Normal synovial fluid is clear, pale yellow, viscid, and does not clot. Studies of mammalian synovial fluid have found considerable similarities among species, although notable differences do exist. The majority of investigative work determining the composition of synovial fluid has been performed on bovine synovial fluid mainly because large quantities of it are available.

Synovial fluid is a plasma dialysate modified by constituents secreted by the joint tissues. The major difference between synovial fluid and other body fluids derived from plasma is the high content of hyaluronic acid (mucin) in synovial fluid. The exact source of the hyaluronic acid has been the subject of debate. It is generally assumed, however, that both fibroblasts beneath the synovial membrane intima and synovial membrane-lining cells produce this mucopolysaccharide constituent of synovial fluid. Hyaluronic acid is a nonsulfated polysaccharide composed of equimolar quantities of D-glucuronic acid and \( \text{N-} \text{acetyl-D-glucosamine} \) residues. It was first identified in joint fluid by acetic acid precipitation. The normal viscosity of synovial fluid is due to the hyaluronic acid.

Synovial fluid is believed to have two main functions: to aid in the nutrition of articular cartilage by acting as a transport medium for nutritional substances, such as glucose, and to aid in the mechanical function of joints by lubrication of the articulating surfaces. Articular cartilage has no blood, nerve, or lymphatic supply. Glucose for articular cartilage chondrocyte energy is transported from the periarticular vasculature to the cartilage by the synovial fluid. Under fasting conditions, the glucose concentration of synovial fluid is usually approximately equal to that of blood. A decreased amount of synovial fluid glucose may be associated with articular diseases, particularly septic and immune-mediated arthritis.

Distribution of synovial fluid electrolytes is in accord with the Donnan theory of membrane equilibrium of solutes, adding credence to the theory that synovial fluid is indeed a plasma dialysate. The distribution ratio of most electrolytes between synovial fluid and serum is 1. The average ratio of total anions of serum to total anions of synovial fluid is 1.99. Ionic calcium ratios are approximately 1.18. This higher ratio is thought to be due to the basecombining power of mucin for calcium.

The normal volume of synovial fluid obviously varies from joint to joint. In the dog, the average is 0.24 ml (0.01 ml – 1.0 ml). The pH ranges from 7.0 to 7.8. Experimental work with dogs has shown that the pH is lowered by exercise and returns to a higher value at rest. As noted above, the viscosity of synovial fluid is due to the hyaluronic acid. Precipitation of synovial fluid mucin with weak acetic acid (mucin clot test) leaves a fluid with a viscosity similar to water. The lubricating ability of synovial fluid is often equated with its normal viscosity. However, experimentally induced tryptic digestion of hyaluronic acid will destroy the synovial fluid lubricating abilities without lowering its viscosity. It has also been shown that hyaluronic acid can be depolymerized without altering its lubricating capacity. Almost all of the protein constituents of synovial fluid are derived from plasma. The passage of plasma proteins to synovial fluid is related to the size and shape of the protein molecule. Most proteins with molecular weights less than 100,000 daltons are readily transferred from one fluid space to another.

Vascular permeability and synovial membrane permeability are altered by inflammation, which accounts for protein content.
changes in diseased synovial fluid. Immunoglobulins, immune complexes, and complement are produced by cells accumulating in the inflamed synovial membrane and periarticular lymph nodes and find their way to the synovial fluid.

Normal synovial fluid complement levels in humans are approximately 10% of the serum values. In the inflamed joints, synovial fluid complement levels will vary. The long-term patterns of variation have some prognostic value in human rheumatoid arthritis patients. (2)

The proteins of coagulation are not found in normal synovial fluid, while proteins of the plasmin system may be found invariable quantities. (20) Normal synovial fluid does not clot but may exhibit thixotropy, (13) the property of certain gels to become fluid when shaken. On standing at room temperature, normalsynovial fluid may assume a gelatin-like appearance. When shaken it will resume its normal fluid nature.

Many enzymes have been found in the normal synovial fluid of domestic animals and humans. Alkaline phosphatase, acid phosphatase, lacticedehydrogenase, and other enzymes are present in detectable quantities. (20) Synovial fluid to serum ratios of these and other enzymes vary with the species studied and the presence of articular disease. Enzymes enter the synovial fluid directly from the plasma or may be produced locally by the synovial membrane or released by synovial fluid macrophages.

**Cellular Constituents**

Cell counts of canine synovial fluid vary from joint to joint but normally are low. Average counts range from 0 to approximately 3000 cells/mm³. (28) Lymphocytes are seen in the greatest numbers, and both B and T lymphocytes have been identified. (20) Monocytes and neutrophils are also present normally, while macrophages are seen only occasionally. Differentiation between mononuclear cells of bone marrow origin and those derived from local tissues may be difficult, since reactive macrophages may assume the characteristics of type A or B synovial cells. Occasionally, aspiration produces clusters of synovial membrane cells that are more readily identifiable. (20)

In pathologic fluids, chondrocytes, osteoblasts, and osteoclasts may be seen. Fragments of articular cartilage may contain chondrocytes within lacunae. Exposed subchondral bonemay give osteoblasts and multinucleated osteoclasts access to the synovial fluid. (20)

It has been suggested that the main function of the mononuclear cells of synovial fluid is the removal of debris that appears in joints in normal use. A direct correlation has been found between synovial fluid leukocytosis and synovial fluid pH. (32) Increasing white blood cell counts and decreasing pH were noted in human patients with various forms of acute and chronic arthritis.

Erythrocytes are rarely seen in normal joint fluid; their presence usually indicates contamination of the sample by peripheral blood at the time of aspiration.

