

# **The Denucleation of the Mammalian Erythrocyte: An Evolutionary Mechanism**

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## **Abstract**

Mammalian red blood cells (RBCs) have no nuclei, a characteristic that sets them apart from other vertebrate orders. It has been hypothesized that increased oxygen needs necessitated the evolution from a larger, nucleated RBC to a smaller, denucleated one. We hypothesize that it was during a period of atmospheric change (from oxygen-rich to oxygen-poor) that the erythrocytes of mammalian ancestors became denucleated. Using a mathematical model to simulate oxygenation of an RBC in the pulmonary capillaries, our research suggests that the absence of a nucleus and decreased cell size can speed oxygenation, and thus be advantageous in a less oxygen-rich atmosphere.

## **Introduction**

About 300 Ma (million years ago), during the Permian Period, atmospheric oxygen levels were much higher than they are today (2). By the early Triassic Period, some 250 Ma, the amount of oxygen in the atmosphere plummeted to below current levels (3). This change in atmospheric PO<sub>2</sub> (partial pressure of oxygen) was accompanied by a mass extinction known as “the great dying.” (4) At the same time, the majority of Synapsids – the class of animals to which mammals belong – became extinct, and lost their position as the dominant terrestrial vertebrates. By the middle Triassic Period (200-250 Ma), the Archosaurs had taken over as the dominant terrestrial vertebrates. The Archosaurs included most of the dinosaurs, as well as many modern reptiles and birds.

The dominance of the Synapsids in the Permian and that of the Archosaurs in the Triassic could be explained, among other factors, as a result of the sudden drop in atmospheric PO<sub>2</sub>. The Synapsid lung – thought to have been similar to the alveolar lung in modern mammals – was perhaps poorly suited to hypoxia, whereas the Archosaurian lung – likely similar to that of modern birds – was better adapted.

Present-day mammalian RBCs have no nuclei since they eject them during maturation. In this respect, mammals are nearly unique. As a possible explanation for this curious trait, it has been hypothesized that the absence of a nucleus could improve oxygenation of RBCs in an alveolar lung (the type of lung present in mammals and thought to have been present in mammalian ancestors) (1).

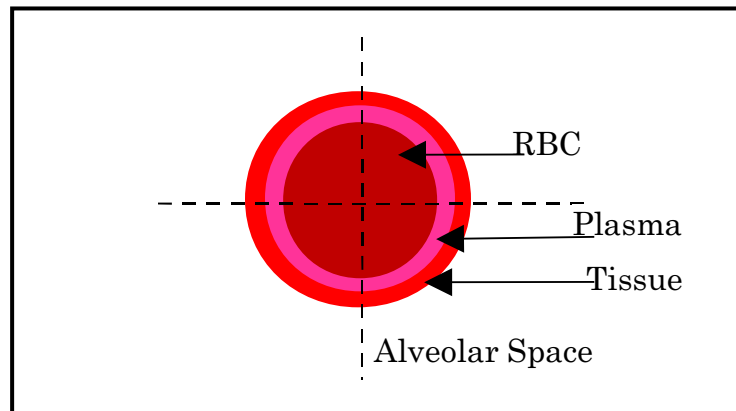
The improvement could result from two factors. In the alveolar lung, very often only one side of a pulmonary capillary is exposed to the oxygen-rich air of an alveolus. The absence of a nucleus would remove a barrier to oxygenation for the side of the RBC opposite the alveolar surface. The absence of a nucleus could also allow the RBC to reduce its size, thus reducing diffusion distances.

Here, we explore the effect of cell size, nucleus size, and hypoxia on the oxygenation of an idealized RBC in the pulmonary capillaries of an alveolar lung: smaller cell and nucleus sizes favor oxygenation and would provide an evolutionary advantage in a hypoxic atmosphere.

## MATHEMATICAL MODEL

### Geometry

A two-dimensional model of the pulmonary blood-air barrier was created. The domain consisted of three subdivisions: the red blood cell, the plasma, and the tissue (capillary wall, see Figure 1). We considered a single RBC suspended in plasma, flowing through the capillary network with diffusion occurring through one side of the blood-air barrier. The RBC cross-section was assumed to be spherical and axisymmetric.



**Figure 1.** Cross-section of the two dimensional blood-air barrier.

## The Red Blood Cell Model

Within the RBC, oxygen transportation relies primarily on the diffusion of free  $O_2$  and the diffusion of  $O_2$  bound to hemoglobin (Hb) with equations (5):

$$\frac{\partial P_{O_2}}{\partial t} = D_{O_2} \nabla^2 P_{O_2} - \alpha^{-1} T$$

$$\frac{\partial S_{O_2}}{\partial t} = D_{Hb} \nabla^2 S_{O_2} - C_{Hb_{tot}}^{-1} T$$

Variables are defined to be:  $t$  time,  $D_{O_2}$  and  $D_{Hb}$  molecular diffusivities of  $O_2$  and  $HbO_2$  respectively,  $\nabla^2$  the Laplacian operator,  $\alpha$  the solubility of free  $O_2$  in the RBC,  $C_{Hb_{tot}}$  the total heme concentration in the RBC, and  $T$  the reaction equation.

