Cell Injury

Cell Death

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The syllabus for Cell Injury and Cell Death covers the material to be presented at the lectures on this topic (Jan. 3-5). The textbook reading for these lectures is Chapter 1 of Robbins and Cotran, 7th edition. Alcohol abuse coverage is Chapter 9, p421-424. The presentation of material in the syllabus and lectures may not follow the exact order of presentation of the material in the textbook. Nevertheless, there are no conflicts in concepts between the syllabus and textbook. The information is easy to locate in Robbins.

As you study the material, use the lecture presentations and the syllabus as a guide for what to emphasize. The material presented in the syllabus and lectures is, however, required knowledge. The most important goal is to gain a general understanding of cellular adaptations, cell injury and the two types of cell death, known as necrosis and apoptosis.

I will not cover in the lectures or syllabus some of the topics presented in the textbook. For these topics, I expect you to know the meaning of some terms (this includes heterophagy/autophagy, cytoskeletal abnormalities, intracellular accumulations of cholesterol, protein, glycogen, pigments and calcification). You should be able to define and recognize these types of injury. I also suggest that you take a look at Chapter 3 p90-94, to become familiar with tissue homeostasis, stem cells and cloning.

The study of cell injury and cell death is the basis for the understanding of disease mechanisms. It is interesting and essential material for medical practice and medical science. I hope that you will enjoy studying these topics, as I do teaching this material, which is both basic science and clinical medicine. At the end of the syllabus (Appendix 1) you will find some clinically relevant questions related to the lectures.

Nelson Fausto, M.D.

"One can be fooled by appearances, which happens only too frequently, whether one uses a microscope or not" (Voltaire)

"...can the human soul be glimpsed through a microscope? May be, but you'd definitely need one of those very good ones with two eyepieces" (Woody Allen)
"You can observe a lot by watching" (Yogi Berra)
1. **Pathology**

   Study of disease process as to:
   1. Causes (etiology)
   2. Mechanisms of development (pathogenesis)
   3. Structural and functional alterations (consequences and clinical significance)

2. **The goals of this series of lectures are:**

   - To define and describe in general terms physiological adaptations, reversible and irreversible injury and cell death.
   - To study the causes and mechanisms of cell death.
   - To distinguish between two patterns of cell death: necrosis and apoptosis (programmed cell death)
   - To study the mechanisms of tissue injury caused by ischemia, free radicals and injury produced by chronic alcoholism.

3. **Physiological adaptations and cell injury**

   1. Normal ("steady state") homeostasis
   2. Adaptations: hypertrophy, hyperplasia, atrophy, metaplasia
   3. Cell injury:
      - Reversible injury (non-lethal)
      - Irreversible injury (cell death): Apoptosis, Necrosis

4. **Homeostasis and cell populations**

   In adult tissues, the size of a cell population is determined by the rates of cell differentiation, proliferation, and death by apoptosis. This dynamic state occurs in tissues such as the bone marrow, skin and gastro-intestinal tract epithelia, in which there is an equilibrium between these various processes. The equilibrium may be disrupted by increased or decreased cell proliferation, or by agents such as irradiation that may induce cell death and inhibit stem cell differentiation.

   Tissues in which there is constitutive cell proliferation are often called **labile tissues**, to contrast them with **stable tissues**, such as the liver, pancreas, kidney and endothelial cells, in which cell proliferation is normally very low. However, cells from stable tissues can readily enter the cell cycle and replicate in response to certain stimuli. The most dramatic example of this type of response is **liver regeneration** after partial hepatectomy. The third type of tissue, called **permanent or non-dividing tissues**, contain cells that have left the cell cycle permanently and are not capable of proliferation. This category includes the brain, and cardiac and skeletal muscle. Nevertheless, skeletal muscle contains stem-like cells called satellite cells that have the capacity to differentiate and regenerate muscle fibers. Recently, stem cells
have been identified in at least 2 areas of mammalian brain. It is not known if these cells may contribute to brain remodeling and regeneration.

5. Cellular adaptations, cell injury and cell death (general definitions)

Cells constantly adapt to physiological demands to maintain a homeostatic steady state. Cells adapt by performing excess work, replicating, decreasing functions, changing its differentiated properties etc. The main adaptations to a persistent stimulus may involve cellular hypertrophy, hyperplasia and metaplasia (see diagram # 6). Atrophy occurs whenever certain normal stimuli (workload, blood supply, etc) are decreased or lost. Depending on the specific condition, adaptive reactions can produce organ damage. Through these adaptations cells maintain their viability. The term cell injury is used to indicate a state in which the capacity for physiological adaptation is exceeded. This may occur when the stimulus is excessive or when the cell is no longer capable to adapt without suffering some form of damage. The capacity for adaptation and the sensitivity to different types of injury varies according to cell type (i.e. myocardial cells and neurons are highly sensitive to ischemic injury; hepatocytes are more sensitive to chemical than ischemic injury). Cell injury may be reversible (non-lethal damage which generally can be corrected by removal of the stimulus) or irreversible (lethal damage). The transition between reversible and irreversible damage, commonly referred to as the "point of no return" is of major importance. Recognition of the point of no return is a key element for devising therapeutic strategies to prevent cell death after injury.

