is apparent from the previous discussions that:

- 1.Many injurious agents may damage the cell and there is as yet no known common pathway of cellular injury.
- 2.Different cell types show major differences in their vulnerability to specific forms of injury.
- 3.The many organelles, biochemical systems, enzymes, etc., within the cell are so closely interdependent that it is difficult to differentiate the primary target of injury from the secondary effects.
- 4.The point at which irreversible damage or cell death occurs is still largely undetermined.

Despite the difficulties encountered, the mechanism and the site of primary attack for a few forms of injury have been fairly accurately elucidated:

- (1) in hypoxic injury, the first point of attack is the cell's aerobic respiratory system **(i.e., oxidative phosphorylation by mitochondria);**
- (2) cyanide selectively affects the terminal respiratory chain in the mitochondria by inactivating cytochrome oxidase, resulting in acute intracellular asphyxiation;

- (3) Clostridium perfringens and certain other anaerobic bacteria elaborate toxins, including lecithinase, which react with the phospholipids of cell membranes, resulting in severe acute increased permeability;
- (4) in mercury chloride poisoning, mercury binds to the cell membrane protein sulfhydryl groups **(as well as those of other proteins)** resulting in increased membrane permeability and inhibition of ATPase-dependent transport;
- (5) carbon tetrachloride (**CCl4)** poisoning is due to the conversion of the molecule (**CCl4)** to CCl3 (which is a high reactive and toxic radical) by an enzyme system in the smooth endoplasmic reticulum. The **CCl3 r**adical causes lipoperoxidation of the endoplasmic reticulum membranes.

HYPOXIA AS A MECHANISM OF CELLULAR INJURY/DEATH:

Since hypoxia may be the final pathway of action of many injurious agents, it is used as a **"model"** in the following discussion to assist the student in correlating and understanding aspects of reversible and irreversible cellular injury.

"Initially, the decreased oxygen supply to any organ altered the normal aerobic respiratory system of organ cells. Oxidative phosphorylation by mitochondria will decrease and the generation of ATP will slow down or stopped -> The decreased cellular ATP and associated increase of cellular [ADP] stimulated the production of phosphofructokinase -> The phosphofructokinase caused an increase rate of anaerobic glycolysis (to maintain the cell's energy sources by generating ATP from glycogen) -> glycogen was rapidly depleted -> The increased anaerobic glycolysis resulted in the intracellular accumulation of lactic acid and inorganic phosphates from the hydrolysis of phosphate esters -> The accumulated lactic acid and inorganic phosphates reduced the intracellular pH (the reduced pH accounted for the early clumping of nuclear chromatin) -> At this point the reduced ATP concentration began to interfere with the sodium-potassium pump at the cell membrane -> Sodium and water began to accumulate within the cell as reflected by early dilatation of the endoplasmic reticulum, whereas potassium diffused out of the cell resulting in increased extracellular K+ (Cellular swelling or cloudy swelling became evident at this time) -> Subsequently, detachment of ribosomes from the rough endoplasmic reticulum and disassociation of polysomes into monosomes occurred -> The continued hypoxic state caused other alterations and these were due to decreased mitochondrial function and increased cell membrane permeability (such as formation of blebs and vesicles at cell surface, increasing concentration of sodium and water within the cell, marked swelling of the entire cell, loss of coenzymes, protein, and ribonucleic acid via the hyperpermeable cell membrane, etc.) -> At this time, the transition across the "point of no return" or cell death began -> Mitochondria exhibited high amplitude swelling and flocculation of the matrical proteins, lysosomes became swollen but did not rupture at this time, nuclear chromatin began to dissolve, denaturation of cellular proteins occurred -> Lysosomal membranes were damaged resulting in leakage and activation of hydrolytic enzymes initiated progressive degradation of all cellular components -> Widespread leakage of cellular enzymes into the extracellular spaces occurred -> Remember, at this point, immediate dissolution of dead cells may or may not occur; regardless, one of the patterns of necrosis described earlier (coagulative, liquefactive, caseous) will become apparent or gross and/or microscopic inspection -> Finally, the dead cells are completely degraded or ingested and removed by phagocytic cells.

POSTMORTEM CHANGES

GENERAL CONSIDERATIONS:

Postmortem changes refer to cell death which accompanies death of the body as a whole **(somatic death).** The term **"antemortem changes"** refer to those alterations that occur in the living body **(prior to somatic death)**. The student must learn to differentiate postmortem changes from antemortem changes in order to correctly interpret those lesions encountered at necropsy.

Somatic death refers to death of the entire body. The absence of heart beat, pulse, respiration, or brain waves have been used to define somatic death. In other words, somatic death is characterized by cessation of all organ function. It is quite difficult, however, to determine the precise moment at which somatic death occurs. In man, this difficulty assumes considerable medical, ethical and legal importance.

Remember, somatic death is clearly different from cellular death.

Following somatic death, cells become ischemic and survive for varying periods of time, depending on cell type, decreasing body temperature and other factors. Thus, it is possible to remove organs for transplantation from individuals who have been pronounced dead **(e.g., fibroblasts may be successfully cultured many hours after somatic death)**. Regardless of the precise moment of death, once all vital body functions have ceased, a sequence of postmortem changes appears.

"The student should be able to distinguish between somatic death, necrosis and necrobiosis. Necrosis predominantly refers to the morphologic changes caused by lysosomal enzymes on the lethally injured cell within the living body. Necrobiosis refers to the death of cells at the end of their normal life-span. It occurs as a part of normal cell turnover. Cell death occurs without harm to the host since cell function has been fulfilled."

Postmortem autolysis refers to self-digestion by enzymes that are present within, or released into, the cytoplasm of cells after death.

Remember, all of the cytopathic changes described for necrosis occur in postmortem autolysis.

However, in postmortem autolysis, the changes are usually uniformly distributed throughout an organ and there is no inflammatory reaction. Postmortem autolysis is due to total diffuse anoxia. Organelles degenerate according to their oxygen requirement. Some tissue undergo autolysis very quickly after death and must be fixed (**formalin, etc.**) rapidly in order to preserve a lesion present at the time of death. The most sensitive are the retina **(which becomes separated from the choroid**), the seminiferous tubules **(in which vacuoles appear within and between cells**) and the intestine (**in which the epithelium over the villi sloughs off**. The rate at which postmortem autolysis occurs in an individual depends on a number of factors, including:

- --Concentration of Proteolytic Enzymes within Cells: Those cells with high concentrations of proteolytic enzymes **(liver, kidney, pancreas**) undergo postmortem autolysis rather quickly. Those with lower concentrations undergo autolytic changes rather slowly **(skeletal muscle, etc.)**.
- --Environmental Temperature: Postmortem autolysis is enhanced by high and retarded by low temperature environmental temperature.
- --Condition of the individual: Postmortem autolysis occurs rapidly in those individuals in good general condition **(well-fattened, etc.**) and is delayed in starved or emaciated individuals.

SPECIFIC TYPES OF POSTMORTEM CHANGES:

A description of some of the more commonly encountered postmortem changes are included below. Remember, it is important to distinguish postmortem changes from those changes that occur prior to death.

- 1.Postmortem putrefaction refers to the decomposition of tissues by bacterial enzymes. After death, bacteria from the digestive tract, etc., are able to invade, multiply and eventually digest tissues with their enzymes. Affected tissues are soft and foul-smelling.
- 2.Rigor Mortis refers to the stiffening of all muscles after death. It is related to a progressive decrease in oxygen, ATP, creatinine phosphate and pH of muscles. Muscle fibers shorten as they pass into rigor. Rigor classically begins in one to six hours after death and disappears in 24-48 hours (**as putrefaction begins**). However, it may be delayed or absent depending on various external factors. For example, rigor is enhanced by high metabolic activity and temperature prior to death. It is delayed by starvation, cachexia and cold. Rigor mortis begins earliest in cardiac muscles. In skeletal muscles, rigor begins in the anterior portion of the body and progresses in a posterior direction (**head, neck, trunk, limbs**). Rigor disappears in the same order as it appears.
- 3.Algor Mortis refers to the loss of body heat as the temperature of the body gradually equilibrates with its environment.
- 4.Hypostatic Congestion refers to the accumulation of blood in the ventral portions of the body due to the influence of gravity. Postmortem hypostatic congestion occurs after death when the heart beat stops and there is no longer a force which will maintain the circulation of blood and overcome gravity. Agonal hypostatic congestion occurs when the failing heart, prior to death, is no longer able to maintain blood pressure and blood accumulates in the veins in the ventral portions of the body.
- 5.Imbibition with hemoglobin refers to the staining of tissues with hemoglobin. After death, hemoglobin is liberated from hemolyzed red blood cells. This soluble hemoglobin diffuses through the abnormally permeable blood vessel wall and stains surrounding tissue pink to red. In addition, the endocardium and intimal surface of vessels are stained.
- 6.Imbibition with bile refers to the leakage of bile through the autolyzed wall of the gall bladder. The adjacent liver tissue is stained a greenish hue.
- 7.Pseudomelanosis refers to the appearance of a gray, green or black pigment in tissues after death. During the process of putrefaction, various bacteria produce hydrogen sulfide which combines with iron in hemoglobin to produce iron sulfide, a black pigment. The concentration of iron sulfide and its combination with other tissue pigments results in a variety of shades of green, gray and black.
- 8.Postmortem emphysema refers to the accumulation of gas in tissues as a result of bacterial fermentation. Hydrogen sulfide is a common gas found in the cadaver. Postmortem emphysema must be distinguished from bloat.
- 9.Postmortem rupture occurs when gases produced by bacterial fermentation cause progressive distention of body structures until they burst (stomach, intestine, diaphragm, etc.). A postmortem rupture must be distinguished from an antemortem rupture.
- 10.Postmortem displacement of organs occurs when the dead animal is rolled over or moved. The intestine is most commonly displaced after death. Postmortem displacement must be distinguished from antemortem displacement (volvulus and torsion).
- 11.Postmortem clotting of blood refers to the coagulation of blood in vessels and/or heart after death. Postmortem clots may be dark red in color (current jelly clot) or the yellow color of plasma (chicken fat clot). Such clots are
    - (1) smooth and shiny on the outside,
    - (2) uniform in texture and
    - (3) unattached to the vessel or heart wall. On the other hand, antemortem clots, thrombi, are
    - (1) friable with a dull color,
    - (2) roughened over the surface and
    - (3) attached to the vessel wall.

- 12.The Heart after Somatic Death: After death, rigor mortis contracts the left ventricle strongly and empties it of blood (blood is forced into the aorta). If the left ventricle contains blood and it is unclotted and not hemolyzed, rigor mortis apparently has not yet taken place (death being quite recent). However, if the left ventricle contains clotted blood, rigor mortis apparently did not develop because body forces were to low at the time of death (seen with lingering illness). If rigor has come and gone (lapse of 24-48 hours), the left ventricle will contain dark, hemolyzed, unclotted blood (blood from the disintegrating clot within the aorta runs back into the left ventricle). In the right ventricle, blood is not forced out of the lumen during rigor mortis. This blood hemolyzes within a few hours and stains the right ventricular wall a lusterless strong red color.

CIRCULATORY DISTURBANCES

In this section, lesions related to circulatory disturbances are considered. Most lesions that develop in the body are influenced directly or indirectly by the blood and/or blood vessels. Those circulatory disturbances common to many types of lesions include hemorrhage, hyperemia, congestion, ischemia, thrombosis, embolism, infarction, edema, shock and disseminated intravascular coagulation.

HYPEREMIA AND CONGESTION

The terms hyperemia and congestion refer to an increased volume of blood in an affected tissue or part. Hyperemia (**also called "active hyperemia"**) occurs when arterial and arteriolar dilatation produces and increased flow of blood into capillary beds (**inactive capillaries are opened**). Congestion **(also referred to as passive congestion or venous congestion**) results from impaired venous drainage. Remember, in both hyperemia and congestion, blood is retained within the vascular system; whereas in hemorrhage, blood is found outside of the vascular system.

ACTIVE HYPEREMIA

Occurs when too much arterial blood is brought to an organ or tissue by dilated arterioles and capillaries. The arteriolar dilatation is brought about by sympathetic neurogenic mechanisms or by the release of vasoactive substances. In most instances, active hyperemia occurs subsequent to an inflammatory reaction (**it is the first stage of inflammation**). Other situations characterized by active hyperemia include:

- (1) heat applied locally to a part and
- (2) increased physiological activity.

Microscopically, the capillaries are dilated and filled with blood. Grossly, the involved organ/part takes on the bright red color of arterial blood (**depending on the original color)**. Clinically, the organ/part is warmer than normal.

Remember, active hyperemia is usually localized (if it was generalized, there would be insufficient blood in major vessels to maintain systemic blood pressure and shock would occur).

PASSIVE CONGESTION

(Congestion) occurs when the flow of blood leaving an organ or part is impeded (impaired venous drainage). Microscopically, congestion is similar to hyperemia (**capillaries and veins are dilated and filled with blood**). Grossly, the involved tissues appear bluish-red because of the poorly oxygenated venous blood.

Remember, congestion of capillary beds is closely related to the development of edema; thus, passive congestion and edema commonly occur together.

Localized Passive Congestion is usually caused by pressure placed on veins leaving an organ or part **(via bandage, rubber band, torsion, etc.**). Oftentimes, the compression on vessels is such that blood still gets in through thick-walled, muscular arteries but pressure on thinner-walled veins restrict the outflow and venous blood accumulates.

