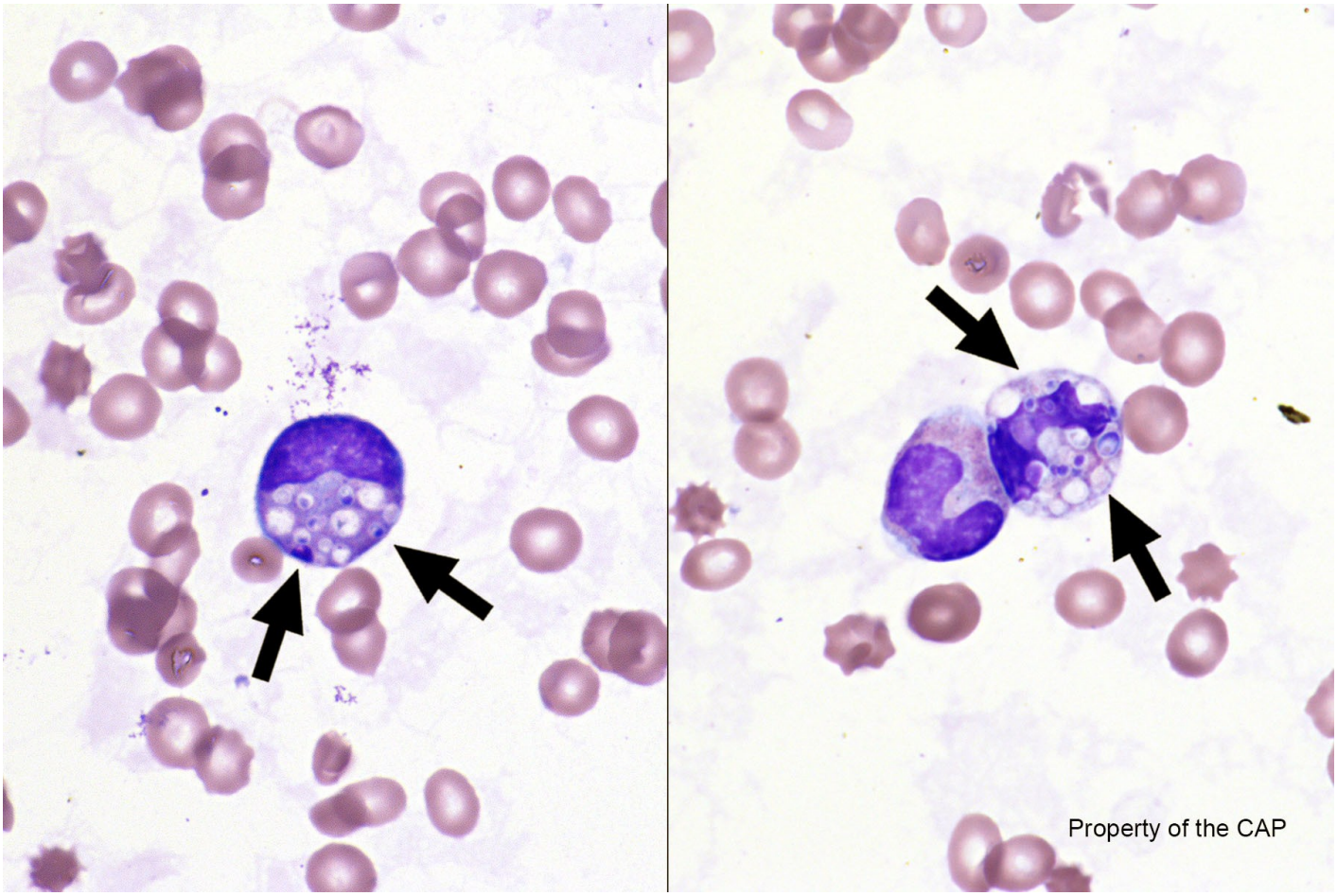
SMJ-FSED missed one of the cell identifications on FH9-C, and so this is the education for that missed identification. Please read the attached information from the Participant Summary as well as the additional comments. I will then add these images to the slide show that was prepared in 2020 (which is still available for review at any time in the G: drive under “ED at Proffit” and “Hematology.” I blew the image up as much as possible.

