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The laboratory part of this course has a large and important online component. All of the exercises and slides can viewed from the computer using the virtual microscope feature. **Please note, you must be online for the virtual scope part of the labs to work.**

Please check to be sure your computer has the plug-ins needed to support the online microscope slide and movie viewing. If you need the plug-ins, they can be found on the accompanying disc and website. Choose ‘system requirements’ to add what you need.

Additionally, at the end of each laboratory you will find a question or two to be answered and emailed to the course’s email account. Don’t overlook this part of the labs.

The web site and your disc are essentially the same. Here’s the URL of the course website:

http://www.opt.indiana.edu/v543/main.html
Defining the issue

Pathology is the branch of medical science that studies the macroscopic, microscopic and physiological aspects of disease and injury. Historically, microscopic analysis has yielded our greatest understanding of the mechanisms of injury and our body’s efforts to repair and heal itself. Even in this day of computer imaging technology and flashy DNA studies, the microscope continues to be the most important tool in understanding disease processes. It should be no surprise, therefore, that we begin our study of the most universal of bodily responses to injury at the cellular level.

It has been observed that “the normal cell, the adapted cell, the injured cell and the dead cell represent hazily delimited states along a continuum of function and structure.” In part, the variability of the body’s response to injury is determined by the nature of the noxious or stressful agent. In the field of pathology, it is often useful to categorize types of injuries or disease by common mechanisms of action. The principal is this: if one understands the mechanism of injury for a particular class of disease, that knowledge can be directly transferred from one organ system to another. For example, if you understand how the lack of oxygen (hypoxia) causes injury to myocardial cells, and what that injury looks like microscopically, that knowledge can be directly transferred to similar situations in the kidney, brain, spleen and so on. In other words, learning a particular mechanism of injury once is going to carry you a long way.

Although there are a number of ways to categorize disease processes and types of injury, the one that makes the most sense to us is the following. Of course, you’re free to come up with your own.

Congenital

- Those things that you are born with, or have the trait that will lead to a disease later in life.
Acquired

- Injury or disease resulting from infectious agents, viruses, bacteria etc.
- Injury or disease brought about by the uncontrolled response of your immune system.
- Disease or injury resulting from problems of nutrition.
- Disease or injury resulting from unregulated cell growth, i.e. neoplastic disease (tumors).
- Disease or injury resulting from inadequate oxygenation (hypoxia) of a particular organ or region of the body, i.e. vascular disease as in the case of a heart or stroke.
- Disease resulting from the action of chemical and toxic agents as well as adverse environmental influences.
- Physical injury and trauma.
- Psychological and emotional factors, which may effect healing and the body’s general ability to maintain a state of good health.

Within each of these categories, there is a predictable and recognizable set of biochemical and functional changes that precede and induce the structural alterations. Keep in mind that the body’s response to injury is part of a “planned” and “normal” physiological process. That is, it is an ongoing, coordinated and active response on the part of the body to eliminate the injurious agent or process, and repair the damage. In its most elemental form, the processes of inflammation and healing are designed with one over riding goal: to keep your genes in the gene pool.

The purpose of this laboratory is to help you become familiar with the appearance of the cellular constituents that are part of the inflammatory response. This is likely to prove a more challenging task than you might think. The cells of the initial inflammatory response are often markedly altered by the processes in which they participate. The typical clean and recognizable form of a neutrophil, such as you might have seen in a histology atlas, is about to become a thing of the past. And even more important than recognizing the individual actors in the inflammatory response will be appreciating the overall picture and milieu of which they are a part. Taking a good look at the microscopic slide on low power is the always the place to start. You need an overall, bird’s eye view of the battle field to know what your warriors are up to. Only after a thorough low power inspection should you move to higher power magnification to determine the principal inflammatory cell in the action. By the time you have finished this laboratory exercise, you should be familiar with several important terms and the corresponding principal cellular constituents: (1) acute inflammation, (2) subacute inflammation and (3) chronic inflammation.
Mechanism of the inflammatory response

OK, here’s the hot and sticky item that few people understand about the inflammatory response: it is a response of VASCULARIZED tissue to injury. In real terms, it is the response of the microscopic blood vessels themselves, in the immediate vicinity of the damage or injury, that identify the area of injury for the inflammatory cells. After all, the blood vessels are the highways that will bring your warriors and agents of repair to the site of the conflict. The blood vessels must signal the inflammatory cells in the flow of blood where the battle is being waged. “This is the place to get out of the blood stream, get into the tissue space and go head to head with whatever is causing the injury.” Clearly, the blood vessels must dilate and become hyperpermeable to allow your warriors (your inflammatory cells) to get out of the vascular flow and trek off into the interstitial space in search of adventure.

All of the local, so called “clinical,” signs of inflammation are actually a direct result of the vascular response to injury. These are signs and symptoms that everyone has seen and felt. Consider, for example, a “zit” (a local skin infection secondary to Staphylococcus) right on the end of your nose. Practically everyone can recite the associated cardinal signs of this type of injury and its inflammatory response, very likely from personal experience. There will be redness (rubor), swelling (tumor), pain (dolor), increased local temperature (calor or heat) and possibly altered or loss of function (funtio laesa). It’s easy to imagine how these local changes in the skin of your nose, brought about as part of the inflammatory response, are all related to the action of the local vasculature. The redness and increased skin temperature are due to increased local blood flow secondary to vascular dilatation. The swelling, and to some extent the pain, is a direct result of increased vascular permeability. In this case it’s desirable to encourage fluid and inflammatory cells to exit the vascular compartment and go into the tissue space. This local alteration of the fluid balance allows for more working room for your inflammatory cells to do battle with the invading bacteria. The purpose, of course, is to stop the invaders at the front door. Remember the prime objective for your inflammatory cells is: to keep your genes in the gene pool.

The outward local manifestations of the inflammatory response are in fact part of complex and well orchestrated set of changes in the local vasculature. The changes occur in the microscopic sized blood vessels, mainly capillaries and venuoles. These vascular changes bring about the cellular response and eventually set the stage for healing and repair. Here’s a basic summary of what happens:

Exudate formation, the local vascular response to injury

- Vasodilatation (this is the big ticket item due largely to local chemical mediators).
- Increased local vascular permeability (let the fluid and inflammatory cells out).
- Exudation of fluid and inflammatory cells at the site of injury.
Response of inflammatory cells

- Margination  (The inflammatory cells floating by in the blood stream begin to stick to the walls of the vessels in the vicinity of the area of injury.)
- Emigration  (The cells exit the vascular compartment and go into the tissue space where the real action is.)
- Chemotaxis  (The first inflammatory cells on the scene notify their buddies this is the site of the battle.)

Healing

- Resolution  (The removal of the debris and dead cells at the site of the battle by the neutrophils and macrophages.)
- Healing typically proceeds along one of two paths:
  1. Healing by regeneration (i.e. the proliferation of parenchymal cells), assuming the underlying frame work of the organ is intact. Examples would include proliferation of the corneal epithelium or regeneration of damaged liver tissue.
  2. Healing by scarring i.e. proliferation of granulation tissue with collagen deposition. (Here the body is simply binding the broken parts back together.)

The process of inflammation is divided into two main categories based on two important factors: (1) the time and duration of its development and (2) its histological make up. The two principal divisions are acute and chronic. You will need to be very familiar with the microscopic appearance and circumstances in which we find these two distinct patterns of the inflammatory reaction.

**Acute inflammation** arises rapidly (minutes), and is characterized by the **exudation** of neutrophils, fluid, electrolytes and proteins at the site of injury. The term **exudate** is important to understand. The exudate is the protein and inflammatory cell rich “soup” that develops at the site of injury as a consequence of the dilatation and increased permeability of the local, microscopic size, vasculature. These changes associated with the inflammatory response are normal and expected. Your genes would not be in the gene pool for very long if it were not for this vital element of our healthy physiology. Yes a zit is painful, red and swollen, but that’s normal, reparative physiology at work on your behalf. The next time you’re aching, thank mother nature, she’s looking out for you.
Chronic inflammation arises and progresses more slowly (days or weeks), and sometimes is an extension of a prolonged acute inflammatory episode. Chronic inflammation is characterized by three important and consistently seen histological features: (1) a cellular infiltrate at the site of injury composed chiefly of mononuclear cells (lymphocytes, plasma cells and macrophages); (2) parenchymal tissue destruction and (3) proliferation of fibroblasts with much collagen production, in other words scar formation.

Our cellular warriors, a brief summary

Our inflammatory cells are categorized largely by the appearance of their nuclei and to a lesser extent by the granularity and staining properties of the cytoplasm. These cells are broadly classified as white blood cells. Obviously, in stained tissue sections or blood smears the “white” cells are shades of blue and pink and not white at all. They come by the name because of their collective gross appearance in a centrifuged sample of unclotted blood. When one centrifuges a small tube of anticoagulated blood, the red blood cells sediment to the bottom rapidly, leaving the clear plasma on top. Right at the red cell and plasma interface one can sometimes see a paper-thin layer of white or cream colored material. This is the concentrated layer of WBC’s (called the Buffy coat). The term “white blood cell” comes about because of this white layer. It’s a term that has been with us for practically a hundred years and predates the general use of the microscope. So much for the history lesson, here’s a brief outline of the major inflammatory cell actors.

Polymorphonuclear leukocytes (abbreviated as PMNLs): The PMNLs (or granular leukocytes) make up 56 to 63 percent of all white blood cells in the circulating blood. They are characterized by a lobated nucleus that is separated into 2 to 3 definitive lobes with a very narrow filament or strand connecting the lobes. They have cytoplasmic granules, the nature of these granules being the basis of their subclassification into groups such as neutrophils, eosinophils, and basophils. In general, these cells are about twice the size of a red blood cell.

1. **Neutrophils (PMNs)** have a characteristic multilobed nucleus and fine, pink-staining cytoplasmic granules. These make up 55 to 60 percent of our peripheral blood white cell count. These cells are actively motile and can pass through the blood vessel walls. The vigorous phagocytic activity of these cells provides the an important method of eliminating injurious material from the body.

2. **Eosinophils** are usually bi-lobed. They contain numerous coarse, bright pink staining cytoplasmic granules and comprise 1 to 3 percent of the white count. Their numbers go up in response to allergic reactions.
3. **Basophils** have a relatively light staining and a V- or S-shaped nucleus. The cytoplasmic granules are unevenly distributed and vary in number, size and shape, but are generally dark blue or black. Basophils are rarely seen and make up only about 0.5 percent of the white count.

4. **Mast cells** (fixed tissue basophils) are found scattered in many tissues. They are 3 to 4 times the size of other PMNLs and are irregular in shape. The nucleus of a mast cell is small and round and usually obscured by the many coarse, dark granules filling the cytoplasm.

**Lymphocytic inflammatory cells:** lack cytoplasmic granules and comprise 25 to 33 percent of the peripheral white blood cell count. Among the lymphocytes' most important functions are: (1) the production of antibodies and the maintenance of immunological "memory," (2) modulation and regulation of some elements of the immune system, and (3) tumor surveillance and fighting complex infectious organisms, such as tuberculosis and fungi.

There are two populations of lymphocytes in the circulating blood: “T” cells (lymphs involved in cellular immune functions) and “B” cells (lymphs involved in antibody production). The B cells, when stimulated by antigen, transform into plasma cells. Plasma cells are not normally present in the circulation, but are formed at the site of injury by the differentiation of the B-lymphocytes.

1. **Lymphocyte** is used generically for both B and T-lymphocytes. The T-lymphocytes vary some in size and are generally round to oval in shape. The cytoplasm of the lymphocyte is often very difficult to see, and generally consist of a thin transparent blue rim around the nucleus. The nucleus is almost always round but may have an indentation. Generally lymphocytes lack cytoplasmic granules and these cells are not phagocytic.

2. **Plasma cells** (end stage B-lymphocytes) are differentiated B-lymphocytes, transformed in response to the presence of an antigen. The plasma cell is round to oval in shape and has an eccentrically located round nucleus and often there is a little half moon shaped area of clearing near the nucleus which identifies the golgi apparatus. Often, chunks of chromatin are redistributed radially along the nuclear membrane, forming a "cartwheel-like" staining pattern. The cytoplasm is often a homogenous pale purple, and the overall appearance of the cell is that of a purple "fried egg" with a dark speckled yolk.
Mononuclear phagocytic cells: Mononuclear phagocytes have few cytoplasmic granules and comprise 3 to 7 percent of the peripheral white blood cell count. These cells are the principal agents of the so-called reticuloendothelial system. The mononuclear phagocytes include (1) the monocyte (found circulating in the peripheral blood) and (2) the macrophage or phagocytic histiocyte found in parenchyma of the organs and the connective tissue (technically speaking “outside” of the blood vessel).

1. **Monocytes** are slightly larger than neutrophils. The cytoplasm of these cells stains weakly and has a dull gray-blue, watery appearance. Its granules are fine, lightly stained, and give the cytoplasm a “ground-glass” appearance. The nucleus is usually round or "kidney bean" shaped, and shows a “net-like” fine reticular staining pattern. The blood monocytes are a population of WBC's that are on their way from the bone marrow to their ultimate sites of activity in the tissues. After leaving the blood and entering various tissue sites, the monocyte transforms into a macrophage and becomes an avid phagocytic cell. These guys are truly the garbage collectors of the immune system.

2. **Macrophages** are divided into "fixed" and "wandering" forms. The macrophages are larger than monocytes and have a bewildering array shapes. Some are round, some are oval and others elongated. It is often the nuclear morphology we use to identify these cells. Their cytoplasm is light blue or bluish-gray and frothy, and the nucleus is eccentric and relatively small and dark staining. The cytoplasm often contains remnants of phagocytized material.

3. **Epithelioid histocytes** (macrophage) are cells which have been transformed into epithelial looking cells. The cell is really not epithelial in nature, it has only taken that appearance. It is a type of cell which is characteristicly identified with granulomatous inflammation. With H & E preparations, the epithelioid cell shows a pale pink granular cytoplasm with indistinct borders that appear to be merging the neighboring cells. The nucleus is not as dense as that of a lymphocyte, is generally oval or elongated and may show folding of the nuclear membrane.

4. **Giant cells** represent the coalescence of many macrophages and the overall appearance is that of one heck of a big macrophage with lots of nuclei. These cells are part of the response to an agent the body cannot eliminate or has difficulty destroying. They are an expected part of the specialized form of the chronic inflammatory pattern known as a “granuloma.” The giant cells may be 40 mm to 50 mm in diameter, and the cytoplasm may contain as many as twenty nuclei. There are two basic varieties:
   - **Langhans type** is said to be characteristic of tuberculosis, but has been seen in other granulomatous reactions. Nuclei, which are very small, are usually arranged at the periphery of the cell producing a horseshoe pattern within the cytoplasm.
• **Foreign body type** giant cells also have numerous nuclei, but unlike the Langhans type, they are more or less scattered randomly throughout the cytoplasm.

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## Classification of Inflammation

### A. According to inflammatory cell type

1. **Acute**: Polymorphonuclear leukocytes make up the majority of the infiltrate.
   
   a. Suppurative: With cellular and nuclear debris (necrosis) and PMNs
   b. Non-suppurative: Diffuse infiltration with PMNs, without debris (without necrosis)

2. **Chronic**: So called round cell (“mononuclear”) infiltrate, consisting largely of lymphocytes, plasma cells and macrophages.
   
   a. Granulomatous: Epithelioid histiocytes, lymphocytes, plasma cells
   b. Non-granulomatous: Diffusely scattered chronic inflammatory cells

3. **Subacute**: A stage of inflammation which, from a histological stand point, falls somewhere between acute and chronic. It usually occurs in low-grade, chronic inflammatory conditions. Both lymphocytes and polymorphonuclear cell infiltrates are present. However, no granulomatous features are seen.

### B. Classification of inflammation by the morphology of the tissue reaction

1. **Exudative** (Classification by the appearance and principal constituent of the exudate.)
   
   a. Serous: Major component—Water, electrolytes and proteins
   b. Fibrinous: Major component—Fibrin
   c. Hemorrhagic: Major component—RBC's
   d. Purulent: Debris and PMN cells, usually seen in bacterial infections

### C. Classification by type of repair and regeneration possible in a particular organ

1. Tissue types and/or organs that normally are undergoing constant regeneration or at least the cells have the capacity to begin dividing again to restore their numbers and function. The critical factor here is that the underlying framework of the organ must still be intact. Examples include epithelial surfaces, RBC’s, hepatocytes and renal tubular epithelium.
2. Repair by scarring or proliferation of granulation tissue. Granulation tissue is a generalized, “all purpose” kind of tissue that is called into existence at the site of injury that has been sufficiently severe that complete restoration of the organ is not possible. It is composed of fibroblasts, newly formed capillaries, and inflammatory cells which eliminate cellular debris. This type of repair is seen in cardiac muscle following a “heart attack.”

D. Classification by special factors unique to the tissue involved; e.g. appendix (appendicitis), iris (iritis), ciliary body (cyclitis). Here we are looking essentially at nomenclature, and not actually a unique form of inflammatory response in a particular organ.

E. Sometimes we even classify patterns on the basis of their source. That is to say whether the primary source of the injury came from within our own bodies, as in the case of autoimmune injury that we see in rheumatoid arthritis.
   1. Exogenous agents
   2. Endogenous agents

After reviewing the morphology of the inflammatory cells, we can begin to associate particular cells with particular functions and types of inflammation. The neutrophils are the primary cells in your body’s response to an acute infection, such as the zit we developed a few pages back. These cells are our front line warriors. They are the most motile of white blood cells and arrive first at the site of inflammation. They vigorously phagocytize all types of injurious materials, but in time disintegrate and may give way to the slower moving, but more methodical, inflammatory cells—the macrophages and lymphocytes. These latter cells often, but not always, appear in the closing stages of acute inflammation. Their purpose is to finish the clean up and set the stage for repair. As you now know, lymphocytes and macrophages are the predominant cell type seen in chronic inflammation.

The lymphocytes' role in chronic inflammation is not well established, though we do know the lymphs have an immunological function. On the other hand, the macrophages are actively phagocytic. They are the "scavenger" cells and act to remove not only the bacteria, but also the dead and dying neutrophils, as well as the dead parenchymal cells of the damaged organ.

It may help to understand why the lymphocytes and plasma cells are the most common cell types of chronic inflammations if we remember that they are the agents of humoral and cell mediated immunity. When there is inflammation of long duration, there is a release of cellular antigens and these antigens provide the immunologic component for the chronic inflammatory response.

