During the course of disease, accumulation of a variety of substances can occur within cells or in the extracellular tissues. These substances can be either endogenous (generated within the body, organ, tissue, or cell) or exogenous (‘foreign’ substances from outside the body that gain access to organs, tissues, or cells). The accumulation can be visualized by microscopic examination and in some instances can be detected grossly. Some accumulations occur in the context of only isolated lesions while others can involve entire organs or can be disseminated in multiple organs in the body. In some, the accumulation represents the primary cause of disease while in others; the accumulated substances are a reflection and component of the underlying disease process. The significance of these accumulated substances varies greatly.

The objectives of Lecture 10 are to acquaint you with: 1) the main types of substances that accumulate; 2) the pathogenesis of intracellular and extracellular accumulations; and 3) the significance of these accumulations with regard to individual lesions or specific disorders.

INTRACELLULAR ACCUMULATIONS (Slides 3 – 18)

The nature of accumulated intracellular substances. The nature of substances that can accumulate within cells is highly diverse but these can generally be grouped into three broad categories (slide 3):

- A normal cellular constituent accumulates in excess. This can be something as simple as water (will be covered in another lecture), proteins, lipids, carbohydrates, or complexes of these.
- An abnormal substance accumulates. The abnormal substance can be generated from within the cell (endogenous) due to faulty synthesis or metabolism. The abnormal substance, by its very nature, is foreign and originates from outside the body (exogenous).
- Pigments, compounds imparting a change in color to cells, tissues, organs. There are a variety of these pigments that can accumulate in cells. These can be endogenous or exogenous. Two endogenous pigments, hemosiderin and lipofuscin, were briefly mentioned in Lecture 8 and pigments will be covered in a separate lecture.

The pathogenesis and significance of intracellular accumulations. Apart from accumulation of water inside cells that results in cellular swelling, a process called hydropic change covered in Lecture 11, there are four general mechanisms that result in accumulation of substances within cells.
• A normal endogenous substance is produced at a normal or increased rate but the rate of metabolism is inadequate. Apart from adipose tissue, the liver is one of the main organs of the body involved in pathways involving metabolism of fats or lipids. Accumulation of lipids in hepatocytes is termed **fatty change** (also known as hepatic lipidosis, hepatic steatosis, fatty liver, etc.) (slides 5, 6). The pathogenesis of fatty change in the liver centers around the inter-related metabolic pathways involving free fatty acids. Free fatty acids can be derived from the diet, from chylomicrons in the blood, or from mobilization of fat stores in adipose tissue. In the liver, fatty acids are converted to triglycerides. The triglycerides are then complexed with apoproteins to form lipoproteins that can be exported from the liver. One of the most common causes of hepatocellular fatty change is **anorexia** or starvation. In this case, mobilization of fat from adipose tissues exceeds the capacity of fatty acid metabolism and fatty acids or triglycerides accumulate in hepatocytes. **Hypoproteinemia** can also be a contributing factor; WHY? **Ketosis** is a complex disease of ruminants that results in severe hepatic fatty change. The disease is associated with the increased energy demands of lactation (dairy cattle) or twin lambs (pregnant ewes). In this disorder, the fatty liver is due in part to mobilization of fat stores for energy. Lactation and fetal growth also preempt the supply of metabolic intermediates such as oxaloacetate in the Kreb’s cycle further degrading the ability of liver cells to metabolize fatty acids. In moderate to severe fatty change (slide 5), the liver is grossly enlarged with rounded margins and becomes progressively pale. In the most severe instances, the fatty liver may float in water. Microscopically, the accumulation of lipids can be seen as discrete, single large or multiple smaller well demarcated clear vacuoles in the cytoplasm of hepatocytes (slide 6). Fatty liver does not seriously compromise hepatic function, per se, but if prolonged, can lead to fibrosis. Another example of accumulation of lipids in cells is **atherosclerosis** (slide 7), a disorder having an even more complex pathogenesis, characterized by the accumulation of lipid-laden foam cells in the walls of arteries. In contrast to humans, atherosclerosis in animals mainly occurs in the context of **hypothyroidism** or is idiopathic. The significance of atherosclerosis in humans is obviously reflected in the high incidence of myocardial and cerebral **infarction** (heart attack and stroke). In animals, this is most often an incidental finding.