Cell death itself is a complex phenomenon that forms the basis for most disease processes. Until a few years ago the term necrosis was used as a synonym to cell death. It is now known that there are at least 2 distinct types of cell death: apoptosis (also known as programmed cell death) and necrosis. The major importance of this distinction between types of cell death is that while necrosis is always a pathological process, apoptosis may take place as a physiological phenomenon that is essential for life. Moreover, necrosis generally elicits an inflammatory reaction while apoptosis is not accompanied by inflammation.

In these lectures we will study the events described above and will give examples of specific conditions or diseases in which these events play a major role. We will study in more detail ischemic and chemically induced injury.

Cells are also subjected to different stresses relate to metabolic alterations, which may be caused by genetic defects or be acquired. These conditions can lead to the accumulation of substances inside the cell (intracellular accumulation), such as fat (steatosis), proteins, pigments and calcium.
6. Tissue response to environmental change

- **Stimulus**
  - **Normal tissue**
    - **Cell response**
      - **Cell survives**
      - **Stimulus abates**
    - **Severe stimulus, or cell is sensitive**
      - **Failure of adaptation**
        - **Cell injury or death**
  - **Stimulus persists**
    - **Increased functional demand**
    - **Reduced functional demand or impaired nutrition**
    - **Hostile environment**
      - **Failure of adaptation**
        - **Cell injury or death**
      - **Change of cell type change of differentiation**
        - **Metaplasia**
7. Cell/Tissue adaptive changes*

<table>
<thead>
<tr>
<th>Change in size of cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertrophy</td>
<td>Increase in the size of cells</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>Increase in the number of cells</td>
</tr>
<tr>
<td>Atrophy</td>
<td>Decrease in the number of cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Change in differentiation of cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Metaplasia</td>
<td>Stable change to another cell type</td>
</tr>
</tbody>
</table>

*Think of examples for each of these states.

8. Examples of metaplasia relevant to human diseases

Metaplasia is the replacement of one tissue by another, for instance, a change in epithelia from columnar to squamous. Both tissues have normal structure, but metaplasia alters the functional capacity of the tissue.

<table>
<thead>
<tr>
<th>Original tissue</th>
<th>Stimulus</th>
<th>Metaplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliated columnar epithelium of bronchial tree</td>
<td>Cigarette Smoke</td>
<td>Squamous epithelium</td>
</tr>
<tr>
<td>Transitional epithelium of bladder</td>
<td>Bladder calculus</td>
<td>Squamous epithelium</td>
</tr>
<tr>
<td>Columnar epithelium in gland ducts (bile ducts, salivary, etc.)</td>
<td>Calculus</td>
<td>Squamous epithelium</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>Chronic trauma</td>
<td>Bone (osseous) tissue</td>
</tr>
<tr>
<td>Esophageal squamous epithelium</td>
<td>Gastric acid</td>
<td>Columnar epithelium (Barrett’s esophagus)</td>
</tr>
</tbody>
</table>
9. Common pathological stimuli causing cell injury

Practically, any conceivable stimulus can cause cell injury. Injury occurs when the adaptive mechanisms already discussed are not sufficient to maintain normal homeostasis. Note, however, that some adaptive mechanisms may become pathologic (for instance, hyperplasia of the prostate and of endometrium). Generally these types of hyperplasia can regress whenever the stimulus for it is withdrawn. Injury can be reversible or irreversible. Irreversible injury leads to cell death by necrosis or apoptosis.

<table>
<thead>
<tr>
<th>Type</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic</td>
<td>Gene defects, chromosomal anomalies</td>
</tr>
<tr>
<td>Nutritional</td>
<td>Deficiency or excess of dietary substances, e.g. iron, vitamins</td>
</tr>
<tr>
<td>Immune</td>
<td>Damage caused by the immune system, e.g. autoimmunity</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Deficient or excessive hormone activity</td>
</tr>
<tr>
<td>Physical agents</td>
<td>Mechanical trauma, thermal damage, irradiation (UV and ionizing)</td>
</tr>
<tr>
<td>Chemical agents</td>
<td>Toxicity due to many agents, e.g. metals, solvents, drugs</td>
</tr>
<tr>
<td>Infective</td>
<td>Infection by viruses, bacteria, parasites, fungi and other organisms</td>
</tr>
<tr>
<td>Ischemia (hypoxia)</td>
<td>Deficit of blood supply or direct oxygen deficit</td>
</tr>
</tbody>
</table>


1. Response depends on nature of injury, duration and severity.
2. Consequences of injury depend on cell type.
3. Morphologic changes detectable by light microscopy may occur much later than functional lesion.
4. Although different agents may have different initial cellular targets, the final pathways are often similar.

11. Main cellular mechanisms of cell injury

1. ATP depletion
2. Loss of calcium homeostasis
3. Oxidative stress (excess Reactive Oxygen Species)
4. Damage to mitochondria, and increased permeability of membranes

12. Cell injury caused by ATP depletion

ATP depletion is particularly important in tissues with low glycolytic activity in which ATP production is solely dependent on oxidative phosphorylation of ADP in the electron transport chain in mitochondria. Neurons and cardiac myocytes are rapidly injured by ATP decreases that occur as a consequence of ischemic injury. A major component of the injury is the alteration of membrane permeability caused by decreased activity of ATP-dependent ionic pumps.
13. Injury produced by loss of calcium homeostasis

Cytosolic free calcium is kept at concentrations that are at least 10-fold lower than the extracellular levels. In the normal cell, most intracellular calcium is sequestered in mitochondria and endoplasmic reticulum. Ca concentration gradients are maintained by membrane-associated Ca/Mg-dependent ATPases. Ischemia and some toxins cause early release of Ca into the cytosol.


