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RESEARCH ARTICLE

INCLUSION BODIES - A REVIEW

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ABSTRACT

The word inclusion means incorporation. Inclusion bodies are nuclear or cytoplasmic aggregates of stainable substances which are usually 'proteins'. They typically represent sites of viral multiplication in bacteria or in a eukaryotic cell and usually consist of viral capsid proteins. Inclusion bodies are typically identified within a cell by their different appearance.

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INTRODUCTION

Inclusion bodies (IB) can also be hallmarks of genetic diseases, as in the case of Neuronal Inclusion bodies in neural disorders, so basically they are Inactive Aggregates of protein known as Inclusion Bodies (IB). In order to identify the types and causes of inclusion bodies in human cells, it is primarily important to know what an inclusion body is. In simple terms, inclusion bodies are abnormal structures that appear in cells. They are foreign invaders and unwelcomed in any living body.

Significance of inclusion bodies (Salvador Ventura and Antonio Villaverde, 2006)

- Inclusion bodies are considered to be formed by unspecific hydrophobic interactions between disorderly deposited polypeptides and are observed as 'molecular dust-balls' in cells.
- Identification of different diseased conditions.
- IBs can be a source of relatively pure protein because they can be easily purified from disrupted cells.

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Classification

Viral inclusion bodies

A. Intracytoplasmic eosinophilic
   - Guarnieri bodies in Small pox
   - Henderson-Peterson bodies in Molluscum contagiosum
   - Negri bodies in Rabies
B. Intranuclear acidophilic-
   - Cowdry type A in Varicella zoster virus
C. Intranuclear basophilic
   - Owl eyes" in cytomegalovirus
D. Both intranuclear and intracytoplasmic-
   - Warthin finkeldey bodies in Measles
   - Mitsoid bodies in Heck's disease

Inclusion bodies in Erythrocytes

Normally a red blood cell does not contain inclusions in the cytoplasm. It may be seen during certain hematologic disorders like

A. Developmental Organelles
   - Howell-Jolly bodies
   - Pappenheimer bodies

- Cabot Rings
- Basophilic stippling

B. Abnormal Hemoglobin Precipitation
- Heinz bodies
- Haemoglobin H Inclusions
- Koilocytes – Term used generally to denote the viral inclusion cell especially for HPV. Large vacuolization in the cell is seen below the granular cell layer with basophilic inclusion bodies (Textbook of Oral Pathology).

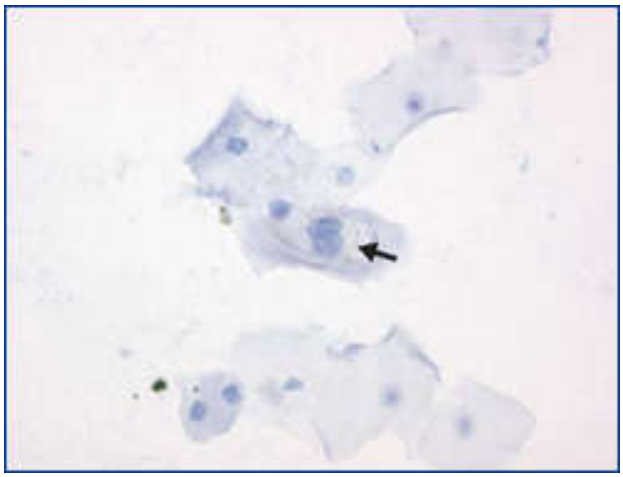


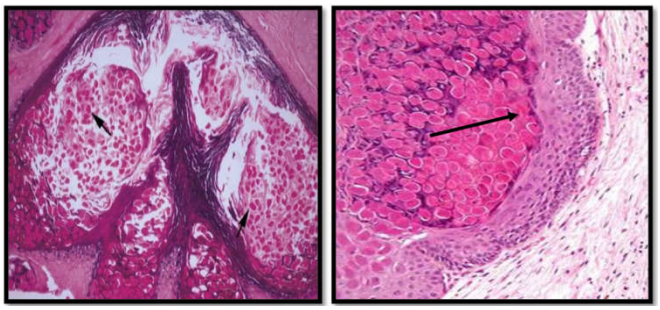
Fig 1. Cytological smear showing Koilocytes with double nucleus

