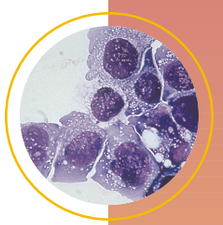
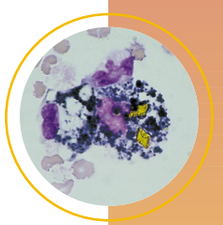
CHAPTER 12





Synovial Fluid

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

1. Describe the formation and function of synovial fluid.
2. Relate laboratory test results to the four common classifications of joint disorders.
3. Determine the appropriate collection tubes for requested laboratory tests on synovial fluid.
4. Describe the appearance of synovial fluid in normal and abnormal states.
5. Discuss the normal and abnormal cellular composition of synovial fluid.
6. List and describe six crystals found in synovial fluid.
7. Explain the differentiation of monosodium urate and calcium pyrophosphate crystals using polarized and compensated polarized light.
8. State the clinical significance of glucose and lactate tests on synovial fluid.
9. List four genera of bacteria most frequently found in synovial fluid.
10. Describe the relationship of serologic testing of serum to joint disorders.

KEY TERMS

arthritis
arthrocentesis
hyaluronic acid
synovial fluid

Physiology

Synovial fluid, often referred to as "joint fluid," is a viscous liquid found in the cavities of the movable joints (**diarthroses**) or synovial joints. As shown in Figure 12–1, the bones in the synovial joints are lined with articular cartilage and separated by a cavity containing the synovial fluid. The smooth articular cartilage and synovial fluid reduce friction between the bones during joint movement. In addition to providing lubrication in the joints, synovial fluid provides nutrients to the articular cartilage and lessens the shock of joint compression occurring during activities such as walking and jogging.

Synovial fluid is formed as an ultrafiltrate of plasma across the synovial membrane. The filtration is nonselective except for the exclusion of high molecular weight proteins. Therefore, the majority of the chemical constituents have concentrations similar to plasma values. Cells lining the synovial membrane (**synoviocytes**) secrete a mucopolysaccharide containing ***hyaluronic acid*** and a small amount of protein into the fluid. This substance causes the noticeable viscosity of synovial fluid. Normal values for synovial fluid analysis are shown in Table 12–1.

Damage to the articular membranes produces pain and stiffness in the joints, collectively referred to as ***arthritis***. A variety of conditions including infection, inflammation, metabolic disorders, trauma, physical stress, and advanced age are associated with arthritis. Laboratory results of synovial fluid analysis can be used to determine the pathologic origin of arthritis. Disorders are frequently classified into four groups, as shown in Table 12–2. Some overlap of test results among the groups may occur (Table 12–3), and the patient's clinical history must also be considered when assigning a category. The most frequently performed tests are the WBC count, differential, Gram stain, culture, and polarized microscopy examination for crystals.[9]

TABLE 12–1 **Normal Synovial Fluid Values**[10]

Volume	<3.5 mL
Color	Pale yellow
Clarity	Clear
Viscosity	Able to form a string 4–6 cm long
Erythrocyte count	<2000 cells/μL
Leukocyte count	<200 cells/μL
Neutrophils	<20% of the differential
Lymphocytes	<15% of the differential
Monocytes and macrophages	65% of the differential
Crystals	None present
Glucose	<10 mg/dL lower than the blood glucose
Lactate	<250 mg/dL
Total protein	<3 g/dL
Uric acid	Equal to blood value

Specimen Collection and Handling

Synovial fluid is collected by needle aspiration called ***arthrocentesis***. The amount of fluid present will vary with the size of the joint and the degree of fluid buildup in the joint. For example, the normal amount of fluid in the large knee cavity is less than 3.5 mL, but can increase to greater than 25 mL with inflammation. In some instances, only a few drops of fluid are obtained, but these can still be used for microscopic analysis or culturing. The volume of fluid collected should be recorded.

