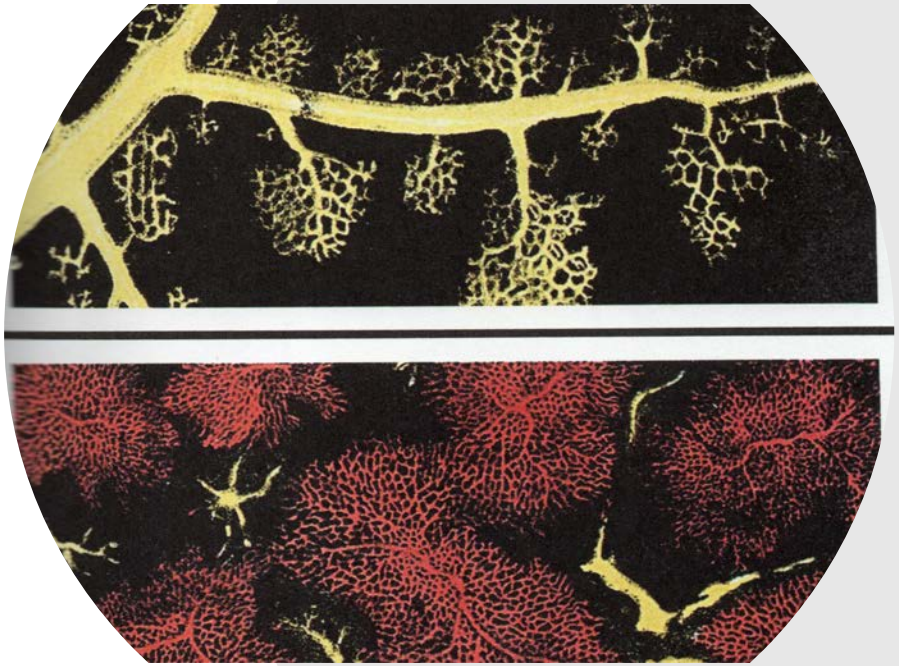

Cell Injury and Cell Death

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Outline

- What is death?
- Reversible cell injury
  - Cellular adaptions to stress
- Intracellular accumulations
- Reversible vs irreversible injury
- Necrosis
  - Classification of necrosis
- Necrosis and apoptosis
- Aging

Reading

- *Robbins Basic Pathology 9th edition*
  - Chapter 1 covers this material
  - Chapter 2 covers the response to injury in greater detail

- *Crowley's An Introduction to Human Disease 11th edition*
  - Recommended for those wanting a more basic text
  - Chapters 2 & 5

Case 1-Why Care About Cell Injury/Death?

- Pathogenesis, how a disease develops
  - What are the mechanisms that result in abnormal cell biology/physiology?
  - How does the body respond to these abnormalities?

- Etiology, the “root” cause of a disease

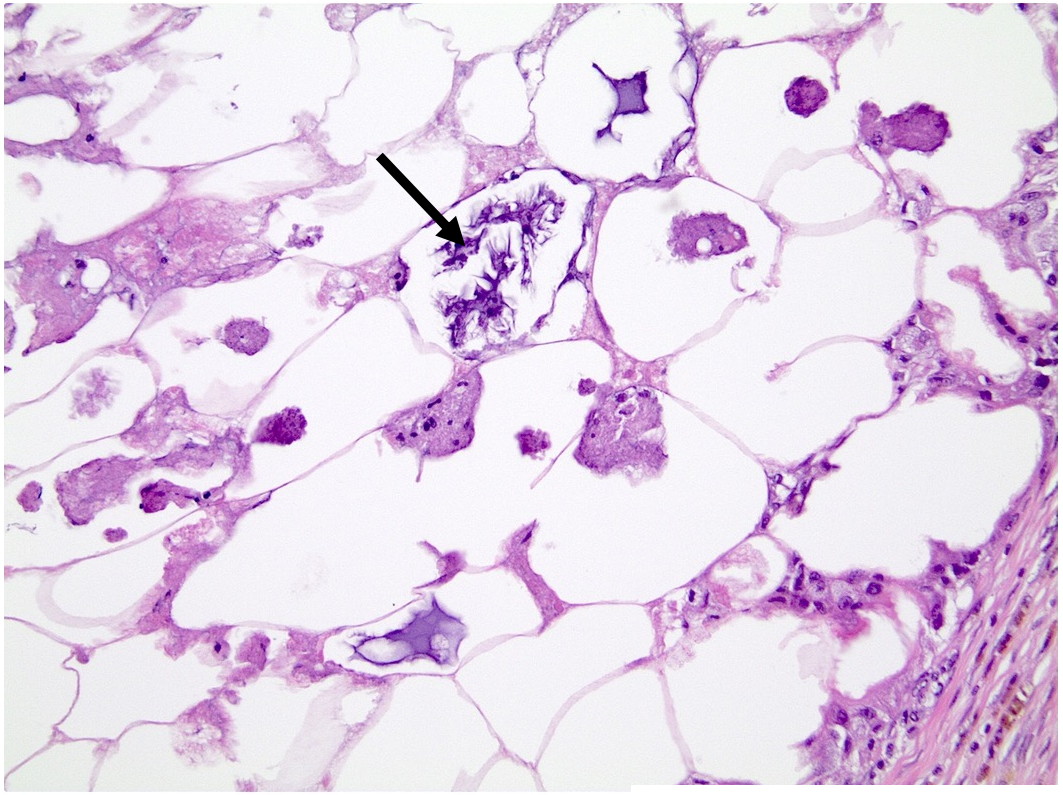
- This case demonstrates how an understanding of the mechanisms of cell injury/death provides the pathologist with insights into disease mechanisms and may help with diagnosis

About Jane

- JW is a Black female 33 years of age
- She is concerned about developing breast cancer
  - Family history with early onset
  - Does not have known genetic risk factors for breast cancer (*BRCA 1 & 2)*
  - Normal mammogram 6 months prior

- JW discovers a single hard 3 cm mass
  - Physical examination and mammography suggest concern for cancer
  - Jane elects to have an excisional biopsy, removal of lesion from a living person to determine nature of lesion(and also be potentially therapeutic)

Results of Biopsy-Microscopic Examination



Necrotic adipocytes show anucleate dead cells (ghost cells).
Contributed by Jing He, M.D.

Pathology Outlines

- Adipocytes are fat containing cells (note clear spaces) common in the breast
- The cells are dead
  - They have no nuclei
  - There are ”crystalline” bodies in some cells (arrow)
    - Calcium may deposit in areas of cell injury or death

Etiology?