Generalized Passive Congestion is associated with impediment of blood flow in the central circulation (**heart, lungs, major vessels, etc.**). It may be acute or chronic. Acute generalized passive congestion is usually associated with a failing heart. Chronic generalized passive congestion is most obviously manifested in the lungs, liver and spleen.

Chronic Generalized Passive Congestion of the Lungs is encountered in all forms of cardiac decompensation that occurs subsequent to reduced left ventricular output (**left-sided heart failure)**. An accumulation of blood and increased hydrostatic pressure occurs in the lungs. Some of the distended lung capillaries may rupture or hemorrhage per diapedesis may occur. Eventually, the breakdown and phagocytosis of erythrocyte debris leads to the appearance of hemosiderin-laden macrophages (**heart-failure cells**) in the alveolar spaces. In time, the alveolar walls become fibrotic. Thus, the fibrosis and hemosiderin pigmentation constitute the basis for the designation **"brown induration of the lung."**

Chronic Passive Congestion of the Liver results from right-sided heart failure (**rarely from obstruction of the posterior vena cava)**.

HEMORRHAGE

Hemorrhage refers to the presence of erythrocytes outside the blood vessels. The vessel may be physically damaged so that erythrocytes flow out through a break in the wall or the erythrocytes may pass through an intact vascular wall by a process called diapedesis. The various etiologic agents that play a role in the development of hemorrhage are discussed in your big textbook. The following are some of the terms used to denote hemorrhage.

- -- Petechiae**: refer** to very tiny hemorrhages which occur as **"tine pin points**" up to 1-2 mm in diameter. Such hemorrhages occur in the skin, mucous membranes and serosal surfaces. Petechiae are commonly observed in septicemia where the endothelium is damaged or destroyed.
- -- Ecchymoses: **refer** to larger hemorrhagic areas up to 2-3 cm in size (**large bruises are ecchymoses**).

- -- Purpura: **refer** to hemorrhages which are slightly larger than ecchymoses. Such hemorrhages are associated most commonly with disturbances of the clotting mechanism.
- -- Agonal hemorrhages: **refer** to small hemorrhages the size of petechiae and ecchymoses that arise just prior to death **(associated with the death struggle)**.
- -- Linear hemorrhages: **refer** to hemorrhages which appear as lines.
- -- Paint-brush hemorrhages: **refers** to extensive streaking with hemorrhages (**several linear hemorrhages which appear side by side).**
- -- Extravasation: **refers** to hemorrhages in tissues spread over considerable areas. Suffusions are diffuse, flat, often irregular-shaped areas of bleeding.
- Hematocyst **refers** to more or less spherical shaped collection of blood in tissues (**size will vary).**
- -- Hemothorax: **refers** to hemorrhage into the thoracic cavity.
- -- Hemopericardium: **refers** to hemorrhage into the pericardial sac.
- -- Epistaxis: **refers** to hemorrhage from the nostrils.
- -- Hemoptysis: **refers** to the expectoration of blood that originates from the respiratory tract.
- -- Enterorrhagia: **refers** to intestinal bleeding.
- -- Metorrhagia: **refers** to uterine bleeding.
- -- Hematuria: **refers** to blood in the urine.
- -- Hemorrhage by rhexis: **refers** to hemorrhage that occurs subsequent to a break in the wall of a vessel **(all constituents of the blood escapes).**
- -- Hemorrhage by diapedesis: **refers** to hemorrhage that occurs when erythrocytes escape through an apparently intact vessel wall.

The significance of hemorrhage depends on:

- (1) the volume of blood loss,
- (2) the rate of blood loss and
- (3) the site of hemorrhage.

About 30% of the total blood volume is the maximum which can be lost and the animal still recover. If more is lost, death is likely to occur subsequent to hypovolemic shock. However, the amount of blood which can be lost depends upon the rapidity with which it leaves the vascular system. ***For example,*** when the rate of hemorrhage is slow **(intestine worm infections**), fluid can be added to the blood rapid enough to maintain near normal blood pressure; thus, the loss of large amounts may have little clinical significance. Also, the site of hemorrhage will influence its effect on the host. A hemorrhage which would be trivial in the subcutaneous tissues may cause death when located in the brain stem.

Repeated or chronic external hemorrhages **(i.e., when blood is shed from the skin, G.I. tract, etc.)** represent losses not only of blood volume but also of valuable iron. Usually, the small but repeated volume losses are rapidly corrected by movement of water from the interstitial spaces into the vascular compartment, but the chronic losses or iron may lead to iron deficiency anemia. In contrast, when erythrocytes are retained, as occurs with hemorrhages into the body cavities, joints or tissues, the iron can be recaptured for synthesis of hemoglobin.

The fate of an area of hemorrhage depends upon the amount of blood that has escaped from the vascular system. If the hemorrhage is relatively small, the fluid portion of the blood is absorbed, the leukocytes move back into the vascular system and the erythrocytes are phagocytized. In a larger hemorrhage, there is disintegration and breakdown of erythrocytes with the formation of hematoidin and hemosiderin. Cholesterol may also be seen in the tissues. The escaped blood also clots with the formation of fibrin and this fibrin and the remaining leukocytes may eventually be phagocytized. In still

larger areas of hemorrhage, fibroblasts and new capillaries may proliferate into the area of clotted blood. This process is known as organization of the area of hemorrhage.

SLUDGED BLOOD

Sludged blood refers to the conglutination or sticking together of erythrocytes within blood vessels and should be distinguished from simple rouleaux formation in which erythrocytes merely stack one on top of another. In the formation of sludged blood, large masses of erythrocytes adhere to each other and may settle to the lower portion of large vessels or even block smaller vessels. The etiologic mechanism is uncertain, but for some reason the erythrocytes lose their ability to repel each other and conglutinate. This condition has been studied extensively by microscopic examination of vessels in the conjuctiva during life. It is postulated that sludged blood flows more slowly, may give rise to blood clots within the vessel and may cause hypoxia of the tissues involved.

ISCHEMIA

Ischemia refers to local anemia or a deficiency of arterial blood to a portion of an organ or part. The chief causes of ischemia are

- (1) external pressure upon an artery,
- (2) narrowing of the lumen of an artery and
- (3) a thrombus or embolus.

However, ischemia may be caused by vasoconstriction as observed in some poisoning. The effects of ischemia are dependent on the organ involved, the size of the vessel, the degree of occlusion and the degree of collateral circulation. If ischemia occurs in an **"end artery,"** as in the kidneys, the result is likely to be acute necrosis of tissue supplied by the vessel. If the obstruction to blood flow is gradual, atrophy may occur.

THROMBOSIS

Thrombosis refers to the formation of a clot from elements of the circulating blood within the vascular system during life. This clot is known as a thrombus **(plural, thrombi).** The development of a clot is life-saving when a large vessel ruptures or is severed. However, when a thrombus develops within the vascular system, it may be life-threatening because:

- -- It may decrease or obstruct vascular flow causing ischemic/hypoxic injury to cells, tissues and organs.
- -- It may become dislodged or fragmented to create emboli (**an embolus is an intravascular mass carried in the bloodstream to some site removed from its origin)**.

The ischemic necrosis created by a thrombus **(embolus)** is referred to as an infarct **(thrombosis and embolism are so closely interrelated as to give rise to the term thromboembolism)**. To a considerable extent, thrombosis is the consequence of inappropriate activation of the processes of normal hemostasis. Therefore, the student should review normal hemostasis as outlined in the big Robbins textbook before considering the pathogenesis of thrombosis.

"Normal Hemostasis" is influenced by components of the blood vessel wall, platelets and the clotting sequence. The integrity of the blood vessel wall is crucial in normal hemostasis as well as in thrombosis. The lining endothelium provides a nonreactive interface between the underlying reactive element of the

vessel wall and the fluid blood. In addition, the endothelial cells serve to protect against thrombi formation by:

- (1) releasing plasminogen activator which initiates fibrinolysis and
- (2) degrading platelet-aggregating agents such as adenosine phosphate and certain forms of prostaglandins. Underlying the endothelial layer is the subendothelial connective tissue which contains collagen fibrils. These collagen fibrils are potent activators of clotting factors, and they promote platelet adhesion. In summary, the endothelial cells of the vessel wall are crucial in the maintenance of normal blood flow. If the endothelial layer is damaged, the subendothelial collagen fibrils will release "tissue factors" that activate the coagulation system.

Platelets are assigned a central role in normal hemostasis and thrombosis. They adhere to sites of endothelial injury, aggregate to form platelet masses, release granules rich in a variety of secretory products and synthesize several types of prostaglandins. In normal hemostasis, platelets adhere to the severed margins of a vessel within seconds to a few minutes. The most important stimulus to such adherence is the exposure of collagen fibrils. Once adhered, platelets release two types of granules:

- (1) alpha granules which contain fibrinogen, beta thromboglobulin, cationic protein and platelet factor 4 (**a heparin neutralizing protein**) and
- (2) dense bodies, which are rich in serotonin, ADP, ATP and ionized calcium.

The release of platelet granules is triggered by a number of substances, including collagen fibrils, thrombin, plasmin, trypsin, endotoxin and antigen-antibody complexes. It is believed that these stimuli to platelet activation inhibit membrane-bound adenyl cyclase **(decreased amounts of cyclic AMP are found in aggregated platelets)**. Within aggregated platelets, there is increased concentration of calcium **(this cation is a potent stimulus to platelet activation).** In addition, platelet factor 3, which participates in the intrinsic pathway of the clotting sequence, becomes activated. Initially, the platelet aggregation forms a temporary hemostatic plug which is friable and easily dislocated in rapidly flowing bloodstreams **(however, at this time, the clotting sequence leads to the formation of thrombin which is the most powerful platelet aggregator yet identified)**. In summary, platelets:

- (1) provide a temporary plug capable of controlling blood flow in small vessels in low pressure systems,
- (2) initiate the development of a permanent plug composed of aggregated platelets and fibrin,
- (3) release serotonin which augments vasoconstriction and
- (4) contributes to the coagulation mechanism.

The coagulation system plays a major role in normal hemostasis. Maintenance of normal fluidity of blood involves the interplay between procoagulants and anticoagulants. When the procoagulants dominate and clotting is triggered inappropriately in the intact cardiovascular system, thrombi result. Concurrent with the formation of the platelet plug, the coagulation system is activated critical events in blood clotting are the conversion of prothrombin to thrombin and the subsequent conversion of soluble fibrinogen into the stable fibrin polymer **(the sequence of interactions among the clotting factors is present on your textbook).** Remember, clotting may be initiated by the intrinsic pathway when blood is exposed to a negatively charged surface, such as collagen. The extrinsic pathway initiates clotting when injury exposes the blood to factors derived from injured cells and tissues. Thus, the evolution of a thrombus begins with the adherence of platelets at sites of vascular injury followed by the build-up, first of a temporary aggregation of platelets, and then the formation of a more permanent platelet mass which in turn leads to the standard clotting sequence, possibly involving both the intrinsic and extrinsic pathways.

Thrombosis is influenced by three major factors:

- (1) injury to vascular endothelium,
- (2) alterations in normal blood flow and
- (3) alterations in the blood (**hypercoagulability**).

Endothelial injury plays a dominant role in the formation of thrombi in arteries and in the heart. Once the endothelium is damaged, subendothelial collagen may be exposed and tissue thromboplastic, etc., is released and the sequence of platelet adherence and activation of the clotting sequence follows.

Alterations in Normal Flow as encountered with stasis and turbulence of blood contributes to the development of arterial and cardiac thrombi and is probably requisite for venous thrombosis. In the normal flowing bloodstream, the larger particles, such as erythrocytes and leukocytes, occupy the central or more rapidly moving axial stream. The smaller platelets are carried in the more slowly moving laminar stream outside the central column. The periphery of the bloodstream adjacent to the endothelial layer moves more slowly and is free of all formed blood elements. If stasis or turbulence occurs, this laminar flow is disrupted and platelets are brought in contact with the endothelium. Evidence suggests that stasis and turbulence assume the greatest degree of importance in the formation of venous thrombi.

Alterations in blood that induce hypercoagulability have been proposed to explain the increased incidence of thrombosis encountered in certain clinical states **(following surgical procedures, parturition, accidental trauma, etc.)** Hyper-coagulability has been defined as **"an altered state of circulating blood that requires a smaller quantity of clot-promoting substances to induce intravascular coagulation than is required to produce comparable thrombosis in a normal host."** Increased numbers of platelets, increased platelet stickiness, elevated levels of fibrinogen, increased generation of thrombin, etc., have been identified as causing hypercoagulability in various clinical conditions.

Grossly, thrombi are friable, a mixture of red and gray in irregular layers, dull, and attached to the endothelium. Arterial thrombi formed in a rapidly flowing bloodstream are usually dry, friable gray masses composed of almost regularly arranged layers of platelets and fibrin, irregularly mixed with small amounts of darker red coagulated blood. The resulting laminations are known as the **"lines of Zahn."** Arterial thrombi are referred to as white or conglutination thrombi. Venous thrombi, formed in a slow-moving bloodstream, appear as an intravascular clot that closely resembles the clotting of blood in a test tube. Such thrombi are red, gelatinous, and they are referred to as stasis or red coagulation thrombi. The following terms are used to describe thrombi:

- -- Mural thrombi - are attached to the wall of the heart or blood vessel.
- -- Occluding thrombi - are attached to the entire circumference of the vessel.
- -- Valvular thrombi - are attached to the heart valves.
- -- Canalized thrombi - occur when new blood channels form in an organized thrombus.
- -- Saddle thrombi - straddle the bifurcation of blood vessels.
- -- Septic thrombi - are those which contain bacteria.
- -- Aseptic thrombi - are those that do not contain bacteria, etc.