Cells generate reactive oxygen forms as byproducts of metabolic reactions that reduce molecular oxygen to water. These reactive forms, called reactive oxygen species, can damage lipids, proteins and DNA. Cells have antioxidant defenses (#15 and 16), but when free radical formation exceeds the cells' neutralizing capacity, free radicals accumulate in the cell, and produce a condition called oxidative stress. Oxidative stress is a very common type of condition that can be caused by inflammation, reperfusion injury, chemical injury and radiation damage.
Depletion of free radical scavengers (vitamins E, C, and A) and other antioxidant defenses, such as glutathione, glutathione peroxidase/reductase, superoxide dismutase and catalase.

Generation of reactive oxygen metabolites:
- $O_2$
- $OH^-$
- $H_2O_2$

Damaging effects on cell:
- Peroxidation of lipids → membrane damage
- Damage of thiol-containing protein → membrane damage
- Mitochondrial damage → apoptosis, decreased respiration
- DNA damage → apoptosis; carcinogenesis (long term)
16. Reactive oxygen species and antioxidant defenses

There are many different types of antioxidant mechanisms. These mechanisms act: 1) directly, by blocking the formation or scavenging free radicals (vitamin A, E, ascorbic acid, glutathione); 2) by binding iron and copper, metals that catalyze ROS formation; 3) through the activity of enzymes such as superoxide dismutase and catalase (breakage of superoxide anion and hydrogenase peroxide, respectively), and glutathione peroxidase.

16. Injury produced by mitochondrial damage and membrane permeability defects
Mitochondria are important primary or secondary targets for most agents that cause cell injury. Alterations in mitochondrial membrane permeability generally lead to apoptosis. Loss of the capacity of the plasma membrane to maintain a proper ionic balance between the intra- and extracellular compartments occurs either as a primary or secondary consequence of practically all types of cell injury. Primary damage of the plasma membrane is caused by viruses, bacterial toxins, complement reactions, cytotoxic lymphocytes (CTL), lipid peroxidation by chemicals such as carbon tetrachloride, etc. Secondary changes in membrane permeability can be caused by ischemia (loss of ATP; decreased activity of ion pumps) and
excess ROS, increased cytosolic Ca++. Damage to lysosome membrane causes release of enzymes into the cytoplasm and digestion of cellular components.

17. Types of cell death: comparisons between apoptosis and necrosis

The most common types of reversible cell injury are manifested by accumulation of fluid (cellular swelling) and of fat (fatty change). Irreversibly injured cells die and have altered morphology. These morphologic patterns are recognized as necrosis or apoptosis. Although necrosis is only recognized by morphologic changes occurring during and after cell death (i.e., enzymatic digestion, “coagulation”, etc.), apoptosis is an active (programmed) form of cell death that can be detected both by morphology and gene expression changes. Necrosis is always pathologic (the end point of irreversible injury). Apoptosis may be physiologic or pathologic.

<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
<td></td>
</tr>
<tr>
<td>Single cells</td>
<td>Groups of cells; disruption of tissue structure</td>
</tr>
<tr>
<td><strong>Cytology</strong></td>
<td></td>
</tr>
<tr>
<td>Shrunken cells</td>
<td>Generally swollen, enlarged cells</td>
</tr>
<tr>
<td>Cell fragmentation (apoptotic bodies)</td>
<td>Pyknotic or fragmented nuclei</td>
</tr>
<tr>
<td>Chromatin condensed in the periphery of nuclei</td>
<td>Dilated ER; high amplitude swelling of mitochondria</td>
</tr>
<tr>
<td>Generally morphologically intact mitochondria</td>
<td>Outline of the cell initially maintained</td>
</tr>
<tr>
<td><strong>Effects on Tissue</strong></td>
<td></td>
</tr>
<tr>
<td>No inflammation</td>
<td>Disrupted membrane permeability; leakage of cellular products into the blood</td>
</tr>
<tr>
<td>Phagocytosis by adjacent cells</td>
<td>Acute inflammatory response</td>
</tr>
<tr>
<td></td>
<td>Possible scar formation</td>
</tr>
</tbody>
</table>

18. Morphological aspects of necrosis

Five main types, recognized morphologically.

- Coagulation necrosis (typical necrosis after myocardium infarct)
- Liquefaction necrosis (necrosis involving tissue digestion; common in the brain)
- Fat necrosis (necrosis involving release of enzymes in tissues containing or surrounded by fat cells, such as the pancreas)
- Caseous necrosis (typical of tuberculosis)
- Gangrene (“dry” gangrene; ischemic injury in fingers, toes)

19. Mechanisms of ischemic/hypoxia cell death

Ischemic injury, i.e. reduced blood flow, is the most common type of injury in clinical medicine. It is generally caused by obstruction of an artery. Hypoxia is reduced availability of oxygen, generally caused by lower saturation or decreased amounts of hemoglobin. In ischemic tissues, there is loss of oxidative phosphorylation and depletion of ATP. Anaerobic glycolysis continues for a while, but stops after glycolytic substrates are exhausted. The switch to anaerobic metabolism is reversible and so are the
changes leading to mild cellular swelling. Prolonged ischemia causes irreversible damage to cell membranes causing cell death.
20. Release of intracellular proteins into the blood in myocardial infarction

- Creatine kinase an enzyme present in brain myocardium and skeletal muscle. Its isoforms containing the M and B subunits are differentially expressed among these tissues. The CK-MB isozyme is found predominantly in myocardium and begins to increase 2-4h after the onset of the infarction (CK-MM is predominantly in skeletal muscle; CK-BB in brain). CK-MB levels decrease 1-3 days after the infarct.
- Lactic dehydrogenase an enzyme released later than CK after infarction
- Troponins are proteins that regulate calcium-mediated muscle contraction. They are not normally found in the circulation, but increase after myocardial infarction, at about the same time as CK-MB. However, elevated levels of troponin persist for 7-10 days after the infarct (the troponin forms are Troponin I and Troponin T).