- The pathologist states the biopsy is consistent with **fat necrosis** a specific form of cell injury/death subsequent to damage to body tissue rich in fat containing cells
  - In the buttocks and breast this may result from trauma, physical injury
  - On questioning Jane remembers she was involved in a minor traffic accident in which she suffered pain and bruising in the area of her breast crossed by the seat belt
- In this case the etiology was determined by the pathologist recognizing a specific type of cell injury/death (distinguished from other more serious breast disease such as cancer)

Pathophysiology- an aside

- Fat necrosis can be a major clinical problem (associated with damage to the pancreas- pancreatitis)
  - Release of lipases from cells release fatty acids from fat cells
  - Fatty acids combine with tissue calcium to produce soap (saponification)
  - Soap is destructive and leads to an inflammatory response and tissue destruction

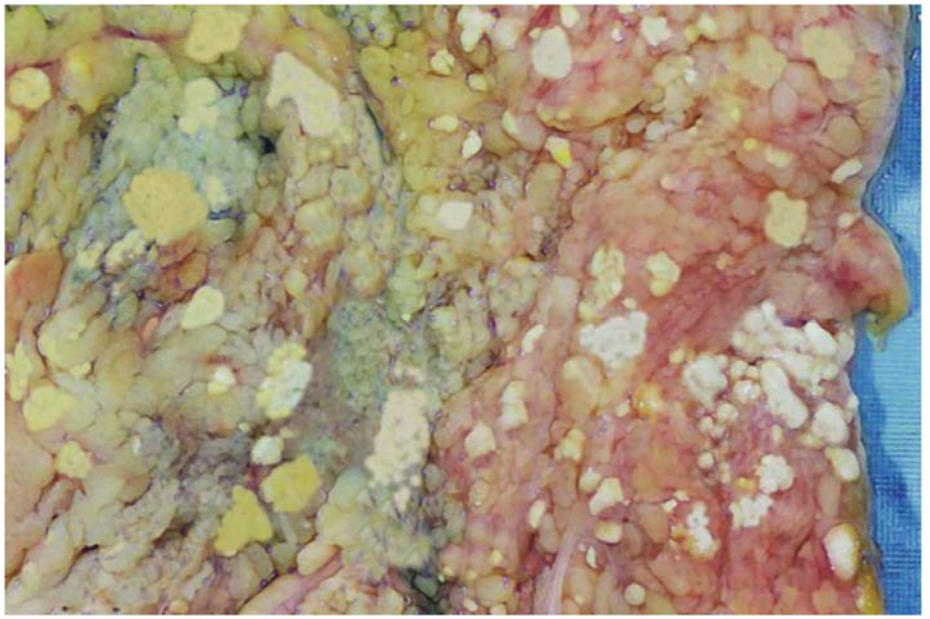


Figure 1–12 Fat necrosis in acute pancreatitis. The areas of white chalky deposits represent foci of fat necrosis with calcium soap formation (saponification) at sites of lipid breakdown in the mesentery.

Discussion breakout-Case 1

- The patient in this case was identified as Black. The question-When are racial identifiers appropriate in research and clinical settings?
  - Are they appropriate in medical records?
  - Are they appropriate in clinical case reports (as in Case 1)?
  - Are they appropriate in clinical research studies ?
  - Pathology texts often give disease frequencies by race. Is this appropriate?
- In what settings might racial identifiers be helpful, harmful or just inappropriate?
- Can you suggest guidelines for use of racial (or ethnic) identifiers
    - N breakout groups-self selected reporter
    - Five-minute discussion
    - Reporter presents group opinions

What is Death?

- Somatic death, death at the level of the organism-can be difficult to define
- Uncertainty led to fear of premature burial and wakes
  - *"Cessation of all life at the cellular level is not a necessary criterion for determination of death"* (World Medical Association)
    - With the cessation of heartbeat oxidative metabolism fails but some cells (muscle, skin and bone) can survive for hours to days
  - *"irreversible cessation of circulatory and respiratory functions and cessation of all functions of the entire brain, including the brainstem"* (AMA, ABA)
    - Depends on clinical judgement, neurological and other tests
    - Respiration fails, blood pressure drops, oxidative metabolism ceases, ATP dependent cellular metabolism fails
    - Calcium transporting ATPases in muscles fail, calcium diffusion inward produces rigor mortis (unrelieved muscle contraction) and ultimately decomposition

Reversible Cell Injury

- Pathologists are now interested in the molecular mechanisms of cell injury/death
- Cellular stress/injury may be potentially reversible (in part)
  - There are recognizable patterns of cellular stress at the tissue level
    - These may be normative and physiological responses
    - They may be indicators of pathogenesis

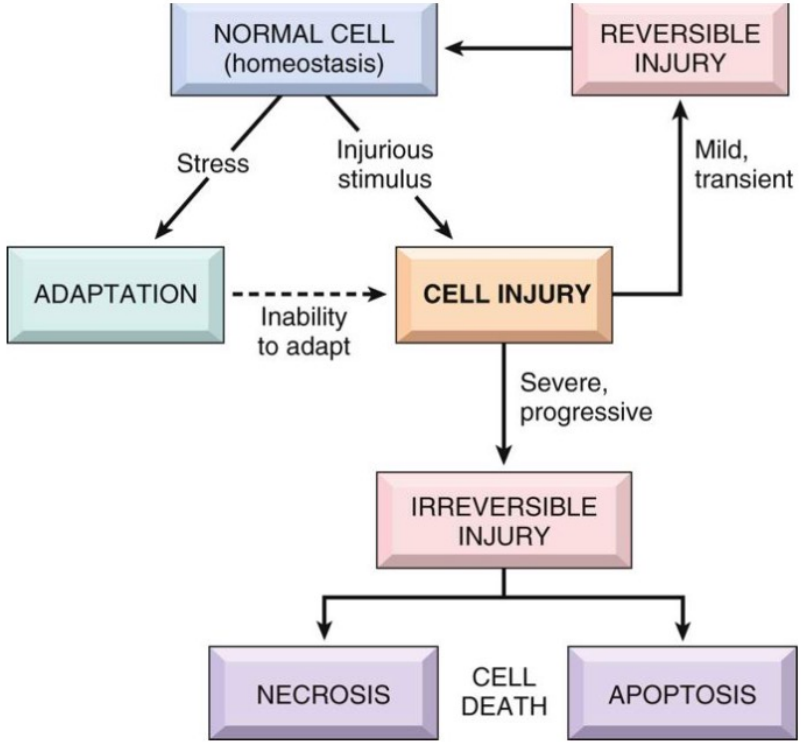


Figure 1–1 Stages in the cellular response to stress and injurious stimuli.