Normal synovial fluid does not clot; however, fluid from a diseased joint may contain fibrinogen and will clot. Therefore, fluid is usually collected in a syringe that has been moistened with heparin. When sufficient fluid is collected, it should be distributed into three tubes—a sterile

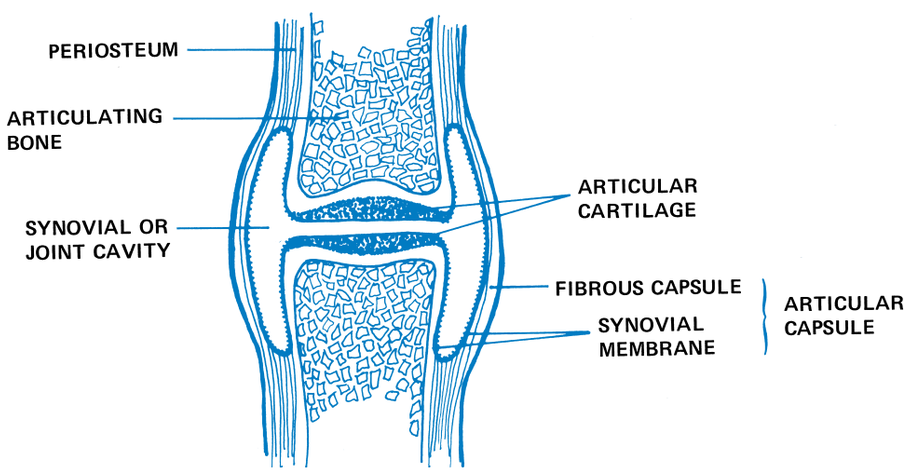


FIGURE 12–1 Diagram of a synovial joint.

TABLE 12–2 **Classification and Pathologic Significance of Joint Disorders**

Group Classification	Pathologic Significance
I. Noninflammatory	Degenerative joint disorders
II. Inflammatory	Immunologic problems, including rheumatoid arthritis and lupus erythematosus
	Crystal-induced gout and pseudogout
III. Septic	Microbial infection
IV. Hemorrhagic	Traumatic injury
	Coagulation deficiencies

heparinized tube for the microbiology laboratory, a liquid ethylenediaminetetraacetic acid (**EDTA**) tube for the hematology laboratory, and a nonanticoagulated tube for other tests. Powdered anticoagulants should not be used because they may produce artifacts that will interfere with crystal analysis. The nonanticoagulated tube must be centrifuged and separated to prevent cellular elements from interfering with chemical and serologic analyses. Ideally, all testing should be done as soon as possible to prevent cellular lysis and possible changes in crystals.

TABLE 12–3 **Laboratory Findings in Joint Disorders**[7,10]

Group Classification	Laboratory Findings
I. Noninflammatory	Clear, yellow fluid
	Good viscosity
	WBCs <2000 µL
	Neutrophils <30%
	Normal glucose (similar to blood glucose)
II. Inflammatory (immunologic origin)	Cloudy, yellow fluid
	Poor viscosity
	WBCs 2000–5000 µL
	Neutrophils >50%
	Decreased glucose level
	Possible autoantibodies present
(crystal-induced origin)	Cloudy or milky fluid
	Poor viscosity
	WBCs up to 50,000 µL
	Neutrophils <90%
	Decreased glucose level
	Elevated uric acid level
	Crystals present
III. Septic	Cloudy, yellow-green fluid
	Poor viscosity
	WBCs 10,000–200,000 µL
	Neutrophils >90%
	Decreased glucose level
	Positive culture and Gram stain
IV. Hemorrhagic	Cloudy, red fluid
	Poor viscosity
	WBCs <5000 µL
	Neutrophils <50%
	Normal glucose level
	RBCs present

Appearance and Viscosity

A report of the gross appearance is an essential part of the synovial fluid analysis.[4] Normal synovial fluid appears clear and pale yellow. The color often becomes a deeper yellow in the presence of inflammation and may have a greenish tinge with bacterial infection. As with cerebrospinal fluid, in synovial fluid the presence of blood from a hemorrhagic arthritis must be distinguished from blood from a traumatic aspiration. This is accomplished primarily by observing the uneven distribution of blood in the specimens obtained from a traumatic aspiration.

Turbidity is frequently associated with the presence of WBCs; however, synovial cell debris and fibrin also produce turbidity. The fluid may appear milky when crystals are present.

Viscosity of the synovial fluid comes from the polymerization of the hyaluronic acid and is essential for the proper lubrication of the joints. Arthritis affects both the production of hyaluronate and its ability to polymerize, thus decreasing the viscosity of the fluid. Several methods are available to measure the viscosity of the fluid, the simplest being to observe the ability of the fluid to form a string from the tip of a syringe, and can be done at the bedside. A string that measures 4 to 6 cm is considered normal.