Henderson- Paterson bodies - Molluscum contagiosum (MC)

Molluscum contagiosum (MC) is a viral infection of the skin or occasionally of the mucous membranes. It is caused by a DNA poxvirus called the molluscum contagiosum virus (MCV) MCV has no animal reservoir, infecting only humans. Molluscum contagiosum lesions are pearly in appearance with a dimpled centre. The central waxy core contains the virus. [2] These lesions appear microscopically as inverted lobules of hyperplastic acanthotic squamous epithelium which is seen pushing into the underlying connective tissue. The center of endophytic bulbous structures are filled with enlarged, altered keratinocytes with basophilic viral inclusions (intranuclear) referred to as Henderson- Paterson bodies. These represent the particles of pox virus in different stages of maturation. Initially, the small viron particles are seen in a cytoplasm of the epithelial cell above basal layer. These eosinophilic particles enlarge at the level of granular layer and occupy the entire cell. In the superficial layers of epithelium, the large cytoplasmic inclusion bodies are seen in the cells along with cresentic compressed nucleus. The Henderson- Paterson bodies are expelled at the center of the crater from the cells of stratum corneum. Foreign body reaction and inflammatory cells may be seen in relation to extrusion of bodies in underlying connective tissue stroma. Molluscum contagiosum has been reported to occur in association with neovascular nevus, a halo nevus and with lupus erythematosus (Textbook of Pathology – Saraf).

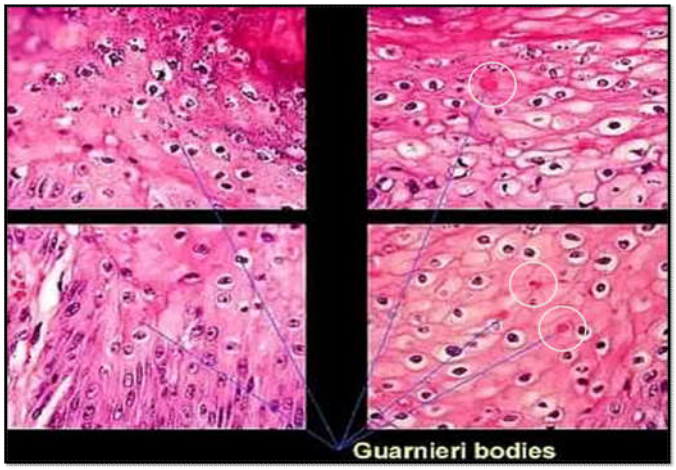
Small pox (Guarnieri bodies)

Small pox (Variola) Variola meaning “pimple” It is a life threatening disorder and was declared that has been eradicated by the World Health Organization in 1977.



Henderson- Paterson bodies

Smallpox pustules have been called “pearls of pus” to help distinguish them from the more delicate “dewdrops on rose petals,” which describes typical Varicella Zoster (Mike bray1 and mark buller, 2004). Histologically cutaneous smallpox lesions may resemble herpetic lesions except that smallpox has intracytoplasmic inclusions (Guarnieri bodies) also known as type-B inclusions. They are named after Italian physician Giuseppe Guarnieri, and appear in the cytoplasm of epithelial cells instead of intranuclear inclusions (Lipschutz bodies) of herpetic lesions (David *et al.,* 2009).



Negri Bodies

They are named after Adelchi Negri who first discovered them. These inclusion bodies are typically found in nerve cells that have been infected by the rabies virus. They probably stem from outside influences which deliver rabies in the first place.

