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Reflections on the crooked timber of red blood cell physiology[1]

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"No end, in wandering mazes lost, vain wisdom all, and false philosophy!"

-Milton (Quoted by G. Gulliver)

1. Introduction

This article reviews a selection of topics on red blood cells, which in large part, have fallen out of the main line of current research. The subjects discussed are in need of understanding at the molecular level. If (or when) forthcoming, the results would provide valuable insight into the organization and properties of the red blood cell membrane. The format used here follows a similar style to that used before [1–4] where the desire for further work on different subjects has also been highlighted.

2. Size and shape of red blood cells

When red blood cells were first observed in the latter part of the 17th century, by Malpighi, Swammerdam and Leeuwenhoek [5], it was not clear what their shape was. This history of descriptions of human red blood cell shape as discussed by Hewson [6] and Gulliver [7] is very varied as described before [8]. This problem persisted until it was definitively settled in 1827 with the publication by Thomas Hodgkin and Joseph J. Lister [9], who showed that it was a biconcave disk. Lister (the father of Lord Lister) was a wine merchant interested in microscopy who improved the resolution of the light microscope [10] such that the physician, Hodgkin (known later for lymphomas) could clearly discern their shape. It may be of interest to note that the polymath, Thomas Young (of "Young's modulus"), carried out an experiment reported in 1813 [11] such that "…(when) human blood, placed near the confine of light and shade…we immediately see an annular shade on the disc, which is most marked on the side of the centre on which the marginal part appears the brightest, and consequently indicates a depression in the centre…" "….(but) I am not perfectly confident that the apparent depression … may not depend on some internal variation of the refractive density of the particle."

The diversity of the size and shape of vertebrate red blood cells has been studied by a number of authors but none more accurately (or thoroughly) than those carried out by Gulliver [12] and Wintrobe [13]. Some of these measurements have been summarized by Ponder [14]. It is remarkable that Gulliver's laboratory notebook, in which his detailed measurements were recorded over many years, in the 19th century, is contained in the Yale University School of Medicine Historical Library. How it got there is not known. It would also be of interest to know how these various authors obtained the blood samples they studied from animals that live in various parts of the earth. In an oft-quoted plate (Plate LV, [12]), Gulliver depicts the known range in size of red blood cells in the five vertebrate Phyla. The range in size varies from that of the mouse deer (*Tragulusjavanicus*, presumably the smallest ungulate in the world) to that of the salamander (*Amphiumatridactylum*), i.e. from 2.1 to 62 μm. The average diameter of the human red blood cell is 7.4 μm [14]. Interestingly, Young [11] describes an instrument, called an eriometer, that was based on the diffraction of light, by which he estimated the size of human red blood cells to be 5.1 μm; while Emmons [15], with a redesigned eriometer, found a value of 7.2 μm.

The impressive paper by Gulliver describes the size (length and width) of the red blood cells of various species from each of the five phyla of vertebrates. Wintrobe's article has some overlap in the vertebrate species covered by Gulliver but with important differences. Thus, Wintrobe provides, in addition to size measurements of the various red blood cells, values of associated blood indices that are also reported

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[1] With apologies to Immanuel Kant and Isiah Berlin.

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with each type of cell studied. Importantly, he shows that the hemoglobin concentration in the cytoplasm of the various non-mammalian species, which varies with cell size, is the *same* when the nuclear volume is excluded. This finding implies that the chloride concentration may also be restricted to the cytoplasm in order to balance the charge on hemoglobin. As a test of this idea that chloride was excluded from the nucleus, it was found that the nucleus of the *Amphiuma* red blood cell was indeed devoid of chloride and that the resulting measurements of the membrane potential were compatible with a chloride equilibrium potential [16].

The original plan for this section was to include measurements of the size (and shape) of the red blood cells of each of the smallest vertebrates in each of the five Phyla. Unfortunately, it turned out that a search of the literature failed to provide any reports of such measurements for the designated species, as presented in Table 1 (in the appendix). It is hoped that in time this information will be forthcoming. One aspect of its importance lies in the parameters associated with the circulatory physiology of each of these species.

It is well known that the size of red cells in the circulation is larger than the size of the capillaries through which they flow. This means that the cells necessarily change shape as they pass through the capillary beds. If, for any reason, the cells are swollen or shrunken, their normal flow would be compromised. This emphasizes the importance of the cell's mechanisms for maintaining their normal cell volume. One such mechanism involves the control of the water content (volume) of the cell by utilizing the ion transport systems to maintain ionic balance [17]. Another mechanism involves factors which also control cell shape. Thus, the cellular constituents (and their arrangements) that are responsible for the shape of the cell must also be regulated. These aspects point to the importance of red cell shape in the physiology of the circulation.

One must also be aware that cell structure varies within the various vertebrate species. Red cells in mammals are enucleated whereas most (but not all) red cells in the other vertebrate Phyla are nucleated. A case in point is the family of the *Plethodontid* salamanders where some species have essentially all enucleated red cells while others are fully nucleated [18,19]. There is also a speculation of the advantage cells have that are enucleated [19]. Red blood cells of the vertebrates, excluding mammals, have marginal bands which may contribute to their structure/shape [20,21]. An important area that is just emerging is the detailed elucidation of mechanisms that underlie the processes responsible for the enucleation in human red blood cells [22,23]. This remarkable event, occurring approximately two million times per second, results in red cell populations whose cell volume distributions are a very narrow Gaussian. The underlying transport pathways that are responsible for this accompanying control of cell volume are unknown. It might prove instructive to compare the proteome of the pyrenocyte to that of the reticulocyte from which it was derived. Red blood cell shape has a long and fascinating history as alluded to above as well as discussed before [24]. There has also been a recurring interest in why the human red blood cell has a biconcave shape. Hartridge [25] and others [26] have speculated that for gases (e.g., O_2) there is an advantage of a disc shape over a sphere of the same volume. However, it was definitively shown that this was not so since the rate of oxygenation of human red blood cell discs versus spheres at the same cell volume was the same [27].