Measurement of the degree of hyaluronate polymerization can be performed using a Ropes, or mucin clot, test. When added to a solution of 2 to 5 percent acetic acid, normal synovial fluid will form a solid clot surrounded by clear fluid. As the ability of the hyaluronate to polymerize decreases, the clot becomes less firm, and the surrounding fluid increases in turbidity. The mucin clot test is reported in terms of good (solid clot), fair (soft clot), poor (friable clot), and very poor (no clot). The mucin clot test is not routinely performed, because all forms of arthritis decrease viscosity and little diagnostic information is obtained. Formation of a mucin clot following the addition of acetic acid can be used to identify a questionable fluid as synovial fluid.

Cell Counts

The total leukocyte count is the most frequently performed cell count on synovial fluid. However, red blood cell counts may be requested unless evidence of a traumatic tap exists. To prevent cellular disintegration, counts should be performed as soon as possible or the specimen should be refrigerated. Very viscous fluid may need to be pretreated by adding a pinch of hyaluronidase to 0.5 mL of fluid or one drop of 0.05 percent hyaluronidase in phosphate buffer per milliliter of fluid and incubating at 37°C for 5 minutes.[5]

Manual counts on thoroughly mixed specimens are done using the Neubauer counting chamber in the same manner as cerebrospinal fluid counts. Clear fluids can usually be counted undiluted, but dilutions are necessary when

fluids are turbid or bloody. Dilutions can be made using the procedure presented in Chapter 10; however, traditional WBC diluting fluid cannot be used because it contains acetic acid, which will cause the formation of mucin clots. Normal saline can be used as a diluent. If it is necessary to lyse the RBCs, hypotonic saline (0.3 percent) or saline that contains saponin is a suitable diluent. Methylene blue added to the normal saline will stain the WBC nuclei, permitting separation of the RBCs and WBCs during counts performed on mixed specimens. Automated cell counters can be used for synovial fluid counts; however, highly viscous fluid may block the apertures, and the presence of debris and tissue cells may falsely elevate counts. As described previously, incubation of the fluid with hyaluronidase will decrease the specimen viscosity. Analysis of scattergrams can aid in the detection of tissue cells and debris. Properly controlled automated counts provide higher precision than manual counts.[8]

WBC counts less than 200 cells/μL are considered normal and may reach 100,000 cells/μL or higher in severe infections.[6] There is, however, considerable overlap of elevated leukocyte counts between septic and inflammatory forms of arthritis.

Differential Count

Differential counts should be performed on cytocentrifuged preparations or on thinly smeared slides. Fluid should be incubated with hyaluronidase prior to slide preparation. Mononuclear cells, including monocytes, macrophages, and synovial tissue cells, are the primary cells seen in normal synovial fluid. Neutrophils should account for less than 20 percent of the differential count and lymphocytes less than 15 percent. Increased neutrophils indicate a septic condition, whereas an elevated cell count with a predominance of lymphocytes suggests a nonseptic inflammation. In both normal and abnormal specimens, cells may appear more vacuolated than they do on a blood smear.[5] Besides increased numbers of these usually normal cells, other cell abnormalities include the presence of eosinophils, LE cells, **Reiter cells** (vacuolated macrophages with ingested neutrophils), and RA cells or **ragocytes** (neutrophils with small, dark, cytoplasmic granules that consist of precipitated rheumatoid factor).[1] Lipid droplets may be present following crush injuries, and hemosiderin granules are seen in cases of **pigmented villonodular synovitis**. The most frequently encountered cells and inclusions seen in synovial fluid are summarized in Table 12–4.

Crystal Identification

Microscopic examination of synovial fluid for the presence of crystals is an important diagnostic test in the evaluation of arthritis. Crystal formation in a joint frequently results in an acute, painful inflammation. Causes of crystal formation include metabolic disorders and decreased renal excretion that produce elevated blood levels of crystallizing chemicals, degeneration of cartilage and bone, and injection of medications, such as corticosteroids into a joint.