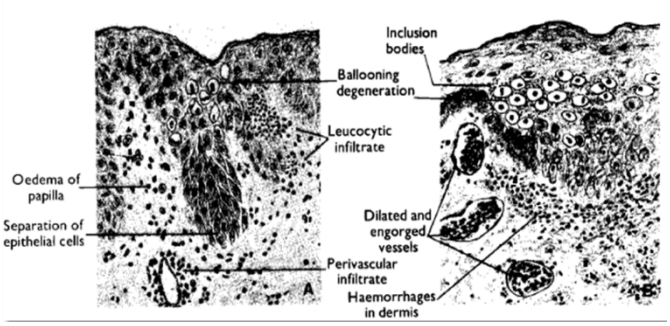
Herpes simplex virus (HSV)

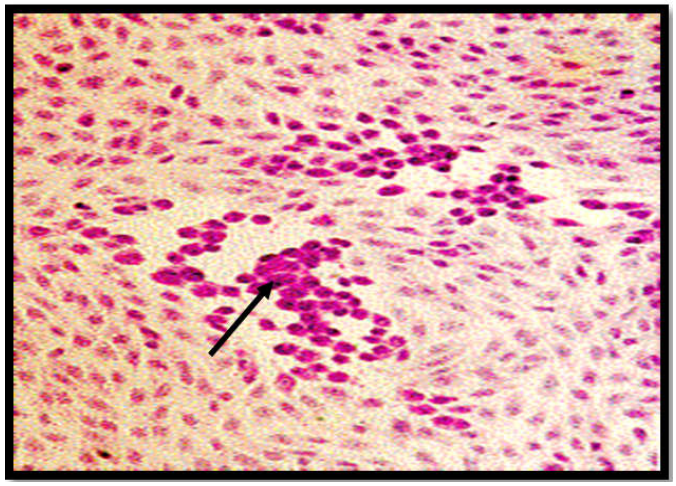
Herpes simplex virus infections are common vesicular eruptions of the skin and mucosa. The virus-infected keratinocytes contain one or more homogeneous, glassy nuclear inclusions. These cells are readily found on cytological preparations (Textbook of Pathology - Regezi). The virus needs cells to replicate and thereby infect it. Over a period of time the cell undergoes a structural and biochemical alterations that ultimately lead to cell death. The cell death is usually due to response to infection and virus replication. The infected cell shows enlarged nucleoli (ballooning) as earliest manifestation which is displaced towards nuclear membrane and eventually it undergoes disaggregation and fragmentation.

The host and other structural changes that are induced by virus in the host cell are:

- Chromosomal margination.
- Duplication and folding of intracellular membrane.
- Fragmentation of golgi stacks.
- Insertation of viral proteins into cellular membranes.
- Polykaryocytosis – cell become round in shape and fuse with each other
- Rearrangement of microtubular network

The light microscopy shows nuclear inclusions of an hour glass appearance. It takes an eosinophilic stain at a later stage. Imunofluroscent experiments using antibodies specific for HSV DNA have shown intranuclear foci and these enlarge to form globular structures and these are called as replication compartment analogus to translucent nuclear inclusion seen by electron and light microscopy. These replication compartments are located in the interior of nucleus, thus the accumulated replication proteins, progeny viral DNA, nucleocapsid may cause nuclear cytopathic effects described as nuclear inclusion effects. The infected cells also manifest acantholysis, such cells are labelled as Tzanck cells (Textbook of Pathology – Saraf).

