LECTURE 39

AN OVERVIEW OF
CLINICAL PATHOLOGY

MAMMALIAN PATHOBIOLOGY
PATB 4130 / 5130

Up till now, your exposure to pathology from photos and illustrations in lectures has dealt primarily with anatomic pathology. Traditionally, anatomic pathology has been divided into gross or necropsy pathology and histopathology (microscopic pathology). The point here is that anatomic pathology deals with the body as a whole and/or with microscopic examination of tissues and organs. There is another whole broad field of pathology that we have touched on only tangentially, that being clinical pathology. For practical purposes, training programs in veterinary pathology have traditionally emphasized either anatomic or clinical pathology. Drs. O’Toole, Fox and I are anatomic pathologists but we have some training, both formal and from experience, in clinical pathology. Clinical pathologists examine blood and body fluids as well as individual cells to further our understanding and diagnosis of disease. In practice, human clinical pathology is farther advanced than veterinary clinical pathology but that is also true of anatomic pathology in many cases.

The purpose of this lecture is to acquaint you with the field of traditional clinical pathology. It will necessarily be superficial and not comprehensive but will introduce you to many terms and acronyms that have not been used in previous lectures. A lot of the nuts and bolts of clinical pathology will be given to you here in the notes. Dr. Fox has already discussed coagulation (clotting of blood) and associated diseases; we will not cover this topic. We will use much of the lecture to go over individual cases that have been submitted to the Wyoming State Veterinary Laboratory where clinical pathology data has been an integral part of or that helped direct us toward the final case diagnosis. These cases were selected to illustrate the value of clinical pathology in several of the diseases that have been covered in past lectures.

HEMATOLOGY

Hematology is the field of clinical pathology that deals with blood and blood-forming organs. For all practical purposes, hematology evaluates primarily the cellular components of blood. A wide variety of parameters can be measured; most are given in slide #3.

- PCV/HCT: Packed cell volume or hematocrit is a measure of the relative volume occupied by blood cells expressed on a percentage basis. This is a crude measure at best but is easy to do and a reliable indicator of the number of red blood cells in circulation. When blood is centrifuged, the erythrocytes spin out of the fluid portion of the blood (plasma). You might also have heard the term buffy coat. The buffy coat is formed by the white blood cells (leukocytes) that separate out of the blood at the interface between the red cells at the bottom of the tube and serum or plasma at the top of the tube.
Anemia, a lower than usual number of red blood cells can be due to increased rate of removal or destruction of erythrocytes and decreased production. Polycythemia, on the other hand is too many red blood cells in circulation. Both parameters can be assessed by PCV and by RBC counts (see below). One of the more common causes of elevated PCV and red blood cell numbers in animals is dehydration where the circulating fluid volume is depressed.

- Hemoglobin: Is a measure of the total hemoglobin present in blood expressed as a weight / defined volume of blood (g/dl).
- MCHC: Mean corpuscular hemoglobin concentration is hemoglobin divided by the packed cell volume X 100. In slide 3, this would be:

\[ \frac{11.1}{32.6} \times 100 = 34 \text{ g/dl} \]

- RBC count is the total number of erythrocytes counted in a defined volume of blood commonly expressed as # of cells per microliter.
- MCV: Mean corpuscular volume is a reflection of the size or volume of individual erythrocytes. Unless you want to deal with conversions and femtoliters \((10^{-15} \text{ liters})\) a simple way to determine MCV is by dividing the PCV by the red blood cell count X 10.

\[ \frac{32.6}{5.94} \times 10 = 55 \]

Having the MCHC and MCV (red blood cell indices) allows classification of the anemias on a morphological basis that can help to categorize or at least narrow down the various etiological possibilities. In microcytic-hypochromic anemia (low MVC, low MCHC), the red blood cells are small in size or volume and the hemoglobin content is less than normal. A possible cause is iron deficiency due to diet or to chronic blood loss. A transitory macrocytic-hypochromic (increased volume of RBCs and relatively low hemoglobin content) may be observed in the bone marrow reaction to anemia. In this response, immature red blood cells are released prematurely into circulation. These immature cells have a greater volume or size but can have relatively less hemoglobin (see slide 4).

There can be considerable variation in the normal range for red as well as white blood cells (below) depending on age, species, and breed.