It has long been known that the biconcave shape of normal human red blood cells is dependent upon the presence of ATP [28,29]. It is also known that there are about 10^6 ATP binding sites/ghost membrane [30]. There is emerging information about which sites are phosphorylated (e.g., [31–34]) and their functional consequences in addition to the Na- and Ca-pumps. Depletion of ATP in human red blood cells by incubation in the absence of substrate results in the transition of the discoid shape to that of echinocytes [29]. These types of shape changes, as well as a variety of other red cell shapes, are well explained on the basis of the 'bilayer couple' hypothesis [35] utilizing in addition some theoretical approaches [36,37]. Even so, the basic question that has existed from the beginning is what red cell constituents are responsible for the biconcave shape of the human red blood cell. The little information available has been recently summarized [24,38]. These two papers also present an approach to defining a basis for the discoid shape. The main result of this work has been to suggest that there is a difference in density between the rim and dimple of the red cell. The experimental evidence supporting this view was that there existed a dense band (presumably the membrane/cytoskeletal components) that oriented the resealed spherical ghosts upon centrifugation. The dense band was deduced as being oriented horizontally to the centrifugal field because, when the spherical ghosts, now attached on the cover slip, were converted to biconcave ghosts (by exposure to an isotonic solution) they collapsed vertically onto the coverslip. The rim of the biconcave ghosts was then observed to be more dense than the dimple. No explanation has so far been forthcoming as to what comprises the dense band or why there was a dense band or why it was oriented horizontally during centrifugation? A recent report [39] indicates that the dimple may be stiffer than the rim in defining the forces responsible for the discoid shape of the red cell.

It is possible that some insight into the density differences might come from studying different types of ghost membranes with recent developments of high resolution microscopy techniques. One such preparation of possible interest describes exposure of human red cells to Triton X-100, where the cells hemolyse and the ghosts, presumably devoid of lipids (and hemoglobin), retain their biconcave shape ([40], p126). A second method utilizes extraction (of horse red cells) with ether, alcohol and toluene, where the ghosts are presumably also disc-shaped [41].

3. Control of cell size

Mention should be made of other aspects of cell size that is in need of molecular explanation. One is the mechanism(s) by which individual cells control their size/volume (e.g., [42]). Another aspect to be identified is the mechanism(s) that determine the final size of individual cells. And a further aspect is what are the mechanisms that define the mature size of individuals - animals and plants: that is the number, size and kind of cells as well as their location? One is reminded that there is a direct correlation between the genome size and the cell volume of vertebrate red blood cells [43]. Another case in point is a report that the size of cells in tetraploid salamanders is larger but fewer in number with mature size of the animals being the same [1,44]. In addition, it is not understood how the inverse relation between red cell size and cell number, with essentially constant hematocrit, during development and in mature species, occurs [45]. The reader is referred to a more recent paper where some of these issues are discussed [46].

4. Red cell membrane structure by electron microscopy

The detailed structure of the human red cell membrane has evolved over time showing, on the one hand, a mostly hexagonal array of the stretched cytoskeletons with junctional evidence for the locations of the ankyrin and actin complexes (e.g., [47]). On the other hand, there is early evidence that there is a "plaque" structure of the shadowed surface [48] as well as intramembrane particles seen in freeze-fracture studies of the membrane [49]. It has never been clear what the relationship is between these two types of observations. In the former study, small fibers were also seen to cover the surface in "unshadowed" preparations. More recently, it was found by polyacrylamide gel analysis of the ghosts used before [48] that essentially all of the major cytoskeleton components were present, although in decreased amounts. It is not known what the protein content was of the freeze-fracture ghosts. More recent work [50,51] has clarified the structure of the inside surface of the membrane but still leaves room for more detailed molecular arrangements to be determined. Problems associated with

understanding the membrane/cytoskeleton structure have recently been delineated [52].

5. Lamentations

The intent here was to mention the current status of problems concerned with the molecular details that underlie and define the mechanism of hemolysis of red blood cells, per se, and the mechanism associated with the resealing of red cell ghost membranes following hemolysis. Characteristics, and problems which attend both of these phenomena, have been reviewed before [53]. It is noteworthy that essentially little progress has been made in the subsequent years, presumably due either to the lack of interest or that the subjects are out of the current mainstream pursuits?

6. Flicker in red blood cells

Vibratory movements of the membrane and cytoplasm of human red blood cells, termed 'flicker', have been seen since it was first reported in 1890 (ref. in [54]). The interpretation of the phenomenon has a curious history in that early on the mechanism was considered as either a metabolically dependent (e.g., [54,55]) or a purely physical process [56]. The arguments for either interpretation have persisted over the years as will become clear below. The process is best seen by phase [55] or by video microscopy [56]. One paper [55] claimed that flicker represented the operation of the Na/K-pump by correlating the vibratory movements with the effects of metabolic inhibitors. This was countered by showing that physical restraint alone prevented the vibratory movements of the membrane/cell [56]. The latter interpretation was based on inhibiting the Brownian bombardment of the membrane and cytoplasm altering the pattern of light coursing through the cell. This was confirmed by analysis of the frequency spectrum of flicker, which depended on zero surface tension, fluidity and inequality of the refractive indices [57]. It is beyond the scope of this review to describe arguments that base their analyses of flicker on the dependence on ATP (e.g., [58–60]) or on the independence from ATP (e.g., [61,62]) being involved in the process. It may be that the bottom line suggests that ATP, instead of being directly involved in driving the flicker process, provides indirectly the membrane properties that supports the phenomenon [63–65].

7. ATP release from red blood cells

While ATP release has been found to happen in a variety of cells and tissues (e.g., [66]), this discussion is focused on the process that has been studied in human red blood cells. It appears that the first work showing that ATP is released from red blood cells was reported in 1992 [66]. This release occurred after a brief exposure of the cells to a combination of hypoxia and hypercapnia. Interestingly, this release of ATP was inhibited by three different band 3 inhibitors including DIDS. The amount of ATP released was about 23% of the ATP in the cell. This was about four times the ATP concentration that could be accounted for by the accompanying degree of hemolysis (see later). The result that hypoxia alone was responsible for the release of ATP from red cells was subsequently confirmed [67]. Other mechanisms responsible for ATP release include shear stress and deformation (e.g., [68–70]).

This subject has not been without controversy. One group in repeating various other studies, in which the prior methods were used, found that hemolysis accounted for the amount of ATP released [71]. This was rebutted [72] and re-rebutted [73] without a consensus being reached. A separate study, in which red cells were exposed to shear stress and hypoxia in a rotating Couette viscometer, reported that the amount of ATP released was significantly more than could be accounted for by hemolysis [74].

Suggestions for the pathway(s) through which the ATP exits the red cell membrane include the possible involvement of G-proteins [75] or pannexin 1 [76,77] or in addition a voltage-dependent anion channel, VDAC [78] or VDAC in combination with a translocase and a nucleotide transporter [79]. A possible source of the ATP that is released might be from the red cell's sequestered membrane pool of ATP rather than from the cell's cytosolic store [80]. Obviously, there is a real need for clarification and definition of the different processes that underlie the mechanism(s) of ATP release from red blood cells.

