

Evolution of Function and Form in Camelid Erythrocytes

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Abstract. The Camelidae, advanced artiodactyls of arid habitats, have unique erythrocytes among mammals. Mammals have advanced erythrocyte features surpassing those of other Vertebrata. Mammalia attained their biconcave disk form from ontogenetic phases recapitulating those in lower vertebrates, beginning with spheroid erythroblast precursors becoming ellipsoid, containing ellipsoid nuclei, mitochondria and other organelles. The mammals, however, continued development by regression of tubulin Marginal Bands, abortion of the nuclei, and DPG production within the erythrocytes instead of ATP produced from mitochondria or by anaerobic respiration. The camels evolved elliptical erythrocytes and kept them, by retaining the MBs. The abortion of the nucleus occurs and, nevertheless, the biconcave disk cannot form. The bilaminar, oval wafer is low in volume, about double in S/V , rich in Hb, and the many thin cells comprise a very low packed volume (and high plasma volume). The erythrocyte resists influx or outflow of water, as does the plasma, and helps the camelids during drastic dehydration. The blood flows normally even when the tissues are dehydrated. The bilaminar oval also helps in hypoxic montane habitats.

Key Words: Erythrocytes Camelidae Ellipsoids Marginal Bands Evolution Morphogenesis

1 Introduction

One marvelous adaptation of vertebrate life was the oxygen-carbon dioxide transport system for respiration. Millions of erythrocytes containing Hemoglobin (Hb) move with the plasma through the body within a pulsating heart, arteries, tiny capillaries, thence returning by way of veins, to thereby transport Hb or Oxyhemoglobin (HbO_2) to the tissues. The evolution of Hb has been conservative, successful early on, and it delivers oxygen, as well does the high oxygen concentration in the blood diffusing it outside the capillaries.

In the Vertebrata there are two patterns of ontogeny (erythropoiesis) and of erythrocyte evolution [1,2,3]. Generally, the spheroid erythroblast becomes ellipsoid, then in mammals the nucleus is always aborted, and the mitochondria vanish when there is no longer a need to manufacture Hb. The mature “definitive” erythrocyte enters the mature circulation as a simple sack of endoplasm and Hb or HbO_2 . The typical shape in the mammals is unique, a radially symmetrical, bulging biconcave disk, deformable,

and high in surface to volume ratio. The loss of the nucleus reduces the use of oxygen in the cell, and effects a loss of volume without even changing the surface area [3, and others]. This leads to other advantages. The cell can either float in the current with low inertia or bend easily to deform and enter the tiny capillaries with ease [4,5, 6]. A typical mammal erythrocyte can enter tiny openings easier than a much smaller sphere, which cannot, of course, bend [6,7]. The phylogeny and ontogeny of non-mammal red blood cells end with the ellipsoid form, which is also invasive and easily penetrates the capillaries. The ellipsoid can bend, has a bilateral aspect facilitating movement forward or back, and has a higher surface than expected [3,8].

The Camelidae erythrocyte is an anucleate, oval cell in an advanced mammal [9]. There are two kinds of camels, such as the two huge Old World desert forms *Camelus dromedarius* and *C. bactrianus*, and the four alpine camels of South America (the vicuna, alpaca, guanaco, and llama). All are unique among mammals, because their

erythrocytes are biscuit- or wafer-shaped ovals, with very little volume, and having little packed volume in whole blood (only 27-28 %) [3]. The hypothesis that they throw back to the ancestral reptiles and other lower vertebrates seemed superficially obvious, and perhaps led to the error often published that camel erythrocytes are nucleated. In fact, they are anucleate, but are unusual and novel in vertebrates. Cohen and his associates found that camels retain primitive or early developing Marginal Bands (MBs) of tubulin, arising from centrioles, and that these seem to constrain the erythrocyte's ellipsoid form. The uncurving nature of the tubulin bands, elasticity of a kind, works against the contraction of the cell membrane, typically underlain by a spectrin network cytoskeleton, uniform in its contraction properties. The depth of the constricted but bowed ellipsoid diminishes as a consequence, and the nucleus bulges the biscuit somewhat centrally. It is interesting that the tubulin effects a plane, developing a bilateral symmetry, which works to flatten the cell.