Microscopically, thrombi are eosinophilic masses in which leukocytes and erythrocytes may be seen. Fibrin is usually obvious, but it is seldom possible to identify platelets.

The significance, effects and outcome of thrombi should be reviewed in your textbook. If an animal survives the immediate ischemic effects of a newly developed thrombus, one of several pathways may be followed. The thrombus may

- (1) increase in size and, by its enlargement, eventually cause obstruction of some critical vessel,
- (2) give rise to emboli (**to be discussed)**,
- (3) be removed by fibrinolytic action or

- (4) become organized.

POSTMORTEM CLOTS:

A thrombus must not be confused with postmortem clotting of blood within the vascular system. The two types of postmortem clots are:

- (1) red or current jelly clots and
- (2) yellow or chicken fat clots.

Red or Current Jelly Clots occur when the components of the blood are evenly distributed throughout the clot. This type develops when there is rapid clotting of blood.

Yellow or Chicken Fat Clots result from a settling and separation of erythrocytes from the fluid phase of the blood. Such clots occur when postmortem clotting is delayed.

The following ***table gives the characteristic features of a thrombus and a postmortem clot.***

	THROMBUS	POSTMORTEM CLOT
CONSISTENCY	Dry and Friable	Moist and Jelly-like
SURFACE	Granular and rough	Smooth and glistening
COLOR	White or Buff	Intense red or yellow
ATTACHMENT	Attached to vessel wall	Not attached to vessel wall
ENDOTHELIUM	Damaged/injured	Undamaged
COMPOSITION	Platelets primarily	Fibrin Primarily
RAPIDITY OF BLOOD FLOW	Formed in flowing stream of blood	Formed in stagnant column of blood
ANIMAL	Formed in living animal	Formed in dead animal
ORGANIZATION	May be partially organized	No organization

DISSEMINATED INTRAVASCULAR COAGULATION

Disseminated intravascular coagulation **(DIC)** refers to widespread microthrombi formation in capillaries, arterioles and venules. The thrombi are composed largely of fibrin and aggregated platelets. The disorder may be a complication of a diverse group of clinical diseases in which there is activation of the intrinsic pathway of blood clotting. During the widespread intravascular coagulation, fibrin is deposited throughout the vascular tree resulting in microthrombi. Although the fibrinolytic system is activated, it cannot effectively deal with the large deposits of fibrin. As a result, there is rapid consumption and eventually a deficiency of clotting factors, including fibrinogen, platelets, prothrombin and factor V, VII, and X (**a deficiency of fibrinogen, platelets and prothrombin is required for the diagnosis of DIC).** Therefore, individuals with DIC have bleeding tendencies on hemorrhagic diathesis. Also the widespread occlusion of the microcirculation may induce signs of shock, acute respiratory distress, central nervous system

depression, heart failure or renal failure. Remember, affected tissues may not necessarily disclose the microthrombi because of prompt activation of the fibrinolytic system.

EMBOLISM

Embolism refers to the process of a foreign body moving through the circulatory system and becoming lodged in a vessel causing obstruction. An embolus **(plural, emboli)** is a detached intravascular solid, liquid or gaseous mass that is carried by the blood to a site distant from its point of origin. Inevitably, emboli lodge in vessels too small to permit their further passage resulting in partial or complete occlusion of the vessel. The majority of all emboli arise from thrombi **(thromboembolism)**. These are pieces of thrombi which have been broken loose by the force of the bloodstream. Less common forms of emboli include fat emboli, gas emboli, bacterial emboli, tumor emboli and parasitic emboli ***(see your textbook).***

Depending on their site of origin, emboli may come to rest anywhere within the cardiovascular system. **(Unless otherwise qualified, the term "embolus" implies thromboembolism throughout this discussion).**

PULMONARY EMBOLISM:

Pulmonary emboli usually originate from thrombi in veins or in the right heart. Dislodgement of venous thrombi, in part or whole, produces an embolus which flows with the venous drainage through progressively larger vessels to the right heart. Unless the embolus is very large, it passes through the spacious chambers and valve openings of the right heart and enters the pulmonary arterial circulation. Lodgement of emboli in major pulmonary vessels is commonly fatal, resulting in sudden death. When pulmonary emboli occlude smaller vessels, they usually cause lung hemorrhage or infarcts. However, in animals without cardiac or circulatory insufficiency, the bronchial circulation suffices to substain the vitality of lung tissue. Remember, pulmonary infarction results only when the bronchial circulation is inadequate to compensate, which is common in animals with impaired cardiovascular function.

SYSTEMIC EMBOLISM:

Systemic embolism **refers** to emboli which travel through the arterial circulation. Such emboli usually arise from thrombi within the left heart. In contrast to venous embolism, arterial emboli travel through vessels of progressively diminishing caliber. The myocardium, spleen, kidneys, brain and lower extremities are commonly the victims of arterial embolism.

Paradoxical embolism refers to emboli which enters the right side of the heart and pass through interatrial or interventricular septal defects to gain access to the arterial side of the circulation.

INFARCTION

An infarct is a localized area of ischemic necrosis in an organ or tissue resulting from occlusion of either its arterial supply or venous drainage. The vascular occlusion is usually caused by thrombosis and/or embolism of the arterial blood supply. More rarely, external compression of vessels by expanding tumors, etc., may result in infarction.

Infarcts are classified on the basis of their color (**red or pale infarcts**) and on the presence or absence of bacterial contamination **(septic or aseptic infarcts).** Pale or anemic infarcts are encountered with arterial occlusion and in solid tissue. When a solid tissue is deprived of its arterial circulation, the infarct may be transiently hemorrhagic, but most become pale in a very short time. The reasons for the development of pale infarcts are as follows:

"The arterial circulation to an area is occluded. Vessels, particularly capillaries, as well as parenchymal cells are destroyed. At the moment of vascular occlusion, blood from anastomotic peripheral vessels flows into the focus of injury, producing the initial hemorrhagic appearance. If the affected tissue is solid, seepage of blood from the anastomotic vessels is minimal. Soon after the initial blood seepage, the erythrocytes are lysed and the released hemoglobin pigment either diffuses out or is converted to hemosiderin. Therefore, in solid organs, the arterial infarct will soon (24 to 48 hours) become pale or anemic. The heart and kidneys are representative of solid, compact organs which tend to have pale infarcts."

Red or Hemorrhagic Infarcts are encountered usually under the following circumstances:

- (1) with venous occlusion,
- (2) in loose tissue,
- (3) in tissue with a dual circulation and
- (4) in tissues previously congested.

Red or hemorrhagic infarcts develop in loose tissue subsequent to arterial obstruction in the following manner.

The arterial circulation to an area is obstructed. If the tissue is loose **(lung, etc.**), large amounts of blood collect in the spongy, loose tissue at the moment of vascular occlusion. This blood remains for long periods; thus, the arterial infarct remains red. The lungs and intestine are sites where red infarcts tend to occur.

Remember, red infarcts may occasionally be encountered in solid tissue or white infarcts in loose tissue.

Factors that influence the severity of damage resulting from infarction include the following:

- 1.General Status of the Blood and Cardiovascular System: Any alteration in the systemic circulation that reduces the oxygen-carrying capacity of the blood or the velocity and volume of blood flow through the tissues predisposes to infarction.
- 2.Anatomic Pattern of Arterial Blood Supply: The various tissues and organs of the body receive their arterial supply through one of several patterns:
    - (1) a dual arterial blood supply,
    - (2) a **"single"** arterial blood supply with few anastomoses **(insufficient to provide adequate bypass channels)**, so-called "end arteries,"
    - (3) a **"single"** arterial blood supply with rich interarterial anastomoses and
    - (4) parallel arterial systems.

A dual blood supply is received by the lungs and liver. In individuals with normal cardiac and cardiovascular status, the bronchial circulation is capable of preventing ischemic necrosis of the lungs when a branch of the pulmonary artery is obstructed. Similarly, infarction is uncommon in the liver because the portal supply of blood may be adequate, even when the hepatic arterial supply is compromised. However, in the presence of cardiac failure, severe anemia, or reduced oxygenation of the blood, occlusion of one system may precipitate ischemic necrosis.

An arterial blood supply with rich interarterial anastomoses is found in the small intestine. Here, blood is able to bypass focal areas of occlusion.

An arterial blood supply with so-called **"end arteries"** is found in the kidneys, for example. The major branches of the renal artery supply well-defined segments of the kidneys. Occlusion of one of the major

branches, or of the main renal artery, is invariably followed by ischemic necrosis. However, if the occlusion occurs at the terminal ramification and involves subcapsular parenchyma, there may be sufficient blood flow from capsular vessels to prevent tissue damage.

Parallel arterial system is encountered in the forelimbs. Either the radial or the ulnar artery is sufficient to sustain the vitality of the tissues when one or the other is occluded.

- Rate of Development of Occlusion: Slowly developing occlusions are better tolerated than those occurring suddenly since they provide an opportunity for alternative pathways and collateral circulation to become activated.

Microscopically, all areas of infarction undergo coagulative necrosis and resorption as discussed.

(Remember, central nervous tissue undergoes liquefactive rather than coagulative type necrosis).

The typical coagulative appearance may be modified by extensive hemorrhage in red infarcts and by bacterial suppuration in septic infarcts. Within a few days after an infarct is initiated, an inflammatory reaction becomes well-defined. Later, a reparative process begins.

Grossly, both red and pale infarcts tend to be wedged-shaped, with the apex of the wedge pointing toward the focus of vascular occlusion.

EDEMA

Edema refers to an abnormal accumulation of fluid **(water)** in the intercellular tissue spaces or body cavities. It may occur as a localized **(e.g. obstruction of venous outflow from the leg)**, or it may be generalized in distribution **(e.g., in chronic congestive heart failure)**. The following terms are used to describe edema:

- --Anasarca: **refers** to generalized edema in which fluid in subcutaneous tissues is especially prominent.
- --Ascites: **refers** to a collection of edematous fluid in the peritoneal cavity.
- --Hydrothorax: **refers** to a collection of edematous fluid in the thoracic cavity.
- --Hydropericardium or Pericardial Effusion: **refers** to a collection of edematous fluid in the pericardial sac.

Edematous fluid may be inflammatory or non-inflammatory. Inflammatory edema is referred to as an exudate and it is associated with an inflammatory reaction. Non-inflammatory edema is referred to as a transudate.

The term **"edema"** refers to non-inflammatory edema throughout this discussion, unless otherwise qualified.

Non-inflammatory edema (**transudate)** can be distinguished from an inflammatory edema **(exudate)** on the basis of the following features.

Mechanisms of Edema Formation:

Before embarking on a study of the pathogenic mechanisms of edema, the normal control and relationships of tissue fluid must be clearly understood.

Under normal physiologic conditions, the main filtration force that expels fluid from the vessel is the hydrostatic pressure at the arterial end of the capillary minus the osmotic pressure of the blood. The main absorption force that draws fluid into the vessel is the osmotic pressure of the blood minus the hydrostatic pressure at the venous end of the capillary. In the normal animal, there is a continuous circulation of fluid from the arterial end of the capillary through the tissues and back into the venous end of the capillary.

Physiologically, blood enters the arterial end of a capillary with a hydrostatic pressure (**blood pressure)** of about 45 millimeters of mercury, which expels fluid and smaller dissolved molecules into the intercellular spaces. However, this hydrostatic pressure **(expulsive force)** is opposed by the osmotic pressure of blood exerted by such molecules as albumin and globulin. The osmotic pressure is about 30 mm of mercury. Therefore, at the arterial end of the capillary, hydrostatic pressure at 45 mm of mercury is overcoming the 30 mm osmotic pressure of the blood plasma, and fluid is forced into the intercellular spaces at the rate of 15 mm of mercury. As blood travels through capillaries, its hydrostatic pressure decreases rapidly to about 15 mm of mercury. Therefore, at the venous end of the capillary, hydrostatic pressure at 15 mm of mercury cannot overcome 30 mm osmotic pressure of the blood, and fluid flows from the intercellular spaces into the bloodstream at the rate of 15 mm of mercury. Since fluid enters tissues at about the same rate as it leaves, there is no accumulation of fluid in the intercellular spaces in the normal animal. However, edema occurs if there is any interference with this normal flow.

Basically, edema is caused by:

- (1) decreased plasma osmotic pressure,
- (2) increased hydrostatic pressure,
- (3) increased permeability of vascular endothelium and
- (4) lymphatic obstruction.

DECREASED PLASMA OSMOTIC PRESSURE:

Occurs when there is a deficiency of blood proteins **(hypoproteinemia).** Thus, hypoproteinemia may result from decreased formation or excessive loss from the blood. Albumin is most important in maintaining osmotic pressure and it exerts four times the osmotic pressure of globulin. A low osmotic pressure in the blood increases the pressure differential at the arterial end of the capillary so that more fluid is pushed into the intercellular spaces. Also, the force available to pull fluid into the bloodstream at the venous end of the capillary is reduced. Thus, there is an accumulation of fluid in intercellular spaces and/or body cavities.

A failure to form blood proteins results from malnutrition **(starvation, emaciation**) in which the **"building blocks"** for blood protein formation are not available. Severe or advanced liver diseases **(cirrhosis, etc**.) may lead to hypoproteinemia since this is the site in which blood proteins (**albumin and globulin**) are synthesized. The loss of plasma proteins from the blood occurs through the intestine and kidneys.