Blood Cell Identification – Ungraded

BCP-28



Before looking at the answer, do you have any guesses?

Identification	Referees		Participants		Evaluation
	No.	%	No.	%	
Leukocyte with intracellular fungi	109	61.9	3009	55.5	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	22	12.5	815	15.0	Educational
Leukocyte with intracellular bacteria	12	6.8	666	12.3	Educational
Immature or abnormal cell, would refer for identification	9	5.1	178	3.3	Educational
Monocyte	6	3.4	202	3.7	Educational
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	5	2.8	194	3.6	Educational
Leukocyte with intracellular *Anaplasma/Ehrlichia*	3	1.7	129	2.4	Educational
Parasite(s) seen, referred for definitive identification	3	1.7	48	0.9	Educational
Neutrophil necrobiosis (degenerated neutrophil)	2	1.1	25	0.5	Educational
Leukocyte containing Alder (Alder-Reilly) anomaly inclusion(s)	1	0.6	6	0.1	Educational
Leukocyte containing Chediak-Higashi anomaly inclusion(s)	1	0.6	20	0.4	Educational
Lymphocyte, reactive (includes plasmacytoid and immunoblastic forms)	1	0.6	24	0.4	Educational
Malignant lymphoid cell (other than blast)	1	0.6	33	0.6	Educational
Protozoa (non-malarial)	1	0.6	7	0.1	Educational

BCP-28 (***these are the explanation pages from the Summary***)

The arrowed cells are leukocytes with phagocytosed fungi, as correctly identified by 61.9% of referees and 55.5% of participants. 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 very important in making the appropriate identification. In this case, leukocytes contain 2 to 4 µm budding yeast forms of Histoplasma capsulatum. 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, Dohle bodies, or large bacterial cocci.

The arrowed cells were incorrectly identified as a neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization) by 12.5% of referees and 15.0% of participants. Toxic granulation is defined by the presence of large, purple or dark blue cytoplasmic granules in neutrophils, bands, and metamyelocytes (monocytes, as seen in the image, do not demonstrate toxic granulation). Vacuoles within the cytoplasm of these same cells define toxic vacuolization. The vacuoles are variable in size (unlike in the image) and may coalesce, sometimes distorting the neutrophil cytoplasm to form pseudopodia. 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 at the periphery of the cytoplasm, near the cell

The arrowed cells were incorrectly identified as a leukocyte with intracellular bacteria by 6.8% of referees and 12.3% of participants. It is very unusual to see bacteria on a routine 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 arranged singly, as diplococci, in clusters or in chains. They can be accentuated and confirmed with a Gram stain.

The arrowed cells were incorrectly identified as a monocyte by 3.4% of referees and 3.7% of participants. While one of the imaged cells is a monocyte, it is not normal and contains phagocytosed fungi.

The arrowed cells were incorrectly identified as leukocytes with intracellular *Anaplasma/Erlichia* by 1.7% of referees and 2.4% of participants. On Wright-stained preparations, Anaplasma species appear as round, dark purple-stained dots or clusters of dots (morulae) in the cytoplasm of either neutrophils (*A. phagocytophilium*) or monocytes and macrophages (*A. chafeensis*). They usually do not fill in the entire cytoplasm.

The arrowed cells were incorrectly identified as parasites seen, refer identification by 1.7% of referees and 0.9% of participants. Plasmodium (malaria) and Babesia infections can be seen as parasites on blood smears within red blood cells (and not leukocytes).