In camelids the abortion of the nucleus dramatically lowers the cell volume, without allowing the constrained and wafer-like ellipsoid to assume any biconcave, radial shape. This cell, however, has perhaps a minimal volume for an erythrocyte to have. Its osmotic toughness [9,10] and rich oxygen capacity serve well in the hypoxia of alpine camelids [11,12] or during dehydration.

The objective here is to describe functions, evolution, and explain the camelid's erythrocyte shape, developing somewhat as in primitive ancestors (but out of sequence). The MBs are retained even in mature definitive red blood cells, whereas in non-mammals they are not lost, but the nucleus seldom aborts.

2 Problem Solution

Long [3,13] discussed the evolution of the mammalian erythrocytes from several aspects, and a probable phylogeny is divorced from living reptiles and birds, but derived from the line of lung fishes to the extinct primitive amphibians, the mammal-like reptiles, and finally the mammals (all probably sharing anucleate erythrocytes. The mammals all evolved biconcave and radial disks, with a strong

reliance on DPG to improve oxygen diffusion to the tissues. It is an evolutionary curiosity that the so-called throwback (MBs, oval form) in camels is not entirely a throwback character used for a new function, but is out of sequence and effects an old morphogenetic function. The developmental change bringing fitness to the camelids in hot, arid habitats is perhaps adaptive. In turning the sequence about, thereby is attained a tiny, flat form of mostly surface, with hardly any water content, hardly any volume, but rich in Hb and even some concentrated surface proteins [12]. Here is an advantage of prolation, an increase of area with no loss of volume [8]. Even an extra loss of volume occurs, by the advanced *mammalian* process of aborting the nucleus. The flattened ellipsoid has been appropriately called a wafer (Fig. 1).

2A. What is the Camelid situation?

What was the new adaptive zone exploited by retaining MBs and oval form, creating an exceptionally thin, bilaminar ellipsis? Since the North American origin of camels, even the early Miocene fossils showed a splayed foot, which is the camel's adaptation for walking on sands [3, 14,15]. One may assume, even in the Miocene time epoch, that the camelids suffered from heat and dehydration. Even South American camelids live in arid habitats. Modern desert camels suffer 25-30% loss of body water, 10% of whole blood, but little cellular water is lost from the red blood cells, which hold water bound to amino acids [12]. The new form of camelid erythrocytes has very low volume, much surface area, facile invasion of the capillaries, and comprises only about 27% of the blood volume. Many tiny cells are a boon in hot dry habitats, even in erythrocytes of such mammals as goats, and are especially helpful to camelids that suffer hypoxia at high elevations.

2B Cassini Models

Models have been devised [3,4,7,6] to describe the mammal biconcave disks and bilaminar ellipses. Long [3,13] has shown that in addition to the wonderful facility for bending, there seems a low intracellular pressure important for the deformable,

biconcave form, which is low in inertia and flows with the plasma, but attains fusiform and fluid qualities of the ellipsoid when entering the capillaries under strain. The typical model depends on a rotation of the longitudinal axis (2a).

If a and b are constants, two foci are in the xy plane located at $(-a, 0)$ and $(a, 0)$. The Cassini oval consists of all points (x,y) for which the product of the distances between (x,y) and each of the foci equals b^2 .

$$[(x - a)^2 + y^2][(x + a)^2 + y^2] = b^4 \quad (1)$$

This describes a circle with radius b and center at $(0,0)$. A Cassini oval which resembles the profile of a mammalian red blood cell is shown in Fig. 2, and for this particular shape its values are $a = 1, b = 1.1$. Shown within is a right triangle showing b and a , and a distance to the periphery at $b(2^{1/2})$. The distance a is the distance from a focus to the center of the oval. To determine the volume and surface area of a form shaped like a mammalian red blood cell we must obtain a solid of revolution,

$$V = 2 \int_0^{\sqrt{(a^2 + b^2)}} 2\pi x [(\sqrt{(b^4 + 4a^2x^2)} - (x^2 + a^2)^{1/2})] dx \quad (2)$$