8. Red blood cell membrane lipids

The major membrane lipids that are present in red blood cells are: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), sphingomyelin (SM) and cholesterol. The distribution of these lipids between the membrane's bilayer leaflets in normal human red cells, places most of the SM and PC lipids in the outer leaflet while some PC and most if not all of the PE are on the inside leaflet [81]. There are approximately 3×10^8 phospholipid and 10^8 cholesterol molecules per red cell [82]. The enzyme flippase (ATP8A2/TMEM30A) moves phospholipids from the outer to the inner leaflet [83], while the enzyme floppase (ABC Transporter) moves phospholipids in the opposite direction. Both of these enzymes utilize ATP. Scamblases (PLSCRs) are independent of ATP and move phospholipids in both directions. Cholesterol is thought to move unassisted between the two bilayers in less than a second [84,85]. Lateral movements of phospholipid analogues in the plane (leaflet) of the membrane occur in less than 1 μm/s [86] while the transfer of phospholipids across the bilayer is measured in minutes to days [81]. The number of scramblase molecules is estimated to be 764/cell [87]; numbers for the other two enzymes are not known. It should be noted that the distribution of different lipids appears to be associated with different parts of the surface of the membrane [88,89]. A study using spin-labeled aminophospholipids found that after hemolysis all of the phospholipids were randomly distributed when the ghosts were resealed in the absence of MgATP. Interestingly, when the ghosts were resealed in the presence of MgATP, the asymmetry in the distribution of the phospholipids was reestablished [90]. This asymmetry was inhibited by vanadate and calcium. This type of study raises interesting questions concerning the mechanisms involved. This brief survey of membrane composition serves in part as a background for the following discussions of other membrane related phenomena.

9. Red cell disc/sphere transformations at constant cell volume

The shape changes that red cells undergo between slide and coverslip or in the presence of surface active agents appear unique in the animal world. Here the shapes that occur change in sequence from biconcave discs to blunt crenated discs (echinocytes) to dusky spheres (very fine crenations) and finally to glistening spheres – all without any change in volume [14]. There is no detailed information on how these changes take place but there has been speculation (models) that alterations in the composition of the bilayer leaflets of the membrane are the root cause of the shape changes (e.g., [91–94]). One type of experimental result that indirectly supports this kind of interpretation is given in Fig. 1. Here cells are exposed to a classical sphering agent, Rose Bengal (RB), at a concentration below which no photodynamic hemolysis takes place. The important point in showing this result is the spontaneous return from spheres to discs in the presence of the agent RB. The shape changes were confirmed by microscopic examination. When the rate of binding of RB to red cells was studied at different concentrations, the time to completion of the uptake was correlated with the return of the sphered cells to discs. This indicated that the shape changes were transient depending on the RB being at disequilibrium in the plane of the membrane - presumably intercalated within the lipid bilayer (e.g., [95] page 74). It is, of course, not known what the shape of the bilayer is or its lipid distributions or what its attendant cytoskeleton is when the cells are not discoid. These are

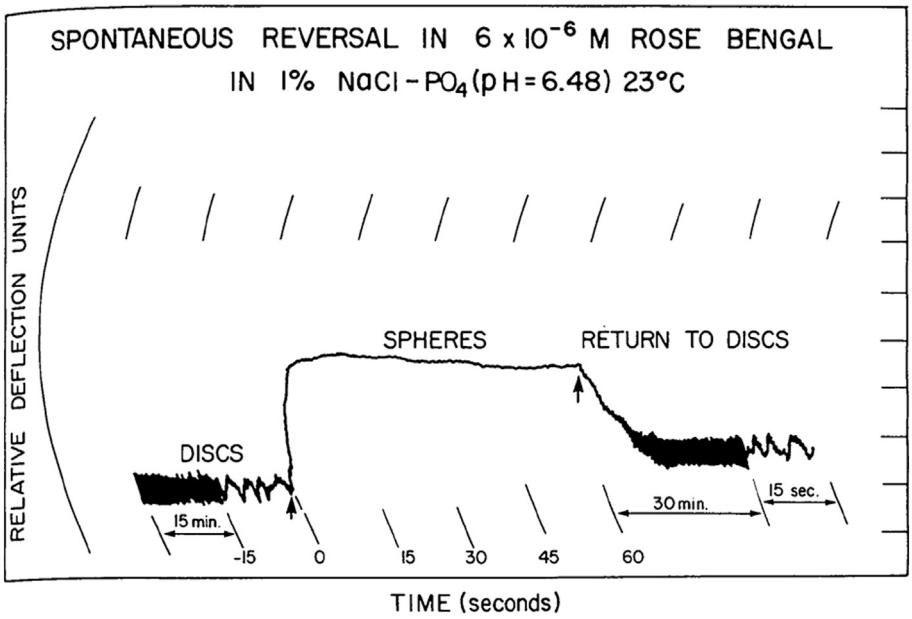

Fig. 1. Spontaneous reversal of red cell shape in 6 × 10–6 M Rose Bengal in 1% NaCl-PO4 (pH = 6.48) at 23 °C.
Measurement by changes in light transmission of disc-sphere shape transformations induced by Rose Bengal (RB), of a stirred suspension of human red blood cells. An upward deflection represents a decrease in transmitted light. Note the difference in timescales (blue) and the changes in the envelopes of fluctuation. RB is added at zero time, with the discs immediately becoming spheres. After 60s the timescale is changed, tracking the cells' return to discs. Adapted from Reference [96].

structural features of the membrane that have yet to be determined. The action of other agents including pH gradients and reversal of shape changes are presented in the paper [96]. Insight into the underlying mechanisms of the disc/sphere transformation might profit from studying red blood cells that have altered cytoskeletal and/or lipid constituents.

10. Tank-treading of red cell membranes

Tank-treading is another unique phenomenon restricted to red blood cells. It actually represents a problem for “all seasons” because it is bristling with enigmas on the horns of a dilemma, unlike any of the foregoing issues. The phenomenon is seen when red blood cells are subjected to experimental sheer/stress flow [97]. In this situation, the cell's membrane, labeled with a particle attached to its outside surface, or by following a Heinz body attached to its inside surface, can be observed to rotate around the cell with the shape of the cell remaining a biconcave disc. The way this is visualized is by use of a specially designed microscope called a ‘rheoscope’ [97]. Its design is such that a red cell lying flat is exposed to oppositely directed sheer forces on its upper and lower sides produced by a modified cone plate Couette viscometer. The red cell can then be observed to be in a stationary state because of a special design of the plates of the viscometer. At a set rate of sheer a membrane with a particle attached on its outside can be seen to migrate around the cell (i.e. tank-treading) all the while with the cell remaining biconcave. Increasing the sheer force increases the rate of membrane circulation until the force is too great and the red cell tumbles. Treatment of the membrane with diamide (i.e., crosslinking) retards cell deformation [98]. Presumably, tank-treading would take place in the capillary circulation given the shape alterations that have been observed [99,100].