**Lubrication of Joints**

Lubrication reduces frictional resistance between bearingsurfaces by keeping them apart. Friction and the resulting wear of two unlubricated surfaces sliding on each other are due to the interaction or contact between the opposing surfaces. In many mechanical bearings lubricated by oil, the relative continuous motion of the surfaces produces a wedge of lubricant that keeps the surfaces apart. This phenomenon is defined as hydrodynamic lubrication and requires uninterrupted motion in the same direction to maintain the integrity of the wedge. (22) Because joints oscillate and change direction of motion, pure hydrodynamic lubrication is not the mechanism by which synovial fluid functions as a lubricant. (13, 22)

Many theories based on extensive investigation of the physical properties and abilities of synovial fluid to act as a lubricant have been presented to explain the mechanisms of joint lubrication. It appears that the low frictional resistance to joint motion is due to a combination of mechanisms. Each mechanism complements the other and depends on the tissues involved and the load imparted to the joint. Resistance to joint motion comes from the stretching of surrounding soft tissues (ligaments, tendons, muscle) and frictional resistance of the joint parts that must slide across each other (cartilage, synovium, tendons in sheaths). (20) Surfaces that contact each other during joint motion and therefore give rise to frictional resistance have been defined as (1) a soft tissue interface—synovium on synovium or synovium on cartilage—and (2) a cartilage-on-cartilage type. (22) Lubrication of synovial surfaces by synovial fluid requires hyaluronate and is due to a boundary phenomenon. Boundary
lubrication occurs when each bearing surface is coated or impregnated with a thin layer of lubricant that keeps the sliding surfaces apart, allowing ease of motion with a low coefficient of friction between the sliding surfaces. (13,22) Hyaluronate sticks to the synovial surfaces. The lubricating properties of synovial fluid in a soft tissue system are directly related to the concentration and molecular weight of the hyaluronate, which is also determined by viscosity. (23) However, it is not the viscosity of synovial fluid that is responsible for lubrication of this system but the stickiness or boundary phenomena exhibited by the fluid. Viscous solutions containing no hyaluronate do not lubricate a soft tissue system nearly as well as solutions containing hyaluronate of equal or even lower viscosity.

The lubricating properties of synovial fluid on articular cartilage were originally attributed to its viscosity, which in turn is due to the presence of hyaluronate or mucin. However, viscosity or the resistance of a fluid to shearing forces is not the same as lubricating effectiveness. (22) Digestion of synovial fluid hyaluronate by hyaluronidase, which totally destroys the viscous nature of the fluid, does not decrease the lubricating properties of synovial fluid on articular cartilage when compared with a nonviscous buffer. (12) This is in contrast to the finding that proteolytic digestion of synovial fluid decreases its lubricating abilities. A glycoprotein has been isolated from synovial fluid. and removal of this fraction from the fluid deprives it of its lubricating properties. (24)

The mechanisms of cartilage-on-cartilage lubrication have been attributed to boundary effects and the presence of a fluid film. The boundary effect of synovial fluid in a cartilage-on-cartilage system is similar to that in a soft tissue system in that synovial fluid readily adheres to the cartilage surfaces, helping to keep them apart and decreasing frictional forces. Unlike the soft tissue system, however, the boundary effect of synovial fluid is not due to the hyaluronate but to the lubricating glycoprotein fraction of synovial fluid. It is this fraction that sticks firmly to the articular cartilage surfaces. Although hyaluronate does not directly decrease the coefficient of friction in a cartilage-on-cartilage system, it may enhance the longevity of the lubricating ability of the protein fraction and act as a spreading factor. (22) Articular cartilage is quite resistant to shear forces but very sensitive to impact loading. (13) Boundary lubrication of articular cartilage is extremely effective in preventing wear due to motion but loses its protective abilities under high loads. (22) Therefore, other lubricating mechanisms must be at work. Fluid-film lubrication is a class of mechanism of lubrication in which a film of fluid separates the opposing sliding surfaces. Squeeze-film lubrication is a form of lubrication in which the approaching surfaces generate pressure in the lubricant as they squeeze it out of the area of impending contact. The resulting pressure keeps the surfaces apart, and the lubricant film that forms in the area of impending contact is referred to as the squeeze film. (22) Electron microscopically, articular cartilage is shown to have depressions and irregularities on its surface. In the early phases of loading these depressions may trap fluid. With increasing load, the articular cartilage surface may deform and the irregularities disappear. The surface deformation and intrinsic elasticity of articular cartilage will tend to make the space of impending cartilage contact narrower at its margin than at its center. More fluid will be trapped in the center of the contact area where it may help to form a squeeze film. This mechanism of lubricant trapping has been called "boosted" lubrication. (31) However, there is disagreement regarding the validity of a "boosted" component in the squeeze-film type of fluid-film lubrication. (13,22)

Compression of articular cartilage produces a watery film on its surface. This wept fluid is composed mainly of water and small ions. (13) Pore size in articular cartilage has been measured to be approximately 60 nm, although occasional large pores (1000 nm) are present. This small pore diameter restricts the passage of large molecules such as the mucopolysaccharides of the cartilage matrix and hyaluronate and synovial fluid protein while allowing passage of interstitial fluid of the cartilage.

It has been suggested that in a highly loaded joint this wept fluid creates a lubricating fluid film referred to as "weeping" or self-pressurized hydrostatic lubrication. The fluid flow onto the cartilage surface probably occurs at the periphery of the area of impending contact where the pressure is lower rather than at the center of the contact area where the pressure is highest. Fluid flow out of the cartilage toward the subchondral area is blocked by the subchondral plate, and sideways flow is retarded by the relatively poor perfusion characteristics of cartilage.

