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# Erythrocyte: Bacteria killer and bacteria pray

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**Abstract:** Erythrocyte is human blood main bactericidal cell. During movement in blood stream erythrocytes are triboelectrically charged by rubbing to each other and vessel walls and this charge automatically attracts and keeps bacteria on erythrocyte surface. Bacteria fixation on erythrocyte membrane activates the receptors of the membrane and stimulates trans membrane releasing of oxygen from oxyhemoglobin that causes bacteria oxidation and killing. If bacteria survive oxidation and enter erythrocyte they are exposed to higher concentration of oxygen (oxygen reactive species). Very few bacteria survive inside erythrocytes, but some can survive because of lack of oxygen inside erythrocyte and/or bacteria resistance to oxygen reactive species. Killed inside erythrocyte bacteria are released back to plasma and are digested in liver and spleen by local macrophages. Erythrocytes that are injured by bacteria and/or contain killed or living bacteria are destroyed in spleen. Erythrocytes out of bloodstream (in case of hemorrhage, extravasation in lobar pneumonia, etc.) after bacteria engulfment can't kill bacteria because of lack of oxyhemoglobin and become bacteria container providing both nutrients (protein, iron, carbohydrates, etc.) for bacteria growth and some defense against phagocytes and antibodies. In bloodstream erythrocyte is bacteria killer, out of bloodstream it is bacteria pray.

**Keywords:** Erythrocyte, Bacteria, Blood, Bacteremia, Sepsis, Liver, Spleen

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## 1. Introduction

Erythrocytes are the main bactericidal cells in human blood. Bloodstream clearance from pathogens is performed by erythrocytes. They attract, engulf, kill and then push killed microorganisms back to blood plasma [1]. Leukocytes can't perform phagocytosis in blood stream and leukocytes do not participate in blood bacteria clearance. Blood velocity prevents phagocytosis because there is no time for leukocyte to recognize and catch bacteria [2]. During motion in bloodstream erythrocytes become charged by triboelectric effect. This charge attracts bacteria and fixes them on the surface of erythrocyte and then bacteria are engulfed and killed by hemoglobin oxygen. In bloodstream leukocyte thin wrinkled elastic membrane can't be charged by triboelectric effect and as a result leukocyte can't catch bacteria by means of electrostatic attraction force. Leukocytes engulf and kill bacteria out of blood circulatory system, particularly, in tissues, lymph nodes, slow velocity lymph, etc. [2]. Erythrocyte and leukocyte are bactericidal partners: the first kills bacteria in bloodstream; the second kills them locally, out of blood circulation. As bacteria killer erythrocytes have many advantages versus phagocytes, for instance, their

volume fraction constitutes 99.9% of blood cells, they kill microorganisms without being injured, they are resistant to pathogens, they live much longer, pathogens can't proliferate in erythrocytes and the latter kill bacteria more effectively than leukocytes. [1].

### 1.1. Triboelectric Charging

Erythrocyte bactericidal power depends upon its membrane triboelectric charge and the amount of oxygen (oxyhemoglobin) inside erythrocyte.

Erythrocyte membrane triboelectric charge provides bacteria attraction, fixation on the surface of the membrane and engulfing. Triboelectric charging is determined by rubbing of erythrocytes to each other and vessel walls. The higher is blood flow velocity and its acceleration and deceleration, the more is erythrocyte triboelectric charging [2]. In systemic circuit blood velocity decreases from aorta to big arteries, small arteries, arterioles, becomes minimal in capillaries and gradually increases from venules to small veins, large veins and venae cava [3, 4]. Blood flow is faster

in systole than in diastole [5]. In the aorta near the valves the blood current varies in rapidity (fast acceleration and fast deceleration) because the flow through the aortic orifice is intermittent. It means that maximal triboelectric charging occurs in heart ventricles (chambers), in aorta and big arteries providing rapid attraction and engulfing of bacteria by erythrocytes. But attracting and engulfing of bacteria are only the first step of erythrocyte bactericidal function: bacteria should be killed inside erythrocyte by means of hemoglobin's oxygen.