The enigmas that attend tank-treading are mainly concerned with explanations of the mechanisms by which the phenomenon occurs. The fact that particle labels on *both* surfaces of the membrane circulate challenges the current concepts of the biconcave structure of the membrane/cytoskeleton complex. The cell's membrane is presumably composed of a glycocalyx (e.g., blood group antigens/sialic acid, band 3, receptors) on its outside attached to a lipid bilayer attached to a cytoskeleton with known proteins extending across the whole complex and attaching to the junctional complexes (e.g. [101]). It is completely unclear how the membrane complex can keep its discoid shape while circulating around the cell? It seems as if the structure responsible for the biconcave shape, acts as a separate and stationary entity sandwiched between the outside and inside parts of the membrane which circulate around it, even though there is no evidence for such an interpretation. Interestingly, it has been observed in an α-spectrin II knockin mouse that spectrin tetramer is critical for tank-treading to occur.[2] The phenomenon also displays a type of hysteresis in the sense that if the tank-treading of a particle is stopped away from its original position, it will in time return to that position [102]. These considerations emphasize the need for studies aimed at producing a molecular explanation of the tank-treading mechanism.

11. Rouleau formation of red blood cells

The first description of red cells (human) being in rouleau (stack of coins) were described by Hewson ([7], p.221,228). Evidently the first depiction of rouleaux formation of red cells was published in 1776 by Father P.D. Giovanni Maria Della Torre ([103] and Fig. 2).

Bessis's picture of rouleaux is noteworthy ([104], p.141). Rouleau is not known to occur in the circulation even in the presence of “slugged blood” or blood cell aggregates ([105], but see [106]). Rouleau formation is non-covalent and is promoted in vitro by neuraminidase treatment of human red blood cells (G.V.F. Seaman, personal communication) which reduces the surface charge of their membranes. In contrast, treatment of the membrane with DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid), which binds specifically to band 3 on the membrane surface, inhibits rouleau formation [107]. Rouleau formation of normal red cells is promoted by fibrinogen [105] and by different molecular weights of dextran [108,109]. Interestingly the different shapes (discoid or cup-like) of the cells in rouleau have been modeled mainly on the differences in the lipid leaflet areas and their relative adhesiveness [110].

The attachment of a single cell to a column of cells in rouleau (poised in a isopycnotic part of the medium) has been observed microscopically in an ultracentrifuge with the cells suspended in an isotonic/Percoll mediun ([111], Fig. 3).

It is not clear why the column of rouleau cells sediments or stays vertical during centrifugation. At the indicated centrifugal force the cells assume a “bag-like” shape due to the sedimentation of the hemoglobin. The upper part of some of the single cells is clear indicating

[2] M. Narla, personal communication.

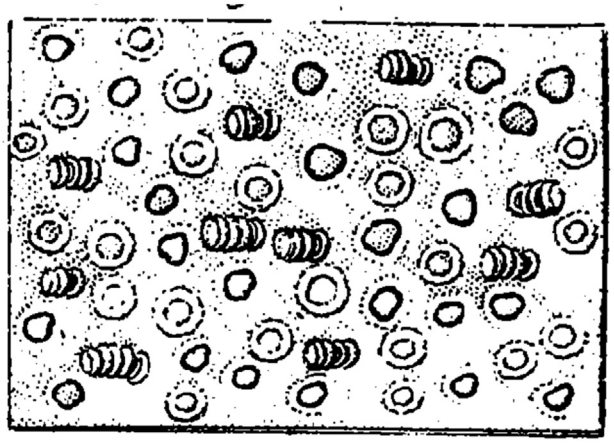

Fig. 2. Rouleaux formation of red cells.
Illustration (plate 2, Fig. IV) taken from [103].

said that it is not known why the phenomenon of rouleau exists or what the rationale is for its occurrence.

12. Short list of subjects that may be of interest for the curious reader

Surface movements of labeled band 3 and glycophorin A molecules on Human red cell membranes [114,115]. Possibly related to understanding membrane movements in tank-treading.

Circadian rhythms of red blood cells [116] are a documented phenomenon with unknown significance.

Implication of a red blood cell abnormality in Chronic Fatigue Syndrome [117].

Changes in cation transport and metabolism when red blood cells are deformed [118], possibly related is the involvement of piezo1 in changes of red cell parameters also upon their deformation [119].

The effect of lactose exposure on human red blood cells which causes changes in their cation permeability [120].

