Cytochemical Stains

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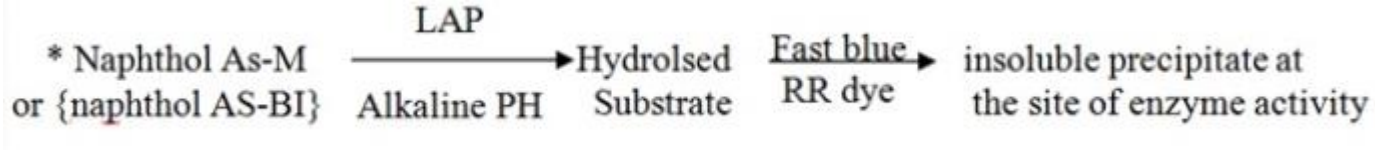
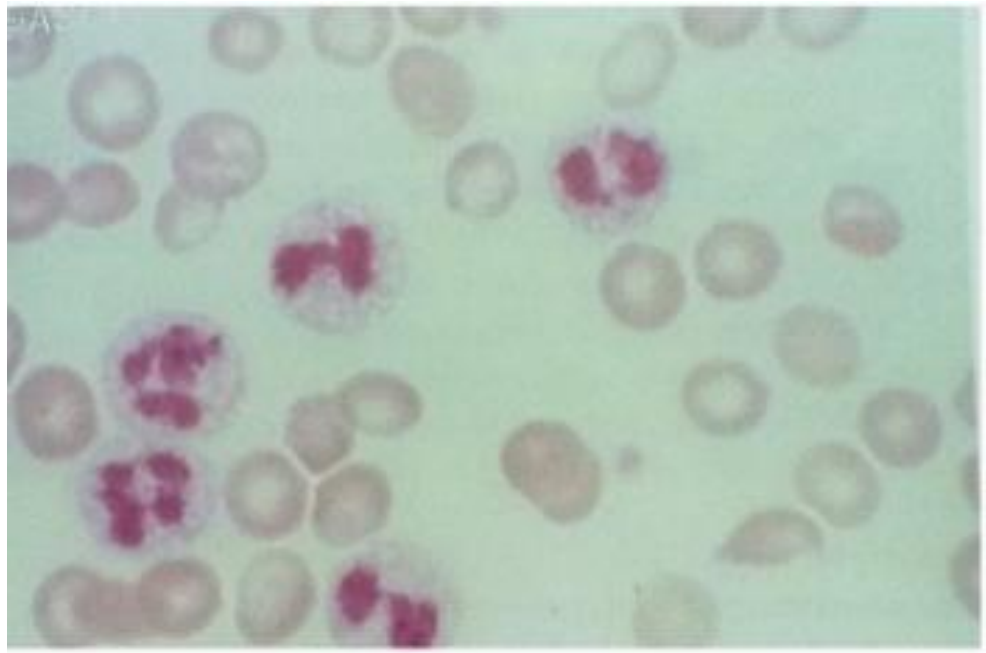
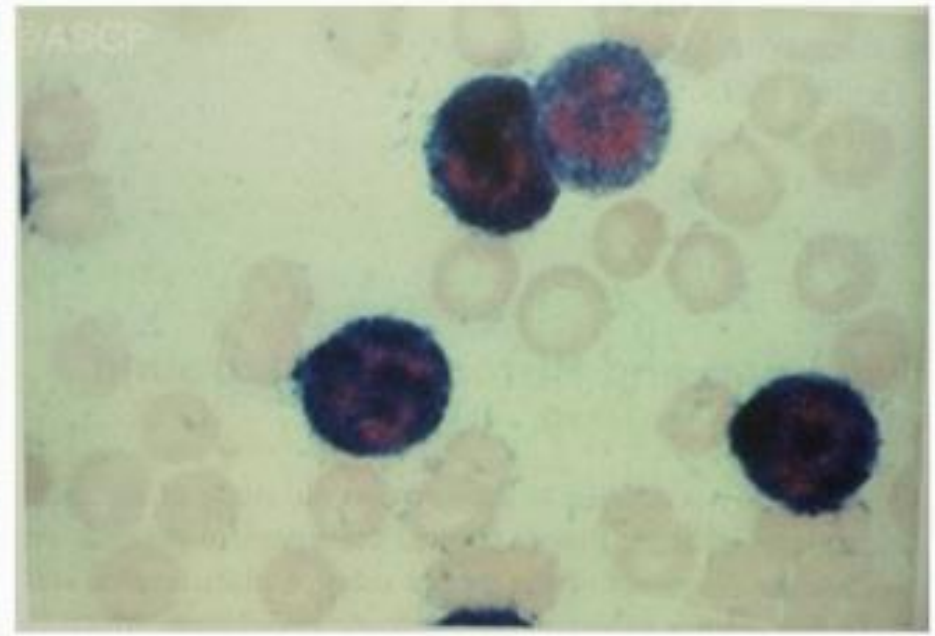
Leukocyte Alkaline Phosphate (LAP)