Example: Normal, Reversibly Injured and Dead Heart Cells (Myocardial Cells)

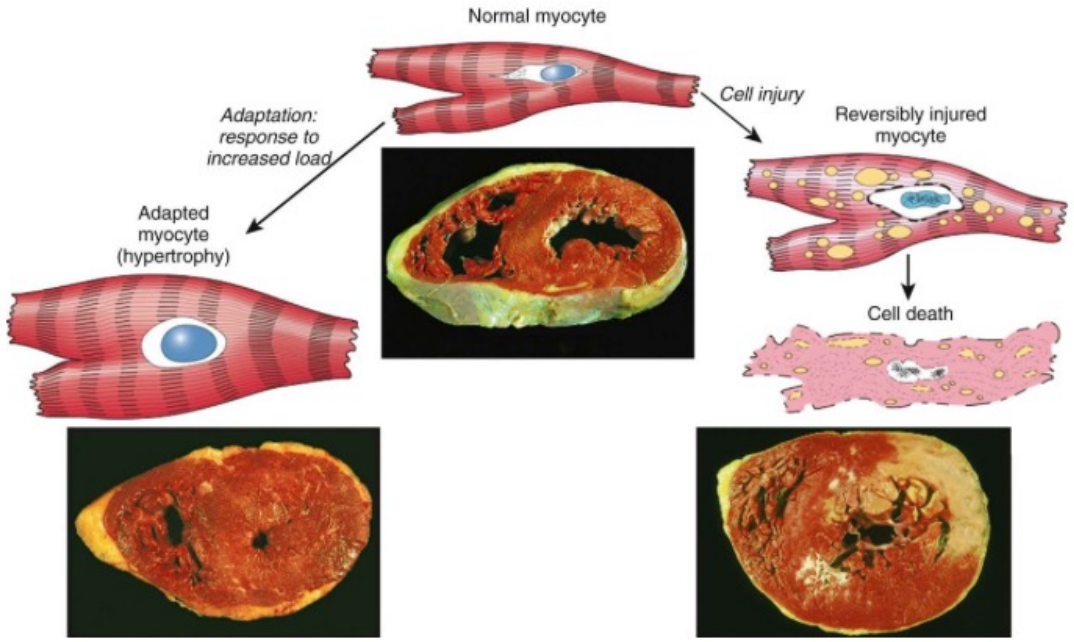
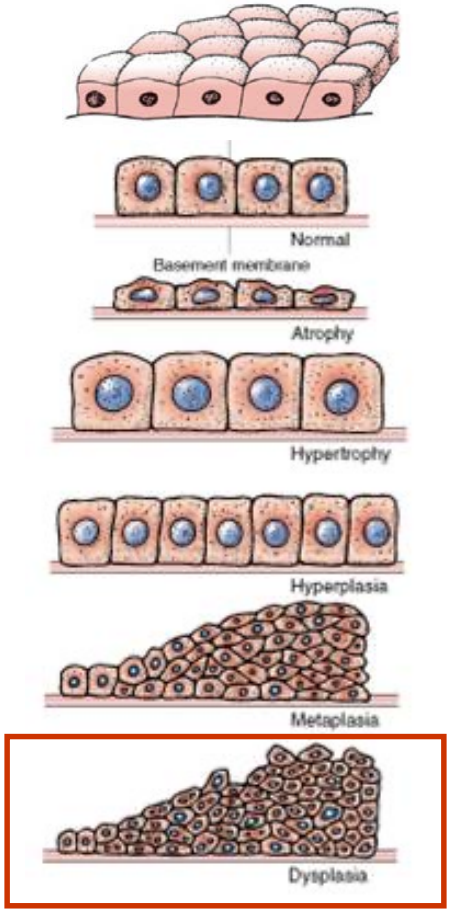


Figure 1–2 The relationship among normal, adapted, reversibly injured, and dead myocardial cells.

Patterns of Cellular Adaptations to Stress

- **Hyperplasia**: The increase in number of cells in an organ or tissue (which may increase in volume)
- **Hypertrophy:** The increase in size of the cells in an organ or tissue (and hence, the increase in organ size)
- **Atrophy:** The decrease in the size of cells in an organ or tissue (and hence, the decrease in organ size)
- **Metaplasia:** A reversible change in which one adult type of cell is replaced by another type. This may not be considered as "normal" by some
- **Dysplasia**: abnormal changes in size, shape, organization and pattern of growth. May be thought of as atypical or abnormal hypertrophy and is a precursor to lesion to neoplasia



Insert Video cellinjury 2020810 9 minutes

Questions

Abnormal Intracellular Accumulations

- Cells may accumulate abnormal amounts of a substance as a result of
  - Exposure to to an exogenous environmental substance-
    - Carbon particles (anthracosis in lung-harmless, Silica particles (silicosis in lung-disease)
  - Production of an abnormal endogenous substance due to a genetic disease
    - Many storage diseases-result of abnormal breakdown of a metabolite
  - Inadequate removal of a normal substance
    - Fatty change (steatosis) in the liver-the liver is the major organ responsible for fat metabolism. Many metabolic abnormalities result in triglyceride accumulation in the liver
      - Most common causes-alcohol abuse, type II (adult onset) diabetes (biochemistry is complex)