## 2. Results

### 2.1. Bactericidal Function of Oxygen and Catalases

Oxyhemoglobin is formed in the pulmonary capillaries when heme binds to oxygen. A gram of hemoglobin binds 1.34 mL O<sub>2</sub> increasing the amount of oxygen in blood seventy-fold. Human hemoglobin molecule binds four molecules of oxygen [6, 7]. There are a taut (tense) and relaxed forms of hemoglobin. Different factors (blood pH, the amount of CO<sub>2</sub> and 2,3-Bisphosphoglyceric acid, partial pressure of oxygen, etc.) determine hemoglobin capacity to bind oxygen [8, 9].

Erythrocytes are exposed to oxygen high concentrations and as a result high concentration of hydrogen peroxide is generated inside erythrocyte. Erythrocyte is tolerant to hydrogen peroxide because erythrocyte is lack of intracellular components and, besides, catalase co-localized with hemoglobin protects the latter from hydrogen peroxide [10]. Catalase deficit causes hemoglobin oxidization and hemolysis or aggregation of erythrocytes [11].

Erythrocyte main weapon for bacteria killing are reactive oxygen species. Different cells protect themselves against reactive oxygen species by means of catalases, lactoperoxidases, superoxide dismutases and other enzymes. Bacteria have various antioxidant defenses for survival [12, 13]. There are three protein families in bacteria that catalyze dismutation. Two of them are typical catalases: the first is found in Archaeobacteria, Eubacteria, Plantae, Protista, Fungi, Animalia, the second - catalase-peroxidases - are absent in animals and plants and have both peroxidatic and catalatic action [14]. Manganese catalases are the third group. Catalyzing the same reaction ( $2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2$ ), these protein families are quite different in structure, localization and reactivity [15, 16]. Manganese catalases are found in many microbes and they are one of alternatives to heme-containing catalases [17, 18, 19]. Catalases are extremely active enzymes: each second their one molecule degrades hydrogen peroxide million molecules to oxygen and water [20]. NADH peroxidase (Npr) may be produced by some bacteria. This enzyme also may be an alternative to catalase when heme is not available [21]. Bacteria that cannot synthesize heme (for example, *E. faecalis*) use heme environmental sources [15]. Thus, bacteria have different mechanisms to withstand reactive oxygen species attack inside erythrocyte, but relatively long term survival of

bacteria in blood plasma and inside erythrocyte also depends upon bacteria metabolism (aerobe, anaerobe, speed of growth and proliferation), capsule production, morphology of bacterial cell membrane (thickness, structure, composition), etc.

The amount of reactive oxygen species in erythrocyte progressively diminishes in capillaries where hemoglobin releases the oxygen into the tissue. Bactericidal potential of erythrocyte is maximal in the lungs, at the alveolar-capillary interface where the partial pressure of oxygen is high and the oxygen binds readily to hemoglobin. In this sense the lungs may be considered the main organ that provides bloodstream clearance from pathogens. Bactericidal potential of erythrocytes is minimal in venae cava superior and inferior. The concentration of venous blood oxygen is dependent upon the amount of arterial blood oxygen, the amount of oxygen taken by tissues and blood cardiac output [22, 23]. The same bacterium may be killed by reactive oxygen species inside erythrocyte in arterial blood and can survive inside erythrocyte in venous blood, but this survival is short term, because one complete cycle of erythrocyte circulation is approximately 20 seconds [24-26]. Triboelectric charging of erythrocytes in venous blood is enough for attraction and engulfing bacteria, but the amount of oxygen (more correctly - reactive oxygen species) inside erythrocyte is not enough for bacteria killing, but this problem is usually successfully solved in the lungs during erythrocyte oxygenation. Venous blood erythrocytes also attract and engulf bacteria from lymph that enters venous blood from terminal lymphatic vessels and then kill them during oxygenation in the lungs. But hemoglobin oxygenation and high concentration of reactive oxygen species inside erythrocyte not always guarantee bacteria killing.

