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2011 Hematology and Clinical Microscopy Glossary

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2011 Hematology, Clinical Microscopy, and Body Fluids Glossary

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BLOOD CELL IDENTIFICATION

Introduction

This glossary corresponds to the master list for hematology and is designed to assist Survey participants in the proper identification of blood cells in photomicrographs. Descriptions are for cells found in blood smears or aspirated bone marrow particles stained with Wright-Giemsa.

Granulocytic (Myeloid) and Monocytic Cells

Myeloblast, with Auer Rods

Myeloblasts are the most immature cells in the myeloid series. They are normally confined to the bone marrow, where they constitute less than three percent of the nucleated cells. They may be present in the blood in leukemic states, myelodysplastic syndromes, myeloproliferative disorders, and, very rarely, in leukemoid reactions. The myeloblast is usually a fairly large cell, 15 to 20 μm in diameter, with a high nuclear-to-cytoplasmic (N:C) ratio, usually 7:1 to 5:1, with cytoplasm that is basophilic.

Myeloblasts may occasionally be smaller, similar to the size of a mature myeloid cell. The cell and nucleus are usually round, although irregularly shaped, or folded nuclei may be present. The nucleus has finely reticulated chromatin with distinct nucleoli present.

Leukemic myeloblasts may exhibit a few delicate granules and/or Auer rods. Distinguishing one type of abnormal blast cell from another is not always possible using Wright-Giemsa stains alone. In the absence of Auer rods, cytochemical data (e.g., myeloperoxidase or Sudan black reactivity), or cell surface marker data (e.g., CD13 and CD33 positivity), it is not possible to define the lineage of a given blast cell.

Dysplastic and Neoplastic Myeloid Changes: Auer Rods

Auer rods are pink or red, rod-shaped cytoplasmic inclusions seen in early myeloid forms and occasionally, in early monocytic forms in patients with myeloid lineage leukemia. These inclusions represent a crystallization of azurophilic (primary) granules. A cell containing multiple Auer bodies clumped together is referred to as a faggot cell (from the English faggot, meaning cord of wood). Faggot cells are most commonly seen in acute promyelocytic leukemia.

Neutrophil, Promyelocyte

Promyelocytes are round to oval cells that are generally slightly larger than myeloblasts; the diameter is 12 to 24 μm . They are

normally confined to bone marrow, where they constitute less than two percent of nucleated cells, but like the myeloblast, can be seen in the blood in pathologic states. The nuclear-to-cytoplasmic ratio is high—5:1 to 3:1. The nucleus is round to oval, has fine chromatin, and contains distinct nucleoli. The cytoplasm is basophilic, more plentiful than in a myeloblast, and contains multiple distinct azurophilic (primary) granules. A paranuclear hof may be present.

Neutrophil, Promyelocyte, Abnormal with or without Auer Rods

The neoplastic cell in acute promyelocytic leukemia is considered to be the neoplastic counterpart of the promyelocyte; however, this leukemic cell differs from normal promyelocytes in several respects. The nucleus is usually folded, bilobed, or reniform, often with overlapping nuclear lobes. A distinct Golgi zone is typically absent. Cytoplasmic granules, while abundant in the classic hypergranular form of this disease, may differ in appearance. They may be coarser or finer than those seen in normal promyelocytes and may also be either slightly darker or more reddish in color. In the microgranular variant, few granules may be visible in the majority of cells and the granules present may be very fine. Finally, the abnormal promyelocytes of acute promyelocytic leukemia frequently contain Auer rods, which may be multiple in an individual cell (faggot cell).

Neutrophil, Myelocyte

The transition from promyelocyte to myelocyte occurs with the end of production of azurophilic (primary) granules and the beginning of production of lilac or pale orange/pink (specific) granules. Myelocytes are usually confined to the marrow where they constitute approximately 10 percent of the nucleated cells. In pathologic states, myelocytes are seen in blood. The myelocyte is smaller than the earlier precursors, usually 10 to 18 μm . The cells are round to oval in shape and have a nuclear-to-cytoplasmic ratio of 2:1 to 1:1. The nucleus is slightly eccentric, lacks a nucleolus, and begins to demonstrate chromatin clumping; one side often shows slight flattening. Sometimes a clear hof is seen adjacent to the nucleus, indicating the location of the Golgi apparatus. The cytoplasm is relatively more abundant than in earlier precursors and is amphophilic. Both azurophilic and specific granules are present in the cytoplasm with specific granules coming to predominate as maturation progresses.

Neutrophil, Metamyelocyte

Metamyelocytes are the first of the post-mitotic myeloid precursors. They constitute 15 to 20 percent of nucleated cells in the bone marrow and may be seen in the blood in pathologic states and in response to stress. They are approximately 10 to 18 μm in diameter. They are round to oval with a nuclear-to-cytoplasmic ratio of 1.5:1 to 1:1. The nuclear chromatin is condensed and the nucleus is indented to less than half of the potential round nucleus (i.e., the indentation is smaller than half of the distance to the farthest nuclear margin). The cytoplasm is amphophilic containing rare azurophilic (primary) granules and many fine specific granules.

Neutrophil, Segmented or Band

Band neutrophils, also known as stabs, constitute 10 to 15 percent of the nucleated cells in the bone marrow and five to 10 percent of the nucleated cells in the blood under normal conditions. Increased numbers of bands appear in the blood in a number of physiologic and pathologic states. The band is round to oval and 10 to 18 μm in diameter. The nuclear-to-cytoplasmic ratio is 1:1.5 to 1:2 and the nuclear chromatin is condensed. The nucleus is indented to more than half the distance to the farthest nuclear margin, but in no area is the chromatin condensed to a single filament. The nucleus can assume many shapes: it can be band-like; sausage-like; S, C, or U-shaped; and twisted and folded on itself. The cytoplasm is similar to that of other post-mitotic neutrophilic cells, with specific granules predominating in the pale cytoplasm.

The segmented neutrophil, the mature cell of the myeloid series and the predominant white cell in blood, mimics its immediate precursors in size (10 to 15 μm), shape (round to oval), and cytoplasmic appearance (pale pink cytoplasm with specific granules). The N:C ratio is 1:3, the lowest of any cell in the neutrophilic series, and the nuclear chromatin is condensed. The nucleus is segmented or lobated (2 to 5 lobes normally). The lobes are connected by a thin filament that contains no internal chromatin, giving it the appearance of a solid, thread-like dark line. The presence of these thread-like filaments is the basis for distinguishing the segmented neutrophil from its precursor, the band neutrophil. However, in repeated proficiency testing studies, it has not been possible to achieve consistent differentiation between bands and segmented neutrophils. Therefore, for the purposes of proficiency testing it is not required that they be differentiated.

(For a detailed guideline for the differentiation of segmented and band neutrophils, see Glassy, 1998).

Neutrophil, Polyploid

Polyploid neutrophils, also referred to as “macropolycytes” or “twin” neutrophils, are twice the size of normal neutrophils and appear to have hypersegmented nuclei. In fact, on careful examination, the apparent hypersegmentation is due to the presence in the cell of two nearly identical segmented nuclei. These cells have been shown to have tetraploid DNA content and appear to represent neutrophils that have undergone DNA replication but have failed to undergo cytoplasmic division. Such cells have been described as a frequent finding after administration of recombinant growth factors, but also may be seen in other conditions such as infections, AIDS, and during recovery from chemotherapy. They are not a result of megaloblastic hematopoiesis and thus should not be confused with hypersegmented neutrophils.

Basophil, Any Stage

Cells in the basophil line have a maturation sequence analogous to the neutrophil line. At the myelocyte stage, when specific granules begin to develop, basophil precursors can be identified. All basophils, from the basophilic myelocyte to the mature segmented basophil, are characterized by the presence of a moderate number of coarse and densely stained granules of varying sizes and shapes. The granules are larger than neutrophilic granules and most are roughly spherical. The predominant color of the granules in Wright-stained preparations is blue-black, but some may be purple to red. The granules are unevenly distributed and frequently overlay and obscure the nucleus. Basophils are increased in the blood in several states including myeloproliferative disorders, hypersensitivity reactions, hypothyroidism, iron deficiency, and renal disease.

Eosinophil, Any Stage

Eosinophils are round to oval leukocytes that are present in the blood, bone marrow, and tissues of normal individuals. They are generally easily recognized due to their characteristic granulation. They are the same size as neutrophilic cells, 10 to 15 μm for mature forms and 10 to 18 μm for immature forms. The N:C ratio ranges from 1:3 for mature forms to 2:1 for immature forms. Their abundant cytoplasm is generally evenly filled by numerous coarse, orange-red granules of uniform size. These granules rarely overlie the nucleus and

exhibit a refractile appearance with light microscopy due to their crystalline structure. This refractile appearance is not apparent in photomicrographs or pictures. Also, due to inherent problems with the color rendition on photomicrographs, which is sometimes imperfect, eosinophilic granules may appear lighter or darker than on a freshly stained blood film. Discoloration may give these granules a blue, brown, or pink tint. Nonetheless, the uniform, coarse nature of eosinophilic granules is characteristic and differs from the smaller, finer granules of neutrophilic cells. Occasionally, eosinophils can become degranulated with only a few orange-red granules remaining visible within the faint pink cytoplasm.

In the most mature eosinophilic form, the nucleus is segmented into two or more lobes connected by a thin filament. About 80 percent of segmented eosinophils will have the classic two-lobed appearance. Typically, these lobes are of equal size and round to ovoid or potato-shaped with dense, compact chromatin. The remainder of segmented eosinophils will have three lobes and an occasional cell will exhibit four to five lobes.

Eosinophils exhibit the same nuclear characteristics and the same stages of development as neutrophilic leukocytes. Immature eosinophils are rarely seen in the blood, but are found in bone marrow smears. They may have fewer granules than more mature forms. The earliest recognizable eosinophilic form by light microscopy is the eosinophilic myelocyte. Eosinophilic myelocytes often contain a few dark purplish granules in addition to the orange-red secondary granules.

Monocytes, Immature (Promonocyte, Monoblast)

For purposes of proficiency testing, selection of the response “monocyte, immature (promonocyte, monoblast)” should be reserved for malignant cells in acute monocytic/monoblastic leukemia, acute myelomonocytic leukemia, chronic myelomonocytic leukemia, and myelodysplastic states. While normal immature monocytes may be identified in marrow aspirates, they are generally inconspicuous and don’t resemble the cells described in this section. The malignant monoblast is a large cell, 15 to 25 μm in diameter. It has relatively more cytoplasm than a myeloblast with the nuclear-to-cytoplasmic ratio ranging from 7:1 to 3:1. The monoblast nucleus is round or oval and has finely dispersed chromatin and distinct nucleoli. The cytoplasm is blue to gray-blue and may contain small, scattered azurophilic granules. Some monoblasts cannot be distinguished morphologically from other blast forms, hence

the need for using other means (e.g., cytochemistry and surface markers) before assigning a particular lineage to a blast cell. Promonocytes have nuclear and cytoplasmic characteristics that are between those of monoblasts and the mature monocyte discussed below. They are generally larger than mature monocytes, but have similar appearing gray-blue cytoplasm that often contains uniformly distributed, fine azurophilic granules. Cytoplasmic vacuolization is not a usual feature. The nuclei show varying degrees of lobulation, usually characterized by delicate folding or creasing of the nuclear membrane. Nucleoli are present but not as distinct as in monoblasts.

Monocyte

Monocytes are slightly larger than neutrophils, 12 to 20 μm in diameter. The majority are round with smooth edges, but some have pseudopod-like cytoplasmic extensions. The cytoplasm is abundant and gray to gray-blue (ground-glass appearance) and may contain fine, evenly distributed, azurophilic granules or vacuoles. The nuclear-to-cytoplasmic ratio is 4:1 to 2:1. The nucleus is usually indented, often resembling a three-pointed hat, but it can also be folded or band-like. The chromatin is condensed, but less densely than that of a neutrophil or lymphocyte. Nucleoli are generally absent, but occasional monocytes may contain a small, inconspicuous nucleolus.

Neutrophil, Toxic (to include Toxic Granulation and/or Döhle Bodies, and/or Toxic Vacuolization)

Toxic changes in neutrophils include toxic granulation, toxic vacuolization, and Döhle bodies. Toxic granulation and Döhle bodies each may be present in an individual cell without the other finding; either change alone is sufficient to designate a neutrophil as “toxic.” Toxic granulation is the presence of large purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes. Vacuoles within the cytoplasm of these same cells constitute toxic vacuolization.

The vacuoles are variable in size and may coalesce, sometime distorting the neutrophil cytoplasm to form pseudopodia. EDTA storage may produce degenerative vacuolization; in this case, only a few, small, punched-out appearing vacuoles are found. However, as it may at times be difficult to distinguish toxic from degenerative vacuoles, it is best not to consider neutrophil vacuoles to be toxic unless accompanied by other toxic changes. Döhle bodies appear as single or multiple blue or gray-blue inclusions of variable size (0.1 to 5.0 μm) and shape

(round, or elongated or crescent shaped) in the cytoplasm of neutrophils, bands, or metamyelocytes. They are often found in the periphery of the cytoplasm, near the cell membrane. These inclusions represent parallel strands of rough endoplasmic reticulum. In the May-Hegglin anomaly, inclusions that resemble Döhle bodies are seen, but in this heritable condition, the inclusion is due to accumulation of free ribosomes and the presence of 7 to 10 nm parallel filaments. Toxic changes result from the action of cytokines released in response to infection, burns, trauma, G-CSF (granulocyte colony stimulating factor) and indicate a shortened maturation time and activation of postmitotic neutrophil precursors.

Neutrophil, Giant Band or Giant Metamyelocytes

Myeloid precursors that are a result of megaloblastic hematopoiesis are increased in size and have nuclei that show aberrant maturation. Although these changes are usually discussed in terms of the neutrophil series, they may also be observed in cells in the eosinophil and basophil cell lines. Larger-than-normal metamyelocytes and bands with decreased chromatin clumping are seen in the marrow. These cells have diameters 1.5 times those of normal metamyelocytes or bands. In the blood, neutrophils show hypersegmentation of the nucleus.

Neutrophil, Hypersegmented Nucleus

To be considered a neutrophil with hypersegmented nucleus, the neutrophil should demonstrate six or more lobes. Hypersegmented neutrophils are uncommon unless there is megaloblastic hematopoiesis. Rarely they have been seen in sepsis, renal disease, and myeloproliferative states. Megaloblastic hematopoiesis occurs when DNA synthesis is impaired. Such conditions include deficiency of cofactors for nucleotide synthesis, such as vitamin B₁₂ and folate, and cases where patients are receiving a nucleotide analog (such as 6-mercaptopurine) or cofactor blocking agents (such as methotrexate) for neoplastic or rheumatologic conditions.

Neutrophil with Dysplastic Nucleus and/or Agranular Cytoplasm

Dysplastic neutrophils are characteristic of myelodysplastic syndromes. Morphologically, the normal synchronous maturation of nucleus and cytoplasm is lost. In the cytoplasm, the primary and secondary granules are often decreased or absent, causing the cytoplasm to appear pale and bluish. The

nucleus shows abnormal lobation with a mature chromatin pattern. In some cases, the nucleus has a “pince-nez” appearance. These cells are known as pseudo-Pelger-Huët neutrophils. For proficiency testing purposes, cells with pseudo-Pelger-Huët nuclei are best defined as Pelger-Huët cells. Dysplastic neutrophils often have abnormal cytochemical reactivity; levels of myeloperoxidase and neutrophil alkaline phosphatase may be low or absent. The dysplastic neutrophils may also exhibit functional defects.

Neutrophil with Pelger-Huët Nucleus (Acquired or Congenital)

Neutrophils with bilobed nuclei in the “pince-nez” conformation (two round or nearly round lobes connected by a distinct thin filament) are designated as neutrophils with Pelger-Huët nuclei or as Pelger-Huët cells. They occur as an inherited autosomal dominant abnormality of nuclear segmentation referred to as Pelger-Huët anomaly. In the heterozygous state of Pelger-Huët anomaly, virtually all of the neutrophils have bilobed nuclei. Individuals with homozygous Pelger-Huët genes contain unilobed nuclei in mature neutrophils. The nuclear chromatin in Pelger-Huët cells is generally denser than in normal cells. Neutrophils with nuclei morphologically indistinguishable from those seen in the congenital abnormality are occasionally observed in association with other conditions, including myelodysplastic syndromes, other myeloid malignancies, sulfonamide therapy, colchicine therapy, mycophenolate mofetil therapy, HIV infection, and *Mycoplasma pneumoniae*. The proportion of nuclei affected in these disorders is variable. These cells are designated as pseudo-Pelger-Huët cells.

Erythrocytic Cells and Inclusions

Erythrocyte (Red Cell), Normal (Normocytic, Normochromic)

An erythrocyte is a mature, non-nucleated red cell of fairly uniform size (6.7 to 7.8 μm in diameter) and shape (biconcave disc, appearing as round or slightly ovoid on the smear). It contains hemoglobin and stains pink-red. A zone of central pallor due to the biconcavity of the cell occupies approximately one third (2 to 3 μm) of the cell diameter.

Acanthocyte (Spur Cell)

Acanthocytes are densely stained spheroidal (lacking central pallor) red cells with multiple (usually three to 20), irregularly distributed, thorn-like spicules of variable size, often with drumstick ends. Acanthocytes are classically described in association with hereditary abetalipoproteinemia (hereditary acanthocytosis). In addition, these cells are often seen in significant numbers in severe (end-stage) liver disease, hepatorenal failure, anorexia nervosa, and chronic starvation. (In the latter two disorders, they appear as irregularly shaped erythrocytes with multiple blunt projections.) A small number of acanthocytes may be seen in other forms of severe hemolytic anemia, particularly after splenectomy. Acanthocytes are rarely encountered in otherwise normal blood smears (one or two per smear). In such smears, they represent older, senescent red cells approaching their extremes of life (120 days). It is logical, therefore, that acanthocytes should readily be found in blood smears in the postsplenectomy state because of diminished splenic activity in removal of such poikilocytes.

Bite Cell

Bite cells are red cells from which precipitated, denatured masses of hemoglobin (Heinz bodies) have been pitted by the spleen. Precipitation is a function of oxidant injury to hemoglobin by certain drugs or denaturation of unstable hemoglobin variants. In particular, patients with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency may be predisposed to such oxidant injury. The net result of the act of pitting is a variety of peripheral red cell defects, ranging from tiny arc-like “nibbles” to large “bites.” “Bitten” red cells may show multiple peripheral defects. Symmetrical equatorial defects result in the formation of “apple-core” poikilocytes. Giant single bites may result in the formation of poikilocytes morphologically indistinguishable from the “helmet” cells of fragmentation anemias. As in the fragmentation anemias, spherocytes are almost invariably present, albeit in small numbers.

Blister Cell/Prekeratocytes

Blister cells are erythrocytes in which the hemoglobin appears to be concentrated on one side of the cell, leaving just a thin membrane on the other side. This produces the appearance of large vacuoles with fuzzy margins. Blister cells are most characteristically seen in sickle cell disease, in which they are considered a sickle cell variant. Similar cells, “eccentricocytes,”

may be seen in the setting of oxidant hemolysis. Blister cells may be similar to prekeratocytes.

Prekeratocytes are red cells containing one or two sharply defined, usually submembranous vacuoles. By electron microscopy, these vacuoles are actually “pseudovacuaes” representing fusion of opposing red-cell membranes with exclusion of intervening hemoglobin. The membrane union is brought about by hemodynamic pressures that have forced red cells to become closely applied to or draped over obstacles, such as non-occlusive thrombi or fibrin strands in small vessels. Dislodgement results in the reappearance of these red cells in the circulation with stigmata of membrane fusion. By light microscopy, the points of fusion appear as crisply demarcated (pseudo) vacuoles. Rupture of peripheral (pseudo) vacuoles of prekeratocytes results in the formation of “keratocytes” or “horned cells.” These cells may be morphologically indistinguishable from (or identical to) classic “helmet” cells. Thus, prekeratocytes and keratocytes are usually found together in the same blood smears and should raise the question of a microangiopathic process. Similar or identical cells are also present in small numbers in iron deficiency anemia.

Echinocyte (Burr Cell, Crenated Cell)

Echinocytes are red cells with 10-30 short, blunt projections distributed evenly over their surfaces. They retain their central pallor. They are the same size or only slightly smaller than normal horned red cells. Their appearance is sometimes the result of an improperly prepared smear (slow drying, thick smears, aged blood and pH alteration of glass slide). Echinocytes that are nonartifactual may be indicative of disease, such as uremia or pyruvate kinase deficiency. Under such circumstances, they should be visible in wet preparations.

Fragmented Red Cell (Schistocyte, Helmet Cell, Keratocyte, Triangular Cells)

Fragmented red cells are red cells that have undergone rips and tears when draped over fibrin strands in the microcirculation or have suffered buffeting against unyielding structures in the macrocirculation. Fragments resulting from such trauma resealed by fusion of opposing ends and persist in the circulation, presumably for a short time. Fragmented red cells include helmet cells, keratocytes (horn cells), triangulocytes and a more indecisive term, schistocytes. Fragments should not have central pallor; such cells are best considered non-specific poikilocytes. Occasional spherocytes

are almost invariably present in association with fragmented cells. Spherocytes are the product of the rounded-up red cell fragments. Fragmented cells are seen in severe burns, disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP), and other microangiopathic hemolytic anemias. When present in large numbers, they may cause the MCV to fall into the microcytic range or interfere with platelet enumeration.