TABLE 12–4 **Cells and Inclusions Seen in Synovial Fluid**

Cell/Inclusion	Description	Significance
Neutrophil	Polymorphonuclear leukocyte	Bacterial sepsis Crystal-induced inflammation
Lymphocyte	Mononuclear leukocyte	Nonseptic inflammation
Macrophage (monocyte)	Large mononuclear leukocyte, may be vacuolated	Normal Viral infections
Synovial lining cell	Similar to macrophage, but may be multinucleated, resembling a mesothelial cell	Normal
LE cell	Neutrophil containing characteristic ingested: "round body"	Lupus erythematosus
Reiter cell	Vacuolated macrophage with ingested neutrophils	Reiter's syndrome Nonspecific inflamation
RA cell (ragocyte)	Neutrophil with dark cytoplasmic granules containing immune complexes	Rheumatoid arthritis Immunologic inflammation
Cartilage cells	Large, multinucleated cells	Osteoarthritis
Rice bodies	Macroscopically resemble polished rice Microscopically show collagen and fibrin	Tuberculosis, septic and rheumatoid arthritis
Fat droplets	Refractile intracellular and extracellular globules Stain with Sudan dyes	Traumatic injury
Hemosiderin	Inclusions within clusters of synovial cells	Pigmented villonodular synovitis

The primary crystals seen in synovial fluid are monosodium urate (uric acid) (**MSU**) found in cases of gout and calcium pyrophosphate (**CPPD**) seen with pseudogout. Increased serum uric acid resulting from impaired metabolism of nucleic acid associated with myeloproliferative disorders and decreased renal excretion of uric acid are the most frequent causes of gout. Pseudogout is most often associated with degenerative arthritis, resulting in cartilage calcification and endocrine disorders producing elevated serum calcium levels.

Additional crystals that may be present include hydroxyapatite (basic calcium phosphate) associated with calcified cartilage degeneration, cholesterol crystals, corticosteroids following injections, and calcium oxalate crystals in renal dialysis patients. The patient history must always be considered. Microscopic characteristics of the commonly encountered crystals are presented in Table 12–5. Artifacts present may include talc and starch from gloves, precipitated anticoagulants, dust, and scratches on slides and coverslips. Slides and coverslips should be examined and cleaned prior to use.

Ideally crystal examination should be performed soon after fluid collection to ensure that crystals are not affected by changes in temperature and pH. Both MSU and CPPD crystals are reported as being located extracellularly and intracellularly (within neutrophils); therefore, fluid must be examined prior to WBC disintegration.

Fluid is examined unstained under polarized and compensated polarized light for detection and identification of MSU and CPPD crystals. Crystals may be observed in Wright's stained preparations (Figure 12–2); however, this should not replace the wet preparation examination. Slides are first scanned under low power with direct polarized light, then focused using high power, and finally examined under compensated polarized light. MSU crystals are seen routinely as needle-shaped crystals, appearing extracellularly and within the cytoplasm of neutrophils (Figures 12–3 through 12–5). CPPD crystals usually appear rhombic-shaped (Figures 12–6 and 12–7) and are found as intracellular inclusions.

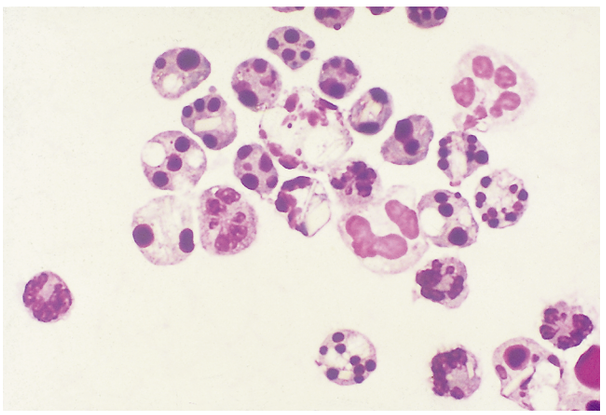
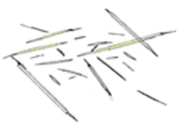
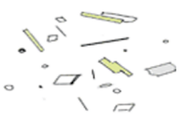
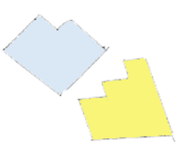


FIGURE 12–2 Wright's stained neutrophils containing CPPD crystals (×1000).