Another consideration in the mechanism of decreased friction between articular cartilage surfaces is the intrinsic elasticity of the cartilage. (13,22) The compliance of articular cartilage may allow its uneven surface to flatten under loading, thus lowering the pressure at impending junction sites. *Elastohydrodynamic lubrication* has been defined as a form of fluid lubrication occurring when bearing surfaces are sufficiently elastic for the lubricant pressure generated by motion under a given load to depress the surfaces a distance greater than their highest peaks, thus facilitating maintenance of a fluid film. (22)

In summary, synovial joints contain two systems that require lubrication: a soft tissue system and a cartilage-on-cartilage system. Lubrication of the soft tissue system is of the boundary type, requiring the hyaluronate of the synovial fluid to stick to the sliding surfaces of the system, thus keeping them apart. In contrast, the cartilage-on-cartilage system is independent of hyaluronate and dependent on a glycoprotein fraction of synovial fluid. At low loads the lubricating action of the glycoproteins of the boundary type. At high loads the cartilage surfaces are kept apart by fluid film composed of fluid and interstitial fluid wept from the articular cartilage itself. The elasticity of articular cartilage may potentiate the fluid-film lubricating mechanisms at high loads.
Arthrocentesis

Synovial fluid analysis should be an integral part of any diagnostic evaluation of an animal with lameness, especially the animal with joint effusion. Properly performed arthrocentesis is a relatively innocuous procedure requiring little time, expertise, or special equipment. General anesthesia of the patient is rarely necessary. It is important, however, to have a cooperative patient. Sedation and restraint can be adequately achieved with tranquilization and analgesic agents.

Arthrocentesis has both diagnostic and therapeutic application. Diagnostic procedures include synovial fluid analysis, cytologic examination, and microorganism culture, as well as intra-articular injection of contrast material for arthrography. Therapeutic benefits of arthrocentesis include decompression of distended joints, removal of fibrin and exudate by lavage, and the instillation of therapeutic agents.

INDICATIONS

There are no absolute contraindications to synovial fluid aspiration. Indications for arthrocentesis and synovial fluid analysis include joint effusion or swelling, chronic or periodic shifting lameness, stiff or altered limb function especially when associated with fever, and joint deformity associated with lameness. Arthrodesis also indicated in those animals who are known to have articular manifestations of disease even if lameness or joint effusion is not yet apparent.

The only significant complication of arthrocentesis is infection. Synovial fluid is readily obtained by needle aspiration. Sterile technique is mandatory to avoid iatrogenic infection and bacterial contamination of samples. Volume requirements for the various laboratory determinations must be known prior to arthrocentesis so that the priority of the tests can be determined if only small quantities of fluid are obtained. The suspected etiology of the condition should indicate which tests have priority. Normal quantities of synovial fluid vary with the joint in question. Even in cats and toy breeds of dogs with effusion of large joints such as the stifle, the quantities of synovial fluid obtained may be small.

EQUIPMENT

Elaborate equipment is not needed for joint aspiration. Sterile hypodermic needles (18 gauge-22 gauge) ranging in length from 1 to 2 1/2 inches are used. Diameter and length of the needle depend upon the joint involved and the size of the patient. The longer needles are more frequently used in large breeds of dogs for aspiration of the coxofemoral or scapulohumeral joints. A 1-inch 22-gauge needle is most commonly used. The long bevel of disposable needles may prevent the needle lumen from fully entering the joint cavity of small articulations such as the carpus and tarsus. In such cases, consistent results can be obtained by using a short beveled disposable spinal needle. The sharp beveled edge of the disposable hypodermic needle may also cut a core of tissue as it is passed toward the joint and thus become occluded. Use of a spinal needle with a stylet will prevent this problem.

Small-capacity syringes (2.5 ml-3 ml) are recommended, especially for use in joints from which only small quantities of synovial fluid are expected. Their use will minimize fluid loss in the syringe barrel. Syringes smaller than 2.5 ml to 3 ml capacity usually do not develop sufficient negative pressure on aspiration to give a successful tap.

Skin over the joint to be aspirated should be prepared as for any sterile invasive procedure. Hair must be clipped and the skin washed with surgical soap. Aseptic technique is important preventing iatrogenic infection and bacterial contamination of aspirated samples. With the patient properly positioned and restrained, the aspiration site is palpated for the appropriate anatomical landmarks and the needle with attached syringe is passed into the joint. Sterile gloves should be worn to prevent contamination. Aspiration should not begin until it is reasonably certain that the needle is in the proper position. In joints with effusion, fluid is usually withdrawn easily. Slight rotation of the needle may facilitate aspiration. Once the desired quantity of fluid has been obtained, the needle and syringe are withdrawn from the joint. It is important that the syringe plunger be released and the negative pressure within the syringe cease before removing the needle from the joint. This will prevent inadvertent contamination of the sample with peripheral blood.

TECHNIQUES

Scapulohumeral Joint

The patient should be in lateral recumbency with the affected joint uppermost and in partial flexion. The acromion process and supraglenoid tubercle of the scapula and the greater tubercle of the humerus are then identified by palpation. The needle enters the joint from its cranialolateral aspect, passing proximal to the lateral aspect of the greater tubercle, lateral to the supraglenoid tubercle, and ventral to the acromion process (Fig. 86-1). In large dogs a 1 1/2-to 2 1/2-inch needle may be needed.
Elbow Joint
With the patient in lateral recumbency and the affected joint uppermost, the elbow is placed in moderate flexion. The joint is entered from its caudolateral aspect. With the joint in flexion, the lateral epicondyle and lateral condyle of the humerus and olecranon process of the ulna are located. The anconeal process of the ulna lies medial to the lateral humeral condyle with its tip approximately in line with the lateral humeral epicondyle. The needle penetrates the skin at the caudolateral aspect of the olecranon process, adjacent to the triceps tendon, and is directed downward and cranially between the olecranon process and the lateral humeral condyle toward the anconeal process (Fig. 86-2). The needle enters the joint space between the anconeal process and the medial surface of the lateral humeral condyle.