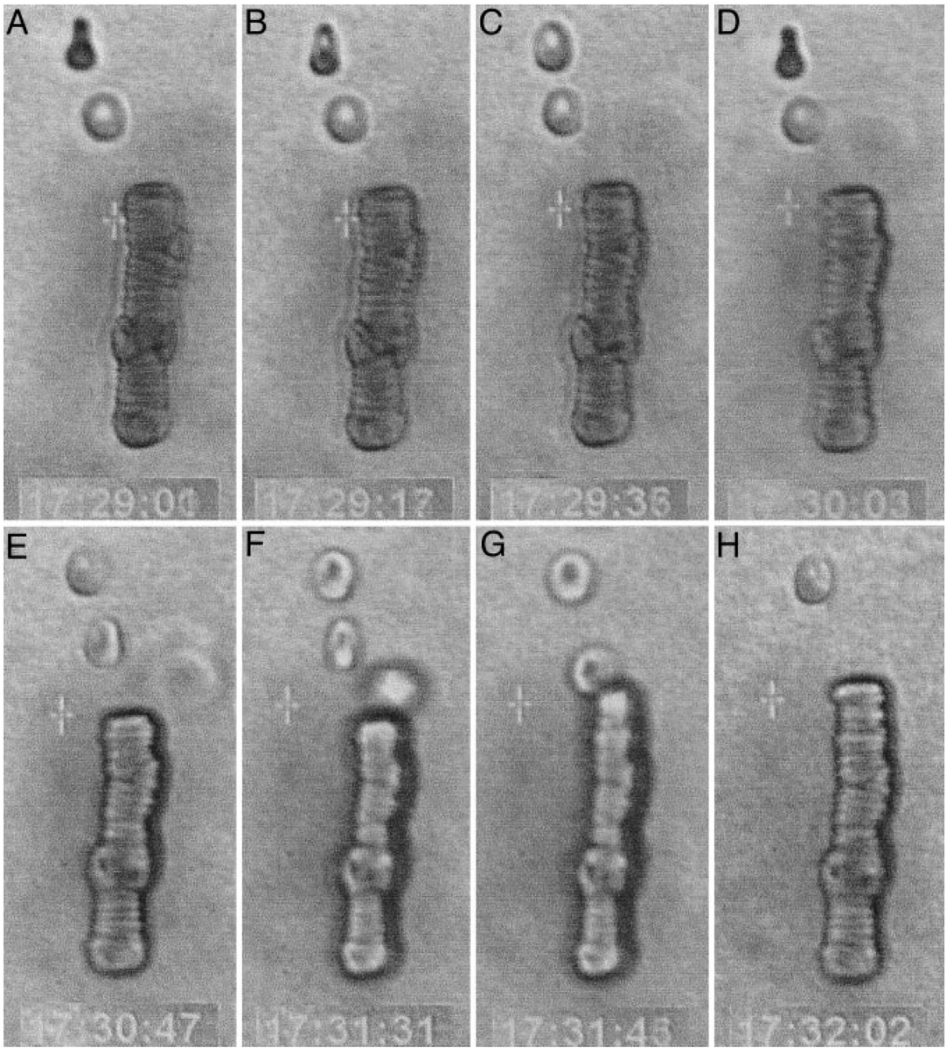

Fig. 3. In Rouleau formation. Shown is a timed sequence of two bag cells adding to the top of a stack of human red cells in rouleau formation. The speed is 4800 rpm and the time of day, shown in the bottom, is in h:min:*sec*. The cells were suspended in an isotonic saline/Percoll medium. It should be noted that this rouleau was only seven cells tall 10.5 min before that seen in A; in the meantime, cells were added to both its top and bottom. In A–G, two bag-shaped cells above the Rouleau can be seen in different views as they turn. (Depending on the focal level, the contrast of the thicker and thinner parts can be reversed.) In E–G, a third cell, which is out of focus, approaches from the right and joins the stack at the top. In G and H, the lower of the two original bag-shaped cells became incorporated into the rouleau. As soon as the bag-shaped cell touches the stack, it loses its asymmetric shape and unequal Hb distribution and resumes its original biconcave disk shape as it flips onto the top. (Taking A as zero time, B–H represent, respectively, 17, 36, 63, 107, 151, 165, and 182 s later.)
Taken from reference [111], see reference for details.

that the biconcave shape is maintained in that part of the cell. It is evident that when a cell touches the column it immediately falls over and assumes a discoid shape. The fact that the cells can slide over the surface of another red cell has been noted before [112]. The force, estimated with an optical tweezer technique, of aggregation and disaggregation between cells in rouleau, has been found to be different with the latter being much stronger than the former [113]. It should be

Deformin, a substance produced by *Bartonella bacilliformis*, that causes Carrion's disease, deforms and alters human red blood cell membranes [121]. The molecular details of its interaction as well as structure have yet to be delineated.

The peculiar effects, details and repetition that the state of transcendental meditation has on human red blood cell glycolysis has yet to be more fully delineated [122].

For those interested in cell shape, here is a nucleated red blood cell, found in a primitive fish (Lamprey), that is biconcave ([123], p175). One wonders if these cells can tank-tread?

13. After thought

The coverage of topics in this article have dealt with what might be construed as being predominately from a biophysical and physiological viewpoint. One hopes that any future review would highlight its bucket list with more molecular biology and biochemical aspects that would include, for instance, the detailed work on hematopoiesis and red cell pathophysiology. This should include hemoglobin switching as well as the changes that occur when a tadpole morphs into a frog.

"Hope is the thing with feathers
That perches in the in the soul,
And sings the tune without the words,
And never stops at all."

-Dickinson

Acknowledgement

It is a pleasure to acknowledge the skilled talents of Mr. Duncan Wong for his help in the preparation of this manuscript.

Appendix A

Table 1
Smallest vertebrates

Phyla	Name	Size or weight	Reference
Pisces	Stout Infant fish (*Schindleria brevipinguis*)	7–8.4 mm	A
Amphibia	Frog (*Paedophryne amanuensis*)	7.7 mm	B
Reptiles	Chameleon (*Brookesia micra*)	23.4–27.6 mm	C
Aves	Bee Hummingbird (*Mellisuga helenae*)	5.5–6.1 g	D
Mammals	Bumble Bee Bat (*Craseonycteris thonglongyai*)	2.0–2.6 g	E
	Pigmy Shrew (*Suncus etruscus*)	36–52 mm 1.5–2.5 g	

[A] W. Watson, H.J. Walker, The world's smallest vertebrate, *Schindleria brevipinguis*, a new paedomorphic species in the family Schindleriidae (Perciformes: Gobioidei), Records of the Australian Museum, 56 (2004) 139–142.
[B] E.N. Rittmeyer, A. Allison, M.C. Gründler, D.K. Thompson, C.C. Austin, Ecological Guild Evolution and the Discovery of the World's Smallest Vertebrate, PLoS One, 7 (2012) 1–8.
[C] F. Glaw, J. Köhler, T.M. Townsend, M. Vences, Rivaling the World's Smallest Reptiles: Discovery of Miniaturized and Microendemic New Species of Leaf Chameleons (Brookesia) from Northern Madagascar, PLoS One, 7 (2012) 1–24.
[D] I.-T.W.C. Union, The IUCN Red List of Threatened Species. Version 2016–1, Birdlife International, http://www.redlist.org, 2016.
[E] J.E. Hill, S.E. Smith, *Craseonycteris thonglongyai*, Mammalian Species, (1981) 1–4.