Exudation and edema (swelling) are characteristic features of the inflammatory response. They are always present in the acute inflammatory reaction, and may persist into the chronic
stage (that is if there is one to follow the acute inflammatory response). The nature and amount of exudate generally depends on the intensity and the type of the injury. A severe injury, especially those involving infectious agents, produce an inflammatory exudate having a high specific gravity (1.020). This “inflammatory soup,” consists of inflammatory cells, electrolytes, water and protein, may have as much as 2 gm to 4 gm percent protein. It is important to remember that the exudate is part of the healthy physiological response to injury, and is going to resolved once the injurious agent has been dealt with. An exudate differs substantially from another kind of edema which is not associated with a local response to injury. This other type of edema is of noninflammatory origin and is referred to as a transudate.

Transudates will be discussed in greater detail when we consider cardiovascular and hemodynamic disorders, but suffice it to say that they are low specific gravity (<1.012), ultra filtrates. A transudate develops when the internal hydrostatic pressure of the circulating blood (that is the blood pressure generated by the pumping action of the heart, or the “back pressure” in the veins) exceeds the capacity of the proteins and other solutes in the blood (the elements producing the counter balancing oncotic pressure) to keep water and electrolytes in the blood. When something alters this normal balanced state, such as vascular congestion seen in a person with heart failure or if the proteins in the blood should drop to a level that they no longer exert a balancing force to the hydrostatic pressure, much of the water in the blood will be literally strained through the capillary and venuole walls, producing tissue swelling. A fast demonstration of this principal would be to eat a whole bag of salty potato chips by yourself and then drink a gallon of water. Wait a few hours and try to take off your rings. I think you get the picture. All this excessive tissue water is a transudate, not an exudate, and the result of shifting water and sodium from the vascular system into the tissue space. In other words, it’s noninflammatory edema.
Inflammation Slides

Keeping in mind the characteristics of acute, subacute, and chronic inflammation, we will examine several slides demonstrating various stages of the inflammatory process. Remember, use low power first to get your bearings and then go for the cellular constituents. All of the cells and stages of inflammation we have discussed are represented in these tissues.

A. General information on viewing slides

1. First, be sure you can identify the tissue you are studying. If you have difficulty or are not sure if you have seen a "normal" example of this tissue, please check with your lab instructor.

2. Identify the different inflammatory cells, but at the same time try keeping the overall picture in mind.

3. Make a list describing the structural changes that you see on the slide. This may sound like a “make work” project, but it’s really instructive. In the process of writing your observations you will have to review what you have seen and do a mental check what might be left out. Besides, when you are a practicing optometrist the written medical record you will generate is going to be real important. This is a good exercise to learn how to render in the King’s English what you see. It’s not as easy as you might think.

4. Compare your observations with your friends.

Today’s slides: Please examine the following slides. Make drawings and label what you see. Photographs are provided to help you orient yourself.

#87 Appendicitis
#95 Pneumococcal meningitis
#76 Sjogren Syndrome
Slide #87 acute appendicitis

For tissue 87, you can only view the round, cross section with the virtual scope. Even so, all the features of acute appendicitis are evident. Look for the PMNs in the muscular wall and serosal fat.

- Yes, there will be plenty of lymphocytes, but remember that they normally live in the submucosa throughout the bowel.

The PMNs may not be easy to recognize in all situations. They are dying too, and as such show changes of necrosis.

Your observations
**Slide #95 acute bacterial meningitis**

This is a very unfortunate case. **Tissue 95** is of the brain and meninges of a young child that died with acute bacterial meningitis. The subarachnoid space is grotesquely expanded with a marked acute inflammatory exudate. PMNs are everywhere. The little square in the adjacent image shows where the picture below came from.

In the combined image to the right, you see the brain tissue in the top, right corner. Most of the field is subarachnoid space containing millions of PMNs. The insert shows a high power view with the typical three lobed appearance to most of the PMNs.

Your observations
**Slide #76 Sjogren syndrome**

*Tissue 76* may be hard to recognize as salivary gland, or much of anything for that matter. The autoimmune infiltrate of lymphocytes has totally destroyed the glandular portion of this tissue. All that remains are a few remnants of ducts. The little box indicates where the image below is from.

Here we see the gland is totally overrun by the aggressive and misdirected lymphocytes of this autoimmune condition. The insert and adjacent image shows the chronic inflammatory infiltrate consists almost exclusively of mature appearing lymphocytes. On the course website and your CD, there is a link telling you about *Sjogren syndrome.*

Your observations
The Repair Part of the Story

As we’ve just learned, the inflammatory reaction is part of the normal physiological response to essentially all forms of injury. But the inflammatory process does not work in isolation and should really be considered the beginning of repair and healing. Obviously, once the injurious agent has been contained and/or eliminated, the damaged organ must be restored to function. And, as we will see, the processes of repair begin almost at the same time as the events of inflammation. In real terms, the actors involved in repair don’t wait for the inflammation to die down or even get out of the way. Repair and restoration of function begin almost immediately after the site of injury has been identified.

The processes of repair may follow one of two paths depending on the organ injured and the extent of damage. Some organs are capable of substantially or completely regenerating themselves, where as others have almost no capacity to replace dead parenchymal cells. Consider red blood cells. Your body is constantly making new ones, so if you should lose some (for example you donate a pint of blood to the Red Cross), in a few weeks you will completely replace your lost red cell buddies. On the other hand, neurons have no capacity to replace themselves. When one dies, it’s lost for good. A person who has sustained a “stroke” has lost the affected brain tissue permanently.

Some organs have parenchymal cells that are normally pretty quiescent, but these cells can be induced to divide and, to a substantial extent, regenerate (or re-populate) the area of damage. The critical factor in this latter scenario is this: the underlying framework of the organ must remain intact. There must remain the reticular structure for the parenchymal cells to re-populate into. If the reticular and fibro-connective tissue framework of the organ is missing (blasted away by the injurious insult), then the dividing parenchymal cells don’t know how to organize themselves into a healthy and functional unit. A good example of successful regeneration is that of the liver following a bout of viral hepatitis. As long as the underlying framework of the liver is intact (and of course some of the liver cells remain, I mean after all, you’ve got to have Adam and Eve to get things restarted), the surviving hepatocytes can be induced to undergo division and effectively regenerate the damaged portion of liver. Unfortunately, not all organs fall into this category.

If regeneration is not an option for an organ, then repair follows another path. Consider damage to cardiac muscle in the event of a coronary artery occlusion. The cardiac muscle deprived of oxygen is going to die within a few minutes. In short order, the processes of inflammation will go to work and the dead muscle will be removed. Unfortunately, cardiac muscle cells lack the capacity to divide and replace lost members. In this case, the continuity of the remaining healthy myocardium will have to be maintained by the development of scar tissue to replace the lost muscle. Effectively, the body is trying to “bind” the “broken” members back together. There is no pretense that full function will ever be restored, rather, the body knows that what remains of the damaged organ is all it has to work with. This type of repair
could be likened to creating a collagen “patch” to fill a gap. Consider again the problem of the heart with dead muscle, things will get pretty “messy” if a hole in the myocardium opens when the dead tissue is removed. The body uses this mechanism of scar formation to optimize the function of the surviving tissue.

Keeping in mind the processes of repair follow on the heels of, if not occur synchronously with, the initial inflammatory reaction, let’s consider the primary cellular actors of the repair process. Essentially, we are looking at three major cell types; the macrophage, the fibroblast and the cells destined to become the new blood vessels (the process is called neovascularization), the angioblast.

As you are probably aware, the macrophage is the quintessential scavenger of the immune system. His job is to clean up all the remaining debris from the battle. Dead bacteria, injured and dying bodily cells, whatever. All the refuse must go to make way for the rebuilding squad. But even as the macrophages are finishing the final clean-up, the other two players of the repair crew are going to work.

The fibroblasts and angioblasts (endothelial cells that are producing new vessels) now begin the processes of producing the viable scar tissue that is the generic glue of the human body. Fibroblasts are induced to proliferate at the site of injury, and they begin to elaborate collagen. The angioblasts are essentially “turned on” endothelial cells from the near by blood vessels. They literally “grow” into the area of damage as little “buds” from the capillaries and venuoles. After all, this newly formed scar tissue is alive itself and will need a blood supply of its own to stay alive. Granulation tissue is the name applied to this newly developing scar tissue.

The term granulation tissue is actually an ancient term, and comes by its name from the gross appearance of a healing wound. The newly forming little capillary buds, that are sometimes visible in the base of a healing skin ulcer, give the appearance of tiny red granules. These little red specks look a bit like the little red sugar crystals you see on sugar cookies, and they develop over a period of a few days in the base of the healing wound. These little red “granules” have been seen by generation upon generation of healers, shamans, doctors, nurses and just about anybody else who takes a close look at the process of repair. We’re going to be using the microscope to examine them as well as the other participants in the repair process.

One aspect of wound healing has remained a bit obscure through the centuries. People have known that the repaired wound, rich in collagen, will undergo considerable contraction and shrinkage over time. In part this feature is ascribed to a population of modified fibroblasts that seem to contain actin and myosin like fibrils. These cells, known as myofibroblasts, seem to have some capacity for contraction and shortening of their length. Some people suggest that under their influence a wound actually shrinks, causing the margins to come closer together. Perhaps more important is the normal “shrinkage” of native collage as it dehydrates. The action of removal of the edema fluid from the wound, as the last stage of healing, also results in some degree of shrinkage of the newly formed collagen. Whatever is taking place, the combined
action of the myofibroblasts and the dehydration of the collagen definitely lead to a shrinking of a wound. Sometimes this shrinkage can have very unpleasant consequences. Should this process occur to excess, a badly scared wound across a joint may render the joint fixed or “frozen.”

Two additional events need to be defined. The following terms apply to wounds exhibiting differing degrees of injury, and importantly, their differing potentials for recovery. The two have different outcomes to a large extent dependent on two factors: (1) extent of injury, but more importantly whether some reasonably effective (2) if an intervention was administered to facilitate healing. The terms you must know are: wound healing by first intention (also called union by primary intention), and wound healing by second intention (also known as union by secondary intention).

Wound healing by first intention applies to a clean, uninfected wound, in which the margins have been brought together to optimally facilitate repair. The best example is a clean surgical wound made under operating room standards, following which the margins of were approximated (held together) by suture. For example, a skin biopsy or the surgical repair of a clean knife wound. Under this circumstance there is practically no necrotic debris left from the injury, no significant chance of infection and no foreign matter left in the wound. Even the edges are being held in position to facilitate the best possible recovery. Wound healing by first intention leads to the best outcome with a small amount of “tidy” scar formation.

Wound healing by second intention is often a problem, and generally leads to a less than satisfactory outcome. An example might be a major evulsion of a portion of skin (say a colossally bad bicycle accident) leaving a gaping evulsion of tissue, In this scenario, there is no chance the edges of the wound can be sewn together, and for good measure throw into this picture an infection of the wound and a few pieces of gravel from the road. Sounds pretty bad, but this is not far from the kinds of injury that are seen with frequency in any large emergency room. This wound will take a long time to heal and there will undoubtedly be a lot of “stewing” and “festering” before the project is complete. Because of the extended period of recovery, complicated by the infectious element, there will be lots of granulation tissue developed and lots of fibrosis (deposition of collagen). The result is likely to be a knobby and ugly scar which is going to contract to a great degree as the collagen dehydrates and myofibroblasts do their thing. This is the kind of wound that can lead to a stiff and almost immovable joint, should it occur across the knee, for example. Wound healing by second intention is what mother nature does on her own when there no better option. For millennia, this is how mankind has healed injuries of war, abuse and accidents, and it’s still the way the body heals wounds such as stomach ulcers.
Repair Exercise

You find it helpful to review several of the inflammation slides first before charging into repair. Once you begin the repair section, be sure you can identify the following cells and understand how they factor into healing.

1. Macrophages
2. Fibroblasts
3. Angioblasts (new blood vessels)

Here are the slides we’ll be looking at.

#87 Appendicitis
#85 Pulmonary abscess
#80 Gastric ulcer
#82 Old myocardial infarction with mural thrombus
Slide #87 acute appendicitis

For tissue 87, you can only view the round, cross section with the virtual scope. Even so, all the features of acute appendicitis are evident. Look for the PMNs in the muscular wall and serosal fat.

- Yes, there will be plenty of lymphocytes, but remember that they normally live in the submucosa throughout the bowel.

The very young granulation tissue is in the area of the serosal fat, and the little box indicates where the picture below is from.

This image to the right shows a good place to look for the changes of acute inflammation and granulation tissue. Truthfully, there isn't much in the way of new vessels yet, but the basic elements of granulation tissue are present.
Slide #85 necrotizing pneumonia with abscess formation

Two factors make Slide 85 challenging. First, there is extensive tissue damage and necrosis, making it hard to find the expected landmarks. Secondly, many of the PMNs themselves are necrotic and have shrunken and pyknotic nuclei. This makes them resemble lymphocytes to a degree. It's best to start at the edge of the slide where the tissue structure is better preserved. The little square indicates the location of the image below.

The image to the right is from near the edge of a microscopic abscess. Although many of the PMNs are necrotic and not recognizable, some still show the typical lobated nucleus. The darker pigment bearing cells contain broken down red blood cells and digested hemoglobin (hemosiderin).

Your observations
Slide #80 gastric ulcer

You'll see plenty of PMNs in the base of this ulcer along with lots of granulation tissue. The little square in the picture to the left shows where the other image is from. You'll see many fibroblasts and angioblasts streaming up to the ulcer base. The appearance is that of schools of fish swimming up from below. You should be see many new blood vessels containing RBCs. Both the fibroblasts and angioblasts are quite plump and 'embryonic' in looking.

In the medium power view to the right, you can see the area of the ulcer damage, with the fibroblasts and angioblasts streaming up to help with repair. Again, PMNs, are throughout the debris in the ulcer base, but now concentrate your efforts on the macrophages and granulation tissue.

Your observations
Slide #82 old myocardial infarction with mural thrombus.

Tissue 82 may be a little hard to recognize for what it is. There isn't much heart muscle here, rather it consists mostly of scar tissue. Much of the scar is very dense, although in the area of the mural thrombus (meaning it forms on the wall), you will find that the clot is being 'organized' and incorporated into what remains of the myocardium. The little box in the adjacent image shows where the picture below came from. Look in this area for the granulation tissue.

In this image, we are just below the area of fresh mural thrombus. Here you will see the ingrowth of new capillaries and the deposition of fairly fluffy looking collagen. This is the new granulation that is forming as the clot becomes organized into the wall of the heart.

Your observations
At the end of most of the laboratory sessions, there will be one or two question to answer. Please answer each question in one short paragraph and email it to the course’s email account. You will find a direct link on your disc and the V543 lab website.

You do not need to go to the library to look up the answer. The intent of these questions is to make you think about general processes in the body and how they might affect the eye.

There are two for this unit, one for inflammation and one for repair.

**Question for the inflammation segment.**

Your patient in the clinic has “pink eye” or conjunctivitis, which is an inflammation of the conjunctiva (white part of the eye). With this condition, the conjunctiva becomes very red because all of the blood vessels are dilated due to the inflammatory condition.

If you made a microscope slide of the discharge (gunk collecting in the inner corner of the eye) from a bacterial conjunctivitis, what cell types do you expect to see?

**Question for repair segment.**

The cornea is an avascular tissue. Most of the oxygen for the corneal epithelium comes from the tear film. When the cornea suffers from hypoxia (under a nonpermeable contact lens, for example), it becomes inflamed and new blood vessels grow into the cornea from the limbus. Using the myocardial infarction as an example, what do you think is the end result (6 weeks to 2 months later) of this condition?
Laboratory 2

Necrosis

The definition of necrosis actually encompasses several elements, but as you can probably see from the root of the word, “necro,” it’s telling us something about death. And in the context of necrosis, it’s important to note we are not dealing with death of the whole organism, just part of it. The need for this distinction stems from the fact that there are no recognizable microscopic differences between a dead and living cell for up to 24 hours following the death of an injured cell. On a cellular level, it’s a bit like the death of the whole organism. When someone dies, they outwardly look pretty much like everyone else, other than the fact that they’re not breathing. The “casual” observer could actually mistaken a dead person for someone who is just sleeping (at least for a little while). The same applies to cellular changes following the death of a cell, or a portion of an organ. Because there is no exacting microscopic method of identifying the moment at which a cell has reached “the point of no return,” or that matter has outright died, we must look to morphological changes that occur over time to distinguish living from dead tissue within the living person. Obviously, dead cells, as well as dead people “decay” or breakdown in order to return to the dust from which we all came.

OK, here comes the important definition, so pay attention. The term necrosis applies to the morphological changes observed as the dead cells dissolve, break down and are removed in preparation for repair. The process of dissolution of the dead cellular elements generally takes several days, and moves through a number of recognizable stages. Importantly, the term necrosis applies only to the situation in which the dead cells, or the portion of dead organ, is contained WITHIN the living individual. That’s right, dead tissue being dismantled and removed within the living person.

OK, the idea of “dead tissue” within the “living person” may sound paradoxical, but consider this. If someone sustains a “heart attack” (that is, a focal occlusion of a coronary artery resulting in hypoxic death of only a portion of the heart), and the person survives the initial insult, the dead cells must be identified and removed before the processes of repair can begin. Remember, myocardial cells do not have the capacity to reproduce, so in the scenario of a heart attack, repair must proceed by the formation of granulation tissue and the production of a “scar.” In this example, there is clearly a period during which dead tissue exists within the living person. If we had some way of taking periodic biopsies from the site of the myocardial infarction (the heart attack), while our patient recovered, we would see characteristic changes in the nucleus and cytoplasm of the dead cells. These changes reflect the condition of the dead cells as they undergo “self” destruction; autolysis, as well as are dismantled and hauled off by body’s inflammatory cells; heterolysis. These are two important terms you must know, so here are their...
definitions:

1. **Autolysis**: The activation of the dead cell’s own lytic enzymes, leading to the denaturation of the cell’s own proteins, thus beginning the process of removal of the defunct cell.

2. **Heterolysis**: The dissolution and removal of dead cells by the action of lytic enzymes brought to the site of damage by the body’s own inflammatory cells.

It’s important to understand that the processes of autolysis (pronounced “ah-tall-oh-sis”) and heterolysis are acting at the same time with a single purpose; to remove the dead tissue so that repair can proceed. There are three time dependent cardinal changes of the nucleus that clue us to the fact that the cell is actually dead and undergoing dissolution. The first of these changes is not even detectable until 24 hours has passed after the lethal injury. These changes require days to evolve and represent a continuum on the way to complete removal of the dead cell. Obviously, it would require serial biopsies of the site of injury to see the process from beginning to end, and not too many myocardial infarction patients would put up with this kind of experiment. But since people with this affliction die at different times in the course of healing an infarction, it is actually possible to document the progression of the process described below. The three nuclear changes you must know and be able to identify are:

1. **Pyknosis**: Shrinking and condensing of the nucleus with markedly increased basophilia. Eventually, the nucleus looks like a little black raisin in the cell.