Carpal Joints
The carpal joints include the proximal, middle, distal, and intercarpal joint surfaces. The proximal or antebrachiocarpal (radiocarpal) joint is the one most frequently entered for arthrocentesis. The middle carpal joint and the carpometacarpal joints are aspirated less often. Each joint is entered from its dorsal surface after the joint has been flexed. Major arteries and veins, tendons, and nerves cross the carpus on its dorsal surface. These structures must be avoided when performing arthrocentesis. The cranial superficial antebrachial artery travels from medial to lateral down the distal limb. Its main trunk is usually midway between the medial and lateral aspects of the limb, just proximal to the antebrachiocarpal joint. At this point, it branches into the dorsal common digital artery. The vessel and the paralleling accessory cephalic vein can be avoided by passing the aspiration needle into the antebrachiocarpal joint just medial or lateral to the midsagittal plane of the joint (Fig. 86-3). Aspiration of the antebrachiocarpal joint will not be diagnostic for the entire carpal articulation, since this joint does not communicate with the middle carpal and carpometacarpal joints, which are contiguous.

Entrance to the middle carpal joint is facilitated by hyperflexion and passage of the needle between the radiocarpal bone and the second and third carpal bones. The carpometacarpal joint space is small and difficult to enter. For this reason and because it communicates with the middle carpal joint, it is rarely aspirated.

Phalangeal Joints
In humans, the interphalangeal joints are frequently affected by degenerative joint disease and rheumatoid arthritis. While these joints are occasionally affected by similar diseases in the dog and by chronic polyarthritis in the cat, they are aspirated infrequently owing to their small size.

Coxofemoral Joint
Both lateral and ventral approaches to the hip joint have been described for arthrocentesis. The ventral approach may prove to be easier. With the animal in dorsal recumbency, the femur is abducted and secured in position perpendicular to the long axis of the body, and the easily identified pectineus muscle is palpated. The ventral aspect of the acetabular fossa is located immediately dorsal to the body of the pectineus muscle. The needle is passed in a caudal to cranial direction at a 45°
angle to the joint. The needle should pass just caudal to the body of the pectineus muscle, lateral to the ventral acetabular rim, and medial to the femoral head (Fig. 86-4, A). Some resistance to needle advancement may be felt if the needle passes through the ligament of the head of the femur. (8)

The lateral approach is obtained by placing the animal on its side with the affected joint uppermost. The limb is grasped at the stifle joint, abducted slightly, and then outwardly rotated. The greater trochanter is identified and the needle is passed just caudal and medial to it. The needle is directed in a caudal to cranial direction toward the hip joint at an angle of approximately 45° (Fig. 86-4, B).

**Stifle Joint**

Owing to its size and ease of entry, the stifle is probably the most frequently aspirated of all the joints. The patient is placed in lateral recumbency with the affected limb uppermost, and the joint is flexed sufficiently to cause tensing of the joint capsule. The needle is passed either medial or lateral to the patellar ligament and directly obliquely and caudally toward the intercondylar space of the distal femur. Entrance to the joint should be made approximately midway between the distal end of the patella and the proximal articular surface of the tibia (Fig. 86-5). Passing the needle directly through the patellar ligament into the intercondylar space has been described for aspiration of the knee in humans (29) and has been used successfully in the dog and cat as well.

**Tarsus**

The tarsalocrural joint may be aspirated from either a dorsal or plantar approach. (8,33) The easier approach is from the proximal plantar-lateral aspect of the joint. With the patient in lateral recumbency and the affected limb uppermost, the space between the distal fibula and tibia is palpated. The needle is advanced in a dorsomedial and distal direction parallel with the fibular tarsal bone (Fig. 86-6). Moderate joint flexion facilitates entrance into the joint.

In the dorsal approach, the needle is passed in a plantar direction between the tibia and tibiotarsal bone adjacent to the flexor tendons.

As with aspiration of the carpal joints unless there is significant joint effusion quantities of synovial fluid greater than 0.1 ml to 0.2 ml will not be obtained.

**Analysis**

The physical characteristics of synovial fluid frequently indicate the disease process affecting the joint. Routine studies...
of synovial fluid analysis are listed in Table 86-1.

**TABLE 86-1 Synovial Fluid Analysis**

### APPEARANCE

The appearance of synovial fluid is characterized by its color, turbidity, viscosity, quantity, and ability to clot. Synovial fluid analysis begins literally the moment fluid is aspirated into the syringe. It is particularly important to note whether the fluid was bloody initially or became so during aspiration. In a traumatic tap, one in which hemorrhage was induced by the tap and was not occurring prior to aspiration, blood in the syringe would usually be distributed unevenly. The sudden appearance of blood in the synovial fluid during aspiration is also a reliable indication of a traumatic tap. In the atraumatic tap yielding bloody synovial fluid, the fluid appearance does not change during the aspiration but remains bloody throughout.

Cytologic examination for platelets may help differentiate intra-articular hemorrhage from a traumatic tap in that platelets are not often found in normal or disease-related samples. Dark yellow or xanthochromic fluids may indicate chronic hemorrhage and erythrocyte breakdown and the formation of bilirubin compounds. (20)

Turbid samples of varied color are commonly associated with inflammatory joint disease, which may be septic or nonseptic. Turbidity is usually due to cells, fibrin, or other debris. When print cannot be read through a sample, it is considered turbid or cloudy, also suggesting inflammation. (20)

Synovial fluid viscosity is easily assessed by slowly expelling one or two drops of fluid from the syringe through the needle. If the fluid flows with the ease of water the viscosity is low. (3) Formation of a tenacious string as the drop leaves the needle indicates normal viscosity. Although quantitative measurements of synovial fluid viscosity can be obtained by the use of a viscometer, such quantitation is not performed routinely. (3) Lowered viscosity, regardless of the synovial fluid appearance, is usually indicative of an inflammatory change.