[Other examples of proteins released into the blood in tissue necrosis ischemic or otherwise: Exocrine pancreas, amylase; striated muscle, creatine kinase (MM isoform); liver damage, alanine aminotransferase (ALT) and aspartate aminotransferase (AST).]

21. Ischemia/reperfusion injury

Paradoxical cell death by restoration of blood flow after ischemic injury

Restoration of blood flow after an ischemic event rescues cells with reversible injury (reperfusion makes no difference if cells are irreversibly damaged). In some situations, however, cells die after reperfusion. Some important features of ischemia/reperfusion injury:

- Cells die after reestablishment of blood flow
- It is clinically important but amenable to therapeutic intervention
- Oxygen free radical and recruitment of polymorphonuclear leukocytes are the main mechanisms of injury, eventually leading to mitochondrial abnormalities
- Apoptosis is a major mechanism of death, although necrosis may also occur

22. Chemical injury

A very large number of chemicals can produce reversible and irreversible injury. The liver is the most common target for toxic injury. Toxic agents include chemicals such as CCl₄ and trichloroethylene, common substances such as alcohol, pharmaceutical agents including hundreds of drugs (for instance antidepressants, anti-convulsants, analgesics, non-steroid anti-inflammatory agents), and foods such as poisonous mushrooms. Chemicals may produce injury by direct interaction with cellular constituents or may require metabolic activation that produces the ultimate toxin. The toxin can be a free radical metabolite produced by the action of cellular enzymes on the chemical, or the metabolism of the chemical itself may generate excess ROS (reactive oxygen species). Examples of direct toxic agents are cyanide poisoning (blockage of mitochondrial cytochrome oxidase and oxidative phosphorylation), mercury chloride injury (binding to cell membranes causing cell permeability changes). Most chemicals are not biologically active but can be converted to toxic metabolites (metabolic activation). This conversion often involves the P-450 mixed function oxidases located in the smooth endoplasmic reticulum, most
prominently in the liver. Examples of indirect acting drugs are carbon tetrachloride and acetaminophen (known as paracetamol and commonly referred to as Tylenol).

23. Examples of clinically relevant chemical injury

<table>
<thead>
<tr>
<th>DRUG</th>
<th>EFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen (Paracetemol, Tylenol)</td>
<td>-Zonal hepatic necrosis</td>
</tr>
<tr>
<td>Halothane; isoniazid</td>
<td>-“viral hepatitis-like” may progress to acute hepatic failure</td>
</tr>
<tr>
<td>Alcohol</td>
<td>-Fatty liver, hepatitis, cirrhosis</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>-Hepatocellular cholestasis hepatic adenomas</td>
</tr>
<tr>
<td>Azathioprine; anti-neoplastic agents</td>
<td>-Hepatic veno-occlusive disease</td>
</tr>
</tbody>
</table>

Antibiotics (amphotericin B, etc.)
Metals (mercury, cadmium, bismuth, etc.)
Solvents (ethylene glycol, etc.)
Iodinated contrast agents
Anti-neoplastic agents (cisplatin, etc.)

24. Drugs metabolized by the P-450 system in humans

<table>
<thead>
<tr>
<th>Drug</th>
<th>Other Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amobarbital</td>
<td>Eucalyptol</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>Glutethimide</td>
</tr>
<tr>
<td>Barbital</td>
<td>Griseofulvin</td>
</tr>
<tr>
<td>Butobarbitone</td>
<td>Halogenated insecticides (primarily lindane and DDT)</td>
</tr>
<tr>
<td>Chloral betaine</td>
<td>Halothane</td>
</tr>
<tr>
<td>Chloral hydrate</td>
<td>Heptobarbital</td>
</tr>
<tr>
<td>Chlordiazepoxide</td>
<td>Meprobamate</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>Phenobarbital</td>
</tr>
<tr>
<td>o,p'-DDD*</td>
<td>Phenylbutazone</td>
</tr>
<tr>
<td>DDT Phetharbital</td>
<td>Phetharbital</td>
</tr>
</tbody>
</table>
25. **Mechanisms of carbon tetrachloride injury in the liver**

$\text{CCl}_4$ is a chemical that a) can produce both reversible injury (fat accumulation) and cell death; b) requires metabolic activation to become toxic; c) the toxic agent is a free radical metabolite ($\text{CCl}_3$) derived from $\text{CCl}_4$. The primary targets of $\text{CCl}_4$ injury are the plasma and ER membranes (lipid peroxidation of membrane fatty acids), mostly in the liver. $\text{CCl}_4$ injury is a prototype for damage produced by tetrachloroethylene and other chlorinated compounds of the same type.