2. **Karyorrhexis**: Fragmentation and eventual leading to complete dissolution of the pyknotic nucleus.

3. **Karyolysis** (pronounced “carry-ALL-oh-sis”): complete dissolving and disappearance of the nucleus.

Within several days the dead muscle cells of our hypothetical myocardial infarction will have lost their nuclei and the proteins of the cytoplasm will have condensed into an intensely eosinophilic, featureless, opaque blob. Microscopically, the dead muscle cells have become bright pink, lifeless little carcasses. Now the inflammatory cells must remove what remains of the dead cells so repair can get underway.

The processes discussed above details the changes of individual cells following their death. But the overall pattern and appearance of necrotic tissue, that is to say a field of dead cells, may vary depending on the type of lethal injury and the contribution of lytic enzymes from outside the dead cells. We will be looking at three basic patterns of necrosis, and keep in mind that now
we are talking about a field change in the dead tissue rather than alterations of a single cell. The patterns you will need to know are:

1. Liquefactive necrosis

2. Coagulative necrosis

3. Caseous necrosis

Liquefactive necrosis occurs in one of two situations: (1) death of cells in which certain classes of bacteria are the culprits. The enzymes that the bugs and the body’s inflammatory cells bring to the scene cause extensive digestion and “liquefaction” of the dead tissue, and (2) death of cells due to hypoxia in the central nervous system. In the case of liquefactive necrosis, the dead tissue is rendered soupy by the action of the lytic enzymes brought to the scene of injury by cells other than the dead parenchymal ones.

An example of bacterially mediated liquefactive necrosis is the pus found in the center of a “zit.” A zit after all is just a small abscess, and at the heart of any abscess is the digested dead cellular material and lots of acute inflammatory cells (polymorphonuclear leukocytes-PMN’s). The “liquefaction” of the dead tissue takes place because of the lytic enzymes contributed by both the bacteria and the body’s acute inflammatory cells responding to the infection. In the case of hypoxic cell death in the central nervous system (a stroke, for example), bacteria are not present, but the overall appearance is still one of liquefactive necrosis, thanks to the action of the lytic enzymes of the body’s inflammatory cells responding to the injury. As you might guess, the lytic enzymes causing liquefaction are digesting not only the dead parenchymal cells, but the inflammatory cells themselves. The life span of a typical acute inflammatory cell is just a few hours, and they give their lives in the battle so your genes will stay in the gene pool. Pretty altruistic wouldn’t you say?

Coagulative necrosis is seen in hypoxic death in those tissues that have an “end organ” vascular supply. That is to say, a branching system of arteries that get smaller and smaller heading into the capillary bed. However, it is important to realize that there is no overlap, or “collateralization,” of the capillary beds. As you can guess, once a vessel is occluded, there will be no blood flow into the affected region of the organ. Since there is no mixing or overlapping of the vascular network, the cells in the affected area are doomed. The heart, kidneys and spleen are organs with this type of vascular arrangement.

The classical microscopic features of coagulative necrosis are the nuclear changes discussed
earlier in this laboratory (karyolysis, pyknosis and karyorrhexis), coupled with the denaturation and congealing of the cytoplasmic proteins into a homogeneous, pink staining goop.

Surprisingly, the cellular outlines of the dead cells will still be recognizable for several days following the lethal hypoxic insult. Thus, with no importation of proteolytic enzymes, such as happens in liquefactive necrosis, the cells are not “digested” and don’t turn into the soup we see with liquefactive necrosis. Eventually, of course, the dead cells are removed by the process of phagocytosis. Typically, macrophages come into the area of coagulative necrosis and dismantle the dead cells, making way for the elements of repair. A good example of this type of necrosis is the heart attack we were using to describe the nuclear changes of hypoxic death.

Caseous necrosis is a distinct form of necrosis, and is seen in only a few circumstances; principally tuberculosis and its kissing cousin, leprosy. The term “caseous” refers to the crumbly, cheese-like nature observed grossly with this type of necrosis. Caseous necrosis is seen in the center of a granuloma (to be described shortly), and is virtually devoid of recognizable cellular matter. It, in fact, results from the action of the host’s over enthusiastic response, by its own immune system, to the tubercular organism.

Granulomatous inflammation and the mature granuloma, represent unique forms of the chronic inflammatory response. They both occur in response to poorly soluble antigens, or organisms bearing poorly soluble or antigens. In addition to infectious processes, we also see this type of response to pieces of inert foreign matter that might have been injected into the body, say like a piece of wood, bullet or fragment of automobile dashboard.

The overall granulomatous infiltrate is composed of lymphocytes, plasma cells, macrophages and importantly epithelioid histocytes. Frequently giant cells will also be part of this chronic inflammatory pattern. Depending on certain circumstances, the more or less diffuse granulomatous reaction may “mature” into a fully developed little “prison cell” we call a granuloma.

As its name indicates, the fully developed granuloma is, in fact, a “small body.” It comes into being because by the response of certain T-lymphocytes to particular antigenic determinants on the surface of some microbial organisms. This “little body” of a granuloma really serves the purpose of a cellular prison, in the instance when the host’s inflammatory cells cannot kill or eliminate the invading organism. This unique chronic inflammatory reaction is composed of all the cellular elements listed above for granulomatous inflammation, but it also includes reactive fibroblasts, and the subsequent production of collagen. In time (several weeks to months) the granuloma forms as a sphere with fibrotic tissue making up the outer wall. Lymphocytes, plasma cells and epithelioid histiocytes are within the core of and throughout the wall of the granuloma.

The center of the granuloma becomes extremely hypoxic and acidic, leading to the death of all cells at its core, eventually producing the characteristic “cheese-like” quality of caseous necrosis. This form of necrosis bares some superficial
resemblance to coagulative necrosis, but no cellular outlines or details can be discerned. Multinucleated giant cells are found within and around the inflammatory milieu of the granuloma. It’s as if the inflammatory cells realize the object to be destroyed simply cannot be engulfed by a single histiocyte, so a number of histiocytes come together to make one really hugamongous phagocyte. Unfortunately, this new giant phagocyte can’t kill the invader either, thus leading to the necessity of the “lock ‘em up and throw away the key” approach.

The granuloma is the result of cell mediated immunity in response to the infectious agent. Although granulomas may form as part of the response to a number of conditions (certain chemicals, some fungal infections, foreign matter such as silica and even suture material), not all develop the caseous, soft and chewy center. This feature of central caseous necrosis is the hallmark of the tubercular granuloma.

The last consideration has to do with what may happen if there is delayed removal of necrotic material, irrespective of the injurious process. As the area of necrosis becomes more acidic, calcium salts will deposit at the site, simply as a function of the pH gradient. This process is referred to as dystrophic calcification, and it sometimes renders areas of old necrosis in soft tissue visible on x-ray. As you might guess, since the core of a tubercular granuloma is so acidotic, the deposition of calcium is a common and predictable feature. It is the deposition of calcium in the granuloma that allows us to make a presumptive diagnosis of quiescent tuberculosis from a chest x-ray.

Although we have discussed in detail three types of necrosis, there are two more seen on occasion.

1. Hemorrhagic necrosis, which as the names implies involves hemorrhage into the necrotic tissue. This type of necrosis is seen with venous occlusions, and in organs with either a “dual” blood supply (the lung), or organs with some degree of overlap in the capillary beds (the gut). In the case of hemorrhagic necrosis, there is not enough blood flowing into the organ to keep it alive, but there is a trickle sufficient to bleed into the dead tissue once the vessels break down.

2. Enzymatic necrosis, which is seen in the pancreas, and develops as a result of activation of the pancreatic enzymes. In this scenario, the pancreas literally digests itself.
Laboratory 2
Exercise

The following slides depict the types of necrosis we have discussed so far:

- Online slide of myocardial infarction  Coagulative necrosis
- #85 Pulmonary abscess  Liquefactive necrosis
- #90 Tuberculosis in the lung  Caseous necrosis

For each slide do the following:

1. Identify the tissue.
2. Systematically describe and sketch the morphological changes evident on the slide; i.e., presence of granulomas, type of cells involved etc.
3. Can you identify all the components of a granuloma?
4. Discuss your observations with your friends.
Online slide of acute myocardial infarction

This slide is exclusively online. In it you will see **two acute myocardial infarctions**. Yes, a person can have a second while recovering from the first. The bright pink staining heart muscle, the cells of which lack nuclei, is the area of the most recent event, an infarction of about 3 days duration. The 'bluer' staining fluffy looking tissue is the original infarction, about 14 days in duration.

The image to the right is from the area of the little square in the other picture. Here you see very **recent coagulative necrosis**. Note the congealed and homogenous staining cytoplasm and **absence of nuclei**. This area is an acute infarction that happened about 3 days before this person's death. The tissue in the upper right shows a resolving infarction that is about 14 days old.

**Your observations**
Slide #85 necrotizing pneumonia with abscess formation

Two factors make Slide 85 challenging. First, there is extensive tissue damage and necrosis, making it hard to find the expected landmarks. Secondly, many of the PMNs themselves are necrotic and have shrunken and pyknotic nuclei. This makes them resemble lymphocytes to a degree. It's best to start at the edge of the slide where the tissue structure is better preserved. The little square indicates the location of the image below.

The image to the right is from near the edge of a microscopic abscess. Although many of the PMNs are necrotic and not recognizable, some still show the typical lobated nucleus. The darker pigment bearing cells contain broken down red blood cells and digested hemoglobin (hemosiderin).

Your observations
Slide 90: Tuberculosis in lung

This slide demonstrates a form of disseminated tuberculosis we call milliary TB. No, it's not military TB, rather the terms refers to the numerous little granulomas that are spread uniformly like millet seeds. In this slide you will see many small, fresh granulomas.

The image to the right is from the little box in the first image. Here we're looking at the edge of one of the granulomas. You can see the caseous necrosis that's at the heart of the tubercle. The wall of fibroblasts and collagen is just beginning to take form. You should see many giant cells in association with the granulomas.

Your observations
**Question for lab 2**

Please answer the following question in one short paragraph and email it to the course’s email account. You will find a direct link on your disc and the V543 lab website.

What type of necrosis do you expect to find in ocular tissue?
Laboratory 3

Neoplasia and Disturbances of Cell Growth

Practically everyone is familiar with the common usage of the word “tumor,” but few actually know what it really applies to. A tumor is simply an area of “swelling or localized enlargement” and thereby applies equally to a cancerous growth as well as swelling secondary to edema fluid around a sprained ankle.

The word “tumor,” is about as generic as you can get. When speaking of a either a malignant or a benign “growth,” we are really talking about a neoplasm. That is to say a “new growth.” A neoplasm is a mass of cells growing in an unregulated manner, and the process of their growth is referred to as neoplasia. Neoplastic proliferation of a tissue can be defined: as abnormal growth which is uncoordinated and exceeds that of normal tissues, and persists in its excessive manner after the removal of the stimulus that evoked the initial change.

But rapid growth and proliferation of the cells of an organ is not necessarily abnormal nor detrimental. There are a number of processes known as non-neoplastic cell growth which are part of our normal physiology and adaptation to environmental stresses. These include (1) regeneration, (2) hyperplasia and (3) metaplasia. In addition to these conditions, there is a form of abnormal cellular proliferation, dysplasia, which embodies some features of true neoplasias, but not the completely unregulated growth.

Regeneration is a process we have already considered when we studied healing and repair. But now, we are reconsidering the process from the perspective of the replicating cell. In the case of an organ undergoing active regeneration we see rapid proliferation of the parenchymal cells, that survived the initial insult, in an effort to restore the functional integrity of the damaged organ. It is important to remember that the underlying framework of the organ must remain intact for the process to eventuate in a “healed” and completely functional organ. If regeneration is not an option, either because the organ is not made of cells that can replication (brain for example) or if the underlying framework has been blasted, then repair proceeds by the formation of abundant granulation tissue and eventually scar formation (for example, heart following myocardial infarction).
Hyperplasia is controlled cell proliferation and is characterized by an increase in absolute cell number in a tissue or organ. Often seen in organs that respond to hormonal stimulation (an example is breast tissue during pregnancy), the hyperplastic cells generally have a slightly larger nucleus and mildly increased nuclear uptake of the histological stains. Because of the increase in number of cells in the organ, the affected tissue or organ increases in size. However, the increased size of an organ can be detrimental in some circumstances. For example, in the case of hyperplasia of the prostate, men will often experience an increased incidence of urinary tract infections because of the accompanying urethral obstruction.

There are two terms sound much alike, but apply to different situations, hyperplasia and hypertrophy; be sure you know the difference. Hypertrophy is a condition in which there is increase in cell size in effort to meet new physiological demands. For example, when you work out and exercise a particular group of muscles, you stimulate the development of additional actin and myosin in those muscle cells. Your muscles become larger because the individual cells contain more muscle protein, not because you have increased the number of muscle cells themselves. This a very important distinction, be sure you understand it.

Metaplasia is an interesting form of controlled growth, and even though the result may meet the needs of new environmental stress, the process is really not reflective of a state of good health. The hallmark of metaplasia is the change of one epithelial type for another, in an effort to meet a new environmental stress. The best example I can think of is the conversion (metaplasia) in a smoker of the typical bronchial epithelial lining (pseudostratified columnar ciliated) for squamous epithelium. The cigarette smoke is clearly injurious to the lining to the trachea and bronchi, resulting loss of ciliary function, chronic inflammation and day by day injury to the epithelium. In an effort to meet this new environmental stress, the more sensitive and vulnerable normal epithelium is replace by a more rugged variety, better able to handle the chronic insult of tobacco smoke. While this is clearly an adaptive move on the part of the body, it certainly can’t be said to represent a state of good health.
Dysplasia is a step in the direction of outright malignancy. Although not malignant itself, in many cases we can trace the cytological alterations of a developing cancer through various stages of dysplasia. The term itself means “sick tissue,” and that is readily seen in the disorderly maturation of the cells exhibiting this process. Dysplasia is most frequently observed in epithelial coverings that are subject to repeat and severe chronic injury. In the case of dysplasia, one sees significant disruption in the maturational sequence of the affected epithelium. Immature basilar cells, from the regenerative lower layers of the epithelium, are carried far up into the more mature portions of the epithelial covering. Moreover, these disturbed cells show profound variation in size and shape, a condition referred to as pleomorphism. Not uncommonly, the rapid rate of proliferation of these dysplastic cells will be manifest by increased numbers of mitotic figures. Again, the best example are the changes brought on by smoking or chewing tobacco. Here, the chronic injury leads to need for more rapid replacement of the epithelial covering, and as we just learned, there will likely be the conversion of one epithelial type for a sturdier one. Now the stage is set for the emergence of a squamous carcinoma to develop in an organ that, under normal circumstances, lacks squamous epithelium.

But the good news is that many of these conditions are reversible once the inciting agent has been removed. Even in the case of metaplasia, and even in some less severe forms of dysplasia, the system will revert to the normal, healthy condition upon removal of the agent causing the chronic injury. As you might have already guessed, more than one of the conditions listed above may co-exist at the same time. This is practically always the case with cigarette smokers. First comes metaplasia, then dysplasia, and finally cancer.

In the first part of the lab, we are going to concentrate our attentions on the non-neoplastic forms of cell growth. As you look at the slides, it should become evident that hyperplasia, metaplasia, and dysplasia demonstrate progressively severe forms of growth derangement. Though these are all forms of abnormal cellular proliferation, they are also controlled forms of cell growth and are reversible, to an extent, when the inciting stimulus is eliminated.

Before launching into your slides sides, consider this brief review of the phases of mitosis.

1. **Prophase**: During this phase, changes occur in the centrosome and nucleus. The chromosomes shorten, become more compact, and stain densely. The centriole divides and each daughter centriole moves to opposite poles of the cell.

2. **Metaphase**: The chromosomes arrange along an equatorial plane midway between the two centrioles, forming the equatorial plate.
3. **Anaphase**: The chromatids move toward their respective centrosomes.

4. **Telophase**: The end of the migration of the chromatids marks the early telophase stage. The chromosomes then become long, loosely-spiral threads, and the nuclear membrane reforms. The cytoplasm separates, forming two complete cells.

The period between two successive divisions is called **interphase**.

**Benign and Malignant Neoplasia**

Now that we’ve studied alterations of cell growth and maturation, we will look at “tumors” and neoplastic lesions that truly represent **unregulated cell growth**. Lesions that behave as a parasite in the body, and in many cases can spread from the site of origin, eventually bring about the death of the afflicted person.

As you will recall, we classified cellular proliferation into categories based on physiological features such as regeneration, adaptive strategies, and finally outright conditions of abnormality. Hopefully, the following terms will not seem too foreign: (1) regeneration, (2) hyperplasia, (3) metaplasia and (4) dysplasia. We considered the latter three to be abnormal conditions, but potentially reversible upon removal of the inciting agent. A true neoplasm is a lesion that will not spontaneously regress, and represents an irreversible end-point. The neoplastic tissue (the tumor) is not going any where unless removed or destroyed in some therapeutic or interventional manner. It is true that we have T-lymphocytes whose job is to fight neoplastic disease, but by the time an actual tumor has developed, we know they were unsuccessful in destroying the neoplasm in its “infant” phase.

The word neoplasm does not equate with malignancy. **Neoplasms** (tumors) come in two basic flavors: **benign** and **malignant**. Before going on, let’s be sure we know how the two are defined.

1. The biological hallmark of a malignancy is its capacity to **invade** and destroy adjacent structures, as well as spread widely (**metastasize**) to involve distant organs. There may be considerable **variation** in the **cytology** (appearance of the individual cells) and **histology** (appearance of the tissue as a whole) between various malignancies and even within an individual tumor. Still, the definition of malignancy rests on the **biological potential and behavior** of a neoplasm.
2. **Benign** tumors lack the capacity to spread widely, but do expand by “radial” growth, and as a consequence may become symptomatic by compressing adjacent structures. This feature of compression, however, is substantially different from “invading” the local structure. Moreover, the overall microscopic appearance of a benign lesion is much like that of the tissue from which it arose. But “biologically benign” tumors may cause the death of person. Consider a “benign” pituitary adenoma. Here a tumor with no propensity to spread may still compress a vital area and cause the death of the individual. This certainly adds a new wrinkle to the term “benign.”