Even in large joints of the dog such as the stifle or hip, normal amounts of synovial fluid are rarely greater than 1 ml. Careful examination of the more superficial joints of the extremities will usually delineate joint effusion and increased amounts of synovial fluid prior to aspiration. However, even in some inflammatory conditions such as degenerative joint disease, the volume of synovial fluid may not be appreciably increased. The amount of fluid present in an affected joint is usually increased when the signs of inflammation are increased. This is particularly true in conditions such as systemic lupus erythematosus in which the clinical signs may wax and wane as does the degree of joint effusion.

Normal synovial fluid does not clot, but as mentioned, it does have thixotropic properties. Synovial fluid that clots suggests the presence of synovitis and is caused by fibrinogen and other clotting factors in the fluid that are not present normally. Clotting of synovial fluid due to the presence of clotting factors should not be confused with the formation of a mucin clot in synovial fluid that has been treated with acetic acid.

The mucin clot test is a qualitative assessment of the degree of polymerization of synovial fluid hyaluronate. In general, the more inflamed the joint the poorer the test results. The test is performed by mixing one part of synovial fluid with four parts of 2% glacial acetic acid in a glass beaker with a glass stirring rod. (3) A clot forms immediately as a result of the precipitation of the hyaluronate and the synovial fluid protein by the acid. The quality of the clot formed reflects the degree of hyaluronic acid polymerization. When the mucin (hyaluronic acid-protein complex) is normal, a firm tight mass forms in a clear solution. This is described as a "good" mucin clot. Formation of a softer, less compact mass with shreds in a cloudy to turbid solution is described as a "flair" mucin clot. A "poor" clot is one that is friable, and thus easily broken up by shaking the beaker, and surrounded by a flocculent cloudy fluid. (3, 20, 29) As noted above, the type of clot formed reflects the degree to which the polymerization of hyaluronic acid has been affected. In immune-mediated articular diseases, fair to poor clots are found most commonly, while good clots are not unusual in degenerative joint disease. Poor clots may be found in some septic arthritides in which the causative bacteria have the capability to produce hyaluronidase.

### CYTOLOGIC EXAMINATION

Cytologic examination of synovial fluid is similar to that of peripheral blood in that total numbers of leukocytes and erythrocytes are counted and a differential count of the white cells is performed. Although particle counters have been used for leukocyte and erythrocyte counts, the results may not be accurate on synovial fluid with low cellularity. (20) Therefore, hemocytometer counts should be made on low-cellularity synovial fluids. The normal acid diluent used for blood samples should be read through a sample, it is considered turbid or cloudy, also suggesting inflammation. (20)

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counts cannot be used for synovial fluid because it will cause mucin precipitation and thus alter the count. (3, 33) Physiologic saline should be used as adiluent. High-power magnification will also ensure accurate counts. Pretreating high-viscosity pathologic synovial fluids with hyaluronidase has been recommended for the even distribution of cells for counting and identification. (20)

Normal cell counts of synovial fluid of the dog range from 0 to 3000/mm³. (28) Mononuclear cells, and lymphocytes in particular, predominate. Occasionally, monocyte-macrophage type cells are seen. Neutrophils are rarely present and when seen are usually the result of blood contamination during sampling. Increased numbers of neutrophils are indicative of disease. An occasional erythrocyte may also be present owing to sampling contamination. (20)

Because of its viscosity, synovial fluid smears for cytologic examination must be made carefully. (21) Clean slides are imperative, and coverglass smears are preferred to glass slides since they produce a thinner fluid film. Direct smears are best made by slow advancement of the spreader slide to ensure a thin smear. Cells and high viscosity fluids are usually evenly distributed throughout the smear and do not accumulate at the feathered edge. Excellent delineation of the cells is obtained by allowing the smears to air dry at room temperature and then staining within a short time after drying. Fixation of the dried smears with methyl alcohol may be less desirable than nonfixed air-dried smears that are to be stained with Wright's stain. Also, the use of methyl alcohol precludes the use of newmethylene blue and Gram's stain, the latter of which is quite important in establishing a diagnosis of septic arthritis. (21)

Cellular characteristics will vary depending upon the stain used. Wright's stain and new methylene blue are commonly employed for synovial fluid smears. (21) Background material such as fibrin and mucin will also be different. Wright's stain imparts a granular appearance to the background mucin with occasional clefts resulting from fibrin formation. (21) Fibrin is more clearly seen in new methylene blue-stained smears.

**BIOCHEMICAL EXAMINATION**

**Glucose**

Synovial fluid glucose levels, when compared with those of serum (plasma), have been used as aids in the diagnosis of joint disease, especially septic arthritis. Under fasting conditions, synovial fluid glucose levels parallel those of serum. Paired samples should always be run. More accurate results are obtained if the patient has been fasted. It has been shown in humans that synovial fluid glucose may be higher than serum glucose 2 to 3 hours after a meal. (3)

Disparity between fasting levels of glucose in synovial fluid and serum in infectious arthritis is due to the glycolytic activities of bacteria. In acute joint infections of humans, a synovial fluid/serum glucose difference of 15 mg/dl is not unusual, and in some instances the amount of glucose in the synovial fluid may be so low that it cannot be measured. (3) Increased numbers of leukocytes in synovial fluid in the absence of bacteria, such as seen in immune-mediated articular disease, can also account for decreased synovial fluid glucose owing to the glycolytic activities of the white cells. (20)

Synovial fluid samples for glucose analysis are handled in a manner similar to serum samples. The standard Somogyi-Nelson glucose method has been recommended for synovial fluid glucose determinations. (3)

**Protein**

Normal synovial fluid protein levels in the dog are approximately 2.0 g to 2.5 g/dl. (20) As with glucose, parallel determinations of serum and synovial fluid protein should be performed. A refractometer may be used to give an estimate of synovial fluid protein. Other methods for synovial fluid protein determinations include the biuret technique and electrophoresis. (3) The electrophoretic pattern of synovial fluid protein will differ from that of serum even in normal subjects owing to the selective permeability of the synovial membrane vasculature.