\[ \begin{align*}
\text{CCl}_4 & \xrightarrow{\text{cytochrome p450}} \text{smooth endoplasmic reticulum} \\
& \xrightarrow{\text{lipid peroxidation}} \text{ER damage} \\
& \xrightarrow{\text{soluble lipid peroxides}} \text{damage to plasma membrane} \\
& \xrightarrow{\text{permeability changes. Influx of Na$^+$ and H}_2\text{O; K$^+$ efflux}}} \text{fatty liver} \\
& \xrightarrow{\text{massive influx of Ca$^{++}$}} \text{Necrosis}
\end{align*} \]
26. Biochemical targets of fat accumulation in the liver in CCL₄ injury

As discussed, CCl₄ induced injury results from the action of a free radical (free radicals are chemicals with unpaired electrons which can remove electrons from other substances). The P-450 system can also act on chemicals by forming electrophiles, substances that form covalent bonds with proteins. Acetaminophen and halothane injury is produced by this type of mechanism. Acetaminophen is metabolized in the liver. While these metabolic reactions involve chemical modifications that allow excretion of the chemical, about 5% of acetaminophen is converted by the P-450 system into a metabolite named NAPQI, which binds to SH groups in mitochondrial proteins and produces cellular damage. A second and important effect of NAPQI is oxidation of glutathione, which greatly increases NAPQI toxic effects. The acetaminophen effect on glutathione levels is the main target for antidote therapy in acetaminophen intoxication.

27. Acetaminophen intoxication

As discussed, CCl₄ induced injury results from the action of a free radical (free radicals are chemicals with unpaired electrons which can remove electrons from other substances). The P-450 system can also act on chemicals by forming electrophiles, substances that form covalent bonds with proteins. Acetaminophen and halothane injury is produced by this type of mechanism. Acetaminophen is metabolized in the liver. While these metabolic reactions involve chemical modifications that allow excretion of the chemical, about 5% of acetaminophen is converted by the P-450 system into a metabolite named NAPQI, which binds to SH groups in mitochondrial proteins and produces cellular damage. A second and important effect of NAPQI is oxidation of glutathione, which greatly increases NAPQI toxic effects. The acetaminophen effect on glutathione levels is the main target for antidote therapy in acetaminophen intoxication.

28. Apoptosis

This form of cell death is also known as **programmed cell death** because it requires the activation of signal transduction pathways and proteases that initiate and execute the process of cell death. Apoptosis can be physiological or pathological and often results in the elimination of abnormal or “unwanted” cells (it is as if these cells commit suicide by eliminating themselves through the activation of the apoptotic machinery).

Physiological apoptosis
- Destruction of cells during embryonic development
• Balance between cell death/proliferation in normal tissues
• Regulation of cellular populations in hormonally sensitive tissues

Pathological apoptosis
• Cell death after DNA damage caused by radiation, cancer treatment drugs
• Cell death caused by cytotoxic T cells; death of B and T lymphocytes
• Cell death caused by many viruses
• Cell death in tumors, in growing tumor but particularly during tumor regression
• Cell death in reperfusion injury

29. Mechanisms of apoptosis I: receptor and non-receptor responses

- Extrinsic Pathway (Death-receptor mediated)  Tumor Necrosis Factor (TNF) system
  Fas ligand/receptor system

- Non-receptor mediated: Radiation, ROS release, toxins, chemotherapeutic agents, among others
(Mitochondrial Pathway)

In receptor-mediated apoptosis, the process is initiated by the binding of the ligand to its receptor in the cell membrane. TNF binds to its type 1 receptor (TNFR1) while the Fas ligand binds to Fas. Both receptors have sequences called “death effector protein domains” that serve as docking sites for binding adapter proteins such as FADD (Fas-associated protein with death domain) and TRADD (TNFR adapter protein with death domain). In non-receptor mediated apoptosis, caspases (see below) are activated without the binding of a ligand to receptors.

Depending on the agent and the cell type, apoptosis is highly dependent on mitochondrial damage. Particularly important is the loss of mitochondrial membrane permeability, creating a “high-conductance channel” that causes the release of cytochrome c into the cytoplasm. Cytochrome c can initiate the cleavage of pro-caspases into active caspases.

30. Mechanisms of apoptosis II: the Bcl-2 gene family

Apoptosis (particularly that induced through the mitochondrial pathway) may be inhibited or promoted by several proteins. Prominent among these are the proteins of the Bcl-2 gene family. The Bcl-2 protein itself is an anti-apoptotic protein that can suppress apoptosis by protecting mitochondrial permeability, and in some cells, by binding to a pro-apoptotic protein Apaf-1 (apoptotic protease activating factor). The Bcl-2 gene was first identified by its overexpression in more than 80% of patients with follicular lymphomas. They carry a 14:18 translocation which brings together the IgH locus on chromosome 14 and the Bcl-2 gene in chromosome 18. Other examples of Bcl-2 family proteins:
- Bcl-X – antiapoptotic protein (the main antiapoptotic protein in hepatocytes
- Bad, Bax – proapoptotic agents

31. Mechanisms of apoptosis III: caspase activation
The adapter proteins bound to death-domain receptor, or the release of proapoptotic molecules such as cytochrome c through the mitochondria pathway, trigger the activation of caspases (cysteine proteases that cleave proteins at aspartic acid residues). There are many different caspases, which are present in the cell in inactive, precursor forms (pro-caspases). Some of these caspases (caspases 8 and 9) initiate the process (initiator caspases) while others, such as (caspase 3), deliver the final blow, and are known as executioner caspases).