Once the presence of the crystals has been determined using direct polarization, positive identification is made using compensated polarized light. A control slide for the

TABLE 12–5 **Characteristics of Synovial Fluid Crystals**

Crystal	Shape		Compensated Polarized Light	Location
Monosodium urate	Needles		Negative birefringence	Intracellular and extracellular
Calcium pyrophosphate	Rod Needles Rhombics		Positive birefringence	Intracellular and extracellular
Cholesterol	Notched rhombic plates		Negative birefringence	Extracellular
Corticosteroid	Flat, variable-shaped plates		Positive and negative birefringence	Primarily intracellular

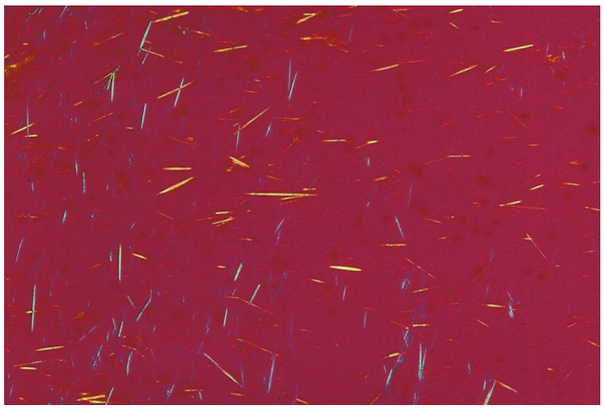


FIGURE 12–3 Extracellular MSU crystals under compensated polarized light: Notice the change in color with crystal alignment (×100).

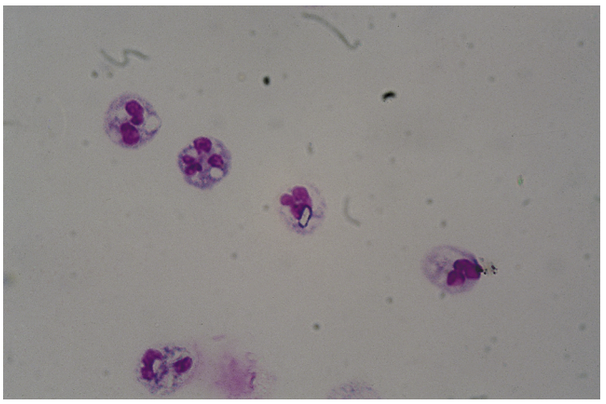


FIGURE 12–6 Weakly birefringent CPPD crystals under polarized light (×1000).

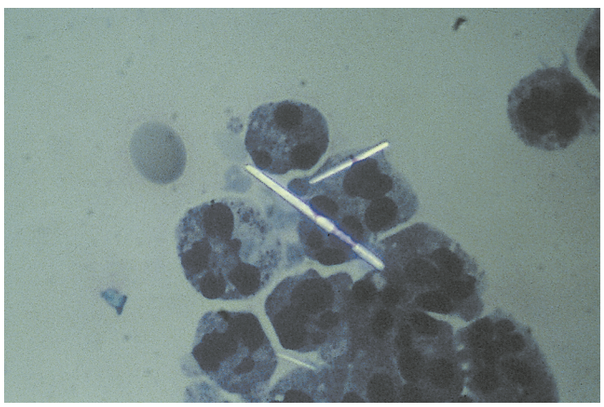


FIGURE 12–4 Highly birefringent MSU crystals under polarized light (×500).

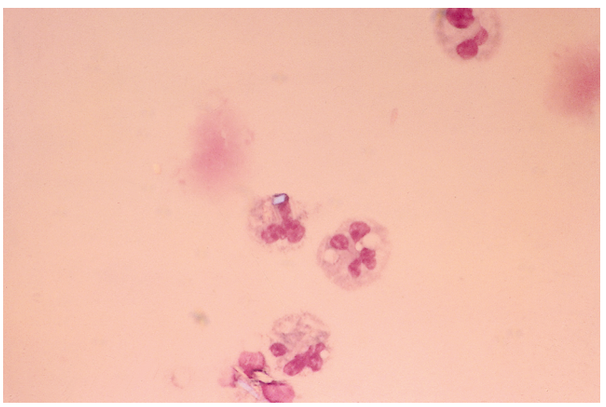


FIGURE 12–7 CPPD crystals under compensated polarized light, the blue crystal is aligned with the slow vibration (×1000).