Even though there may be great differences in the biological temperament of benign and malignant neoplasms, there are some common microscopic features we need to consider.

1. All tumors have a **stroma**, or supportive network of fibro-connective tissue and vascular elements. In the case of some malignant neoplasms, the malignant cells can induce the formation of large amounts of collagen and cause considerable vascular growth.

2. The actual “tumor” cells are referred to as **parenchymal** cells, and represent the proliferating cell line from the organ that gave rise to the lesion in the first place. Depending on the degree of maturation and differentiation of the tumor, these cells will reflect the cytology and histology of the mother tissue to a greater or lesser extent.

Nomenclature can be a little confusing, but there are some basic rules. As a general rule, the name of the mother tissue or organ is incorporated and modified with a suffix. Here are the basics

1. The suffix “-oma” is often applied to indicate we are dealing with a mass or space occupying lesion. The term really means “body.” The term generally, but not always, implies a benign lesion.

- schwannoma; a benign tumor of schwann cells
- glioma; a tumor of variable biological potential of glial cell origin
- adenoma; generic term used for a benign tumor of glandular tissue origin
- carcinoma; generic term for malignant tumor of epithelial cell origin
- adenocarcinoma; generic term used for malignant lesion of glandular tissue origin
2. Descriptive terms are used to indicate the type of tissue in which the tumor arose.

- carcinoma; malignant tumors of epithelial cell origin, for example: squamous cell carcinoma of lung
- adenocarcinoma; malignant tumors of glandular epithelial origin, for example: adenocarcinoma of colon
- sarcoma; malignant tumors of mesenchymal cell origin, for example: chondrosarcoma; a malignant tumor of cartilage origin
- osteogenic sarcoma; a malignant tumor of bone origin
- teratoma; either benign or malignant tumor of germ tissue, (generally reproductive tissue, i.e. ovary or testis), composed of more than one germ line. Examples: - benign cystic teratoma of the ovary and malignant teratoma of testis

Distinguishing malignant and benign lesions on a microscopic level is not as straightforward as it might seem. Here are some of the terms and microscopic features used to describe neoplasms. Keep in mind that we are referring only to the parenchymal cells, as they are really the “tumor” cells. The stroma of the neoplasm, even of a biologically malignant neoplasm, is actually just benign connective tissue the tumor uses to grow on.

**Benign** tumors are, for the most part, well differentiated and faithfully demonstrate the cytology and histology of the tissue of origin. Mitosis are rare if seen at all, and there is frequently an investing capsule separating the tumor from the surrounding normal tissue.

**Malignant** tumors lack a capsule and often show a wide range of parenchymal cell differentiation. Mitosis are often present and the cells generally show some degree of anaplasia. The term anaplasia implies the backward form, primitive or more embryonic appearance of the cells and tissue. Anaplasia is an important term to understand. Anaplastic cells often show marked pleomorphism (that is variation is size and shape). The nuclei of anaplastic cells often show hyperchromasia, are generally much larger than their normal counter parts, have prominent nucleoli and have a lopsided nucleus to cytoplasm ratio (very much favoring the nucleus). Abnormal mitosis, that is tripolar and even quadrupolar, may be seen.

The foregoing are general rules that apply to most, but not all, situations. On occasion malignant neoplasms are very well differentiated and may be “under-diagnosed.” The real nature of the lesion is learned only when it recurs or is found to have metastasized. Here’s a brief review of what we are looking for to make the distinction between benign and malignant neoplasms.

The two principal criteria of therefore cancer are:
1. Anaplasia
2. Evidence of invasion of normal tissues or spread to distant sites.
To summarize, the basics true neoplasms are:

**Benign**

1. Extremely well differentiated.
2. Mitosis scant and normal.
3. Stays localized; does not spread. Most develop an enclosing fibrous capsule, although lack of capsule does not mean malignancy.
4. Growth rate is low.

**Malignant**

1. Anaplastic (hallmark of cancer).
2. Some degree of undifferentiation.
3. Invasion of normal tissue (hallmark of cancer).
5. Cancers grow rapidly by progressive infiltration, invasion, destruction, and penetration of surrounding tissues.
Laboratory 3

Growth disturbances and benign lesions first

Please examine the following slides:

#58: Pituitary adenoma
#92: Benign prostatic hyperplasia
#86: Bronchus with metaplasia
#97: Fibroadenoma of the breast
Slide 58: Pituitary adenoma

You have just pieces of this tumor to look at. Biologically speaking this is a benign lesion; that is from the standpoint of potential to metastasize. Even so, it's at the base of the brain, not a place that's very accessible. Most pituitary adenomas don't produce much in the way of hormones, but they may be symptomatic with regard to visual changes. What visual deficit would you expect in a patient with a space occupying lesion of the sella turcica?

How about the size of the tumor at the right? Think there might have been some visual field changes? Click the image for the big picture.

In the microscopic section to the right, note the cord-like and glandular organization of the tumor cells. They pretty much look like the normal cells of the pituitary, they just don't know not to keep slowly proliferating.

Your observations

[Image of Adenoma and Pituitary remnant]
Slide 92: Benign prostatic hyperplasia

In Slide #92, here we are looking at a section from a prostate gland that demonstrates nodular hyperplasia. Normally in the prostate, we see many glands separated by an abundant fibromuscular stroma. The glands are lined by two layers of cells, a basal layer of low cuboidal cells covered by a layer of columnar mucous-secreting cells. There are many patterns of nodular hyperplasia. Ultimately, all are differentiated on the basis of whether nodularity is due to:

1. Glandular proliferation or dilation
2. Fibrous or muscular proliferation of the stroma.

Though both elements are involved in most cases, one element may predominate over the others. Usually, it is the epithelial element that predominates in the form of aggregations of small to large dilated glands lined by both layers of epithelium. Again, it’s a matter of quantity and not quantitative change. The epithelium of the hyperplastic glands is characteristically thrown up into papillary buds and enfoldings. Frequently, these microscopic glands of the prostate contain inspissated secretion, granular desquamated epithelial cells, and numerous corpora amylacea. It is also common to find aggregations of lymphocytes within the stroma.

Your observations
Slide 86: Bronchus with metaplasia

In this cross section we see all the structures of a bronchus, even a tiny bit of alveolar lung tissue at the edge. You will notice the bronchus is in large part lined by the typical columnar, pseudostratified, ciliated epithelium. But in several areas the epithelium has transitioned to something sturdier form namely squamous epithelium. There may even be a little dysplasia in some areas. What sort of insult would have lead to this modification? Would you suspect it's a result of an acute or chronic type of injury?

The image to the right shows the area we need to pay attention to. You will also see that the changes of squamous metaplasia are not confine exclusively to the lining mucosa, but in several areas even extends down into the gland necks of the submucosal mucus secreting glands. If the chronic injury that produced this change were to continue, what would be an expected outcome?

Your observations
Slide 97: Fibroadenoma of the breast

Breast tissue consists of glandular tissue and a fibrous and fatty connective tissue stroma. Each mammary gland is divided into 15 to 20 lobes. Each lobe is a compound gland with a separate lobar duct opening at the nipple. The connective tissue forming the septa between the lobes (interlobar) and the lobules of each lobe (interlobular) is of the dense fibrous type. While the connective tissue found within the lobules (intralobular) is fine and cellular, the epithelial cells lining the glands are generally cuboidal. This distinction may vary with the functional state of the gland.

Lesions affecting the breast usually take the form of palpable masses that are sometimes painful. Except in rare instances these are limited to woman, but men do suffer from disorders of the breast, including cancer (very rare, but it does happen). Some of the more common breast pathologies and their differentiating characteristics are presented in this lab. Fibroadenoma is the most common benign tumor of the breast. It may be due to an increased sensitivity to estrogen at some focal area within the breast. These tumors are composed of both fibrous and glandular tissue and are encapsulated and freely moveable. Histologically, there is a loose, delicate fibroblastic stroma containing pleomorphic glandular and cystic spaces. When the glands take the form of round to oval shape, the lesion is termed pericanalicular. And when the glands are compressed so that they appear slit-like or star shaped, it is termed intracanalicular. Again, these lesions are usually benign and only rarely become malignant.

In slide 97 it's easy too see the benign nature of this little tumor. It's extremely well demarcated, in fact, it's encapsulated. There is no hint of spread into the surrounding breast tissue, because there is none. The little box indicates the location of the image to the right. You can see part of the capsule and the two tissue types that make up the benign neoplasm itself; stromal and ductal elements.

In the picture to the lower right, you can see the numerous wiggling and twisting ducts with stromal connective tissue between them. Both the stromal connective tissue and ductal epithelium are benign. If you look carefully at the ductal cells, you will see they are no different than the regular ducts in the surrounding health breast tissue.
Your observations of slide 97
Now for the malignant tumors.

Use the characteristics mentioned in the introduction and identify neoplastic changes in the following slides:

- #93 Squamous cell carcinoma
- #78 Carcinoma of the rectum
- #79 Malignant melanoma of the eye
- #84 Scirrhous carcinoma of the breast

**Slide #93: Squamous cell carcinoma**

1. **Acanthosis**: Greatly thickened epidermis
2. **Hyperkeratosis**: An overgrowth of the keratin layer of the epidermis
3. Epidermal cells showing pleomorphism, hyperchromatic nuclei, mitoses, and a loss of orientation of the dermis and connective tissue
4. Also note the ulceration and inflammation present

![Image of squamous cell carcinoma](image)

**Your observations**
Slide #78: Adenocarcinoma of the rectum

Here we see a section of bowel with a relatively common malignancy, an adenocarcinoma. The tumor has a 'glandular' appearance microscopically because the cell of origin thinks it's supposed to make little rings and tubes, in a manner similar to the healthy epithelium. This section is full thickness and shows the mucosa, muscle layers and serosal surface. The malignancy is that big lump in the middle, and there is normal mucosa on either side.

In the iamge to the right we see the hallmark of an adenocarcinoma; malignant epithelium making little gland- like forms. Most characteristic of all is the gland-within-gland growth pattern. Gland-within-gland refers to the very close arrangement of the glands, such that there is no intervening connective tissue stroma.

Your observations
**Slide #79: Malignant melanoma of the eye**

1. Key feature is the proliferation of pigment cells and fibrovascular tissue within the choroid—choroidal tumor

2. Identify the different cell types present:
   - (1) Spindle A
   - (2) Spindle B
   - (3) Epithelioid

**Your observations**
Slide #83: Scirrhouos carcinoma of the breast

Carcinoma of the breast is the leading cause of death among women due to cancer. Breast cancer has bimodal pattern of incidence with a peak in women in their late 20s and early 30s and another peak among women of menopausal age. Ninety percent of breast carcinomas arise from ductal epithelium and 10 percent from lobular epithelium. Both of the types can be further classified by whether or not they have penetrated their basement membranes and are thus named infiltrating or noninfiltrating. Breast carcinomas are outlined below and their differentiating characteristics are given.

1. Arising from lobular epithelium
   (1) Infiltrating: Lobar carcinoma
   (2) Noninfiltrating: In-situ lobar carcinoma

2 Arising from ductal epithelium
   (1) Noninfiltrating: Intraductal carcinoma
   (2) Infiltrating: Most common form and is manifested in one of the following forms

Scirrhouos carcinoma accounts for 75 percent of carcinomas of the breast. The lesion has a stony hard consistency and a gritty texture. Histologically, it is composed of dense fibrous tissue with widely scattered cords of tumor cells. This stromal overgrowth is called the desmoplastic response.

On your slide, you should observe:
1. Dense collagenous stroma (desmoplastic)
2. Scattered cords of tumor cells (often the cords of tumor cells will be a single strand of tumor cells and are called Indian file or soldier column tumor cells)
3. Infiltration through the basement membrane of the epithelium as well as some cystic and dilated ducts

Your observations
Questions for lab 3

Remember you are to email your responses to the course email account.

1. Because we accept the fact that both benign and malignant lesions can cause life-threatening situations, give an example and explain how a benign lesion can be fatal.

2. While performing ophthalmoscopy on your patient, you observe three small nodules in his/her right eye (vitreous or retina). If this tumor has metastasized to the eye from some other location in the body, where would be the most likely site of origin if your patient is a woman? What if your patient is a man?

3. Is there any way to differentiate on the basis of clinical or histological appearance a tumor that has originated in the eye from one that has metastasized to the eye from another organ?

The surface of the eye is a mucous membrane and should be kept moist for its proper function. There are numerous glands in the conjunctiva that lubricate the ocular surface and produce mucins. In patients with "dry eye" (keratoconjunctivitis sicca), the surface of the eye is poorly lubricated. The relative dryness induces metaplasia of the epithelial surface cells of the conjunctiva.

1. What type of metaplasia (original to resultant tissue) do you expect?

2. How might the function of the ocular surface be affected by the metaplasia?
Laboratory 3
Ocular Case Study

History:
Jesse is a 54 year-old ranch-hand who comes today because he’s having trouble reading small print. The labels on the vaccines he has to inject into his cattle have become a problem, to say nothing of the fine print on maps. Worst of all, he’s an avid reader of fantasy fiction, and he’s having real trouble following the adventures of Frodo and Gandalf, although he’s read the books so many times he knows the story by heart.

He thinks his distance vision is good, he just needs help with things up close.

External exam:
- The patient has no phorias or tropias. Pupils are normal and versions are full and smooth.
  - There is a painless nodular growth just below the right eyebrow, superior nasal to the globe.

Unaided Visual Acuities
- Distance: 20/20 O.U.
- Near:
  - O.D. 20/80
  - O.S. 20/60
Slit Lamp Examination

- Cornea: Normal exam.
- Conjunctiva:
  - Raised yellowish, discoloration of the conjunctiva (pinguecula).
  - A pinguecula is evidence of solar damage. (Roll cursor over image if you don’t see it.)
  - Click the image for a bigger picture.

- Crystalline lens:
  - There are opacities of the crystalline lens, indicative of an early cortical cataract.

Dilated Fundus Examination:

- The fundus is normal.
- Intraocular pressures are within the normal range (15mmHg O.U.)
How about another look at that eyelid:

- Note the lesion in the medial aspect of the right eyelid.
  - Pearly and glistening surface.
  - Raised and rolled borders.
  - Maybe a small central area of ulceration.
  - No obvious keratin accumulation on its surface.

Microscopic section from biopsy.

- Note clusters and groups of cells.
- ‘Picket fence’ arrangement at edge of clusters.
- Solar degenerative changes of the dermis.
- Epidermis is largely intact.

What do you think?

- Skin tumor arising on sun exposed skin.
- Epithelial verses connective tissue origin?
- How bad can it be?

Make a list of what you know, and especially, what additional information would be helpful.

Consult the V543 CD, or website, to see what the bigshots though about this case. Be sure you understand why it’s important that you can recognize lesions of this type.
Laboratory 4
Infectious Diseases

In spite of the fact that we know a great deal about microbiological agents and their actions, infectious diseases continue to be the major cause of death and disease world wide. Consider this. One third of the world’s population is currently infected with tuberculosis and malaria remains the undisputed leading cause of death among our species. As for other, seemingly banal, infectious processes we will never the know the tremendous number of newborns that die in the first days of life from infantile diarrhea. Moreover, parasitic diseases are still rampant in most developing countries, and even among the affluent nations of the world infectious diseases due to uncommon organisms are on the rise as a result of immunosuppressive therapy and acquired immunodeficiency syndrome. And to compound matters, antibiotics are losing their effectiveness.

Our contact and symbiosis with the microbial world starts as soon as we are born. A person is infected, or more appropriately “colonized,” in the first few days of life with bacteria we rely on to help us maintain good health. For example, the bacteria found in our gastrointestinal tract make vitamins we require as well as create a local environment that is hostile to pathogenic organisms. As is probably apparent, the mere presence of bacteria does not imply a pathological state of affairs.

We live in a world of bacteria and viruses.

- So why is it sometimes we get sick?
- We know three things are necessary for an infectious disease to develop.
  - A susceptible host.
  - The agent of the disease.
  - Proper environmental factors.
- So, if the bugs are on our skin, in our GI tracts and up our noses, what constitutes an infection?

A reasonable working definition might be this:

- An infection results when a microbial agent causes disease or injury in the course of its growth, cellular metabolism or reproduction.
The three elements needed for an infection.

**Here are a few host factors.**

- Immune susceptibility
- Nutritional status
- Breaks in skin or mucosa
- Being in the wrong place at the wrong time

**Here are a few things the bugs bring to the scene.**

- Size
- Adhesive proteins
- Toxins
- Antibiotic resistance

**Environmental factors**

- Reservoir and/or vector, if needed.
- People in close proximity.
- The proverbial ‘dirty toilet seat.’
- Inappropriate use of antibiotics.

**Mechanisms of microbial injury.**

They can **kill the host cell directly**

- Proliferate within the cell, eventually causing cell rupture.
- Elaborate and release enzymes locally that cause cell death.
- An example: Staph brain abscess

**Toxins**

**Exotoxins:** Cellular metabolites released into the environment

- *C. difficile* enteritis (picture right)
- *B. anthracis*

**Endotoxins:** Cell wall fragments

- Gram negative shock
Mechanisms of microbial injury, continued

Hypersensitivity (the host’s immune system goes wild, destroying host tissue)

- Type I: IgE mediated (anaphylaxis)
- Type II: antibodies cross react with host
- Type III: antigen-antibody complexes
- Type IV: cell mediated (tuberculosis)

Sure, we've got a few tricks of our own.

Barriers and filters

- Skin and mucous membranes
- Nose hairs for filtration

Physical removal

- Ciliary action of respiratory epithelium
- Flushing action, tears, urination…

Normal flora

- GI track
- Skin
- Vagina

Local environmental changes

- Lysozyme in tears
- IgA

Immune system

- PMN inflammatory cells (immediate response)
- T and B cell lymphocytes
The bugs have their own tricks.

Rapid growth rate

- Thirty minutes in some cases
- Rapid mutation rate, flu and HIV
- Information sharing (plasmids)

Adhesive proteins

- Just like Velcro, they stick to target cells
- Increases infectivity

Encysted or dormant portion of life cycle

- *M. Tuberculosis*, waxy coat protects the bug
- Sporulation, *C. difficile* (wait for thing to improve)
- Malaria

Disguise themselves

- Intracellular pathogens
- Shed antigens (syphilis)
- Coat themselves with host proteins

Capsule surrounding the bug

- Antibodies bind a great distance from the organism
- Complement is too far to cause damage

- Capsule interferes with phagocytosis and bacterial killing

- With TB, the cell wall elicits the destructive inflammatory response

Ease of spread from one person to the next

- Survives in droplets
- People to people contact
- Survives in a cell leading to cell borne transmission
Evaluating a patient with an infection.