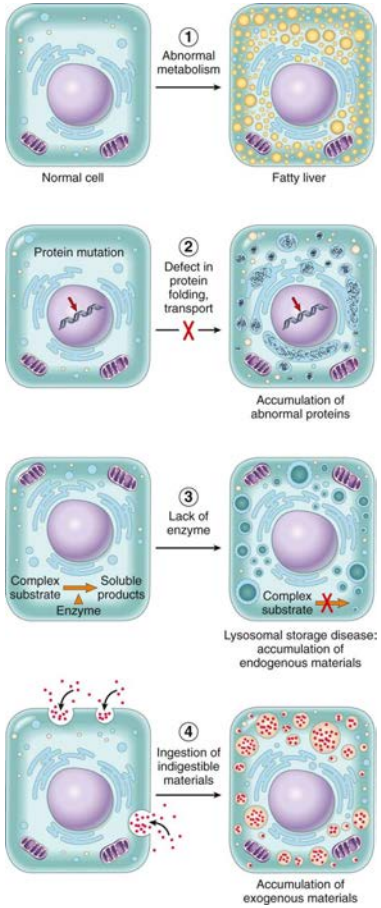


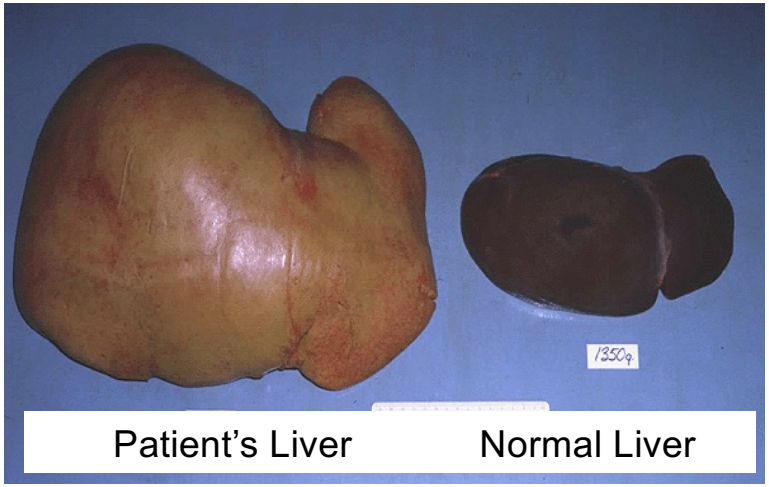
Fig. 1-26

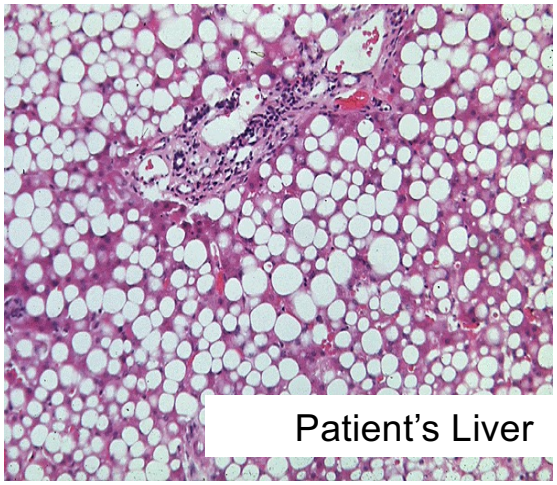
Case 2 Steatosis (Fatty Liver)

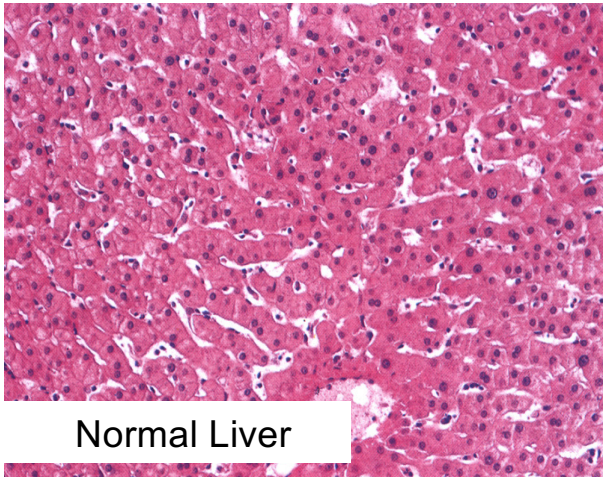
- A college student who is 22 years of age is seen in the ED for upper right quadrant pain and gastritis in association with drinking. He was noted to have
  - A mild increase in girth
  - Mild elevation in *serum aspartate aminotransferase (AST) & gamma glutamyl transferase (GGT)* in a blood sample
    - The above are enzymes found in liver cells. They "leak" into the blood when liver cells are injured and hence are indicators of liver injury. He is in a fatal automobile accident (while under the influence) and comes to autopsy
    - Two days post he is in a fatal automobile accident (while under the influence) and comes to autopsy

Case 2 : Autopsy Results (1)

- His liver is hypertrophic (this image is a bit exaggerated)
- Under the microscope his cells seem to be swollen and filled with something

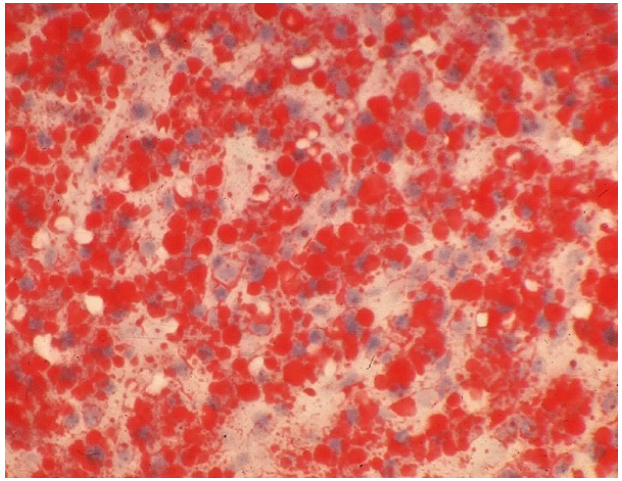




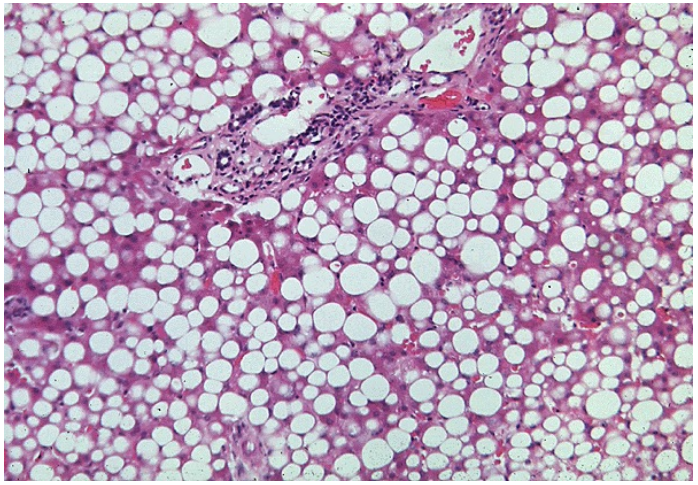


Case 2 : Autopsy Results (2)

- What is in the clear spaces?
  - Standard tissue processing extracts lipid & water from tissue
  - Frozen sections may be used to rapidly process tissue and maintain lipids
  - Special “fat stains” can be used to identify lipids



Frozen Section (Oil Red O)



Paraffin Section (Hematoxylin & Eosin

Reversible & Irreversible Cell Injury

- There is no absolute point when cell injury becomes irreversible however at the cellular level certain changes correlate with cell death
  - Microscopic signs of cell death tend to lag the degree of injury
- There are two major types of cell death necrosis and apoptosis (simplistically unplanned or inadvertent and planned or programmed)-More later