BCP-28, cont'd

The arrowed cells were incorrectly identified as neutrophil necrobiosis (degenerating neutrophil) by 1.1% of referees and 0.5% of participants. 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, in association with inflammatory disorders, and in malignancies). It is a non-diagnostic and non-specific finding. Degenerated neutrophils are generally easily identified because they resemble normal segmented neutrophils: they are round to oval cells ranging from 10 to 15 μm in diameter 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 pattern in these karyorrhexic or pyknotic states is also characteristic: dense and homogeneous, without visible parachromatin or nucleoli. The nuclear lobes may fragment into numerous small particles of varying size that can resemble microorganisms suchas bacteria or fungi. Also, the nuclear outlines may become indistinct and blurred. As cellular degeneration continues, the cytoplasm will become hypogranulated, then agranular, and the cytoplasmic borders may become frayed and indistinct.

Clinical Presentation:

This peripheral blood smear is from a 46-year-old man with HIV/AIDS, who presents with severe abdominal pain, pancytopenia, and hepatosplenomegaly. Laboratory data include: WBC = 3.0 × 10E9/L; RBC = 0.90 × 10E12/L; HGB = 2.6 g/dL; HCT = 9.0 %; MCV = 87 fL; MCHC = 29.2 g/dL; PLT = 20 × 10E9/L; and RDW = 19 %.

(PERIPHERAL BLOOD, WRIGHT-GIEMSA)

Case discussion: Disseminated histoplasmosis

Histoplasma capsulatum var. capsulatum is the most widely distributed of the endemic mycoses, being present in many parts of the world. In North America, *H. capsulatum* is endemic to the Mississippi and Ohio River valleys but is also present in other areas. The fungus is associated with bat and bird guano, nitrogen-rich substrates that support fungal growth.

The clinical manifestation of *H. capsulatum* infection depends on the degree of the exposure and the immune status of the host, ranging from an asymptomatic infectious process to disseminated life-threatening disease. Exposure to low concentrations of spores from the environment in a normal host is typically asymptomatic, while immunocompromised individuals, particularly those with advanced HIV disease and those receiving tumor necrosis factor inhibitors, are at risk for disseminated histoplasmosis. Individuals at the extremes of age who do not have a recognized immunosuppressive condition but may have an immune system that is incompletely developed or is diminished by age are also at a higher risk for disseminated infection, however, the mechanism for this group of patients is not completely understood.

Disseminated histoplasmosis may affect any system with the hallmark of disease being an oropharyngeal ulcer, which may cause hoarseness, dysphagia, or a painful lesion on the tongue or gingiva. Infection of the reticuloendothelial system results in lymphadenopathy, hepatosplenomegaly and/or thrombocytopenia. Central nervous system infection may manifest as chronic meningitis, intracerebral granulomas, or both. Destruction of the adrenal cortex by the granulomatous process may be sufficiently extensive to cause hormonal insufficiency. Endovascular infection includes endocarditis with large, bulky vegetations. Any part of the gastrointestinal tract may be affected, and ulcerating lesions may suggest a neoplasm macroscopically.

H. capsulatum is a facultative intracellular pathogen and found predominantly in macrophages and monocytes. In affected tissues, pathologic lesions consist of collections of infected macrophages, non-necrotizing granulomas, or necrotizing granulomas and intracellular organisms (2-4 μm yeast forms with narrow based budding) are visible by H&E staining (with PAS and GMS stains being more sensitive). In the profoundly immunocompromised patients *H. capsulatum* are found in circulating monocytes and neutrophils on peripheral blood smears using Wright-Giemsa stain, as seen in our case. Phagocytosed fungi appear in cytoplasm as round forms with clearing around the yeast giving an appearance of a cell wall. However, this clearing represents an artefact due to the poorly staining yeast wall and retraction of its cytoplasm during fixation.

The diagnosis of Histoplasma infections requires a combination of culture, cyto- and histopathology, serology, and antigen testing. A urinary antigen test is the most sensitive in active and disseminated disease. However, this test is known to have some cross-reactivity in patients with blastomycosis. Culture, including blood culture, is generally required for disease confirmation.

The prognosis for patients with histoplasmosis depends on status of their immune system, as well as the presence of comorbid conditions. Patients with disseminated disease require systemic antifungal treatment andsupportive care. Patients with endocarditis require surgical valve replacement in conjunction with antifungal therapy.