Macrocyte, Oval or Round (excluding Polychromatophilic Red Cells)

Macrocytes are abnormally large red cells (volume >100 fL). They are best detected by comparing to other red cells in a smear in the context of the MCV. They may be oval or round. The hemoglobin concentration is normal; cells lack significant polychromasia. (If polychromasia is readily identified, the term polychromatophilic red cell is preferred for proficiency testing purposes). Round macrocytes are associated with reticulocytosis, liver disease, hypothyroidism, and post-splenectomy states. Oval macrocytes are most commonly associated with vitamin B₁₂ or folic acid deficiency. Abnormal red cell maturation (dyserythropoiesis) may also cause oval macrocytosis. Examples include myelodysplastic syndromes and chemotherapy. Oval macrocytes may be mistaken for ovalocytes (elliptocytes). Ovalocytes are often longer than normal red cells and are significantly narrower. The sides of the cells are nearly parallel, unlike the much more rounded edges of oval macrocytes. The hemoglobin of ovalocytes is often concentrated at the ends, unlike the even peripheral distribution of oval macrocytes. Also, oval macrocytes are much larger than ovalocytes.

Microcyte (with Central Pallor)

Microcytes are smaller than normal red cells, measuring less than 80 fL. On the blood film, they generally appear smaller than the nucleus of a small lymphocyte, although morphologic evaluation is a subjective way of evaluating red cell size and is not reliable. Red cell size is more accurately assessed by instrument-generated MCVs. On a peripheral blood film, microcytes retain central pallor, appearing either normochromic or hypochromic. Although other poikilocytes, such as spherocytes and fragmented red cells, can be very small in size, these red cells lack central pallor and should be specifically identified rather than classified as “microcytes.” Microcytes commonly are seen in iron deficiency anemia, thalassemias, and lead poisoning.

Ovalocyte (Elliptocyte)

The terms “elliptocytes” and “ovalocytes” are used to describe the red cells appearing in the shape of a pencil or thin cigar, with blunt ends and parallel sides. Hemoglobin is often concentrated at the ends, producing a “dumbbell appearance.” A small number of elliptocytes/ovalocytes may be present on the smears of normal individuals (<1%), whereas a moderate to marked elliptocytosis/ovalocytosis (>25%) is observed in patients with hereditary elliptocytosis, an abnormality of erythrocyte skeletal membrane proteins. Elliptocytes are also commonly increased in number in iron deficiency and in the same states in which teardrop cells are prominent (see teardrops). Some ovalocytes may superficially resemble oval macrocytes but they are not as large and tend to be less oval with sides that are nearly parallel. The ends of ovalocytes are always blunt and never sharp, unlike those of sickle cells.

Polychromatophilic Non-Nucleated Red Cell

A polychromatophilic red cell is a non-nucleated, round or ovoid red cell that represents the final stage of red cell maturation after exiting the bone marrow. It is larger than a mature erythrocyte and lacks central pallor. It primarily contains hemoglobin with a small amount of RNA, and thereby stains homogeneously pink-gray or pale purple with Romanowsky stain. These cells can be stained as reticulocytes and enumerated by using supravital stains, such as new methylene blue. When supravitally stained, reticulocytes reveal deep blue granular and/or filamentous structures. This reticulin network is called the “substantia reticulofilamentosa.” The amount of precipitated RNA varies with the age of the reticulocyte. Automated technologies for assessing reticulocytes improve the accuracy of determining absolute reticulocyte numbers.

Sickle Cell (Drepanocyte)

Red cells appearing in the shape of a thin crescent with two pointed ends are called sickle cells. The polymerization/gelation of deoxygenated hemoglobin S may cause red cells to appear in one or more of the following forms: crescent-shaped, boat-shaped, filament-shaped, holly-leaf form, or envelope cells. These cells usually lack central pallor. Sickle cells may be seen particularly in the absence of splenic function or after splenectomy in patients with sickle cell anemia, hemoglobin SC disease, SD disease, and S-beta-thalassemia.

Spherocyte

Spherocytes are identified as densely staining, spherical, or globular red cells with normal or slightly reduced volume (MCV) and increased thickness (more than 3 μm), but with decreased diameter (usually less than 6.5 μm) and without central pallor. Such cells are commonly found in hereditary spherocytosis and immune hemolytic anemias. Microspherocytes (spherocytes measuring 4 μm or less in diameter), frequently seen in severe burns, probably represent rounded-up fragments of red cells.

Stomatocyte

Stomatocytes are red cells in which the central pallor is straight or appears as a curved rod-shaped slit, giving the red cells the appearance of a smiling face or a fish mouth. Stomatocytes are commonly seen in hereditary stomatocytosis, liver disease, and acute alcoholism; however, a small number of stomatocytes often form as an artifact resulting from the slow drying of smears.

Target Cell (Codocyte)

Target cells are thin red cells with a greater-than-normal surface membrane-to-volume ratio. They are often flattened-out on the smears, revealing sometimes a greater-than-normal diameter. These cells are characterized by a central hemoglobinized area within the surrounding area of pallor, which in turn is surrounded by a peripheral hemoglobinized zone. These morphologic features give target cells the appearance of a Mexican hat or a bull’s-eye. Target cells associated with hemoglobin C may have a slightly reduced or normal MCV, whereas those associated with hemoglobin E disorders or hemoglobin H disease exhibit microcytosis of varying degree. Target cells are usually seen following splenectomy or in patients who are jaundiced or have liver disease. In these conditions, the MCV may be normal or greater than normal. Target cells may also appear as artifacts from slow drying the slides in a humid environment or made from specimens anticoagulated with excessive EDTA. The drying artifact results in the presence of numerous target cells in some fields, but none or few in other fields.

Teardrop Cell (Dacrocyte)

Red cells appearing in the shape of a teardrop or a pear with a single, short or long, often blunted or rounded end are called teardrop cells. These are commonly seen in chronic idiopathic myelofibrosis (primary myelofibrosis) but may also be seen

in pernicious anemia, anemia of renal disease, hemolytic anemias, and other forms of severe anemia. These cells are often associated with an abnormal spleen or bone marrow. Bone marrow infiltration with hematologic and nonhematologic malignancies may also be accompanied by dacrocytosis. Teardrop cells may be seen as an artifact of slide preparation; such dacrocytes are usually easily recognized from the fact that their “tails” all point in the same direction.

Basophilic Stippling (Coarse)

Basophilic stippling may be either fine or coarse. Fine stippling is seen in reticulocytes. It is barely discernible in the red cell and is not of any clinical consequence. Coarse stippling, on the other hand, is clinically significant and suggests impaired hemoglobin synthesis. The punctuation is readily visible and made up of relatively evenly distributed blue-gray granules. Coarse stippling results from abnormal aggregates of ribosomes and polyribosomes in reticulocytes. Iron-containing mitochondria in the aggregates may further accentuate the stippling. Lead poisoning, thalassemia, and refractory anemia are disorders commonly associated with coarse basophilic stippling.

Heinz Body (Supravital Stain)

Heinz bodies appear as large (1 to 3 μm or greater), single or multiple, blue-purple (depending on the stain used) inclusions often attached to the inner surface of the red cell membrane. They characteristically are seen at the edge of the red cell, stuck to the interior of the membrane and protruding into the cytoplasm. They are visible only with the help of supravital stains such as new methylene blue, Nile blue, crystal violet, or methyl violet. They are almost never visible in Wright-Giemsa-stained blood films, although bite cells are markers of their presence. Depending on the disease, the Heinz body is composed of precipitated normal hemoglobin (e.g., G-6-PD deficiency) or structurally defective hemoglobin (e.g., unstable hemoglobin).

Hemoglobin C Crystal

Hemoglobin C crystals within red cells are dense structures with rhomboidal, tetragonal, or rod shapes. They often distort the cell and project beyond its rim. The classic shape resembles the Washington monument. The crystals are often surrounded partly by a clear area or blister devoid of hemoglobin. Hemoglobin C crystals are readily seen after splenectomy in patients with hemoglobin C disease or SC disease.

Hemoglobin H Inclusions

Hemoglobin H inclusions represent precipitated excess beta hemoglobin chains, seen only after supravital staining. They are found in hemoglobin H disease, a form of alpha thalassemia (three alpha-gene deletion). Excess beta hemoglobin chains form tetramers that precipitate with the addition of brilliant cresyl blue stain. The deposits are small and evenly dispersed within the red cell, producing a “golf ball” or peppery appearance. The fine, deep-staining deposits are numerous, varying from 20 to 50 per cell. They are much smaller and more numerous than classic Heinz bodies. They are not visible with Wright-Giemsa stain.

Howell-Jolly Body (Wright Stain)

Howell-Jolly bodies are small round objects about 1 μm in diameter. They are larger than Pappenheimer bodies and are composed of DNA. They are formed in the process of red cell nuclear karyorrhexis or when an aberrant chromosome becomes separated from the mitotic spindle and remains behind when the rest of the nucleus is extruded. Normally, the spleen is very efficient in removing Howell-Jolly bodies from red cells, but if the spleen is missing or hypofunctioning, they may be readily found in the peripheral blood. Howell-Jolly bodies are usually present singly in a given red cell. Multiple Howell-Jolly bodies within a single red cell are less common and typically seen in megaloblastic anemia.

Pappenheimer Body

Pappenheimer bodies are small, angular, dark inclusions appearing either singly or in doublets. They are less than 1 μm in diameter and thus are smaller than Howell-Jolly bodies. Unlike Heinz bodies, they are visible on Wright-Giemsa-stained smears. These tiny, generally angular inclusions stain positively with Prussian blue, indicative of the presence of iron. Wright-Giemsa-stain does not stain the iron, but rather the protein matrix that contains the iron. Pappenheimer bodies are formed as the red cell discharges its abnormal iron-containing mitochondria. An autophagosome is created that digests the offending organelles. If the autophagosome is not discharged out of the cytoplasm or removed by the pitting action of the spleen, the inclusions will be visible on Wright-Giemsa-stained blood films. Their true nature is confirmed with an iron stain. Heinz bodies and Howell-Jolly bodies do not contain iron.

Red Cell Agglutinates

Red cell agglutination occurs when red blood cells cluster or clump together in an irregular mass in the thin area of the blood film. Usually, the length and width of these clumps are similar (14 by 14 μm or greater). One must distinguish this abnormality from rouleaux formation. Individual red cells often appear to be spherocytes due to overlapping of cells in red cell agglutinates. This misperception is due to obscuring of the normal central pallor of the red cells in the clump. Autoagglutination is due to cold agglutinins, most commonly an IgM antibody. Cold agglutinins can arise in a variety of diseases and are clinically divided into cases occurring after viral or *Mycoplasma* infections, cases associated with underlying lymphoproliferative disorders or plasma cell dyscrasias (cold agglutinin disease), and chronic idiopathic cases that are more frequently seen in elderly women. Red cell agglutinates can also be found in cases of paroxysmal cold hemoglobinuria that exhibit a similar clinical pattern and can occur after viral infections. This disorder is caused by an IgG antibody that binds to the red cells at low temperature and then causes hemolysis when the blood is warmed to 37°C.

Rouleaux

Rouleaux formation is a common artifact that can be observed in the thick area of virtually any blood film. This term describes the appearance of four or more red blood cells organized in a linear arrangement that simulates a stack of coins. The length of this arrangement (18 μm or more) will exceed its width (7 to 8 μm), which is the diameter of a single red cell. The central pallor of the red cells is generally apparent, but it may be obscured due to overlapping of the cells' cytoplasm. When noted in only the thick area of a blood film, rouleaux formation is a normal finding and not associated with any disease process. True rouleaux formation is present when this artifact is seen in the thin area of a blood film. It is often associated with a proteinaceous, blue-staining background. True rouleaux formation is due to increased amounts of plasma proteins, primarily fibrinogen, and globulins. It is seen in a variety of infectious and inflammatory disorders associated with polyclonal increases in globulins and/or increased levels of fibrinogen. Rouleaux formation associated with monoclonal gammopathies can be seen in multiple myeloma and in malignant lymphomas such as Waldenstrom's macroglobulinemia.

Nucleated Red Cells

Pronormoblast (Proerythroblast)

Pronormoblasts, morphologically the most immature cells of the erythrocytic series, are large round or ovoid cells measuring 14 to 24 μm in diameter. The nucleus is round or slightly oval and contains one or more prominent nucleoli. The chromatin is finely reticulated or lacy and blast-like without clumping. A perinuclear halo is usually evident. The cytoplasm stains pale blue and is lighter blue than basophilic normoblasts. The N:C ratio is approximately 8:1.

Normoblast, Basophilic

Basophilic normoblasts are smaller (10 to 17 μm in diameter) than pronormoblasts, but similar in cellular and nuclear shape. The chromatin, however, is open but thickened or "beady." The chromatin should not show any significant condensation or clumping (if it does, the cell is best designated a polychromatophilic normoblast). The nuclei of large or early basophilic normoblasts may reveal single nucleoli, but those of small or late basophilic normoblasts lack nucleoli. A perinuclear halo is often visible. The cytoplasm is more abundant than pronormoblasts and it is intensely basophilic, imparting a royal blue color. The N:C ratio is approximately 6:1.

Normoblast, Polychromatophilic

Polychromatophilic normoblasts are round or ovoid cells, but are slightly smaller (10 to 15 μm in diameter) than earlier erythroid precursors. The nucleus is round and may have a cartwheel appearance. It lacks nucleoli and may be placed centrally or eccentrically. The chromatin is clumped (early forms mildly so, later forms markedly so). A perinuclear halo is visible. The cytoplasm is abundant and stains as admixtures of blue-gray and pink-gray, depending upon the relative proportions of RNA and hemoglobin in it. The N:C ratio is approximately 4:1.

Normoblast, Orthochromic

Orthochromic normoblasts are round or ovoid cells and are even smaller than the polychromatophilic normoblasts (8 to 12 μm in diameter). The nucleus is also very small, often pyknotic, and sometimes appears as a homogeneous mass of dense chromatin. It is often eccentrically placed and at times may be extruding or fragmented. The cytoplasm is greater in

relative amount than in the polychromatophilic normoblast, and usually stains uniformly pinkish orange with little or no basophilia and lacks the variegated appearance of polychromatophilic normoblasts. The cytoplasmic color is uniform, unlike the variegated coloration of the polychromatophilic normoblast. The N:C ratio is approximately 1:2.

Nucleated Red Cell, Megaloblastic

Megaloblastic nucleated red blood cells are larger than the corresponding normal cells of the erythrocytic series and are characterized by asynchronous nuclear-cytoplasmic development, manifested by delayed or incomplete nuclear maturation relative to cytoplasmic development (hemoglobinization). This results in cells having an immature chromatin pattern compared to the degree of hemoglobinization or cytoplasmic maturation. Red cells with megaloblastic changes are classified into similar stages of development as their normal counterpart cells, based primarily on the stage of cytoplasmic maturation. Megaloblastic cells are larger in size than their normoblastic counterparts. Megaloblastic changes may also be found in other hematopoietic cell series.

Nucleated Red Cell (Normal or Abnormal Morphology), Peripheral Blood

The term nucleated red cell (NRBC) is used to state the presence of normoblasts in the peripheral blood and includes all normoblasts regardless of the stage of maturation. Typically, the circulating nucleated red cell is at the orthochromic stage of differentiation. Both megaloblastic and dysplastic changes can be seen in these circulating red cells, reflecting simultaneous erythroid maturation abnormalities present in the bone marrow. Caution should be used in classifying a circulating nucleated red cell as dysplastic on the basis of abnormal nuclear shape (lobated or fragmented), as these changes may occur during their egress from the marrow space and may not be present in the maturing erythroids precursors present in the marrow. For the purposes of proficiency testing, it is adequate to identify a cell as a nucleated red cell when it is present in the peripheral blood, be it normal or abnormal (i.e., exhibits megaloblastic or dysplastic changes). For bone marrow photographs, nucleated red cell is insufficient identification; identification of maturational stage and assessment of dyserythropoietic changes are necessary.

Nucleated Red Cell, Dysplastic

Dyserythropoiesis or abnormal red cell differentiation encompasses both megaloblastic maturation and dysplastic maturation. Megaloblastic maturation is characterized by varying degrees of nuclear-cytoplasmic maturation dyssynchrony. Vitamin B₁₂ and folate deficiencies are the classic examples of megaloblastic maturation, but stem cell abnormalities associated with myelodysplasia, toxins, drugs, or any number of other extrinsic factors may also alter DNA production, resulting in lesser degrees of maturation dyssynchrony or megaloblastoid change. True megaloblastic red cells exhibit dramatic maturation differences between the nucleus and cytoplasm. This dyssynchrony is not as obvious in megaloblastoid red cells. The chromatin is more clumped and the chromatin strands are much coarser than in a corresponding megaloblastic nucleated red cell. The clear spaces between the dense chromatin strands are more prominent in megaloblastoid red cell nuclei. The changes are most noticeable in the later stages of red cell maturation as hemoglobin production in the cytoplasm is more demonstrable. Pronormoblasts are too young to display overt nuclear-cytoplasmic dyssynchrony.

Dysplastic nucleated red blood cells will exhibit strikingly abnormal nuclear features. The nucleus may be enlarged, grotesquely shaped, lobated, fragmented, or multinucleated. The cytoplasm may be vacuolated and contain multiple Howell-Jolly bodies or exhibit coarse basophilic stippling. If the red cell is severely dysplastic, a PAS stain may show clumped cytoplasmic positivity. Megaloblastoid and dysplastic nucleated red cells are found in many different conditions, such as acute myeloid leukemias (including erythroleukemia), myelodysplasia, chronic myeloproliferative disorders, and congenital dyserythropoietic anemias. Megaloblastoid change and lesser degrees of dysplasia may also develop in states of erythroid hyper-proliferation, as well as following exposure to certain toxins, heavy metals, antibiotics, antimetabolites and excess alcohol.

Sideroblast, Ringed Sideroblast, Siderocyte, Siderotic Granules

Red cells containing inclusions that react with Prussian blue or Perl's stain for iron are known as siderocytes. These inclusions are recognized as Pappenheimer bodies on Wright-Giemsa-stained smears. Nucleated red cells staining positive for iron (nonheme) granules are termed sideroblasts

and the granules staining positive for iron are designated as siderotic granules. Approximately 30 – 50% of erythroid precursors in normal bone marrow are sideroblasts. Neither sideroblasts nor siderocytes are found in normal peripheral blood. If five or more granules appear in a ring formation around the nucleus (siderotic granules in mitochondria), covering at least one half of the nuclear periphery, the term *ringed sideroblast* is used. Ringed sideroblasts are not present in normal marrow. Ringed sideroblasts in the blood and/or bone marrow are seen in sideroblastic anemias and other dyserythropoietic states. Increased siderocytes may be seen in many conditions, including hemosiderosis, hemoglobinopathies, and sideroblastic anemia.

Megakaryocytic Cells and Platelets

Megakaryocyte or Precursor, Normal

Megakaryocytes are the largest bone marrow hematopoietic cell. They are derived from bone marrow stem cells and are responsible for platelet production. During development, the cell does not divide, but instead the nucleus undergoes nuclear replication without cell division (endomitosis or endoreduplication) giving rise to a hyperdiploid nucleus with several lobes and each lobe roughly containing a normal complement of chromosomes. The cytoplasm becomes granular and eventually fragments into platelets. The nucleus is left behind to be phagocytized by marrow histiocytes. For proficiency testing purposes, the term *normal megakaryocyte* almost always refers to a mature cell rather than one of the maturation stages. Typically, the mature megakaryocyte measures at least 25 to 50 μm in diameter. The numerous nuclear lobes are of various sizes, connected by large bands or fine chromatin threads. The chromatin is coarse and clumped to pyknotic. The abundant cytoplasm stains pink or wine-red and contains fine azurophilic granules that may be clustered, producing a checkered pattern.

Megakaryocyte or Precursor, Abnormal

Megakaryocytic dysplasia may manifest as abnormalities in cell size, nuclear shape, and cell location. Micromegakaryocytes, also known as dwarf megakaryocytes, are abnormally small megakaryocytes that usually measure 20 μm or less in diameter. The N:C ratio is 1:1 or 1:2. The nucleus

may be hypolobated or may have multiple small lobes reminiscent of the PMNs in megaloblastic anemia. The cytoplasm is pale blue and may contain pink granules. Micromegakaryocytes may be found in the marrow or circulating in the peripheral blood. Larger abnormal megakaryocytes are highly variable in morphology. Some show increased nuclear lobation, while others are hypolobated or mononuclear. Normal megakaryocyte nuclei are connected in series. Dysplastic nuclei may be separated or form masses of chromatin and nuclei. The finding of triple nuclei may be a particularly useful marker of dysplasia. Pyknotic megakaryocytes are also abnormal. The naked or near-naked nuclei are composed of dark masses of chromatin. These cells are undergoing apoptosis (programmed cell death). On biopsy specimens, abnormal megakaryocytes may cluster together, sometimes close to bony trabeculae. Normal megakaryocytes are usually well separated from each other and located away from the trabeculae.