History and physical

Look and listen
Specific signs of inflammation
  Local: rubor, dolor, tumor…
  Systemic: fever, malaise..

Laboratory and X-Ray

Blood count, number and shift

Organ specific enzymes
  Hepatitis
  Myocarditis

Direct evidence of the bug

Culture: eye, throat, sputum, stool, urine..
  Specimen quality is critical

Tissue biopsy for culture or direct identification

*P. carinii*
  Blood smear for malaria

Rounding up the usual suspects.

Although *culture* is most obvious, it's *not the only way* of identifying the offending agent, or monitor the progression of an infectious disease.

*Gram stain.* This is pretty obvious, but often overlooked. Be suspicious of the specimen if no PMNs are present. If it's *acutely inflamed* there should be
If you’re going to culture, be sure the specimen is collected properly and transported to the lab promptly.

For aerobic bugs, the lab should provide you

Identification of potential pathogens
An antibiotic sensitivity profile for the bug(s).

Anaerobes are a special case

They require special transport media
Swabs are not the same. Rayon or Dacron are the best
Natural fibers like cotton or wool contain fatty acids that inhibit some bugs.
Sometime even tissue is needed.
Antibiotic sensitivities are hard to come by on anaerobes.

Blood and cerebral spinal fluid is easy to contaminate, often leading to confusing results

It’s virtually impossible to sterilize the skin
Skin flora may contaminate the specimen
In the case of blood cultures, several sets are recommended.

*H. Pylori*, for instance, we don’t culture

We take advantage of the urease the bug makes.
It starts with a gastric mucosal biopsy.
The biopsy is placed in agar containing urea.
If the bug is present, the urease will hydrolyze the urea, causing a pH shift and color change.

PCR and nucleic probes

Very rapid ID, gonorrhea for example
Viral loads, HIV (PCR)
Forensic and ancient DNA samples (PVR)

Cytopathic effect (CPE) and immunofloresence

Herpes
Chlamydia
Still more of identifying the usual suspects

Direct visualization

Syphilis (dark-field of primary lesion)
Malaria

P. carinii

Serological conversion (the appearance of diagnostic antibodies)

Syphilis
Hepatitis
Rickettsia
Lyme disease

Not every infectious organism is a bacteria or what even we might view as a “microbial agent.”

Here’s a list of infectious agents.

- Prions
- Viral diseases
- Rickettsial disorders
- Chlamydial disorders
- Bacterial diseases
- Fungal diseases
- Protozoal (parasitic) diseases
  - unicellular
  - multicellular
- Helminthic diseases (worms)
- Ectoparasites, lice and other cooties
- Disorders of uncertain etiology or mechanism
  - Sarcoid agent?
Our body's inflammatory response to an infection depends on a number of factors.

- Type of organism.
- Does the bug prefers an intra- or extracellular environment?
- Is toxic or enzymatic destruction part of the process?
- Does T-cell mediated hypersensitivity become part of the mechanism of injury?
- Sometimes even duration of the condition becomes a factor.

Depending on the type of microorganism and its actions, the inflammatory response may be

- **Acute** or
- **Chronic**

There may even be a characteristic structure such as a granuloma or an abscess. What about the X-ray to the right?

If you're unclear on what these terms mean, it might be a good time to review some of these patterns described in laboratories 1, and 2

Now it’s time to go to either the lab CD or website for a series of short cases that will illustrate important aspects of a variety of infectious conditions. Then, there’s a real ocular case.

Case 1: Vomiting
Case 2: Cough & fever
Case 3: Bruising
Case 4: Sore throat
Case 5: Jaundice
Case 6: Flu & fever
Case 7: Diarrhea
Case 8: Black Robe
Case 9: Back Pain
Laboratory 4
Exercise

Today’s slides:

Slide 68: malaria positive blood smear
Slide 69: crab louse
Slide 74: Neisseria gonorrhoeae in human urethral smear
Slide 75: vaginal smear with Candida albicans
Slide 90: tuberculosis
Slide 95, acute bacterial meningitis.

Slide 68, Blood smear positive for Plasmodia falciparum.

Only a small portion of the blood smear is scanned, but even so, you'll have no trouble finding the bugs. Don't go to the highest power to start, rather begin with the 31.5 power and find the RBCs that contain little blue smudges. Then magnify the area to 60 power.

We are looking at a heavily parasitized individual. There is no trouble finding infected RBCs, and you will even be able to get multiple positive cells in one field. This is the leading cause of death worldwide today. P. falciparum is an especially bad actor. This species also has a characteristic, banana-shaped, gametocyte, pictured to the right.
You can even see the little critter developing in the egg case. For this slide, you can't go much more than about 5 power magnification, but then these are infectious agents that can be seen with the unaided eye. This infectious agent is *Phthirius pubis*.
Slide 74, *Neisseria gonorrhoeae* in human urethral smear

The *Neisseria gonorrhoeae* organisms can be seen both intra and extracellularly. They are little kidney bean looking creatures that are seen in pairs, standing up next to each other.

Note that the entire slides is not scanned in, so you need to be sure the little red + is squarely in the green box when you right click for the magnification change. The black box in the image to the right indicates where the image bellow came from.

Keep in mind that this is a gram stain, and the staining you are used to seeing in the inflammatory cells won't be the same. The large tri-lobed body in the left lower are of the image is indeed a PMN. Remember this is a very high power scan.

The bacteria in question are a little dark staining, but are indeed gram negative diplococci; *N. gonorrhoeae*. Many will be within the polys, and a few will be seen scatter about in the background.
Slide 75, *Candida albicans* in a vaginal smear

As with the previous slide, only a small amount has been scanned. You may need to explore some, clicking with the right mouse button, to find the area that can be magnified. Again, the little red + needs to be pretty squarely in the green box on the insert in the upper left corner.

As before, the black box in the lower center portion of the image is what is magnified below.

Here you can see both the hyphal and budding growth phases. The adjacent image shows a smear from a culture, which makes a little easier to find what you are looking for.
Slide 90, pulmonary tuberculosis

This slide demonstrates a form of disseminated tuberculosis we call milliary TB. No, it's not military TB, rather the terms refers to the numerous little granulomas that are spread uniformly like millet seeds. In this slide you will see many small, fresh granulomas.

This image is from the little box in the above image. Here we're looking at the edge of one of the granulomas. You can see the caseous necrosis that's at the heart of the tubercle. The wall of fibroblasts and collagen is just beginning to take form. You should see many giant cells in association with the granulomas.
Slide 95, acute bacterial meningitis

This is a very unfortunate case. **Tissue 95** is of the brain and meninges of a young child that died with acute bacterial meningitis. The subarachnoid space is grotesquely expanded with a marked acute inflammatory exudate. PMNs are everywhere. The little square in the adjacent image shows where the picture below came from.

In this combined image, you see the brain tissue in the top, right corner. Most of the field is subarachnoid space containing millions of PMNs. The insert shows a high power view with the typical three lobed appearance to most of the PMNs.
Laboratory 4
Ocular Case Study

This is an example of a real life problem. Although the images in this manual are black and white, they are in living color on the website and your CD. You really need to see the pictures in color to make the diagnosis, so after reading through the lab exercise, check out one of the other sources for the color photos.

History

Jennifer S. is an 18 year old female college student comes to your clinic complaining that her right eye is red and irritated, and her left eye is slightly red. She has worn AcuVue soft disposable contact lenses for about 3 years with no problems until now. When she takes her lenses out at night, she cleans and stores them in ReNu (a cleaning and disinfecting solution).

Yesterday, she was fine but when she woke up this morning, her right eye was red and somewhat painful. It watered a lot. She couldn’t see very well. Unfortunately, she had no glasses, so she was forced to continue wearing her contact lenses. Her left eye is also irritated, but not as much as the right eye.

Further questioning: She admits that she went to a party and had a bit too much to drink the night before all this happened. In fact, she passed out and doesn’t remember too much until she woke up with her right eye red. She put in some Visine eye drops to make her eyes less red (while her contact lenses were in), but it didn’t help much.

Further questioning: Some of the girls in her sorority house have “pink eye.” She also borrowed and used a friend’s contact lens case and contact lens solutions the day before her eyes got red.
Objective findings


Pupils: normal

Slit lamp examination: Conjunctiva: 3+ injection (figure 1)

Lids: palpebral conjunctiva injected; OD much more injected than OS (figure 2).
Cornea:

OD: numerous infiltrates (figures 3a and 3b).

Also, microcystic edema of the OD

OS shows a few infiltrates.
Consider the four following diagnoses.

1. bacterial keratoconjunctivitis  
2. viral keratoconjunctivitis  
3. allergic keratoconjunctivitis  
4. contact lens associated red eye (C.L.A.R.E.)

Write down what you think to be the salient features of each of the above diagnoses. What might a conjunctival smear show? Want to take a stab at outcomes or treatments for each?

Consult with the web, your friends or whatever, and when done checkout the V543 website, or your CD, for more pictures and descriptions of these conditions.

By the way, what’s your diagnosis?
Laboratory 5
Arteriosclerosis and Hypertension

Arteriosclerosis is actually an “umbrella” term meaning "hardening of the arteries." More specifically it refers to three types of vascular disease which have in common thickening and loss of elasticity of the arterial walls. These conditions are:

1. Atherosclerosis: Characterized by the formation of focal intimal plaques in large and medium sized elastic arteries.
2. Mönckeberg's medial calcific sclerosis: Characterized by calcification of the media of muscular arteries.
3. Arteriolosclerosis (note the emphasis on arteriole size blood vessels here): Marked by proliferative fibromuscular or endothelial thickening of the walls of small arteries and arterioles.

Atherosclerosis, and by extensions its major clinical consequences, is the leading killer in the Western world. Although any elastic artery may be affected, the aorta, the coronary and cerebral systems are the prime targets. The result is that myocardial infarcts (heart attacks) and cerebral infarcts (strokes) the two most frequent lethal complications of this condition.

Specifically, atherosclerosis begins in the intima and may secondarily involve the tunica media of the medium size and large elastic arteries. Elevated plaques or thickenings, called atheromas, appear within the intima and media, and project into the lumen of the vessel. The atheroma gets its name because of the yellow color of the lesion seen grossly. The earliest visible signs in the intima of the atherosclerotic vessel are the proliferation of myointimal cells and the appearance of small lipid vacuoles within these cells. With progression, focal clusters of myointimal cells become “ballooned” with lipid accumulation, creating "foam cells." In time, the foam cells die, become necrotic and the center of the atheroma takes on the appearance of fatty debris with needle-like cholesterol crystals.

Interestingly, atherosclerosis doesn’t even score in the top ten world-wide. In virtually all developing countries, infectious disease remains the leading cause of death.
Atheromas tend to increase in size and may undergo a variety of changes, including calcification, internal hemorrhage and even ulceration with subsequent embolization of the fatty material from the core of the atheroma. Major complications include thrombus formation on the surface of the plaque, leading to complete lumenal occlusion, and in some situations, even aneurysmal dilatation of the wall of the diseased vessel.

Mönckeberg's medial calcific sclerosis is characterized by ring-like calcifications within the media of medium to small sized muscular arteries. These medial lesions do not encroach on the vessel lumen and the endothelium and intima remain intact. The calcification is not associated with any inflammatory reaction, and the adventitia is unaffected.

Arteriolosclerosis represents a “reactive” thickening and strengthening of the wall of arteriole sized vessels as a consequence of hypertension. The changes are divided into several major categories based on the microscopic changes of the vessel walls. The two most frequently seen conditions are: hyaline and hyperplastic arteriolosclerosis.

Hyaline arteriolosclerosis is most often encountered in diabetics and aged patients with mild to moderately high blood pressure. The vascular “lesion” is not an isolated or focal thickening of the vascular wall (as seen in atherosclerosis), rather it consists a generalized change in the muscular portion of the wall of these little vessels. A homogenous, pink, hyaline thickening of the walls of arterioles is seen with loss of underlying structural detail. Consequences of this type of vascular change are not terribly severe, but they may be accumulative. One observes minimal narrowing of the diameter of the affected vessels as well as some loss of compliance of the vessel wall.

The development of hyperplastic arteriolosclerosis is related to more severe degrees of hypertension, and definitely indicates the vessels are in trouble. In this form of arteriolar disease, we observe the reactive and progressive grow of the smooth muscle cells of the wall of the arteriole, giving rise to an “onionskin” concentric thickening of the muscular wall. As you might guess, in this more advanced condition there will be more progressive narrowing of the lumen of the arteriole, leading to a greater and more progressive degree of tissue hypoxia.

Ophthalmic examination of the fundus allows a unique opportunity to directly visualize the development of arteriosclerosis. The central retinal artery and its first branches are true arteries; that is, they have an internal elastic lamina and a complete tunica muscularis. As a result, these vessels can demonstrate atherosclerosis. Most of the retinal arterial vessels, however, are really arterioles, and thus are subject to arteriolosclerosis.
Arteriolosclerosis of the retinal vessels has been classified (by Scheie) into stages typified by an increased arteriole light reflex and arteriovenous crossing phenomena. In later stages, the arterioles are described as resembling burnished copper wire and finally thin white, threadlike silver wires. These changes correspond to a hyaline thickening and lipoidal infiltration of the intima and media.

Other changes of hypertension can also be visualized on a fundus examination. Most investigators would agree that hypertension can be defined as a sustained diastolic pressure above 90 mmHg. This elevated blood pressure, whether relatively mild or accelerated, will, in time, produce generalized narrowing of the arterioles and small arteries throughout the body. Arteriolar narrowing is also seen in hypertensive retinopathy. Microaneurysms, dilated capillaries, hemorrhages, and/or edema may be present as well.

**Pulmonary Embolization and Pulmonary Arteriosclerosis**

An embolus is a simple a space occupying body (“lesion”) moving along within the vascular system. Emboli can be blood clots, foreign matter such as a bullet, bone chips, amniotic fluid or even air. The usual source of the typical blood clot embolus is a thrombus from one of the deep leg veins. For all practical purposes, both the blood clot embolus and thrombus are the same thing, a “pathological” blood clot; the thrombus is fixed and the embolus is on the move. In the case of deep leg vein thrombosis, if the clot should break free, it can only go as far as the lungs before coming to a vascular bed too small to pass through. But in rather rare situations, a venous embolus may actually cross into the arterial circulation and cause a stroke or other evidence of arterial occlusion. Can you imagine a scenario in which this is possible? It does happen, and the blood clot **does not** enter the arterial circulation by going through the pulmonary vasculature.

On occasion, however, primary thrombi may arise in the lung all on their own due to vascular sclerosis of the pulmonary vasculature itself. Pulmonary vascular sclerosis is associated with pulmonary hypertension. In primary pulmonary vascular sclerosis, there is no observable cause for the changes that occur. It has been postulated that overactive sympathetic innervation causes prolonged vasoconstriction. This induces pulmonary hypertension and, in time, arterial thickening. A second theory proposes that multiple small pulmonary emboli are the cause of the pulmonary hypertension. The emboli arise as thrombi at sites of vascular injury in the lung.

In secondary pulmonary vascular sclerosis, some underlying pulmonary disease leads to vascular narrowing (or loss of vascularized lung tissue) and the subsequent development of pulmonary hypertension. The most frequent underlying cause is chronic obstructive pulmonary disease (COPD) secondary to cigarette smoking. The common term is emphysema, and it applies to the loss of pulmonary parenchyma which leads to reduced numbers of vascular channels in the lung, and thereby increased pulmonary vascular resistance. The changes that occur in both primary and secondary pulmonary vascular sclerosis involve the entire pulmonary arterial tree.
In the larger elastic pulmonary arteries, atheromatous plaques can even form, just like in the systemic circulation. In medium-sized muscular arteries, marked thickening of the media occurs, which leads to narrowing of the vascular lumen. The intima may become thickened and fibrotic, and even the adventitia may become sclerotic. In both medium and small-sized arteries, the internal and external elastic membranes generally exhibit some degree of thickening. It’s the arterioles, however, that are most significantly affected. These vessels show marked thickening of the media which in some instances may narrow the vascular lumens to pinpoint channels.

**Infarction and Emboli**

An infarction is a localized area of ischemic necrosis within a tissue or organ resulting from occlusion of either its arterial supply or its venous drainage. Nearly all infarcts result from thrombotic or embolic occlusion. Infarcts are classified as either anemic (white) or hemorrhagic (red).

**White** infarcts (that is the absence of hemorrhage in the area of dead tissue) are seen in organs that have a so called “end artery” vascular system. They develop following arterial obstruction. In this situation, there is no significant overlap of the capillary beds, so when the blood flow stops and the tissue dies an anoxic death, there is no source of blood to leak into the necrotic region. The absence of blood gives the pale appearance of the dead tissue. The heart, kidneys and spleen are organs that develop white, or anemic, infarcts.

**Red** (hemorrhagic) infarcts are in organs that have either a dual blood supply or substantial overlapping of the capillary beds. However, the degree of overlap is not enough to keep the organ or tissue alive when one of the vascular elements has become occluded. The lung is an organ with a dual blood supply, having both the pulmonary artery system (which surprisingly does bring some nutrients and oxygen to the pulmonary tissue) and the bronchial arterial system which is the “arterial” blood supply to the pulmonary tissue itself.

What one observes in the case of a pulmonary embolus, is that the pulmonary parenchyma dies following the pulmonary artery occlusive event with a pulmonary embolus, and the bronchial arterial blood flow is not adequate to keep the lung tissue alive. Generally, there is enough bronchial arterial flow to bleed into the infarct once the vascular tissue in the necrotic region breaks down. This often takes a few days after the initial vascular occlusive event.

In red infarcts, the suffusion of red cells seems to obliterate the underlying structure of the tissue. Ischemic coagulative necrosis still results and the effect is hemorrhage within the dead tissue. From this point, the elements of repair do the best they can and the process looks much like the recovery of other infarcts, apart from the obvious problem of removing all the broken down red blood cells. In essence, an inflammatory exudation begins to form at the margins of the area of infarction, eventually giving way to fibrosis and replacement by scar tissue.
As you might guess, hemorrhagic infarcts are also seen in the case of venous obstruction, and this is especially so in the bowel. Consider this, it doesn’t matter whether the artery or vein is occluded for infarction to develop. If there is no blood flowing through an affected organ, the lack of oxygen and nutrients will result in the death of the tissue.