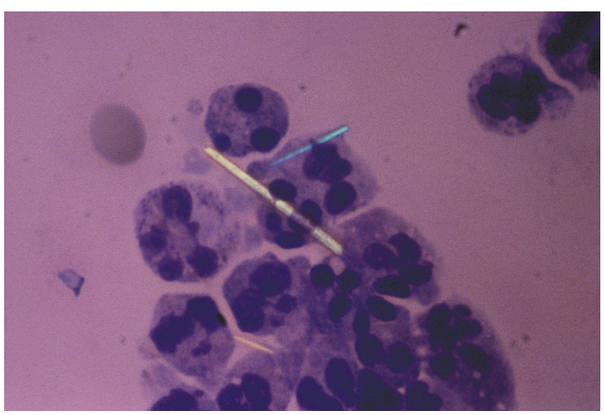


FIGURE 12–5 MSU crystals under compensated polarized light, the yellow crystal is aligned with the slow vibration (×500).

polarization properties of MSU can be prepared using betamethasone acetate corticosteroid.

Both MSU and CPPD crystals have the ability to polarize light as discussed in Chapter 6; however, MSU is more hightly birefringent and will appear brighter against the dark background.

When compensated polarized light is used, a red compensator crystal is placed in the microscope between the crystal and the analyzer (Figure 12–8). The compensator separates the light ray into slow-moving and fast-moving vibrations and produces a red background.

Owing to differences in the linear structure of the molecules in MSU and CPPD crystals, the color produced by each crystal when it is aligned with the slow vibration can be used to identify the crystal. The molecules in MSU crystals run parallel to the long axis of the crystal and, when aligned with the slow vibration, the velocity of the slow light passing through the crystal is not impeded as

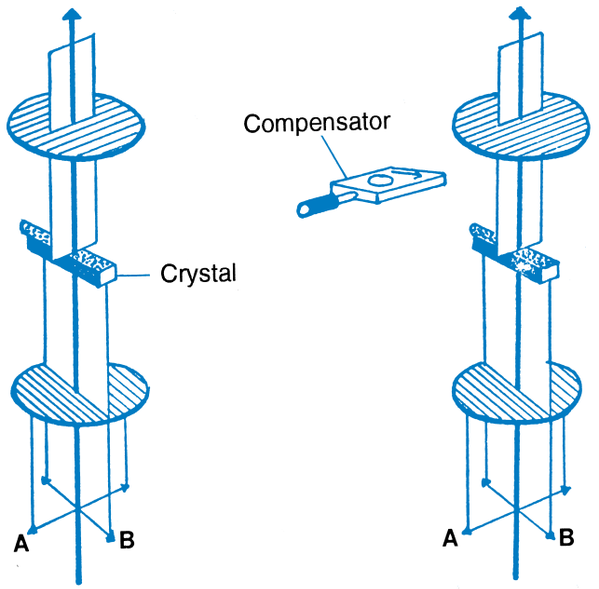


FIGURE 12–8 *(Left)* Direct polarized light. *(Right)* Compensated polarized light. (Adapted from Phelps, Steele, and McCarty: Compensated polarized light microscopy: Identification of crystals in synovial fluid from gout and pseudogout. JAMA 203(7):167, 1969.)

much as the fast light, which runs against the grain and produces a yellow color. This is considered negative birefringence (subtraction of velocity from the fast ray). In contrast, the molecules in CPPD crystals run perpendicular to the long axis of the crystal and when aligned with the slow axis of the compensator, the velocity of the fast light passing through the crystal is much quicker, producing a blue color and positive birefringence.[2] Care must be taken to ensure crystals being analyzed are aligned in accordance with the compensator axis. Notice how the colors of the MSU crystals in Figure 12–9 vary with the alignment.

Crystal shapes and patterns of birefringence that vary from the standard MSU and CPPD patterns may indicate the presence of one of the less commonly encountered crystals and further investigation is required.

Chemistry Tests

Because synovial fluid is chemically an ultrafiltrate of plasma, chemistry test values are approximately the same as serum values. Therefore, few chemistry tests are considered clinically important. The most frequently requested test is the glucose determination, because markedly decreased values are indicative of inflammatory (group II) or septic (group III) disorders. Because normal synovial fluid glucose values are based on the blood glucose level, simultaneous blood and synovial fluid samples should be obtained, preferably after the patient has fasted for 8 hours to allow equilibration between the two fluids. Under these conditions, normal synovial fluid glucose should not be more than 10 mg/dL lower than the blood value. To prevent falsely decreased values caused by glycolysis, specimens should be analyzed within 1 hour or preserved with sodium fluoride.