### 2.2. Anaerobe and Aerobe Bacteria in Bloodstream

Reactive oxygen species effectively kill anaerobe bacteria and as a result bacteremic episodes caused by anaerobes are unusual [27-29]. Anaerobes are the cause of bacteremia in adults only from 8% to 11% of cases and they are rare present in blood cultures [30]. Even in ulcerative colitis with the opportunity of massive penetration of anaerobes to bloodstream suppurative pylephlebitis with bacteremia is rare. A review of 783 cases of chronic ulcerative colitis and ileitis did not reveal bacteremia even in a case [31]. Later studies also showed that extracolonic *Clostridium difficile* infections, particularly bacteremia, occurs very rarely [32-36]. *Clostridium ramosum*, *Clostridium perfringens* and other clostridia also cause bacteremia very rare [37-38]. There is no data regarding *C. tetani* bacteraemia [39].

After tooth extractions more than 120 anaerobe species were found in blood [40-43]. The frequency of anaerobe bacteremia after tooth extraction is 39-100% [44-47]. All these bacteria are cleared from bloodstream during an hour [43, 44]. Eating, chewing, brushing the teeth, using toothpicks, etc. also cause short-term bacteremia [45]. During tooth brushing in 23% - 57% of adults billions of bacteria enter the bloodstream [42].

Nontyphoidal Salmonella causes bacteremia in 1% of enteric infections. Among different serotypes, the most invasive are Salmonella typhimurium, Salmonella cholerae suis and Salmonella Virchow [48, 49]. At the same time if a bacterium is facultatively anaerobic and produces catalase, it may better withstand reactive oxygen species action and often cause bacteremia. *S. enterica* serovar Typhi (*S. Typhi*) and *S. Paratyphi A* cause typhoid fever and bacteremia without septic shock and neutrophilia [50, 51].

Aerobes are more resistant to erythrocyte reactive oxygen species attack, but catalase production is more important for survival inside erythrocyte. Catalase provides bacteria survival by hydrogen peroxide neutralization [52]. All Staphylococcus species (except Staphylococcus saccharolyticus and Staphylococcus aureus subsp. anaerobius) produce catalase [53, 54]. Facultative anaerobe Staphylococcus aureus causes community-acquired and hospital-acquired bacteremia with highest morbidity and mortality [55, 56]. Another, may be no less effective (than catalase production) mechanism of erythrocyte killing evasion is capsule production.

### **2.3. Bacterial Capsule as an Electric Insulator**

Producing capsule and capsular polysaccharides pathogens simultaneously affect two factors that provide pathogen attraction and fixation on erythrocyte membrane. Acting as an insulator, the capsule prevents: (a) pathogen body triboelectric electrification; (b) interaction and attraction of pathogen charge (zeta potential) and erythrocyte surface electric charge. The thicker is the capsule, the more significant is its protective effect against erythrocyte attraction and fixation. The capsule of some pathogens acts as insulating tape wrapped around bacterial body whereas the capsules of other pathogens provide more prolonged but less intensive electron exchange between bacterium and erythrocyte as a result decreasing the attraction between them. Pathogens may lose their capsules in high velocity bloodstream because of capsule mechanical tearing away. In case of electric fixation on erythrocyte surface bacteria may escape fixation via leaving the capsule. The capsule provides bacteria survival in bloodstream and escaping erythrocyte attraction and engulfment, so invasive bacteria as a rule are protected by a capsule [57, 58]. In bloodstream the capsule predominantly acts as an insulator regarding pathogen's own and erythrocyte electric charge interaction, out of blood circulatory system it acts as an antiphagocytic and serum-resistance factor. All pathogens that cause bacteremia and sepsis are able to escape erythrocyte bactericidal action by means of capsule formation. Bacterial strains capable of capsular production are *Escherichia coli* [59, 60], *Staphylococcus aureus* [61- 63], *Klebsiella pneumonia* [64], *Haemophilus influenza* [65, 66], *Neisseria meningitidis* [67], *Streptococcus pneumonia* [68, 69], *Pseudomonas aeruginosa* [70, 71] and some others. Oral microbiota (*Porphyromonas gingivalis*, *Bacteroides forsythus*, *Streptococcus sanguinis*, *Streptococcus mutans* and others) that enter bloodstream and participate in various systemic diseases ((subacute bacterial

endocarditis, glomerulonephritis, rheumatoid arthritis, myocardial infarction, carotid and coronary atheromas, etc.) also produce a capsule for escaping erythrocyte engulfing [72-75].