Remember, decreased plasma osmotic pressure always leads to generalized edema.

INCREASED HYDROSTATIC PRESSURE:

Is influenced mainly at the venous end of the capillary and it usually results from venous stasis (severe passive congestion that results in increased back pressure in the venous circulation). The

increased hydrostatic pressure at the venous end of the capillary which pushed fluid out of the bloodstream counterbalances the osmotic pressure which pulls fluids into the bloodstream. Therefore, fluid fails to return to the vessel from the intercellular tissue. Subsequent to venous stasis, the capillaries become more permeable to large molecules **(albumin and globulin)**, since they are deprived of their normal supply of oxygen and other nutrients.

Remember, the usual causes of venous stasis and subsequent increased hydrostatic pressure are impaired heart function or a lesion in which the venous flow is obstructed.

INCREASED PERMEABILITY OF CAPILLARY ENDOTHELIUM:

Occurs subsequent to venous stasis **(resulting in increased hydrostatic pressure),** as well as from direct damage, as in inflammation. Increased vascular permeability is the most important mechanism in the formation of inflammatory edema **(exudate).**

LYMPHATIC OBSTRUCTION:

Occurs when any lesion impedes normal lymphatic drainage by pressure or obstruction. Under normal conditions, the lymphatics constantly drain small amounts of fluid from the intercellular spaces. Thus, in the absence of lymphatic drainage from a area, fluid accumulates.

In summary, decreased plasma osmotic pressure produces generalized edema, whereas increased hydrostatic pressure may induce either localized or generalized edema. Increased permeability of capillary endothelium and lymphatic obstruction almost always lead to localized edema.

Microscopically, when well-defined, edema appears as a granular, eosinophilic interstitial precipitate that separates the cellular and fibrillar elements of tissue **(the pink-staining appearance is due primarily to the presence of albumin in the edematous fluid)**. In the absence of albuminous precipitate, edema is represented by empty spaces in the interstitial areas.

Grossly, edematous tissues are swollen, firm, doughy and pit on pressure. There is no redness and not sign of pain. If the edematous part is external, it is cool to the touch.

Edema of the brain and lungs is the most life-threatening form of abnormal fluid retention.

SHOCK

Shock is a clinical term which refers to peripheral circulatory failure with pooling of the blood in the terminal circulatory beds (**small capillaries**). The fundamental disturbance is that blood volume is too small to fill the vascular system, resulting in a fall of blood pressure and cell damage due to anoxia.

NOTE:If all capillary beds in the body were to open up, there would not be enough blood to fill the major vessels and/or heart; thus, blood pressure falls and the flow of blood is decreased. Also, the loss of massive amounts of blood via hemorrhage, etc., would have a similar effect. Subsequently, oxygen and nutrient delivery to cells and removal of waste products are decreased. Therefore, depending on the severity and duration of the shock state, the cells of many vital organs suffer injury and even death.

The clinical signs of shock are inconsistent and vary with the precipitating cause. However, individuals with shock are usually inactive and unresponsive to external stimuli. Muscle weakness is prominent and there is pallor and coolness of the skin. Body temperature is subnormal and the heart rate is increased in most types of shock **(but it may be slow and irregular)**. Depression of renal function and urine production often occur.

The causes of shock may be classified as hypovolemic, septic, cardiogenic and neurogenic.

HYPOVOLEMIC SHOCK:

Is due to loss of blood volume (**hemorrhage, trauma, loss of fluids in burns, etc.)** which directly induces inadequate perfusion of organs and tissues.

Remember, extensive blood loss is required before individuals develop hypovolemic shock. The following sequence of changes is associated with hypovolemic shock (as well as other forms).

Severe blood loss occurs. The arterial blood pressure drops and venous return to the heart decreases. The heart rate may increase but stroke volume and cardiac output are decreased. Arterial vasoconstriction occurs rapidly with the drop in blood pressure and increased peripheral resistance is produced which shunts blood from the skin and viscera to the heart and brain. In the kidneys, vasoconstriction reduces perfusion and causes activation of the juxtaglomerular apparatus with the release of the enzyme renin into the plasma. Renin acts on an unidentified plasma protein substrate converting it to a polypeptide angiotensin I. Angiotensin I is converted to the potent vasoactive polypeptide angiotensin II by another converting enzyme. Also, the pituitary gland is stimulated to release the antidiuretic hormone **(vasopressin**) which acts to conserve water normally lost from the lower nephrons. Aldosterone secretion by the adrenal cortex is augmented which leads to increased resorption of salt and water by the renal tubules. All of the above mechanisms conserve fluid and support blood volume.

Remember, progressive deterioration of the circulatory system may occur despite the above compensatory mechanisms. The term "irreversible shock" implies the refractory state of circulatory failure with inability to clinically control the condition.

SEPTIC SHOCK:

Implies septicemia or an overwhelming infection with gram-negative **(endotoxic shock**) or gram-positive (**exotoxic shock)** organisms. In toxic and septicemic conditions, there is oftentimes peripheral dilatation of the capillary beds which subsequently lead to shock. When capillary beds are fully dilated **(vasodilation**), they have the capacity to accommodate nearly the total blood volume. If this occurred,

blood pressure would drop to zero (**normally, continual vasoconstriction of the terminal arterioles prevents this from happening).**

CARDIOGENIC (CARDIAC) SHOCK:

Can be viewed as **"pump failure.**" It occurs subsequent to the sudden decrease in cardiac output which accompanies sudden extensive damage to the heart. However, most individuals succumb directly to the myocardial failure. In those that do not, shock may ensue because of the pooling of the blood.

NEUROGENIC SHOCK:

Implies a shock state mediated by the nervous system which induces peripheral dilatation **(dilatation of the capillary bed)**. It occurs in individual with severe fright, pain and trauma (**without hemorrhage).**

The manifestations of shock involve many vital organs (**depending on the severity and duration of the shock state**). The sequences of changes at the cellular and subcellular levels are those described for hypoxic injury. In general, the brain and heart are highly susceptible to hypoxia generated by the shock state.

Acute Inflammation

Acute inflammation is a rapid response to an injurious agent that serves to deliver mediators of host defense—leukocytes and plasma proteins—to the site of injury. Acute inflammation has three major components: (1) *alterations in vascular caliber that lead to an increase in blood flow*; (2) *structural changes in the microvasculature that permit plasma proteins and leukocytes to leave the circulation*; and (3) *emigration of the leukocytes* from the microcirculation, *their accumulation* in the focus of injury, *and their activation* to eliminate the offending agent

STIMULI FOR ACUTE INFLAMMATION

Acute inflammatory reactions are triggered by a variety of stimuli:

- Infections (bacterial, viral, parasitic) and microbial toxins
- Trauma (blunt and penetrating)
- Physical and chemical agents (thermal injury, e.g., burns or frostbite; irradiation; some environmental chemicals)
- Tissue necrosis (from any cause)
- Foreign bodies (splinters, dirt, sutures)
- Immune reactions (also called hypersensitivity reactions)

Each of these stimuli may induce reactions with some distinctive features, but all inflammatory reactions share the same basic features. We first describe the characteristic reactions of acute inflammation, and then the chemical mediators responsible for these reactions.

VASCULAR CHANGES

Since the two major mechanisms of host defense against microbes—antibodies and leukocytes—are normally carried in the bloodstream, it is not surprising that vascular phenomena play a major role in acute inflammation. Normally, plasma proteins and circulating cells are sequestered inside the vessels and move in the direction of flow. In inflammation, blood vessels undergo a series of changes that are designed to maximize the movement of plasma proteins and circulating cells out of the circulation and into the site of injury or infection.

Changes in Vascular Flow and Caliber

Changes in vascular flow and caliber begin early after injury and develop at varying rates depending on the severity of the injury. The changes occur in the following order:

- *Vasodilation* is one of the earliest manifestations of acute inflammation; sometimes, it follows a transient constriction of arterioles, lasting a few seconds. Vasodilation first involves the arterioles and then results in opening of new capillary beds in the area. Thus comes about *increased blood flow*, which is the cause of the heat and the redness. *Vasodilation is induced by the action of several mediators, notably histamine and nitric oxide, on vascular smooth muscle*; these mediators are described later in the chapter.

- Vasodilation is quickly followed by *increased permeability of the microvasculature*, with the outpouring of protein-rich fluid into the extravascular tissues; this process is described in detail below.

- The loss of fluid results in concentration of red cells in small vessels and increased viscosity of the blood, reflected by the presence of dilated small vessels packed with red cells and slower blood flow, a condition termed *stasis*. With mild stimuli, stasis may not become apparent until 15 to 30 minutes have elapsed, whereas with severe injury, stasis may occur in a few minutes.

- As stasis develops, leukocytes, principally neutrophils, accumulate along the vascular endothelium. Leukocytes then stick to the endothelium, and soon afterward they migrate through the vascular wall into the interstitial tissue, in processes that are described later.

Increased Vascular Permeability (Vascular Leakage)

A hallmark of acute inflammation is increased vascular permeability leading to the escape of a protein-rich fluid (exudate) into the extravascular tissue. The loss of protein from the plasma reduces the intravascular osmotic pressure and increases the osmotic pressure of the interstitial fluid. Together with the increased hydrostatic pressure owing to increased blood flow through the dilated vessels, this leads to

a marked *outflow* of fluid and its accumulation in the interstitial tissue. The net increase of extravascular fluid results in *edema*.

Normal fluid exchange and microvascular permeability are critically dependent on an intact endothelium. How then does the endothelium become leaky in inflammation? The following mechanisms have been proposed :

- *Formation of endothelial gaps in venules.* This is the most common mechanism of vascular leakage and is elicited by histamine, bradykinin, leukotrienes, the neuropeptide substance P, and many other classes of chemical mediators. It occurs rapidly after exposure to the mediator and is usually reversible and short-lived (15 to 30 minutes); it is thus known as the *immediate transient response*. *Classically, this type of leakage affects venules 20 to 60 µm in diameter*, leaving capillaries and arterioles unaffected. The precise reason for this restriction to venules is uncertain; it may be because there is a greater density of receptors for the mediators in venular endothelium. Parenthetically, many of the later leukocyte events in inflammation—adhesion and emigration—also occur predominantly in the venules in most organs. Binding of mediators, such as histamine, to their receptors on endothelial cells activates intracellular signaling pathways that lead to phosphorylation of contractile and cytoskeletal proteins, such as myosin. These proteins contract, leading to *contraction of the endothelial cells* and separation of intercellular junctions. Thus, the gaps in the venular endothelium are largely intercellular or close to the intercellular junctions. Cytokines such as interleukin-1 (IL-1), tumor necrosis factor (TNF), and interferon-γ (IFN-γ) also increase vascular permeability by inducing a structural reorganization of the cytoskeleton, such that the endothelial cells retract from one another. In contrast to the histamine effect, the cytokine-induced response is somewhat delayed (4 to 6 hours) and long-lived (24 hours or more).

- *Direct endothelial injury, resulting in endothelial cell necrosis and detachment.* This effect is usually encountered in necrotizing injuries and is due to direct damage to the endothelium by the injurious stimulus, as, for example, in severe burns or lytic bacterial infections. Neutrophils that adhere to the endothelium (discussed below) may also injure the endothelial cells. In most instances, leakage starts immediately after injury and is sustained at a high level for several hours until the damaged vessels are thrombosed or repaired. The reaction is known as the *immediate sustained response. All levels of the microcirculation are affected, including venules, capillaries, and arterioles.* Endothelial cell detachment is often associated with platelet adhesion and thrombosis.

- *Delayed prolonged leakage.* This is a curious but relatively common type of increased permeability that *begins after a delay of 2 to 12 hours, lasts for several hours or even days, and involves venules as well as capillaries*. Such leakage is caused, for example, by mild to moderate thermal injury, x-radiation or ultraviolet radiation, and certain bacterial toxins. Late-appearing sunburn is a good example of a delayed reaction. The mechanism of such leakage is unclear. It may result from the direct effect of the injurious agent, leading to delayed endothelial cell damage (perhaps by apoptosis), or the effect of cytokines causing endothelial retraction, as described earlier.

- *Leukocyte-mediated endothelial injury.* Leukocytes adhere to endothelium relatively early in inflammation. As discussed later, such leukocytes may be activated in the process, releasing toxic oxygen species and proteolytic enzymes, which then cause endothelial injury or detachment, resulting in increased permeability. In acute inflammation, this form of injury is largely restricted to vascular sites, such as venules and pulmonary and glomerular capillaries, where leukocytes

adhere for prolonged periods to the endothelium.

- *Increased transcytosis* across the endothelial cytoplasm. Transcytosis occurs across channels consisting of clusters of interconnected, uncoated vesicles and vacuoles called the *vesiculovacuolar organelle*, many of which are located close to intercellular junctions. Certain factors, for example, vascular endothelial growth factor (VEGF), appear to cause vascular leakage by increasing the number and perhaps the size of these channels. It has been claimed that this is also a mechanism of increased permeability induced by histamine and most chemical mediators.

In summary, *in acute inflammation, fluid loss from vessels with increased permeability occurs in distinct phases*: (1) an immediate transient response lasting for 30 minutes or less, mediated mainly by the actions of histamine and leukotrienes on endothelium; (2) a delayed response starting at about 2 hours and lasting for about 8 hours, mediated by kinins, complement products, and other factors; and (3) a prolonged response that is most noticeable after direct endothelial injury, for example, after burns.