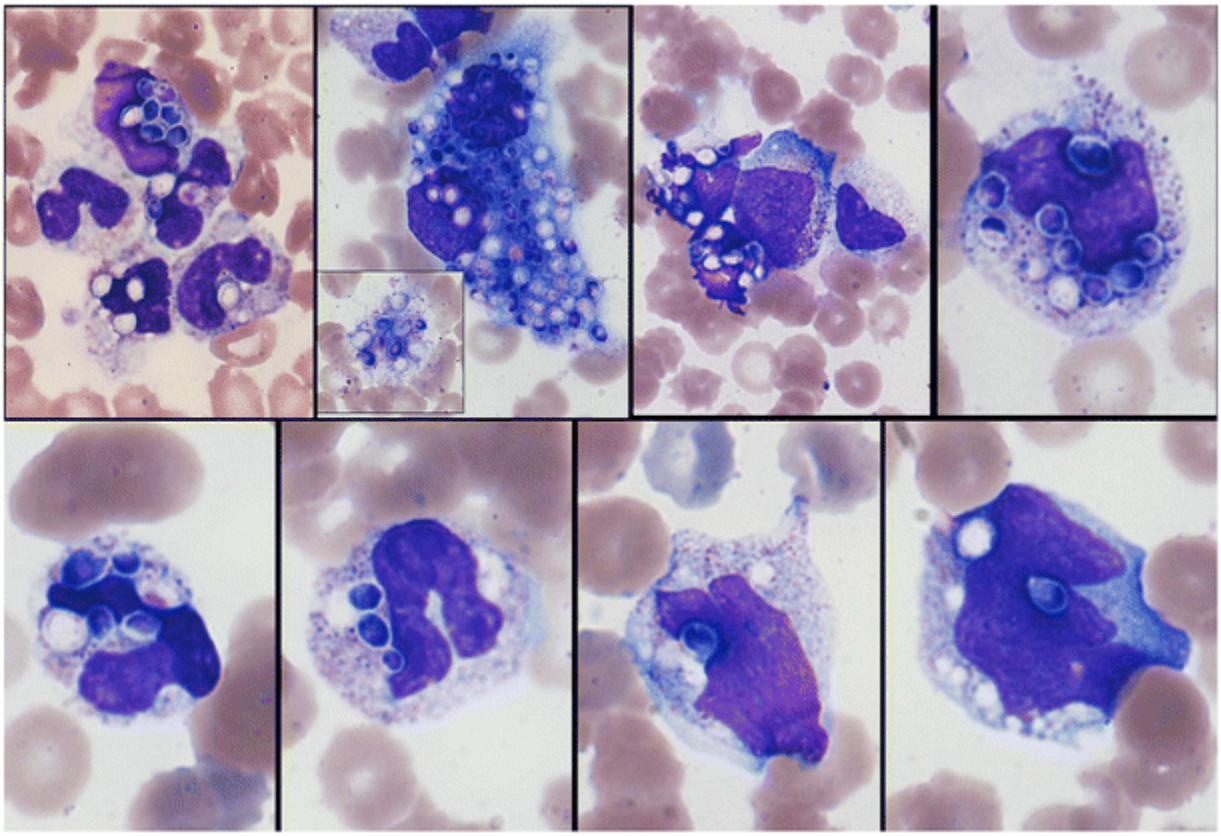
Olga Pozdnyakova, MD, PhD Hematology and Clinical Microscopy Committee