Another example of accumulation of an endogenous substance, this time proteins, produced in quantities that exceed the metabolic capacity of cells is the accumulation of protein in tubular epithelial cells of the kidney known as **hyaline droplet change** (slide 8). A function of the renal tubular epithelium is to absorb and conserve proteins that pass through the glomerular filter. When too much protein passes the filter (**proteinuria**), as is the case in **renal amyloidosis** (to be discussed later in this lecture) the excess is absorbed as pink proteinaceous droplets in the cytoplasm of the epithelial cells. Other proteins can occur as droplets in renal tubules including **myoglobin** (myoglobinuria) in the case of **rhabdomyolysis** and **hemoglobin** (hemoglobinuria) following intravascular **hemolysis**. Another example of protein accumulation due to excess production would be the **Russell bodies** in **Mott cells** (lecture 3). Accumulation of protein in these instances would not seriously affect the function of cells. The underlying cause of the proteinuria is often significant, however.
The last example we will cover is accumulation of glycogen in cells. Glycogen is stored normally in many types of cells, most obviously in hepatocytes, brain cells, and striated muscle to be used for energy. In some diseases, such as diabetes mellitus and Cushing’s disease (excess glucocorticoids), both associated with defective metabolism of glycogen or glucose, glycogen accumulates in hepatocytes (slide 9) and sometimes in other cells. Grossly, the liver is pale but certainly not to the extent as a severe fatty liver. Microscopically, the change is often not uniform but affected hepatocytes are swollen, pale, and vacuolated or have clear cytoplasm. The cytoplasm is sometimes referred to as ‘ground glass’ or the change as cloudy swelling. Glycogen can be demonstrated in cells at the light microscopic level using the periodic acid-Schiff (PAS) stain. The accumulation of glycogen in the cytoplasm of cells causes an ‘osmotic load’ and water content also increases. In these disorders of glucose metabolism, there is often mobilization of fat from adipose tissue for energy so that in diabetic and glucocorticoid hepatopathies, pallor and cell swelling can be due to accumulation of glycogen, water, as well as lipids.

- An abnormal substance accumulates due to faulty synthesis. A good example here would be the accumulation of protein in the endoplasmic reticulum (slide 11) of liver cells due to faulty synthesis of alpha-1 antitrypsin covered in lecture 3. Additionally, there are a host of neurodegenerative diseases in humans that are due to abnormal intracellular processing of proteins (largely protein misfolding) with accumulation in cells of the central nervous system. Examples include the Lewy bodies in Parkinson’s disease, neurofibrillary tangles in Alzheimer’s disease, and huntingtin in Huntington’s disease. These diseases are significant but for various reasons. With alpha-1-antitrypsin deficiency, significance lies not in the accumulation of the defective protein but in the more generalized deficiency in activity of the defective protein. You are all familiar with at least one of the neurodegenerative diseases mentioned and most of you have or will have personal experience with an affected individual.

- Normal endogenous substances accumulate due to faulty catabolism or degradation. The best examples here include the lysosomal storage diseases (slide 13) covered in lecture 3. There are a whole host of these hereditary diseases that are due to a deficiency of specific lysosomal enzymes (hydrolases) allowing intracellular accumulation of non-digested substrates. These are sometimes broken into categories based on the dominant material that accumulates.
  - Sphingolipidoses (glycosphingolipids are components of cell membranes) – Niemann-Pick disease
  - Glycoproteinoses (defective degradation of the carbohydrate component of glycoproteins) - mannosidosis, fucosidosis
  - Mucopolysaccharidoses (defective degradation of glycosaminoglycans) – Hurler’s disease
  - Glycogenoses (only one lysosomal disease is associated with defective catabolism of glycogen in lysosomes) – alpha-1,4-glucosidase deficiency. This enzyme breaks down the glycogen that accumulates in autophagosomes. The disorder in humans is also known as Pompe’s disease. There are at least seven
other glycogenoses associated with defective catabolism of glycogen with storage in the cytoplasm of cells that have been recognized in humans and some have an animal counterpart.

- Mucolipidoses (have features of both sphingolipidoses and mucopolysaccharidoses) – I-cell disease mentioned in lecture 3.

All of these diseases are serious. Many of these diseases affect the central nervous system, liver, and muscles or musculoskeletal system. Life expectancy is shortened and the quality of life is affected. As noted in lecture 3, some toxins can also result in an acquired lysosomal storage disease. Can you give an example?