The integral is well known to be difficult, but by standard algorithms the approximate value for specified values of a and b provide the value for V , which for the biconcave shape above ≈ 6.789 . The integral for the surface area is also complex, as follows:

$$S = \int_0^{\sqrt{(a^2 + b^2)}} 2\pi x b^2 [b^4 - a^4 + 3a^2x^2 + x^2 \sqrt{(b^4 + 4a^2x^2)}]^{1/2} dx \quad (3)$$

Similarly we obtain for the biconcave spheroid a value $S \approx 19.854$. The $S/V = 2.92$.

The theoretical biconcave shapes obtained are not to be considered so much the path of a moving point on an ellipsoid, but instead a mathematical consequence of adjusting the product of two variables. These are the distance from the center of the biconcave disk to a focal point on x at a , and the distance from that same focal point to the lowest point on the dimple of the upper side of the biconcave disk [or on the underside dimple]. If one reduces the inner volume, either the value a diminishes or the value along y diminishes. To maximize the surface area of the biconcave disk, or

the periphery of the Cassini oval, the reduced volume or area requires a dimpling, usually a double dimpling. Or to maximize surface, without changing the volume during development or evolution, it could be resolved by prolation to an ellipsoid.

2C A Continuous Model

Q-H Liu et al. [16] presented a continuous (analytical) model which produced several useful forms from a sphere [Biconcave disk \leftrightarrow Spheroid \Rightarrow ellipsoid or flattened ellipsoid.], including both typical biconcave mammal form and the typical non-mammal ellipsoid. Unfortunately, the model does not show how the camelid form might evolve, derived from one phylogeny or the other. Thus, the ontogeny for the camelid erythrocyte and the modeling for it are inexplicable, without an explanation. The camel cell is a compromise, but it does lose significant volume by abortion of organelles, and retains Hb from erythropoiesis. MBs flatten the ellipsoid, limiting its potential area slightly, and the surface retains typical levels of band III and spectrin [12]. The erythrocyte is so small because it is a flattened bilaminar ellipse (Fig. 1). How could it possibly have any more surface to volume, being so thin? Even the camel cell's length, almost typical, suggests an ellipsoid origin of the flattened form.



Fig.1. Alpaca bilaminar ellipses (after Jain & Keeton).

2D. Camelid Erythrocyte Features

The length of camelid cells is not as short as one

might expect in artiodactyls of hot, dry habitats (Table 1). The measured parameters of some mammals including camels reveal that the tiny camel red blood cell, incredibly thin, has

**CASSINI OVAL WITH $a = 1.0$, $b = 1.1$.
THIS RATIO OF a TO b APPROXIMATES
THOS VALUES IN A MAMMAL ERYTHROCYTE.**

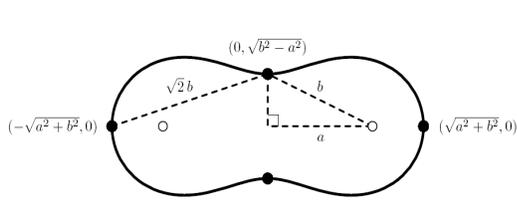


Fig. 2. The Cassini biconcave ellipse. enormous surface area for water and gas exchange. The cell volumes of camelids are exceedingly small. Spheres cannot bend, but are compared with bilaminar ovals of camelids and biconcave disks of other mammals. Since the depth in all camelids is approximately one, the formulation of the camel cell volume is not altered by the depth value. The surfaces double the volume (Table 2).