## The Reaction Equation

The reaction equation is more accurately represented as the equilibrium of  $O_2$  and Hb,  $Hb_n + nO_2 \rightleftharpoons Hb_n(O_2)_n$  (6). Since Hb contains four heme groups (each heme capable of binding with one oxygen molecule), the dissociation rate was determined by Michaelis-Menton kinetics:  $T_d = k_d C_{Hb_{tot}} S_{O_2}$  (5), where  $k_d$  is the dissociation rate constant and the latter product is the bound heme concentration. Furthermore, the Hill expression was used for the dissociation curve:  $S_{O_2} = \frac{P_{O_2}^n}{P_{50}^n + P_{O_2}^n}$ , where  $P_{50}$  is the  $O_2$  saturating 50% of the Hb at equilibrium and  $n$  is the Hill coefficient (5). The final reaction term is then given to be:

$$T = k_d C_{Hb_{tot}} \left[ (1 - S_{O_2}) \left( \frac{P_{O_2}}{P_{50}} \right)^n - S_{O_2} \right]$$

Parameter values for  $k_d$  and  $n$  were  $200 \text{ sec}^{-1}$  and 2.6, respectively.

## The Plasma and Tissue

Outside the RBC, transport of oxygen is based only on the diffusion of free  $O_2$  with the equation (5):

$$\frac{\partial P_{O_2}}{\partial t} = D_{O_2} \nabla^2 P_{O_2}$$

Variables are similar to the RBC transport equations although  $D_{O_2}$  can differ between plasma and tissue layers.

### The Diffusion Term

The Laplacian can be easily transcribed for both equations by directly using the definition. The diffusion term for P is:

$$\nabla^2 P = \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial P}{\partial r} \right) + \frac{1}{r^2} \frac{\partial^2 P}{\partial \theta^2}$$

The diffusion term for S is also the same translation. The Laplacian can furthermore be discretized to form the basis of the coding model.

### Model Assumptions

When forming the model we made the following assumptions:

1. The geometry of the model is as described.
2. At  $t=0$  the cell enters the capillary network.
3. Initial  $P_{O_2}$  is uniform and in equilibrium with initial  $S_{O_2}$ .
4. No flux conditions are imposed within the nucleus:

$$\frac{\partial P_{O_2}}{\partial n} = 0, \frac{\partial S_{O_2}}{\partial n} = 0$$

and on the RBC surface:

$$\frac{\partial P_{O_2}}{\partial n} = 0.$$

Where  $\partial n$  denotes a normal derivative.

### Results

We found that RBC size strongly influences the time required for the hemoglobin carried in the RBC to saturate with oxygen (Fig. 1), with smaller cells reaching saturation more quickly. This is natural, given that a larger RBC will contain more Hb (hemoglobin) and thus require more oxygen to saturate. Under conditions of exercise, however – and particularly if conditions are hypoxic even when at rest – short saturation times could provide a substantial advantage to

large endotherms.

We also found that the absence of a large nucleus improved oxygenation (Fig. 2). In Fig. 2 all cells, regardless of nucleus size, have the same volume of Hb solution at constant concentration, thus allowing geometry to be the only factor determining relative oxygenation efficiency. For very small nucleus sizes, little or no difference was observed, but a realistic lower limit on RBC nucleus size casts doubt on the relevance of these data. For large nucleus sizes, a significant decrease in oxygenation efficiency was observed.

Finally, we explored the effect of capillary wall thickness on oxygenation (Fig. 3). It was found that decreased wall thickness corresponded to increased oxygenation efficiency. Furthermore it is possible that, in addition to increasing oxygen efficiency, smaller cell sizes are advantageous because they exert less stress upon the capillary wall. This would be an interesting avenue to explore in the future.

## Conclusion

The results of our research suggest that smaller RBC size and the absence of a nucleus favor oxygenation in the alveolar lung. The hypoxic atmosphere of the Triassic Period could have provided the selective pressure to favor the small size and denucleated character observed in modern mammalian RBCs.