- **An exogenous substance accumulates because it cannot be catabolized.** For these substances to accumulate within cells, they must gain access to the body, tissues and cells. A major natural portal of entry is the lungs. Dust particles, if in sufficient quantities can overwhelm the respiratory defenses that include 1) the filtering effect of nasal hairs, 2) the mucous secretion that traps particulate matter, and 3) the cilia on epithelial cells that beat in a rhythmic fashion to move particular matter out of the respiratory tract. What detrimental effects might you expect for individuals with severe vitamin A deficiency (lecture 8) or ciliary dyskinesia (lecture 3)? These defenses can be overwhelmed depending on the size and amount of the particles. When these particles are inhaled to the level of the lung, macrophages located in alveoli phagocytose the material and migrate into the interstitium. The inhalation and accumulation of such material goes by various names depending on the nature of the substance and include such terms as **anthracosis, asbestosis, silicosis,** and **pneumoconiosis.** These substances can be irritating and cause significant damage (inflammation and scarring) if present in sufficient amounts such as in coal miner’s lung (slide 15) and **mesothelioma** associated with asbestos inhalation. Lead is another exogenous substance that can accumulate in the body from various portals including inhalation and, more commonly, ingestion. In chronic lead poisoning (**plumbism**), lead complexed with protein forms inclusion bodies in the nucleus of cells that are bright red when acid-fast stains are used but that are difficult to visualize with routine H&E (slide 16).

Another broad category of intracellular accumulation that doesn’t readily fit into the previous categories (but more akin to the fourth mechanism just covered) includes various microorganisms that infect, replicate, and reside within cells. Although viruses cannot be visualized with the light microscope, some viruses form **inclusion bodies**, factories for viral production and assembly, within cells. As a general rule, DNA viruses form intranuclear inclusions and RNA viruses form cytoplasmic inclusions. To every rule there are often many exceptions. Canine distemper (morbillivirus, Paramyxoviridae) is an RNA virus that can form intranuclear as well as cytoplasmic inclusions (slide 17). Poxviruses are DNA viruses but form cytoplasmic inclusions. Other classes of microorganisms including rickettsia, bacteria, and protozoa also reside and accumulate in cells (slide 18).

**EXTRACELLULAR ACCUMULATIONS** (slides 19 – 43)
A variety of endogenous substances can accumulate extracellularly. Water accumulating in extracellular spaces is called edema (lecture 11); hyalin membranes that form in pulmonary alveoli are composed of proteins that leak from the alveolar septae and capillaries after diverse types of acute lung injury (slide 20); thickening of renal glomeruli due to accumulation of extracellular matrix proteins (lecture 3), and accumulation of advanced glycation end products (abnormally glycated proteins) in diabetics (lecture 26) are all examples. This lecture will focus on one other extracellular deposition.

**Amyloid.** As mentioned previously, protein misfolding can result in the intracellular accumulation of proteins. Amyloid is an extracellular, abnormally folded glycoprotein with a rich β-sheet component that is resistant to degradation. Amyloid is not a single substance; it can arise from a variety of endogenous precursor proteins. The deposition of amyloid within extracellular spaces of tissues and organs results in a diverse group of conditions, seemingly having little in common, and called **amyloidosis.** All have in common, however, production of proteins that are inherently prone to undergo a conformational change to a β-sheet configuration and that are deposited in tissues as fibrils. There are various and sometimes confusing ways to classify these diverse disorders: primary versus secondary, systemic versus localized, based on the type of precursor protein, or combinations of these schemes. We will do away with the concept of primary versus secondary as it is especially confusing and has little practical significance. Three main categories of amyloidosis are:

- **Immunocyte-associated amyloidosis.** This form of amyloidosis can be localized or systemic (affecting more than 1 tissue). The precursor protein is the immunoglobulin light chain produced in excess by plasma cells, hence the name “immunocyte-associated.” The light chain proteins composing the amyloid fibrils are designated as AL (amyloid, light chain). The systemic form of the disease can be associated with a form of bone cancer known as multiple myeloma. In this form of cancer, malignant plasma cells proliferate in the bone marrow causing osteolytic lesions. Up to 15% of patients with multiple myeloma may have systemic deposits of amyloid. The localized form of immunocyte-associated amyloidosis is sometimes associated with intense plasmacellular inflammation or localized neoplasms called plasmacytomas. The latter are most common in dogs, are usually solitary, and occur in the skin or oral cavity. Sometimes the amyloid deposits are so extensive that they form the bulk of the mass.

- **Reactive systemic amyloidosis.** This form of amyloidosis is often associated with diverse chronic inflammatory or wasting conditions (cachexia) but in some cases, predisposing conditions cannot be defined of identified (idiopathic). Common to all, however, is a change in patterns of protein synthesis in the liver to production of acute phase proteins. One such acute phase protein, whose function is unknown, is designated as serum amyloid A (SAA), the amyloidogenic precursor protein. Deposits of amyloid in reactive systemic amyloidosis can affect multiple organs such as kidney (glomeruli), liver (spaces of Disse), spleen, (central arterioles), and intestine (mucosa, submucosa).