Megakaryocyte Nucleus

After discharging their cytoplasm to form platelets, megakaryocyte nuclei or nuclear fragments may enter the peripheral blood stream, particularly in conditions associated with marrow myelofibrosis. The cell nucleus is single-lobed or less commonly, multilobated. The chromatin is smudged or “puddled” and is surrounded by a very scant amount of basophilic cytoplasm or no cytoplasm at all. If a small amount of cytoplasm is present, it is often wispy, frilly, or fragmented. Rarely, there may be a few localized areas of cytoplasmic blebs or adherent platelets. Small cells with more abundant cytoplasm are best termed *micromegakaryocytes*. If the nuclear characteristics are not appreciated, megakaryocyte nuclei may be mistakenly identified as lymphocytes. Finding megakaryocyte cytoplasmic fragments and giant platelets in the field are helpful clues to the origin of the nucleus. It is important to remember that these cells are not degenerating cells and therefore, the chromatin pattern does not have the characteristics of basket cells. For CAP proficiency testing purposes, megakaryocyte nuclei are almost always seen in the blood whereas micromegakaryocytes may be seen in blood or marrow.

Platelet, Normal

Platelets, also known as thrombocytes, are small, blue-gray fragments of megakaryocytic cytoplasm typically measuring 1.5 to 3 μm in diameter. Fine, purple-red granules are aggregated at the center or dispersed throughout the cytoplasm.

Platelets are quite variable in shape but most are round or elliptical. Some have long cytoplasmic projections or ruffled margins. They are typically single but may form aggregates.

Platelet, Giant (Macrothrombocyte)

Most normal-sized platelets are 1.5 to 3 μm in diameter. Small platelets are less than 1.5 μm in diameter. So-called large platelets usually range from 4 to 7 μm . Giant platelets are larger than 7 μm and usually 10 to 20 μm in diameter. For proficiency testing purposes, the term *giant platelet* is used when the platelet is larger than the size of the average red cell in the field, assuming a normal MCV. The periphery of the giant platelet may be round, scalloped, or stellate. The cytoplasm may contain a normal complement of fine azurophilic granules, or the granules may fuse into giant forms. Giant platelets may be seen in many different reactive, neoplastic, and inherited conditions including myeloproliferative and myelodysplastic disorders, autoimmune thrombocytopenia, in association with severe leukemoid reactions, May-Hegglin anomaly and Bernard-Soulier syndrome.

Platelet, Hypogranular

Hypogranular platelets have reduced if any, of the purple-red granules found in normal platelets. The cells may be normal in size, shape, and configuration, or they may be enlarged and misshapen. The cytoplasm stains pale blue or blue-gray. If no granules are present, zoning is needed to identify the structure as a megakaryocyte fragment or platelet. Zoning refers to the normal alternation of lighter and darker areas within the cytoplasm. Cytoplasmic fragments from cells other than megakaryocytes generally do not show zoning. Hypogranular and other dysplastic platelet forms are typically seen in myeloproliferative and myelodysplastic disorders. Rarely, prominent platelet degranulation resulting in platelet hypogranularity may be seen as an EDTA-induced artifact.

Platelet Satellitism

Platelet satellitism, also known as “platelet rosettes,” is a rare peripheral blood finding that is due to the clumping and adherence of four or more platelets to a neutrophil or band, or very rarely, to a monocyte. Platelet phagocytosis may occasionally occur. The platelets and neutrophils are normal in morphology and function. The phenomenon is due to the interaction of EDTA and immunoglobulin, which nonspecifically binds to platelets. The antibody-coated platelets then bind to the surface of neutrophils or monocytes. Platelet satellitism is

a cause of false thrombocytopenia with automated cell counters because the cellular aggregates are counted as leukocytes rather than platelets.

Lymphocytic and Plasmacytic Cells

Lymphoblast

Lymphoblasts are the most immature cells of the lymphoid series. They are most commonly seen in acute lymphoblastic leukemia (ALL) and lymphoid blast crisis of chronic myelogenous leukemia (CML). These round to oval cells range in size from 10 to 20 μm . The N:C ratio varies from 7:1 to 4:1. Morphologically, lymphoblasts are variable in appearance, even at times within a single case. On one end of the spectrum, L-1 lymphoblasts are small cells with dense but not clumped chromatin, inconspicuous or absent nucleoli, and extremely scanty cytoplasm. On the other end are L-2 lymphoblasts that are large cells with finely dispersed chromatin, variable numbers of distinct basophilic nucleoli, and moderate amounts of cytoplasm, closely resembling myeloblasts. The nuclear contours of lymphoblasts range from round to convoluted. The cytoplasm is typically slightly to moderately basophilic, and is usually agranular. Auer rods are absent.

Because lymphoblasts are quite variable in appearance, it is often impossible to correctly classify an individual cell based on the morphology alone. Lymphoblasts can be indistinguishable from other types of blasts and lymphoma cells. Lacking additional confirmatory information from cytochemical stains or immunophenotyping, one should identify individual cells exhibiting this immature type of morphology as blast cells.

Lymphoma Cell (Malignant)

Lymphoma cells can exhibit a variety of appearances dependent on the subtype of the underlying tumor and definitive diagnosis can be difficult. These cells can exhibit a variety of sizes, shapes, and nuclear and cytoplasmic characteristics. While lymphoma cells are usually round to oval, they can be irregular. Cell size ranges from 8 to 30 μm and the N:C ratio varies from 7:1 to 3:1. It is critical to obtain an accurate clinical history, since knowledge of a previous diagnosis of lymphoma greatly aids in the identification of these cells. Supplemental studies,

such as immunophenotyping, are often necessary to arrive at a diagnosis.

In chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), the cells are generally small with round to oval nuclei, compact and coarse chromatin, and scant basophilic cytoplasm. They may be the same size as normal lymphocytes or slightly larger. A small nuclear indentation may be present. Nucleoli are inconspicuous. For the purposes of proficiency testing, a single CLL/SLL cell cannot be distinguished from a normal lymphocyte; either lymphocyte or lymphoma cell is a reasonable response in this context. Scattered prolymphocytes, which are larger cells with a centrally placed nucleus, more reticular chromatin, a prominent single nucleolus, and moderate basophilic cytoplasm, often are seen.

In low grade follicular lymphomas, the cells are slightly larger than normal lymphocytes and have an angulated or twisted appearance. The majority of nuclei have clefts, indentations, folds, convolutions, and possibly lobation. The chromatin is moderately coarse and one or more nucleoli may be present. The cytoplasm is scant to moderate and is often basophilic.

The cells in Burkitt lymphoma are generally moderate in size (10 to 25 μm), have a round to oval nucleus with moderately coarse chromatin, and contain one or more prominent nucleoli. The cytoplasm is moderate in amount, is darkly basophilic, and may contain numerous small vacuoles.

Large cell lymphomas may exhibit some of the most abnormal morphology. These cells are large (20 to 30 μm) and have scant to moderate amounts of basophilic cytoplasm. The nuclei are generally round to oval, but may be angulated, folded, indented, or convoluted. Nucleoli are prominent and may be single or multiple. Vacuoles can occasionally be seen in the cytoplasm. These cells can be easily confused with blasts, and additional studies such as immunophenotyping may be necessary to make the correct diagnosis.

T-cell lymphomas have a broad spectrum of morphologies and may appear similar to any of the above types of lymphoma or two Sézary cells (described below).

Sézary Cell

Sézary cells are classically found in patients with leukemic manifestations of mycosis fungoides, a form of primary cutaneous T-cell lymphoma. These cells are usually round to oval, but can be irregular. They range in size from 8 to 20 μm and their N:C ratio varies from 7:1 to 3:1. Smaller Sézary cells are slightly bigger than normal lymphocytes and have folded,

grooved, or convoluted nuclear membranes that may give them a cerebriform appearance. The chromatin is dark and hyperchromatic without visible nucleoli. Larger Sézary cells can be more than twice the size of normal lymphocytes. The nucleus is also convoluted and cerebriform appearing with hyperchromatic chromatin. Often, the nuclear membrane is so folded that the nucleus may appear lobulated or even like a cluster of berries. Some cells may exhibit a small nucleolus, although this is not a prominent feature. Both large and small Sézary cells have scant, pale blue to gray agranular cytoplasm and may contain one or several small vacuoles that lie adjacent to the nucleus. While the appearance of Sézary cells is distinctive, other T-cell lymphomas and some cases of B-cell lymphoma can mimic Sézary cells. Some authors have described small populations of Sézary-like cells comprising up to 6 percent of lymphocytes in normal, healthy individuals.

Hairy Cell

Hairy cells, typical of hairy cell leukemia, are round to ovoid lymphoid cells that measure 12 to 20 μm and are larger than normal, mature lymphocytes. Their N:C ratio ranges from 4:1 to 2:1 and they contain moderate to abundant pale blue to grayish blue cytoplasm. The cell borders are often indistinct secondary to the presence of characteristic elongated, fine (hairy), cytoplasmic projections. These projections are frequently irregular and may be thick, blunted, smudged, serrated, or short. Occasional cases lack these projections and have a smooth cytoplasmic border. Most cells lack granules however, occasional fine azurophilic granules may be seen in some cases. Small vacuoles can be present and often give a mottled appearance to the cytoplasm. The nuclei of hairy cells are usually oval to indented. They may also be folded, bean-shaped, angulated, or dumbbell-shaped. In some cells they are centrally located, while in others they may be eccentrically placed. The chromatin is usually homogeneous and finer than in normal lymphocytes or chronic lymphocytic leukemia cells but may be slightly to moderately coarse. It is evenly distributed with scant, evenly dispersed intervening parachromatin. Nucleoli, if present, are generally small and single. Multiple small nucleoli can be found, and occasional cells may have a single larger nucleolus.

Lymphocyte

While most lymphocytes seen in a blood film are fairly homogeneous, they do exhibit a range of normal morphology. Lymphocytes are small, round to ovoid cells ranging in size from 7 to 15 μm . Their N:C ratio varies from 5:1 to 2:1. Some

normal lymphocytes are medium-sized due to increased amounts of cytoplasm. Most lymphocytes have round to oval nuclei that may be slightly indented or notched. Some normal lymphocytes are medium-sized due to an increased amount of cytoplasm. The chromatin is diffusely dense or coarse and clumped. Nucleoli, if present, are small and inconspicuous. Some cells may exhibit a small, pale chromocenter that may be mistaken for a nucleolus.

Most lymphocytes have a scant amount of pale blue to moderately basophilic, agranular cytoplasm. Occasionally, the edges may be slightly frayed or pointed due to artifacts induced during smear preparation. Some cells show a perinuclear clear zone or halo that surrounds the nucleus. Occasional lymphocytes will have a small clear zone, or hof, adjacent to one side of the nucleus.

Lymphocyte, Large Granular

Large granular lymphocytes are medium to large cells with round nuclei, dense chromatin, and no visible nucleoli. The cytoplasm is moderate to abundant, clear or lightly basophilic, and contains several coarse, unevenly distributed, azurophilic granules. Cell surface marker studies show that these cells are either suppressor/cytotoxic T-lymphocytes (CD3+, CD8+) or natural killer cells (CD3-, CD56+).

Lymphocyte, Reactive (to include Plasmacytoid and Immunoblastic Forms)

The key distinguishing feature of reactive lymphocytes is their wide range of cellular sizes and shapes, as well as nuclear sizes, shapes, and chromatin patterns. These cells are reacting to an immune stimulus and are frequently increased in viral illnesses. The classic example is infectious mononucleosis (acute Epstein-Barr virus infection). Reactive or atypical lymphocytes can also be found in a variety of other viral infections (including cytomegalovirus, adenovirus, or acute HIV infection) protozoal infections (such as toxoplasmosis) some drug reactions, connective tissue diseases, and after a major stress to the body's immune system. A variety of reactive lymphocyte forms have been described and they are often seen concurrently in the same blood film. These round to ovoid to irregular cells range from 10 to 25 μm and the N:C ratio varies from 3:1 to 1:2.

The most common type of reactive lymphocyte resembles a large lymphocyte and corresponds to a Downey type II cell. These cells have round to oval nuclei, moderately condensed

chromatin (giving it a "smeared" appearance), and absent or indistinct nucleoli. They contain abundant pale gray-blue, cytoplasm. Granules, if present, are usually small and few in number. Frequently, these reactive lymphocytes have an amoeboid cytoplasm that partially surrounds adjacent red cells and has a darker-staining, furled margin. Basophilia radiating out from the nucleus may also be present.

Immunoblasts and immunoblastic-like reactive lymphocytes are large cells (15 to 20 μm) with round to oval nuclei. They have finely to moderately dispersed chromatin with abundant parachromatin and one or more prominent nucleoli. These may resemble lymphoma cells or blasts. Their cytoplasm is moderately abundant and stains deeply basophilic. The N:C ratio is high (3:1 to 2:1). These reactive lymphocytes correspond to Downey type III cells.

Another type of reactive lymphocyte is referred to as a Downey I cell. These cells are rare. These cells possess scant to moderate amounts of basophilic cytoplasm. The nuclei often appear indented, folded, or lobulated. The chromatin is condensed. A few small vacuoles may be present. Granules may also be apparent.

Plasmacytoid lymphocytes resemble plasma cells and are intermediate in size (10 to 20 μm) and round to oblong in shape. They have round nuclei that are centrally placed or slightly eccentric. The chromatin is slightly to moderately coarse and forms small dense masses or a meshwork of strands resembling that of plasma cells. Nucleoli are generally not visible, but some cells may have one or two small irregular nucleoli. The cytoplasm is moderately abundant, homogeneous, light blue to deep slate-blue, and may show a perinuclear clear zone, or hof.

Plasma Cell, Morphologically Mature

Normal plasma cells are seen in the bone marrow, lymph nodes, spleen, gastrointestinal tract, and connective tissues, but occasionally are encountered in blood films. Most commonly they are seen in association with either reactive neutrophilia or reactive lymphocytoses of various etiologies. Plasma cells are medium-sized, round to oval cells with moderate to abundant cytoplasm and eccentric nuclei. These cells range in size from 10 to 20 μm and the N:C ratio is 1:2. Their nuclei are usually round to ovoid with prominently coarse and clumped chromatin that is often arranged in a cartwheel-like or clock-face pattern. Nucleoli are absent. The cytoplasm stains gray-blue to deeply basophilic. A prominent hof or perinuclear zone of pale or lighter-staining cytoplasm

is typically seen toward one side of the nucleus and corresponds to the Golgi zone (which is prominent in cells that produce large amounts of protein such as immunoglobulins in the case of plasma cells). Granules are absent, and scattered vacuoles of varying size may be seen. In some cases, plasma cells may show a pink-red cytoplasm. These cells are called “flame cells.”

Plasma Cell, Abnormal (Malignant, Myeloma Cell, Plasmablast)

Immature or atypical plasma cells in the bone marrow or, rarely, in the blood are associated with a variety of plasma cell dyscrasias, including multiple myeloma (plasma cell myeloma), plasmacytoma, and amyloidosis. Immature plasma cells can range from those that are easily recognized as plasma cells to those that are difficult to classify without special studies. Plasmablasts are the least mature form of plasma cell. They are moderate to large, round to oval cells, measuring 25 to 40 μm . They have central to eccentrically placed, round or oval nuclei with finely dispersed chromatin and variable amounts of distinct parachromatin. One or more prominent nucleoli may be present. The nuclei may be eccentric or centrally placed. The N:C ratio is 2:1 to 1:1. Plasmablasts contain scant to moderate amounts of pale to deep blue cytoplasm. Malignant plasma cells, as seen in multiple myeloma, may show a variety of morphologic features and may include some or all forms of plasmablasts, immature plasma cells, and mature plasma cells. Binucleated and multinucleated forms may be frequent and, when present, often display immature nuclear characteristics. Malignant plasma cells may be seen in the peripheral blood in plasma cell leukemias.

Plasma Cell or Precursor with Inclusion Body

Plasma cells normally produce and secrete immunoglobulins. This protein product may appear in different forms within the cytoplasm. When production within a particular plasma cell is increased or when there is a blockage in its secretion, accumulation of immunoglobulin occurs. This finding can occur in mature, immature or malignant plasma cells (as described above). These plasma cells range from 10 to 25 μm and the N:C ratio varies from 1:2 to 1:3.

Accumulations of immunoglobulin often appear as large cytoplasmic eosinophilic globules called Russell bodies. Sometimes these globules appear as intranuclear inclusions called Dutcher bodies. While Dutcher bodies appear to be within the nucleus, they are actually pseudo-inclusions that

occur when a cytoplasmic globule invaginates through the nucleus or is surrounded by the nucleus. When multiple Russell bodies are present, these cells are called “Mott cells.” Occasionally, immunoglobulin inclusions in plasma cells may form crystalline structures in the cytoplasm.

Prolymphocyte

Prolymphocytes are larger lymphoid cells that are seen in cases of chronic lymphocytic leukemia (CLL), where they usually comprise less than 10 percent of lymphoid cells. They can also be found in prolymphocytoid transformation of CLL and B-cell prolymphocytic leukemia (PLL). These round to ovoid cells range from 10 to 18 μm and the N:C ratio varies from 5:1 to 3:1. They are larger than normal lymphocytes and the typical CLL cells and are similar in size to lymphoblasts. A centrally placed, oval to round nucleus, and a moderate amount of homogeneously staining, blue cytoplasm are typical. The cytoplasm is more abundant than in normal lymphocytes and blasts and may contain a few azurophilic granules. The nucleus shows somewhat condensed chromatin (coarser than in lymphoblasts and more open than in mature lymphocytes) with indistinct parachromatin and, typically, a single, prominent nucleolus. Occasionally, these cells may exhibit more than one nucleolus.

Microorganisms

Babesia

Babesia microti and related organisms are intracellular parasites that are often confused with malaria. The organisms range in size from 1 to 5 μm , mimicking the ring forms of malaria. They may be round, oval, elongate, ameboid, or pyriform. Pyriform organisms form a “Maltese cross” after division into four organisms. *Babesia* will form teardrop-shaped organisms that occur in pairs at right angles to one another. The tetrad arrangement of the merozoites and the lack of other findings on the peripheral blood smear are most helpful in distinguishing these organisms from malaria. In addition, Schüffner’s granules are absent, as are the schizont and gametocyte forms of malaria. Organisms are smaller and more commonly extracellular with *Babesia* than with *Plasmodium* species. Other potential look-alikes include platelets or stain precipitate overlying erythrocytes. Thick blood films are preferred for diagnosis, where one will see tiny chromatin dots and wispy cytoplasm.

Bacteria (Cocci or Rod), Extracellular

Although bacteremia is relatively common, it is quite unusual to identify bacteria on a random blood film. In most cases, this finding represents an overwhelming infection. When present, individual organisms are typically 1 μm in size, although there is considerable variation in size and shape—organisms can range from cocci to bacilli and can occur singly, in clusters, or in chains. A Gram stain can be useful in confirming the presence of bacteria and in separating organisms into Gram-positive and -negative groups.

The most likely error in interpretation is to misidentify stain precipitate as microorganisms. This error can be avoided by remembering that bacteria tend to be relatively uniform in size and shape, while stain precipitate is often irregular in shape and individual grains vary considerably in size. In addition, extracellular bacteria may represent a stain contaminant. Careful search should be made for intracellular organisms, as this finding indicates a true bacteremia.

Bacteria (Spirochete), Extracellular

Pathogenic spirochetes include members of the genera *Leptospira*, *Borrelia*, and *Treponema*, but only *Borrelia*, is encountered on peripheral blood films. These bacteria are 5

to 25 μm long and 0.2 to 0.5 μm wide, with 4 to 30 helical coils. The organisms can be seen in fresh wet-mount preparations, on thin Giemsa-stained blood films, or on thick Giemsa-stained blood preparations. A concentration technique can be used in mildly infected persons. Fibers, thread, or hair contamination may mimic spirochetes, but should be easily distinguished as artifacts, given their lack of uniform coiling.

Leukocyte with *Ehrlichia/Anaplasma*

Recognized as an arthropod-borne infectious agent in humans, members of the genus *Anaplasma* (previously *Ehrlichia*) are small, Gram-negative, obligate intracellular organisms currently classified as rickettsiae. On Wright-stained preparations, *Anaplasma* species appear as round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either PMNs (*A. phagocytophilum*) or monocytes and macrophages (*A. chafeensis*). The morulae are microcolonies of elementary bodies.

Fungi, Extracellular

Extracellular fungi are most commonly seen in the bone marrow, but fungi such as *Histoplasma capsulatum* can rarely be identified in peripheral blood films in an extracellular location. The organisms are usually associated with intracellular organisms as well. When visualized, they indicate a serious infection. Probably the most frequently seen fungus in the bone marrow is *Histoplasma capsulatum*, but the organisms are nearly always exclusively present within macrophages as 1- to 2- μm budding yeast forms. They are only rarely seen in an extracellular location, usually when the cell membranes of the macrophages have ruptured. The other organisms, such as *Coccidioides*, *Cryptococcus*, *Candida*, and *Aspergillus*, occur less frequently but are more commonly extracellular. The appearance of the fungal form is dependent upon the specific organism. *Coccidioides* typically shows mature spherules ranging between 20 to 60 μm , and contains endospores ranging from 2 to 4 μm . *Cryptococcus* is a round to oval yeast-like fungus ranging from 3.5 to 8 μm or more in diameter, usually with a thick mucopolysaccharide capsule, and demonstrating a narrow neck when budding. *Candida* can appear in bone marrow as either yeast-like organisms with budding or as pseudohyphae. *Aspergillus* is typically identified by its septate 4- μm -wide hyphae with characteristic 45° branching. Most organisms will stain with a periodic acid-Schiff (PAS) stain, but are accentuated by Gomori’s methenamine silver (GMS) staining.