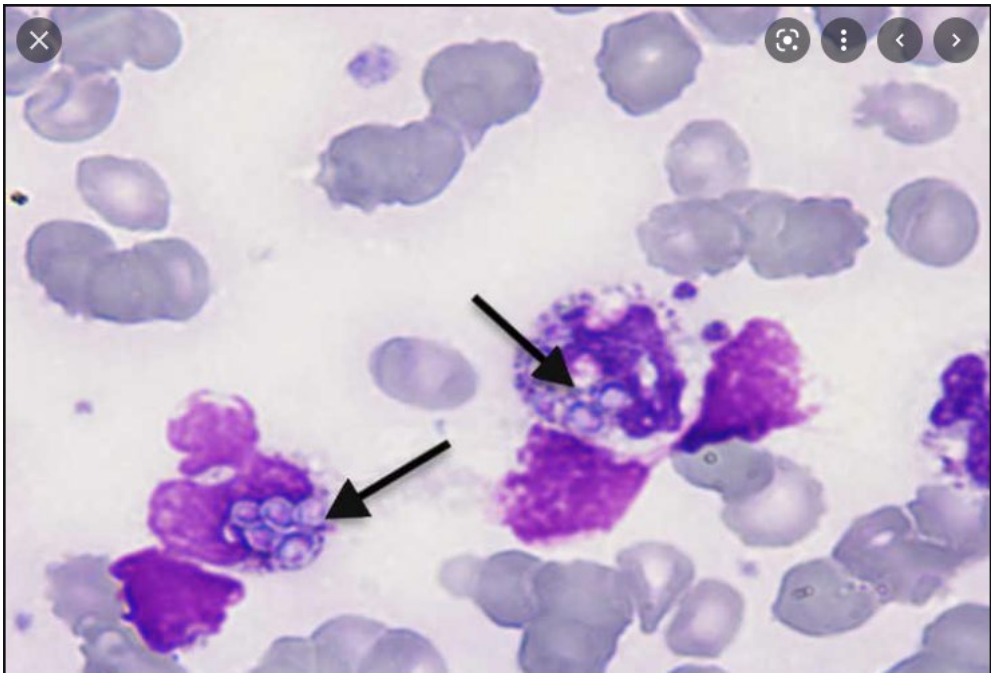
References:

1. Glassy EF, ed. *Color Atlas of Hematology: An Illustrated Field Guide Based on Proficiency Testing*. 2nd ed. Peripheral Blood. College of American Pathologists; 2018.
2. Procop Gary W and Pritt Bobbi. *Pathology of Infectious Diseases: A volume in the Series: Foundations in Diagnostic Pathology*, 1st ed. Saunders; 2014.
3. McPherson Richard A and. Pincus Matthew R. *Henry's Clinical Diagnosis and Management by Laboratory Methods,* 23rd ed. Elsevier; 2016.

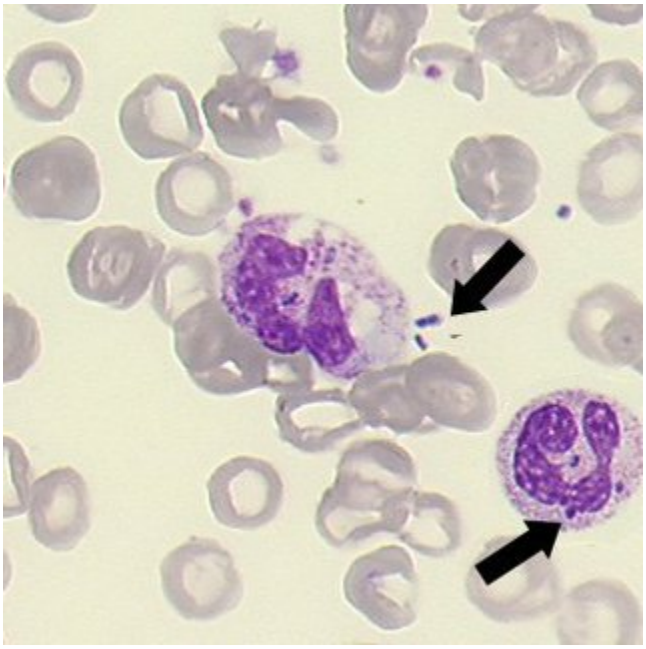
--I wanted to gather a few more images. After reading the descriptions, I can see how this would be intracellular yeast. I think it would have been clearer with an image bank to compare against (hence this education). I don't think it is clear, but I could at least tell there was something within the white cell that was abnormal, but I called them Ehrlichia.