### **2.4. Bacteria Slow Metabolism Provides “Electric Invisibility”**

Slow metabolism and growth make bacteria “invisible and uncatchable” for erythrocyte because the latter rapidly attracts and engulfs bacteria by means of interaction of its surface static electric charge with zeta-potential of bacterial cell membrane. Bacteria slow metabolism does not generate enough electric potential for interaction with erythrocyte electric charge and as a result bacteria are not attracted and fixed on the surface of erythrocyte. Tuberculosis and leprosy are good illustration of this phenomenon. In the past *Mycobacterium tuberculosis* bacteremia was asymptomatic and was providing infection dissemination [76, 77]. At present the situation is the same [78, 79]. *M. tuberculosis* cell wall contains 60% of lipids that include cord factor, wax-D and mycolic acids. Although the wall of *M. tuberculosis* is thick and dense, it is hydrophobic because of lipids and so it is not triboelectrically charged enough for attraction by erythrocyte. The lipids of *M. tuberculosis* cell wall provide the bacterium survival inside erythrocyte by means of protecting from lethal oxidation. *M. tuberculosis* can survive in H<sub>2</sub>O<sub>2</sub> mmol concentrations [80, 81]. But not only cell wall lipids protect the bacterium from oxidation. Protective effect is also provided by mycobacterial catalase-peroxidase protein (KatG) and the alkyl hydroperoxide reductase protein (AhpC) [82]. It should be taken into account that *M. tuberculosis* is obligate aerobe and its metabolism is accommodated to oxygen high concentrations.

Continuous bacteremia is present in leprosy but often it is difficult to reveal because *Mycobacterium leprae* grows and multiplies extremely slowly, besides it is an intracellular parasite and circulates in bloodstream in limited amount [83-85].

### **2.5. Bacteria wall Features Affect Bloodstream Survival**

Bacterial wall thickness and structure also influences attraction, engulfment and killing of bacteria by erythrocyte. Gram stain is determined by bacteria wall structure, composition and thickness. The wall of Gram-positive bacteria is thicker (20-80 nm), porous, with single lipid bilayer (monoderm) and contains 90% of peptoglycan, whereas Gram-negative bacteria wall is thinner (10 nm), chemically more complex, less permeable (with two layers - diderms), it is composed predominantly from lipopolysaccharides and contains only 5 – 20% of peptidoglycan [86-88]. Despite their thicker peptidoglycan layer (it is relatively porous and so can't be permeability barrier for reactive oxygen species), gram-positive bacteria are more easily killed inside erythrocyte than Gram-negative bacteria. Bacterial wall of gram-positive cells is denser and so it is more triboelectrically charged in bloodstream. As a

result, gram-positive bacteria are more attracted by erythrocytes than Gram-negative bacteria.

Some bacteria (for example, *S. aureus*) form conglomerates in site of infection but entering bloodstream the cells of conglomerate are mechanically separated by inertia and rubbing force and travel in plasma as a group of a few cells or a cell. Triboelectric charging of bacterial wall inhibits active transport and passive diffusion of nutrients and waste products through bacterial wall and metabolism and growth of bacteria stops or slows down.