Concentrations of synovial fluid protein are known to vary with the degree of joint inflammation. The amount of plasma-derived synovial fluid protein is a function of the molecular size of the protein, its plasma concentration, and local vascular permeability. With increasing joint inflammation, total synovial fluid protein levels rise to those approaching those of plasma. In humans the albumin decreases while the alpha2-globulin and globulin fractions increase. (3) Clotting factors are also found in increasing amounts. While electrophoretic patterns of synovial fluid protein are not normal in joint inflammation, they are not characteristic of specific causes of inflammation.

**MICROBIOLOGIC EXAMINATION**

To avoid iatrogenic joint infection or contamination of the aspirate, the precautions of sterile technique are mandatory when obtaining synovial fluid by aspiration. Synovial fluid from suspected infectious arthritis is handled by following the standard practices of specimen staining and culturing used for other infectious biologic fluids. (20) It must be remembered that blood collection tubes are not sterile and therefore cannot be used for the transfer of fluids for culture.
Aerobic and anaerobic bacterial cultures should always be requested. While mycoplasma and viral isolation studies are not performed routinely, they may be necessary in some cases. Antibiotic sensitivities should also be determined. In some cases of septic arthritis, aerobic and anaerobic bacterial cultures will show no growth, thus hampering the establishment of a diagnosis and the institution of appropriate antibiotic treatment. Gas chromatography of synovial fluid to identify bacterial organisms may prove quite useful in such cases. (1)

Gram's stains of synovial fluid smears may be of particular value in establishing a diagnosis of septic arthritis. A chronic progressive polyarthritis of cats has been described that may be due in part to the immunosuppressive effects of feline syncytia-forming virus and feline leukemia virus, both of which can be isolated from the synovial fluid of affected cats. (18)

**IMMUNOLOGIC STUDIES**

In humans with immune-mediated articular disease, rheumatoid factor, complement levels, antinuclear factors, and lupus erythematosus (LE) cell tests are often performed on synovial fluid. However, except for the prognostic value derived from differences between paired serum and synovial fluid complement levels, (2) there is probably little advantage of conducting these immunologic studies on synovial fluid since similar findings are obtained from serum. (20) These studies are routinely performed on the sera of dogs.

**ENZYMES**

Enzyme levels in synovial fluid have been studied extensively in humans and increases have been found to accompany various articular diseases. However, increases (or decreases) in specific enzymes that would allow a differentiation of one disease entity from another have not been demonstrable.

Enzymes are released into the synovial fluid from damaged tissue during phagocytosis and from the general circulation about the inflamed joint. Although their diagnostic significance is not great, the role of enzymes play in destruction of articular tissue is important. Of particular importance are the collagenases, which specifically break down collagen of articular cartilage. Collagenases have been found in various inflammatory synovial fluids and especially in synovial fluid of animals with rheumatoid arthritis, where they are produced by the synovial membrane cells and by the polymorphonuclear leukocytes. (9) These enzymes probably account for the invasive properties of rheumatoid pannus.

Lactate dehydrogenase isoenzymes in synovial fluid have been studied in horses, and differences between normal and diseased joints have been found. (20) Apparently little work has been done to date on enzyme levels in canine and feline synovial fluid. Until such work is done, the presence of these substances in the synovial fluid of diseased joints in these animals can only be assumed.

**PROSTAGLANDINS**

Prostaglandins are a family of compounds comprising 20 carbon aliphatic unsaturated fatty acids. (26) Although first described in 1933, it is only recently that their physiological significance has begun to be appreciated. In relation to articular disease, it is now known that prostaglandin E2 (PGE-2) plays a significant role in aiding or promoting the tissue destruction of rheumatoid arthritis. (25, 26) Synovial fluid levels of prostaglandins in human rheumatoid arthritis patients are elevated far beyond those in normal joint fluid or joint fluid from patients with osteoarthritis. In vitro studies have shown increased prostaglandin synthesis by rheumatoid synovium and the promotion of bone resorption and articular cartilage destruction by prostaglandins. (27)

To date, data are not available describing prostaglandin levels in synovial fluid from a large number of normal dogs or cats or in those with disease. However, increased amounts of prostaglandins have been found in the joints of dogs injected with known inflammation producing substances, suggesting that, as in humans, synovium of the canine joint produces prostaglandins when inflamed. (10) Prostaglandin levels of synovial fluid have no great diagnostic significance as yet, but they may prove to be valuable in differentiating early immune-mediated synovitis from the synovitis of osteoarthritis.

**CRYSTALS**

Crystals are present in the synovial fluid of human patients with gout, and their detection aids significantly in establishing a diagnosis. (3) Synovial fluid cholesterol crystals have also been found. Repeated intra-articular injections of corticosteroids are also known to cause crystal-induced synovitis. Crystals may be detected in the synovial fluid by the use of wet mount preparations and water-soluble stains and regular light microscopy. However, phase microscopy or polarized light microscopy are better for crystal identification. (3)

While naturally occurring crystal-induced synovitis has not been reported in the dog or cat, synovitis due to intra-articular crystal injections is known to occur in dogs. (5) A case of pseudogout has been reported in the dog; however, synovial fluid analysis was not performed. (6)

Although intra-articular crystal formation is apparently not common in the dog or cat, the clinician should be aware of the
Pathologic Changes Of Articular Tissues

Synovial fluid from diseased joints often serves as a mirror reflecting the pathologic changes of synovial membrane and articular cartilage. Based on its pathologic changes, synovial fluid can be classified as belonging to one of several broad, yet differing, groups. There is often overlap between the characteristics of one group and another.