CELLULAR EVENTS: LEUKOCYTE EXTRAVASATION AND PHAGOCYTOSIS

A critical function of inflammation is to deliver leukocytes to the site of injury and to activate the leukocytes to perform their normal functions in host defense. Leukocytes ingest offending agents, kill bacteria and other microbes, and get rid of necrotic tissue and foreign substances. A price that is paid for the defensive potency of leukocytes is that they may induce tissue damage and prolong inflammation, since the leukocyte products that destroy microbes and necrotic tissues can also injure normal host tissues.

The sequence of events in the journey of leukocytes from the vessel lumen to the interstitial tissue, called extravasation, can be divided into the following steps[16](Fig. 2-6):

1. In the lumen: margination, rolling, and adhesion to endothelium. Vascular endothelium normally does not bind circulating cells or impede their passage. In inflammation, the endothelium has to be activated to permit it to bind leukocytes, as a prelude to their exit from the blood vessels.

2. Transmigration across the endothelium (also called diapedesis)

3. Migration in interstitial tissues toward a chemotactic stimulus

The recruitment of leukocytes to sites of injury and infection is a multistep process involving attachment of circulating leukocytes to endothelial cells and their migration through the endothelium. The first events are the induction of adhesion molecules on endothelial cells, by a number of mechanisms

The next step in the process is migration of the leukocytes through the endothelium, called transmigration or *diapedesis*. Chemokines act on the adherent leukocytes and stimulate the cells to migrate through interendothelial spaces toward the chemical concentration gradient, that is, toward the site of injury or infection. Certain homophilic adhesion molecules (i.e., adhesion molecules that bind to each other) present in the intercellular junction of endothelium are involved in the migration of leukocytes. One of these molecules is a member of the immunoglobulin superfamily called PECAM-1 (platelet endothelial cell adhesion molecule) or CD31. *Leukocyte diapedesis, similar to increased vascular permeability, occurs predominantly in the venules* (except in the lungs, where it also occurs in capillaries). After

traversing the endothelium, leukocytes are transiently retarded in their journey by the continuous basement membrane of the venules, but eventually the cells pierce the basement membrane, probably by secreting collagenases. The net result of this process is that leukocytes rapidly accumulate where they are needed.

Outcomes of Acute Inflammation

The discussion of mediators completes the description of the basic, relatively uniform pattern of the inflammatory reaction encountered in most injuries. Although hemodynamic, permeability, and leukocyte changes have been described sequentially and may be initiated in this order, all these phenomena may be concurrent in the fully evolved reaction to injury. As might be expected, many variables may modify this basic process, including the nature and intensity of the injury, the site and tissue affected, and the responsiveness of the host. In general, however, *acute inflammation may have one of three outcomes* :

1. *Complete resolution.* In a perfect world, all inflammatory reactions, once they have succeeded in neutralizing and eliminating the injurious stimulus, should end with restoration of the site of acute inflammation to normal. This is called *resolution* and is the usual outcome when the injury is limited or short-lived or when there has been little tissue destruction and the damaged parenchymal cells can regenerate. Resolution involves neutralization or spontaneous decay of the chemical mediators, with subsequent return of normal vascular permeability, cessation of leukocytic infiltration, death (largely by apoptosis) of neutrophils, and finally removal of edema fluid and protein, leukocytes, foreign agents, and necrotic debris from the site.

2. *Healing by connective tissue replacement (fibrosis).* This occurs after substantial tissue destruction, when the inflammatory injury involves tissues that are incapable of regeneration, or when there is abundant fibrin exudation. When the fibrinous exudate in tissue or serous cavities (pleura, peritoneum) cannot be adequately cleared, connective tissue grows into the area of exudate, converting it into a mass of fibrous tissue—a process also called *organization*. In many pyogenic infections there may be intense neutrophil infiltration and liquefaction of tissues, leading to pus formation. The destroyed tissue is resorbed and eventually replaced by fibrosis.

3. Progression of the tissue response to *chronic inflammation* (discussed below). This may follow acute inflammation, or the response may be chronic almost from the onset. Acute to chronic transition occurs when the acute inflammatory response cannot be resolved, owing either to the persistence of the injurious agent or to some interference with the normal process of healing. For example, bacterial infection of the lung may begin as a focus of acute inflammation (pneumonia), but its failure to resolve may lead to extensive tissue destruction and formation of a cavity in which the inflammation continues to smolder, leading eventually to a chronic lung abscess. Another example of chronic inflammation with a persisting stimulus is peptic ulcer of the duodenum or stomach. Peptic ulcers may persist for months or years and, as discussed below, are manifested by both acute and chronic inflammatory reactions.

c. Some of these activated cells so formed enter into the circulation and remain in the memory pool of T cells for long period of time.
d. An intracutanous injection of the tuberculin for example to a person previously exposed individual to the tubercle bacilli , the memory TH1 cells interact with the antigen on the surface of APC and are activated with formation of granulomatous reactions

2. **T-cell mediated cytotoxicity**

In this variant of type IV reaction, sensitized CD8+T cells kill antigen-bearing cells. Such effector cells are called cytotoxic T lymphocytes (CTLs). CTLs are directed against cell surface of MHC type l antigens and it plays an important role in graft rejection and in resistance to viral infections. It is believed that many tumour-associated antigens are effected by CTLs. Two mechanisms by which CTLs cause T cell damage are:

 Preforin-Granzyme dependant killing where perforin drill a hole into the cell membrane with resultant osmotic lysis and granzyme activates apoptosis of the target cells

 FAS-FAS ligand dependant killing which induce apoptosis of the target cells

Amyloidosis

COMPOSITION OF AMYLOID

Amyloidosis is a group of diseases characterized by the deposition of an extracellular protein that has specific properties.

• Individual molecular subunits form β-pleated sheets. Amorphous eosinophilic extracellular deposits of amyloid are seen on the H&E stain. These deposits stain red with the Congo red stain, and apple green birefringence of the amyloid is seen on the Congo red stain under polarized light.
• The fibrillary protein of amyloid varies with each disease. Also present in amyloid are serum amyloid P (SAP) and glycosaminoglycans (heparan sulfate).

SYSTEMIC TYPES OF AMYLOID

Primary amyloidosis has amyloid light chain (AL) amyloid, whose fibrillary protein is made of kappa or lambda light chains. Primary amyloidosis may be seen in plasma cell disorders (multiple myeloma, B-cell lymphomas, etc.) but most cases occur independent of other diseases.

Reactive systemic amyloidosis (secondary amyloidosis) has amyloid-associated (AA) protein, whose precursor is serum amyloid A (SAA), an acute phase reactant produced by the liver which is elevated with ongoing chronic inflammation and neoplasia. Reactive systemic amyloidosis can be seen with a wide variety of chronic diseases, including rheumatoid arthritis, systemic lupus erythematosus, tuberculosis, bronchiectasis, osteomyelitis, inflammatory bowel disease, and cancer.

Familial Mediterranean fever has AA type amyloid with fibrillary protein composed of serum amyloid A (SAA). This autosomal recessive disease is characterized by recurrent inflammation, fever, and neutrophil dysfunction. Gain of function mutations of *pyrin* are present.

Hemodialysis-associated amyloidosis has Aβ2M type amyloid with precursor protein β2-microglobulin. This form of amyloidosis may cause carpal tunnel syndrome and joint disease.

LOCALIZED TYPES OF AMYLOID

Senile cerebral amyloidosis (Alzheimer disease) has Aβ type amyloid with fibrillary protein composed of β-amyloid precursor protein (βAPP). It is found in Alzheimer

plaques and in cerebral vessels. The gene for βAPP is located on chromosome 21.
Senile cardiac/systemic amyloidosis has ATTR type amyloid with fibrillary protein composed of transthyretin. This type of amyloidosis is seen in men older than 70 years and may cause heart failure as a result of restrictive/infiltrative cardiomyopathy. Four percent of African Americans have a transthyretin (TTR) V1221 mutation with 1% being homozygous, serving as a risk for cardiac disease.
Endocrine type amyloidosis is seen in medullary carcinoma of the thyroid (procalcitonin), adult-onset diabetes (amylin), and pancreatic islet cell tumors (amylin).
CLINICAL FEATURES
In **systemic forms** of amyloidosis, the kidney is the most commonly involved organ, and patients may experience nephrotic syndrome and/or progressive renal failure. Cardiac involvement may cause restrictive cardiomyopathy and conduction disturbances. Other clinical features include hepatosplenomegaly and involvement of the gastrointestinal tract, which may produce tongue enlargement (macroglossia, primarily in AL type) and malabsorption.
Diagnosis in systemic forms of amyloidosis can be established with biopsy of the rectal mucosa, gingiva, or the abdominal fat pad; Congo red stain shows apple green birefringence under polarized light of amyloid deposits. The prognosis of systemic amyloidosis is poor. AL amyloidosis is diagnosed by serum and urinary protein electrophoresis and immunoelectrophoresis. Proteomic analysis is another diagnostic tool.
TTR = transporter of thyroxine and retinol

Neoplasia Definitions

Neoplasia literally means the process of "new growth," and a new growth is called a *neoplasm*. The term *tumor* was originally applied to the swelling caused by inflammation. Neoplasms also may induce swellings, but by long precedent, the non-neoplastic usage of *tumor* has passed into limbo; thus, the term is now equated with neoplasm. *Oncology* (Greek *oncos* = tumor) is the study of tumors or neoplasms. *Cancer is the common term for all malignant tumors.* Although the ancient origins of this term are somewhat uncertain, it probably derives from the Latin for crab, *cancer*—presumably because a cancer "adheres to any part that it seizes upon in an obstinate manner like the crab."

Nomenclature of Tumors

Tissue of Origin	Benign	Malignant
Composed of One Parenchymal Cell Type		
Tumors of mesenchymal origin		
Connective tissue and derivatives	Fibroma	Fibrosarcoma
	Lipoma	Liposarcoma
	Chondroma	Chondrosarcoma
	Osteoma	Osteogenic sarcoma
Endothelial and related tissues		
Blood vessels	Hemangioma	Angiosarcoma
Lymph vessels	Lymphangioma	Lymphangiosarcoma
Synovium		Synovial sarcoma
Mesothelium		Mesothelioma
Brain coverings	Meningioma	Invasive meningioma
Blood cells and related cells		
Hematopoietic cells		Leukemias
Lymphoid tissue		Lymphomas
Muscle		
Smooth	Leiomyoma	Leiomyosarcoma
Striated	Rhabdomyoma	Rhabdomyosarcoma
Tumors of epithelial origin		
Stratified squamous	Squamous cell papilloma	Squamous cell or epidermoid carcinoma
Basal cells of skin or adnexa		Basal cell carcinoma
Epithelial lining of glands or ducts	Adenoma	Adenocarcinoma
	Papilloma	Papillary carcinomas
	Cystadenoma	Cystadenocarcinoma
Respiratory passages	Bronchial adenoma	Bronchogenic carcinoma
Renal epithelium	Renal tubular adenoma	Renal cell carcinoma
Liver cells	Liver cell adenoma	Hepatocellular carcinoma
Urinary tract epithelium (transitional)	Transitional cell papilloma	Transitional cell carcinoma
Placental epithelium	Hydatidiform mole	Choriocarcinoma
Testicular epithelium (germ cells)		Seminoma

Tissue of Origin	Benign	Malignant
		Embryonal carcinoma
Tumors of melanocytes	Nevus	Malignant melanoma
More Than One Neoplastic Cell Type—Mixed Tumors, Usually Derived from One Germ Cell Layer		
Salivary glands	Pleomorphic adenoma (mixed tumor of salivary origin)	Malignant mixed tumor of salivary gland origin
Renal anlage		Wilms tumor
More Than One Neoplastic Cell Type Derived from More Than One Germ Cell Layer—Teratogenous		
Totipotential cells in gonads or in embryonic rests	Mature teratoma, dermoid cyst	Immature teratoma, teratocarcinoma

The nomenclature of tumors is important because specific designations have specific clinical implications, even among tumors arising from the same tissue. *Seminoma* is a form of testicular carcinoma that tends to spread to lymph nodes along the iliac arteries and aorta. Further, these tumors are extremely radiosensitive and can be eradicated by radiotherapy; thus, few patients with seminomas die of the neoplasm. By contrast, the embryonal carcinoma of the testis is not radiosensitive and tends to invade locally beyond the confines of the testis and spread throughout the body. There also are other varieties of testicular neoplasms, and so the designation *cancer of the testis* tells little of its clinical significance.

Comparisons Between Benign and Malignant Tumors

Characteristics	Benign	Malignant
Differentiation/anaplasia	Well differentiated; structure may be typical of tissue of origin	Some lack of differentiation with anaplasia; structure is often atypical
Rate of growth	Usually progressive and slow; may come to a standstill or regress; mitotic figures are rare and normal	Erratic and may be slow to rapid; mitotic figures may be numerous and abnormal
Local invasion	Usually cohesive and expansile well-demarcated masses that do not invade or infiltrate surrounding normal tissues	Locally invasive, infiltrating the surrounding normal tissues; sometimes may be seemingly cohesive and expansile
Metastasis	Absent	Frequently present; the larger and more undifferentiated the primary, the more likely are metastases

Biology of Tumor Growth: Benign and Malignant Neoplasms

The natural history of most malignant tumors can be divided into four phases: (1) malignant change in the target cell, referred to as transformation; (2) growth of the transformed cells; (3) local invasion; and (4) distant metastases.