- WBC count is the total number of white blood cells (leukocytes) in a defined volume of blood commonly expressed as # of cells per microliter. Total white blood cell counts can become elevated with stress and in certain infections (often bacterial), a situation termed leukocytosis. The WBC count can also be dramatically increased in cases of leukemia. Leukopenia, reduced numbers of leukocytes, can be observed with diseases of the bone marrow and with certain viral infections.
- The differential white blood cell count is the relative percentage of neutrophils (including immature band cells), lymphocytes, monocytes, eosinophils, etc. in circulation. If you know the WBC count, you can figure the total numbers (absolute cell count) of each cell type in a given volume of blood.

\[ .74 \text{ neutrophils} \times 10,740 \text{ WBCs} = 7,948 \text{ neutrophils} \]
The differential white cell count can also provide some general information as to the underlying cause of disease. A high neutrophil count, known as **neutrophilia**, (including some immature band cells) can be an indication of bacterial infection. Some viral infections cause a drop in lymphocyte numbers (**lymphopenia**). A high eosinophil count (**eosinophilia**) can be seen with certain types of parasitic infections.

- Nucleated red blood cells in circulation are abnormal. This could reflect a bone marrow response to severe anemia or a defect in maturation and premature release of red blood cells from the bone marrow.
- The WSVL hematology report also estimates the number of platelets (thrombocytes) and makes any relevant comment concerning platelet morphology.
- The microscopic appearance of erythrocyte and leukocyte morphology on blood smears is an important part of hematology. This allows confirmation of the values noted above as well as to detect other possible scenarios. As you know, erythrocytes in most species have the morphology of biconcave discs. **Spherocytes** are abnormal spherical rather than biconcave erythrocytes and this change may be associated with **autoimmune hemolytic anemia**. Slide #4 is a good example, albeit probably exaggerated, of a response to anemia. Here you can observe anisocytosis (variation in RBC size), polychromasia (variation in staining), spherocytosis, and nucleated red blood cells. **Schistocytes** are fragmented erythrocytes. **Schistocytes** may be observed when there is fibrin deposition in small blood vessels such as occurs in disseminated intravascular coagulation and in splenic disorders such as **hemangiosarcoma**, a cancer of endothelial or blood vessel-forming cells. For other cells such as leukocytes, immature cells (such as band neutrophils) may be an indication of a normal response to inflammatory disease or can be abnormal. Neoplastic cells in circulation (**leukemia**) can be uncovered by microscopic examination of blood smears.

**Case #1 – Hematology** (slides 5-8 will be covered in class)

**FLUID ANALYSIS**

Much like blood, clinical pathologists use other body fluids to aid in the diagnosis of disease. These include the fluids that accumulate when there is abnormal fluid redistribution (**hydrothorax, ascites**), cerebrospinal fluid in cases of neurological disease, synovial fluid in cases of joint disease, and urine. Unlike many of the fluids, evaluation of urine can give some indication of systemic disease as well as disorders of the urogenital system. Parameters that can give an indication of systemic disease include glucose (remember, **glucosuria** is a manifestation of diabetes mellitus), bilirubin (**bilirubinuria** can be an indication of **cholestatic** liver or **hemolytic** disease), ketones (**ketonuria** in diseases associated with negative energy balance). Can you name two disorders mentioned in class that are associated with ketosis or ketoacidosis? Another parameter that would suggest a systemic disorder is the **hemoglobinuria** that can be seen following intravascular destruction of red blood cells as in autoimmune hemolytic anemia. The other parameters that can be measured in urine largely reflect diseases of the urinary or urogenital system. Of these, measurement of urine **specific gravity** is important. As you will remember, the osmolarity of urine can be regulated by
several hormones acting on the kidney to resorb or eliminate metabolites as the body maintains normal fluid balance, electrolyte concentrations, and pH. What happens in a severely diseased kidney? The kidney can no longer concentrate urine leading to polyuria. Thirst is stimulated leading to polydipsia. Hence, a primary manifestation of severe renal disease is polyuria/polydipsia (PU/PD). Urine specific gravity is a direct measure of the ability of the kidney to concentrate urine. A persistently low urine specific gravity, usually on the order of 1.005, can be an indication of kidney disease. Can you name another disease we talked about where PU/PD can be a prominent clinical sign? Could you explain the pathogenesis of the PU/PD? Protein is also a potential measure of renal health. There should be relatively little protein in the urine, usually only trace amounts. Proteinuria can be an indication of kidney disease, usually diseases involving leakage of protein through the glomerular filter such as amyloidosis. A variety of other parameters can be assessed in urine. These will be covered in class.