- **Familial (localized or systemic) amyloidosis.** Hereditary or familial forms of amyloidosis are also recognized. In dogs and cats, the precursor protein is SAA, the same as in reactive systemic amyloidosis. Why the liver increased production of SAA in these animals is unknown. It is proposed that the breeds affected may have some
underlying predisposition to inflammation. The patterns of amyloid depositions vary. In the Abyssinian cat, deposits occur in renal glomeruli, liver in the Siamese cat, and renal medullary interstitium in Shar pei dogs. In humans, the precursor protein is termed transthyretin, a transport protein in blood for thyroxin and retinol (vitamin A). Deposits occur in peripheral nerves. Another human disorder is familial Mediterranean fever. In this disorder, there are periodic attacks of inflammation affecting a variety of tissues related to a specific mutation on chromosome 16. The precursor protein is SAA similar to the familial diseases in dogs and cats. Deposits of amyloid occur in a variety of tissues including kidney, heart, spleen, gastrointestinal tract, and thyroid glands. Colchicine (mentioned in lecture 3) reduces the periodic attacks of inflammation and can help prevent the deposition of amyloid. Colchicine is also a treatment for acute attacks of gout (see PPT presentation).

In addition to these forms of amyloid deposition, there are other types of amyloidosis.

- **Amyloid of aging.** Two age-related forms of amyloidosis include deposits that occur in the brain of Alzheimer’s disease and other forms of senile dementia and in the cardiovascular system of aged humans. In Alzheimer’s, amyloid deposits occur in the walls of small blood vessels called congophilic angiopathy (see staining of amyloid below) and as senile plaques within the neuropile. The precursor is amyloid precursor protein (APP), a membrane protein in most cell types. The amyloid fibrils are termed Aβ (amyloid beta). In the heart and aorta of aged humans, there is a relatively high incidence of amyloid deposits but the clinical significance is uncertain. Autopsies have shown an incidence of approximately 30% in people over 70 increasing to 50% in 90-year-olds. The precursor protein is transthyretin.

- **Endocrine amyloidosis.** Localized deposits of amyloid can affect the thyroid gland and pancreatic islets. Calcitonin is a hormone produced by C-cells in the thyroid gland that regulates calcium balance in the body (see lecture 12). Localized amyloid deposits are seen in some malignant tumors (carcinomas) of C-cells. Of much more clinical significance is the amyloid deposition that occurs in the pancreatic islets. The precursor protein, termed islet amyloid or islet-associated polypeptide (IAPP) is secreted by β-cells of the islets. As you will recall, β-cells also secrete insulin, a major hormone controlling glucose balance in the body. Deposition of amyloid in the pancreatic islets is associated with type 2 diabetes mellitus (covered in lecture 26) in cats, non-human primates, and in humans.

- **Prion diseases.** Will be covered in another lecture.

Despite all these variations in the chemical composition and the various tissues affected, the appearance of amyloid is the same. Amyloid can be visualized grossly, if in sufficient amounts, when iodine is applied to the tissue followed by a weak acid solution (slide 28). Microscopically with H&E stains, amyloid forms pink amorphous, homogeneous to somewhat fibrillar deposits (slide 29). Congo red is a more specific stain for amyloid (slide 30) but alone is not definitive. When Congo red stained sections are viewed with polarized light, apple-green birefringence is characteristic of amyloid (Slide 31). Slides 32 and 33 show amyloid deposits in other tissues.
The significance of amyloid varies considerably depending on the extent of deposition and the organ or tissue. Small localized deposits are largely innocuous. The renal glomerulus is an entirely different story. Renal glomeruli serve as a selective filter of the blood, wastes are removed based largely on particle size and electrical charge and are eliminated from the body as urine. Deposits of amyloid disrupt this selective filter, making the glomerulus leaky. As a result, large molecules pass through the glomeruli into the renal tubules and are lost in the urine. A major effect is protein loss in the urine (proteinuria) that can be visualized as pink-staining homogeneous ‘casts’ in the lumens of tubules. Renal glomerular amyloidosis is a serious and often fatal disorder. Deposits of amyloid in the medullary interstitium on the other hand, as occurs in the Shar pei dog, are often not clinically significant. Additionally, as already mentioned, deposition of amyloid in pancreatic islets is associated with diabetes mellitus. We will briefly discuss the significance of amyloid deposits as we go through some of the photos.

In the PPT presentation are examples of 3 others types of extracellular accumulations:

- Fibrinoid change (slides 34, 35)
- Gout (slides – 36 – 41)
- Accumulation of cholesterol (slides 42 – 43)

If time permits, we will cover these accumulations briefly in class. but you will not be held responsible for this material on the tests.