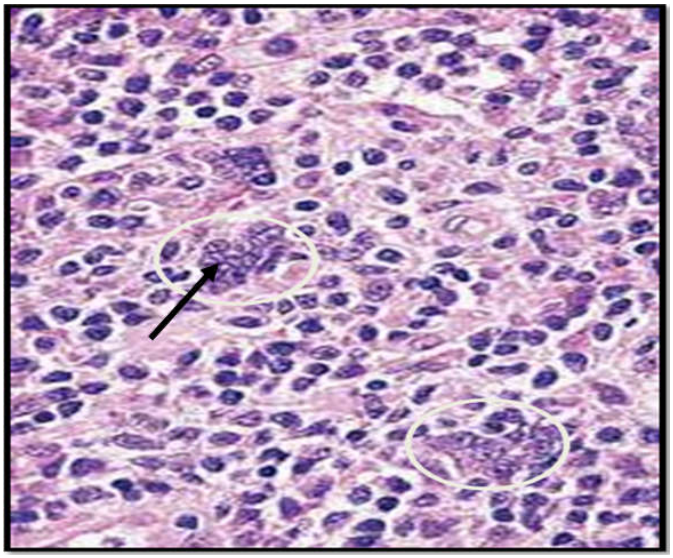




Balooning of the cells

Warthin-Finkeldey - Measles (Rubeola, morbilli)

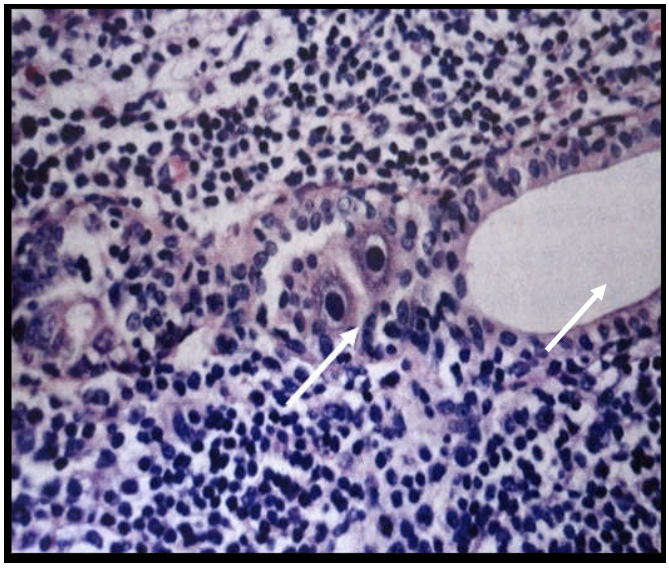
Measles is a highly contagious viral infection caused by paramyxovirus. The virus is spread by airborne droplets through the respiratory tract. Infected epithelial cells, which eventually become necrotic, overlie an inflamed connective tissue that contains dilated vascular channels and a focal inflammatory response. In lymphoid tissues, large characteristic multinucleated macrophages, known as Warthin-Finkeldey giant cells, are seen (Textbook of Pathology - Regezi). Mainly infected cell is monocyte (Textbook of Pathology – Saraf).



Warthin-Finkeldey

"Owl eyes" inclusion bodies in cytomegalovirus

Cytomegalovirus (from the Greek cyto- "cell" and -megalo "large") is commonly known as HCMV or Human Herpes virus 5 (HHV-5). HCMV infections are frequently associated with salivary glands though they may be found throughout the body. The infection rarely manifests intra orally in patient who are compromised. CMV infection can be demonstrated microscopically by the detection of intra nuclear inclusion bodies. On H&E staining the inclusion bodies stain dark pink and are called "owl's eye" inclusion bodies. Histological analysis of the biopsied lesion shows intranuclear inclusions and prominent nucleoli. These nuclear inclusions involve half of the nuclear diameter and usually set off from the nuclear membrane by a clear halo. Affected cells are strikingly enlarged often to a diameter of 40um in size and show cellular and nuclear polymorphism (Textbook of Pathology - Robbins) these cellular inclusions take up PAS stain and Gomori's stain (Textbook of Pathology – Saraf).