Classification has been based on characteristics of the examined fluid or on etiology of the articular disease. A classification for the arthropathies of the dog has been proposed that divides articular disease into two main categories, inflammatory and noninflammatory. This classification system, based on etiology mechanisms, is valuable clinically in evaluating arthritic conditions but is not consistent with the histopathologic findings of affected articular cartilage, synovial membrane, or the alterations found in synovial fluid.

A classification system of articular diseases and synovial fluid that adheres more closely to the pathologic findings of diseased articular tissue has been reported. This system has three main categories: type 1, normal; type 2, inflammatory nonpurulent; and type 3, inflammatory purulent. The inflammatory nonpurulent category encompasses those conditions that previously had been classified as noninflammatory and includes degenerative joint disease (osteoarthritis) and traumatic and neoplastic joint disease. The inflammatory purulent category includes two main subcategories, infectious and noninfectious.

Inflammatory Nonpurulent Synovial Fluid
Inflammatory nonpurulent synovial fluid is generally characterized by a mild to moderate increase in cellularity, mainly monocytes and lymphocytes, and, depending on cause, increases in erythrocytes, variable volume changes, and changes in viscosity. The mucin clot test is usually normal. Turbidity and color will be changed in relation to the presence of cells, cell types, and hemorrhage.

Degenerative Joint Disease
is a broadly descriptive category encompassing many etiologic factors, all of which ultimately produce what appear to be similar changes of articular cartilage and synovial membrane. Degenerative joint disease, also called osteoarthritis, is a chronic condition characterized by fibrillation of articular cartilage, hypertrophy of synovial membrane, and in some cases sclerosis of subchondral bone and osteophyte formation. Common conditions leading to degenerative joint disease include osteochondritis dissecans, anterior cruciate ligament rupture, ununited anconeal process, articular fractures, and hip dysplasia.

In many cases of degenerative joint disease, synovial fluid analysis will be essentially normal. However, in the great majority of cases the findings, particularly those seen on cytologic examination, will confirm a low-grade but ongoing inflammatory process. Total white cell counts rarely exceed 5000 cells/mm3. The predominant cell types are lymphocytes and monocyte-macrophages. On close examination phagocytic vacuoles will be seen within the macrophages. In some cases intracellular accumulation of debris such as cartilage fragments may be seen (Figs. 86-7 and 86-8).

The synovial fluid from joints with degenerative joint disease is usually clear, although haziness or flocculence due to cells and cartilaginous debris may be found. The fluid is usually pale yellow to straw-colored.

Unless significant joint effusion is present, viscosity is normal, and the mucin clot is usually rated fair to good. Protein and glucose values usually remain unchanged, although a slight reduction in glucose can occur in fluids with increased cellularity due to glycolytic activity of the cells. Low glucose values must be interpreted in light of any delays in synovial fluid analysis.

Although enzyme and prostaglandin levels of canine and feline synovial fluid have not been studied extensively as yet, in other species (e.g., human, horse) enzyme levels of synovial fluid from joints with degenerative joint disease are usually within normal ranges. Certain isoenzymes of lactic dehydrogenase have been found to be increased in both humans and horses. Prostaglandin levels (PGE-1) are usually normal or only slightly increased in humans with degenerative joint disease.

![FIG. 86-7 Synovial fluid of secondary degenerative joint disease. Mononuclear cells of the macrophage-monocyte type indicate reactivity within the joint. (original magnification, x 125)]
TRAUMATIC JOINT DISEASE

The finding of blood components in synovial fluid is the significant change from normal in traumatic joint disease, especially in the acute situation. (20) However, extensive hemorrhage is not always present in the traumatized joint. Acute intra-articular ligamentous rupture will usually produce small amounts of intra-articular hemorrhage and a synovial fluid that is serosanguineous in appearance, while acute intra-articular fractures almost always produce frankly bloody synovial fluid. Traumatic joint disease (i.e., ruptured anterior cruciate ligament) will often progress to more chronic states, and the results of later synovial fluid analysis will be similar to those of degenerative joint disease (Fig. 86-9).

In traumatic joint disease the synovial fluid volume may be increased, and unless the effusion is extensive, viscosity usually remains normal. Mucin clot quality is most often classified as good.

Protein levels are often increased in bloody fluids, and the fluid will clot owing to the presence of coagulation factors.

White blood cell counts usually do not exceed 5,000/mm³, with lymphocytes and monocytes being the predominant cell types. (20) The monocyte-macrophage type cell often displays phagocytic vacuoles. Neutrophils rarely account for more than 25% of the total leukocyte count. (20)

Fat droplets within the synovial fluid and synovial fluid leukocytes in the presence of hemorrhagic synovial fluid have been reported as an aid in establishing a diagnosis of acute traumatic arthritis. (7) It is speculated that fat from bone marrow and subchondral bone enters the joint secondary to profound cartilage or ligamentous injury and that intra-articular lipid droplet phagocytosis may be a stimulus for a profound inflammatory arthritis associated with traumatic arthritis.

FIG. 86-8 Synovial fluid of secondary degenerative joint disease. Multinucleated cells, found in concentrated samples, may indicate cartilaginous damage. (original magnification x 200)

FIG. 86-9 Synovial fluid from the elbow joint of a dog with a 3 day-old Salter type IV fracture of the distal humerus. Many erythrocytes are present, in addition to occasional neutrophils and macrophage-monocyte-type cells. (original magnification, x 125)

Inflammatory Purulent Synovial Fluid

The classification of purulent synovial fluid may be subdivided into two main categories, infectious and noninfectious. (20) Bacterial, fungal, mycoplasmal, viral, rickettsial, and protozoal organisms have been incriminated as agents of infectious articular disease in humans and in several species of animals. (16,19) In the dog, bacterial infections are probably most common. Recent reports suggest that a chronic progressive polyarthritis in the cat may be due to viral infection. (18)

