

Recent Advances in Synovial Fluid Analysis*

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Synovial fluid analysis is a frequently ignored examination except in suspected cases of septic conditions. It has been shown that it is an extremely valuable procedure in making rapid and accurate diagnoses in many types of joint diseases.

Table 1 illustrates the findings in ten separate joint states. The gross appearance, "wet-prep" microscopic examination, leukocyte count and sugar content are procedures that are extremely important and can be performed with a paucity of equipment. We have not found the mucin-clot test or protein content to be very helpful. Of course, if infection is a possibility, Gram's stain and appropriate cultures should be instituted. It should be remembered, however, that only 25–30% of the cases of gonococcal arthritis will be associated with a positive Gram's stain or culture. The culture yield may possibly improve with the increasing use of Thayer-Martin media. The anticoagulant should be either heparin or EDTA instead of oxalate because the examiner may confuse oxalate crystals with urate or pyrophosphate crystals.

Cell Counting. The white blood cell count is of particular importance in suspected septic conditions. Methods have been outlined in detail in an excellent book by Ropes and Bauer. The fluid should be collected in an anticoagulant tube. The diluent should be physiologic saline because acetic acid will cause precipitation of mucin and make an accurate count practically impossible. The addition of methylene blue to the diluent will help differentiate the cell types. If the fluid is hemorrhagic, a hypo-

tonic diluent of 0.3% sodium chloride can be tried which theoretically will disrupt the red blood cells but not the nucleated cells.

A differential white count using Wright's stain is performed in the same manner as a peripheral blood smear. If the total white blood count in the synovial fluid is less than 5,000/mm³, it is best to centrifuge the fluid at 2,000–3,000 rpm for ten minutes. The supernatant is then removed and physiologic saline added to the sediment until the original volume is obtained. The solution should then be recentrifuged which will remove most of the mucin. The sediment can then be easily smeared on a slide, air dried, and stained. In cases of systemic lupus erythematosus (SLE), the LE cell may be seen.

Glucose. The glucose value of normal synovial fluid usually parallels that of serum. The results are of greater value if the patient has been fasting for about six hours. It is even possible for normal synovial fluid sugar to be higher than that of the serum for several hours postprandially.

In any suspected septic joint condition, a glucose determination should always be performed. We have found the glucose levels to be low in almost all cases of septic arthritis observed at the Medical College of Virginia during the past seven years. It is important to have the glucose level determined immediately after aspiration. Because of the glycolytic action of white cells, falsely low levels may be observed in cases of rheumatoid arthritis and gout, for example, if the white cell count of the fluid is high and the fluid is not tested for one or more hours. The falsely low sugar level can also be prevented by placing the fluid to be tested in a sodium fluoride tube. A synovial glucose of 50 mg %

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TABLE 1
SYNOVIAL ANALYSIS

Disease	Appearance	Mucin Clot	Viscosity	Leukocytes per mm ³	Sugar: serum-synovial difference (mg/100ml)	Crystals	Comment
Normal	Clear, amber	Good	High	<200 <25% polys	<10	None	
Gouty Arthritis	Milky or yellow	Poor	Low	15,000+ 70% polys	10±	Sodium urate	Strongly negative birefringent intra- and extracellular urate crystals.
Pseudogout	Turbid, amber, or yellow	Fair to Poor	Fair or Low	25,000± 90% polys±	10±	Calcium pyrophosphate	Few-to-many weakly positive intra- and extracellular calcium pyrophosphate crystals.
Rheumatoid Arthritis	Turbid, amber to light green	Poor	Low	15,000+ 70% polys	25	Occasional cholesterol	WBC inclusions frequently present. Rheumatoid factor usually present.
Septic Arthritis	Very turbid, gray	Poor	Low	70,000± 90% + polys	70	None	Culture positive in only 20-30% of GC cases.
Tuberculous Arthritis	Turbid, amber, or yellow	Poor	Low	30,000± 60%± polys	50+	None	Biopsy frequently helpful.
Osteoarthritis	Clear, amber	Good	High	1000± 10% polys	<10	None	Cartilage fibrils may be present.
Acute Traumatic Arthritis	Bloody or turbid	Good	High	<2000 <25% polys	<10	None	Occasional cartilage fibril. Many RBC's.
Systemic Lupus Erythematosus	Slightly turbid, amber	Good	High	3000± <10% polys	10±	None	Examine for LE cells.
Rheumatic Fever	Slightly turbid, amber	Good	Fair to Low	15,000 50% polys	10±	None	Occasional WBC inclusion

less than fasting serum should be considered as strongly indicating an infectious process until proved otherwise.

"Wet Drop" Preparation Studies. The microscopic examination of a drop of fresh or anticoagulated synovial fluid may yield diagnostic information, especially in cases of crystal deposition diseases (gout and pseudogout). This examination is performed by placing a small drop of synovial fluid on a glass slide, covering with a cover slip and sealing the edges with clear fingernail polish. Only a small drop is used to prevent individual cells from

moving across the field of view. Sealing the slide prevents the formation of artifacts while drying and will preserve the specimen for one or more hours.