Laboratory 5
Exercise

Today’s slides:

- #70  Coronary artery atherosclerosis
- #99  Pulmonary artery atherosclerosis
- #96  Diabetic nephropathy
- #98  Pulmonary arteriosclerosis
- #100 Pulmonary infarction

1. Draw a cross section of a large artery (such as the aorta), labeling the intima, media, adventitia, internal elastic lamina, external elastic lamina, and layers of smooth muscle and elastic fibers.
Slide #70: atherosclerosis of coronary vessel

There are several cross sections of the same coronary artery on this slide. You will be able to see a fairly mature plaque, and in several sections there is the thrombus that killed this man. Note the complexity of the atheroma. You will see tiny slits where the cholesterol was before it was washed out during the processing of the tissue in order to make the slide.

In the higher power view, lower right, the plaque shows a collagen cap, with areas of calcification just beneath. The needle-like slits contained oxidized cholesterol when this man was alive. The cholesterol was removed, inadvertently, when the tissue was passed through the xylene as your slide was being stained. The little image (upper right) shows what happened to cause the clot to form. Click it for an enlarged view.

Your observations
Slide #99: pulmonary atherosclerosis

On the slide, the atheroma shows two main zones. There is a fibrous cap consisting of smooth muscle cells or fibroblasts that produce collagen, and there is a central myxomatous core. Also, because this atheroma is relatively old, there is granulation tissue at its margins.

Your observations
Slide #96: diabetic nephropathy

Diabetic nephropathy is a vascular disaster. It involves virtually every vessel of the kidney, including the capillary tuft of the glomerulus. In this slide you will see almost every glomerulus is diseased. Note the marked basement membrane thickening of the small vessels and the capillaries of the glomerulus. Additionally, you will see loads of lymphocytes in the interstitial tissue. Chronic pyelonephritis is a pretty consistent part of this condition.

The most dramatic changes are seen in the cortex. In the image to the right you will note that most glomeruli are scarred and show profoundly thickened basement membranes. If you're lucky enough to find a glomerulus with both afferent and efferent arteriole in section, you will see how profoundly diseased both vessels plus the glomerular capillaries really are.

Your observations
Slide #98: Pulmonary arteriosclerosis, emboli

There are several things happening in this slide. The major things is the advanced small vessel disease throughout the lung parenchyma. In addition, there happens to be a clot in one of the larger vessels, but that's not the surprising, consider the vascular back pressure created by the increased resistance from the small vessel narrowing. Recall, there are several reasons for this condition to develop, among them infection with HSV-8.

In the image to the right, the vessels may not look markedly thickened, but they are. Normally the arterioles of the lung have very thin walls and are very compliant and resilient. Not here. Compare the vessel above to this normal one. The wall is so thin in the healthy condition that you can hardly see it. Not so with pulmonary hypertension.

Your observations
Slide #100: Pulmonary infarction with embolus

There are several large structures to catch your eye in this slide. One is the bronchus with its cartilage rings, and the other is the vessel plugged with an embolus. Although the center of the clot is missing in the section, it was present before the processing of the tissue to make your slide. Big clots often don't fix well, and their centers will chip out when the tissue is cut super thin for the slide.

In the picture to the right we're looking at the edge of the clot and vessel wall. You can see some of the platelet and fibrin rich areas distinguished from the RBC rich areas. This effect creates the lines of Zahn that can sometimes be seen in with the unaided eye. Also in this slide you will see some black particulate matter in the lymphatics surrounding the vessel. This is junk that was inhaled, possible cigarette smoke residue. It'll be there for life.

Your observations
I. Review of vascular anatomy and histology

A. Capillaries

B. Arteries
   1. Arterioles and small arteries
   2. Medium-sized arteries
   3. Large arteries

C. Veins

II. Normal Aging Changes

III. Arteriosclerosis ("hardening of arteries")

A. Atherosclerosis
   1. Histopathology
   2. Complications
   3. Predisposing factors

B. Arteriolosclerosis
   1. Histopathology
   2. Complications
   3. Predisposing factors

C. Mönckeberg's medial calcification

IV. Hypertension and optometry

V. Cardiovascular disorders

A. Infarct
B. Mural thrombus
C. Dissecting aortic aneurysm
Question for lab 5

You are looking in a patient's eye with your ophthalmoscope and note signs of arteriosclerosis of the retinal vessels. How should you advise your patient? Support your answer.

Remember to email your answer to the V543 email account.
Laboratory 6
Cardiovascular Disorders

Myocardial Infarction

A myocardial infarct (MI) results when there is inadequacy oxygenation of the myocardial muscle. Typically this occurs when there is an acute cessation of coronary blood flow. Practically always this means there has been thrombotic occlusion of the coronary vessel in association with pre-existing atherosclerosis. On rare occasions, a thrombus is not identified, leading some investigators to suggest that a severe temporary episode of coronary spasm (with co-existing atherosclerosis) may be sufficient to cause focal necrosis of the myocardium. Even so, myocardial infarction in the absence of coronary atherosclerosis is virtually unheard of.

The diagnosis of acute myocardial infarction is based on several factors:

1. Clinical history
2. EKG changes indicating a region of “electrically” inert myocardium
3. Alteration of serum enzymes reflecting the necrosis of myocardial cells

Typically, the onset is sudden and devastating with severe, constricting substernal or precordial pain that often radiates to the left shoulder, arm, or jaw. I once heard the pain described as if the person were swallowing a “a flaming softball.” It is often accompanied by sweating, nausea, vomiting, or breathlessness. Occasionally, the symptoms are less specific and may be interpreted as "heartburn" or "indigestion." The EKG changes usually become evident from the outset of the.

Alterations in serum enzymes is strong evidence in support of the diagnosis, however, the source of the enzyme elevation must be confirmed as cardiac. Enzymes released by the dying myocardial cells are similar to those found in skeletal muscle, making confirmation of the source a necessity.

The enzyme most specific for myocardial necrosis, however, is an elevated level of serum creatine phosphokinase (CPK), specifically its MB isoenzyme. As CPK is released from the necrotic cells in the myocardium, it will appear in elevated levels within hours of the infarction. The CPK levels peak within 24 to 48 hours and then rapidly decline. As you might guess from the timing of the CPK peak, if a person were to seek medical help several days after the coronary
occlusive event, levels of this important enzyme would have returned to the reference range (normal range), making the diagnosis a little tricky.

One of the less specific serum enzymes associated with myocardial death is serum glutamic oxalacetic transaminase (SGOT). This enzyme starts to rise within 6 to 8 hours following an MI, and reaches a peak in 24 to 48 hours. The levels then fall to normal within 4 to 8 days. Even less specific, but still helpful to a degree, is a measured rise in serum levels of lactic dehydrogenase (LDH). This enzyme is usually the last to rise, but remains measurable for 8 to 12 days following a myocardial infarct. Even though the total LDH is not terribly specific for myocardium, there is an isotype of the enzyme which comes largely from cardiac muscle. Elevated levels of LDH5 can be particularly helpful in making a diagnosis of myocardial in a person who has presented late in the course of the development of the infarction, and the other earlier detectable enzymes have fallen back to the reference range.

The histopathologic changes of a myocardial infarction follow a fairly predictable and orderly progression. Viewing H&E stained tissue with the light microscope, the cellular changes of coagulative necrosis are not detectable for the first 12 to 18 hours. Usually, within 24 hours, the myocardial fibers undergo sufficient enzymatic changes to yield the expected features of coagulative necrosis. These changes are almost always accompanied by some interstitial edema (after all, this is a site of developing inflammation). A scant amount of fresh hemorrhage may be present and the beginnings the neutrophilic exudate will begin to appear by end of the first day. During the subsequent days, the neutrophilic exudation increases, the cytoplasm of dead myocardial cells becomes more distinctly “coagulated,” nuclei become pyknotic and disappear. The ingrowth of granulation tissue becomes evident by the end of the first week, and usually completely replaces the area of necrosis by six weeks.

**Thrombus formation**

A thrombus is a pathological blood clot. Clearly, if you have damaged tissues and your blood is running out on the ground, you want your blood to clot so that your valuable genes stay in the gene pool. On the other hand you don’t want the clotting system to become confused and produce a clot in the middle of one of your blood vessels, potentially “turning” off the blood flow to some vital part of your
body. A thrombus is this kind of vascular malfunction, in other words a **pathological** blood clot (that is one within the fixed vascular system). Just like a flower out of its place is a weed, a blood clot out of its place is a thrombus.

A thrombus may form anywhere in the cardiovascular system. However, there are some interesting and therapeutically important differences between those that arise on the arterial side of the circulation and those that form on the venous side. I know this may sound like we are splitting hairs for the sake of academic interest (or to preserve our jobs), but not so.

**Arterial thrombi** develop because of platelet activation and adhesion to the wall of a vessel (or even the ventricle of the heart), typically at the site of endothelial damage. These thrombi are dry, friable, grayish in appearance, have darker gray lines of platelets and fibrin running through them. Platelet activation is the important feature here.

**Venous thrombi** form because of stasis of the blood flow and the subsequent activation of the clotting proteins. These thrombi have a deep red-purple color, and often form in the deep leg veins of people with poor venous return (such as is seen in chronic heart failure) or those who are confined to bed rest (such as patients following orthopedic surgery).

We use a special term to describe a thrombus that has formed on the wall of the heart or major vessel; **mural thrombus**. Mural thrombi develop following injury to the endothelium of a chamber of the heart, or even the aorta. As the name implies, this is a pathological blood clot that has developed on the wall of the injured structure. In the case of the heart, mural thrombus can form on the endothelial surface of the ventricle in a matter of hours following a myocardial infarction.

There are several potential outcomes for a thrombus:

1. May propagate (increase in size) to cause an obstruction of a vital vessel,
2. May embolize (that is they may break free and float off to become wedged in another vessel),
3. May be destroyed by fibrinolysis and the original vessel is reopened, or
4. They may go through a process of **organization** and eventually may **recanalize**.

In this laboratory exercise, we shall consider the **organization** of a thrombus. In an organizing thrombus, its surface becomes covered by endothelial cells and there is ingrowth of
subendothelial smooth muscle cells along with mesenchymal cells into the fibrinous thrombus. These cells form capillary channels through which blood may flow. This process of forming these channels is known as recanalization.

The reason it’s important to keep in mind the difference between the mechanism of formation of arterial and venous thrombi has to do with treatment and prevention strategies. If you know an arterial thrombus results from platelet activation, then doing something to prevent platelet adhesion to the endothelial cells would be a smart move. For this reason, people who have a history of coronary thrombosis or bypass surgery are often put on daily aspirin. Whereas those who have a history of deep leg vein thrombosis, and pulmonary embolization, are put on an anticoagulant that inhibits coagulation factor activation.

**Dissecting Aortic Aneurysm**

The dissecting aneurysm is considered to be the most common catastrophic illness involving the aorta. As the name implies, these aneurysms are characterized by dissection of the laminar planes of the aortic media. Because these aneurysms are not usually associated with marked dilatation of the aorta, the term "acute aortic dissection" has largely replaced the term "dissecting aneurysm." Hypertension is almost always antecedent to the condition (seen in 94 percent of cases) and may well play an important role in initiating the intramural hemorrhage.

The hemorrhage in a dissecting aneurysm generally occurs between the middle and outer thirds of the media. The intimal tear, generally representing the origin of the dissection, is found in the ascending portion of the arch in 90 percent of cases. Many times the dissection extends into the great vessels of the neck, and in other instances, into the coronary, renal, mesenteric, and iliac arteries. The dissection may extend through the wall of the aorta, essentially “blowing out” into the surrounding structures or hollow spaces. Needless to say this outcome is almost uniformly fatal.

Two questions arise concerning the pathogenesis of dissecting aneurysms. First, what causes the media of the aorta to weaken, allowing blood to dissect into the wall, and second, what causes the hemorrhage and intimal tear?
The most widely known lesion which is thought to weaken the wall of the aorta is cystic medial necrosis. This lesion involves changes in the musculoelastic media of the aorta and, less commonly, the coronary arteries. It is evidenced by the accumulation of a basophilic amorphous material in the media, often with the formation of mucoid cysts. Once the vessel wall has been weakened by cystic medial necrosis (or by other biochemical, ischemic, or hydrodynamic factors), an intimal tear occurs, often due to hemodynamic factors accentuated by hypertension. Once the tear has occurred, the increased blood pressure present in most of these patients enhances dissecting either through or along the length of the aortic wall.

There is, however, a genetic condition in which a defect in collagen synthesis results in a weakened and dilated aorta, often leading to rupture. Marfan’s syndrome is a condition that you as an optometrist may actually diagnosis before anybody else because of a characteristic abnormality of the optic lens. Patients with Marfan’s syndrome have a decentered crystalline lens resulting from the malformation of the zonular fibers. Marfan’s patients often have co-existing hypertension and are great risk of dying from an acute aortic dissection. The decentered crystalline lens causes a high levels of astigmatism and can easily be seen by slitlamp biomicroscopy when the pupils are dilated.
Laboratory 6
Exercise

Today’s slides:

Online slide of acute myocardial infarction
#82    Myocardial infarction
#84    Dissecting aortic aneurysm

Acute and healing myocardial infarction.

This slide is exclusively online. It represents an unfortunate state of affairs for the person who died, but it is a good teaching case. There are two stages of an acute myocardial infarct in this one slide. The first an acute infarct of about 3 days duration. You will note the bright pink are of coagulative necrosis. The second area is an infarction of about 14 days duration. It is lighter in color and has a blue tint.

In the picture to the lower right we are looking at an area with both the very recent infarction and the one that is two weeks old. In the area of healing infarction, there has already been almost complete removal of the dead myocardial cells. The area of 3 day old infarction shows all the features of coagulative necrosis.

Your Observations
Slide #82: Myocardial infarction with mural thrombus

It's hard to imagine this is even heart. There is a little tag of cardiac muscle at one side, but for the most part this tissue now consists largely of scar tissue and mural thrombus. Virtually all of the muscle in the area of the old infarct is gone, and replaced by dense collagenized connective tissue. The ventricular wall in this area is only about 10-15% of the normal thickness. There is a large fragment of clot, mural thrombus, adherent to the endothelial surface. It's even becoming organized into the old infarct.

In the image to the right we are looking at the region of the old infarct with organization of the mural thrombus stuck on the endothelial surface. The infarct is composed of dense connective tissue, whereas the organizing thrombus shows younger, delicate granulation tissue with many new blood vessels.

Your observations
Slide #84: dissecting aortic aneurysm.

In this section, it's easy to see the advancing hemorrhage and dissection of the muscle wall of the aorta. In this condition, there is a preexisting weakness in the layers of the media (muscle), which easily separates once the blood, under pressure, gets through a tear in the intimal lining. People experiencing this disorder complain of excruciating pain as the muscle layers are torn apart by the advancing wave of blood.

In the picture to the right we're looking at the advancing wave of hemorrhage and separation of the muscle layers. Note the changes of cystic medial necrosis (little image below) indicating the intrinsic weakness in the muscular wall of the aorta.

Your observations
Question for lab 6

Describe the difference in retinal appearance between a central retinal vein occlusion (CRVO) and central retinal artery occasion (CRAO) by a thrombus or embolus.

Remember to send your answer to the V543 email account.
Diseases of the kidney are among the most challenging of all conditions to diagnose. The reason kidney disease can be so baffling is that the kidney is involved in the functioning of so many other physiological systems. By and large, most people consider the kidney to have just one job, namely the processing of Budweiser into urine. But in fact, the kidney is involved in electrolyte balance, red blood synthesis and even indirectly in our mental health. Clinical presentations include everything from hypertension to anemia and if the nitrogenous wastes are not cleared from the blood effectively, the individual with renal failure may present with psychiatric symptoms. But the interesting thing about renal disease is that much of it can be traced to the malfunction of one segment of the renal anatomy: the microvasculature. We will see time and again that diseases producing damage in arterioles and capillary size vessels will have a profound effect on the kidney’s ability to function properly. But before considering the pathological processes, a review the micro-architectural and functional units of the kidney is in order.

Functionally, the kidney can be divided into two regions: the cortex and medulla. In the cortex, one finds the filtration apparatus and part of the recovery system of the kidney. These elements consist of the glomerulus for “straining” or filtering the blood, as well as the proximal and distal convoluted tubules, responsible for reabsorbing things your body really intends to keep. In the medulla we find the Loops of Henle which are responsible for creating the concentration gradient used to recover free water from the urine. Without this concentrating mechanism, our urine would be extremely dilute, and we would become seriously dehydrated in a matter of hours.
The glomerulus is the real business end of the kidney. Essentially, it’s a ball of capillaries, with a few specialized cells called mesangial cells. This little filtration structure is tucked into a hollow space designed to capture and send the newly filtered urine into the tubular system. This hollow space, Bowman's capsule, actually consists of a “folded” or two-layered capsule and is connected directly to the proximal convoluted tubule.

The capillaries of the glomerulus are the terminal branches of the afferent arteriole, and as such are subject to levels blood pressure not found in any other capillary bed. It is this higher than usual pressure that serves as the driving force to filter the newly formed urine through the capillary basement membrane. At the end of the capillary bed, these little vessels re-coalesce to form the efferent arteriole. As the efferent arteriole leaves the glomerulus, it brushes past some specialized cells in the distal convoluted tubules that are responsible for monitoring blood pressure, salt concentration and even hemoglobin content of the blood. Depending on the status of the blood, these cells send signals to the appropriate organs to make adjustments in sodium content and hemoglobin level of the blood, as well as determine the amount of water the kidney should recover from the newly formed urine.

In addition to the capillary loops of the glomerulus, one finds structural connective tissue elements as well as a few very specialized cells known as mesangial cells. These mesangial cells have the important function of “cooling down,” on a local basis only, the immune and coagulation systems in the capillary bed of the glomerulus. These little fellows seem to have the ability to keep the immune system from going over board when antigen-antibody complexes, that we all have from time to time in our circulating blood, stick in the capillary membrane of the glomerulus. If the effect of these antigen antibody complexes is not tamed immediately, your immune system will likely end up destroying the glomerulus in an “innocent bystander” manner as it responds to the complement activation brought about by these immune complexes.

The proximal convoluted tubules can be differentiated histologically from the distal convoluted tubules as follows:

1. Proximal convoluted tubules
   - low columnar cells
   - irregular shape
   - eroded lumen = "brush border"
   - cell borders difficult to make out
2. Distal convoluted tubules
   - cuboidal cells
   - regular lumen
   - cell borders easier to see

**Disorders of the Kidney**

**I. Acute Pyelonephritis**

Acute pyelonephritis is a relatively common, suppurative inflammatory disease of the interstitial tissue of the kidney. It is usually caused by gram negative bacteria that actually come from the affected individual’s own body flora. The most common etiologic agent is *Escherichia coli* which ascends to the kidney from the lower urinary tract. The organism causes suppurative necrosis and abscess formation within the renal interstitial tissue. In the early stages, many neutrophils are found in the interstitium, but later, abscess formation may involve the tubules also. **Necrotizing papillitis**, ischemic and suppurative necrosis of the medullary “papillae,” may also occur in diabetics.