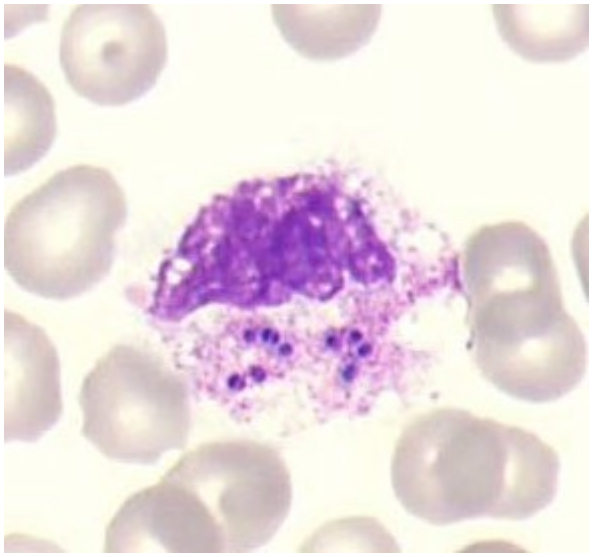
Here are more images of intracellular fungi (specifically histoplasma capsulatum).

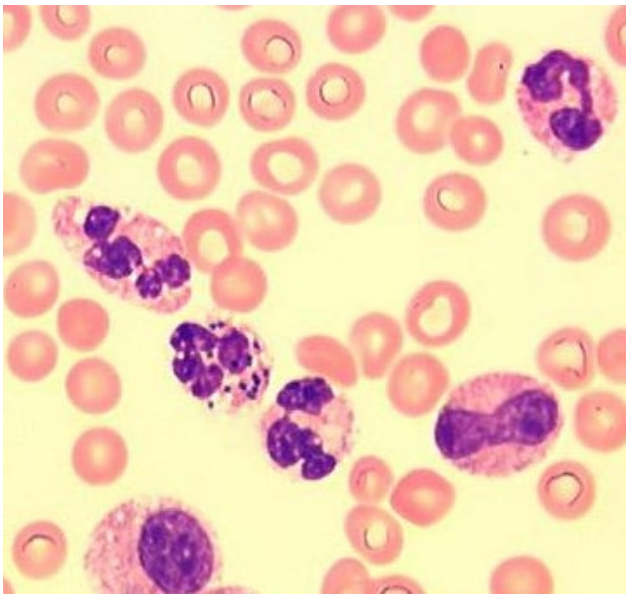


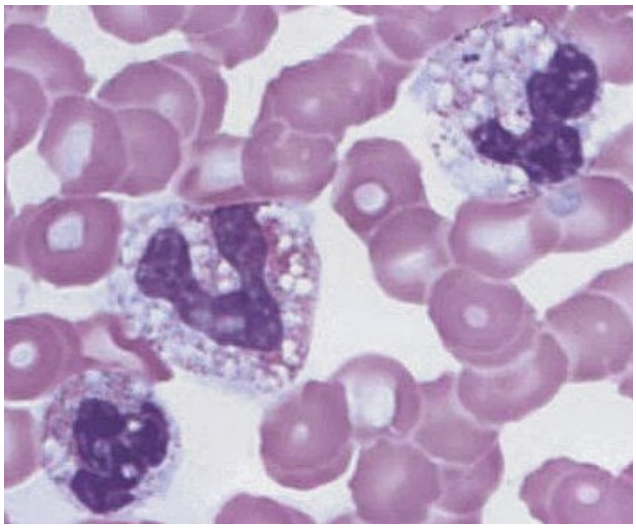


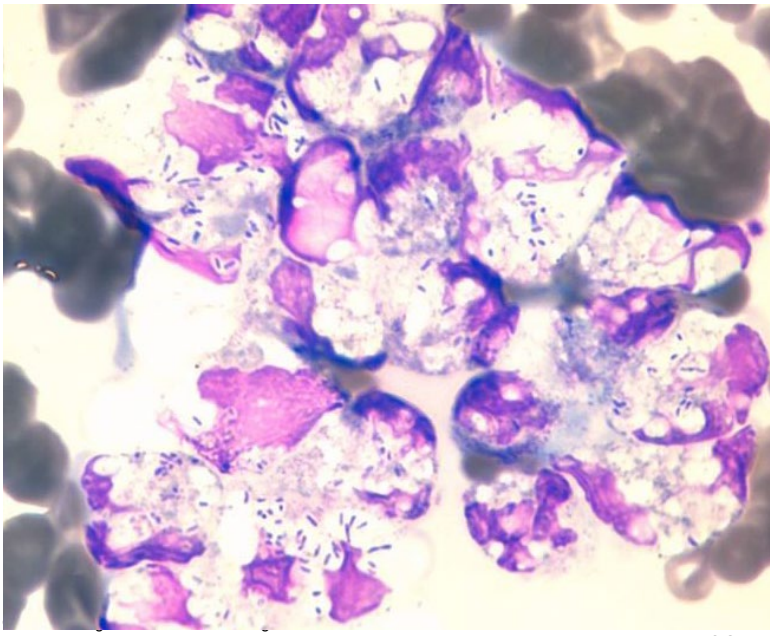
In Contrast, here are a few images of Leukocytes with intracellular bacteria.