- Distinguish the cells of leukemoid reactions with increase activity from CML with decreased activity
- The LAP enzyme is in the tertiary granules of segmented neutrophils, bands and metamyelocytes
- The LAP within the neutrophil hydrolyzes the substrate (naphthol-AS-BI phosphate)
- hydrolyzed substrate then couples with the dye (fast blue BB salt) and precipitates at the site of activity
- ↑staining ↑enzyme activity





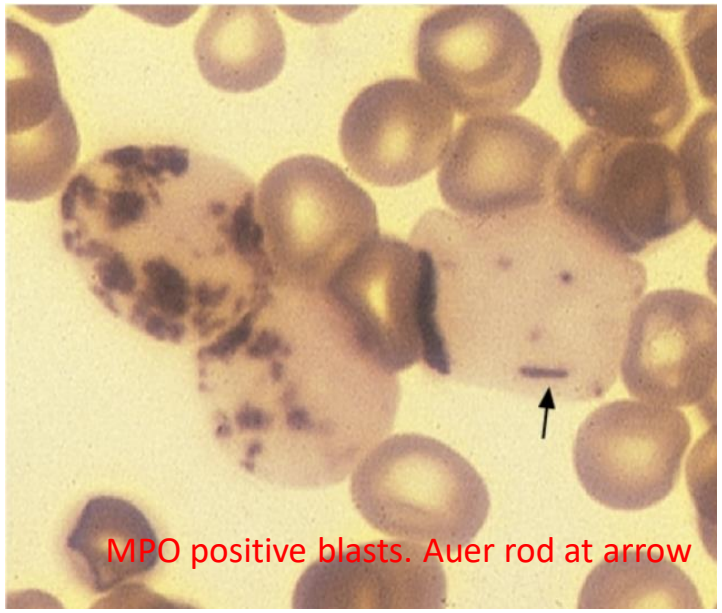
Negative LAP reaction

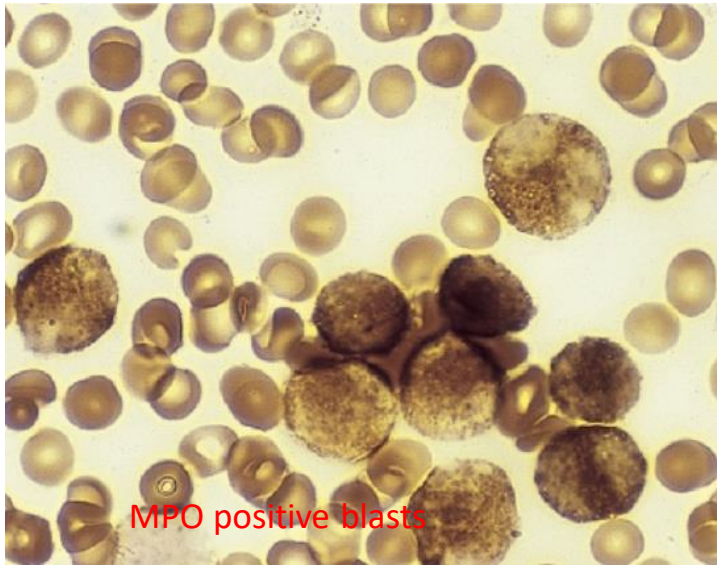


Positive LAP reaction

Myeloperoxidase (MPO)