**Case 2 – Urinalysis (slides 10 – 13)**

**CLINICAL CHEMISTRY**

Clinical chemistry measures a variety of analytes in the fluid component of blood (serum or plasma). These measurements can give an indication of the health of the body as a whole or pointing to diseases involving specific organs.

- **Total serum protein** and **albumin**. Measurements of total serum protein and albumin are a gauge of the protein status of an animal. Low levels of protein can be observed in starvation. More importantly, low protein levels can point to increased loss of protein in urine such as occurs with renal glomerular amyloidosis or in the gastrointestinal tract as would occur through maldigestion or intestinal malabsorption. Since the liver is the primary organ producing albumin and other proteins, hypoproteinemia and hypoalbuminemia may also be an indication of chronic liver disease. Elevations of total serum protein can be observed in dehydration. Elevations in total protein can also occur when there is chronic inflammation or antigenic stimulation leading to increased production of inflammatory proteins and immunoglobulins.

- **Elemental / electrolyte analyses**
  - **Calcium**
  - **Phosphorus**

Disturbances of calcium and phosphorus were covered for the most part in lecture 12. There are also additional more acute forms of hypocalcemia including milk fever of ruminants and syndromes in other animals typically associated with parturition and lactation. Hypophosphatemia is recognized in cattle. One syndrome is known as postparturient hemoglobinuria. There is another syndrome called “creepers” in which cattle on a phosphorus deficient diet experience bone pain and spontaneous bone fractures. Hyperphosphatemia can be dietary and, as you will remember, can also be associated with renal failure.
- **Sodium**
- **Potassium**
- **Chloride**
- **Magnesium**

As you will also remember, a number of hormones involved in regulation of water balance ultimately regulate the absorption or elimination of electrolytes including sodium, potassium, and chloride in the kidney (see lecture 11). Diseases associated with some of these hormones can also result in electrolyte imbalances. You might review aldosterone and Addison’s disease from lecture 11. Additionally, electrolytes (sodium and chloride) can be lost in cases of severe or prolonged diarrhea. **Hyponatremia** is a common condition in chronic alcoholics admitted to hospitals but it can be observed in a variety of clinical settings. **Grass tetany** is a neurological syndrome associated with hypomagnesemia in cattle. Drugs can also be associated with electrolyte imbalances.

- **Glucose.** Hypoglycemia (low blood glucose) can be observed in various metabolic conditions. This is a major abnormality in pregnant ewes during the last stages of pregnancy, a disorder known as pregnancy toxemia. Hypoglycemia can also be a life-threatening consequence of insulinoma, an insulin-secreting neoplasm of the pancreatic islets. What about blood glucose in diabetes mellitus covered in lecture 26?

- **Blood urea nitrogen (BUN).** Ammonium is a bi-product of protein catabolism in the body. Mainly in the liver, the urea cycle converts two molecules of ammonium to urea. Most of the urea in the body is excreted by the kidney. Failure to excrete urea results in an elevated BUN. Elevations in BUN can be pre-renal, renal, or post-renal. A high protein diet can cause modest elevations in BUN. Other pre-renal causes include increased protein catabolism, hemorrhaging into the intestine, and decreased blood flow to the kidney. Post-renal causes include obstruction to the elimination of urine. Significant elevations in BUN suggest kidney disease in most species.

- **Creatinine.** Creatinine is a non-protein product of muscle metabolism. Like BUN, creatinine is a pretty good indication of kidney function in most mammals but does suffer from the same pre- and post-renal elevations as BUN.

- **Bilirubin.** Bilirubin is derived from the breakdown of hemoglobin. In the liver, bilirubin is conjugated and excreted into the bile. Elevation of bilirubin is responsible for jaundice (icterus), yellow discoloration of the tissues in the body. Elevations in bilirubin can indicate severe liver disease, cholestasis, or increased destruction of erythrocytes such as would occur from hemolysis.