**II. Chronic Pyelonephritis**

Chronic pyelonephritis is a common cause of chronic renal failure. It develops for a combination of reasons, among them chronic and/or persistent bacterial infections as well as chronic ischemia due to small vessel disease typically associated with hypertension and/or diabetes.

One or both kidneys may be involved. The affected kidney may weigh as little as 50 gm. There is usually scarring in the pelvis and/or the calyces. Microscopically, interstitial fibrosis is evident with the infiltration of lymphocytes, plasma cells, and few neutrophils. "Colloid casts" may be present in the tubules. "Thyroidization" of the kidney and concentric fibrosis around the parietal layer of Bowman's capsule is seen. Inflammation and fibrosis of the calyceal mucosa and wall are often present. Hypertension changes in the vessels are quite obvious.
III. Diabetes Mellitus

Diabetes is the leading cause of renal failure in the United States. Because the major anatomic lesions in diabetes mellitus include renal disease, microangiopathy, atherosclerosis, retinopathy, and neuropathy, we will spend a fair amount of time to learn about the lesions that are present in a diabetic kidney. Some of the vascular changes that are apparent in the kidney are quite similar to that seen in the arterioles in the fundus (eye).

Diabetes mellitus is a chronic disorder of carbohydrate and fat metabolism, but in the long run, it becomes a vascular disease. Although increased incidence and severity of atherosclerosis is virtually always a long term problem, it is the smaller vessel (arteriolar size vessels) that are the most severely affected and lead to the greatest number of problems.

We divide diabetes into two forms: "juvenile-onset" diabetes, which accounts for about 5 to 10 percent of diabetics, while "maturity-onset" diabetes represents the remaining 90 to 95 percent of all cases. While attention is often focused on the disordered carbohydrate metabolism of diabetics, it is important not to overlook the fact that all pathways of intermediary metabolism are disrupted. Insulin is a major anabolic hormone in the body, and derangement of insulin affects fat and protein metabolism as well as glucose.

Due to the wide range of metabolic derangement found in diabetes mellitus, the disease can take several forms. Overt or manifest diabetes refers to the fully expressed clinical syndrome which is characterized by fasting hyperglycemia, glycosuria, and usually the three “P”s (polyuria, polydipsia, polyphagia). Both latent and subclinical diabetes refer to a patient who is generally asymptomatic, but may progress to clinical diabetes under stressful conditions (such as obesity, pregnancy, or infection).

A. Microangiopathy and basement membrane thickening (BMT):

In long-term diabetics, the basement membrane of the vessels becomes markedly thickened and stains as a homogenous, eosinophilic "hyaline" material. These changes develop throughout the body, but they are most easily seen in the afferent and efferent arterioles as well capillary tufts of the glomeruli. The microangiopathy resulting from this thickening of the capillary basement membrane leads to tissue hypoxia and compromise of organ function. The tissue ischemia has far-reaching implications, inducing serious lesions in the renal glomeruli and retina, just to name a few.

B. Atherosclerosis:

All diabetics who have had the disease for at least 10 years, whatever the age of onset, are likely to have clinically significant atherosclerosis. In most diabetics, the atheromas are similar qualitatively to those found in nondiabetics, but they are much more numerous in diabetics, and tend to undergo complications (ulceration, calcification, and thrombosis).
Thus, relatively early in the diabetic's life, atherosclerosis may result in arterial narrowing or occlusion and/or aneurysmal dilatation. The latter is seen most often in the aorta and accounts for the higher rate of myocardial infarction, stroke, and gangrene of the extremities often leading to amputation. Both hyperlipidemia and derangement of the metabolism of the endothelial cells themselves have been proposed as the cause for the increased incidence of atherosclerosis found in diabetics.

C. Neuropathy:
Peripheral nerves, brain, and spinal cord may all be damaged in longstanding diabetes. This neuronal damage is characterized by myelin degeneration, and with time, the axon processes may be damaged as well. Most commonly, symmetrical peripheral neuropathy is encountered, affecting both the motor and sensory nerves of the lower extremities.

D. Renal disease:
The kidneys are usually the most severely damaged organs in the diabetic, and renal failure accounts for many of the diabetic deaths in both juveniles and adults. The following lesions may be found.

1. Glomerular lesions
   a. Diffuse glomerulosclerosis is the most common form of nephropathy. It is present in at least 90 percent of patients who have had the disease for more than 10 years, but is also encountered in patients with atherosclerosis and hypertension. An overall thickening of the basement membranes of the glomerular capillaries occurs along with the deposition of a diffuse eosinophilic hyalin matrix surrounding the capillaries. When seen in microscopic section, the overall effect is one in which a diffuse eosinophilic (pink) haze is seen within the glomerulus. With progression of this lesion, the luminae of the glomerular capillaries are narrowed, and eventually, the entire glomerulus may undergo sclerosis.

   b. Nodular glomerulosclerosis (Kimmelstiel-Wilson disease) is, on the other hand, considered to be pathognomonic of diabetes mellitus. Here, the glomerular lesions take the form of ovoid or spherical deposits of a homogenous hyaline material within the central regions of each glomerular tuft. One, several, or all of the lobules in the individual glomerulus may be involved. As Kimmelstiel-Wilson disease advances, the individual nodules enlarge and eventually compress and engulf capillaries, obliterating the glomerular tuft.

   c. Fibrin cap appears as a homogenous, brightly eosinophilic crescentic deposit found between the visceral epithelial cells of Bowman's capsule and the basement
membrane of the capillaries. It appears to represent a condensate of plasma proteins and thus may reflect the heavy proteinuria sometimes encountered in the diabetic. Fibrin caps can also be found in the nondiabetic.

d. **Capsular drop** is virtually diagnostic of diabetes. The capsular drop appears as an eosinophilic focal thickening of the parietal layer of Bowman's capsule, which apparently hangs in the uriniferous space. It also contains plasma proteins, but it may be only an expression of the widespread BMT. Neither the capsular drop nor the fibrin cap has significance in terms of causing renal functional impairment. Both the fibrin cap and the capsular drop are exudative lesions composed of plasma proteins and fibrin. They are caused by excessive leakage from the glomerular capillaries that have been damaged by either diffuse or modular glomerulosclerosis.

2. **Vascular lesions** often involve the kidney more severely than other organs.

   a. **Atherosclerosis** of the renal arteries may cause generalized renal ischemia or focal infarcts.

   b. Advanced arteriolosclerosis (thickening of the arteriole wall) of both afferent and efferent arterioles. Efferent arteriolosclerosis is also considered pathognomonic of diabetes mellitus.

3. **Pyelonephritis**, both the acute or chronic variety, is much more common in diabetes than in nondiabetics. **Necrotizing papillitis**, a form of acute pyelonephritis, is particularly prone to develop in diabetics. In this disease, an acute bacterial infection develops within the renal pyramids (papillae), inducing an infarct-like necrosis of the distal segment. Cortical suppuration and abscesses usually accompany necrotizing papillitis.

4. **Tubular lesions**

   a. **Glycogen deposition** may occur within the renal tubular epithelial cells of a diabetic who had marked hyperglycemia and glycosuria in the last days of life. It is a reversible change (before death), believed to be caused by the reabsorption of urinary glucose followed by the accumulation of glycogen within the epithelial cells of both the distal portions of the proximal convoluted tubules and the descending limb of the loop of Henle. This lesion, called the Armanni-Ebstein lesion, is virtually diagnostic of diabetes, but rarely appears today because it is usually found only in the diabetic who has been medically out of control for some time before death.

   b. **Fatty changes** may also occur in the epithelial cells of the proximal convoluted tubules. This is probably due to tubular reabsorption of lipoproteins in diabetic lipoproteinuria.
Diabetic retinopathy is the fourth leading cause of legal blindness (20/200 or worse) in the United States today. The fundus abnormalities seen in diabetic retinopathy consist of changes within the retina, in front of the retina, and within the vitreous. The intraretinal changes compose the nonproliferative or simple phase of the disease, while the preretinal and vitreous alterations make up the proliferative or malignant phase.

The **nonproliferative** changes are the first to occur and are most often manifestation of retinal ischemia. The earliest sign of diabetic retinopathy is the retinal capillary microaneurysm, which, in the beginning, appears as a dark red spot on the retina and then may gradually hyalinize and appear as a yellow or white spot. Retinal hemorrhages, capillary occlusion, and large arteriole obstruction may also occur in this phase. Localized areas of venous pathology (such as venous kinking, looping, and duplication) can also be found frequently in nonproliferative diabetic retinopathy.

The **proliferative** phase of diabetic retinopathy is characterized by neovascularization and fibrosis of the retina. The proliferation begins with the formation of fibrotic new retinal vessels which are abnormally "leaky" and form dense adhesions to the vitreous body. The adhesions are extremely important because they are responsible for transmitting the forces of vitreous traction to the retina. The visual symptoms of this proliferative phase are the result of two processes: (1) opacification of the vitreous due to neovascular hemorrhage and fibrous tissue growth and (2) the detachment of the retina due to traction around vitreous adhesions. Both of these conditions can result in legal blindness if the macula is involved.
Slide #96: Diffuse diabetic nephropathy

The diabetic vascular changes are seen throughout this slide. You will see markedly thickened basement membranes in virtually every glomerulus, as well as thickened arteriole walls. (Click this little image for a larger view.) You will also see chronic inflammation and fibrosis of the interstitial tissue.

Chronic inflammation and interstitial fibrosis (chronic pyelonephritis) is seen throughout this slide. You will even find areas of thyroidization with congealed, eosinophilic stained material in the tubular lumens. The tubular epithelial cells themselves look to be in pretty good shape.

Be sure you can find the features listed below.

1. Glomerular lesions
   a. Diffuse glomerulosclerosis
   b. Nodular glomerulosclerosis (Kimmelstiel-Wilson lesion)
c. Fibrin caps
d. Capsular drops

Both the fibrin cap and the capsular drop are exudative lesions composed of plasma proteins and fibrin. They are caused by excessive leakage from the glomerular capillaries that have been damaged by either diffuse or nodular glomerulosclerosis.

2. Vascular lesions
   a. Atherosclerosis
   b. Hyaline arteriolosclerosis

3. Pyelonephritis
   a. Acute pyelonephritis is a manifestation of the vulnerability of the diabetic patient to bacterial infection. A bacterial suppurative inflammation which may cause abscesses is seen. Many PMN's are visible.
   b. Chronic pyelonephritis may be a progression of acute pyelonephritis, or may have other complex etiologies. Lymphocytes predominate and a few plasma cells are seen.

4. Tubular lesions
   a. Glycogen deposition
   b. Fatty change

Your observations
Slide #91: Acute and chronic pyelonephritis

1. Acute pyelonephritis: The most common etiologic agent is *Escherichia coli* which ascends to the kidney from the lower urinary tract. The organism causes suppurative necrosis and abscess formation within the renal substance. In the early stages, many neutrophils are found in the interstitium, but later, abscess formation may involve the tubules also. Necrotizing papillitis (common among diabetics) may also occur. This is ischemic and suppurative necrosis of the apical two-thirds of the renal pyramids (papillae).

In the slide, many PMN's are evident in the interstitium.

2. Chronic pyelonephritis
Microscopic findings visible on the slide are:
   a. Uneven interstitial fibrosis
   b. An inflammatory cell infiltrate in the interstitium consisting of lymphocytes and plasma cells
   c. Dilatation or contraction of the tubules with atrophy of the lining epithelium
   d. A glassy, pink material in the tubules known as a "colloid cast," and neutrophils in the tubules
   e. Fibrosis of the parietal layer of Bowman's capsule
   f. Vascular changes similar to arteriolosclerosis
Your observations of slide 91.

Question for lab 7

If your patient shows signs of advanced diabetic retinopathy in one or both eyes, what lesions would you expect to find in a biopsy of the kidney?

Remember to email your response to the course email account.
History

Marge S. is a 46 year-old house wife who comes complaining of a recent change in visual acuity and 'drop outs'. She was last seen two years ago and at that time had unaided vision of 20/40 bilaterally. To the right is how Marge saw things as she approached the building for her appointment today. She normally requires correction for distance only and has never required glasses for reading. Over the past three months she has noticed progressive trouble reading signs and clearly seeing oncoming cars while driving.

Today's unaided visual acuities

- Distance:
  - 20/200

- Near:
  - 20/80
  - 20/80

Dilated Fundus exam

- This is definitely not normal.
- What sort of abnormalities do you see?
- Check the CD for a normal retina and to get a heads up on what’s wrong here.

Marge's blood pressure: 164/98 mmHg
Marge has been an insulin-dependent diabetic since the age of 16.

- She presented in diabetic ketoacidosis (DKA) as a junior in high school, the problem was everyone thought it was the flu. She almost died.
- Two uncles have maturity onset diabetes (DM type II), but otherwise no one has type I diabetes.
- Today she closely monitors her blood sugar and even has an insulin pump, but for the first 20 years of her disease, her control was spotty.

**Diabetes mellitus** is a chronic disorder of carbohydrate, protein and fat metabolism, and it can explain all of Marge's problems. It's the *microvascular injury* over the long term that accounts for it.

- Keeping blood glucose as close to normal is important, but.....
- The *long-term complications* of diabetes are *vascular* in nature.
  - Non-enzymatic glycosylation of proteins in the vessel walls leads to thickening of the arterioles and diminished blood flow.
- The picture to the right shows the accumulation of the altered proteins in the wall of the diabetic arteriole.
- The diminished blood flow leads to low-grade chronic ischemia and irreparable damage to many organs.
  - Retina
  - Kidney
  - Peripheral nerves
  - Vascular supply to extremities
- Atherosclerosis involving large elastic arteries is substantially accelerated in diabetes, but..... It's the microscopic *arteriole-sized* blood vessels that are the most *severely affected*.
- Arterioles are the vessels that regulate blood delivery at the tissue level.
All parts of the kidney are damaged with diabetes.

- Large and small vessels
- Glomerulus
- Interstitial tissue
  - Possibly leading to papillary necrosis
- Tubular epithelium

To the right is a picture of a diabetic glomerulus

- Below is a normal glomerulus for comparison.
- What's different?

- Note the marked thickening of the afferent and efferent arterioles with diabetes.
- See how thickened and irregular the glomerular basement membrane has become.
  - In the healthy condition, you can barely see the basement membrane.
- The basement membrane thickening can be come quite pronounced and even nodular.
  - Kimmelstiel-Wilson lesion
In addition to glomerular disease, diabetic patients develop advanced chronic pyelonephritis because of:

- Chronic ischemia and repeated infections.
- This kidney shows numerous cortical scars, and the microscopic shows chronic advanced pyelonephritis with thyroidization.
- Eventually, the glomerular injury, coupled with the pyelonephritis, leads to complete renal failure.

The poet tells us the eyes are the windows to the soul.

This statement may or may not be true, but the eyes can certainly tell us a lot about the health of the microvasculature of the body.

As Marge's case shows, the condition of her retinal vessels directly correlates with the health and function of her kidneys, but many other systems are at risk as well.

As an optometrist, you'll be on the frontlines and may well be the first health-care giver to see these changes.

- Marge's eyes and kidneys are both in serious trouble, and the problem is only going to get worse.
- Excellent glucose control will slow the process, not reverse it.
- Diabetes needs to be thought of as a small vessel disease.
- In time, more sophisticated monitoring systems, linked with automatic insulin injections will help reduce the long-term consequences.
Laboratory 8
Hematological Disorders

Disorders of the hematopoietic system (blood and lymphocyte forming tissues) have recently come before the public eye, most notably in the form of Acquired Immune Deficiency Syndrome (AIDS). Almost everyone today seems to know something about T-lymphocytes and the effects of a low CD4 count. But as important as AIDS has become in our society, it represents only a tiny percentage of all hematologic disease. It’s very likely that many people reading this laboratory exercise have had a hematologic problem, or knows someone with one. The most characteristic and common, of course, being a case of anemia. Our purpose with this laboratory is to look at some of the more common, and representative disorders of the hematopoietic system, and hopefully understand mechanisms of injury that are transferable to other parts of the hematopoietic tissues.

Conditions related to abnormalities of hematopoietic activity cut across all social and ethnic boundaries, and often pose a significant diagnostic dilemma for the practicing physician. Diseases of the hematopoietic system are difficult to diagnose because the symptoms are not always specific and often overlap with other clinical problems. Moreover, if the condition is acquired, and develops over a period of time (as in the case of a chronic anemia), a person’s physiology may adapt somewhat to the new state of affairs, further obscuring the underlying problem until it becomes a major concern.

But to simplify matters, it can be said that the basics of a hematological malfunction boil down to just two issues: (1) quantity versus (2) quality. That is to say, do you have enough of a particular bone marrow product (RBC’s, WBC’s and platelets), and if so are those cells working to their full potential. A good example of the quality vs. quantity issue is the case of sickle cell disease. In this scenario, a person may have a sufficient number of RBC’s, but the hemoglobin they carry is defective. Quantity is good, but quality is the problem.

Dealing strictly on a statistical, or occurrence basis, the three most common disease categories of the hematopoietic system in the United States are:

1. Anemias
2. Myeloproliferative syndromes and leukemias
3. Malignancies of the lymphoid system, including diseases such a Hodgkin’s disease and plasma cell neoplasms

The groups listed above are fairly specific diagnostic categories and indeed represent the
tallying of clinical diagnoses nationwide. Clearly, however, people don’t stroll into a physician’s office with a diagnosis already made, so grouping conditions by clinical presentations is really necessary to get the diagnostic process going. The major hematological symptom complexes that most physicians work from are these: (1) lethargy and fatigability (suggesting anemia), (2) problems of blood clotting related to platelet problems, often manifested by ease of bruising, (3) problems handling infections and, less commonly, (4) bone pain. These clinical forms are obviously not completely specific, and sometimes are seen in conditions not related to the hematopoietic system at all. Still they’re really the best place to start.

What’s really wrong?