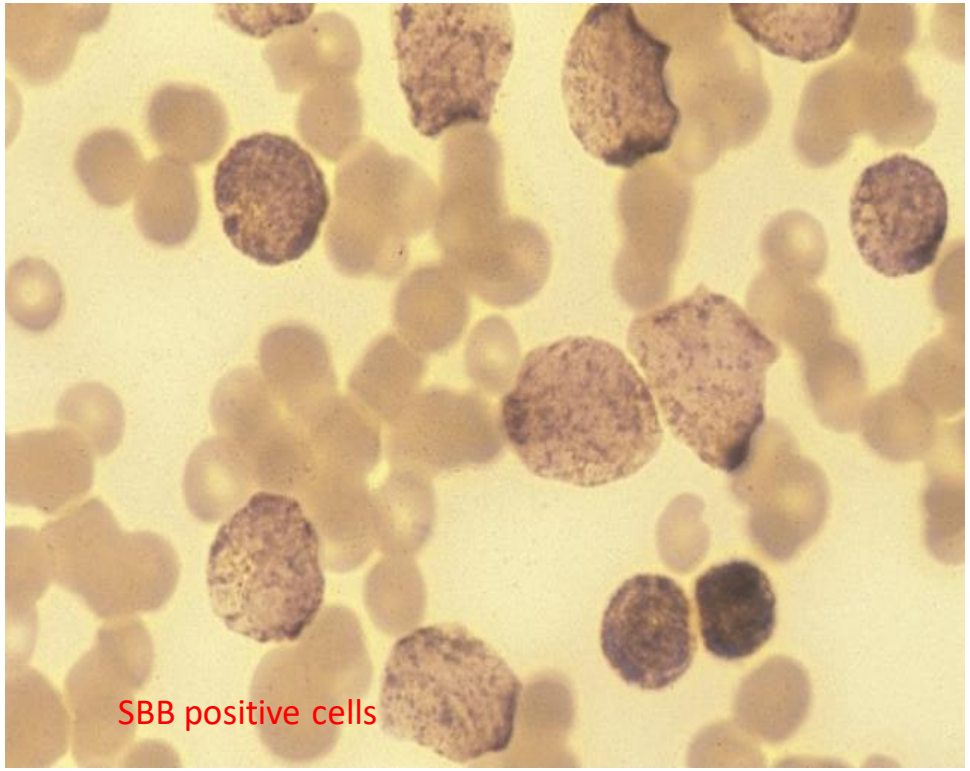
- Peroxidase enzyme is present in the primary granules of neutrophils(beginning promyelocyte stage and continuing throughout maturation),eosinophils and to certain extent monocytes
- Red cells and lymphocytes do not exhibit MPO activity
- Useful in differentiating myeloblasts from lymphoblasts