### **2.6. Bacteria Survival Inside Erythrocyte**

After bacterium engulfment by erythrocyte two scenarios are possible: (a) bacterium is killed by reactive oxygen species inside erythrocyte; (b) bacterium survives and starts to grow and multiply, using erythrocyte as some kind of Petri dish with cultivation media. The second scenario is probable in venous blood because of lack of oxyhemoglobine there and it is quite often in case of hemorrhage because erythrocytes in tissues and cavities are void of oxyhemoglobine and become a good source of nutrients (protein, iron, etc.) for bacteria. A good example is bacterial pneumonia. In the past (before antibiotics discovery) *Streptococcus pneumoniae* had four stages. One of the stages called the stage of red hepatisation was characterized by extravasation of erythrocytes which gave red color to consolidated lung and were colonized by *S. pneumoniae*. These erythrocytes were giving bacteria protein, iron and other nutrients for growth and multiplication. If bacteria are attracted and engulfed by erythrocyte and survive inside erythrocyte the latter becomes bacteria pray. The same happens in case of hemorrhage: the lack of oxygen inside erythrocytes makes them vulnerable for pathogens and the hunter (erythrocyte) may become the pray (for pathogens) as it happens with leukocyte in case of incomplete phagocytosis. Deoxygenated hemoglobin cannot provide bacteria killing and as a result after attracting and engulfing a pathogen the latter may start to multiply having some advantages inside the erythrocyte, for instance, protection from the immune system, action of antibiotics, etc. Surviving inside erythrocyte pathogens also have access to all essential nutrients including hemoglobin iron - a very specific metabolic trophy for the pathogen (iron is indispensable for growth and proliferation of many bacteria and other microorganisms) [89-91].

### **2.7. Bloodstream Killed Bacteria Decomposition and Digestion**

In bloodstream erythrocytes can rapidly engulf and kill bacteria but they can't decompose and digest them because they have no appropriate enzymes, intracellular components and mechanisms for that. Erythrocytes are very effective "devices" for bacteria killing but their antibacterial function is limited by killing only. After releasing killed bacteria back to plasma decomposition and digestion of killed bacteria take place in reticuloendothelial system (RES). Two main organs

that perform this function are liver and spleen. The liver provides 80% of RES function [92, 93]. Kupffer cells are the major macrophage population of human body that has direct contact with the blood [94].

The spleen removes older and injured during bacteria killing erythrocytes from the circulation as well as cellular debris and microorganisms in case of their resistance to erythrocyte killing. The spleen is one of important immune organs [95]. The spleen is also unique because it is the only lymphoid organ specialized in the filtration of blood (the rest of lymphoid organs filter lymph). Additionally, the spleen contains the largest single aggregate of lymphoid tissue in the body, housing approximately one third of total circulating lymphocytes, thus with a vast number of them migrating through the spleen at any given time, surpassing the combined traffic of all lymph nodes in the body [96].

Bacteria often invade macro organism in billions. Killing and digesting living bacteria in RES is time consuming process whereas killing bacteria by erythrocyte takes not so much time and is more effective. Erythrocyte is the cell that provides oxygen to all other cells of human body and so erythrocyte contains more oxygen and reactive oxygen species than any other cell. High concentration of reactive oxygen species determines rapid killing of pathogens and so there is no another cell in human body that can kill bacteria as fast and effective as erythrocyte. It should be also taken into account that erythrocytes are the most numerous cells in human body: they are approximately a quarter of all cells [97] and considerably surpass the number of RES cells. Erythrocyte also spends less energy for bacteria killing in comparison with the cells of the liver and the spleen: erythrocyte reactive oxygen species are some kind of byproducts of respiratory function of erythrocyte and only a very little amount of energy and oxygen is spent for reactive oxygen species production. The last circumstance explains why reactive oxygen species production and bacteria killing doesn't interfere O<sub>2</sub> – CO<sub>2</sub> transport function of erythrocytes. Usually erythrocytes kill bacteria that then are digested in RES; in case of bacteria resistance to erythrocyte reactive oxygen species the cells of RES become the second line of antibacterial defense killing and digesting bacteria simultaneously. Too many bacteria may overload the liver and spleen causing their enlargement and injury.

## **3. Conclusion**

Thus, erythrocyte is bloodstream main bacterial killer. Out of bloodstream erythrocyte may become bacteria pray providing the latter nutrients for growth and multiplication. Erythrocyte bactericidal effectiveness is maximal in arterial blood and minimal in venous blood. Bactericidal power of erythrocyte depends upon the amount of reactive oxygen species inside erythrocyte whereas bacteria resistance to reactive oxygen species is determined by the type of bacterial metabolism (aerobe, anaerobe), catalase production, capsule production, bacterial wall thickness, structure, chemical complexity, permeability, etc. Bacteria killed by erythrocyte

and released back to plasma are engulfed and digested by the cells of RES, particularly, in the liver and the spleen. . In comparison with RES cells erythrocytes are more numerous and more effective and rapid bacteria killers.

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