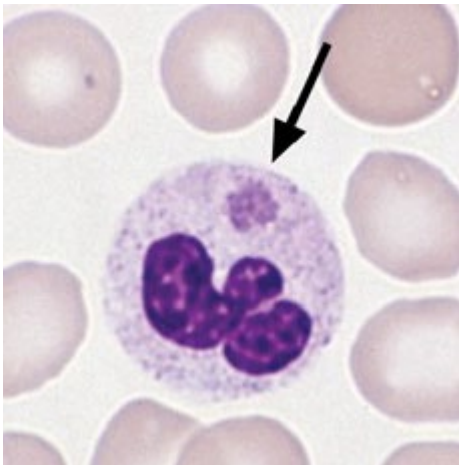


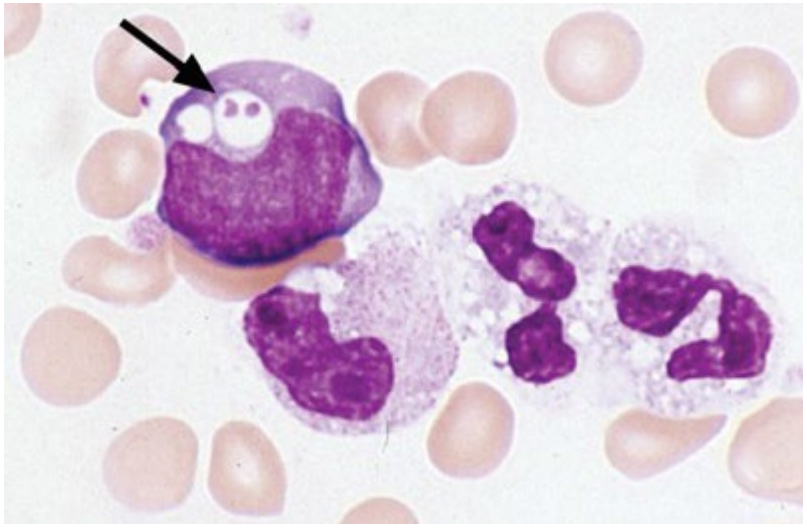


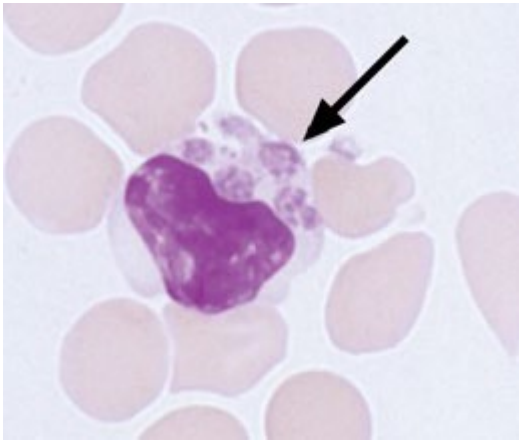


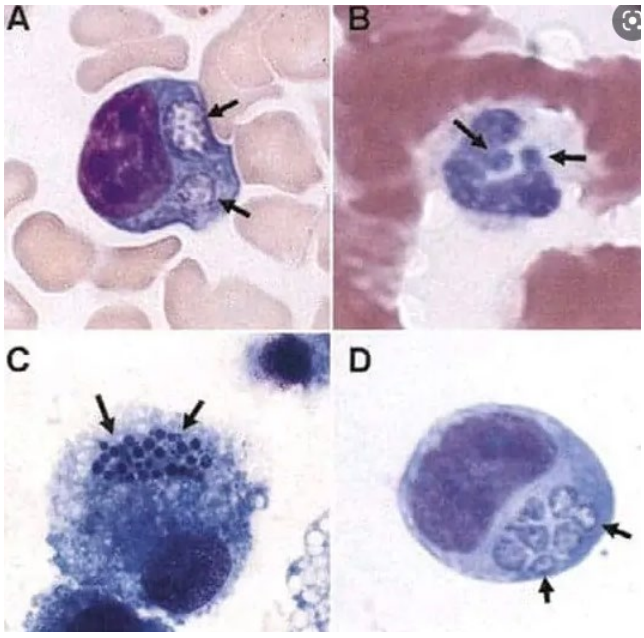
Note the major difference between the bacteria and fungi besides appearance of the particle itself is the vacuole that creates a clearing around the fungi/yeast. The bacteria typically do not have as large a vacuole or cleared space around it when intracellular. The fungi/yeast are also slightly larger than the bacteria. The bacteria also tend to be solely seen in neutrophils, and you will note some of the fungi are seen in monocytes.