Leukocyte (Blood) with Phagocytized Bacteria

As noted under “Bacteria (cocci or rod), Extracellular,” it is very unusual to see bacteria on a random blood film. This finding usually represents an overwhelming infection. When present, the bacteria may be ingested by neutrophils or monocytes and can be seen within the cytoplasm of these cells. Although leukocytes with phagocytized bacteria are rare in the blood film, they are commonly seen in infected body fluids. When present within neutrophils, bacteria can be difficult to distinguish from toxic granulation. However, toxic granulation tends to involve nearly all of the cytoplasm of the neutrophil, whereas engulfed bacteria are usually few in number. In addition, bacteria are typically larger than toxic granules, measuring around 1 μm in size, and are more defined in shape, ranging from cocci to bacilli and occurring singly, as diplococci, or in clusters or chains. They can be accentuated and confirmed with a Gram stain.

Leukocyte (Blood) with Phagocytized Fungi

Fungi are only rarely visualized in peripheral blood. When present, the fungi are usually seen within the cytoplasm of monocytes, macrophages, or neutrophils. Phagocytized fungi are usually localized within a vacuole that forms a clear halo around the organism. Usually the number of organisms present is scant. Clinical history and blood cultures are also very important in making the appropriate identification.

Histoplasma capsulatum is most frequently seen; *Candida albicans* can be seen, but is exceptionally rare. Although other fungi can be grown from blood cultures and therefore are present in the circulation, the level of fungemia is so low that they are virtually never visualized on a blood film. Intracellular fungi can be confused with precipitated stain overlying a leukocyte, large toxic granules, Döhle bodies, or large bacterial cocci.

Macrophage (Bone Marrow) with Phagocytized Fungi, *Leishmania*, *Toxoplasma*

Histoplasma capsulatum is a 1- to 2- μm budding yeast that typically is present in large numbers within the cytoplasm of macrophages within the bone marrow of infected patients. The organisms may manifest a crescent or ring shape, and also may be surrounded by a small halo, both of which are artifacts, but helpful in diagnosis. The amastigote form of the parasite

Leishmania has a similar size and appearance within marrow macrophages, but is recognized by the additional presence of a dot-like kinetoplast associated with each organism. The unicellular tachyzoites of *Toxoplasma gondii* also imitate *Histoplasma* morphologically, but do not stain positively with the Gomori’s methenamine silver (GMS) stain. The biggest diagnostic problem with this group of organisms is their ability to imitate each other, but they can also be confused with other budding yeast organisms, large bacterial cocci, or phagocytized material, particularly cells. If the macrophage has ruptured, extracellular organisms may be mistaken for platelets.

Macrophage with Phagocytized Mycobacteria

The mycobacteria are responsible for a variety of clinical infections, with tuberculosis and leprosy being the best known. At least 25 species of mycobacteria are causative agents of human disease and several species can infect the bone marrow. The two species that most commonly involve the bone marrow are *Mycobacterium tuberculosis* and *Mycobacterium avium* complex. *M. tuberculosis* elicits a granulomatous response with or without caseous necrosis, while *M. avium-intracellulare* is usually seen in large numbers within bone marrow macrophages with or without a granulomatous response. When a granulomatous response is present, organisms may be rare and difficult to find. The mycobacteria are straight to slightly curved bacilli varying from 0.2 to 0.6 μm in width and 1 to 10 μm in length. They are acid-fast (due to the high lipid content in the cell wall) and may appear beaded on acid-fast stain. The organisms appear as nonrefractile “negative images” or clear or red refractile beaded rods on Romanowsky-stained preparations. The incidence of disseminated *M. avium-intracellulare* infection has greatly increased as the population of patients with HIV/AIDS has expanded. Because this organism often does not elicit a granulomatous response, some authors have advocated routine use of the acid-fast stain (and the Gomori’s methenamine silver stain for fungi) on marrow biopsies in all patients with HIV.

Plasmodium Sp. (Malaria)

There are four species of *Plasmodium* that cause the clinical disease known as malaria: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. The different shapes and appearance of the various stages of development and their variations between species are distinctive. The ring forms of all four types of malaria are usually less than 2 μm in diameter. Trophozoites range from 3 to 8 μm , depending on the species. Schizonts

and gametocytes range from approximately 5 to 11 μm . Two species have enlarged infected erythrocytes (*P. ovale* and *P. vivax*). Schüffner stippling (a golden brown to black pigment in the cytoplasm of the infected erythrocyte) is most conspicuous in infections with *P. ovale* and *P. vivax*. Multiple stages of organism development are seen in the peripheral blood with all species except *P. falciparum*, where the peripheral blood usually contains only ring forms and gametocytes (unless infection is very severe). Multiple ring forms within one erythrocyte are also most common with *P. falciparum*, and are not seen with *P. malariae*. Mixed infections occur in 5 to 7 percent of patients. Potential look-alikes include platelets overlying red blood cells, clumps of bacteria or platelets that may be confused with schizonts, masses of fused platelets that may be confused with a gametocyte, precipitated stain, *Babesia* infection, and contaminating microorganisms (bacteria, fungi, etc.).

Microfilaria

There are eight main species of filariae that infect humans. The microfilariae of five of the species circulate in the blood, some on a regular periodicity and others sporadically. The other three species do not circulate and are identified from small biopsies of skin and subcutaneous tissue. All microfilariae are elongate cylindrical bodies with one tapered end, one rounded end, and smooth contours. Nuclei are arranged in a chain, filling most of the body. Some species have a thin-covering transparent sheath. They vary from 160 to 315 μm in length and 3 to 10 μm in width on a stained blood film. When microfilariae circulate in the peripheral blood, it is in low number, and, as a result, they can be difficult to detect on a thin blood film stained with Wright-Giemsa. In order to decrease the number of false-negative results, thick smears (such as those used in diagnosing malaria), concentration methods, or membrane filtration are used. Once the organisms are identified in the blood, speciation is usually possible using various morphologic parameters, including size, shape, presence or absence of an investing sheath, and the disposition of nuclei in the tail. The patient's travel history is also helpful, as various species occur in different parts of the world. These morphologic and geographic features have been reviewed in many texts. Microfilariae should not be confused with trypanosomes, chains of bacteria or fungi, nor with artifacts such as fibers or threads.

Trypanosomes

The trypanosomes are protozoan hemoflagellates, along with *Leishmania*, and are characterized by the presence of a kinetoplast. The trypomastigote stage is seen in the peripheral blood and shows a long, slender body with a kinetoplast at the posterior end, an undulating membrane and axoneme extending the entire length, and a flagellum at the anterior end, representing an extension of the axoneme. Trypomastigotes of the *Trypanosoma brucei* group are up to 30 μm long with graceful curves and a small kinetoplast; trypomastigotes of *T. cruzi* are shorter (20 μm), with S and C shapes and a larger kinetoplast. Trypanosomes should not be confused with artifacts, such as fibers or threads, or microfilarial organisms.

Artifacts

Basket Cell/Smudge Cell

A basket cell or smudge cell is most commonly associated with cells that are fragile and easily damaged in the process of making a peripheral blood smear. The nucleus may either be a nondescript chromatin mass or the chromatin strands may spread out from a condensed nuclear remnant, giving the appearance of a basket. Cytoplasm is either absent or indistinct. Smudge cells are usually lymphocytes, but there is no recognizable cytoplasm to give a clue to the origin of the cell. They are seen most commonly in disorders characterized by lymphocyte fragility, such as infectious mononucleosis and chronic lymphocytic leukemia. Basket cells should not be confused with necrobiotic neutrophils, which have enough cytoplasm to allow the cell to be classified.

Neutrophil Necrobiosis (Degenerated Neutrophil)

Neutrophil necrobiosis is a common phenomenon that can be seen both in normal individuals and in patients with a variety of medical conditions, including infections, inflammatory disorders, and malignancies. It is nondiagnostic and nonspecific. Degenerated neutrophils are generally easily identified because they resemble normal segmented neutrophils. They are round to oval cells ranging from 10 to 15 μm and their N:C ratio is 1:3 or less. The major distinguishing feature is that the nucleus shows karyorrhexis and/or pyknosis. These changes are appreciated when a cell with neutrophilic granules (pale pink cytoplasm with fine lilac

granules) contains multiple, unconnected nuclear lobes (karyorrhexis) or a single, dark, round to oval nucleus (pyknosis). The chromatin is dense and homogeneous without visible parachromatin or nucleoli. The nuclear lobes may fragment into numerous small particles of varying size that can resemble microorganisms such as bacteria or fungi. Also, the nuclear outlines may become indistinct and blurred. As the cellular degeneration continues, the cytoplasm will become hypogranulated, then agranular, and the cytoplasmic borders may become frayed and indistinct. Sometimes, the cells will contain scattered larger azurophilic or dark blue granules (toxic granulation). Vacuolation is frequent. If a cell is too degenerated to be recognized as a neutrophil and lacks recognizable cytoplasm, one should identify it as a basket/smudge cell. On occasion, necrobiotic neutrophils can contain ingested bacteria or fungi. However, the microscopist must be very careful when making this identification since nuclear fragments may appear similar and deceive the observer. Other cells that may resemble degenerated neutrophils are nucleated red cells in the blood and orthochromic normoblasts in the bone marrow. These cell types have pinkish orange, agranular cytoplasm and a single, often eccentric nucleus with dense chromatin and very little to no parachromatin.

Erythrocyte with Overlying Platelet

In preparing a wedge smear of the peripheral blood, platelets may adhere to or overlap red cells, suggesting a red cell inclusion or parasite. A correct interpretation depends on carefully examining the morphology of the platelet and comparing the size, staining characteristics, and granularity with known platelets in the same field as well as determining if the platelet is in the same plane of focus as the red cell. Many times the platelet is surrounded by a thin clear zone or halo, which is not a feature of most genuine red cell inclusions.

Stain Precipitate

Stain precipitate on a Wright-Giemsa smear is usually due to unclean slides or improper drying of the stain on the smear. Oxidized stain appears as metachromatic red, pink, or purple granular deposits on and between cells. The stain may adhere to red cells and be mistaken for inclusions, parasites, or infected cells. The size of the stain droplets is variable and this can be helpful in discerning their origin. Yeast and bacteria have a more uniform morphology than precipitated stain. Organisms are usually rare and dispersed throughout the slide;

they do not circulate in large aggregates. Stain deposits, on the other hand, may be very focal and intense.

Miscellaneous

Alder Anomaly Inclusion

Alder anomaly inclusions are large, purple or purplish black, coarse, azurophilic granules resembling the primary granules of promyelocytes. They are seen in the cytoplasm of virtually all mature leukocytes and, occasionally, in their precursors. At times, the granules may be surrounded by clear zones or halos. The prominent granulation in lymphocytes and monocytes distinguishes these inclusions from toxic granulation, which only occur in neutrophils. Alder anomaly inclusions are seen in association with the mucopolysaccharidoses (MPS), a group of inherited disorders caused by a deficiency of lysosomal enzymes needed to degrade mucopolysaccharides (or glycosaminoglycans).

Blast Cell

A blast is a large, round to oval cell, 10 to 20 μm in diameter. In the blood film, the cell may appear flattened or compressed by adjacent red cells. The nuclear-to-cytoplasmic ratio is high, varying from 7:1 to 1:1. The blast often has a round to oval nucleus, but sometimes is indented or folded with fine, lacy to granular chromatin; one or more prominent nucleoli may be present. The cytoplasm is basophilic and agranular. In the absence of lineage-associated findings, such as Auer rods, cytoplasmic granules, cytochemical data (e.g., peroxidase or Sudan black B reactivity), or cell surface marker data, it is not possible to define the lineage of a given blast cell. Therefore, blastic cells without definitive cytoplasmic inclusions should be identified as “blast, not otherwise specified” unless ancillary data are available to aid in classification as “myeloblast” or “lymphoblast.”

Chediak-Higashi Anomaly Inclusion

Giant, often round, red, blue, or greenish gray granules of variable size are seen in the cytoplasm of otherwise typical leukocytes (granulocytes, lymphocytes, and monocytes) and sometimes normoblasts in patients with Chediak-Higashi syndrome. These may be single or in aggregates. Platelets and megakaryocytes are unaffected. A poorly understood membrane abnormality results in fusion of primary (azurophilic) and, to

a lesser extent, secondary (specific) lysosomal granules, resulting in poor function in killing phagocytized bacteria.

Cryoglobulin

Cryoglobulins are immunoglobulins that precipitate when cooled. They may cause a clinical syndrome that can include joint pain, Raynaud's phenomenon, skin lesions, and renal abnormalities. Rarely, cryoglobulins may be observed in routine peripheral blood smears. Typically these immunoglobulin precipitates take the form of cloud-like, extracellular masses of blue, amorphous material. The intensity of staining of these aggregates varies from case to case, such that they range from very pale, barely visible deposits to obvious, dense masses. Rarely, cryoglobulins may be diffusely distributed in a blood smear as fine droplets. Also rare is phagocytosis of cryoglobulin by neutrophils or monocytes, producing pale blue to clear cytoplasmic inclusions that mimic vacuoles.

Squamous Epithelial Cell/Endothelial Cell

Squamous epithelial cells are large (30 to 50 μm), round to polyhedral-shaped cells with a low nuclear-to-cytoplasmic ratio (1:1 to 1:5). The nucleus is round to slightly irregularly shaped, with dense, pyknotic chromatin and no visible nucleoli. The abundant cytoplasm is lightly basophilic and may show keratinization or a few blue kerato-hyaline granules. Epithelial cells from deeper layers of the epidermis have larger nuclei with a high nuclear-to-cytoplasmic ratio. In contrast to squamous carcinoma, contaminant squamous epithelial cells lack nuclear atypia. Squamous epithelial cells (derived from the skin) rarely may contaminate peripheral blood, particularly when smears are obtained from finger or heel punctures.

Endothelial cells are a normal component of the bone marrow, lining capillaries and sinuses. They have an elongated or spindle shape, approximately 5 μm wide by 20 to 30 μm long, with a moderate nuclear-to-cytoplasmic ratio (2:1 to 1:1). The oval or elliptical nucleus occasionally is folded and has dense to fine, reticular chromatin. One or more nucleoli may be visible. The frayed cytoplasm tapers out from both ends of the nucleus and may contain a few azurophilic granules.

Endothelial cells have a similar, if not identical, appearance to fibroblast-like cells (reticulum cells) that make up the skeletal framework of the bone marrow. Endothelial cells (lining blood vessels) rarely may contaminate peripheral blood, particularly when smears are obtained from finger or

heel punctures. When present as a contaminant in blood smears, endothelial cells may occur in clusters.

Lipocyte (Adipocyte, Fat Cell)

The lipocyte, a normal constituent of yellow or fatty marrow, is a large (25 to 75 μm in diameter) round to oval cell with a very small, densely staining, eccentric or flattened nucleus (N:C ratio <1:10). Occasionally, a globular body, thought to be fatty material, is seen in the nucleus. The fat-laden cytoplasm is abundant and often consists of a single, colorless fat vacuole, giving the cell a signet-ring appearance. Alternately, it may appear to contain numerous large fat vacuoles, separated by delicate, light blue or pink cytoplasm. Eosinophilic fibrils may be present, both within the cytoplasm and extending outward from the cell margins. The lipocyte, a fat-producing cell, is to be distinguished from a macrophage with phagocytized fat (or "lipophage"). The lipid-laden macrophage contains small, uniform lipid particles, giving the cytoplasm a foamy or bubbly appearance.

Macrophage (Histiocyte)

A macrophage is a large (15 to 80 μm in diameter) phagocytic cell. It is irregular in shape, frequently with shaggy margins and bleb-like or filiform pseudopodia. The nucleus usually is round or oval, but occasionally may be indented. The nuclear membrane is distinct, and the nuclear chromatin is fine with a spongy, reticular pattern. One or more small nucleoli may be seen. The abundant frayed, streaming cytoplasm (N:C approximately 3:1) is abundant, pale gray-blue, and often granulated (coarse, azurophilic granules).

Phagocytized material (white cells, red cells, platelets, nuclei or their remnants, and microorganisms) may be present in native or degraded form within the cytoplasm. Cytoplasmic vacuoles may be abundant, and may contain phagocytized material or appear empty. Iron is stored in bone marrow macrophages as ferritin or hemosiderin (demonstrated with Prussian blue stain). The stored iron derives almost exclusively from phagocytosis and degradation of senescent or defective erythrocytes.

Less phagocytic macrophages sometimes are referred to as "histiocytes." They have fewer lysosomal granules and may play a role in antigenic presentation to lymphocytes, cell-cell interactions in the immune system, and production of mediators important in inflammatory and immune responses. Histiocytes may cluster together, forming an epithelioid agglomeration, or fuse to form multinucleated giant cells.

These aggregated, epithelioid histiocytes or Langhans' giant cells often are prominent components of marrow granulomas, a finding best appreciated in the bone marrow biopsy.

Macrophage with Phagocytized Red Cell (Erythrophagocytosis)

The cytoplasm of macrophages may contain one or more intact erythroid cells as well as degraded erythroid forms within vacuoles. With further digestion, dark blue hemosiderin granules may be evident. Phagocytosis of erythrocytes often occurs concomitantly with macrophage ingestion of neutrophils and/or platelets (hemophagocytosis). Erythrophagocytosis is not common in peripheral blood smears but can be observed.

Gaucher Cell and Pseudo-Gaucher Cell

A Gaucher cell is a form of histiocyte (macrophage) that is ovoid and measures 20 to 90 μm in diameter with a low nuclear-to-cytoplasmic ratio (less than 1:3). It contains a small, round or oval nucleus with indistinct nucleoli. The chromatin is coarse. The cytoplasm is abundant, lipid-laden (containing glucosylcerebroside), and stains gray to pale blue. A fibrillar, reticular, "crumpled cellophane," or "wrinkled tissue paper" appearance of the cytoplasm is characteristic. This distinctive linear striation results from lamellar bodies stacked within secondary phagolysosomes.

A morphologic variant shows less striking linear striation and contains a small number of fine blue cytoplasmic granules. The cells stain for PAS and lysosomal enzymes such as acid phosphatase (tartrate-resistant) and nonspecific esterase. Gaucher's disease is an inherited deficiency of beta-glucocerebrosidase, leading to accumulation of glucosylcerebroside in a variety of tissues, including bone, liver, lung, and brain.

Pseudo-Gaucher cells are indistinguishable from true Gaucher cells on light microscopy, although they differ ultrastructurally. They are phagocytic cells engaged in catabolism of glycoside from the membranes of dead cells. These macrophages have normal amounts of beta-glucocerebrosidase enzyme and are postulated to arise from excessive cell breakdown with an overload of glucoceramide. Pseudo-Gaucher cells have no diagnostic significance.

Histiocyte, Sea Blue

These bone marrow cells are macrophages (histiocytes) that have abundant cytoplasm filled with variably sized bluish or

bluish green globules or granules of insoluble lipid pigment called ceroid. Ceroid, a Latin term for wax-like, is a pigment of uncertain identity thought to represent partially digested globosides derived from cell membranes. In H&E-stained marrow sections, the histiocytes appear foamy or slightly eosinophilic and contain a variable number of yellow to yellow-brown granules. They are distinguished from hemosiderin-laden macrophages (siderophages) by a negative Prussian blue stain. Small numbers of sea-blue histiocytes may be seen in normal marrows and should not be considered a pathologic finding. Large numbers occur in marrow, spleen, and liver in an inherited disorder of unknown cause called the "sea blue histiocyte syndrome." Occasional to moderate numbers of sea-blue histiocytes can be seen in other lipid storage diseases, hyperlipidemias, chronic myeloid leukemia, and in any disorder with massively increased intramedullary cell destruction.

Niemann-Pick Cell, Foamy Macrophage

Niemann-Pick disease is an inherited deficiency of the lysosomal enzyme sphingomyelinase, leading to extensive accumulation of sphingomyelin in a variety of tissues, including the bone marrow. The Niemann-Pick cell is a sphingomyelin-laden histiocyte of variable size (20 to 90 μm diameter) with abundant cytoplasm (nuclear-to-cytoplasmic ratio less than 1:10). The cell has one or more small, round nuclei with coarse chromatin. The cytoplasm is vacuolated and foamy with a mulberry-like appearance. Some variants of Niemann-Pick disease have mixtures of foamy macrophages and sea-blue histiocytes, probably representing breakdown of the stored sphingomyelin to ceroid. Blood lymphocytes and monocytes also may display cytoplasmic vacuoles containing sphingomyelin. Although foamy macrophages characterize Niemann-Pick disease, they may be seen in other conditions, including inherited deficiencies in the metabolism of lipid materials (e.g., gangliosidoses, Fabry's disease, and lactosyl ceramidosis) or excess accumulation of lipid material in bone marrow macrophages (e.g., hyperlipidemias, thalassemias, rheumatoid arthritis, sickle cell anemia, thrombocytopenic purpura, infectious mononucleosis, chemotherapy-induced marrow aplasia, hepatitis, and chronic renal failure). The foamy macrophages in these disorders differ slightly from Niemann-Pick cells in that their vacuoles may be larger and are more irregular in size.