32. Apoptosis – Summary Points

- Apoptosis is a actively regulated form of cell death.
- It has a role in biological processes, including embryogenesis, normal homeostasis and aging. It is an important component of many diseases, including cancer and immune-mediated processes.
- Most of the molecular pathways involved in death signals, and the regulation and activation of the effectors have been identified.
- Many existing and new therapies under development target the modulation of apoptosis

33. Alcohol-induced cell injury and chronic disease

Statistics – Excessive alcohol (ethanol) consumption leads to more than 100,000 deaths annually in the U.S. About 25% of these are from accidents caused by drunken driving; alcohol-related homicides and suicides accounted for about 20%. Alcohol is associated with the development of squamous cell carcinoma of the esophagus, chronic gastritis and pancreatitis and most particularly fatty damage and cirrhosis of the liver. In addition, alcohol consumption causes nervous system diseases including cerebellar degeneration and peripheral neuropathies. The mortality rate from liver cirrhosis generally parallels alcohol consumption by the population. It is estimated that there are more than 10 million chronic alcoholics in the U.S. and more than 5 million individuals who suffer some consequences of excessive drinking.

What is “alcohol abuse”? – it has been estimated that ingestion of more than 80g of alcohol/day constitutes alcohol abuse and that this dose can cause liver injury. Chronic liver disease (cirrhosis) is present in more than half of individuals who consume 200g or more of alcohol per day for about 15 years.

Note: 1 ounce serving of hard liquor (whiskey, gin, vodka, etc) contains about (11g) of alcohol. A 1 glass serving of wine (4 ounces or more) contains approximately 9-18g of alcohol. Beer cans generally contain 12 ounces with servings ranging form 6–16 ounces (6-16gm of alcohol). Using these data some researchers have calculated a cirrhosis dose50 (CD50), i.e. the daily alcohol dose that will cause cirrhosis in 50% of individuals. The estimated CD50 is roughly “15 pint-years” (“pint-year” being the consumption of a pint of whiskey or about 200gm of alcohol per day for one year). It has been suggested that for women the CD50 might be less, perhaps “15 half-pint-years. A concentration of 100mg/dl in the blood is considered as the legal definition for drunk driving in many states (in some it is 80mg/dl). To reach this level consumption of about 8 bottles of beer, 12 ounces of wine and 6 ounces of 100-proof whiskey is needed.
34. Diseases resulting from alcohol (ethanol) abuse
   1. liver damage (fatty liver, alcoholic hepatitis and cirrhosis)
   2. carcinoma of the esophagus, pharynx and oral cavity
   3. pancreatitis, gastritis
   4. impaired small intestinal absorption
   5. degenerative brain damage (particularly in cerebellum) and peripheral neuropathy
   6. muscle damage (skeletal and cardiac) and cardiomyopathy
   7. testicular atrophy, spontaneous abortion
   8. fetal alcohol syndrome (birth defects, mental and growth retardation)

35. Metabolism of ethanol
Most of ingested ethanol is metabolized in the liver, although some metabolism may occur in the gastric mucosa or distant organs such as the placenta or heart. There are 2 major biochemical pathways for metabolism of ethanol:
   a) alcohol dehydrogenase - a cytosolic enzyme
   b) microsomal ethanol oxidizing system (MEOS) – the main component is cytochrome P450 (isozyme CYP2E1) in the smooth endoplasmic reticulum
In both systems alcohol is oxidized to acetaldehyde and further downstream to acetate.

**Ethanol** \( \text{CH}_2 \text{CH}_2 \text{OH} \)

HEPATOCYTE

SMOOTH ENDOPLASMIC RETICULUM

CYTOSOL
Alcohol dehydrogenase

Increased function of drug metabolizing systems
Increased:
- Smooth endoplasmic reticulum
- P450
- Drug metabolism
Increased activation of:
- Hepatotoxins

NAD
NADH

- 2H

NADPH
NADP

Acetaldehyde

Aldehyde dehydrogenase
- Mitochondria
- Cytosol

Covalent binding to proteins: ROS release, membrane peroxidation

FAT Accumulation
↓↓Fatty acid oxidation
↑Fatty acid synthesis and esterification

36. **Metabolism through alcohol dehydrogenase (ADH)**
Microsomal induction explains the increased susceptibility of alcoholics to toxicity of other compounds metabolized to active by-products in the smooth endoplasmic reticulum - industrial solvents (carbon tetrachloride, bromobenzene), drugs (anesthetics, isoniazid, phenylbutazone, acetaminophen), carcinogens (aflatoxin, nitrosodimethylamine), and other toxic agents (cocaine). Similarly, drug catabolism may be accelerated in chronic alcoholics reducing the efficacy of these agents (coumadin, tolbutamide, propranol, rifampin). In contrast, acute use of alcohol inhibits drug catabolism thereby potentiating the effects of tranquilizers and barbiturates.
38. Drug alcohol interaction: chronic ethanol ingestion leads to increased metabolism of many drugs

Increased activity of the mixed function oxidase system (MFOS) is associated with increased rates of drug metabolism by the microsomal cell fraction in vitro and by increased rates of drug clearance in vivo. In man chronic ethanol ingestion has been found to increase rates of metabolism of pentobarbital, antipyrine, tolbutamide, warfarin, and meprobamate, the latter shown on this slide. The increased rates of drug clearance in vivo relate to ethanol-induced increases in the MFOS and perhaps to other factors, such as increased liver blood flow and increased supply of NADPH produced by ethanol.