DIFFERENTIATION AND ANAPLASIA

Differentiation refers to the extent to which neoplastic cells resemble comparable normal cells, both morphologically and functionally; lack of differentiation is called anaplasia. Well-differentiated tumors are composed of cells resembling the mature normal cells of the tissue of origin of the neoplasm. Poorly differentiated or undifferentiated tumors have primitive-appearing, unspecialized cells. In general, benign tumors are well differentiated. The neoplastic cell in a benign smooth muscle tumor—a leiomyoma—so closely resembles the normal cell that it may be impossible to recognize it as a tumor by microscopic examination of individual cells. Only the massing of these cells into a nodule discloses the neoplastic nature of the lesion. One may get so close to the tree that one loses sight of the forest.

Lack of differentiation, or anaplasia, is marked by a number of morphologic changes.

- *Pleomorphism.* Both the cells and the nuclei characteristically display *pleomorphism*—variation in size and shape. Cells may be found that are many times larger than their neighbors, and other cells may be extremely small and primitive appearing.

- *Abnormal nuclear morphology.* Characteristically the nuclei contain an abundance of DNA and are extremely dark staining *(hyperchromatic)*. The nuclei are disproportionately large for the cell, and the nucleus-to-cytoplasm ratio may approach 1:1 instead of the normal 1:4 or 1:6. The nuclear shape is very variable, and the chromatin is often coarsely clumped and distributed along the nuclear membrane. Large nucleoli are usually present in these nuclei.

- *Mitoses.* As compared with benign tumors and some well-differentiated malignant neoplasms, undifferentiated tumors usually possess large numbers of mitoses, reflecting the higher proliferative activity of the parenchymal cells. *The presence of mitoses, however, does not necessarily indicate that a tumor is malignant or that the tissue is neoplastic.* Many normal tissues exhibiting rapid turnover, such as bone marrow, have numerous mitoses, and non-neoplastic proliferations such as hyperplasias contain many cells in mitosis. More important as a morphologic feature of malignant neoplasia are atypical, bizarre mitotic figures, sometimes producing tripolar, quadripolar, or multipolar spindles .

- *Loss of polarity.* In addition to the cytologic abnormalities, the *orientation of anaplastic cells is markedly disturbed (i.e., they lose normal polarity)*. Sheets or large masses of tumor cells grow in an anarchic, disorganized fashion.

- *Other changes.* Another feature of anaplasia is the formation of *tumor giant cells,* some possessing only a single huge polymorphic nucleus and others having two or more nuclei. These giant cells are not to be confused with inflammatory Langhans or foreign body giant cells, which are derived from macrophages and contain many small, normal-appearing nuclei. In the cancer giant cell, the nuclei are hyperchromatic and large in relation to the cell. Although growing tumor cells obviously require a blood supply, often the vascular stroma is scant, and in many anaplastic tumors, large central areas undergo ischemic *necrosis*.

RATES OF GROWTH

A fundamental issue in tumor biology is to understand the factors that influence the growth rates of tumors and the role of these factors in clinical outcome and therapeutic responses. One can begin the consideration of tumor cell kinetics by asking the question: How long does it take to produce a clinically overt tumor mass? It can be readily calculated that the original transformed cell (approximately 10 μm in diameter) must undergo at least 30 population doublings to produce 10^9 cells (weighing approximately 1 gm), which is the smallest clinically detectable mass. In contrast, only 10 further doubling cycles are required to produce a tumor containing 10^{12} cells (weighing approximately 1 kg), which is usually the maximal size compatible with life. These are minimal estimates, based on the assumption that all descendants of the transformed cell retain the ability to divide and that there is no loss of cells from the replicative pool. This concept of tumor as a "pathologic dynamo" is not entirely correct, as we discuss subsequently. Nevertheless, this calculation highlights an extremely important concept about tumor growth: *By the time a solid tumor is clinically detected, it has already completed a major portion of its life cycle.* This is a major impediment in the treatment of cancer, and underscores the need to develop diagnostic markers to detect early cancers.

LOCAL INVASION

Nearly all benign tumors grow as cohesive expansile masses that remain localized to their site of origin and do not have the capacity to infiltrate, invade, or metastasize to distant sites, as do malignant tumors. Because they grow and expand slowly, they usually develop a rim of compressed connective tissue, sometimes called a fibrous *capsule,* which separates them from the host tissue. This capsule is derived largely from the stroma of the native tissue as the parenchymal cells atrophy under the pressure of expanding tumor. Such encapsulation does not prevent tumor growth, but it keeps the benign neoplasm as a discrete, readily palpable, and easily movable mass that can be surgically enucleated. Although a well-defined cleavage plane exists around most benign tumors, in some it is lacking. Thus, hemangiomas (neoplasms composed of tangled blood vessels) are often unencapsulated and may appear to permeate the site in which they arise (commonly the dermis of the skin).

The growth of cancers is accompanied by progressive infiltration, invasion, and destruction of the surrounding tissue. In general, malignant tumors are poorly demarcated from the surrounding normal tissue, and a well-defined cleavage plane is lacking. Slowly expanding malignant tumors, however, may develop an apparently enclosing fibrous capsule and may push along a broad front into adjacent normal structures. Histologic examination of such apparently encapsulated masses almost always shows rows of cells penetrating the margin and infiltrating the adjacent structures, a crablike pattern of growth that constitutes the popular image of cancer.

METASTASIS

Metastases are tumor implants discontinuous with the primary tumor. *Metastasis unequivocally marks a tumor as malignant because benign neoplasms do not metastasize.* The invasiveness of cancers permits them to penetrate into blood vessels, lymphatics, and body cavities, providing the opportunity for spread. *With few exceptions, all cancers can metastasize.* The major exceptions are most malignant neoplasms of the glial cells in the central nervous system, called *gliomas,* and basal cell carcinomas of the skin. Both are locally invasive forms of neoplasia (the latter being known in the older literature as *rodent ulcers* because of their invasive destructiveness), but they rarely metastasize. It is evident then that the properties of invasion and metastasis are separable. At the molecular level, however, invasion and metastases represent a continuum of changes.

In general, the more aggressive, the more rapidly growing, and the larger the primary neoplasm, the greater the likelihood that it will metastasize or already has metastasized. There are innumerable exceptions, however. Small, well-differentiated, slowly growing lesions sometimes metastasize widely; conversely, some rapidly growing, large lesions remain localized for years. No judgment can be made

about the probability of metastasis from pathologic examination of the primary tumor. Many factors relating to both invader and host are involved.

Pathways of Spread

Dissemination of cancers may occur through one of three pathways: (1) direct seeding of body cavities or surfaces, (2) lymphatic spread, and (3) hematogenous spread

Protein-Energy Malnutrition

Severe PEM is a disastrous disease. It is far too common in third world countries, where up to 25% of children may be affected; in these countries, it is a major factor in the high death rates among children younger than age 5 years.

PEM refers to a *range of clinical syndromes* characterized by an inadequate dietary intake of protein and calories to meet the body's needs. From a functional standpoint, there are two protein compartments in the body: the *somatic protein compartment*, represented by the skeletal muscles; and the *visceral protein compartment*, represented by protein stores in the visceral organs, primarily the liver. These two compartments are regulated differently, and as we shall see, the somatic compartment is affected more severely in marasmus (calorie deficiency), while the visceral compartment is depleted more severely in kwashiorkor (protein deficiency). Before the clinical presentations of the two polar forms of severe malnutrition (marasmus and kwashiorkor) are discussed, some comments are made on the clinical assessment of undernutrition and some of its general metabolic characteristics.

The diagnosis of PEM is obvious in its most severe forms. In mild to moderate forms, the usual approach is to compare the body weight for a given height with standard tables; other parameters are also helpful, including evaluation of fat stores, muscle mass, and serum proteins. With a loss of fat, the major storage form of energy, the thickness of skin folds (which includes skin and subcutaneous tissue) is reduced. If the somatic protein compartment is catabolized, the resultant reduction in muscle mass is reflected by reduced circumference of the midarm. Measurement of serum proteins (albumin, transferrin, and others) provides a measure of the adequacy of the visceral protein compartment. The most common victims of PEM worldwide are children. A child whose weight falls to less than 80% of normal is considered malnourished.

Malnutrition can present in many forms. Marasmus and kwashiorkor are two ends of a specimen and considerable overlap exists. *Marasmus* refers to malnutrition caused primarily by severe reduction in caloric intake. It results in greater than 60% reduction in body weight adjusted for height and sex. A child with marasmus suffers growth retardation and loss of muscle. The loss of muscle mass results from catabolism and depletion of the somatic protein compartment. This seems to be an adaptational response that serves to provide the body with amino acids as a source of energy. Interestingly, the visceral protein compartment, which is presumably more precious and critical for survival, is depleted only marginally, and hence *serum albumin levels are either normal or only slightly reduced*. In addition to muscle proteins, subcutaneous fat is also mobilized and used as a fuel. With such losses of muscle and subcutaneous fat, the *extremities are emaciated*; by comparison, the head appears too large for the body. Anemia and manifestations of multivitamin deficiencies are present, and there is evidence of *immune deficiency*, particularly of T cell-mediated immunity. Hence, concurrent infections are usually present, and they impose an additional stress on an already weakened body.

Kwashiorkor, in contast to marasmus, occurs when protein deprivation is relatively greater than the reduction in total calories. This is the most common form seen in African children who have been weaned (often too early, owing to the arrival of another child) and are subsequently fed an exclusively carbohydrate diet. The prevalence of kwashiorkor is also high in impoverished countries of Southeast Asia. Less severe forms may occur worldwide in persons with chronic diarrheal states in which protein is not absorbed or in those with conditions in which chronic protein loss occurs (e.g., protein-losing enteropathies, the nephrotic syndrome, or after extensive burns).

Kwashiorkor is a more severe form of malnutrition than marasmus. Unlike marasmus, marked protein deprivation is associated with severe loss of the visceral protein compartment, and the resultant hypoalbuminemia gives rise to generalized, or dependent, edema. The weight of children with severe kwashiorkor is typically 60% to 80% of normal. However, the true loss of weight is masked by the increased fluid retention (edema). In further contrast to marasmus, there is relative sparing of subcutaneous fat and muscle mass. The modest loss of these compartments may also be masked by edema. Children with kwashiorkor have characteristic skin lesions, with alternating zones of hyperpigmentation, areas of desquamation, and hypopigmentation, giving a "flaky paint" appearance. Hair changes include overall loss of color or alternating bands of pale and darker hair, straightening, line texture, and loss of firm attachment to the scalp. Other features that differentiate kwashiorkor from marasmus include an enlarged, *fatty liver* (resulting from reduced synthesis of carrier proteins) and a tendency to develop early apathy, listlessness, and loss of appetite. As in marasmus, other vitamin deficiencies are likely to be present, as are *defects in immunity* and *secondary infections*. The latter add to the catabolic state, thus setting up a vicious circle.

Secondary PEM is not uncommon in chronically ill or hospitalized patients within the United States. Both marasmus-like and kwashiorkor-like syndromes (with intermediate forms) may develop. Table 9-21 summarizes the secondary forms of these two syndromes.

Table 9-21 -- Comparison of Severe Marasmus-Like and Kwashiorkor-Like Secondary Protein-Energy Malnutrition

Syndrome	Clinical Setting	Time Course	Clinical Features	Laboratory Findings	Prognosis
Marasmus-like protein-energy malnutrition	Chronic illness (e.g., chronic lung disease, cancer)	Months	History of weight loss Muscle wasting Absent subcutaneous fat	Normal or mildly reduced serum proteins	Variable; depends on underlying disease
Kwashiorkor-like protein-energy malnutrition	Acute, catabolic illness (e.g., severe trauma, burns, sepsis)	Weeks	Normal fat and muscle Edema Easily pluckable hair	Serum albumin <2.8 gm/dL	Poor

Cytogenetics is a branch of genetics that is concerned with the study of the structure and function of the cell, especially the chromosomes[1]. It includes routine analysis of G-Banded chromosomes, other cytogenetic banding techniques, as well as molecular cytogenetics such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH).

cytogenetics: The study of the structure, function, and abnormalities of human chromosomes

Cytogenetics = the study of metaphase chromosomes**,** their abnormalities, and the physiological/phenotypic consequences of those abnormalities.
Karyotype = Microscopic visualization of the metaphase chromosomes from an Organism
karyotype: A photographic representation of the chromosomes of a single cell, cut and arranged in pairs based on their size and banding pattern according to a standard classification

Example: A human karyotype is made of 23 chromosome pairs arbitrarily arranged from largest (chromosome 1) to smallest (chromosome 22), and one pair of sex chromosomes (X and Y)*.
*If X were included in the size lineup it would fall between chromosome 7 and 8.
If Y were included it would be number 24, the smallest.
Chromosomes 1-22 are called **autosomes**
Chromosome X & Y are called **sex chromosomes**
Genetic abnormalities resulting from aberrant numbers of chromosomes
~15% of all recognized pregnancies are spontaneously aborted
~50% of those are due to major chromosome abnormalities
Aneuploid – unbalanced chromosome complement, gene dosage is upset, usually caused by non-disjunction during meiosis

aneuploidy: The occurrence of one or more extra or missing chromosomes leading to an unbalanced chromosome complement, or, any chromosome number that is not an exact multiple of the haploid number

Trisomy 1, 5, 3, exceedingly rare or never observed = probably results
6, 9, 19, 20 in a very early spontaneous abortion before implantation
Trisomy 2, 4, miscarried, usually during first trimester
7, 8, 10,
11, 12, 14,
15-17, 22