Mast Cell

The mast cell is a large (15 to 30 μm) round or elliptical connective tissue cell with a small, round nucleus and abundant cytoplasm packed with black, bluish black, or reddish purple metachromatic granules. Its relationship to circulating basophils or their precursors is unclear. Mast cells are differentiated from blood basophils by the fact that they are larger (often twice the size of blood basophils), have more abundant cytoplasm, and have round rather than segmented nuclei. The cytoplasmic granules are smaller, more numerous, more uniform in appearance, and less water-extractable than basophil cytoplasmic granules. Although both mast cells and basophils are primarily involved in allergic and anaphylactic reactions through degranulation, the content of their granules is not identical. Both mast cell and basophil granules can be differentiated from neutrophilic granules by positive staining with toluidine blue in the former.

Systemic mast cell disease, best appreciated in the bone marrow biopsy, usually shows focal marrow lesions, frequently perivascular or paratrabeular. The aggregates of mast cells often are accompanied by eosinophils and can be associated with prominent lymphoid aggregates, with marrow fibrosis, and occasionally with osteoporosis or osteosclerosis. In some cases, the uninvolved marrow is hypercellular with a concomitant myeloproliferative or myelodysplastic process. Cytologically, the neoplastic mast cell may exhibit an irregular, elongated spindle shape with cytoplasmic extensions, nuclear atypia, and degranulation. Diffuse infiltration of the marrow by malignant mast cells is less frequently observed, and may be associated with mast cell leukemia. Mast cells can be highlighted by immunostaining for tryptase, a marker specific for mast cells.

Metastatic Tumor Cell

Metastatic tumor cells are larger than most bone marrow cells, except megakaryocytes, varying from approximately 15 μm to 100 μm in diameter, with a highly variable nuclear-to-cytoplasmic ratio (7:1 to 1:5). They frequently adhere in tight clusters, forming syncytial sheets or mulberry-like aggregates (“morulae”), best detected in the periphery of the aspirate smear. Within a given sample, the tumor cells often are polymorphous, varying in cell size and shape. Likewise, nuclei are round, spindle-shaped, or pleomorphic, and multiple nuclei of unequal size and shape may be present. The nuclear chromatin usually is finely reticulated, often with prominent parachromatin spaces; one or more large nucleoli

may be seen. Rapidly proliferating tumors can show many mitotic forms and many small autolytic cells with nuclear pyknosis or karyorrhexis. The amount of cytoplasm is variable, scant in small cell tumors (e.g., oat cell carcinoma, neuroblastoma, retinoblastoma, rhabdomyosarcoma, and Ewing’s sarcoma) and plentiful in others, particularly adenocarcinoma. The cytoplasm may be intensely basophilic, may contain granules or fine vacuoles, may contain bluish cytoplasmic debris, or may contain large vacuoles (especially adenocarcinoma). The cytoplasm often appears frayed on the aspirate smear due to pulling apart of cohesive tumor cells. Keratin formation may be apparent in squamous carcinoma.

Nonhematopoietic malignant cells frequently are unrespirable (“dry tap”); thus, biopsy sections are preferred for the detection of metastatic tumors. In addition, the organization of tumor cells into glandular or rosette structures and tumor-associated fibrosis may not be detected in marrow smears. In the bone marrow biopsy, an inflammatory response (macrophages, lymphocytes, and plasma cells) may be seen adjacent to the metastatic site. Nests or cords of tumor cells may be trapped and compressed within dense fibrous tissue, making it difficult to discern cytological features. Cytochemistry and immunohistochemistry are useful in distinguishing metastatic neoplasia from hematopoietic malignancy and in determining tumor origin. The presence of a leukoerythroblastic reaction (i.e., immature neutrophils plus nucleated red cells) in the blood is associated with involvement of bone marrow by metastatic tumor.

Mitotic Figure

A cell containing a mitotic figure is variable in size; it may or may not be larger than the surrounding cells. The cytoplasm has color and granulation characteristic of the resting cell. When a cell undergoes mitosis, typical nuclear features no longer are present. Instead, the nucleus appears as a dark, irregular mass, often with a clear central zone. It may take various shapes, including a daisy-like form or a mass with irregular projections. In metaphase, the individual chromosomes become visible; arranged equatorially, they begin to separate and to move toward opposite poles.

Rarely, the anaphase or telophase of mitosis may be seen, with two separating masses of chromosomes forming two daughter cells. A mitotic cell can be distinguished from a degenerating cell by a relatively compact nucleus (or nuclei); a degenerating cell often displays a pyknotic nucleus that has been fragmented into numerous purple, roundish inclusions. Although the bone

marrow is normally a rapidly dividing tissue, only small numbers of mitoses are found in normal marrow aspirates. Cells in mitosis are rarely seen in the blood smear, usually associated with hematopoietic malignancy.

Osteoblast

The osteoblast is a bone-forming cell, producing bone matrix (osteoid), which, when mineralized, becomes lamellar bone. It is large (25 to 30 µm) in diameter, often elliptical, and contains a round or ovoid nucleus with one or more nucleoli. The nucleus may be partially extruded from the cell. The cytoplasm is abundant, stains blue-gray, and may have an indistinct, streaming border. A prominent clear zone (hof or Golgi zone) is usually evident a small distance away from the nucleus. Although they resemble plasma cells, osteoblasts may be distinguished by their larger size (at least twice as large as plasma cells), elliptical shape, lightly basophilic cytoplasm, prominent clear zone away from (rather than next to) the nucleus, fine reticular nuclear chromatin, and one or more nucleoli. Osteoblasts often occur in clusters in the marrow of growing children; small numbers may be seen in adult specimens. In bone marrow biopsies, they are located along the margins of the bone trabeculae.

Osteoclast

Osteoclasts are involved in bone resorption, frequently located along the bone trabeculae. They are very large cells, approximately 100 µm in diameter. Though osteoclasts resemble megakaryocytes, they can be differentiated by the presence of an even number of multiple round to ovoid nuclei, relatively uniformly shaped, but widely separate. The nuclear chromatin may be dense or reticular, and each nucleus usually contains one or more small, prominent nucleoli. The cytoplasm is abundant, with frayed margins, stains blue or purple to pale pink, and contains many fine reddish purple granules. Osteoclasts are most frequently seen in marrow samples from children or from patients with Paget's disease or hyperparathyroidism.

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URINE SEDIMENT CELL IDENTIFICATION

Introduction to Urine Sediment

The following descriptions of objects found in the urine are intended only as a guide to photomicrograph-based proficiency testing. More complete descriptions of the subject are available in standard atlases and textbooks, some of which are listed in the references to this section. In clinical practice it is recommended that all urine specimens with positive reagent strips for hemoglobin, protein, leukocyte esterase, or nitrite, and all turbid or abnormally colored urines be examined microscopically.

The adjective “broad” may be attached to all of the specific casts described below. Broad casts are defined as being wider than twice the length of a renal tubular epithelial cell. While this is a nonspecific term, as renal tubular epithelial cells are not often found in the same field as the cast in question, it is a helpful reference standard to have in mind when evaluating casts. Broad casts are important as they are considered to originate in dilated, atrophic tubules and the term “renal failure casts” is often applied to them. Thus, it is possible to recognize “broad granular casts,” “broad waxy casts,” etc. They are important to identify and report as their presence suggests chronic renal disease.

The photomicrographs presented in this Survey are typically unstained wet preparations, but split-screen photomicrographs may be used to demonstrate other techniques. Microscopic imaging includes bright-field, phase-contrast, and polarizing techniques. The use of polarized microscopy in the analysis of urine sediment is recommended. The procedure is easily and quickly performed and provides a ready means of distinguishing many birefringent crystals, oval fat bodies, and fibers from nonbirefringent casts and other structures.

Urinary Cells

Erythrocyte

Under high power, unstained red blood cells in wet preparations appear as pale yellow-orange discs. They vary in size, but are usually about 7 to 8 μm in diameter. With dissolution of hemoglobin in old or hypotonic specimens, cells may appear as faint, colorless circles or “ghosts.” These ghost membranes are more defined with phase-contrast microscopy.

Red blood cells may become crenated in hypertonic urine and appear as small, rough cells with irregular edges and surfaces. Smooth, shrunken, and crenated cells may all be seen in the same urine specimen. Surface crenations on erythrocytes may suggest the presence of granules and the cells may be confused with small granulocytes. Red blood cells may be confused with

oil droplets or yeast cells. Oil droplets (mineral oil or vaginal creams) show a great variation in size and are usually highly refractile. Endogenous lipid droplets also vary in size. Yeast cells are oval to round, generally smaller than erythrocytes, nearly colorless, and often show budding.

Small numbers of erythrocytes, less than three per high power field, may be found in the urine sediment of otherwise normal patients. Hematuria, or the presence of increased numbers of RBCs in the urine, suggests possible disease anywhere in the kidney or urinary tract. Generalized bleeding disorders, trauma, and the use of anticoagulants also may produce hematuria. Contamination of the urine by menstrual blood frequently causes falsely positive test results. Nucleated red cells and sickle cells are only rarely seen in the urine of patients with sickle cell disease. Macrophages containing ingested red cells may be seen in the urine of patients with chronic hematuria.

Erythrocyte, Dysmorphic

Dysmorphic red cells are strongly suggestive of glomerular bleeding, typically glomerulonephritis. As described by Birch and Fairley and confirmed by others, these are red cells that, when examined by phase-contrast microscopy, demonstrate loss of the limiting membrane or the presence of cytoplasmic blebs (“Mickey Mouse ears”). Subsequent publications have reduced the specificity for glomerular hematuria by loosely applying the term “dysmorphic red cells” to include abnormal poikilocytes found in air-dried Wright-Giemsa-stained blood smears (codocytes, stomatocytes, acanthocytes, etc.), which may occur in patients without renal disease. A specific type of dysmorphic erythrocyte known as the “G1 Cell” was described by Dinda, 1997, and may be more specific for glomerular hemorrhage. It is described as “doughnut-shaped with one or more membrane blebs.”

Neutrophil, Unstained

In unstained wet preparations, neutrophil leukocytes appear as colorless granular cells about 12 μm or nearly twice the size of a red cell. Dense granular neutrophils, not much larger than a red cell, and large swollen neutrophils may occur in the same specimen. Ingested bacteria or yeast in the cytoplasm occasionally crowds the nucleus and enlarges the cell by two to three times. In freshly voided urine, nuclear detail is well-defined. With cellular degeneration, nuclear segments fuse into a single, round nucleus, and cytoplasmic granules may be lost, making distinction from renal tubular cells difficult or impossible.

In dilute or hypotonic urine, neutrophils swell. There also may be small intracytoplasmic vacuoles and loss of nuclear segmentation. Cytoplasmic granules wiggle or “dance” due to Brownian movement. Neutrophils containing these refractile “dancing” granules are called “glitter” cells. Neutrophils are actively phagocytic and can often be seen to extend pseudopods and show amoeboid motion. These cells stain poorly.

Increased numbers of leukocytes in the urine, principally neutrophils, are seen in most urinary tract disorders. Leukocytes from secretions of the male and female genital tracts can also be present. The presence of many neutrophils and/or clumps of leukocytes in the sediment is strongly suggestive of acute infection. However, small numbers of neutrophils, usually less than five per high power field (hpf), may be found in the urine of normal persons.

Neutrophil, Stained

The neutrophil is usually easy to identify. The nucleus often is segmented or lobulated into two to five lobes which are connected by a thin filament of chromatin. The abundant, pale pink cytoplasm contains many fine, lilac-colored granules. The nuclear lobes may appear eccentric and the cytoplasm may be vacuolated. Nuclear pyknosis and fragmentation in degenerating neutrophils can make recognition difficult. Cyto centrifuge (cytospin) preparation may reveal artifacts, cellular distortion, and cellular degeneration.

Eosinophil, Unstained

In unstained wet preparations, eosinophils appear slightly larger than neutrophils and may be oval or elongated. Cytoplasmic granules are less prominent. In fresh specimens, two or three large nuclear segments are apparent.

Eosinophil, Stained

Eosinophils are recognized by their characteristic bright orange-red spherical granules. These granules are larger than primary or secondary granules in neutrophils. The nucleus typically has two or more lobes separated by a thin filament. Urinary eosinophils, unlike those found in blood smears, may not stain with the Wright-Giemsa stain, but Hansel's stain may enhance their visibility. Increased numbers (greater than one percent) are found in patients with interstitial nephritis. In general, eosinophils are not normally seen in the urine; more than one percent is considered significant.

Lymphocyte, Unstained

Rare lymphocytes are normally present in urine, but are difficult to recognize. Only slightly larger than erythrocytes, they have round nuclei and a small amount of smooth nongranulated cytoplasm. Increased numbers of small lymphocytes may occur in the urine during the first few weeks after renal transplant rejection. Plasma cells and atypical lymphocytes are rare in urine and should be reported.

Lymphocyte, Stained

Normal lymphocytes are small cells with dense chromatin. Their round to ovoid nuclei may be notched or slightly indented. The scant to moderately abundant light blue cytoplasm may contain a few fine azurophilic granules. Urine lymphocytes prepared by cyto centrifugation may differ morphologically from those in blood films. The “mature” or

quiescent lymphocyte appears slightly larger and often contains more abundant cytoplasm than is found in blood smears. Sometimes a small nucleolus may also be seen in cytocentrifuge preparations.

Other Mononuclear Cells, Unstained

Monocytes, histiocytes, and macrophages are phagocytic cells of variable size. In urine sediment, monocytes are slightly larger than neutrophils. The nucleus is often indented and may be oval or round. Cytoplasm is usually abundant, sometimes frayed, and usually contains vacuoles and granules. Histiocytes may be large and multinucleated. They occur in the presence of chronic inflammation and with radiation therapy.

Macrophages may show evidence of ingested lipid, hemosiderin, red cells, or crystals. The nucleus is oval, indented, relatively small, and sometimes pyknotic. Granular cytoplasm may be filled with multiple vacuoles, creating a foamy appearance that obscures the nucleus. The cell border is often indistinct and irregular when compared with transitional or squamous epithelial cells. Disintegrating macrophages without a nucleus contain particles that resemble ingested nuclei. Macrophages containing lipid globules may form “oval fat bodies” identical to those formed by renal tubular cells.

Monocyte/Macrophage, Stained

The continuum of monocyte/macrophage morphology can range from the typical blood monocyte to the vacuolated, activated stage of a macrophage. The cells are usually large (14 to 30 μm), with abundant blue-gray cytoplasm containing sparse azurophilic granules. The nucleus may be round or oval, indented, lobulated, band-like, or folded. The chromatin is fine and lacy and may contain small nucleoli. Binucleated forms may be seen. Sometimes there is evidence of active phagocytosis, such as ingested material, postingestion vacuoles, or remnants of digested products. Occasionally, a single large cytoplasmic vacuole displaces the nucleus, suggesting the signet ring appearance of some tumor cells.

Neutrophil/Macrophage with Phagocytized Bacteria, Stained

Bacteria within a neutrophil or macrophage usually appear dark blue to black on Wright-Giemsa stain, but may be better defined using a Gram stain. They are of uniform appearance, round or rod-shaped, single, diploid, or forming small chains, depending upon the particular organism. It is important to

distinguish bacteria from the normal cytoplasmic granules present within a neutrophil or macrophage. Bacteria of similar appearance may also be present extracellularly. Phagocytosed bacteria are a significant indicator of infection and should be characterized as completely as possible.

Epithelial Cell, Stained

Squamous, transitional, cuboidal, and columnar epithelial cells may be found in cytocentrifuge urine preparations. Squamous cells are the most common epithelial cells in the urine. All have a low nuclear-to-cytoplasmic ratio. Binucleated cells are occasionally seen. Squamous and transitional cells have a small, round nucleus with dense nuclear chromatin and abundant blue cytoplasm. Small keratohyaline granules may be found in squamous cells. Transitional cells tend to be more rounded and appear in clusters. Cuboidal and columnar epithelial cells have eccentric, round to oval nuclei, moderately coarse chromatin, and abundant blue cytoplasm which may contain vacuoles.

Renal Tubular Epithelial (RTE) Cell

RTE cells are derived from the epithelium lining all segments of the nephron. Their presence indicates tubular damage. They vary in size from approximately two to five times the size of red cells, up to twice as large as a neutrophil (20 to 35 μm). Typically, they are polyhedral in shape, and elongated or ovoid with granular cytoplasm. The single nucleus is round and sometimes eccentric. Renal tubular cells originating from the proximal tubule may show a microvillous border, which is visible with brightfield microscopy. Disintegrating RTE cells become swollen and frayed, and the cytoplasm is often indistinct. In wet preparations, RTE cells may be difficult to distinguish from degenerating neutrophils, mononuclear leukocytes, or transitional epithelial cells. Increased numbers of RTE cells are found in many diseases affecting the kidney, especially in cases of acute tubular necrosis, viral infections involving the kidney, and renal transplant rejection.

In viral infections, such as rubella and herpes, RTE cells may contain inclusion bodies. Especially large intranuclear inclusions are seen in cytomegalovirus disease. Cytoplasmic inclusions may be found in cases of lead poisoning. These inclusions are most obvious in Papanicolaou-stained preparations.

The glomerular filtrate of patients with nephrosis or lipiduria contains large amounts of lipids, such as cholesterol and/or triglycerides, which are partially reabsorbed by the renal

tubular cells. These lipids are toxic and accumulate in the cytoplasm of degenerating tubular epithelial cells. Enlarged, lipid-laden RTE cells are called oval fat bodies. Spherical intracytoplasmic lipid droplets, rich in cholesterol esters, form a “Maltese cross” when viewed with the polarizing microscope. Triglyceride-rich fat droplets stain positively with Oil Red O or Sudan dyes. Several days after an episode of hemoglobinuria, RTE cells containing orange-yellow to colorless intracytoplasmic hemosiderin granules may appear in the urine. The hemosiderin granules stain positively with Prussian blue.

Spermatozoa

Spermatozoa may be found in the urine of males who have undergone prostatectomy and have retrograde ejaculation, or in voided specimens obtained from males shortly after ejaculation. In wet preparations, the sperm head is about 4 to 6 μm long, usually tapering anteriorly. It is smaller and narrower than a red cell. The slender tails are about 40 to 60 μm long. The head may be separated from the tail, making identification more difficult.

Squamous Epithelial Cell

These large (30 to 50 μm), flat cells are derived from the lining of the female urethra, the distal male urethra, or from external skin, or vaginal mucosa. Increased numbers of epithelial cells in urine suggest perineal, vaginal, or foreskin contamination. They may also be seen in males with prostatic disease, or after administration of estrogen. In wet preparations, squamous cells are about five to seven times as large as a red cell and larger than most transitional epithelial cells. A single small, condensed, round, polygonal, or oval central nucleus about the size of a small lymphocyte (10 to 12 μm) is seen in flat, round, or rectangular cells. Binucleation occurs, although less frequently than in transitional epithelial cells, and is often associated with reactive or inflammatory changes. The cell membrane is usually well-defined, with occasional curled or folded edges, and there may be fine cytoplasmic granulation. Degenerating squamous cells have granular swollen cytoplasm with a frayed cell border and a pyknotic nucleus. Sheets of squamous epithelial cells, accompanied by many rod-shaped bacteria and/or yeast, occur with contamination of the urine by vaginal secretion or exudates. Columnar or polyhedral cuboidal epithelial cells, with or without cilia, are occasionally found in urine and cannot be distinguished from RTE cells. They originate in the prostate gland, seminal vesicles, or periurethral glands. Columnar epithelial cells from gut mucosa

can also be found in urine containing fecal material as a result of fistula formation, and in fluid from ileal “bladders.”

Transitional Epithelial Cell (Urothelial Cell)

Urothelial cells line the urinary tract from the renal pelvis to the distal part of the urethra in the male, and to the base of the bladder in the female. They vary in size (40 to 200 μm), usually averaging about four to six times the size of a red blood cell. They are usually round or pear-shaped and smaller than a squamous cell. The nucleus is well-defined, oval or round, usually central. Binucleate cells may occur. Transitional epithelial cells can occur singly, in pairs, or in small groups (syncytia). In wet preparations, they appear smaller and plumper than squamous epithelial cells and have a well-defined cell border. They may be spherical, ovoid, or polyhedral. The smaller cells resemble renal tubular epithelial cells. Some, called “tadpole cells,” have elongated cytoplasmic processes, indicating a direct attachment to the basement membrane. Small vacuoles and/or cytoplasmic inclusions may be present in degenerating cells.

Small numbers of transitional epithelial cells are normally present in the urine. Increased numbers, usually accompanied by neutrophils, are seen with infection. Clusters or sheets of transitional cells are found after urethral catheterization or with urinary tract lesions.

Urinary Casts

Urinary casts are cylindrical objects that form in the distal tubules and collecting ducts as a result of solidification of protein within the tubule lumen. Any material present within the tubules is trapped in the matrix of the cast. Casts are sub-classified based on their appearance and composition (e.g., White cells, red cells, granules, bacteria). Casts must be distinguished from mucous threads and rolled up squamous epithelial cells. Filtered Polarized light microscopy is helpful in distinguishing highly birefringent synthetic fibers from the true casts that are usually non-birefringent.