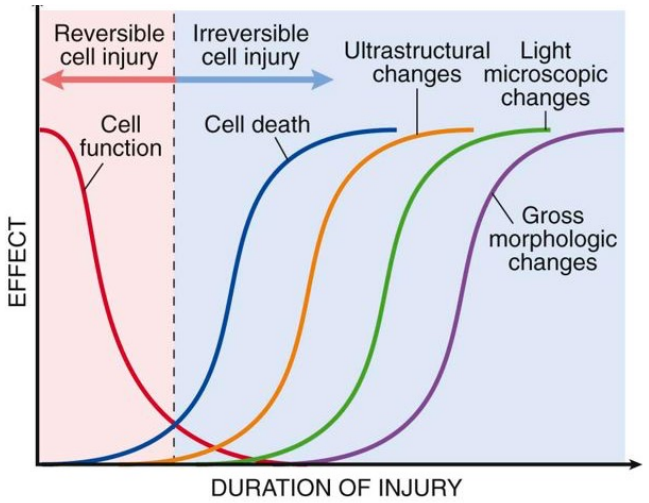


Figure 1–7 The relationship among cellular function, cell death, and the morphologic changes of cell injury. Note that cells may rapidly become nonfunctional after the onset of injury, although they are still viable, with potentially reversible damage; with a longer duration of injury, irreversible injury and cell death may result. Note also that cell death typically precedes ultrastructural, light microscopic, and grossly visible morphologic changes.

Mechanisms of Necrosis-Cellular Features (1)

- Certain cellular features distinguish reversible cell injury from irreversible injury resulting in necrosis
  - Reversible injury is associated with
    - Cell swelling and blebbing, swelling of the endoplasmic reticulum and mitochondria and the formation of myelin figures indicative of membrane damage
  - Irreversible injury is associated with
    - Breakdown of the plasma membrane,, organelles and major changes to mitochondria
    - Nuclear damage and loss

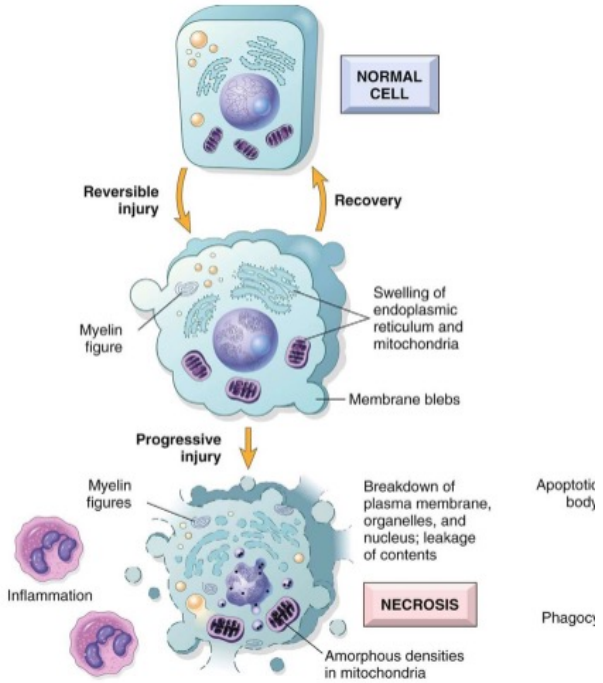


Figure 1–6 Cellular features of necrosis

Mechanisms of Necrosis-Cellular Features (2)

- Examples of reversible and irreversible cell injury in rer tubular cells
  - Injury initially associated wit ischemia and loss of ATP
  - Details follow

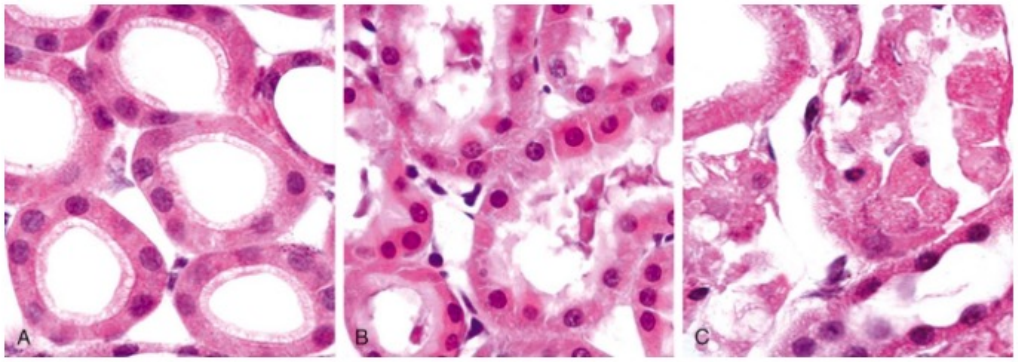


Figure 1–8 Morphologic changes in reversible and irreversible cell injury (necrosis). A, Normal kidney tubules with viable epithelial cells. B, Early (reversible) ischemic injury showing surface blebs, increased eosinophilia of cytoplasm, and swelling of occasional cells. C, Necrotic (irreversible) injury of epithelial cells, with loss of nuclei and fragmentation of cells and leakage of contents.

Mechanisms of Necrosis-Biochemistry

- Most injurious agents damage cells by disrupting one of four cellular pathways
  - Aerobic respiration (mitochondrial oxidative phosphorylation) and the production of ATP
  - The integrity of membranes
  - Protein synthesis
  - Integrity of the genetic apparatus (DNA replication and repair). This is indicative of irreversible damage
- The pathways are interrelated

Ischemic Injury

- Ischemia is the interference with blood supply to a tissue (tissue hypoxia or anoxia)
  - Clinically this is often related to inadequate transport of oxygen related to compromise of blood flow to a tissue (such as by a thrombus in a vessel)
  - The result is a tissue infarct (as in myocardial infarct-heart attack)
- The key is impairment of oxidative phosphorylation and decrease in ATP and a cascade of biochemical abnormalities