Thenoninfectious purulent category includes those conditions thought to be immune-mediated such as systemic lupus erythematosus and rheumatoid arthritis, as well as the changes produced by intra-articular deposition of crystals. (20) Synovial fluid changes associated with intra-articular neoplasia are not well described in animals. It has been suggested that synovial fluid would generally be of an inflammatory nonpurulent nature. (20) However, in humans periarticular malignant bone tumors produce in the synovial fluid a definitely purulent change such that the synovial fluid contains many neutrophils and a mild to moderate synovitis characterized by plasma cell, lymphocyte, and neutrophilic invasion. (11)

INFECTIONOUS

Synovial fluid of septic (bacterial) arthritis is generally characterized by a leukocyte count in excess of 5,000 cells/mm³, at least half of which are neutrophils. (20) Counts as high as 100,000 cells/mm³ have been reported. (16) As a result of the high cellularity, the clarity and color of the fluid are altered. Color may vary from yellow gray to creamy. The fluid is most often opaque or turbid. Increased numbers of phagocytes are also present, and variable numbers of erythrocytes may be seen. Depending upon the type of bacterium and the degree of joint effusion, viscosity and muffin clot will be altered. In general, the mucin clot test is rated fair. This may be due to the production of hyaluronidase by the offending organism and subsequent breakdown of hyaluronic acid. Viscosity is usually decreased. Glucose levels are often decreased and are commonly less than one half of the corresponding blood level. This is due primarily to glucose utilization by the invading bacteria. Paired blood and synovial fluid glucose determinations should be performed. Synovial fluid protein levels are often increased, in some
cases to twice normal values. This is probably due to leakage through the vessels of the highly inflamed synovial membrane.

The degree of synovial fluid change and the nature of the inflammatory exudate of septic arthritis are due mainly to the type of offending bacterium. Aerobic and anaerobic cultures of synovial fluid should be made. In some cases, however, bacteria will be identified only by careful cytologic examination of synovial fluid smears. Gram stains of well-prepared smears are valuable in this regard. As mentioned, proper aseptic handling of arthrocenteses samples is important to avoid sample contamination.

Bacteria may gain direct entrance to a joint by trauma, arthrocentesis, or at the time of surgery. However, any systemic infection may localize in the synovial membrane and produce a septic arthritis such as that occasionally seen with bacterial endocarditis. It has also been noted that systemic infections can produce a sterile arthritis resulting from immune-mediated mechanisms. Anonerosive immune-mediated arthritis has been reported in dogs with a variety of chronic bacterial disease processes. (16, 19) The findings of synovial fluid examination and analysis in such cases are often similar to those of other immune-mediated arthritides. It has also been noted that unless bacteria can be identified in the synovial fluid or synovial membrane, synovial fluid analysis may be unable to distinguish between septic arthritis and immune-mediated arthritis. (20)

Feline chronic progressive polyarthritis has been linked etiologically with feline leukemia virus and feline syncytia-forming virus. (18) Synovial fluid leukocytosis is a common feature in this disease, with counts ranging from 4,000 cells to 70,000 cells or more/mm³. Polymorphonuclear neutrophils may account for between 25% and 99% of the cells, with the remainder being large and small mononuclear cells.

As the name suggests, feline chronic progressive polyarthritis affects many joints simultaneously. Generally speaking, septic arthritis usually affects only one joint. However, those arthritides that develop subsequent to primary infections elsewhere in the body may be seen in several joints, such as in the case of articular disease associated with bacterial endocarditis. Immune-mediated arthropathies usually affect two or more joints simultaneously.

**NONINFECTIOUS.**

The cardinal features of noninfectious purulent synovial fluid are the great number of neutrophils and the failure to establish an infectious etiology for the disease process. (20) The immune-mediated articular diseases, rheumatoid arthritis and systemic lupus erythematosus, are the primary conditions in this classification. While crystal-induced polyarthropathy (i.e., gout) may also be placed into this group, its occurrence in domestic animals is unknown. (20) Also, as mentioned above, articular disease associated with neoplastic processes may be placed in this group as well.

In general, the immune-mediated arthropathies affect two or more joints. The appearance of the synovial fluid will vary from normal to an obvious purulent exudate. Appearance and findings will be affected to some extent by the duration and degree of joint injury, the presence of superimposed degenerative joint disease changes, and the erosive or nonerosive character of the disease.

The erosive type of immune-mediated articular disease is one in which the articular cartilage is being destroyed, thus releasing articular cartilage fragments and subchondral bone material onto the synovial fluid. The erosive type of immune-mediated arthropathy has been likened to rheumatoid arthritis of humans. (15, 17) In contrast, the nonerosive immune-mediated arthropathy there is not extensive articular cartilage destruction; this condition is similar to the arthropathy often seen in humans with systemic lupus erythematosus. (19)

Synovial fluid color will vary depending upon the type and number of cellular constituents. Large numbers of erythrocytes impart a serosanguineous color to the fluid. White blood cell counts in excess of 5000/mm³ are usually found and may range up to several thousand with at least 50% of the cells being neutrophils. (20) Phagocytic cells are often found, some of which may contain debris. Scavenged deoxyribonucleic acid (DNA) has been seen in phagocytic cells from synovial fluid of dogs with systemic lupus erythematosus, and typical LE cells have also been identified. Lymphocytes, monocytes, and macrophage-type cells predominate in fluids of low cellularity (Fig. 86-10).

Synovial fluid viscosity is usually decreased, and the mucin clot is rated as fair to poor in the immune-mediated articular diseases. While extensive studies of synovial fluid complement have not been performed on the dog or cat, in humans with immunearticular disease levels are usually decreased. Protein, glucose, and enzyme levels are frequently altered as well.

![FIG. 86-10 Synovial fluid of immune-mediated articular disease in which neutrophils are the predominant cells. (original magnification, x200)](image-url)
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