## Figures

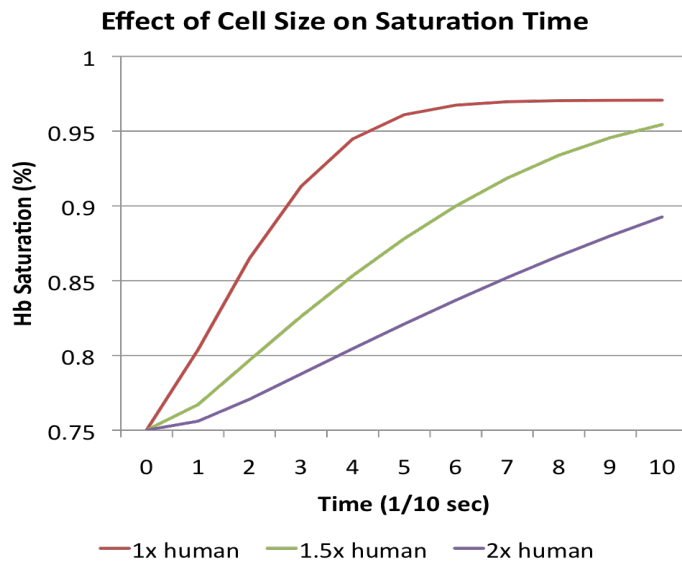


Figure 1. Relative system distances were held constant while the system size was increased from human dimensions (5 micrometers) to 2 times human dimensions. Smaller RBCs hold less Hb and saturate much faster than larger RBCs.

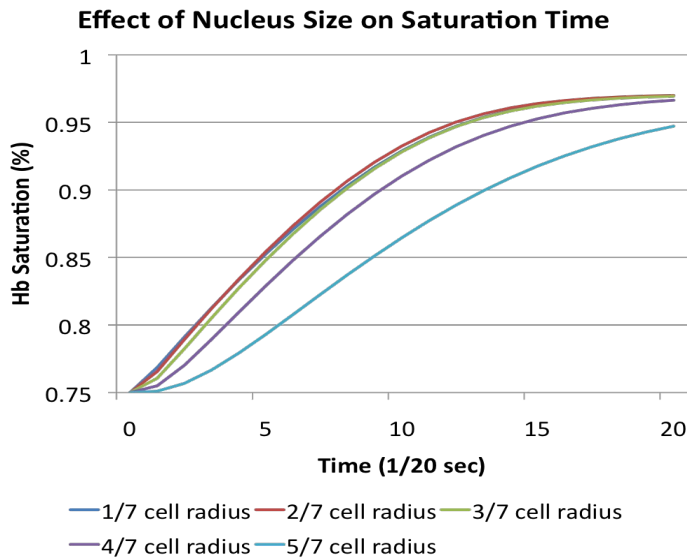


Figure 2. The amount of Hb contained in each cell (carrying capacity) was held constant while the size of the nucleus within each cell was varied from 1/7 to 5/7 of the total cell radius. For very small nuclei, no substantial effect was observed on oxygenation. For large nuclei, a substantial decrease in oxygenation efficiency was

observed.

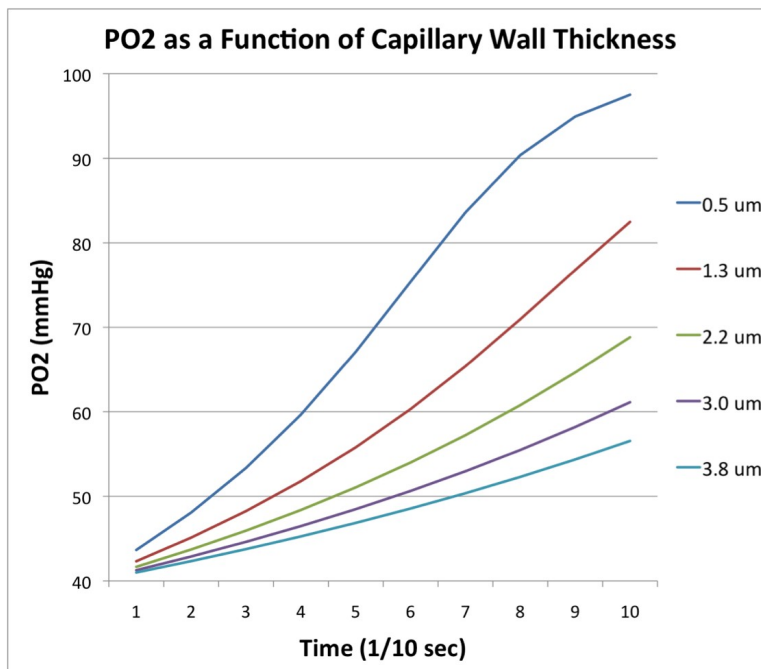


Figure 3. The barrier to diffusion (the capillary wall) was increased from roughly human dimensions (0.5 micrometers) to 3.8 micrometers (um). Thinner barriers correlate to more efficient oxygenation. Larger cells require thicker barriers to prevent tearing, so smaller cells could be more efficient in the alveolar lung.

## References and Notes

1. Dr. Colleen G. Farmer of the University of Utah first suggested this hypothesis to our group in September of 2008.
2. P. D. Ward, *Out of Thin Air: Dinosaurs, Birds, and Earth's Ancient Atmosphere* (Joseph Henry Press, 2006).
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