References

[1] J.F. Hoffman, Questions for red blood cell physiologists to ponder in this millenium, Blood Cells Mol. Dis. 27 (2001) 57–61.
[2] J.F. Hoffman, Some red blood cell phenomena for the curious, Blood Cells Mol. Dis. 32 (2004) 335–340.
[3] J.F. Hoffman, Further musings on some red blood cell problems for perspicacious physiologists, Blood Cells Mol. Dis. 39 (2007) 56–62.
[4] J.F. Hoffman, Red blood cells, compasses and snap shots, Blood Cells Mol. Dis. 71 (2018) 67–70.
[5] M. Bessis, G. Delpech, Discovery of the red blood cell with notes on priorities and credits of discoveries, past, present and future, Blood Cells 7 (1981) 447–480.
[6] W. Hewson XXXIII. On the figure and composition of the red particles of the blood, commonly called the red globules, Phil. Trans. R. Soc. A, (1773) 303–323.
[7] W. Hewson, G. Gulliver, The Works of William Hewson, F.R.S, Printed for the Sydenham Society, London, 1846.
[8] F. Gerber, G. Gulliver, Elements of the General and Minute Anatomy of Man and the Mammalia, Chiefly After Original Researches, H. Bailliere, London, 1842.
[9] T. Hodgkin, J.J. Lister, XXVI. Notice of some miscroscopic observations of the blood and animal tissues, Philos. Mag. 2 (1827) 130–138.
[10] J.J. Lister, On some properties in achromatic object-glasses applicable to the improvement of the microscope, Proc. R. Soc. Lond. 2 (1830) 187–200.
[11] T. Young, An Introduction to Medical Literature, Including a System of Practical Nosology, Intended as a Guide to Students, and an Assistant to Practitioners, Printed by B. R. Howlett for Underwood and Blacks, London, 1813.
[12] G. Gulliver, Observations on the sizes and shapes of the red corpuscles of the blood of vertebrates, Proc. Zool. Soc. London (1875) 474–495.
[13] M.M. Wintrobe, The size and hemoglobin content of the erythrocyte: methods of determination and clinical application, Transl. Res. 115 (1990) 374–387.
[14] E. Ponder, Hemolysis and Related Phenomena, Grune & Stratton, 1948.
[15] W.F. Emmons, The clinical eriometer, QJM (1927) 83–90 os-21.
[16] J.F. Hoffman, J.P. Geibel, Fluorescent imaging of Cl^- in *Amphiuma* red blood cells: how the nuclear exclusion of Cl^- affects the plasma membrane potential, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 921–926.
[17] D.C. Tosteson, J.F. Hoffman, Regulation of cell volume by active cation transport in high and low potassium sheep red cells, J. Gen. Physiol. 44 (1960) 169–194.
[18] M. Villolobos, P. León, S.K. Sessions, J. Kezer, Enucleated erythrocytes in plethodontid salamanders, Herpetologica 44 (1988) 243–250.
[19] R.L. Mueller, Genome size, cell size, and the evolution of enucleated erythrocytes in attenuate salamanders, Zoology (Jena) 111 (2008) 218–230.
[20] W.D. Cohen, Observations of the marginal band system of nucleated erythrocytes, J. Cell Biol. 78 (1978) 260.
[21] W.D. Cohen, The cytomorphic system of anucleate non-mammalian erythrocytes, Protoplasma 113 (1982) 23–32.
[22] D.G. Konstantinidis, S. Pushkaran, J.F. Johnson, J. Cancelas, S. Manganaris, C. Harris, D.A. Williams, Y. Zheng, T.A. Kalfa, Signaling and cytoskeletal requirements in erythroblast enucleation, Blood 119 (2012) 6118–6127.
[23] R.B. Nowak, J. Papoin, D.S. Gokhin, C. Casu, S. Rivella, J.M. Lipton, L. Blanc, V.M. Fowler, Tropomodulin 1 controls erythroblast enucleation via regulation of F-actin in the enucleosome, Blood 130 (2017) 1144–1155.
[24] J.F. Hoffman, Evidence that asymmetry of the membrane/cytoskeletal complex in human red blood cell ghosts is responsible for their biconcave shape, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 1641–1645.
[25] H. Hartridge, Proceedings of the Physiological Society, J. Physiol. 53 (1920) lxxv–cvii.
[26] D.A. Jones, The important of surface area/volume ratio to the rate of oxygen uptake by red cells, J. Gen. Physiol. 74 (1979) 643–646.
[27] E. Carlsen, J.H.J. Comroe, The rate of uptake of carbon monoxide and of nitric oxide by normal human erythrocytes and experimentally produced spherocytes, J. Gen. Physiol. 42 (1958) 83–107.
[28] M. Nakao, T. Nakao, S. Yamazoe, Adenosine triphosphate and maintenance of shape of the human red cells, Nature 187 (1960) 945–946.
[29] C. Feo, N. Mohandas, Clarification of role of ATP in red-cell morphology and function, Nature 265 (1977) 166–168.
[30] B.E. Haley, J.F. Hoffman, Interactions of a photo-affinity ATP analog with cation-stimulated adenosine triphosphatases of human red cell membranes, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 3367–3371.
[31] C.M. Cohen, P. Gascard, Regulation and post-translational modification of erythrocyte membrane and membrane-skeletal proteins, Semin. Hematol. 29 (1992)