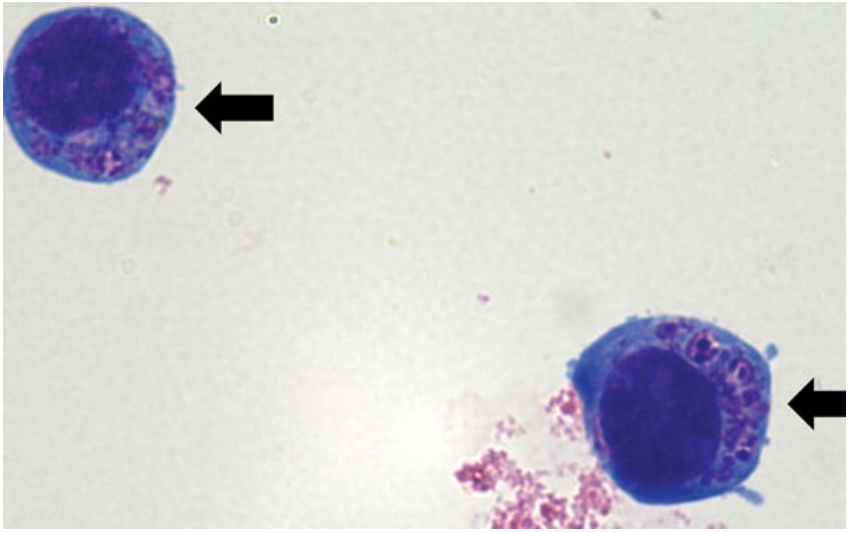
And below are images of leuikocytes with intracellular Ehrlichia and Anaplasma (tick bourne illnesses).











With Ehrlichia there is sometimes a larger vacuole around the organism, but the size and coloration is different than fungi or bacteria. Note that it almost resembles a platelet, but more purple and diffuse, and within the matrix of the cytoplasm. The anaplasma (bottom pictures) are slightly more obvious based on the way they gather within the cell. Note that these can be seen in neutrophils, monocytes, or lymphocytes (will never see fungi or bacteria within a lymphocyte).