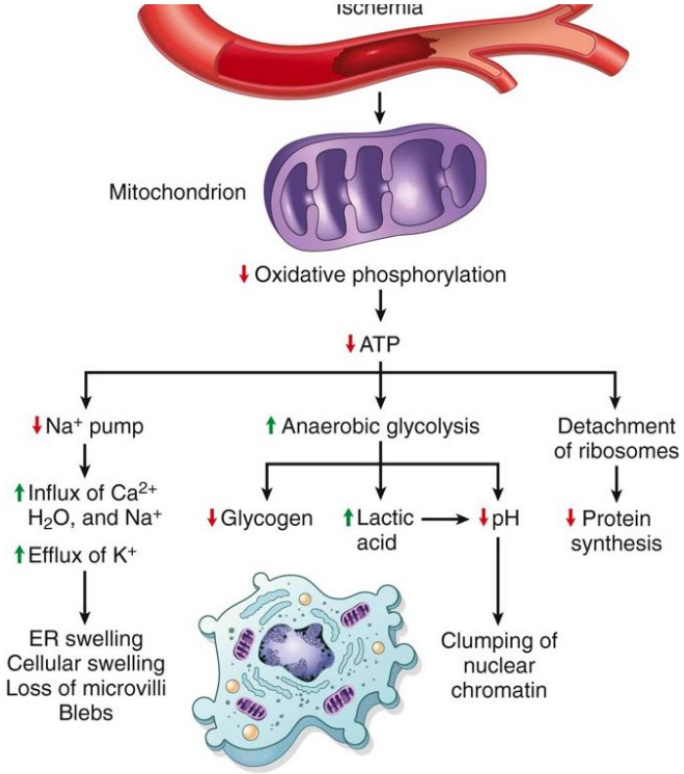
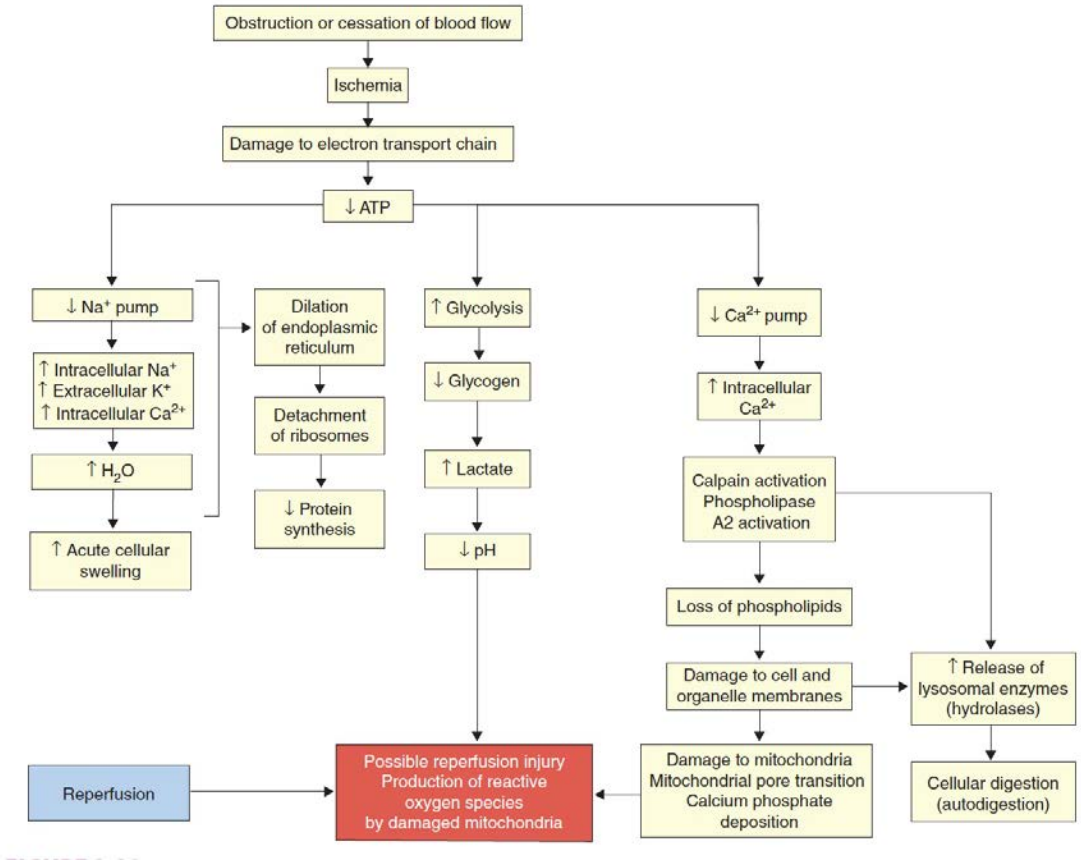


Figure 1–15 The functional and morphologic consequences of depletion of intracellular adenosine triphosphate (ATP). ER, endoplasmic reticulum

Ischemic Injury: Summary of Mechanisms

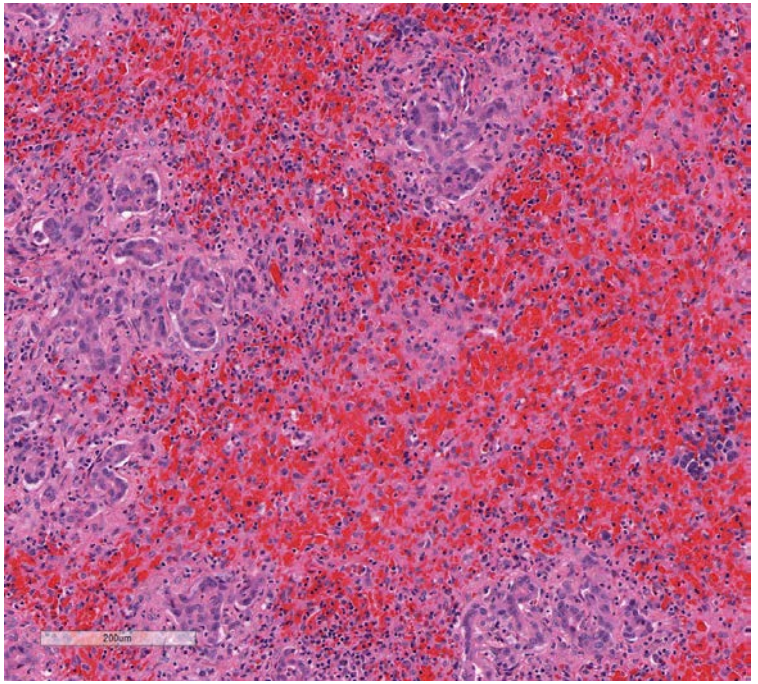


Causes of Cell Injury Other than Ischemia

- Chemical (toxin)
  - Direct-dependent on agent and cellular target
  - Indirect-often a liver targeted chemical which is activated to form a reactive species (free radical)
- Pathogen (very complex)
  - Direct: viral cell lysis, DNA damage, bacterial endo/exotoxin
  - Indirect: Host response immune mediated injury
- Genetic/congenital (very complex)
  - Many single gene defects, structural defects, developmental defects
- Reactive oxygen species-free radicals (may be important in aging)
  - Produced by damaged mitochondria (leaks in electron transport, reperfusion injury), part of host response (inflammation)
- **The pattern of cell injury/necrosis may be indicative of the injurious agent**

Indirect Chemical Injury (Acetaminophen)