244–292.
[32] A. George, S. Pushkaran, L. Li, X. An, Y. Zheng, N. Mohandas, C.H. Joiner, T.A. Kalfa, Altered phosphorylation of cytoskeleton proteins in sickle red blood cells: the role of protein kinase C, Rac GTPases, and reactive oxygen species, Blood Cells Mol. Dis. 45 (2010) 41–45.
[33] G. Bouyer, L. Reininger, G. Ramdani, L.D. Phillips, V.P. Sharma, S. Egee, G. Langsley, E. Lasonder, Plasmodium falciparum infection induces dynamic changes in the erythrocyte phospho-proteome, Blood Cells Mol. Dis. 58 (2016) 35–44.
[34] H. Chu, M.M. McKenna, N.A. Krump, S. Zheng, L. Mendelsohn, S.L. Thein, L.J. Garrett, D.M. Bodine, P.S. Low, Reversible binding of hemoglobin to band 3 constitutes the molecular switch that mediates O_2 regulation of erythrocyte properties, Blood 128 (2016) 2708.
[35] M.P. Sheetz, S.J. Singer, Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions, Proc. Natl. Acad. Sci. 71 (1974) 4457–4461.
[36] G. Lim H. W, M. Wortis, R. Mukhopadhyay, Stomatocyte–discocyte–echinocyte sequence of the human red blood cell: evidence for the bilayer– couple hypothesis from membrane mechanics, Proc. Natl. Acad. Sci. 99 (2002) 16766.
[37] S. Svetina, D. Kuzman, R.E. Waugh, P. Ziherl, B. Žekš, The cooperative role of membrane skeleton and bilayer in the mechanical behaviour of red blood cells, Bioelectrochemistry 62 (2) (2004) 107–113.
[38] J.F. Hoffman, Biconcave shape of human red-blood-cell ghosts relies on density differences between the rim and dimple of the ghosts plasma membrane, Proc. Natl. Acad. Sci. 113 (2016) 14847.
[39] H. Alimohamadi, A.S. Smith, V.M. Fowler, P. Rangamani, Non-Uniform Distribution of Myosin-Mediated Forces Governs Red Blood Cell Curvature Through Tension Modulation, bioRxiv, (2019), p. 668582.
[40] M.P. Sheetz, Integral membrane protein interaction with Triton cytoskeletons of erythrocytes, Biochim. Biophys. Acta 557 (1979) 122–134.
[41] F. Haurowitz, Zdenko, Über das Gerüst der Erythrocyten, Med. Klin. (1928) 19.
[42] A.R. Kay, M.P. Blaustein, Evolution of our understanding of cell volume regulation by the pump-leak mechanism, J. Gen. Physiol. 151 (2019) 407–416.
[43] T.R. Gregory, The bigger the C-value, the larger the cell: genome size and red blood cell size in vertebrates, Blood Cells Mol. Dis. 27 (2001) 830–843.
[44] G. Fankhauser, The effects of changes in chromosome number on amphibian development, Q. Rev. Biol. 20 (1945) 20–78.
[45] J.W. Boylan, J.B. Van Liew, P.U. Feig, Inverse changes in erythroid cell volume and number regulate the hematocrit in newborn genetically hypertensive rats, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 9848–9852.
[46] M.B. Ginzberg, R. Kafri, M. Kirschner, On being the right (cell) size, Science 348 (2015) 1–19.
[47] S.C. Liu, L.H. Derick, J. Palek, Visualization of the hexagonal lattice in the erythrocyte membrane skeleton, J. Cell Biol. 104 (1987) 527–536.
[48] J. Hillier, J.F. Hoffman, On the ultrastructure of the plasma membrane as determined by the electron microscope, J. Cell. Comp. Physiol. 42 (1953) 203–247.
[49] A. Elgsaeter, D. Branton, Intramembrane particle aggregation in erythrocyte ghosts. I. The effects of protein removal, J. Cell Biol. 63 (1974) 1018–1036.
[50] J.A. Ursitti, Ultrastructure of the human erythrocyte cytoskeleton and its attachment to the membrane, Cell Motil. Cytoskeleton 19 (1991) 227–243.
[51] L. Pan, R. Yan, W. Li, K. Xu, Super-resolution microscopy reveals the native ultrastructure of the erythrocyte cytoskeleton, Cell Rep. 22 (2018) 1151–1158.
[52] S.E. Lux, Anatomy of the red cell membrane skeleton: unanswered questions, Blood 127 (2016) 187–199.
[53] J.F. Hoffman, On red blood cells, hemolysis and resealed ghosts, in: M. Magnani, J.R. DeLoach (Eds.), The Use of Resealed Erythrocytes as Carriers and Bioreactors, Springer US, Boston, MA, 1992, pp. 1–15.
[54] R.J.V. Pulvertaft, Vibratory movement in the cytoplasm of erythrocytes, J. Clin. Pathol. 2 (1949) 281–283.
[55] R. Blowers, E.M. Clarkson, M. Maizels, Flicker phenomenon in human erythrocytes, J. Physiol. 113 (1951) 228–239.
[56] A.K. Parpart, J.F. Hoffman, Flicker in erythrocytes; vibratory movements in the cytoplasm, J. Cell. Comp. Physiol. 47 (1956) 295–303.
[57] F. Brochard-Wyart, J.F. Lennon, Frequency spectrum of the flicker phenomenon in erythrocyte, J. Phys. 36 (1975) 1035–1047.
[58] N.S. Gov, S.A. Safran, Red blood cell membrane fluctuations and shape controlled by ATP-induced cytoskeletal defects, Biophys. J. 88 (2005) 1859–1874.
[59] R. Rodríguez-García, I. Lopez-Montero, M. Mell, G. Egea, N.S. Gov, F. Monrov, Direct cytoskeleton forces cause membrane softening in red blood cells, Biophys. J. 108 (2015) 2794–2806.
[60] H. Turlier, D. Fedosov, B. Audoly, T. Auth, N.S. Gov, C. Sykes, J.-F. Joanny, G. Gompper, T. Betz, Equilibrium physics breakdown reveals the active nature of red blood cell flickering, Nat. Phys. 12 (2016) 513–519.
[61] J. Evans, W. Gratzer, N. Mohandas, K. Parker, J. Sleep, Fluctuations of the red blood cell membrane: relation to mechanical properties and lack of ATP dependence, Biophys. J. 94 (2008) 4134–4144.
[62] A.A. Evans, B. Bhaduri, G. Popescu, A.J. Levine, Geometric localization of thermal fluctuations in red blood cells, Proc. Natl. Acad. Sci. 114 (2017) 2865.
[63] M. Puckeridge, Membrane flickering of the human erythrocyte: constrained random walk used with Bayesian analysis, Eur. Biophys. J., 43 157–167.
[64] M. Puckeridge, B.E. Chapman, A.D. Conigrave, P.W. Kuchel, Membrane flickering of the human erythrocyte: physical and chemical effectors, Eur. Biophys. J. 43 (2014) 169–177.
[65] D. Boss, A. Hoffmann, B. Rappaz, C. Depeursinge, P.J. Magistretti, D. Van de Ville, P. Marquet, Spatially-resolved eigenmode decomposition of red blood cells membrane fluctuations questions the role of ATP in flickering, PLoS One 7 (2012) e40667.
[66] G.R. Bergfeld, T. Forrester, Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia, Cardiovasc. Res. 26 (1992) 40–47.