Measurement of synovial fluid lactate levels has been shown to provide rapid differentiation between inflammatory and septic arthritis and does not require equilibration and comparison with blood lactate levels. Synovial fluid lactate levels greater than 250 mg/dL are found consistently with septic arthritis, but may also be seen in rheumatoid arthritis.[3,10]

Other chemistry tests that may be requested are the total protein and uric acid determinations. Because the large protein molecules are not filtered through the synovial membranes, normal synovial fluid contains less than 3 g/dL of protein (approximately one-third of the serum value). Increased levels are found in inflammatory and hemorrhagic disorders; however, measurement of synovial fluid protein does not contribute greatly to the classification of these disorders. When requested, the analysis is performed using the same methods used for serum protein determinations. The elevation of serum uric acid in cases of gout is well known; therefore, demonstration of an elevated synovial fluid uric acid level may be used to confirm the diagnosis when the presence of crystals cannot be demonstrated in the fluid.

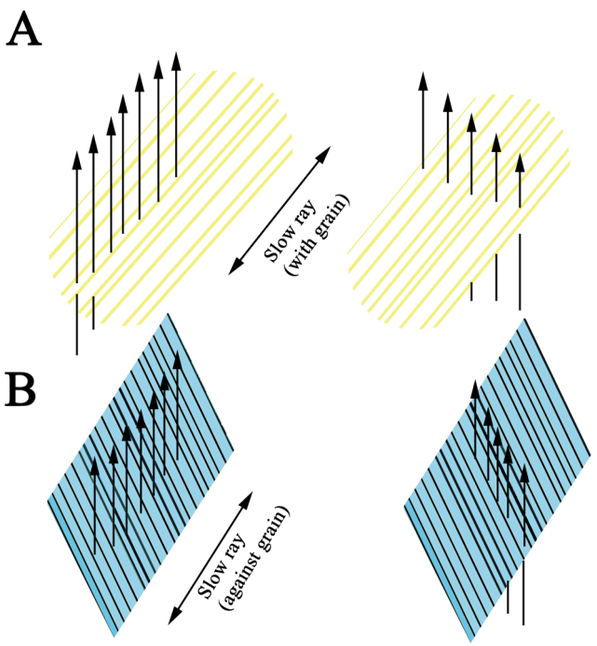


FIGURE 12–9 Diagram of negative and positive birefringence in MSU and CPPD crystals. *(A)* MSU crystal with grain running parallel to the long axis. The slow ray passes with the grain producing negative (yellow) birefringence. *(B)* CPPD crystal with grain running perpendicular to the long axis. The slow ray passes against the grain and is retarded producing positive (blue) birefringence.

Microbiologic Tests

An infection may occur as a secondary complication of inflammation; therefore, Gram stains and cultures are two of the most important tests performed on synovial fluid. Gram stains should be performed on all specimens. Bacterial infections are most frequently seen; however, fungal, tubercular, and viral infections also can occur. When they are suspected, special culturing procedures should be used. Routine bacterial cultures should always include an enrichment medium, such as chocolate agar, because in addition to *Staphylococcus* and *Streptococcus*, the most common organisms that infect synovial fluid are the fastidious *Hemophilus* species and *Neisseria gonorrhoeae*.

Serologic Tests

Because of the association of the immune system to the inflammation process, serologic testing plays an important role in the diagnosis of joint disorders. However, the majority of the tests are performed on serum, with actual analysis of the synovial fluid serving as a confirmatory measure in cases that are difficult to diagnose. The autoimmune diseases rheumatoid arthritis and lupus erythematosus cause very serious inflammation of the joints and are diagnosed in the serology laboratory by demonstrating the presence of their particular autoantibodies in the patient's serum. These same antibodies can also be demonstrated in the synovial fluid, if necessary. Arthritis is a frequent complication of Lyme disease. Therefore, demonstration of antibodies to the causative agent *Borrelia burgdorferi* in the patient's serum can confirm the cause of the arthritis.