- Acetaminophen (Tylenol) is toxic when overdosed
  - More than 100 deaths /year. Responsible for half of all cases of acute liver failure (28% mortality)
  - A proportion of the drug is converted to a highly active compound (NAQPI) by cytochrome oxidases which damages liver cells



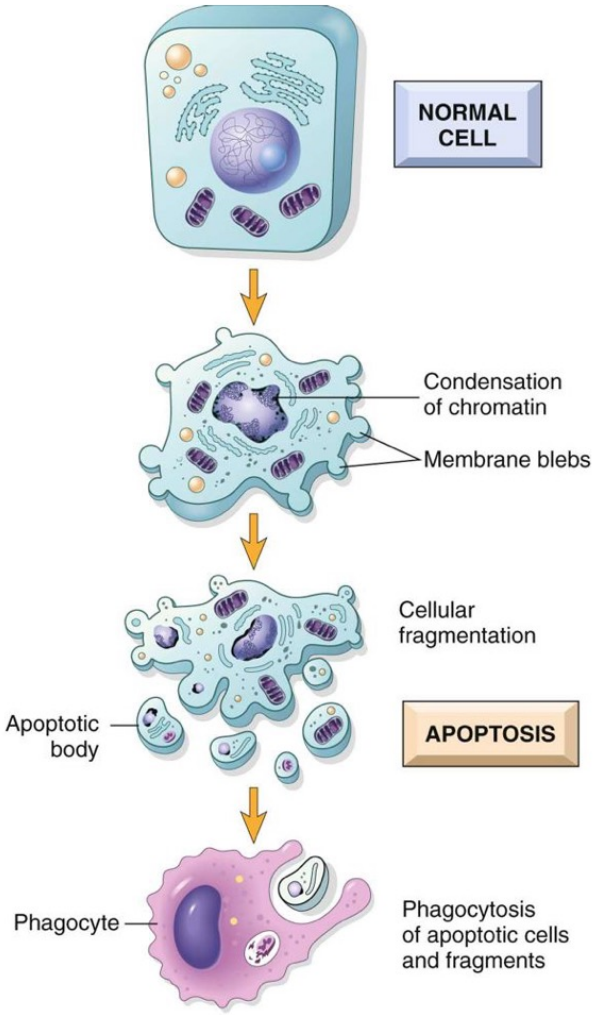
Acetaminophen toxicity in the liver (necrosis and hemorrhage)

Classification of Necrosis

- Insert Video Necrosis2 4.5 minutes
- Questions 2?

Apoptosis versus Necrosis

- Necrosis is disorderly and often provoked by events external to the cell
  - Necrotic cells swell and contents are dispersed to tissue provoking host response (inflammation)
- Apoptosis (Greek falling off) is programmed and orderly form of cell suicide
  - It may be provoked by external of internal signals
  - Apoptosis is an energy requiring process
    - Apoptotic cells shrink, organelles remain “intact”
    - Chromatin condenses, fragments (in a specific manner)
    - Cells bleb and fragment into membrane bound apoptotic bodies (which have membrane signals for clearance by inflammatory cells)
- Apoptosis limits response by other cells (inflammation)



Role of Apoptosis

- Important in many physiological process (programmed cell death)
  - Programmed cell death important in embryogenesis
  - Hormone dependent loss of cell mass (regression of lactating breast, endometrial breakdown in menses)
  - Immune development and function. (Immune killing, deletion of self-reactive clones)
- Important in controlling disease
  - Cell death in tumors
  - Death of cells with defects in DNA synthesis and repair
  - Killing of virally infected cells (see figure)
- Some injurious agents induce both apoptosis, necrosis and other "intermediate" forms of cell death

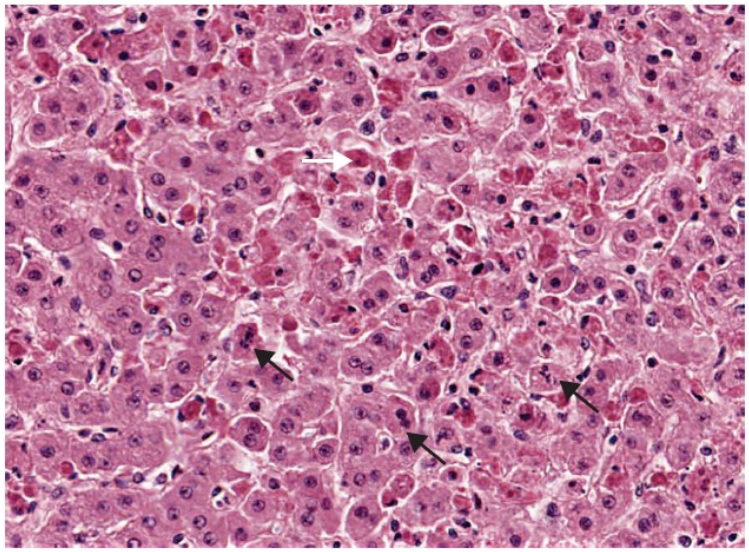


FIGURE 2-30 **Apoptotic bodies.** Hepatocytes in the liver from a case of yellow fever. Many of the cells are in various stages of apoptosis. For example, some cells (arrows) demonstrate a condensed fragmented nucleus. The eponymous term "Councilman body" refers to an anucleate eosinophilic remnant of a hepatocyte that has undergone apoptosis (apoptotic body) (white arrow).

Apoptosis: Biochemical Features (1)

- The key to apoptosis are caspases, enzymes with active site cysteine that cleave target proteins after aspartic acids.
  - Inactive caspases are activated –often by another caspase (in a chain)
  - The issue is the initial trigger-which defines pathways of apoptosis
    - Extrinsic pathway-death receptor on cell and extracellular ligand (TNF-receptor/TNF-alpha for example
    - Intrinsic pathway-involves the balance of of antiapoptotic and proapoptotic proteins causing mitochondrial damage & release of contents (proapoptotic proteins are upregulated by p53 system responding to DNA damage)
  - Ultimately both pathways converge with the activation of effector caspases responsible for the cellular changes characterizing apoptosis such as cleavage of DNA strands

Apoptosis: Biochemical Features (2)