Bacterial Cast

Bacterial casts often are misclassified as granular or cellular casts. However, bacterial forms can be seen on close inspection using phase or differential interference contrast (Nomarski) microscopy. Gram staining of the sediment is also helpful. Most of these casts contain segmented neutrophils. Urine containing large numbers of WBCs and granular or WBC casts

is pathognomonic for acute pyelonephritis and should be carefully examined for the presence of bacterial casts. Yeast forms may be seen in casts from patients with fungal pyelonephritis.

Cellular Cast, Neutrophil

These cellular casts are most prevalent in pyelonephritis. The cast may be crowded with cells, or have only a few clearly defined cells present in the matrix, often at one end. They contain predominantly intact segmented neutrophils, with cell membranes and nuclei clearly visible in most of the cells. The nucleus of the segmented neutrophil may be degenerated and rounded, precluding categorization of the cell.

Cellular Cast, Renal Tubular Epithelial (RTE)

These casts contain RTE cells within their matrix that are usually intact and irregularly dispersed over the surface. However, in some RTE casts, the cells may be “lined up” in columns or rows, indicating sloughing of the epithelium of an entire tubule. RTE cells have a large single central nucleus, and relatively sparse agranular cytoplasm. As RTE cells degenerate, their nuclei become pyknotic and dense. The cast matrix may contain granules thought to arise from degenerated RTE cells. While the cast matrix may be scant or difficult to visualize due to overlying RTE cells, it must be present in order to diagnose a cast. RTE casts are found in a wide variety of kidney diseases, but are most prominent in diseases that cause damage to the kidney tubules.

Fatty Cast

Fatty casts contain large numbers of spherical, highly refractile fat droplets of varying size in the cast matrix or within oval fat bodies in the cast. Fat may be stained with Sudan stain or examined with polarized light to demonstrate the birefringent “Maltese-cross” pattern of cholesterol esters. Fatty casts often are associated with marked proteinuria and the nephrotic syndrome.

Granular Cast

Granular casts may contain many fine or coarse granules that are most often evenly dispersed over the cast, but may be confined to one area or loosely scattered. They may also include degenerated cell remnants. Distinction between coarsely and finely granular casts has no clinical relevance. Granular casts are found in normal urine as well as in urine from individuals with renal disease.

Hyaline Cast

Hyaline casts are colorless, homogeneous, and translucent, and have a low refractive index. They have a smooth or finely wrinkled surface and may appear tortuous or coiled. Inclusion granules may occasionally be seen in the cast matrix. These casts are usually present in small numbers in normal urine, but may be more prevalent after strenuous physical exercise or physiological stress.

Pigmented Cast (Nonhemoglobin Pigment)

Large quantities of pigmented material may be adsorbed into the cast matrix, transforming a transparent hyaline cast into a colored one. For example, large quantities of urinary bilirubin or urobilinogen give a yellow color to bile casts.

RBC/ Pigmented/ Muddy Brown Casts

The predominant cells are intact erythrocytes, densely or loosely covering the hyaline or granular matrix. The red cells may be shrunken or crenated when compared with those in the surrounding urine. A yellow or red-brown color is seen when a large number of red cells fill the cast. Red cells are of uniform size within the cast, as opposed to fat globules which vary in size. Numerous causes of acute nephritis, particularly with glomerular injury, may produce blood casts or red blood cell casts.

The term muddy brown cast is used for a specific variant of granular cast with a dark brown or red-brown muddy color and a granular interior. The casts are usually multiple and tend to vary somewhat in length. They are narrower than waxy casts. They are seen in 70-80% of cases of acute tubular necrosis and are a very significant clinical finding.

Waxy Cast

Waxy casts are usually broad and stubby, with blunt ends that may appear “broken-off.” They have well-defined parallel margins that may be serrated or notched. The colorless or waxy yellow interior is dense and homogeneous. They are thought to arise from the degeneration of cellular casts, and are frequently associated with severe or progressive renal disease.

Urinary Crystals

At Acid pH

Ampicillin crystals appear in the urine following large intravenous doses of the antibiotic ampicillin. They are long, slender, colorless crystals that aggregate into irregular sheaves after refrigeration.

Cystine crystals are clear, colorless, and hexagonal. There may be a wide variation in crystal size. They demonstrate weak birefringence when viewed with polarized light. The reduction of cysteine to cystine in the cyanide-nitroprusside test produces a cherry-red color, supporting the crystal morphology. However, the nitroprusside test is also positive with cysteine and homocystine, and in urines with large amounts of ketones, although the latter generally produces a dark red color. These crystals are present in large numbers in patients with cystinosis, a congenital autosomal recessive condition that has a homozygous incidence of about 1:10,000 to 1:13,000. It is the most common cause of aminoaciduria. Definitive diagnosis is dependent upon chromatography and quantitative amino acid analysis. Only cystine forms crystals. One or two percent of all renal calculi are composed of radiopaque cystine, which may produce obstruction and infection at any level of the urinary tract.

Sulfonamide crystals may form renal calculi, especially in a dehydrated patient, but with the use of water-soluble sulfonamides, this is infrequently seen today. They are colorless to yellow-brown or green-brown and precipitate at a low acid pH. Small brown acid urate crystals found in slightly acid pH may be confused with sulfonamide crystals. Sulfadiazine crystals appear as bundles of long needles with eccentric binding that resemble stacked wheat sheaves, fan shapes, or spherical clumps with radiating spikes. Sulfamethoxazole crystals are dark brown, divided or fractured spheres.

Uric acid crystals occur at low acid pH. They are usually yellow to brown in color and birefringent. Common forms are four-sided, flat, and whetstone. They vary in size and shape, including six-sided plates, needles, lemon-shaped forms, spears or clubs, wedge shapes, and stars.

Amorphous urate crystals are often referred to as “brick dust.” These colorless or red-brown aggregates of granular material occur in cooled standing urine, and must be distinguished from bacteria.

At Neutral or Acid pH

Bilirubin crystals are occasionally seen in urine containing large amounts of bilirubin and usually accompany bile-stained cells. Small brown needles cluster in clumps or spheres, or on cells or hyaline casts.

Calcium oxalate crystals vary in size and may be much smaller than red blood cells. The dihydrate form appears as small colorless octahedrons that resemble “stars” or “envelopes.” They are sometimes described as two pyramids joined at the base. Oval, elliptical, or dumbbell monohydrate forms are less commonly seen. All calcium oxalate crystals are birefringent. Patients who consume foods rich in oxalic acid, such as tomatoes, apples, asparagus, oranges, or carbonated beverages, may have large numbers of calcium oxalate crystals in their urine. Although oxalate crystals are usually not an abnormal finding, they may suggest the cause of renal calculi.

Cholesterol crystals are large, flat, clear, colorless rectangular plates or rhomboids that often have one notched corner. They are frequently accompanied by fatty casts and oval fat bodies. Cholesterol crystals polarize brightly, producing a mixture of many brilliant hues within each crystal. They may be confused with radiographic contrast media, but are not associated with a high urinary specific gravity.

Hippuric acid crystals are a rare component of acid urine. They are typically found in persons who eat a diet rich in benzoic acid, such as one rich in vegetables, but may also be seen in patients with acute febrile illnesses or liver disease. Hippuric acid crystals are colorless to pale yellow and, unlike uric acid, may occur as hexagonal prisms, needles, or rhombic plates. They are birefringent when examined with polarized light, but lack the interference colors usually seen with uric acid. While both types of crystals are soluble in NaOH, only hippuric acid is also soluble in alcohol.

Leucine crystals may be found in the urine in hereditary disorders of amino acid metabolism and in severe liver disease. These highly refractile brown, spherical crystals have a central nidus and “spokelike” striations extending to the periphery. Leucine spherules are birefringent, demonstrating a pseudo “Maltese cross” appearance with polarized light.

Tyrosine crystals may be seen in hereditary tyrosinosis or with hepatic failure. They appear as silky and fine, colorless to black needles, depending on focusing. Clumps or sheaves form after refrigeration.

At Neutral to Alkaline pH

Ammonium biurate crystals may be associated with phosphate crystals in alkaline urine. Biurates appear as crystalline yellow-brown smooth spheres, with radial or concentric striations. The “thorn apple” variety has projecting horns. These crystals should not be confused with sulfonamide crystals.

Amorphous phosphate crystals form colorless or brown granular aggregates. They are similar in appearance to amorphous urates, but occur in alkaline, rather than acid, urine.

Ammonium magnesium (triple) phosphate crystals are typically colorless, often large monoclinic crystals with a “coffin-lid” appearance. Triple phosphate crystals assume a characteristic four-armed, feathery appearance as they dissolve. They are birefringent and are often accompanied by amorphous phosphates and bacteria.

Organisms

Bacteria

Rod-shaped bacteria (bacilli), most commonly Gram-negative enteric organisms, are identified in wet mounts as rod-shaped organisms of medium size. Large, longer bacilli seen in urine are likely to be Gram-positive lactobacilli from vaginal or fecal contamination. Cocci are more difficult to identify in wet mounts and must be distinguished from amorphous phosphates and amorphous urates.

Abnormal elongated bacillary forms, about the size of yeast cells with swollen centers, are occasionally seen in urine. Their appearance is due to bacterial cell wall damage induced by antibiotics, typically of the penicillin group, in patients being treated for urinary tract infections.

Stained bacteria may be round or spherical (cocci), or rod-shaped (bacilli). They can appear singly or in groups, clusters, pairs, or chains of variable length and may be seen in both intracellular and extracellular locations. They stain deeply basophilic with Wright-Giemsa. Gram stain may be helpful for further classification. If found within a cell, the more specific diagnosis of “neutrophil/macrophage with phagocytized bacteria, stained” should be used. The fact that bacteria are regular and uniform in appearance is helpful in distinguishing them from cellular constituents, especially

granules and phagocytized debris, and from crystals such as amorphous urates.

Yeast/Fungi

Candida albicans is characteristically a colorless ovoid form with a single bud. The 5 to 7 μm , thick-walled cells stain poorly with aqueous stains in wet preparations but are strongly positive with Gram staining. *Candida* species form elongated cells (pseudohyphae) up to about 50 μm long, resembling mycelia. They are branched and may have terminal budding forms. These pseudomycelia may be found in urine from immunocompromised patients or those with serious underlying illnesses.

Stained yeast and fungi may assume a variety of forms. They are regular in contour and usually basophilic on Wright-Giemsa stain. They may be within or outside of cells, and may have a clear capsule surrounding them. The most commonly encountered yeast is *C. albicans*. The spores may form pseudohyphae, up to 50 μm in length, that branch and may have terminal budding. If found within a cell, the more specific diagnosis of “neutrophil/macrophage with phagocytized fungi, stained” should be used.

Protozoa

Trichomonas vaginalis primarily causes vaginal infections, but is also capable of infecting the urethra, periurethral glands, bladder, and prostate. The normal habitat of *T. vaginalis* is the vagina in women and the prostate in men. This protozoan flagellate has only a trophozoite stage. It is pyriform, or pear-shaped, with a length of 7 to 23 μm . There is a single nucleus and a stout central axostyle protruding from the posterior end of the body. Additional morphologic features include four anterior flagella and an undulating membrane in the anterior half, from which projects a single posterior flagellum. In wet mounts, it demonstrates a jerky, rotating, nondirectional leaf-like motion. This is a required diagnostic feature that obviously cannot be illustrated in the photomicrographs used for proficiency surveys. “Rippling” of the undulating membrane can be seen for several hours after cessation of motility.

Degenerating forms resemble large oval cells, without visible flagella, and may be easily confused with neutrophils or other leukocytes.

Helminths

Schistosoma haematobium is a trematode that inhabits the veins of the bladder, prostate, vagina, and uterus. It is most often present in the urine of patients from Africa and the Middle East who have schistosomiasis. Large oval eggs, about 150 μm long, with a distinct terminal spine, accumulate in the bladder wall. Eggs containing embryos eventually pass into the urinary bladder, usually accompanied by neutrophils and many red blood cells.

Miscellaneous/Exogenous

Fat Droplets

Free, highly refractile droplets in urine or stool are seen as dark spherules under low power, and clear spheres of varying size under high power. Fat droplets may represent endogenous triglycerides, neutral fats, cholesterol esters, or combinations of all three. In urine, they may be observed in association with fat-laden cells or casts, and are usually seen in patients with the nephrotic syndrome. In stool samples, fat droplets may be associated with malabsorption. Exogenous mineral oil, catheter lubricant, or vaginal creams also appear as fat globules, sometimes assuming large, amorphous, irregular shapes.

Fecal Contamination of Urine

Fecal material in the urine may be due to a fistula between the colon and urinary tract, or caused by contamination of the urine with feces during collection. Plant structures, muscle fibers, and microorganisms can be seen. Plant material may include aggregates of starch granules, each about 10 μm in diameter; larger vegetable fibers with a regular spiral structure; multiple thick-walled plant cells; or leaf cells that are somewhat similar in structure to wood applicator stick fibers. There may also be small smooth single plant cells, pollen grains, and vegetable hairs. Vegetable hairs are long (30 μm or greater), slender, and pointed at one end, and have a long thin central canal. Skeletal muscle fibers, yellow-brown in color, often are seen as remnants of undigested meat in stool specimens. They are two to four times the size of a broad waxy cast, and may show distinctive cross-striations or appear smooth and amorphous. Columnar epithelial cells from gut mucosa and squamous epithelial cells from anal mucosa are rarely seen. Columnar cells have a distinct cell border, round nucleus, and smooth cytoplasm, and may be vacuolated.

Ileal urinary bladders are formed from a segment of ileum to which the ureters are attached. Ileal bladder urine usually contains large numbers of degenerating columnar cells, neutrophils, macrophages, and bacteria. Cells are not stained yellow-brown, as in urine contaminated with fecal material.

Fibers

Hair, and synthetic and natural fibers from clothing, cotton balls, dressings, and disposable diapers can be found in urine or stool specimens. Most fibers are large, long, and sometimes twisted. Short cellulose fibers from disposable diapers resemble large, broad, waxy casts but, unlike waxy casts, they are birefringent. Fibers are well-defined, flat, refractile, and colorless and often contain fissures, pits, or cross-striations.

Mucus

Mucus strands or threads arising from glands in the lower urinary and vaginal tracts are frequently found in urinary sediments. Translucent delicate strands may form long, wavy, intertwined aggregates. They constitute the background material in the field and are more obvious with phase microscopy.

Pollen Grains

Pollen grains contaminate urine and urine containers, often on a seasonal basis. They are usually large, about 20 μm or greater in diameter, tend to be rounded or regularly shaped, and have a well-defined thick cell wall. They may have short, regular, thorny projections. Some are yellowish tan. They may resemble worm ova.

Starch Granules

Starch granules from surgical gloves or other sources are a frequent contaminant of body fluids. Granule size varies from that of a red cell to four to six times larger. The usual form is colorless and irregularly rounded with a central slit or indentation, often described as looking like a “beach ball.” With crossed polarizing filters, the granules form white “Maltese crosses” against a black background.

Stain

Crystal violet-safranin and similar stains, such as Sternheimer-Malbin, which are used for wet urinary sediments, crystallize, especially at alkaline pH. They form brown to purple needle-shaped crystals that sometimes aggregate in star-shaped clusters. Wright-Giemsa stain precipitate appears as

metachromatic granular deposits on and between cells, and may be confused with bacteria, yeast, or other parasites. The size of stain droplets varies, unlike bacteria and yeast, which have a more uniform morphology.

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CEREBROSPINAL FLUID (CSF) AND BODY FLUID CELL IDENTIFICATION

Introduction

The value of routine evaluation of body fluids has been amply documented. Concentration by cytocentrifugation allows for the evaluation of fluids with low cell counts, as well as adequate preservation of cytologic detail. The following descriptions are based primarily on fluids that are prepared by cytocentrifugation, air-dried, and stained with Wright-Giemsa. Most of the material used for preparation of CAP Surveys cell identification images has been processed in a similar manner.

Erythroid Series

Erythrocyte, Nucleated

These cells are found uncommonly in body fluids and are usually derived from peripheral blood contamination in which circulating nucleated red cells are present. Occasionally, they may arise from accidental aspiration of the bone marrow in an infant or adult with osteoporosis. When the nucleated red cells are a result of accidental marrow contamination they are earlier stages (polychromatophilic and basophilic normoblast) and may also be associated with immature myeloid cells. The cytoplasm should be carefully evaluated to distinguish these cells from necrobiotic cells. Nucleated red blood cells due to peripheral blood contamination tend to be a later stage of development (orthochromatophilic normoblast).

Erythrocyte, Mature

These are typical blood erythrocytes without nuclei and similar to those present in the peripheral blood. They are not typically found in normal body fluid samples and reflect hemorrhage or traumatic contamination. They may also be seen in association with many disease states, such as malignancy or pancreatitis. Erythrocytes may appear crenated in certain fluids, but that finding is not clinically significant.

Lymphoid Series

Lymphocyte

The cytologic features of lymphocytes prepared by cytocentrifugation may differ from those in blood smears. Changes induced by cytocentrifugation may include cytoplasmic spreading, nuclear convolutions and nucleolar prominence. The “mature” or quiescent lymphocyte appears slightly larger than its counterpart on blood smears, often with more abundant cytoplasm but usually smaller than neutrophils and monocytes. Because of the high speed used in cytocentrifugation, a small nucleolus may be seen, and this should not be interpreted as indicating a lymphoma. A few azurophilic granules may be noted in the lymphocytes on slides prepared by cytocentrifugation, and do not of themselves denote abnormality. Large granular lymphocytes are medium to large lymphocytes, with a round to oval nucleus, clumped basophilic chromatin, inconspicuous nucleolus and light blue cytoplasm containing numerous small azurophilic granules.

Lymphocyte, Reactive (Atypical)

All of the lymphocyte variants seen in peripheral blood smears may be seen in body fluids. Reactive lymphocytes tend to be larger with increases in volume of both nuclei and cytoplasm. Most reactive lymphocytes in viral illnesses type as T-lymphocytes. However, plasmacytoid lymphocytes are also frequent. Plasmacytoid lymphocytes are medium-sized cells

with irregular densely clumped nuclear chromatin, absent to indistinct nucleoli, abundant basophilic cytoplasm often with a paranuclear clear zone (hof).

Immunoblasts are large cells with round to oval nuclei, fine delicate chromatin, prominent nucleoli and moderate amounts of deeply basophilic cytoplasm.

The distinction between normal and reactive lymphocytes is often difficult and subjective; however, it is more important to distinguish reactive lymphocytes from lymphoma cells. The reactive lymphocyte usually has a distinct, smooth nuclear membrane in contrast to the often irregular nuclear membrane of lymphoma cells. Also in contrast to malignant lymphoproliferative disorders, there is usually a spectrum of lymphocyte morphology present in reactive conditions.

In some situations, differentiation of reactive from malignant lymphocytes may require the use of ancillary techniques including flow cytometry and molecular analysis.

Lymphoma Cell

The morphology of lymphoma cells is dependent upon the specific nature of the lymphoproliferative process. Large cell lymphomas may be distinguished from reactive lymphocytes by noting some or all of the following features in lymphoma cells: high nuclear-to-cytoplasmic ratio; immature nuclear chromatin pattern; irregular nucleus; prominent, large nucleoli; lack of a clear Golgi region next to the nucleus; and monotonous morphologic appearance. Lymphoma cells are usually unaccompanied by other inflammatory cells.

Follicular lymphoma cells (formerly known as small cleaved lymphoma cells) are slightly larger than normal lymphocytes, and the nuclear-to-cytoplasmic ratio is high; the nuclear chromatin pattern may appear dense or hyperchromatic; and some of the nuclei may show large clefts or irregularities in contour.

Lymphoblastic lymphoma cells appear similar to the blasts described in the Miscellaneous Cells section and sometimes contain a more folded or convoluted nuclear pattern. With chronic lymphocytic leukemia or small lymphocytic lymphoma, a uniform population of small lymphocytes is present that often cannot be distinguished morphologically from normal resting lymphocytes. Sometimes, however, they are slightly enlarged with prominent parachromatin clearing, and occasional prolymphocytes may be present. Prolymphocytes are large cells with clumped nuclear chromatin, abundant basophilic

cytoplasm, and a characteristically prominent central nucleolus.

While lymphoma cells typically occur singly, cyto centrifugation artifact may result in small cell aggregates. Large clumps of tightly cohesive cells with continuous outer borders are more characteristic of carcinoma.

Immunocytochemical studies and flow cytometric immunophenotypic studies are very useful in difficult cases to distinguish malignant from reactive lymphocytes, and lymphoma from nonhematopoietic neoplasms.

Plasma Cell

Plasma cells are terminally differentiated forms of reactive B-lymphocytes. Plasma cells can be seen in body fluid but are not normally present. They may be seen in infectious, inflammatory, or neoplastic processes. They have round to oval, eccentrically placed nuclei with condensed, clumped chromatin. The cytoplasm is deeply basophilic, often with a paranuclear clear zone or Golgi region. Occasionally, the cytoplasm may contain immunoglobulin-filled vacuoles that may appear clear. Binucleate plasma cells occasionally can be seen. Mesothelial cells may resemble plasma cells, but are usually larger in size, have more centrally placed nuclei with smooth rather than rosey nuclear chromatin, and usually lack the perinuclear clear zone.

Plasma Cell, Abnormal

Plasma cell neoplasms such as plasma cell myeloma (multiple myeloma) are B-cell neoplasms. In most situations, malignant plasma cells resemble normal plasma cells, but also have prominent nucleoli, irregularly shaped nuclei, more open chromatin, absent perinuclear halo and high nuclear/cytoplasmic ratio. Special studies such as immunophenotyping or immunocytochemistry may be necessary to confirm the monoclonal nature of the proliferation, indicating malignancy.