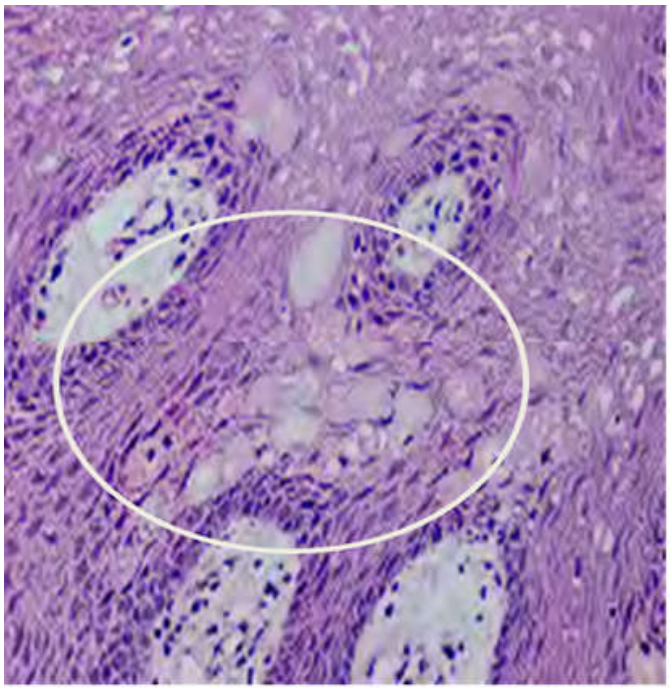


Owl eyes" inclusion bodies

Oral Focal Epithelial Hyperplasia (Heck's Disease)

Oral focal epithelial hyperplasia (FEH) Heck's Disease is relatively rare benign condition seen predominantly in children

caused by human papilloma virus and presents as multiple small white or pink papules on the mucosal surface of lips, buccal mucosa and tongue. Biopsy reveals epithelial hyperplasia, acanthosis, parakeratosis, bulbous extension of rete ridges called as "bronze age" battle axe appearance and focal or diffuse ballooning degeneration of cells in the spinous layer with koilocytotic changes are seen and also abnormal nuclear chromatin with nuclear inclusions. Ultrastructurally crystalline arrangements of virus like particles are seen located within the superficial spinous layer which measures approximately 50 nm in diameter. Virus may be found in the cytoplasm and nucleus of spinous cell layer (Textbook of Pathology - Regezi). Virally affected cells are seen in superficial layer and collapsed nucleus which resembles a mitotic figure called as Mitsoid Cell (Textbook of Oral Pathology - Shafers).



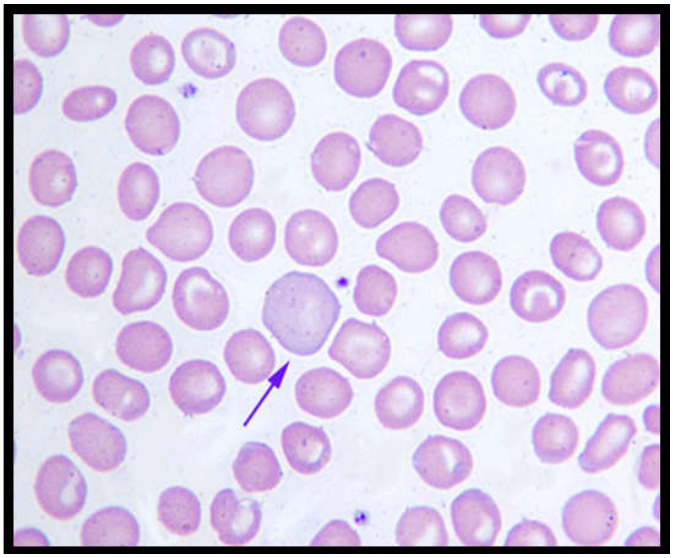
Mitsoid bodies

Erythrocytes and inclusions

Studies have indicated that the percentage of mature erythrocytes containing inclusions ranged from 3 to 82%. Each erythrocyte contained only one round inclusion body. J. Exp. Zool. 315:416–423, 2011.