MPO positive blasts. Auer rod at arrow


MPO positive blasts

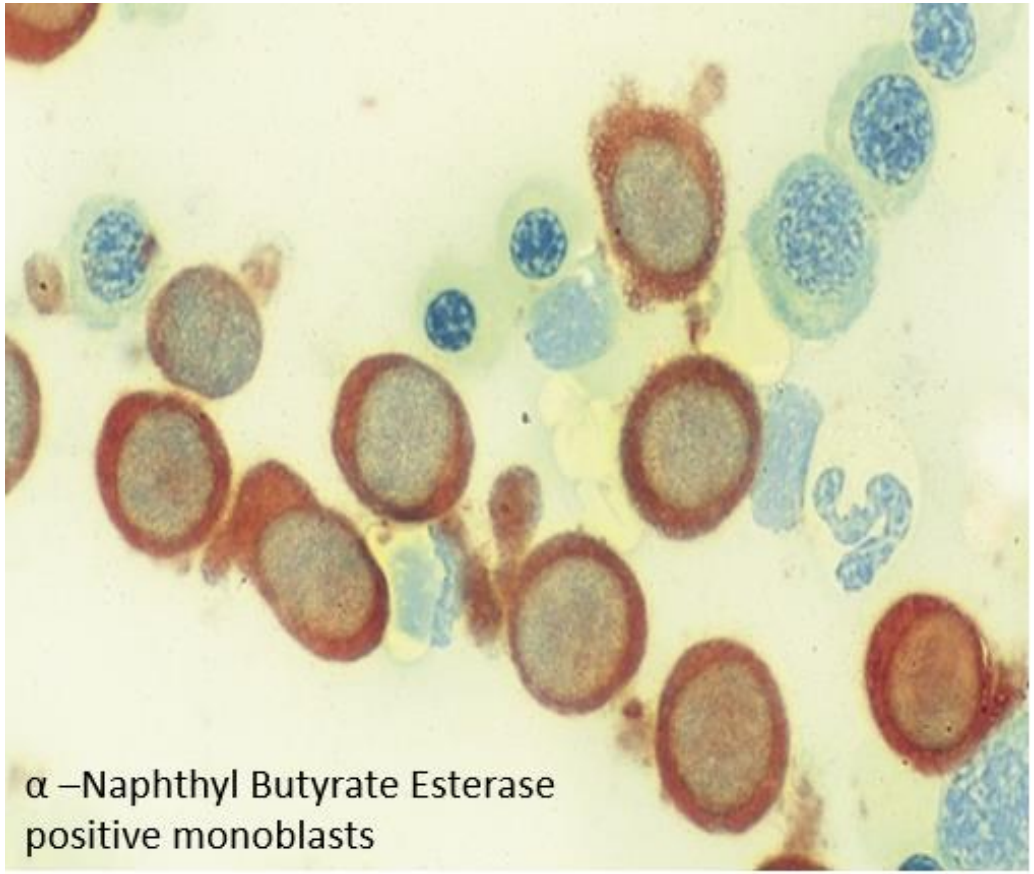
Sudan Black B (SBB)

- Distinguish acute myelocytic and monocytic leukemia from acute lymphocytic leukemia
- SBB dye is fat soluble, stains fat particles present in the primary and secondary granules of myelocytic and monocytic cells.
- Lymphoid cells are unable to stain
- The reaction increases with the maturity of the granulocytic cell