Plasma cells may be bi-nucleated and even multinucleated. In some rare situations, the nuclear:cytoplasmic ratio may be so altered and the cytologic features so atypical, that it is difficult to recognize the cells as of plasma cell origin.

Myeloid Series

Basophil, Mast Cell

Basophils and mast cells are recognized by characteristic granules that stain dark blue to black with Wright-Giemsa which overlap or may obscure the nucleus. The nucleus of the basophil is segmented and the chromatin is condensed or smudged. The granules of a basophil are larger than the azurophilic granules of a promyelocyte and are often irregular in shape. Mast cells are usually larger than basophils with a low nuclear to cytoplasmic ratio and a round or oval nucleus usually obscured by abundant red-purple granules. These granules are smaller and more round and regular than basophilic granules and release histamine upon stimulation, they are derived from separate progenitor cells.

Mast cells are usually found in tissues. Basophils and mast cells are not normally found in body fluids, but when present, are most commonly associated with inflammatory conditions, foreign body reactions, and parasitic infestations.

Eosinophil, Any Stage

The eosinophil is recognized by its characteristic round, orange-pink to orange-red granules. These are larger than primary or secondary granules in neutrophils. Particularly large numbers of eosinophils may be seen in foreign body reactions, parasitic infection, and introduction of air into a body cavity.

Neutrophil, Immature (Promyelocyte, Myelocyte, Metamyelocyte)

Immature stages of the myeloid series are less often found in body fluids, unless there is an accompanying increase in those same cells in the peripheral blood. Patients with chronic myeloid leukemia may have soft tissue involvement and increased numbers of immature myeloid cells may be seen in fluids from these patients. Immature granulocytic (and erythroid) cells can be found when there is marrow contamination of the fluid, most commonly in CSF.

Neutrophil, Segmented or Band

Usually the segmented or band neutrophil is easily recognized. Often, the nuclear lobes appear eccentric in cytocentrifuge preparations. In inflammation, the cytoplasm may contain toxic granules or be vacuolated. Intracellular bacteria, crystals, or

debris may be seen in pathologic conditions. If inclusions are present, the more specific identifications such as “neutrophil/macrophage with phagocytized bacteria” or “neutrophil/macrophage containing crystal” should be used.

Neutrophils in body fluids can show morphologic change due to autolysis, including nuclear pyknosis and fragmentation, making recognition of cell type difficult. In particular, these autolytic neutrophils can be mistakenly identified as nucleated red cells; however, persistence of a few azurophilic granules in the cytoplasm provides a clue to the neutrophilic origin. Neutrophils in samples from the stomach, intestine, or stool often show striking degenerative changes. For the purpose of proficiency testing, the identification “degenerative cell, NOS” should be chosen if the cell of origin can no longer be recognized.

Mononuclear Phagocytic Series

Monocyte/Macrophage

Monocytes are bone marrow derived cells that circulate in the blood. Macrophages arise from bone marrow derived cells that migrate into tissues and evolve morphologically. Monocyte/macrophage morphology in fluids is quite variable, ranging in continuum from the typical blood monocyte of the peripheral blood to a vacuolated, activated stage with the morphology of a typical macrophage. Monocytes are usually large (12 to 20 μm) with abundant blue-gray cytoplasm containing and often contain sparse azurophilic granules. The nucleus is round to oval and may show indentation, giving it a kidney bean or horseshoe shape. The chromatin is lacy and small nucleoli may be apparent.

Macrophages are larger cells (15 to 80 μm) with abundant cytoplasm showing evidence of active phagocytosis. This includes ingested material such as other blood cells or bacteria, hemosiderin, fungi, and remnants of digested materials as well as cytoplasmic vacuoles post-ingestion. One or more round to oval nuclei are present and occasionally prominent nucleoli may be seen.

Alveolar macrophages normally are the predominant cells in bronchoalveolar lavage fluid, which is obtained by instilling sterile saline into the alveolar spaces and then removing it through a fiberoptic bronchoscope. These cells often appear

similar to macrophages in pleural or peritoneal fluids, with an eccentric, round nucleus, light blue cytoplasm, and variable numbers of cytoplasmic azurophilic granules. Bluish black cytoplasmic carbon particles may be prominent, particularly in people who inhale smoke. Macrophages can at times be difficult to differentiate from mesothelial cells. Mesothelial cells are usually larger than monocytes/macrophages and usually show more biphasic staining cytoplasm and surface microvilli.

Macrophage Containing Erythrocyte(s) (Erythrophage)

The erythrophage is a macrophage that has ingested red blood cells usually due to hemorrhage from trauma or a bleeding disorder. As phagocytic activity may persist following acquisition of the specimen, the presence of erythrophagocytosis does not always imply *in vivo* erythrophagocytosis. However, it can be an important clue to prior hemorrhage. Erythrophagocytosis is also seen in hemophagocytic syndromes where it is usually accompanied by leukophagocytosis.

Macrophage Containing Abundant Small Lipid Vacuole(s)/Droplet(s) (Lipophage)

The lipophage is a macrophage containing uniform, small lipid vacuoles that completely fill the cytoplasm. These fat-filled inclusions may originate from extracellular fatty material or from the membranes of ingested cells. Lipophages may be present in CSF following cerebral infarcts, injections of intrathecal chemotherapy, or post-irradiation. They may be present in pleural fluid associated with chylothorax or with extensive cell membrane destruction.

Macrophage Containing Neutrophil(s) (Neutrophage)

The neutrophage is a macrophage containing one or more phagocytosed neutrophils. Initially, the segmented nucleus of the neutrophil will be evident. The nucleus is surrounded by a large, clear zone of cytoplasm. As digestion of the neutrophil proceeds, the nucleus becomes round and pyknotic. Finally, remnants of digested nuclei of neutrophils and other white cells may appear as smaller, purple, homogeneous inclusions. However, these inclusions are larger than the small azurophilic lysosomal granules characteristic of macrophages. These inclusions should be distinguished from bacteria and yeast,

which are usually much smaller and have a more uniform appearance. Bacteria display either a coccal or bacillary morphology; yeast often display budding forms. Darkly staining blue-black hemosiderin granules (from breakdown of red cells) should also be distinguished from digested leukocyte debris.

For purposes of identification in CAP Surveys, a macrophage should be termed a neutrophage when the phagocytized nuclear inclusion is clearly identifiable as originating from a segmented neutrophil. If a macrophage contains microorganisms, the identifications of “neutrophil/macrophage with phagocytized bacteria” or “neutrophil/macrophage with phagocytized fungi” should be used.

Neutrophages may be found in fluids following any cause of neutrophilia. The “Reiter” cell in synovial fluid is a neutrophage and is not specific for Reiter’s syndrome; it may be seen with any cause of infection or inflammation affecting the synovial cavity.

Macrophage Containing Hemosiderin (Siderophage)

The siderophage is a macrophage containing the coarsely granular iron-protein complex known as hemosiderin. They are granules which are dark blue with the Wright stain, arising from iron by-product from digested red cells. These cells are seen, for example, after a CSF hemorrhage and may remain for up to four months. These cells may also be seen in other conditions leading to hemorrhage in any body cavity. The Prussian blue stain can confirm the identity of intracytoplasmic iron and stains hemosiderin a vivid lighter blue. Hemosiderin pigment should be differentiated from melanin and anthracotic pigment.

Neutrophil/Macrophage Containing Crystal

Crystals may be present within the cytoplasm of a neutrophil/macrophage and are most frequently seen in synovial fluids. They may vary in shape, size, and color. Crystals can be seen in conditions such as gout, pseudogout, or hemorrhage (hematoidin crystals). As they may not be readily apparent on Wright-Giemsa stain, further evaluation with polarized light microscopy is required if the presence of crystals is suspected. For proficiency testing, when crystals are present within a neutrophil or macrophage, this more specific identification should be chosen.

Neutrophil/Macrophage with Phagocytized Bacteria

Bacteria within a neutrophil or macrophage are notable for their uniform appearance - round or rod-shaped, single, diploid, or in small chains depending upon the species present.

They usually appear dark on Wright-Giemsa stain; Gram stain may be helpful. Bacteria of similar appearance may also be present extracellularly. It is important to distinguish bacteria from the normal cytoplasmic granules or debris present within a neutrophil or macrophage. For proficiency testing, when bacteria are present within a neutrophil or macrophage, this more specific identification should be chosen.

Neutrophil/Macrophage with Phagocytized Fungi

Fungi or yeast may occur within a neutrophil or macrophage. Their shape is distinctive and regular, occasionally showing budding, and a clear capsule may be present around them. They appear basophilic when stained with Wright-Giemsa stain. Fungi may also be present in an extracellular location. As with intracellular bacteria, fungi should be distinguished from normal or degenerating intracellular granules and other constituents. For proficiency testing, when fungi/ yeast are present within a neutrophil or macrophage, this more specific identification should be selected.

Lining Cells

Bronchial Lining Cell

Ciliated bronchial lining cells may be obtained as a contaminant in bronchoalveolar lavage fluid, indicating sampling from the bronchial tree. These cells have a unique appearance with a columnar shape, a basally placed oval to round nucleus, coarsely stippled chromatin, inconspicuous nucleolus, and amphophilic to pink cytoplasm with a row of cilia at one end. They are seen as single cells or in small clusters.

Endothelial Cell

Endothelial cells line blood vessels. They are a normal component of tissue and are rarely found in body fluids. They have an elongated or spindle shape, measure approximately 5 μm wide by 20 to 30 μm long, and have a moderate nuclear-to-cytoplasmic ratio (2:1 to 1:1). The oval or elliptical

nucleus occasionally is folded and has dense to fine, reticular chromatin. One or more nucleoli may be visible. The frayed cytoplasm tapers out from both ends of the nucleus and may contain a few azurophilic granules. Occasionally, an intact capillary may contaminate a fluid, and in this case the endothelial cells are arranged in a longitudinal overlapping pattern in two rows, sometimes with a visible lumen. Isolated capillary fragments appear similar to the capillary segments seen in tissue fragments.

Mesothelial Cell

The mesothelial cell (20 to 50 μm) normally lines pleural, pericardial, and peritoneal surfaces. These cells can be shed individually or in clusters. When found in pairs or clusters, mesothelial cells have articulated or coupled cell borders with a discontinuous outer border (clear spaces or "windows") between many of the cells. The nucleus is round to oval in shape with a definitive nuclear membrane and regular contour. Nuclear chromatin varies from dense to fine, but it is evenly distributed. Multiple nucleoli may occur and the nuclei may overlap; however, the nuclei remain of approximately equal size and shape. One or more nucleoli may be present. The nuclear-to-cytoplasmic ratio is low (less than 1:1) and the nucleus may be central or eccentrically placed. The cytoplasm is light to dark blue and may have a grainy texture, typically dense grainy basophilia or even a crystalline/ground glass appearance to the perinuclear area. With some staining techniques, the periphery and perinuclear cytoplasmic regions may appear as very lightly stained areas. With degeneration, additional small vacuoles may occur throughout the cell. Cytoplasmic budding or fragmentation may also occur. In chronic effusions or during inflammatory processes, mesothelial cells proliferate and become very large. Mitotic figures occasionally are seen within mesothelial cells. The nuclear chromatin is less condensed and nucleoli may be prominent; however, the nucleus still retains a definitive, smooth, nuclear membrane. Mesothelial cells can be phagocytic and resemble macrophages, resulting in forms that have a morphology intermediate between mesothelial cells and macrophages.

Synoviocyte (Synovial Lining Cell)

Synovial lining cells cover the non-articular surface of the joint cavity. By electron microscopy, different subtypes can be recognized. This large (20 to 40 μm) cell has a round to oval shape. The nucleus is round to oval with a distinct nuclear membrane and regular nuclear contour. Occasional

multinucleate forms occur, but nuclei typically are similar in size. The nuclear chromatin varies from dense to finely granular and one or more nucleoli may be present. Cytoplasm is abundant, basophilic and agranular. Often it shows an uneven or grainy texture. Degenerative changes may occur including multiple small vacuoles or cytoplasmic blebs. Overall, the appearance of synovial lining cells is similar to that of mesothelial cells in serous fluids. Their presence in synovial fluid is expected and has no diagnostic significance.

Ventricular Lining Cell (Ependymal or Choroid Cell)

Cells lining the ventricles (ependymal cells) or choroid plexus (choroidal cells or choroid plexus cells) may be shed into the CSF, particularly in neonates or in the presence of a ventricular shunt or reservoir. Choroidal and ependymal cells are not diagnostically significant but must be distinguished from malignant cells.

These large (20 to 40 μm) cells may occur singly or in clumps. Clumps may be loose aggregates or may be tissue with indistinct cell borders. Nuclei are eccentrically placed and are round to oval with a definitive smooth nuclear membrane and regular nuclear contour. Chromatin is distributed evenly and is reticulated or dense; occasionally the nucleus may appear pyknotic. Nucleoli are inconspicuous. The cytoplasm is typically amphophilic and grainy but occasionally is blue (a feature of ependymal cells). Microvilli may be present (a feature of choroidal cells). Extensive degeneration of choroidal and ependymal cells may occur so that only naked nuclei remain.

Miscellaneous Cells

Blast Cell

A blast is a large, round to oval cell, 10 to 20 μm in diameter, with a high nuclear-to-cytoplasmic ratio. The blast often has a round to oval nucleus, but it is sometimes indented or folded. In addition, cytocentrifugation artifact may result in an irregular nuclear contour. The nuclear chromatin is typically fine, lacey, or granular and one or more nucleoli may be present. Nucleoli are more prominent in cytocentrifuge slides. The cytoplasm is basophilic and often agranular; however, when cytoplasmic granules occur, they are more easily visualized in the cytocentrifuge slide than in peripheral blood or bone marrow smears. In the absence of lineage-associated findings, such as Auer rods, cytoplasmic granules, cytochemical data, or cell

surface marker data, it is not possible to further characterize a given blast cell. This is particularly true for body fluids, where cyto-spin preparation artifact may alter or obscure morphologic details. Degenerative changes also may occur if the fluid specimen is not processed promptly.

Chondrocyte (Cartilage Cell)

Rarely, chondrocytes are obtained during lumbar puncture, probably when the needle nicks the vertebral cartilage. This is a more common occurrence in infants of adults with a narrow intervertebral space. Chondrocytes are typically seen in the synovial fluid of patients with osteoarthritis, but also may occur after joint trauma or surgery.

The cells have round or oval, dark nuclei which are typically centrally placed. The cytoplasm is dense and wine-red. A cytoplasmic clear zone adjacent to the nucleus is often present and it may completely surround the nucleus.

Degenerating Cells (Not Otherwise Specified)

Degenerating cells with pyknotic (highly condensed) nuclei or nuclear karyorrhexis (fragmentation) may occasionally be seen in body fluids. Autodigestion or autolysis of neutrophils may occur as they attempt to remove foreign material.

The nucleus becomes pyknotic and fragments and with further autolysis, may appear as one or more indistinct, light purple inclusion(s). The nuclear lobes may fragment into numerous small particles of varying sizes that resemble microorganisms. Cytoplasmic granules may become less prominent or may fuse (particularly with toxic granulation). The cytoplasmic borders may become frayed and indistinct. Cytoplasmic vacuole formation is common.

Autolytic neutrophils with eccentric, dense, round nuclei and pale cytoplasm may resemble nucleated red cells, but differ from them in the persistence of cytoplasmic granules.

Actively dividing cells such as malignant cells, reactive lymphocytes and mesothelial cells, may more readily undergo degenerative changes in body fluids. The cytoplasm may show a swollen, vacuolated, or frayed appearance. The nuclear chromatin may show coarse condensations separated by enlarged parachromatin spaces (salami-like appearance).

Ventricular lining cells often will not appear intact when shed into CSF or ventricular fluid; only bare nuclei with pieces of frayed cytoplasm will be seen.

All cell types may undergo degenerative changes in fluids with prolonged storage or after infusion of sclerosing agents into a body fluid cavity.

Germinal Matrix Cell

Germinal matrix cells are also known as undifferentiated leptomeningeal cells and are small blast-like cells that typically occur in clusters. They have a high N:C ratio, delicate nuclear chromatin and may have a single small nucleolus. Nuclear molding may occur. Immunophenotypically, these cells are of neural origin. Germinal matrix cells originate from the subependymal cell layer in the lateral ventricles. These cells are pluripotent and can give rise to mature neuronal and glial cells. Significant amounts of vascular germinal matrix persist until about 32-weeks of gestation. As migration of neuronal and glial precursors proceeds into the cerebral cortex, the germinal matrix layer progressively thins and breaks into small islands, which may persist through the first post-natal year of life. The germinal matrix has a thin, fragile microvasculature, often prone to hemorrhage in premature infants. Germinal matrix cells may be found in neonatal CSF in association with hydrocephalus after intraventricular hemorrhage or following ventriculostomy or placement of a ventricular-peritoneal shunt.

Lupus Erythematosus (LE) Cell

Spontaneous (LE) cell formation occasionally is seen in pleural, peritoneal, pericardial and synovial fluids. Characteristically, an intact neutrophil contains a large, homogeneous, pink inclusion (denatured or degenerated nucleus) that distends the cytoplasm and displaces the nucleus. Although assessing effusions for LE cells is no longer considered a sensitive or specific test for the diagnosis of lupus erythematosus or other autoimmune diseases, identifying an LE cell in a patient with an unknown diagnosis is useful in guiding further laboratory evaluation. (LE cells may form in vitro and serous fluids standing at room temperature for a prolonged period of time may have more LE cells.)

Tart cells should be distinguished from LE cells. Tart cells are macrophages that have phagocytized the nucleus of another cell but in contrast to the true LE cell, the ingested nucleus remains intact and non-homogenized. Their occurrence is not associated with lupus erythematosus or other autoimmune disorders. Tart cells are found more frequently in serous fluids and should not be confused with LE cells.

Malignant Cell (Nonhematopoietic)

A variety of neoplastic cells may be found in body fluids, although their presence in synovial fluid is rare. The morphology is dependent upon the underlying malignancy. Malignant cells may be numerous or only a rare cell may be identified.

Tumor cells may exfoliate from primary CNS tumors and be found in the CSF. Medulloblastoma has a propensity to invade the ventricular system and is the most common primary CNS tumor associated with malignant cells in the CSF. Metastatic carcinoma and melanoma may also result in malignant cells in the CSF. Immunocytochemistry can be used to confirm the nonhematopoietic nature of the neoplastic cells.

Virtually any neoplasm can invade the serous cavities, resulting in malignant cells in fluid specimens. Cytologic features of malignant cells on cytocentrifuge preparations include: high nuclear-to-cytoplasmic ratio, increased cell and nuclear size, irregularly shaped nuclei, atypical nuclear chromatin patterns, large nucleoli, and a tendency to form large clusters, frequently with nuclear molding. Occasionally, a cell cluster may recapitulate an organoid structure, such as pseudo-gland formation with adenocarcinoma. With malignant tumors, a distinct population of abnormal cells is usually present. Transitional cell forms resembling more typical monocytic, lymphocytic, and mesothelial elements should suggest a reactive process.

Megakaryocyte

Occasionally, hematopoietic elements of the bone marrow may accidentally be obtained, particularly in CSF. Megakaryocytes appear as large cells with a multilobated nucleus and distinctly granular cytoplasm.

Neural Tissue/Neurons

Neural tissue consists of capillary fragments, neurons (ganglion cells), glial cells or fragments of these cells within fibrillar cerebral cortex tissue. They can be seen in the CSF of patients who have experienced an intracranial hemorrhage, had significant head CNS trauma, recently had neurosurgery, or have a ventricular shunt in place. The tissue fragments in Wright-Giemsa-stained preparations appear as basophilic or pink, fibrillar, finely granular matrix containing nuclei without apparent cytoplasm; the fragments also may be acellular. Acellular neural tissue fragments may be indistinguishable from fragments of pia mater, a tightly adherent membrane

composed of sparsely cellular, loose fibrovascular stroma that lines the subarachnoid space covering the spinal cord and brain. Pial membrane fragments may also be found in similar clinical situations as fragments of neural tissue.

Occasionally, intact pyramidal-shaped neurons with round to oval nuclei, reticulated nuclear chromatin, a single nucleolus and basophilic cytoplasm occur within the fragment or as isolated cells. Neurons can be identified by their pyramidal shape and axonal processes. Isolated glial cells resemble monocytes and hence are more difficult to identify.

Inflammatory cells also may be seen within degenerating neural tissue. If necessary, immunocytochemistry can be used to confirm the suspected nature of such elements, using markers such as glial fibrillary acidic protein (GFAP), S-100 protein and neuron-specific enolase (NSE).

When CSF is collected from the ventricles through a shunt or reservoir device, neural tissue and/or neurons are more frequently encountered.

Squamous Epithelial Cell

Squamous cells derived from skin may be found in fluids as contaminants. Squamous epithelial cells are large (30 to 50 μm), round to polyhedral-shaped cells with a low nuclear to cytoplasmic ratio (1:1 to 1:5). The nucleus is round to slightly irregular, with a dense, pyknotic chromatin pattern and no visible nucleoli. The abundant cytoplasm is lightly basophilic and may show evidence of keratinization or contain a few blue keratohyaline granules. Epithelial cells from deeper layers of the epidermis have larger nuclei with a high nuclear-to-cytoplasmic ratio. In contrast to squamous carcinoma, contaminant squamous epithelial cells lack nuclear atypia.