39. Drug alcohol interaction: Decreased drug metabolism after acute alcohol

Drug metabolism in alcoholics is complex. For example, an acute large dose of ethanol may decrease the rate of metabolism of some drugs, as shown here. Therefore, the chronic heavy user of ethanol, also accustomed to using large amounts of sedatives, may inadvertently take a fatal overdose if the same amount of drug is ingested with a large dose of ethanol.
Furthermore, levels of cytochrome P-450 and activities of MFOS fall in actively inflamed or cirrhotic livers. If chronic heavy use of ethanol produces serious liver injury, tolerance to drugs, once enhanced by ethanol, may progressively decline.

**Note:** The effects of alcohol/drug interactions result in more rapid or slower drug metabolism. The biological effect produced by these interactions depends on whether the metabolism of the drug leads to detoxification or activation.

### 40. Alcohol induced liver disease: pathogenesis and pathologic consequences

- fatty liver
- alcoholic hepatitis
- cirrhosis

### 41. Fatty liver

- reversible liver injury; intracellular accumulation of fat in hepatocytes causes liver enlargement with no clinical symptoms.
There are several biochemical mechanisms responsible for fat accumulation induced by alcohol (see diagram, next page). The most important factor is decreased oxidation of fatty acids:

### 42. Alcoholic hepatitis and fibrosis

- potentially reversible liver injury; localized cell death of hepatocytes; intracellular accumulation of fat and alcoholic hyalin around central veins (Mallory bodies) in hepatocytes. Neutrophils around foci of necrosis. Symptoms - fever, liver tenderness, jaundice.
There are several suspected mechanisms responsible for hepatocyte necrosis (nutritional deficiency has been eliminated as a cause): mitochondrial injury, toxicity due to acetaldehyde (protein cross-linking, and formation of...
free radicals). Hepatocyte cell death might occur through an immune mediated mechanism which activates the FAS apoptotic pathway.

43. **Alcoholic cirrhosis** – a stage of irreversible liver damage, generally in the form of micronodular cirrhosis (fibrosis between small regenerating nodules of hepatocytes) generally with fatty change. This is a serious disease accompanied by muscle wasting, weakness, ascites, and a tendency for massive gastrointestinal hemorrhage (esophageal varices).

Fibrosis may develop starting around central veins. The mechanisms responsible for the fibrous scarring of cirrhosis are not well known. The earliest lesions begin around the central vein. This is followed by perisinusoidal fibrosis, perhaps due to the activation of stellate cells (mesenchymal cells located in the space of Disse). These cells are the major source of collagen in liver fibrosis and cirrhosis.

44. **Survival and outcome of chronic alcoholism**
SURVIVAL AFTER DIAGNOSIS OF ALCOHOLIC CIRRHOSIS

- 93 Patients stopped EtOH (63.0% survival)
- 185 Patients continued EtOH (40.5% survival)

Percent Survival vs. Years Following Diagnosis
Clinically Relevant Questions

1. A 17-year-old male infected with hepatitis A experiences some mild nausea for about a week and has very mild scleral icterus. Laboratory findings include elevations in the blood levels of the hepatic enzymes aspartate transaminase (AST) and alanine transaminase (ALT). What is the source of the enzymes and the injury that causes their increase in the blood?

2. A 54-year-old male experienced the onset of severe chest pain. An electrocardiogram demonstrated changes consistent with an acute myocardial infarction. He was given thrombolytic therapy with tissue plasminogen activator (tPA). However, his serum creatine kinase increased after this therapy. What is the most likely explanation for the enzyme activity increase?

3. A 51-year-old male has a blood pressure of 150/95 mm Hg. If this condition remains untreated for years, which cellular alterations may occur in the heart?

4. A 38-year-old woman experienced severe abdominal pain with hypotension and shock that led to her death within 36 hours. At autopsy fat necrosis was found in the mesentery. What is the most likely disease condition in this patient?

5. Absorption of radiant energy, such as x-rays, can result in cell injury. What are the cellular mechanisms that may protect against this injury?

6. A 32-year-old male experiences “heartburn” with substernal pain from reflux of gastric contents into the lower esophagus. After many months, a biopsy shows changes in the esophageal epithelium. What pathologic alterations may have occurred?

7. A young patient is admitted to the Emergency Room 24h after ingesting 15g of acetaminophen. The patient is treated with N-acetyl-cysteine (NAC) and recovers. Why was NAC given?

8. A young adult male patient is admitted to the Emergency Room 96h after ingesting 15g of acetaminophen. The ER doctors decide that treatment with NAC is not appropriate. Why?

9. In a chronic alcoholic would the effects of 1) meprobamate (a tranquilizer) and 2) acetaminophen be increased or decreased?

10. What is the relationship between the Bcl-2 gene, a gene that can prevent apoptosis, and follicular B-cell lymphomas with chromosome translocation?
PERSONAL HEALTH; A Price to Pay as Autopsies Lose Favor

By JANE E. BRODY

A Roto-Rooter operator collapses and dies while working on a drain. Assumption; a heart attack. A seemingly healthy baby dies in his sleep. Assumption: sudden infant death syndrome. An elderly Kentucky woman known from C.T. scans to have malignant-looking lesions in her brain dies before undergoing exploratory surgery. Assumption: metastatic cancer. A 43-year-old Connecticut man with shortness of breath, a cough, positive skin test for tuberculosis and multiple clots in his lungs dies before a biopsy can be performed. Assumption: tuberculosis.