Trisomy 13, 18, 21 can go full term, the incidence of these increases with maternal age
13 – Patau syndrome
infants live on average 3 months
Thought to be deaf, polydactic, most organs have severe congenital defects which usually means defects originated as early as the 5th week of gestation
18 – Edward's syndrome
infants survive less than 4 months

skull is elongated, ears are malformed, webbed neck, severe organ defects
21 – Down's syndrome (1/800 live births)
characteristic "look" and facial expression partially due to reduced muscle tone, short stature, reduced mental capacity, can live into 50's
Triplo-X Syndrome (XXX) 1/1200 female births
Most are normal, fertile females.
XXXX & XXXXX have been observed. They are physically normal females but with severe mental deficiencies
Klinefelter's (XXY) 1/1000 males
Appearance is male, there are descriptions of female secondary sexual characteristics in Klinefelter's individuals but many are normal yet sterile males
XXXY and XXXXY have been described but they consistently have severe mental deficiencies
XYY syndrome (1/1000)
Initially described as very tall, aggressive and impulsive (?) but this is exaggerated. There is a slightly higher percentage of XYY men in prisons and mental facilities than in the general population.
Fertile – produce children with a normal number of sex chromosomes
XYYY & XYYYY have been observed
XXYY – similar to klinefelter's except taller
Monosomy Never observed among autosomes,
Only one human example…
Turners - XO - 9% of miscarriages
1/2000 female births, Often very short and appear prepubescent even in adulthood – sterile. No mental deficiency is associated with Turner's syndrome
Partial monosomy – A piece of one chromosome is lost. An individual will have one complete chromosome but its homologous partner is missing some genes.
Cri-du-chat "cry of the cat" syndrome.
A portion of the small arm of chromosome 5 is lost.
Multiple developmental abnormalities including malformation of the larynx and epiglottis.
Can live to adulthood – the severity of the syndrome depends upon the amount of chromosome 5 that was lost
Fragile X syndrome.A portion of X chromosome is lost.
Created from the improper folding and condensation of the X chromosome resulting in a region that can break during chromosome movements. Most common form of inherited mental impairment.
Euploid – extra genome copies, same relative gene dosage (aka **polyploidy**)
Polyploidy = multiple complete sets of chromosomes.
Monoploid = 1x
Diploid = 2x
Triploid = 3x
Tetraploid = 4x

Pentaploid = 5x
Among humans - Monoploid never observed
Triploid 1.2 % of pregnancies, 8.5% of miscarriages
Tetraploid 0.4 % of pregnancies, 3% of miscarriages

Main article: Chromosome abnormalities

Chromosome abnormalities can be numerical, as in the presence of extra or missing chromosomes, or structural, as in translocations, inversions, large-scale deletions or duplications. Numerical abnormalities, also known as aneuploidy, often occur as a result of nondisjunction during meiosis in the formation of a gamete; trisomies, in which three copies of a chromosome are present instead of the usual two, are common numerical abnormalities. Structural abnormalities often arise from errors in homologous recombination. Both types of abnormalities can occur in gametes and therefore will be present in all cells of an affected person's body, or they can occur during mitosis and give rise to a genetic mosaic individual who has some normal and some abnormal cells.

Chromosomal abnormalities that lead to disease in humans include:

- Turner syndrome results from a single X chromosome (45, X or 45, X0).
- Klinefelter syndrome, the most common male chromosomal disease, otherwise known as 47, XXY is caused by an extra **X** chromosome.
- Edwards syndrome is caused by trisomy (three copies) of chromosome 18.
- Down syndrome, a common chromosomal disease, is caused by trisomy of chromosome 21.
- Patau syndrome is caused by trisomy of chromosome 13.
- Also documented are trisomy 8, trisomy 9 and trisomy 16, although they generally do not survive to birth.

Some disorders arise from loss of just a piece of one chromosome, including

- Cri du chat (cry of the cat), from a truncated short arm on chromosome 5. The name comes from the babies' distinctive cry, caused by abnormal formation of the larynx.
- 1p36 Deletion syndrome, from the loss of part of the short arm of chromosome 1.
- Angelman syndrome – 50% of cases have a segment of the long arm of chromosome 15 missing.

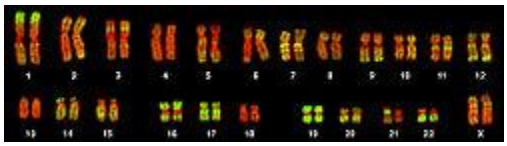
Chromosomal abnormalities can also occur in cancerous cells of an otherwise genetically normal individual; one well-documented example is the Philadelphia chromosome, a translocation mutation commonly associated with chronic myelogenous leukemia and less often with acute lymphoblastic leukemia.

Types of banding

Cytogenetics employs several techniques to visualize different aspects of chromosomes:[48]

- G-banding is obtained with Giemsa stain following digestion of chromosomes with trypsin. It yields a series of lightly and darkly stained bands - the dark regions tend to be heterochromatic, late-replicating and AT rich. The light regions tend to be euchromatic, early-replicating and GC rich. This method will normally produce 300-400 bands in a normal, human genome.
- R-banding is the reverse of G-banding (the R stands for "reverse"). The dark regions are euchromatic (guanine-cytosine rich regions) and the bright regions are heterochromatic (thymine-adenine rich regions).
- C-banding: Giemsa binds to constitutive heterochromatin, so it stains centromeres.
- Q-banding is a fluorescent pattern obtained using quinacrine for staining. The pattern of bands is very similar to that seen in G-banding.
- T-banding: visualize telomeres.

[edit] Classic karyotype cytogenetics

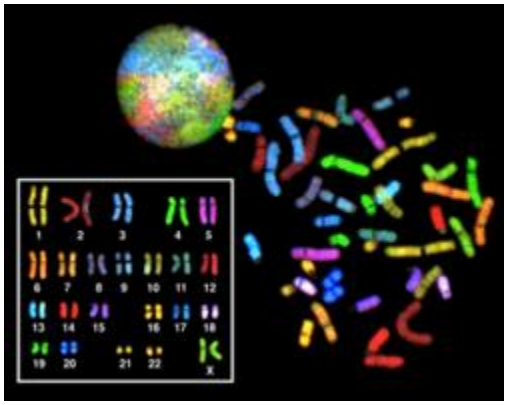


Karyogram from a human female lymphocyte probed for the Alu sequence using FISH.

In the "classic" (depicted) karyotype, a dye, often Giemsa *(G-banding)*, less frequently Quinacrine, is used to stain bands on the chromosomes. Giemsa is specific for the phosphate groups of DNA. Quinacrine binds to the adenine-thymine-rich regions. Each chromosome has a characteristic banding pattern that helps to identify them; both chromosomes in a pair will have the same banding pattern.

Karyotypes are arranged with the short arm of the chromosome on top, and the long arm on the bottom. Some karyotypes call the short and long arms *p* and *q*, respectively. In addition, the differently stained regions and sub-regions are given numerical designations from proximal to distal on the chromosome arms. For example, Cri du chat syndrome involves a deletion on the short arm of chromosome 5. It is written as 46,XX,5p-. The critical region for this syndrome is deletion of 15.2, which is written as 46,XX,del(5)(p15.2).[49]

[edit] Spectral karyotype (SKY technique)



Spectral karyogram of a human female

Spectral karyotyping is a molecular cytogenetic technique used to simultaneously visualize all the pairs of chromosomes in an organism in different colors. Fluorescently-labeled probes for each chromosome are made by labeling chromosome-specific DNA with different fluorophores. Because there are a limited number of spectrally-distinct fluorophores, a combinatorial labeling method is used to generate many different colors. Spectral differences generated by combinatorial labeling are captured and analyzed by using an interferometer attached to a fluorescence microscope. Image processing software then assigns a pseudo color to each spectrally different combination, allowing the visualization of the individually colored chromosomes.[50]

This technique is used to identify structural chromosome aberrations in cancer cells and other disease conditions when Giemsa banding or other techniques are not accurate enough.

[edit] Digital karyotyping

Digital karyotyping is a technique used to quantify the DNA copy number on a genomic scale. Short sequences of DNA from specific loci all over the genome are isolated and enumerated. This is method is also known as virtual karyotyping.

Fluorescent in situ hybridization refers to using fluorescently labeled probe to hybridize to cytogenetic cell preparations.

In addition to standard preparations FISH can also be performed on:

- bone marrow smears
- blood smears
- paraffin embedded tissue preparations
- enzymatically dissociated tissue samples
- uncultured bone marrow
- uncultured amniocytes
- cytospin preparations

[edit] Slide preparation

This section refers to preparation of standard cytogenetic preparations

The slide is aged using a salt solution usually consisting of 2X SSC (salt, sodium citrate). The slides are then dehydrated in ethanol, and the probe mixture is added. The sample DNA and the probe DNA are then co-denatured using a heated plate and allowed to re-anneal for at least 4 hours. The slides are then washed to remove excess unbound probe, and counterstained with 4',6-Diamidino-2-phenylindole (DAPI) or propidium iodide.

[edit] Analysis

Analysis of FISH specimens is done by fluorescence microscopy by a clinical laboratory specialist in cytogenetics (CLSp(CG)). For oncology generally a large number of interphase cells are scored in order to rule out low level residual disease, generally between 200 and 1000 cells are counted and scored. For congenital problems usually 20 metaphase cells are scored.

[edit] Future of cytogenetics

Advances now focus on molecular cytogenetics including automated systems for counting the results of standard FISH preparations and techniques for virtual karyotyping, such as comparative genomic hybridization arrays, CGH and Single nucleotide polymorphism-arrays.

1. **Sample Collection**

   The first step in performing a karyotype is collecting the “sample.” In newborns, a blood sample which containes red bloods cells, white blood cells, serum and other fluids is collected. A

karyotype will be done on the white blood cells which are actively dividing (a state known as mitosis). During pregnancy, the sample can either be amniotic fluid collected during an amniocentesis or a piece of the placenta collected during a chorionic villi sampling test (CVS). The amniotic fluid contains fetal skin cells which are used to generate a karyotype.

2. Transport to the Laboratory

A karyotype is a specialized test that is done in a specific laboratory called a cytogenetics lab. Not all hospitals have cytogenetics labs. If your hospital or medical facility doesn't have it's own cytogenetics laboratory (most don't), the test sample will be sent to a lab that specializes in karyotype analysis. The test sample is analyzed by specially trained cytogenetic technologists, Ph.D cytogeneticists, or medical geneticists. 'Cytogenetics' is a word for the study of chromosomes.

3. Separating the Cells

In order to analyze chromosomes, the sample must contain cells that are actively dividing (or in mitosis). In blood, the white blood cells are actively dividing cells. Most fetal cells are actively dividing. Once the sample reaches the cytogenetics lab, the non-divided cells are separated from the dividing cells using special chemicals.

4. Growing Cells

In order to have enough cells to analyze, the dividing cells are grown in special media or a cell culture. This media contains chemicals and hormones that enable the cells to divide and multiply. This process of "culturing" the cells can take 3 to 4 days for blood cells, and up to a week for fetal cells.

5. Synchronizing Cells

Chromosome are long string of human DNA. In order to see chromosomes under a microscope, chromosomes have to be in their most compact form. This compact form occurs at a specific stage of mitosis called metaphase. In order to get all the cells to this specific stage of cell division, the cells are treated with a chemical which stops cell division at the point where the chromosomes are the most compact.

6. Releasing the Chromosomes from their Cells

In order to see these compact chromosomes under a microscope, the chromosomes have to be out of the white blood cells. This is done by treating the white blood cells with a special solution that causes them to burst. This is done while the cells are on a microscopic slide. The leftover debris from the white blood cells is washed away, and the chromosomes are now fixed (or stuck) to the slide.

7. Staining the Chromosomes

Chromosomes are naturally colorless. In order to be able to tell one chromosome from another, a special dye called Giemsa dye is applied to the chromosomes on the slide. Giemsa dye stains regions of chromosomes that are rich in the bases adenine (A) and thymine (T). When stained, the chromosomes look like strings with light and dark bands. Each chromosome has a specific pattern of light and dark bands which enables cytogeneticist to tell one chromosome from another. Each dark or light band actually encompasses hundreds of different genes.

8. Analysis

Once chromosomes are stained, the slide is put under the microscope and the analysis of the chromosomes begins. A picture is taken of the chromosomes and at the end of the analysis, the total number of chromosomes will be known and there will be a picture of the chromosomes arranged by size.

9. Counting Chromosomes

The first step of the analysis is counting the chromosomes. Most humans have 46 chromosomes. People with Down syndrome have 47 chromosomes. It is also possible for people to have missing chromosomes or more than one extra chromosome. By looking at just the number of chromosomes, it is possible to diagnose different conditions including Down syndrome. However, cytogeneticist don't stop there.

10. Sorting Chromosomes

After determining the number of chromosomes present, the cytogeneticist will start sorting the chromosomes. To sort the chromosomes, the cytogeneticists will compare chromosome length, the placement of centromeres (the areas where the two chromatids are joined), and the location and sizes of G-bands. The chromosomes pairs are numbered from largest (number 1) to smallest (number 22). There are 22 pairs of chromosomes, called autosomes, which match up exactly. There are also the sex chromosomes, two X's is a female and an X and a Y is a male.

11. Looking at the Structure

In addition to looking at the total number of chromosomes and the sex chromosomes, the cytogeneticist will also look at the structure of the specific chromosomes to make sure that there is no missing or additional material, no structural abnormalities like translocations and a variety of other possible chromosome abnormalities.