Basophilic stippling

Basophilic stippling refers to an observation found when observing a blood smear, where erythrocytes display small dots at the periphery. These dots represent accumulations of rRNA and are always pathological. More recent studies suggests that basophilic stippling occurs only in young erythrocytes. The stipple material is composed exclusively of ribonucleo proteins whereas others have suggested that stipple material is composed of ribonucleoproteins plus mitochondria and portions of the red cell membrane and also have suggested that non heme iron may constitute a part of the basophihic stipple substance (Wallace *et al.,* 1995). The recognition of basophilic stippling in erythrocytes depends largely on subjective factors such as visual acuity and experience. An expert may observe stippling in the ordinary blood film stained with Leishman stain diluted with water at pH 6.8 to 7.0 if one could increase contrast between the stippling and its background it would become easier to recognize. This increase in contrast can be achieved by dark-ground illumination. A stained particle under dark-ground illumination reflects that light which would be absorbed were it to pass through the stain. The thionins and thionols which constitute the basic components of all Romanowsky stains in their blue form absorb orange light, so that particles stained with them appear orange under dark-ground illumination. The red cell, if properly stained has a pale, yellowish-green outline, within which the orange-coloured dots of light represent basophilic stippling. These particles can be easily differentiated from debris (Jacques mallarmé, 1948). It is associated with several conditions, including, Sideroblastic anemia, lead poisoning (microcytic anemia), beta thalassemia particularly in impaired haemoglobin synthesis.

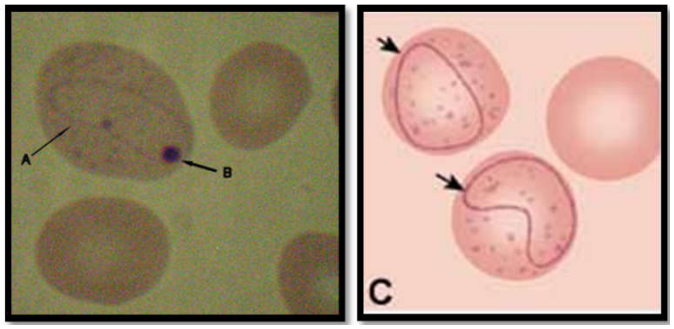


Basophilic stiplling

Cobot rings

Cabot rings are thin, red-violet stained, threadlike strands in the shape of a loop or figure-8 that are found rarely in erythrocytes. They are believed to be microtubules which are remnants from a mitotic spindle. Using the ammoniacal silver stain, Cabot rings can be identified in peripheral blood erythrocytes from patients with severe untreated pernicious anaemia. Ultrastructural studies of these erythrocytes showed silver deposits in partial loops and figure-eight forms, indicating that arginine rich histone may be a prominent component of the cabot ring. Cabot's rings have been described in pernicious anaemia, lead poisoning, leukemia, disordered erythropoiesis and in alcoholic jaundice. In case of pernicious anemias the orthochromic megaloblasts have an orange colour but may contain basophilic remnants in the form of basophilic areas or granules as in lead poisoning.

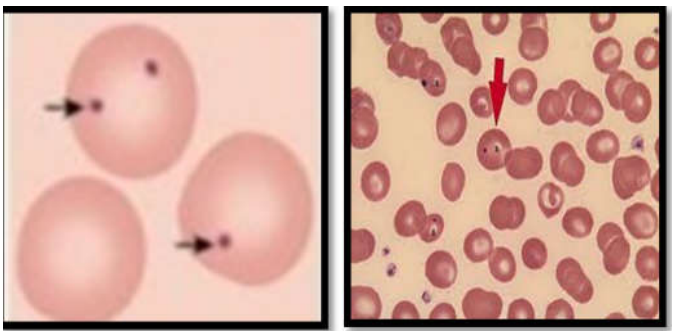
The nucleus may be round and regular as the nucleus of the orthochromic normoblast, but more often it is dumbell-shaped or has the shape of a clover leaf. The orthochromic megaloblasts vary in size and some may be rather small. When they lose their nucleus they become remnants, Cabot's rings, and Jolly's bodies. The megaloblasts in pernicious anaemia often show atypical mitotic figures at all phases (Wallace *et al.,* 1965).