Crystals

Calcium Pyrophosphate Dihydrate (CPPD) Crystals

Found in synovial fluid of patients with arthritis, pseudogout, as well as in association with other diseases (e.g., metabolic disorders such as hypothyroidism), these intracellular crystals are most often confused with monosodium urate (MSU) crystals. The intracellular crystals are rod-shaped, rhomboid, diamond, or square forms, usually 1 to 20 μm long. They are only truly distinguished from MSU crystals by use of a polarizing microscope with a first-order red compensator. The CPPD

crystals are blue when the long axis of the crystal is parallel to the slow ray of light from the color compensator (positive birefringence); MSU crystals are yellow (negative birefringence).

Cholesterol Crystals

These crystals are extracellular and are one of the larger crystals found in fluids. The most common form is flat, plate-like with a notch in one corner. Occasionally they may be needle-like. They are transparent and appear as a negative impression. They are strongly birefringent when viewed with polarizing filters and are found in chronic effusions, especially in rheumatoid arthritis patients. They are believed to have no role in causing the arthritis.

Hematin/Hematoidin Crystals

Hematin and hematoidin crystals both result from the breakdown of hemoglobin in tissue. Hematin is a porphyrin compound. Hematoidin is similar to bilirubin. The crystals may be found anywhere in the body approximately two weeks after bleeding/ hemorrhage. The crystal may be either intra- or extracellular. The crystals are bright yellow and have a rhomboid shape. They do not stain with iron stains.

Monosodium Urate (MSU) Crystals

Pathognomonic of gout, monosodium urate crystals are found in synovial fluid. They are found either intra- or extracellularly and are described classically as needle-like. They are 2 to 20 μm in length and 0.2 to 1 μm thick. Intracellular crystals are said to be present in acute attacks of gout. The biggest mimic of MSU crystals is calcium pyrophosphate dihydrate (CPPD) crystals. They are reliably distinguished by use of a polarizing microscope and a first-order red compensator. The MSU crystal is yellow when the long axis of the crystal is parallel to the slow ray of light from the color compensator (negative birefringence); the CPPD crystal is blue (positive birefringence).

Crystals, Not Otherwise Specified

Steroid crystals may occasionally be seen, especially in synovial fluids. For example, betamethasone acetate occurs as blunt-ended rods, 10 to 20 μm long. Steroid crystals may be either positively or negatively birefringent and interfere with the diagnosis of crystal associated arthritis. Other structures that can be confused with crystals include fragments of

degenerated cartilage and "foreign" material from prosthetic devices.

Microorganisms

Intracellular and extracellular organisms such as bacteria and yeast may be found in body fluids, particularly during the acute stage of an infection. The organisms are uniform in structure and staining characteristics. Bacteria must be differentiated from nonspecific phagocytic debris commonly found in neutrophils and macrophages and from precipitated stain. This can be easily done with a gram stain. A wide variety of parasites may be found in body fluids. The organisms usually have characteristic features that allow identification.

Bacteria, Extracellular

A wide variety of bacteria can be seen in body fluids, including bacilli, cocci, and filamentous bacteria. All are best seen under oil immersion magnification, and may be seen in an intracellular or extracellular location. However, when they are intracellular, the more specific identification of "neutrophil/macrophage with phagocytosed bacteria" should be used.

Bacilli are rod-shaped bacteria, while cocci are spherical. Filamentous bacteria are bacilli that grow in a branching, filamentous pattern, reminiscent of a tree. They can be mistaken for fungal hyphae, but are typically smaller and narrower.

Most bacteria have a basophilic hue on Wright-Giemsa stain. A Gram stain can be useful in separating these microorganisms into Gram-positive (blue/purple) and Gram-negative (pink) groups. An acid-fast stain is also useful in identifying certain filamentous bacteria. The most likely error in interpretation is to misidentify stain precipitate as microorganisms. This error can be avoided by remembering that bacteria tend to be relatively uniform in size and shape, while stain precipitate is often irregular in shape and individual grains vary considerably in size.

Ehrlichia/Anaplasma

Only recently recognized as an arthropod-borne infectious agent in humans, members of the genus *Anaplasma* (previously *Ehrlichia*) are small, Gram-negative obligate intracellular organisms currently classified as rickettsiae. On Wright-stained preparations, *Anaplasma* species appear as

round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either PMNs (*A. phagocytophilia*) or monocytes and macrophages (*A. chafeensis*). The morulae are microcolonies of elementary bodies.

Parasites

A wide variety of parasites may be found in body fluids. The organisms usually have characteristic features that allow identification. Both unicellular (e.g., amoeba, *Giardia*) and multicellular (e.g., tapeworm, roundworms) can be encountered. Proficiency testing identification of a parasite should be performed in accordance with defined laboratory policy for patient samples.

Yeast/Fungi, Extracellular

Yeast and fungi may assume a variety of forms. They are regular in contour and usually basophilic on Wright-Giemsa stain. They may be within or outside of cells and can have a clear capsule surrounding them. If located intracellularly, the more specific identification of "neutrophil/macrophage with phagocytosed fungi" should be used. The most commonly encountered yeast is *Candida albicans*. It is ovoid, 5 to 7 µm, and has a thick wall. The spores may form pseudohyphae that branch and may have terminal budding forms. These pseudohyphae may be up to 50 µm in length. These microorganisms can be accentuated, as can most fungal organisms, by GMS (Gomori methenamine silver) staining. The pseudohyphae may be encountered in immunocompromised patients with severe infection.

In the cerebrospinal fluid, *Cryptococcus* is the most commonly encountered fungus. This microorganism is a round to oval yeast-like fungus ranging from 3.5 to 8 µm or more in diameter, usually with a thick mucopolysaccharide capsule. Budding forms display a narrow neck. These microorganisms are often lightly basophilic on Wright-Giemsa stain, and the capsule is accentuated by staining with mucicarmine.

Miscellaneous Findings

Fat Droplets

Fat droplets are found free in the fluid as translucent or nearly translucent spheres of varying size. They are quite refractile and are anucleate. Fat droplets may be endogenous or exogenous in origin. In CSF fat droplets suggest injected dyes or fat emboli. They are seen in body cavities in pancreatitis

and dyslipidemia. In synovial fluid they suggest an articular fracture.

Mitotic Figure

When a cell undergoes mitosis, the regular features of a nucleus are no longer present. Instead, the nucleus appears as a dark, irregular mass. It may take various shapes, including a daisy-like form or a mass with irregular projections. On rare occasion, the telophase of mitosis may be seen as two separating masses of irregularly shaped nuclear material (chromosomes).

A cell containing a mitotic figure may or may not be larger than the cells around it. A mitotic figure may on occasion be difficult to distinguish from a degenerating cell, but in a degenerating cell, the nucleus is often fragmented into a single or multiple purple, round, dark-staining, homogeneous cytoplasmic object(s), without discernable chromosomal structures.

Stain Precipitate

Wright-Giemsa stain precipitate appears as metachromatic granular deposits on and between cells, and may be confused with bacteria, yeast, or other parasites. The size of the stain droplets varies in contrast to bacteria and yeast, which have a more uniform morphology.

Starch Granule

Starch granules are best thought of as contaminants from the powder on gloves that are worn by the physician during the procedure used to obtain the sample. Size varies from the diameter of a red cell to four to six times larger. With Wright-Giemsa stain, they are blue to blue-purple and irregularly rounded with a central slit or indentation. When polarizing filters are used, starch granules form white "Maltese crosses" against a black background.

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CLINICAL MICROSCOPY MISCELLANEOUS CELL

Introduction to Vaginal Wet Preparations

Wet preparations of vaginal secretions are often examined to diagnose causes of vaginal discharge. The nature of the discharge, its pH and odor, and the presence or absence of characteristic organisms in wet preparations are key to the evaluation process. For microscopic evaluation, a sample of vaginal secretions from the posterior vaginal pool, obtained by a speculum that has not been lubricated with petroleum jelly, is used. The secretions are collected on a cotton or dacron-tipped swab and mixed with a few drops of nonbacteristatic saline on a slide. The slide is studied with brightfield or phase microscopy. In cases where identification of fungi is a major consideration, some authors have suggested that placing a drop of vaginal fluid in a drop of 10-percent potassium hydroxide solution, covering it with a cover slip, and examining it with brightfield or phase microscopy enhances detection. Another type of vaginal wet preparation, the post-coital test, is performed in the preovulatory period, two to 12 hours following intercourse to assess the interaction between the sperm and cervical mucus. In this case the sample is of cervical mucus. The number of sperm and sperm motility are evaluated. For the purpose of photomicrograph-based proficiency testing, unstained wet-preparation photomicrographs are presented. The following descriptions are provided as a guide and are not exhaustive. A number of elements identifiable in vaginal wet preparations (erythrocytes, leukocytes, bacteria, fibers, mucus strands, pollen grains, spermatozoa, squamous cells, starch granules, and yeast/fungi) have the same appearance as in urinary sediment and their description can be reviewed in that section if not repeated below.

Vaginal Cells

Squamous Epithelial Cells with Bacteria (Clue Cell)

Clue cells are vaginal epithelial cells encrusted with the bacterium *Gardnerella vaginalis*. Clue cells have a heavy stippled or granular, very refractile cytoplasm with shaggy or bearded cell borders due to the heavy coating of the coccobacilli. Most of the cell surface should be covered by bacteria for it to be identified as a clue cell. The presence of occasional irregular keratohyalin granules in the cytoplasm of squamous cells should be distinguished from adherent bacteria.

Parabasal Cell, Basal Cell

Parabasal cells and basal cells are located in the deeper layers of the squamous epithelium in the vaginal tract. Vaginal smears

from women in child-bearing years usually contain less than five percent parabasal cells and rarely contain basal cells. Smears obtained from post-menopausal or postpartum women will show a higher proportion of parabasal cells. These cells are increased in numbers when the upper layers of the squamous epithelium have been damaged or lost due to injury, trauma, or an inflammatory process. Parabasal cells are also increased in numbers in direct cervical smears and are derived from areas of squamous metaplasia of the endocervical epithelium.

Parabasal cells vary in size from 12 to 30 μm in diameter, about a quarter to half the size of superficial squamous cells. They tend to have a round to oval shape with smooth borders and occasional small vacuoles in the cytoplasm. They can appear in clusters and may be angulated and have irregular polygonal shapes. Their nuclei are round to oval and the nuclear- to-cytoplasmic ratio is higher than seen in superficial squamous cells.

Basal cells are rarely seen in vaginal smears unless a pathologic process has damaged the squamous epithelium. These cells are smaller than parabasal cells and are round to oval in shape. They resemble very small parabasal cells. They have scanty cytoplasm and their nuclei are about the same size as those of parabasal cells. However, due to their smaller size, basal cells have a higher nuclear-to-cytoplasmic ratio than parabasal cells.

Squamous Epithelial Cell

These large (30 to 50 μm) flat cells are derived from the lining of the female vagina and cervix. In wet preparation, squamous cells are about five to seven times as large as a red cell and larger than parabasal and basal cells. A single, small, condensed, round or oval central nucleus about the size of a small lymphocyte (10 to 12 μm) is seen in flat, round, or rectangular cells. There may be fine cytoplasmic granulation. The edges of the cell may be curled. The cell membrane is usually well-defined in brightfield and phase microscopy. Degenerating squamous cells show granular swollen cytoplasm and eventual fraying; the nucleus becomes pyknotic and then lyses, and the cell may eventually resemble an amorphous disintegrating mass.

Spermatozoa

In wet preparations, the sperm head is about 4 to 6 μm long, usually tapering anteriorly. It is smaller and narrower than red cells. Slender tails are about 40 to 60 μm long. The head may be separated from the tail, making identification more difficult.

Fern Test

Evaluation of an air-dried slide prepared from the vaginal pool is one of the most widely used tests to detect rupture of the amniotic membranes and the early onset of labor. When properly performed and, particularly if used in conjunction with another widely used test such as the nitrazine test, this is highly sensitive and specific for the detection of ruptured membranes. The “fern test” was initially described in 1955 and its ease of use and clinical utility has been confirmed by multiple published studies.

A sample of fluid is collected from the vaginal pool and allowed to air dry on a microscope slide for 5-7 minutes. This is then examined under the microscope at low power. A positive test, indicating the presence of amniotic fluid, consists of an elaborate arborized crystallization pattern (ferning) best visualized when the substage condenser is lowered to accentuate the diffraction pattern. The test may be positive as

early as 12 weeks of gestation. Common contaminants such as blood, urine, meconium (by itself indicative of ruptured membranes), semen, or alkaline antiseptic solutions that may be present in the vagina do not usually cause a falsely negative result unless present in very high concentrations. Inadvertent contamination of the specimen by cervical mucus may cause a falsely positive result but the arborization pattern is less elaborate and normally will not form after the first trimester of pregnancy due to high levels of progesterone present.

Organisms

Trichomonas

Trichomonas vaginalis primarily causes vaginal infection, but also is capable of infecting the urethra, periurethral glands, bladder, and prostate. The normal habitat of *T. vaginalis* is the vagina in women and the prostate in men. In women, the organism feeds on the mucosal surface of the vagina, ingesting bacteria and leukocytes.

T. vaginalis is a protozoan flagellate with only a trophozoite stage. It is pyriform or pear-shaped with a length of 7 to 23 μm . There is a single nucleus and a stout central axostyle protruding from the posterior end of the body. Additional morphologic features include four anterior flagella and an undulating membrane in the anterior half from which projects a single posterior flagellum. In wet mounts, it demonstrates a jerky, rotating, nondirectional leaf-like motion. “Rippling” of the undulating membrane can be seen for several hours after cessation of organism motility.

Yeast/Fungi

Candida albicans is a colorless, ovoid, 5 to 7 μm , thick-walled cell. A cell with a single bud is characteristic. The cells stain poorly with aqueous stains in wet preparations, but are strongly positive with Gram staining. *Candida* species form elongated cells (pseudohyphae) up to about 50 μm long, resembling mycelia. These are branched and may have terminal budding forms. These pseudomycelial forms may be seen in patients with severe *Candida* infections. *Candida* species are a common cause of vaginitis, which is characterized by itching, burning, and a thick, “cottage cheese-like” discharge. This infection invokes an inflammatory response that is composed of lymphocytes and neutrophils.

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Introduction to Stained Stool and Nasal Smears For Eosinophils

It is sometimes useful to characterize the cellular elements in a bodily product. In stool, the presence of neutrophils is suggestive of certain enteric pathogens. *Shigella* dysentery will have neutrophils present in approximately 70 percent of cases; *Salmonella* and *Campylobacter* will demonstrate neutrophils in 30 to 50 percent of cases; and noninvasive organisms, such as Rotavirus and toxigenic *Escherichia coli*, will show neutrophils in only five percent of cases. Smears are prepared by selecting flecks of mucus from fecal material with a cotton swab that is then rolled across a glass slide. The smear is allowed to air dry and is then stained with a Wright-Giemsa stain.

Nasal smears for eosinophils are useful in distinguishing the nature of a nasal discharge. In nasal smears, the identification of eosinophils is a correlate of allergic rhinitis. In discharges due to allergy, the predominant cell is the eosinophil. In contrast, nasal discharge due to nonallergic causes will show either a predominance of neutrophils or acellular mucus. Infectious processes show predominantly neutrophils. The slide is prepared by having the patient blow his/her nose in a nonabsorbent material (e.g., waxed paper, plastic wrap). A swab is then used to transfer the mucus to a glass slide. A thin smear (one through which newspaper can be read) is essential. Cytologic detail is lost if the smear is too thick. The smear is then allowed to air dry and is stained. In nasal smears, usual Wright-Giemsa blood stains may yield bluish rather than red granules in eosinophils. Many use a Hansel stain instead, as eosinophils stain bright red whereas neutrophils and mucus debris have a blue color.

Since the characteristics of eosinophils and neutrophils are the most important features in stool and nasal smears, these are described below.

Neutrophil, Stained

Usually the neutrophil is easily recognized. The nucleus often is segmented or lobulated (two to five lobes) and is connected by a thin filament of chromatin. The abundant, pale pink or colorless cytoplasm contains many fine, lilac neutrophilic granules.

In smears, artifacts, cellular distortion, and cellular degeneration are common. The nuclear lobes may appear eccentric and the cytoplasm may contain toxic granules or be vacuolated. Neutrophils may show morphologic changes due to autolysis, including nuclear pyknosis and fragmentation, making recognition of the cell type difficult.

Eosinophil, Stained

Eosinophils are recognized by their characteristic bright orange-red spherical granules. They typically have a bilobed nucleus separated by a thin filament. Occasionally, more than two lobes may be seen. The granules are larger.

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KOH Preparations for Fungi

Hair, nails, and skin scrapings can be examined using a 10 percent KOH (potassium hydroxide) solution for the presence of fungi. KOH acts to disrupt cellular sheets or clumps of proteinaceous material and dissolves cellular material at a more rapid rate than fungi because of their chitinous cell wall. The result is a cleared background in which hyphal elements (prolonged branching filaments often divided into chains of cells by the presence of transverse walls or septa), yeast cells, and arthrospores (structures resulting from a hyphae fragmenting into individual cells) can be detected.

To make a KOH smear, a drop of 10 percent KOH solution is placed in the center of a clean glass slide. The specimen to be examined (hair, skin flake, piece of nail, etc.) is placed in the KOH. A coverslip is then placed over the material and the slide is gently heated for five to 10 minutes. The coverslip is then compressed to spread the material and is examined with a brightfield microscope with the condenser lowered to increase contrast. In laboratories where it is available, phase microscopy or interference microscopy can be used to increase detection. If fluorescent microscopy is available, Calcofluor White can be added to enhance detection.

Several species of fungi cause infection of the skin. Tinea versicolor consists of areas of depigmented to brown-red areas of skin on the trunk. It is due to growth of *Malassezia* in the cells of the stratum corneum. In the KOH prep, one sees many short, stubby hyphal segments (3 to 5 μm in diameter) admixed with budding, spheroidal yeast cells (4 to 6 μm in diameter). *Microsporum*, *Epidermophyton*, and *Trichophyton* species can cause several types of infection depending on the structures involved. Tinea corporis (ringworm) consists of circular patches with a red vesiculated border and central scaling that results from infection of nonhairy, smooth skin. Tinea pedis (athlete's foot) consists of red, scaling areas in the interdigital spaces of the feet due to infection of these areas. Tinea capitis consists of scaling, bald patches on the scalp due to infection of the hair by fungal elements that either invade (endothrix) or surround (ectothrix) the hair shaft. In all these conditions, if a preparation is made at the active border of advancing infection, one would see slender hyphal forms (3 to 5 μm in diameter), often breaking into arthrospore-like segments.

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Pinworm Preparations

Helminths - including Pinworm

Humans are a common host for *Enterobius vermicularis* (pinworm), and the number of human infections is estimated at 209 million cases worldwide, with the highest prevalence of infestation in children ages five to 14 in temperate, rather than tropical, zones. Adult pinworms inhabit the human appendix, cecum, and ascending colon without invasion of the intestinal mucosa. The gravid female descends the human colon nocturnally, emerging from the anus and crawling over the perianal/perineal/vaginal areas to deposit her eggs; each female worm harbors about 11,000 eggs. The eggs are not usually shed within the lumen of the human intestine, in contrast with those of other parasites; thus, the standard “O&P” stool exam is unlikely to reveal pinworm eggs.

Ova are laid in the perianal region of the human host by the gravid female pinworm and embryonate to the infective first stage within four to six hours. Infection is usually by direct transmission of eggs to mouth by hands or through fomites (dust particles containing infective eggs). As anal pruritus is a common symptom due to migration of the egg-laying female worm through the anus, and since children are the most common hosts, scratching with subsequent finger-sucking produces autoinfection. Some eggs may hatch in the perianal region, with these larvae reentering the rectum and maturing into adults (retroinfection).

Egg morphology is highly characteristic for *Enterobius*. They are elongate or ovoid, with a thick, colorless shell, 50 to 60 μm long and 20 to 32 μm wide. Typically, they are conspicuously flattened on one side, which helps distinguish them from hookworm eggs, which also have thinner shells. The egg of the whipworm (*Trichuris trichiura*), another human colonic nematode, is about the same size as a pinworm egg, but is barrel-shaped with a transparent plug at each end.

Specimen collection is by cellophane tape or Graham technique (adhesive cellophane tape is firmly applied to the uncleaned perianal area in the morning). The tape is then applied to a glass microslide on which a small amount of toluidine has been placed to partially clear the tape and eliminate distracting air bubbles. Alternatively, there is an anal swab technique using paraffin/petroleum jelly-coated cotton swabs, or the surface of stool specimens may be gently scraped to remove adherent

Enterobius eggs. Multiple samples over several days may be necessary to establish the diagnosis.

Strongyloides stercoralis (rhabditiform larva) is a tiny intestinal nematode where the mature form and eggs are rarely seen. However, the rhabditiform larvae can be found in the duodenal contents and stool and comprise the diagnostic form. The larva is small and slender, measuring about 225 by 16 μm . The head has a short buccal cavity, distinguishing it from hookworm larva which have long buccal cavities. The tail is notched, in contrast to the pointed tail of hookworm larvae.

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