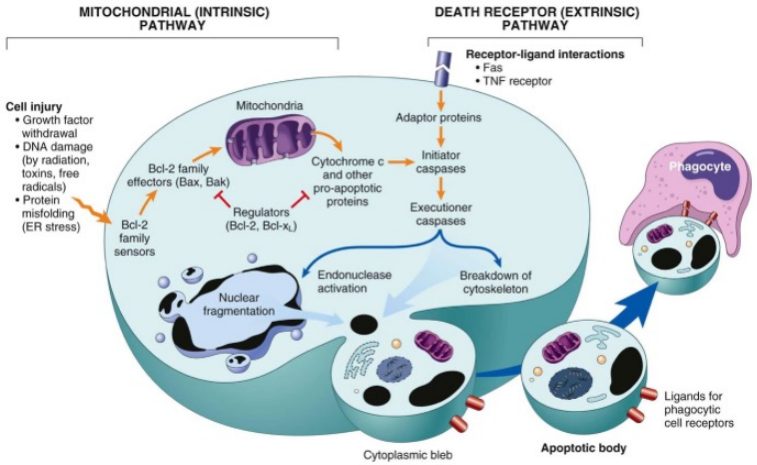


Figure 1–22 Mechanisms of apoptosis. The two pathways of apoptosis differ in their induction and regulation, and both culminate in the activation of caspases. In the mitochondrial pathway, proteins of the Bcl-2 family, which regulate mitochondrial permeability, become imbalanced and leakage of various substances from mitochondria leads to caspase activation. In the death receptor pathway, signals from plasma membrane receptors lead to the assembly of adaptor proteins into a "death-inducing signaling complex," which activates caspases, and the end result is the same.

Aging: Determination of Lifespan

- Maximal lifespan: Likely constant through history ~100-125 years. Does not vary with populations
- Life expectancy: (average life span or more accurately - how long you can expect to live at a given age) has increased, particularly for the elderly
  - Depends on population, sex, lifestyle etc. This is influenced by endogenous and exogenous factors
  - Some are inevitable results of metabolism and intrinsic genetic factors-modification of proteins & cellular components-by oxidation (?), "clock genes" telomere shortening-limitation to 20-40 mitotic divisions
  - Extrinsic factors-lifestyle (nutrition) environmental chemicals, radiation, motorcycles

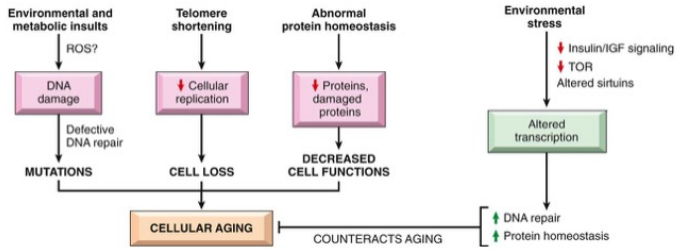


Figure 1–29 Mechanisms that cause and counteract cellular aging. DNA damage, replicative senescence, and decreased and misfolded proteins are among the best described mechanisms of cellular aging. Some environmental stresses, such as calorie restriction, counteract aging by activating various signaling pathways and transcription factors. IGF, insulin-like growth factor; TOR, target of rapamycin.

Aging and Telomere Length

- In the absence of an active telomerase complex the length of telomeres *telomere is a region of repetitive nucleotide sequences associated with specialized proteins at the ends of linear chromosomes*.
  - Germ cells and stem cells have active telomerase and can divide repeatedly
  - Somatic cells are limited to about 40 mitotic divisions (Haflick limit)
  - Telomerase is reactivated in some cancers allowing unlimited replication

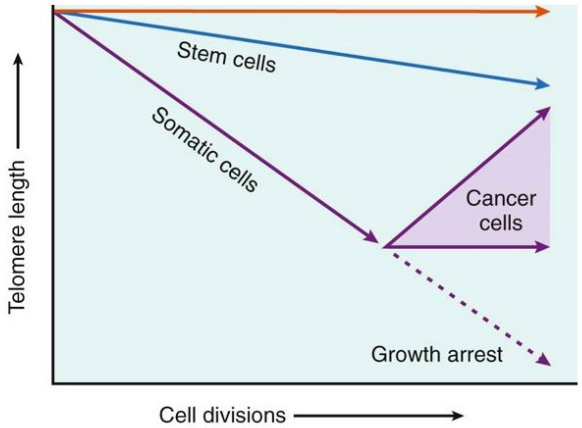


Figure 1–30 The role of telomeres and telomerase in replicative senescence of cells. Telomere length is plotted against the number of cell divisions. In most normal somatic cells there is no telomerase activity, and telomeres progressively shorten with increasing cell divisions until growth arrest, or senescence, occurs. Germ cells and stem cells both contain active telomerase, but only the germ cells have sufficient levels of the enzyme to stabilize telomere length completely. In cancer cells, telomerase is often reactivated.

Effect of Telomere Erosion

- With aging (or in the case of uncommon genetic defects in telomerase in stem cells-)-
  - Telomeres erode
  - Free chromosome ends can
    - Result in chromosome fusion and chromosome defects (cancer?)
    - Increase the p53 system sensing DNA damage and induce apoptosis

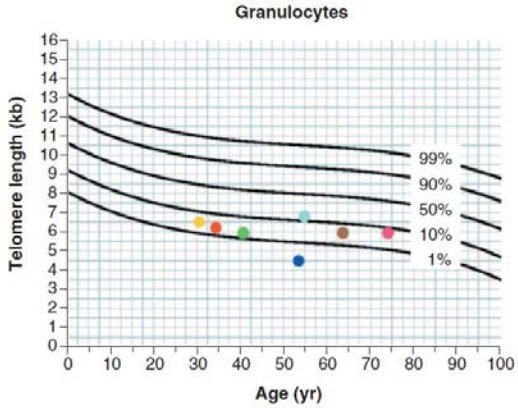


FIGURE 2-64 **Telomere length in healthy individuals by age.** Solid lines indicate percentile distribution of 400 control subjects. Colored solid points indicate telomere length is seven individuals with mutations in the TERT gene who have marrow

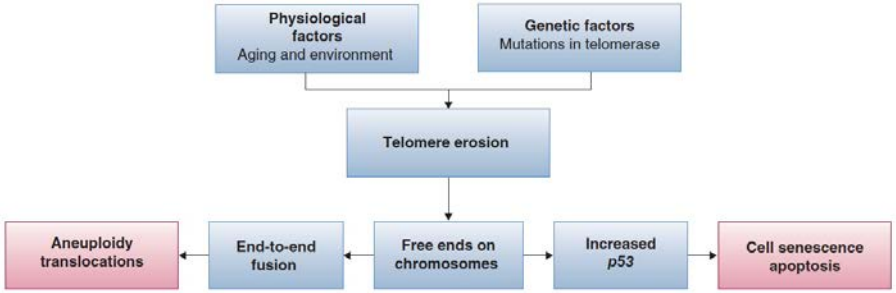


FIGURE 2-65 **Telomere erosion and aging.** Telomere shortening beyond a critical point leads to uncapped chromosome ends and ultimately cellular senescence or apoptosis.