The synovial fluid findings in the crystal deposition diseases, gout (monosodium urate crystal deposition disease) and pseudogout (calcium pyrophosphate dihydrate crystal deposition disease), were described in the early 1960's. The discovery and identification of these crystals in synovial fluid, using the compensated polarizing microscope, led to great enthusiasm in making rapid and accurate diagnoses of crystal-induced synovitis. Unfortunately,

the compensated polarizing microscope is an expensive piece of equipment, which is not readily available in the office of most physicians and it is infrequently found in general hospitals.

Simple Polarization. A simple polarizing microscope can be made from an ordinary microscope by inserting a polarizing filter below the condenser, usually on top of the light source (the polarizer), and using one filter above the objective, usually in the barrel or above the eye piece (the analyzer). The polarizer is then rotated until the darkest field possible is obtained. The monosodium urate (MSU) crystals usually appear as bright needles against this black background. The calcium pyrophosphate dihydrate (CPPD) crystals usually appear in bright monoclinic, triclinic, rectangular or rhomboid forms, and may be quite difficult to differentiate from MSU crystals in synovial fluid. Figure 1 illustrates the placement of simple plastic polarizing filters in a laboratory microscope.

Compensated Polarization. The compensated polarizing microscope utilizes a first-order red filter, the compensator, which is inserted between the objective and the analyzer. The compensator retards red light and the polarized background becomes red instead of black. The axis of the line of slow vibration of the compensator is then defined. The MSU and CPPD crystals will be either yellow or blue, depending upon their respective positions to this axis. The MSU crystal is brightly yellow when parallel to the axis and indicates that it is strongly negatively birefringent. The CPPD crystal is faintly blue when in this position, indicating weakly positive birefringence.

On occasion, both MSU and CPPD crystals are noted in the same fluid indicating coexisting disease. Previously injected adrenocorticosteroids, oxalate anticoagulant, cholesterol crystals, and scratches on the slide or coverslip should not be confused with either MSU or CPPD crystals.

New and Simple Technique. We have found a very adequate substitute for the first-order red filter which can be used with an ordinary light microscope which has been adapted with simple polarizing filters. One needs only a clean microscopic glass slide and simple transparent cellophane tape. One piece of the cellophane tape is carefully applied to the top side of the slide and then another piece of tape is applied over this. Wide tape is preferable but two pieces of narrow tape carefully applied

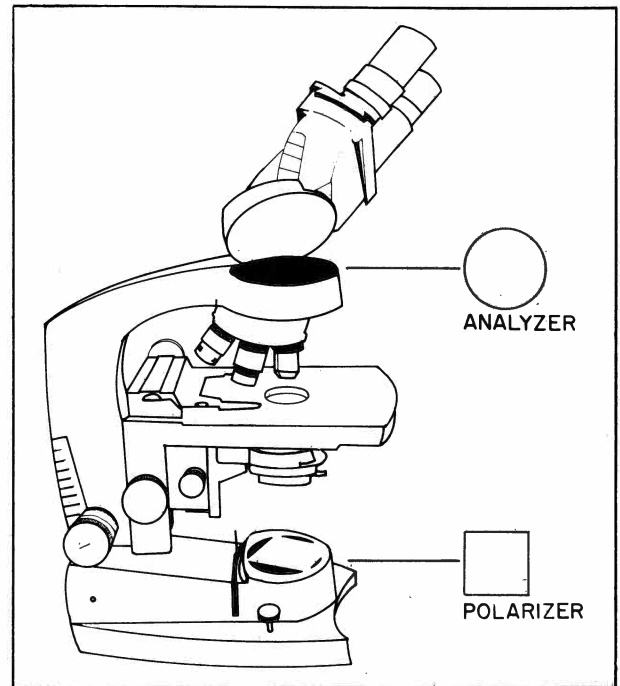


Fig. 1—Illustration of simple plastic polarizing filters in barrel of microscope (analyzer) and above light source (polarizer).

beside two other pieces is satisfactory. The microscope is then adapted for simple polarizing microscopy as previously described. (One may first wish to focus on the material to be examined.) The cellophane-taped slide is then placed over the polarizer and carefully rotated until the background is quite red. We have discovered that the long axis of the taped slide substitutes amazingly well for the axis of slow vibration of the first-order red compensator. The examiner can then mentally project the axis of the taped slide on the stage. Figure 2 illustrates the cellophane-taped slide on top of the polarizer. With the stage arranged to permit free movement of the "wet-prep" slide, the examiner can then rotate the "wet-prep" slide and define whether the observed crystals are negatively or positively birefringent.

It should be emphasized that cellophane is closely controlled for thickness in its manufacturing process but not for its refractive index. Therefore, there may be a different retardation with each supply of cellophane tape. We have tested numerous batches of cellophane tape. Approximately 50% will give the red background and the other 50%

a blue background. The interested examiner may have to purchase a few rolls of tape until a satisfactory one is found.