- **Alkaline phosphatase.** A variety of enzymes are released into the blood following cellular injury. Alkaline phosphatase (ALP) is one such enzyme that is present in almost all cell types. Its diagnostic usefulness includes its role as an indicator primarily of liver or bone disease. ALP is an inducible enzyme; the levels of the enzyme can increase following treatments with a variety of endogenous compounds and drugs in dogs. One such drug or endogenous compound includes the corticosteroids (either as drugs or produced by the adrenal cortex). In canine Cushing’s disease (hyperadrenocortisolism), whether it is from overzealous use of the drug, from hyperplasia of the adrenal cortex, or from secretory neoplasms of the adrenal cortex,
levels of ALP can be quite high, over and above what would be expected for liver or bone injury.

- **Alanine aminotransferase (ALT, also known as SGPT) and aspartate aminotransferase (AST, also SGOT).** These enzymes are largely nonspecific but have been used as indicators of striated muscle and liver injury. **Lactate dehydrogenase (LDH)** is similar. With these enzymes, there is considerable species variation. For instance, ALT and LDH are of very little use in horses.

- **Gamma-glutamyltransferase (GGT, γ-GT, also gamma glutamyltranspeptidase).** This enzyme is fairly specific for liver injuries. Increases can occur following damage to the bile ducts or hepatocytes.

- **Creatine kinase (CK, also creatine phosphokinase or CPK).** Creatine kinase isoenzyme activity is found mainly in striated muscle (heart and skeletal muscle) and brain. The enzyme is composed of two subunits designated as M (muscle) and B (brain). The BB homodimeric form of the enzyme is found in brain, MM in skeletal, and the heterodimer MB, in cardiac muscle. In humans, measurement of these isomeric forms has allowed differentiation of cardiac disease, i.e. heart attack, from skeletal muscle damage. The utility of the isoenzymes is not as great in animals due to species variations but CK is considered mainly as a sensitive indicator of skeletal muscle damage.

- **Amylase and lipase.** These pancreatic enzymes, which aid in digestion, increase following inflammation of the pancreas (pancreatitis) and following pancreatic necrosis.

- **Cholesterol and lipoproteins.** Compared to humans, these analytes have not been used to the same extent in animals. Cholesterol is often elevated in cases of hypothyroidism in animals, notably dogs.

**Case #3 (slides 16-18)**

**Case #4 (slides 19-21)**

**Case #5 (slides 22-28)**

**ENDOCRINOLOGY**

Sometimes hematology, clinical chemistry, and urinalysis as well as other tests such as histopathology can give us an indication of diseases related to the endocrine system but not a confirmation. Measurements of hormones and trophic substances do provide a more specific means of arriving at a solid diagnosis. Time does not allow an exhaustive coverage but below are some of the tests that are available:

- **Thyroid gland**
  - Triiodothyronine (T3)
  - Thyroxine (T4)
  - Thyroid stimulating hormone (TSH). TSH is released by the pituitary and stimulates the thyroid gland to release thyroid hormone. Direct measurement of TSH evaluates the pituitary-thyroid axis. Another application is the TSH stimulation test that measures the ability of the thyroid gland to release T3 or T4.
• Pancreatic islets
  - Insulin. As discussed in lecture 26, in type 2 diabetes mellitus there may not be an absolute deficiency of insulin. In some cases of diabetes mellitus, it is necessary to measure the body’s response to glucose, i.e. the glucose tolerance test. After a test dose of glucose is administered, the rate of decline of serum glucose is measured. Direct measurement of insulin can however, be an aid in the diagnosis of an insulin-secreting neoplasm, insulinoma.

• Adrenal gland (aids in the diagnosis of hyperadrenocorticism, Cushing’s disease)
  - Adrenocorticotropic hormone (ACTH). The levels of ACTH secreted by the pituitary gland and ultimately blood glucocorticoids (cortisol) produced by the adrenal cortex are controlled via negative and positive feedback mechanisms. With neoplasms of the adrenal cortex that secrete cortisol, ACTH levels would be expected to be low due to feedback inhibition. Likewise, with iatrogenic Cushing’s disease due to administration of exogenous corticosteroids, ACTH levels would be expected to be low. Conversely, dogs with pituitary neoplasms secreting ACTH or hyperplasia of corticotrophic cells (pituitary dependent hyperadrenocorticism) would obviously have elevated levels of ACTH.
  - Cortisol (cortisone). Cortisol would be expected to be elevated in cases of pituitary dependent hyperadrenocorticism or with secretory neoplasms of the adrenal cortex. Endogenous cortisol levels would be low in cases of iatrogenic Cushing’s disease. There is also an ACTH response test that helps to further evaluate the pituitary-adrenocortical axis. ACTH is administered and cortisol is measured. Similarly, there is a dexamethasone (a synthetic corticosteroid) suppression test that also measures cortisol. In normal dogs, dexamethasone suppresses ACTH release resulting in low cortisol levels. What would you expect to see with ACTH secreting pituitary or cortisol secreting adrenal cortical neoplasms?