But when autopsies were performed on these people, quite different stories emerged. The Roto-Rooter operator was electrocuted, a finding that may have saved the lives of others using the faulty equipment and that resulted in double indemnity insurance for his survivors. The baby succumbed to previously undiagnosed meningitis, prompting protective vaccinations of other children in the family and the neighborhood. The woman turned out not to have cancer at all but rather abscesses in her brain resulting from an advanced case of periodontal disease. But the Connecticut man did have cancer that had spread from his pancreas.

Forensic pathologists, the physicians who perform autopsies, are quick to relate numerous tales of mistaken diagnoses, missed diseases and wrong assumptions, any of which can, if undetected, undermine the quality of future medical care and leave survivors racked with guilt, deprived of deserved compensation or vulnerable to preventable diseases.

Yet the rate of autopsies in the nation’s hospitals has been declining for decades. In many institutions, the rate is so low — no more than 5 percent to 10 percent of all deaths — that it is no longer possible for the living to know what has killed the dead. As the value of autopsies is described. In many institutions, fewer than 3 percent of deaths prompt an autopsy, and the autopsy rate is even lower in nursing homes, where at most 1 percent of deaths result in autopsy. Many new hospitals do not even have autopsy rooms.

When an out-of-hospital death occurs suddenly, unexpectedly or results from unusual or violent circumstances, in most states it is up to the county coroner or medical examiner to decide whether an autopsy is warranted, although some states mandate that autopsies be done in certain situations. Dr. Grigory Davis, a forensic pathologist at the University of Kentucky, says about half of unusual or suspicious deaths turn out to be due to natural causes. That information can provide valuable medical information as well as emotional relief for survivors.

Why the Decline

Financial pressures have prompted many hospitals, which traditionally have covered the cost of autopsies as an operating expense, to discourage or abandon them entirely. As Dr. Davis put it, “in a lot of modern hospitals, doctors are told indirectly that doing lower autopsies means more money is available for other things.”

When survivors request an autopsy, some hospitals now charge the family, which may think twice when told that the minimum cost is $2,500.

Another factor is the advent of advanced diagnostic tools like C.T. scans, M.R.I.’s and P.E.T. scans. That technology sometimes causes people to assume falsely that nothing more of value can be learned from an autopsy. But, as the Kentucky woman’s case demonstrated, no test can replace the accuracy of “looking for oneself,” the literal meaning of the Greek-derived word autopsy.

Time pressures also are a factor. Dr. Davis says hospital-based pathologists today are dealing with a workload at least 50 percent greater than that of two decades ago. A proper autopsy takes a minimum of two hours, which may not seem like a hardship when families of living patients are waiting impatiently for biopsy results.

Some doctors may be reluctant to request an autopsy for fear that the findings may result in a lawsuit. But Dr. Davis said: “Ninety-five percent of the time, a good autopsy dispels any notion of malpractice suspected by the family. In 15 years, I’ve seen many more lawsuits thrown out because of autopsy findings than won.”

Survivors may be reluctant to request an autopsy because of mistaken beliefs about what is involved. An autopsy does not disturb the body in any way apparent to mourners viewing an open coffin. An autopsy cannot increase the suffering of someone who is dead. And an autopsy does not interfere with embalming and need not delay a funeral by more than a few hours.

Value of Autopsies

At the societal level, accurate data on the causes of death are critical for planning a health care system responsive to the needs of the people. Without knowing why people die, it is not possible to provide the facilities needed to care for the living.

Through autopsies, Legionnaire’s disease and AIDS were discovered, and the “cold coronary” was revealed to be a choking death that could be averted by the Heimlich maneuver. Autopsies of crash victims have led to improved safety standards in cars and planes. Autopsies have also alerted officials to growing threats of infection or drug addiction. And sometimes car “accidents” are discovered in autopsies to have been suicides or even homicides.

“A good autopsy uncovers the true disease process,” Dr. Davis said. “In 20 to 40 percent of cases, the autopsy finds a diagnostic discrepancy between the medical diagnosis and the actual disease that may help improve the care of future patients.”

For example, in a Connecticut study of 272 randomly selected autopsies and their corresponding death certificates, Dr. William H. Harriman, executive vice president of the American Board of Pathologists, said, “In 28 percent of the deaths, a major disagreement on the underlying cause of death led to a reclassification of the death in a different major disease category.”

He added: “An additional 28 percent of deaths were attributed to a different specific disease. And of those, 14 percent would in all probability have led to a change in clinical management that might have resulted in cure or prolonged survival.”

For the survivor, knowing the real cause of death may help them avert a similar fate. For example, a man who dies in an automobile accident may have suffered a heart attack before losing control of the car. He may alert his friends and offering to an increased risk of heart disease and prompt them to pursue heart-healthy living habits. Or the autopsy may reveal a hereditary condition that may influence family decisions about future childbearing.

If malpractice is a possibility in the death, the autopsy can provide the facts the family needs to win a lawsuit or negotiate a settlement. Or the findings may increase an insurance award, as in the case of the Roto-Rooter worker.

In other cases, autopsy findings can save the guilt family members and friends may harbor. For example, when a man dies while shoveling snow, his spouse may berate herself for not having cleaned the walk, until the autopsy reveals an advanced heart condition that could have caused his death at any time.