The improvised compensated polarizing microscope techniques described above have been a tremendous help in making a rapid and accurate diagnosis of crystal-induced synovitis. Faculty, housestaff, and students are encouraged to carry polarizing filters and a cellophane-taped microscope slide with them. A great deal of enthusiasm has been expressed. For the first time, individuals are really learning the true meaning of the planes of birefringence. In the past, many individuals memorized, in preparation for written examination, which crystals were negatively or positively birefringent. It is hoped that others will discover these techniques to be helpful to them. Examiners will be greatly helped in establishing their own personal techniques by smearing some MSU crystals from a tophus on a clean microscopic slide. They can then experiment with the filters until the simple maneuvers are mastered.

Ragocytes (RA cells, inclusion body cells, raisin seed cells) in synovial fluid of patients with rheumatoid arthritis were originally described by Dr. Joseph Hollander and associates. These cells

are leukocytes containing inclusion bodies. They appear as dark granules under regular light microscopy at high dry magnification and as clear vacuolated areas with phase microscopy. It was assumed that these bodies were phagocytosed rheumatoid factor and they were noted in from 5–95% of the total leukocyte population of rheumatoid synovial fluid. It was believed that phagocytosis of rheumatoid factor played a role in the pathogenesis of rheumatoid arthritis in a similar fashion to the phagocytosis of sodium urate crystals in the pathogenesis of gouty arthritis. This concept was supported with the production of acute synovitis by the injection of autologous γ G-globulin into inactive joints of patients with rheumatoid arthritis. Subsequent studies have shown that the findings of these leukocyte inclusions are not specific for rheumatoid arthritis. They may be found in other types of inflammatory joint disease and may be scavengers which have phagocytosed products of inflammation including γ -globulins.

Osteoarthritis fluid may reveal a few-to-many cartilage fibrils and fragments. These may also be seen in cases of pseudogout.

Cholesterol crystals are noted rarely in the fluid of cases of chronic rheumatoid arthritis. These crystals are large, rhomboid, have punched-out corners and are birefringent.

An inexperienced examiner may confuse previously injected corticosteroid ester crystals with urate or pyrophosphate crystals. The steroid crystals may be phagocytosed by white cells and apparently can precipitate a "postinjection flare" of the injected joint. The crystals are usually negatively birefringent.

Reiter's syndrome synovial fluid has been reported to show large mononuclear cells containing many vacuoles. Some of these cells appear to contain polymorphonuclear cells. The specificity of this is unclear.

Table 2 illustrates the complement (C') levels in various disease entities. It is most helpful, however, to know the normal values and type of complement determination for each laboratory. For example, if total complement is determined on a viscous synovial fluid and if a diffusion technique is used, poor diffusion may occur due to viscosity and erroneously low level results.

Summary and Conclusions. Examination of synovial fluid is a most helpful test for making a

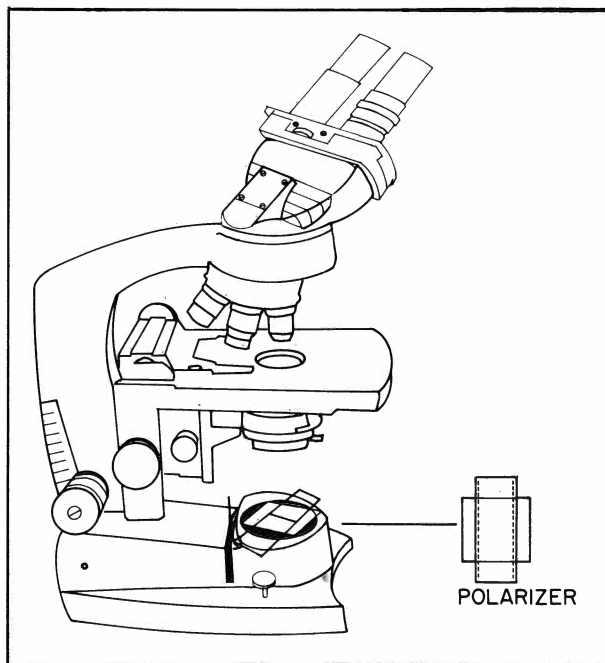


Fig. 2—Illustration of "cellophane tape compensator" above the polarizer.

TABLE 2
COMPLEMENT

	Serum	Synovial Fluid
Rheumatoid arthritis	N (rarely ↓)	↓
Systemic lupus erythematosus	↓	↓
Reiter's	N	↑↑
Septic	N (occ. ↑)	↑
Crystal disease	N	N or ↑
Spondylitis & psoriasis	N	N or ↑

definitive diagnosis of one of the various types of arthritis, especially gout, pseudogout and septic arthritis. Unfortunately, it is one of the most frequently ignored tests. It should be remembered that only a few drops of fluid are needed for a "wet drop" examination for crystals, ragocytes, Gram's stain and a Wright's stain.

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