Cabot rings

Howell jolly bodies

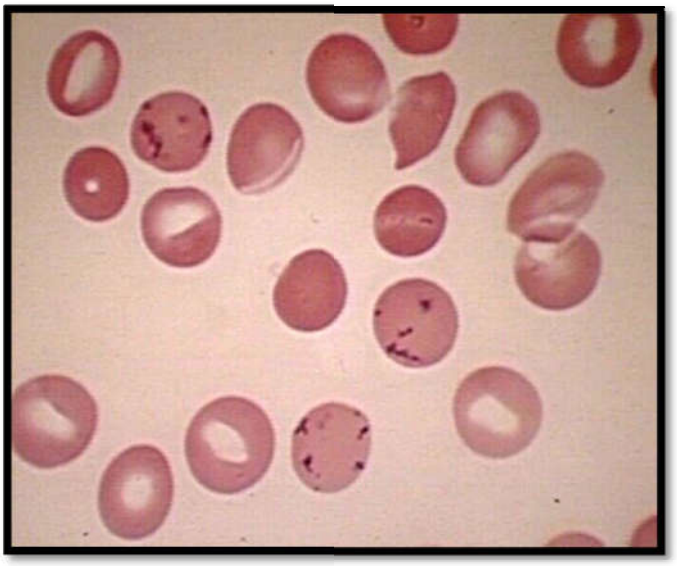
It is named for William Henry Howell and Justin Marie Jolly. Howell-Jolly bodies are histopathological findings of basophilic nuclear remnants (clusters of DNA) in circulating erythrocytes. During maturation the bone marrow erythrocytes normally expel their nuclei but in some cases a small portion of DNA remains. Nuclear fragments in the erythroid precursors are probably due to disordered nucleic acid synthesis resulting from vitamin B12 or folic acid deficiency. Similar inclusions are also found in cases of iron-deficiency anaemia. Howell-Jolly bodies are not refractile, a feature that distinguishes them from artefacts. Since occasional Howell-Jolly bodies occur in normal marrows and in conditions in which deficiency of B12 or folic acid can be excluded, less than 1% of Howell-Jolly bodies has no significance of the etiology such as anaemia but more than this number or the presence of multiple bodies in one cell can be appreciated but not pathognomonic of vitamin B12 or folic acid deficiency. [10] The fourth stage in the developmental series is that of polychromatophilic megaloblasts. In which asynchromism between nucleus and protoplasm is seen, which manifest a big nucleus in conjunction with a small cytoplasm or a small nucleus in conjunction with a large cytoplasm. The cytoplasm shows vivid colours, purple or greenish with heterogeneous areas of variable shapes. The nucleus still has a partly reticulated structure and may undergo mitotic division or fragmentation which produces an aspect of a petalled flower. The nucleus is still young as shown by its transparent and pearl-like aspect. But the tendency toward the formation of fragments of the nucleus classifies the cell as an old type of cell. The nuclear fragments appearing early in the cell are the future jolly's bodies (Jacques mallarmé, 1948).



Howell jolly bodies

Pappenheimer bodies

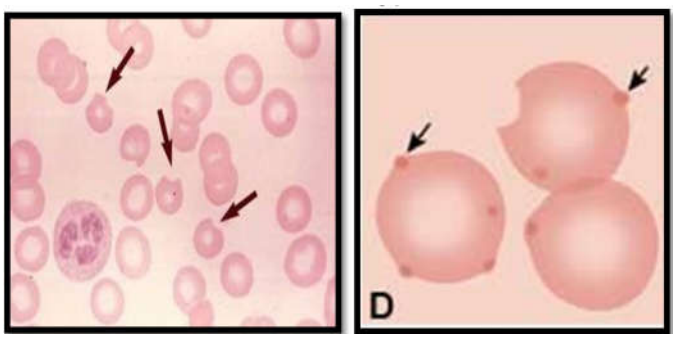
Introduced by in 1945 Pappenheimer. These are abnormal granules of iron found inside the red blood cells on routine blood stain. They are a type of inclusion body formed by phagosomes that have engulfed excessive amounts of iron. They appear as dense, blue-purple granules within the red blood cell and are usually only one or two, located in the cell periphery. They are seen in diseases such as sideroblastic anemia, hemolytic anemia, and sickle cell disease (Textbook of Pathology - Robbins).