Although the ontogeny of mammals suggest their biconcave disk is derived from the ellipsoid, the typical trend during ages of evolution toward small erythrocyte size *and* high density or proliferation of blood cells in desert mammals raises problems with getting too small, surely there is a minimal threshold. One sees in the range of measurements for blood cells that if the ratio of the radius of a diminishing sphere approaches three, then the surface value (in square units) exceeds the volume (in cubic units). Thus, the S/V ratio becomes greater than one. This would seem to be important, I do not know how, in gas and water exchanges, especially during the dehydration of cells and whole blood. But there is another impact made on the ratio, which occurs as the nucleus is aborted. That automatically lowers the S/V ratio in biconcave cells and bilaminar camelids cells as well. In attaining ellipsoid form, the volume can be constant as the area increases. But the abortion of the nucleus might be a

dramatic drop in volume, and in fact camelids show it. However, this cell can contain the same amount of Hb, and much less water. The flat oval cells make such a small proportion of the whole blood, and the camelids show no diminished blood flow, no decrease in fluidity, even when the camel is drastically dehydrated (up to 27% water loss). Camels achieve a low volume cell, exceptionally tough osmotically. It contains little free water. As a consequence, the plasma is high in volume.

Table 1. Length, width, surface area, thickness, volume, MCV of camels, human, & pigmy goat. After Yamoguchi et al*, & others. Camel Area $2\pi a b$. Thickness, approx. 1.0, to calculate camel volume $\pi a b X 1.0$.

Sp.	L.	W.	S Ar.*	Th.	Vol.	MCV
Llama	7.7	3.87	187.2	1.05	98.3	25
Vicuna	7.1	3.87	172.6	1.01	87.2	31
Alpaca	7.2	3.8	171.9	1.01	86.8	30
SAmer	7.3	3.8	174.3	1.02	88.9	--
Dromedary	7.9	4.4	218.4	1.11	121.2	40-51
Bactrian Camel	76.6-8.1	3.8-4.3	179.0	1.11	88.9-1203	40
Human	7.6	7.6	~134	~1.7	~145	80-96
Pigmy goat	3.1-4.0	3.1-4.0				17-22

• Estimates are less than half these.

Table 2. The curious S/V when $r = 3$.

Comparison of spheres, camel cells, and biconcave disks

Spheres	Camels	Cassini form
$r = 100, S/V = 0.27$	L, b 8.1, 4 $S/V = 2.01$	
= 8, 0.37	= 7.9, 4.4 = 1.8	
= 6, 0.5	7.7, 3.87 = 1.91	
= 5.0, 0.6	7.2, 3.8 = 1.97	
= 3.8, 0.79	7.1, 3.87 = 1.98	Human 4., $S/V = 1.6$
= 3.1, 0.97	arbitrary values:	
= 2, 1.5	5.0, 2.63 = 1.94	Goat 2.1, "low"
= 1.5, 2.0	3.0, 1.58 = 1.94	
= 1.1, 2.5	2.0, 1.05 = 1.93	
= 1.0, 3.0		

The dehydrated camel may lose 25 % or even more of its tissue water and only an estimated 10 % water [< 10] from the blood. If the erythrocytes seem low in water content, “osmotically unresponsive”, its water seems bound to amino acids [12] then since the volume of plasma or erythrocytes may vary around 50%, but in camels more nearly 70-73% plasma, the camel can afford more water loss from its whole blood. Therefore, the blood remains functional, not viscous..

If one multiplies $0.1 \times 73/27 \rightarrow 27\%$, where 10% is raised to the fraction of plasma/PCV [compare that to 52/50 for human blood] that value approximates the water lost from camel body fluids. The osmotic problem of dehydrating whole blood, which causes explosive heat rise in dehydrated or overheated humans, seems no problem for dehydrated camels. They also suffer little by drinking to replenish water loss, the erythrocytes may double volume [17].

Dehydration in camels does not much effect blood fluidity or blood flow (or oxygen transport). The percent of water in plasma (10% less) is similar to the surrounding dehydrated body fluids.

Table 3. Adaptation hypothesized for three shapes. Spheres would be unfit with low r and S/V .