As you’ve probably guessed, there are a number of ways in which the bone marrow, and other segments of the hematopoietic system, can become damaged. But the good news is that the basic mechanisms of injury are just the same as those we have studied in every other organ system this semester. Yep, everything you’ve learned about mechanisms of injury in other systems applies right here. In the case of hematopoietic tissue some categories of injury are more significant than others, so here are the biggies:

- nutritional deficiency states (anemias of various sorts, iron deficiency being most common)
- inherited conditions (anemias such as sickle cell; inherited WBC malfunction; inherited bleeding disorders)
- infectious processes (AIDS; Epstein-Bar Virus causing mononucleosis and certain forms of malignant lymphoma)
- malignancies (both primary and metastatic disrupting the normal function of the bone marrow, such as leukemias and lymphomas)
- immunological injury (hemolytic anemias such as in the case of the development of a “rogue” antibody that reacts with your own RBC’s)
- iatrogenic (an undesirable byproduct of efforts to make someone well, such as immune suppression with steroids)

Given this wide spectrum of conditions and mechanisms of injury, it’s clearly not feasible to take on all of hematological disease in one laboratory. So, mercifully, we are going to limit ourselves to several representative examples of red blood cell and white blood cell disorders.
Red Blood Cell Disorders

Disorders of red blood cells manifest themselves as symptoms related to either (1) problems of oxygen carrying capacity, or (2) blood viscosity. In the case of inadequate oxygen carrying capacity, we are talking about anemias. One of the main thrusts of this laboratory is to help you understand the basic categories of anemia, especially from a mechanistic standpoint. Blood viscosity, on the other hand, is directly related to the peculiar situation of too many RBC’s in the circulating blood. You might think the more red cells the better, but not so. When the blood becomes too “thick” the heart has to work so hard that heart failure may ensue. What’s more, there are often serious consequences relative to blood stasis and poor transit time through the tissues. It may seem paradoxical, but in the case of too many RBC’s (here we mean 20% more than normal), the tissue beds are actually hypoxic because of poor circulation and stasis of the blood. An interesting problem, but our main purpose here is to understand anemias.

An anemia can be broadly defined as a deficiency of oxygen carrying capacity of the blood. Almost always this means an absolute deficiency in the number of RBC’s in the circulating blood, although it could indicate an abnormality of the hemoglobin itself. The diagnosis of anemia always starts with the history and physical, but at some point the doctor’s suspicions must be confirmed by examining the peripheral blood for total hemoglobin and RBC content. These are the quantitative measurements, but remember, quality has to be assessed as well.

Today measurements of total hemoglobin and hematocrit (percentage of RBC’s in a given volume of blood) are done by laboratory equipment, but one aspect of the assessment still requires the human eye: examination of the peripheral smear. The peripheral blood smear helps to assess two important features of the “injured or inadequate” RBC’s: (1) size and (2) shape. These two aspects of red blood cell morphology are critical to understanding what’s happening to the RBC’s as well as gauging the bone marrow’s capacity to make new red cells. By extension, a physician is often able to make a good guess as to why the person is anemic just by knowing something about the size and shape of the RBC’s. The “why” is the critical factor here, after all, it’s not enough to simply make the diagnosis of anemia. If the underlying reason for the anemia isn’t discovered, it’s going to be impossible to fix the problem.

An interesting byproduct of knowing the size and shape of the RBC’s in establishing the “mechanism” of an anemia is that the descriptive terms used for a diagnosis often reflect some
aspect of the abnormal RBC morphology. For example, common types of anemias include the following descriptive modifiers: microcytic (small RBC’s) anemia, macrocytic (large RBC’s) anemia, anemia secondary to spherocytosis, anemia of sickle cell disease, and so on. But morphological changes only open the door to understanding the anemia, it’s the mechanism that must be uncovered. At the risk of being too repetitive, once a diagnosis of anemia is made, the search for why begins. Here’s a brief outline of anemias organized by what may go wrong.

Classification of Anemias by Mechanism

I. Hypoproliferation (your marrow isn’t making them)
   A. Decreased erythropoietin
      - renal disease
   B. Specific nutritional deficiency
      - iron deficiency leads to microcytic anemia
   C. Bone marrow damage
      - toxic injury (industrial volatiles and some pesticides)
      - bone marrow replacement by fibrosis or metastatic cancer (this is referred to as a “myelophthisic” process)
      - Aplastic anemias, as with infectious conditions killing off the RBC precursors (parvo virus infection in young children)

II. Maturational abnormalities
   A. Macrocytic anemias, also known as Megaloblastic anemias (large precursors in the marrow and larger than expected mature RBC’s in peripheral blood)
      - Vitamin B12 deficiency
      - Folic acid deficiency
   B. Normocytic anemia (normal size RBC’s)
      - bone marrow replacement by fibrosis or metastatic cancer (this is referred to as a “myelophthisic” process)
   C. Microcytic not related to iron deficiency
      - Vitamin B6 deficiency
      - alcohol related problems of porphyrin synthesis
      - Inherited abnormalities of porphyrin synthesis (Sideroblastic anemias)
III. Hemolytic anemias (shortened life span of RBC’s)

A. Phagocytosis by reticuloenthelial system (so called “extravascular” hemolysis)
   - Autoimmune (non-complement binding antigen antibody reactions)
   - Inherited RBC membrane and cytoskeletal structural defects (Spherocytosis)

B. Fragmentation and traumatic injury to RBC’s
   - some types of artificial heart valves
   - Intravascular clotting
   - Vasculitis
   - Sickle cell disease

C. Intravascular hemolysis
   - Complement binding antigen antibody reaction with RBC membrane
   - G6PD deficiency in the face of the triggering drug
   - Severe burns

IV. Blood loss (hemorrhage)

A. Acute loss, with no time to compensate (if severe shock may develop)

B. Chronic loss with sufficient time for compensation (generally becomes an iron deficiency anemia with time)

Disorders of white blood cells.

Trying to figure out what’s wrong with a person’s white blood cells is just the same as for the red cells: quantity verses quality. It’s the same old story, do you have enough of them, and if you do, are they really working? And, as is the case for the evaluation of an anemia, every physician begins with a history and physical to try and establish a differential diagnosis, that is a list of causative possibilities, consistent with the unique clinical scenario of each patient. But at some point, it’s essential to look at the peripheral blood to assess (you guessed it), the quantity and quality of the circulating white blood cells.

Matters of Quantity

One thing a physician has to keep in mind when trying to evaluate the state of a person’s
bone marrow health is that the number of WBC’s in the peripheral blood changes all the time. If you have an infection, there is a greater need for inflammatory cells, so your bone marrow makes more and releases them into the blood stream. Because of this physiological response, it’s sometimes hard to tell the difference between the expected rise in the white blood cell count in response to an infection, and a truly “pathological proliferation” of WBC’s. In the case of the “run away,” sick bone marrow, carefully studying the quality of the circulating WBC’s is called for. Under “normal” circumstances, the circulating white blood cell count is roughly between 4,000 and 9,000 cells per cubic milliliter of blood. In the case of a rip roaring infection, this count may shoot to 50,000 per cubic milliliter in just a few hours. A figure this high in an otherwise healthy person, with no infection or other apparent cause, would be quite troubling.

Breaking the code for what might happen when you don’t have enough white blood cells doesn’t take a Sherlock Holmes. If a person has a disastrously low white blood cell count, one would expect serious problems with recurrent infections and, depending on the segment of the immune system damaged, there’s even the possibility of the development of tumors in other organ systems. But simply knowing a person has a dangerously low white count is only helpful to a point. As we have emphasized, it’s the underlying mechanism or problem that must be uncovered. In order to actually correct the problem, if it can be fixed at all, you’ve got to know what’s broken in the first place. But before considering all of the possibilities for abnormalities of the white blood cells, we would like to look at one of the more serious conditions in some detail.

**Leukemias**

The term leukemia applies to the malignant proliferation of one of the bone marrow elements, most commonly the precursors of the myeloid (granulocyte) series. But on rare occasions “leukemias” develop from the red blood cell precursors and even the megakaryocytes. And to confuse matters even more, the term leukemia doesn’t necessarily mean there’s going to be an elevation of the peripheral white blood cell count. Nor does it mean that the other normal or healthy “formed” elements of the bone marrow (platelets and RBC’s) will not be effected in some manner. Things can get dicey when the malignant cells occupy the same cramped space in the bone marrow along with the other cell lines.

One must keep in mind that the leukemia is essentially a disease of the bone marrow. All of the malignant cells produced in the marrow space may not necessarily leave and get into the peripheral blood. However, in most cases of leukemia malignant will be recognized in the

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2 Keep in mind that your immune system is checking every day for the development of malignant cells in every organ of your body. Once found, your T-lymphocytes kill the abnormal cells before they can grow into a full fledged tumor. You might be surprised to learn that the average person generates four malignant cells each day, and the immune system successfully sniffs them out and destroys them.
circulating blood. On occasion, we actually see a patient with active leukemia who has a circulating WBC count below the expected value. But even in these atypical situations, frequently some of the circulating WBC’s will be malignant and identifiable as such. The percentage of malignant cells in the peripheral blood of someone with active leukemia may vary form as little as 1% to as much as 99%, but they should be recognizable. The importance of recognizing these malignant cells in the circulating blood goes beyond just making the diagnosis of leukemia. The malignant cell’s type and its degree of maturation are the key factors in classifying the variety of leukemia, and by extension determining its therapy.

But the white cell line is not the only part of the bone marrow effected by the proliferation of the malignant clone of WBC precursors. In fact, most people with leukemia present with symptoms of either anemia or a newly developed bleeding disorder. How can this be? The fact is that in a leukemia many of the proliferating malignant cells stay in the bone marrow, and effectively crowd out all of the other normal and healthy cellular constituents. There’s only so much room in the bone marrow, and eventually it’s all occupied by the malignant cells. The normal RBC precursors and megakaryocytes are squeezed out of the picture, thus leading to the symptoms related to these other formed elements.

The diagnosis of leukemia is not equivalent to a death sentence. There is no question, it is a serious and potentially life threatening condition, but many people survive years with the diagnosis and some are cured outright. Today, we enjoy extraordinary success with childhood leukemias. Most are curable, although sometimes serious complications develop as a result of the therapy. Among adult leukemias, however, the success rate is not nearly so good. In the long run cures of adult leukemias are achieved only with bone marrow transplant; a risky yet manageable procedure. Since the therapies are different for the different varieties of leukemias, it is incumbent upon the pathologist to interpret the blood and bone marrow smears carefully.

To aid in the understanding of white blood cell disorders, we have provided the following outline organized by basic problem effecting the manufacture of WBC’s. Since most laboratory evaluations of a person’s white cell status begin with the number of circulating white blood cells, we have hybridized the outline, intermixing the quantity and quality aspects of the WBC’s.

Disease States and Normal Physiological Responses of WBC’s

I. Leukopenia (decreased WBC’s in peripheral blood, this is not normal nor healthy)
A. Decreased production
- “Space occupying” lesion of marrow causing death of healthy marrow
- metastatic cancer
- marrow fibrosis
- malignant proliferation of marrow elements themselves (leukemias)

B. Peripheral destruction or consumption of WBC’s
- Antigen antibody mediated destruction
- Consumption in the process of fighting an overwhelming infection

C. Peripheral sequestration
- Large spleens traps the cells, not necessarily destroying them, but holding them in jail.

II. Leukocytosis (high WBC count, but cells themselves are healthy and not malignant)

A. Leukemoid reaction (a very enthusiastic response to infection)

B. Specific elevation of a cell type depending on infectious agent
- Lymphocytes in the case of viruses
- PMN’s in the case of bacteria
- Eosinophils in the case parasites and response to allergens

C. Myeloproliferative processes (not malignant but loss of controlled reproduction
- the equivalent of a benign tumor in other organs)

III. Leukemia (true malignancy of one of the bone marrow elements)
Several overlapping classifications schemes are used for leukemias

A. Classified by malignant cell type
- lymphocytic (lymphocyte origin)
- myeloid (a malignancy derived from granulocyte precursors)

B. Also classified by degree of maturation of the malignant cells
- Acute leukemia (little maturation, cells look for primitive and embryonic, people with leukemias of this type die quickly if not treated)
  - example is “Acute lymphoblastic leukemia”
  - example is “Acute myelogenous leukemia”

- Chronic leukemia (malignant cells show varying degrees of maturation, leukemias of this type tend have longer survivals).
  - example is “Chronic lymphocytic leukemia”
  - example is “Chronic myelogenous leukemia”
IV. Infectious processes

A. AIDS

B. Others

V. Space occupying lesions of the bone marrow that effectively squeeze out the normal elements

A. Metastatic cancer (look for nucleated RBC’s in peripheral blood)

B. Myelofibrosis (scarring away of the bone marrow space, reason unknown) (look for nucleated RBC’s in peripheral blood)

C. Primary proliferative lesions of the bone marrow
   - leukemias
   - malignancies of lymphoid origin

VI. Toxic injury

A. Medications

B. Industrial materials (toluene)

C. Renal failure

VII. Inherited enzyme deficiencies of WBC such that they can’t kill bacteria

A. Chronic granulomatous disease

B. Many others
Laboratory 8
Exercise

Today’s slides are all online

OK, this is important, so please read it before you try looking at the slides on your microscope.

Before launching into the blood smear slides, you need to know where to look to be able to see the individual cells clearly. A peripheral blood smear is not like a regular slice of solid of tissue. To see what’s important in a blood smear, you don’t want to be in the middle of the slide, rather you have to be out on the thinnest part of the smear, a region we refer to as the “feathered edge.” Look for the diminishing staining and the long pointed ends of the blood smear. You want to be out where the sample is about to disappear entirely. In other words, the thinnest part of the sample. Here, the cells should not be overlapping or piled on top of one another. You want the individual RBC’s widely separated and clearly recognizable as single cells. Otherwise, the staining is too intense and the cells heap up on one another and obscure their nuclear detail.
Iron deficiency anemia, online slide only.

This 69-year-old man complains of hematuria (blood in the urine) of several days duration. He claims to have had bloody urine several times over the past 3 years, but nothing like he is experiencing now. He also has arthritis and claims to take up to 8 aspirin a day. His stool tests positive for blood, although none is grossly visible. Check your disc or the CD for his lab values.

This guy has given himself a large gastric ulcer, thanks to all the aspirin he's been taking. His urinary tract bleeding is related to his malfunctioning platelets (again due to aspirin) and a chronic urinary tract infection he has developed because of his enlarged prostate. The prostatic problem is unrelated to aspirin use, rather comes with his age. His arthritis is the basic osteoarthritis that most elderly people suffer with.

His RBCs are small and very pale staining, owing to the reduced hemoglobin. His iron levels are low because the low-grade, chronic GI bleeding has depleted his iron stores.

Your observations
Chronic lymphocytic leukemia, blood smear

This 70 year-old white man complains of fatigue, decreased exercise tolerance and what describes as a "pulling" sensation in his abdomen. He has always been in good health, is able to walk several miles a day and lives alone.

Physical reveals enlarged and non-tender cervical lymphadenopathy and spleen tips is easily palpated at the level of the umbilicus. The liver seems enlarged.

This slide shows lots of lymphocytes. They are a little odd looking, but not outright embryonic or blast like. He is moderately anemic because of the bone marrow replacement with the malignant lymphocytes. Often there is lymph node, spleen and even liver involvement with the leukemic cells. The pulling sensation in his abdomen is due to very large spleen. The image to the right shows the outline of a markedly enlarge liver and spleen.

Your observations
Chronic lymphocytic leukemia in the liver

Here's our man's liver section. Note the leukemic infiltrate in the region of the triads. (If you're having trouble seeing this in the picture to the right, roll the cursor over the image.) The image below is of the area in the box. Here you can see the relatively innocent looking lymphocytes. The only thing is that there is a blue million of them.

In this view, you can see a little bile duct of the triad, with the leukemic infiltrate in the surrounding tissue. The malignant cells here really don't look bad, but they are definitely malignant. Recall, the criteria is based on invasion and distant spread, not necessarily the cytological appearance.

Your observations
Mononucleosis

This 20 year-old female college student has been in good health all her life, but developed a fever and sore throat 2 days ago. Physical exam reveals a red and injected throat with tonsilar enlargement and exudate. There are several large and tender cervical lymph nodes.

As you probably know, mononucleosis is caused by an infection by the Epstein-Bar virus. The infection begins in the epithelium of the throat, but quickly spreads to regional lymph nodes. The virus specifically infects the B-lymphocytes, resulting in a production of some pretty stimulated T-lymphocytes in response to the virus. These stimulated, or 'atypical' lymphocytes, are the hallmark of the infection. Here's an example of one. Lots of lymphoid tissue gets stirred up, including the spleen, which can become quite large acutely. The danger here is that with minimal trauma, the spleen can rupture, resulting in fatal hemorrhage.

A potential long-term problem is the development of lymphoid tumors, and head and neck cancer. Remember that Epstein-Bar is a transforming virus, meaning it can cause chromosomal breaks and translocations.

Your observations
Sickle cell crisis, blood smear

This 22-year-old black man complains of severe **abdominal pain** and fever. He has a history of similar episodes, some requiring hospitalization. He also believes he has some form of 'kidney' problem.

Physical exam reveals a slight man of small stature who is in considerable distress. No significant physical findings are present, and **no spleen** is palpated.

The genetic defect of sickle cell disease was one of the first to be studied. A mutation occurs in the gene that codes for the beta chain of the hemoglobin, resulting a protein that will **clump and polymerize with others when deoxygenated**. The result is a huge hemoglobin crystal that deforms the RBC, resulting in it being removed early from circulation. On occasion, many of the red cells rupture at once, producing a sickle crisis.

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Your observations
Sickle cell crisis, spleen section

Here's another example of sickle cell disease, but in a 7 year-old little girl. She died during sickle crisis, in which there was massive and diffuse sickling in just about all organs of her body. Your section is from the spleen.

You will see the vessels packed with what looks like little red worms. These are her sickle deformed RBCs. Had she lived, she would have in time completely infarcted her spleen, just as the young man from the previous case did. (That's why he had not palpable spleen.) This is known as an autosplenectomy.

The spleen is lost in bits and pieces over the years due to many repeat episodes of sickling within it. The problem that this presents in the long run is that the affected person will be subject lots of bacterial infections, particularly with dangerous, encapsulated organisms, such as Streptococcus and Meningococcus.

Your observations
Please give us your thoughts and suggestions.

Thanks to all for working so hard in the pathology course. We hope you've learned a lot from these online laboratories, and that the experience wasn't too painful. We're interested in your ideas and views.

If you have time, and there's something you want to say regarding the online laboratories, here's your opportunity. We are particularly interested in knowing how the virtual microscope part of the lab assignments worked for you, but anything you want to say will be helpful.

Basically, the questions you might want to address are these:

- What worked?
- What didn't work, and what might have helped?
- What would you liked to have seen more of?

Email is the way to get your comments and suggestions back to us to the V543 email account.

Thanks to all and best wishes for the future.

Drs Begley and Braun