12. The Final Result

In the end, the final karyotype test shows the total number of chromosomes, the sex of the person being studied, and if there are any structural abnormalities with any of the individual chromosomes. A digital picture of the chromosomes is generated with all of the chromosomes arranged by number.

When diagnosing Down syndrome, the focus tends to be on the total number of chromosomes, but in reality, a karyotype gives you information on the number, the structure and many other facets of an individual's chromosomes.

A **karyotype** is the characteristic chromosome complement of a eukaryote species.[1][2] The preparation and study of karyotypes is part of cytogenetics.

Karyogram of human male using Giemsa staining.

The basic number of chromosomes in the somatic cells of an individual or a species is called the *somatic number* and is designated *2n*. Thus, in humans 2n=46. In the germ-line (the sex cells) the chromosome number is *n* (humans: n=23).[1]

So, in normal diploid organisms, autosomal chromosomes are present in two copies. There may, or may not, be sex chromosomes. Polyploid cells have multiple copies of chromosomes and haploid cells have single copies. The study of whole sets of chromosomes is sometimes known as *karyology*. The chromosomes are depicted (by rearranging a microphotograph) in a standard format known as a *karyogram* or *idiogram*: in pairs, ordered by size and position of centromere for chromosomes of the same size.

Karyotypes can be used for many purposes; such as, to study chromosomal aberrations, cellular function, taxonomic relationships, and to gather information about past evolutionary events.

Karyotype Test

Karyotype is a test to identify and evaluate the size, shape, and number of chromosomes in a sample of body cells. Extra, missing, or abnormal positions of chromosome pieces can cause problems with a person's growth, development, and body functions.

Why It Is Done

Karyotype is done to:

- Determine whether the chromosomes of an adult have an abnormality that can be passed on to a child.
- Determine whether a chromosome defect is preventing a woman from becoming pregnant or causing miscarriages.
- Determine whether a chromosome defect is present in a fetus. Karyotyping also may be done to determine whether chromosomal problems may have caused a fetus to be stillborn.
- Determine the cause of a baby's birth defects or disability.
- Help determine the appropriate treatment for some types of cancer.
- Identify the sex of a person by determining the presence of the Y chromosome. This may be done when a newborn's sex is not clear.

Genetic Disease

What is a genetic disease?

A genetic disease or disorder is any disease that is caused by an abnormality in an individual's genome. The abnormality can range from minuscule to major -- from a discrete mutation in a single base in the DNA of a single gene to a gross chromosome abnormality involving the addition or subtraction of an entire chromosome or set of chromosomes.

What are the different types of inheritance?

There are a number of different types of genetic inheritance, including the following four modes:

1. **Single gene inheritance --** Also called Mendelian or monogenic inheritance. This type of inheritance is caused by changes or mutations that occur in the DNA sequence of a single gene. There are more than 6,000 known single-gene disorders, which occur in about 1 out of every 200 births.

   Some examples of single gene inheritance are cystic fibrosis, sickle cell anemia, Marfan syndrome, Huntington's disease, and hemochromatosis. Single-gene disorders are inherited in recognizable patterns: autosomal dominant, autosomal recessive, and X-linked.

2. **Multifactorial inheritance --** Also called complex or polygenic inheritance. This type of inheritance is caused by a combination of environmental factors and mutations in multiple genes. For example, different genes that influence breast cancer susceptibility have been found on chromosomes 6, 11, 13, 14, 15, 17, and 22. Some common chronic diseases are multifactorial disorders.

   Examples of multifactorial inheritance include heart disease, high blood pressure, Alzheimer's disease, arthritis, diabetes, cancer, and obesity. Multifactorial inheritance also is associated with heritable traits such as fingerprint patterns, height, eye color, and skin color.

3. **Chromosome abnormalities --** Chromosomes, distinct structures made up of DNA and protein, are located in the nucleus of each cell. Because chromosomes are the carriers of the genetic material, abnormalities in chromosome number or structure can result in disease.

   For example, Down syndrome or trisomy 21 is a common disorder that occurs when a person has three copies of chromosome 21. There are many other chromosome abnormalities including Turner syndrome (45,X), Klinefelter syndrome (47, XXY), the cat cry syndrome (46, XX or XY, 5p-), and so on.

4. **Mitochondrial inheritance --** This type of genetic disorder is caused by mutations in the nonchromosomal DNA of mitochondria. Mitochondria are small round or rod-like organelles that are involved in cellular respiration and found in the cytoplasm of plant and animal cells. Each mitochondrion may contain 5 to 10 circular pieces of DNA.

Examples of mitochondrial disease include an eye disease called Leber's hereditary optic atrophy; a type of epilepsy called MERRF which stands for Myoclonus Epilepsy with Ragged Red Fibers; and a form of dementia called MELAS for Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like episodes

A **chimera** is an animal that has two or more different populations of genetically distinct cells that originated in different zygotes; if the different cells emerged from the same zygote, it is called a mosaicism.

Tetragametic chimerism is a less common cause of congenital chimerism. It occurs through the fertilization of two ova by two sperm, followed by the fusion of the zygotes and the development of an

organism with intermingled cell lines. This happens at a very early stage of development, such as that of the blastocyst. Such an organism is called a tetragametic chimera as it is formed from four gametes — two eggs and two sperm. Put another way, the chimera is formed from the merger of two nonidentical twins in a very early (zygote or blastocyst) phase. As such, they can be male, female, or hermaphroditic.

As the organism develops, the resulting chimera can come to possess organs that have different sets of chromosomes. For example, the chimera may have a liver composed of cells with one set of chromosomes and have a kidney composed of cells with a second set of chromosomes. This has occurred in humans, and at one time was thought to be extremely rare, though more recent evidence suggests that it is not as rare as previously believed. Most will go through life without realizing they are chimeras. The difference in phenotypes may be subtle (*e.g.,* having a hitchhiker's thumb and a straight thumb, eyes of slightly different colors, differential hair growth on opposite sides of the body, etc) or completely undetectable . Another telltale of a person being a chimera is visible Blaschko's lines.

Affected persons are identified by the finding of two populations of red cells or, if the zygotes are of opposite sex, ambiguous genitalia and hermaphroditism alone or in combination; such persons sometimes also have patchy skin, hair, or eye pigmentation (heterochromia). If the blastocysts are of the same sex, it can only be detected through DNA testing, although this is a rare procedure. If the blastocysts are of opposite sex, genitals of both sexes are often formed, either ovary and testis, or combined ovotestes, in one rare form of intersexuality, a condition previously known as *true hermaphroditism*. As of 2003, there were about 30-40 documented human cases in the literature, according to *New Scientist*. Since hermaphroditic chimeras would be expected to be the one half of all chimeras, with purely male and purely female chimeras being one-quarter each, this would suggest that the condition is not particularly common.

Natural chimeras are almost never detected unless the offspring has abnormalities such as male/female or hermaphrodite characteristics or skin discolouring. The most noticeable are some male tortoiseshell cats or animals with ambiguous sex organs.

Chimerism can be detected in DNA testing. The Lydia Fairchild case, for example, was brought to court after DNA testing showed that her children could not be hers, since DNA did not match. The charge against her was dismissed when it became clear that Lydia was a chimera, with the matching DNA being found in her cervical tissue. Another case was that of Karen Keegan.[1]

The tetragametic state has important implications for organ or stem-cell transplantation. Chimeras typically have immunologic tolerance to both cell lines. Thus, for a tetragametic human, a wider array of relatives and other persons may be eligible to be an organ donor.[*citation needed*]

CLONING

The possibility of human cloning, raised when Scottish scientists at Roslin Institute created the much-celebrated sheep "Dolly" (*Nature* **385,** 810-13, 1997), aroused worldwide interest and concern because of its scientific and ethical implications. The feat, cited by *Science* magazine as the breakthrough of 1997, also generated uncertainty over the meaning of "cloning" --an umbrella term traditionally used by scientists to describe different processes for duplicating biological material.

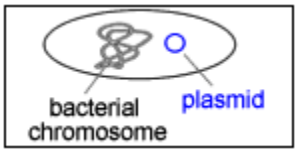
What is cloning? Are there different types of cloning?

When the media report on cloning in the news, they are usually talking about only one type called reproductive cloning. There are different types of cloning however, and cloning technologies can be used for other purposes besides producing the genetic twin of another organism. A basic understanding of the

different types of cloning is key to taking an informed stance on current public policy issues and making the best possible personal decisions. The following three types of cloning technologies will be discussed: (1) recombinant DNA technology or DNA cloning, (2) reproductive cloning, and (3) therapeutic cloning.

Recombinant DNA Technology or DNA Cloning

The terms "recombinant DNA technology," "DNA cloning," "molecular cloning," and "gene cloning" all refer to the same process: the transfer of a DNA fragment of interest from one organism to a self-replicating genetic element such as a bacterial plasmid. The DNA of interest can then be propagated in a foreign host cell. This technology has been around since the 1970s, and it has become a common practice in molecular biology labs today.



Scientists studying a particular gene often use bacterial plasmids to generate multiple copies of the same gene. Plasmids are self-replicating extra-chromosomal circular DNA molecules, distinct from the normal bacterial genome (see image to the right). Plasmids and other types of cloning vectors were used by Human Genome Project researchers to copy genes and other pieces of chromosomes to generate enough identical material for further study.

To "clone a gene," a DNA fragment containing the gene of interest is isolated from chromosomal DNA using restriction enzymes and then united with a plasmid that has been cut with the same restriction enzymes. When the fragment of chromosomal DNA is joined with its cloning vector in the lab, it is called a "recombinant DNA molecule." Following introduction into suitable host cells, the recombinant DNA can then be reproduced along with the host cell DNA. See a diagram depicting this process.

Plasmids can carry up to 20,000 bp of foreign DNA. Besides bacterial plasmids, some other cloning vectors include viruses, bacteria artificial chromosomes (BACs), and yeast artificial chromosomes (YACs). Cosmids are artificially constructed cloning vectors that carry up to 45 kb of foreign DNA and can be packaged in lambda phage particles for infection into *E. coli* cells. BACs utilize the naturally occurring F-factor plasmid found in *E. coli* to carry 100- to 300-kb DNA inserts. A YAC is a functional chromosome derived from yeast that can carry up to 1 MB of foreign DNA. Bacteria are most often used as the host cells for recombinant DNA molecules, but yeast and mammalian cells also are used.

Reproductive Cloning

Celebrity Sheep Died at Age 6

Dolly, the first mammal to be cloned from adult DNA, was put down by lethal injection Feb. 14, 2003. Prior to her death, Dolly had been suffering from lung cancer and crippling arthritis. Although most Finn Dorset sheep live to be 11 to 12 years of age, postmortem examination of Dolly seemed to indicate that, other than her cancer and arthritis, she appeared to be quite normal. The unnamed sheep from which Dolly was cloned had died several years prior to her creation.

Dolly was a mother to six lambs, bred the old-fashioned way.

Image credit: Roslin Institute Image Library

Reproductive cloning is a technology used to generate an animal that has the same nuclear DNA as another currently or previously existing animal. Dolly was created by reproductive cloning technology. In a process called "somatic cell nuclear transfer" (SCNT), scientists transfer genetic material from the nucleus of a donor adult cell to an egg whose nucleus, and thus its genetic material, has been removed. The reconstructed egg containing the DNA from a donor cell must be treated with chemicals or electric current in order to stimulate cell division. Once the cloned embryo reaches a suitable stage, it is transferred to the uterus of a female host where it continues to develop until birth.

Dolly or any other animal created using nuclear transfer technology is not truly an identical clone of the donor animal. Only the clone's chromosomal or nuclear DNA is the same as the donor. Some of the clone's genetic materials come from the mitochondria in the cytoplasm of the enucleated egg. Mitochondria, which are organelles that serve as power sources to the cell, contain their own short segments of DNA. Acquired mutations in mitochondrial DNA are believed to play an important role in the aging process.

Dolly's success is truly remarkable because it proved that the genetic material from a specialized adult cell, such as an udder cell programmed to express only those genes needed by udder cells, could be reprogrammed to generate an entire new organism. Before this demonstration, scientists believed that once a cell became specialized as a liver, heart, udder, bone, or any other type of cell, the change was permanent and other unneeded genes in the cell would become inactive. Some scientists believe that errors or incompleteness in the reprogramming process cause the high rates of death, deformity, and disability observed among animal clones.

Therapeutic Cloning

Therapeutic cloning, also called "embryo cloning," is the production of human embryos for use in research. The goal of this process is not to create cloned human beings, but rather to harvest stem cells that can be used to study human development and to treat disease. Stem cells are important to biomedical researchers because they can be used to generate virtually any type of specialized cell in the human body. Stem cells are extracted from the egg after it has divided for 5 days. The egg at this stage of development is called a blastocyst. The extraction process destroys the embryo, which raises a variety of ethical concerns. Many researchers hope that one day stem cells can be used to serve as replacement cells to treat heart disease, Alzheimer's, cancer, and other diseases. See more on the potential use of cloning in organ transplants.

In November 2001, scientists from Advanced Cell Technologies (ACT), a biotechnology company in Massachusetts, announced that they had cloned the first human embryos for the purpose of advancing therapeutic research. To do this, they collected eggs from women's ovaries and then removed the genetic material from these eggs with a needle less than 2/10,000th of an inch wide. A skin cell was inserted inside the enucleated egg to serve as a new nucleus. The egg began to divide after it was stimulated with a chemical called ionomycin. The results were limited in success. Although this process was carried out with eight eggs, only three began dividing, and only one was able to divide into six cells before stopping.