REFERENCES

1. Broderick, PA, et al: Exfoliative cytology: Interpretation of synovial fluid in disease. J Bone Joint Surg Am 58(3):396–399, 1976.
2. Cornbleet, PJ: Synovial fluid crystal analysis. Lab Med 28(12): 774–779, 1997.
3. Gratacos, J, et al: d-lactic acid in synovial fluid: A rapid diagnostic test for bacterial synovitis. J Rheumatol 22(8):1504–1508, 1995.
4. Hasselbacher, P: Variation in synovial fluid analysis by hospital laboratories. Arthritis Rheum 30(6):637–642, 1987.
5. Kjeldsberg, CR, and Knight, JA: Body Fluids: Laboratory Examination of Amniotic, Cerebrospinal, Seminal, Serous and Synovial Fluids: A Textbook Atlas. ASCP, Chicago, 1993.
6. Naib, ZM: Cytology of synovial fluids. Acta Cytol 17(4):299–309, 1973.
7. Rippey, J: Synovial fluid analysis. Lab Med 10(3):140–145, 1979.
8. Salinas, M, et al: Comparison of manual and automated cell counts in EDTA preserved synovial fluids. Am Rheum Dis 56(10):622–626, 1997.
9. Shmerling, RH: Synovial fluid analysis. A critical reappraisal. Rheum Dis Clin North Am 20(2):503–512, 1994.
10. Smith, GP, and Kjeldsberg, CR: Cerebrospinal, synovial, and serous body fluids. In Henry, JB (ed): Clinical Diagnosis and Management by Laboratory Methods. WB Saunders, Philadelphia, 1996.

STUDY QUESTIONS

1. State three functions of synovial fluid.
2. List the four classifications of joint disease and a pathologic cause of each.
3. List the five most frequently performed laboratory tests on synovial fluid.
4. What procedure is performed to collect synovial fluid?
5. Why is synovial fluid collected in liquid rather than powdered anticoagulant?
6. What is the clinical significance of the following synovial fluid colors: dark yellow, milky, blood streaked, green?
7. Why is hyaluronic acid an important constituent of synovial fluid? How is its presence determined?
8. How is synovial fluid diluted when performing a WBC count? Why?
9. What is the significance of the following in a synovial fluid differential: increased neutrophils, increased lymphocytes, ragocytes?
10. Name the two primary crystals seen in synovial fluid, and state the pathologic significance of each.
11. Under what conditions might calcium oxalate crystals be seen in synovial fluid? Corticosteroid crystals?
12. What is the recommended slide preparation for the detection and identification of synovial fluid crystals?
13. Under polarized light, do MSU or CPPD crystals appear brighter? Which appears blue when aligned with the slow axis of the red compensator?
14. Are fast light rays more impeded when passing through MSU or CPPD crystals? Why?
15. If an MSU crystal is aligned perpendicular to the slow axis, what color will it be?
16. Why are synovial fluid glucose levels compared with serum glucose levels and synovial fluid protein levels not compared?
17. What is the significance of an elevated synovial fluid lactate level?
18. Why is chocolate agar routinely included in synovial fluid cultures?
19. Name two causes of arthritis in which autoantibodies may be found in the patient's serum.
20. What is the significance of a patient's serum containing antibodies to *Borrelia burgdorferi*?

CASE STUDIES AND CLINICAL SITUATIONS

1. A 50-year-old man presents in the emergency room with severe pain and swelling in the right knee.

Arthrocentesis is performed and 20 mL of milky synovial fluid is collected. The physician orders a STAT Gram stain and crystal examination of the fluid and a serum uric acid. He requests that the fluid be saved for possible additional tests.

a. Describe the tubes into which the fluid would be routinely placed.
b. If the patient's serum uric acid level is elevated, what type of crystals and disorder are probable?
c. Describe the appearance of these crystals under direct and compensated polarized light.
d. Why was the Gram stain ordered?

2. A medical technology student dilutes a synovial fluid prior to performing a WBC count. The fluid forms a clot.

a. Why did the clot form?
b. How can the student perform a correct dilution of the fluid?
c. What two concerns might the student have when performing the count using an automated cell counter? How can they be prevented?

3. Fluid obtained from the knee of an obese 65-year-old woman being evaluated for a possible knee replacement has the following results:

APPEARANCE: Pale yellow and hazy
WBC COUNT: 500 cells/μL
GRAM STAIN: Negative
GLUCOSE: 110 mg/dL (SERUM GLUCOSE: 115 mg/dL)

a. What classification of joint disorder do these results suggest?
b. Under electron microscopy, what crystals might be detected?
c. How does the glucose result aid in the disorder classification?
d. Are the test results consistent with a probable candidate for a knee replacement? Why or why not?