Adaptive evolution of three shapes attaining very small sizes and concomitant red cell proliferation

- | | |
|---|---|
| <ul style="list-style-type: none"> • Diminished V of sphere or biconcave disk.: • 1. Less conservation of mass & energy eg O_2.. • 2. In higher densities, great collective surface area. • 3. Numerous cells reach all the fractal capillary network. • 4. Enucleation keeps surface, ends oxygen use, allows bending, easy movement. • 5. $r < 3$, hence surface $> V$. | <ul style="list-style-type: none"> • Diminished V of Camels' bilaminar ellipse • 1. Least conservation of mass & energy eg O_2. • 2. In high densities, double collective surface area. • 3. Tiny cells invade all capillaries in dehydration. • 4. Enucleation keeps surface, ends oxygen use, allows bending, easy movement. Increases S/V. • 5. $r < 3$, surface $> V$, and in camels is double V. • 6. Resists osmotic problems. |
|---|---|

2E Non-mammal Morphogenesis

The only way to understand, it seems, morphogenesis of the camel cell is to study that of

the non-mammals, which have MBs. Wonderful studies have been made by W. D. Cohen and his associates, mostly on salamanders [18-25].

Cohen and his associates (Mary F. Ginsburg, L. H. Twersky, and lately Li-Fang Huang and Liat Levinhair have traced the cellular morphogenesis of microtubules within developing flattened ellipsoids in *Ambystoma*. Two advances were demonstrated, one that the pointed ends at one or both ends were not aberrant cells but were normal developmental features, and the other was that the growth of tubulin bands from its origin at one end extending to the other led to the flattening and prolation of the ellipsoid. The twin extensions of MBs around either side of the nucleus to the opposite end in some cases continued around the end, encircling the nucleus, and leading to an odd discoid coin like form.

Years before Trotter [26] had suggested that flattening and discoid form was induced by the MBs. MBs now have been described in embryonic mammal red blood cells, adult camelid cells, mammal blood platelets, and in some invertebrate erythrocytes. Wang (Huang) et al.[25] summarize morphogenesis in *Ambystoma* red cell cultures. Daughter cells remained adjacent to one another, i. e., in pairs, and become pointed in 1-2 hr development. (a) The end opposite the cytokinetic furrow became pointed, a spheroidal and singly pointed stage; (b) The furrow end subsequently became pointed too; (c) The furrow-end points then disappear to again produce a singly-pointed end concomitant with flattening [and doubtless prolation]; and (d) in time the points often disappeared.

Wang et al. [25] using time-course photo sequences for developmental stages proposed an advanced morphogenetic model, more or less as follows: Following spherical cell progeny from cytokinesis, the centriole pairs pull toward opposite margins thereby establishing the long axis for the prolated ellipsoid, then at the centrioles tubulin rays and anchoring tubulin bundles extend, especially the elongate tubes that will form the MBs. At this time, opposite the adjoined spheroids contact the centriole ends point

themselves. How this happens is not known, but it is opposite the movement of the tubes moving around the nucleus in these nucleated cells. As the daughter cells separate and the two elongate tubes approach one another, that end also becomes pointed. Then the cell is football shaped, and is said to be two-pointed. Flattening takes place as the tubules extend and pass each other, circling back toward their origins. At this time that the two tubules are passing each other, the first point rounds out. And if the tubules encircle the spheroid, returning to the origin, to the site of the two centrioles, then that end also may round out. The flattened ellipsoid becomes a flattened disk, but it of course is not biconcave; it remains swollen by the amphibian nucleus.

In the camel [21-22,26-27] the nucleus is voided before the MBs disintegrate, and indeed they may not disintegrate. The pointed ends seem to be pressure related, as the prolotion increases by constriction of the cell affected by the elastic bands. The resulting erythrocyte is either a biscuit-shaped ovoid or has a thin oval coin-like shape (Fig. 1). It can be described as “all area”, since the thin cell has about a depth of 1 unit, and its S/V is nearly constant, 2.0 or “double”. The tiny flattened cells show a low PCV as mentioned. If one presumes the MBs are like a rack on which the cytoskeleton and membrane are stretched flat, one must wonder why the tubulin effects such a plane, why the ellipsoid flattens as if the MBs function as two strung bows. Evolutionary adaptations are summarized in Table 3.

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