Case #6 (slides 29-32)

Case #7 (slides 33-37)

CYTOLOGY

Cytology literally means the study of cells which, you will all agree, is pretty vague. Perhaps a better but still nonspecific term would be cytopathology, the study of cells in disease. As used today, cytology and cytopathology are used synonymously to indicate the examination of individual cells, but not in the context of tissues or organs, in various disease states. What then is the source of the cells that cytologists or cytopathologists examine? The cells can 1) be aspirated with a syringe and needle from solid tissues (organs, skin lumps and bumps, bone marrow), from various body fluids (synovial fluid, cerebrospinal fluid), from abnormal fluid accumulations (hydrothorax or ascites), as well as from other samples such as tracheal or bronchial lavages. The cells are then routinely placed on a slide, usually stained, and viewed microscopically. The major difference between histopathology and cytopathology is that histopathologists view the cells in the context of the surrounding tissue and tissue or organ architecture while cytopathologists typically have only individual cells to examine.
Histopathologists view this as a major shortcoming and most would agree that the practice of cytology is less precise than histopathology for most applications. What then is the value of cytopathology?

1. Aspiration of fluid from any site or cells from solid lesions is a less invasive, less time-consuming, and a less costly procedure, generally requiring only minimal sedation. The value of PAP smears in the diagnosis of cervical cancer in women is well recognized.
2. Cytology allows examination of cells from various fluid compartments such as cerebrospinal fluid, joints, and body cavities that simply cannot be done otherwise.
3. Bone marrow is a special case. From a practical standpoint, the morphology of aspirated bone marrow cells is far superior to those obtained from bone biopsy specimens. Remember, tissues destined for histopathology undergo a variety of rigorous treatments such as fixation and dehydration before embedding in paraffin wax, sectioning, and staining for microscopic examination; all of these treatments introduce artifacts that cannot be overcome when it comes to bone marrow cells.

Apart from bone marrow evaluation, major reasons for performing cytologies are to better define the nature of space-occupying lesions, as a step in evaluating body fluids, and to identify microorganisms if the lesions are inflammatory in nature.

**Space occupying lesions** can be evaluated by aspiration of cells with a needle and syringe. These ‘tumors’ can commonly be differentiated into neoplastic or inflammatory in nature by cytology. Other space occupying lesions can be due to localized fluid accumulation in soft tissues and can include **cysts, hematoma, and seroma**. These need not be mentioned further. Differentiation of neoplastic from inflammatory lesions is usually straightforward but inflammation and necrosis can occur in neoplastic lesions which add some degree of difficulty. Another degree of difficulty is added by the fact that cell recovery with mesenchymal neoplasms such as those of fibrous tissue is typically poor following needle aspiration. This is also true of chronic inflammatory lesions with extensive fibroplasia or scarring. Once the lesion is identified as neoplastic, the major challenge is to identify the type of neoplasm and whether it is benign or malignant. Some neoplasms such as lymphoma (a malignant neoplasm of lymphocytes) are pretty straightforward and recovery of neoplastic lymphocytes is usually easy with needle aspiration. For most neoplasms, however, knowledge and experience are required for a reliable diagnosis. These nuances are beyond the scope of this lecture but two examples will be given to illustrate the pitfalls (slides 38 and 39).

**Evaluating body fluids**, much like observing the morphology of red and white blood cells in hematology, is an integral part of the examination. Again, a major purpose is to identify if the fluid shows evidence of inflammation or, less commonly, neoplasia. The morphology of the cells in fluid accumulations, such as in ascites, or with others such as joint fluid and cerebrospinal needs to be carefully correlated with other parameters such as total nucleated cell count and total protein. As an example, ascitic fluid of low total nucleated cell count and low protein is typically called a **transudate**; it could be due to right-sided congestive heart failure or hypoproteinemia.
Microorganisms can be readily identified in cytological preparation if present in sufficient numbers and/or with careful/fortuitous examination. These can also be observed in blood smears (slides 41 and 42).

Case #8 (slides 43-46)