Pappenheimer bodies

Heinz bodies

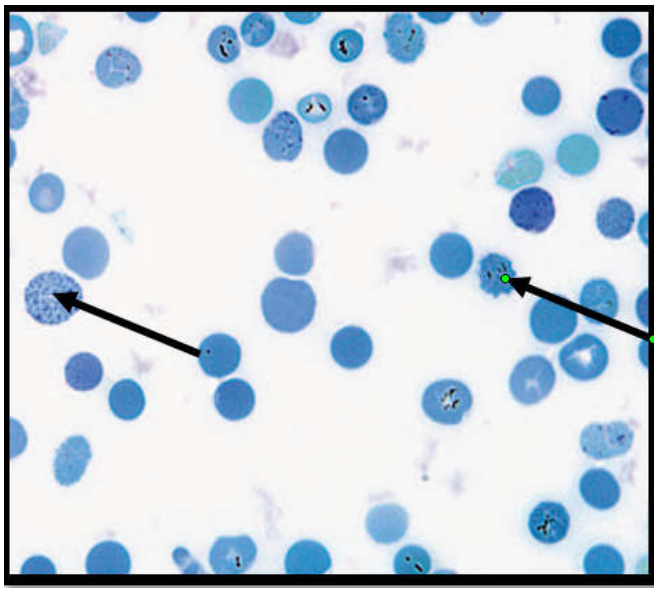
They are named after a German physician Robert Heinz in 1865-1924. G6PD deficiency cause episodic intravascular and extravascular hemolysis, which seems to involve the following sequence. When G6PD deficient cell exposed to high levels of oxidant there is oxidation of reactive sulphahydryl groups on globin chains, which will become denatured and form membrane bound precipitates called as Heinz bodies. They get stained with crystal violet and appear as dark inclusion. They can damage the membrane sufficiently to cause intravascular hemolysis. Due to membrane damage these cells retain abnormal shape appearing to have a bite of cytoplasm removed and termed as "bite cell" and these cells get removed by erythrophagocytosis. Morphologically and physically they are globular, refractile and irregular in shape and are insoluble in water (Robert *et al.,* 1958). Negative Feulgen staining indicated that the inclusion bodies are not derived from DNA fragmentation. Various cytochemical stains can be used like brilliant cresyl blue, a basic dye used to identify reticulocytes and Heinz bodies, and neutral red, a supravital dye that shifts from yellow under alkaline conditions to red under acidic conditions and to blue under strongly acidic conditions (Filomena basile *et al.*).



Heinz bodies

Hemoglobin H Inclusions

Hemoglobin H (Hb H) inclusions is seen in α-thalassemia syndromes and is characterized by chronic haemolytic anemia of variable severity and a clinical picture of thalassemia (George *et al.,* 2001).



Hemoglobin H Inclusions

The inclusion bodies are greenish blue in appearance and could be easily differentiated from the darkly-stained and filamentous structure of the reticulocyte (Munib, 1974). Inclusion bodies can appreciate in erythrocytes by incubation of four drops of blood with 0.5ml of Brilliant cresyl blue for 20 minutes at 37°C. These inclusion bodies are numerous and can easily be seen by light microscopy in most circulating erythrocytes. In addition, Hb H ranging between 5% and 30% can be detected by haemoglobin electrophoresis, isoelectric focusing (IEF), or high-performance liquid chromatography (HPLC) (David *et al.,* 2003). In the presence of iron deficiency, the appearance of haemoglobin H may be masked due to the decrease in heam synthesis (Munib, 1974).

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