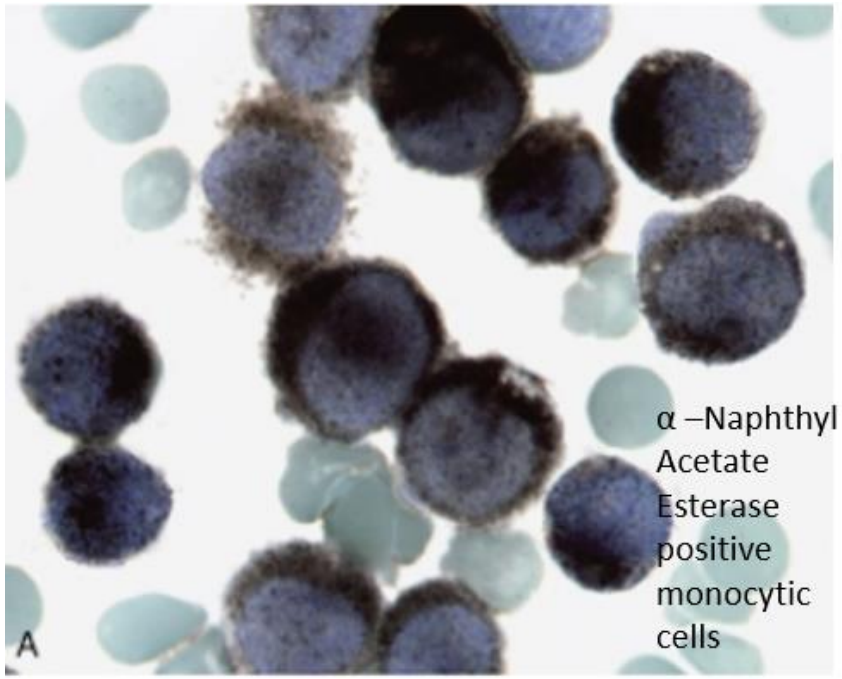


Esterases

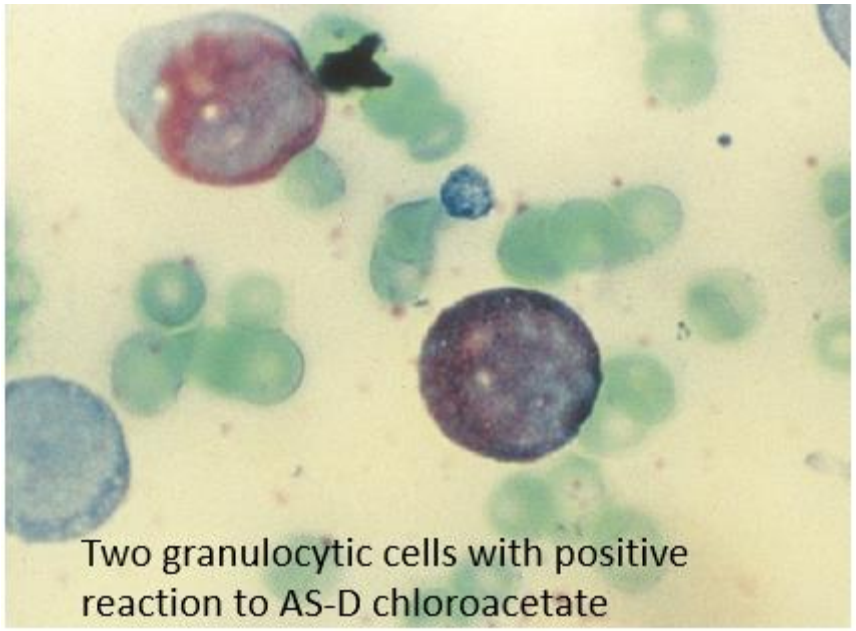
- Differentiate myelocytic and monocytic leukemia
- WBCs contain esterases, a group of lysosomal enzymes
- Two substrate esters
  - α –naphthyl acetate and α- naphthyl butyrate
    - both nonspecific and strong activity with cells of monocytic origin
  - Naphthol ASD chloroacetate
    - specific and positive reaction with granulocytic cells



α –Naphthyl Butyrate Esterase positive monoblasts



α –Naphthyl Acetate Esterase positive monocytic cells



Two granulocytic cells with positive reaction to AS-D chloroacetate

Tartrate Resistant Acid Phosphatase- TRAP

- Acid phosphatase is lysosomal enzyme present in many tissues and hematopoietic cells
- Seven isoenzymes have been classified as nonerythrocytic
- Isoenzyme 5 is tartrate resistant, produced in abundance by hairy cells

Acute Leukemia Cytochemical Reaction Chart

Condition	MPO	SBB	NASDA	ANBE	ANAE
ALL	–	–	–	–/+ (focal)	–/+ (focal)
AML	+	+	+	–	–
AMML	+	+	+	+ (diffuse)	+ (diffuse)
AMoL	–	–/+	–	+ (diffuse)	+ (diffuse)
Megakaryocytic leukemia	–	–	–	–	+ (localized)