[67] M.L. Ellsworth, C.G. Ellis, R.S. Sprague, Role of erythrocyte-released ATP in the regulation of microvascular oxygen supply in skeletal muscle, Acta Physiol (Oxford) 216 (2016) 265–276.
[68] J. Wan, W.D. Ristenpart, H.A. Stone, Dynamics of shear-induced ATP release from red blood cells, Proc. Natl. Acad. Sci. 105 (2008) 16432–16437.
[69] A.M. Forsyth, J. Wan, P.D. Owrutsky, M. Abkarian, H.A. Stone, Multiscale approach to link red blood cell dynamics, shear viscosity, and ATP release, Proc. Natl. Acad. Sci. 108 (2011) 10986–10991.
[70] H. Zhang, Z. Shen, B. Hogan, A.I. Barakat, C. Misbah, ATP release by red blood cells under flow: model and simulations, Biophys. J. 115 (2018) 2218–2229.
[71] J. Sikora, S.N. Orlov, K. Furuya, R. Grygorczyk, Hemolysis is a primary ATP-release mechanism in human erythrocytes, Blood 124 (2014) 2150–2157.
[72] B.S. Kirby, P.J. Schwarzbaum, E.R. Lazarowski, F.A. Dinenno, T.J. McMahon, Liberation of ATP secondary to hemolysis is not mutually exclusive of regulated export, Blood 125 (2015) 1844.
[73] J. Sikora, S.N. Orlov, K. Furuya, R. Grygorczyk, Response: hemolysis is a primary and physiologically relevant ATP release mechanism in human erythrocytes, Blood 125 (2015) 1845.
[74] H. Mairbäurl, F.A. Ruppe, P.D. Bärtsch, Role of hemolysis in red cell adenosine triphosphate release in simulated exercise conditions in vitro, Med. Sci. Sports Exerc. 45 (2013) 1941–1947.
[75] R.S. Sprague, E.A. Bowles, J.J. Olearczyk, A.H. Stephenson, A.J. Lonigro, The role of G protein beta subunits in the release of ATP from human erythrocytes, J. Physiol. Pharmacol. 53 (2002) 667–674.
[76] S. Locovei, L. Bao, G. Dahl, Pannexin 1 in erythrocytes: function without a gap, Proc. Natl. Acad. Sci. 103 (2006) 7655–7659.
[77] M. Sridharan, S.P. Adderley, E.A. Bowles, T.M. Egan, A.H. Stephenson, M.L. Elsworth, R.S. Sprague, Pannexin 1 is the conduit for low oxygen tension-induced ATP release from human erythrocytes, Am. J. Physiol. Heart Circ. Physiol. 299 (2010) H1146–H1152.
[78] M. Sridharan, E.A. Bowles, J.P. Richards, M. Krantic, K.L. Davis, K.A. Dietrich, A.H. Stephenson, M.L. Elsworth, R.S. Sprague, Prostacyclin receptor-mediated ATP release from erythrocytes requires the voltage-dependent anion channel, Am. J. Physiol. Heart Circ. Physiol. 302 (2012) H553–H559.
[79] I. Marginedas-Freixa, C.L. Alvarez, M. Moras, M.F.L. Denis, C. Hattab, F. Halle, F. Bihel, I. Mouro-Chanteloup, S.D. Lefevre, C. Le Van Kim, P.J. Schwarzbaum, M.A. Ostuni, Human erythrocytes release ATP by a novel pathway involving VDAC oligomerization independent of pannexin-1, Sci. Rep. 8 (2018) 1–13.
[80] H. Chu, E. Puchulu-Campanella, J.A. Galan, W.A. Tao, P.S. Low, J.F. Hoffman, Identification of cytoskeletal elements enclosing the ATP pools that fuel human red blood cell membrane cation pumps, Proc. Natl. Acad. Sci. 109 (2012) 12794–12799.
[81] C.W.M. Haest, Distribution and movement of membrane lipids, in: I. Bernhardt, J.C. Ellory (Eds.), Red Cell Membrane Transport in Health and Disease, Springer-Verlag Berlin Heidelberg, 2003, pp. 1–25.
[82] L.L.M. Van Deenen, J. De Gier, Chapter 4 - lipids of the red cell membrane, in: D.M. Surgenor (Ed.), The Red Blood Cell, Second edition, Academic Press, 1974, pp. 147–211.
[83] J.P. Andersen, A.L. Vestergaard, S.A. Mikkelsen, L.S. Mogensen, M. Chalat, R.S. Molday, P4-ATPases as phospholipid Flippases-structure, function, and enigmas, Front. Physiol. 7 (2016) 1–23.
[84] T.L. Steck, J. Ye, Y. Lange, Probing red cell membrane cholesterol movement with cyclodextrin, Biophys. J. 83 (2002) 2118–2125.
[85] T.L. Steck, Y. Lange, Transverse distribution of plasma membrane bilayer cholesterol: picking sides, Traffic 19 (2018) 750–760.
[86] J.A. Bloom, W.W. Webb, Lipid diffusibility in the intact erythrocyte membrane, Biophys. J. 42 (1983) 295–305.
[87] E.-F. Gautier, M. Leduc, S. Cochet, K. Bailly, C. Lacombe, N. Mohandas, F. Guillonneau, W. El Nemer, P. Mayeux, Absolute proteome quantification of highly purified populations of circulating reticulocytes and mature erythrocytes, Blood Adv. 2 (2018) 2646–2657.
[88] C. Leonard, L. Conrard, M. Guthmann, H. Pollet, M. Carquin, C. Vermylen, P. Gailly, P. Van Der Smissen, M.P. Mingeot-Leclercq, D. Tyteca, Contribution of plasma membrane lipid domains to red blood cell (re)shaping, Sci. Rep. 7 (2017) 1–16.
[89] C. Leonard, Tuning of differential lipid order between submicrometric domains and surrounding membrane upon erythrocyte reshaping, Cell. Physiol. Biochem. 48 (2018) 2563–2582.
[90] S.L. Schrier, A. Zachowski, P. Hervé, J.C. Kader, P.F. Devaux, Transmembrane redistribution of phospholipids of the human red cell membrane during hypotonic hemolysis, Biochim. Biophys. Acta 1105 (1992) 170–176.
[91] W. Helfrich, Blocked lipid exchange in bilayers and its possible influence on the shape of vesicles, Z. Naturforsch. 29 (9–10) (2014) 510–515 2014 v.29.
[92] S. Svetina, B. Zeks, Membrane bending energy and shape determination of phospholipid vesicles and red blood cells, Eur. Biophys. J. 17 (1989) 101–111.
[93] K. Khairy, Minimum-energy vesicle and cell shapes calculated using spherical harmonics parameterization, Soft Matter 7 (2011) 2138–2143.
[94] Y. Lange, A. Gough, T.L. Steck, Role of the bilayer in the shape of the isolated erythrocyte membrane, J. Membr. Biol. 69 (1982) 113–123.
[95] T.M. Fischer, Human Red Cell Shape and the Mechanical Characteristics of the Membrane, (2003), pp. 61–82.
[96] J.F. Hoffman, On the mechanism and measurement of shape transformations of constant volume of human red blood cells, Blood Cells 12 (1987) 565–588.

[97] T.M. Fischer, M. Stohr-Lissen, H. Schmid-Schonbein, The red cell as a fluid droplet: tank tread-like motion of the human erythrocyte membrane in shear flow, Science 202 (1978) 894.
[98] T.M. Fischer, C.W. Haest, M. Stöhr, D. Kamp, B. Deuticke, Selective alteration of erythrocyte deformabiliby by SH-reagents: evidence for an involvement of spectrin in membrane shear elasticity, Biochim. Biophys. Acta 510 (1978) 270–282.
[99] M.M. Guest, T.P. Bond, R.G. Cooper, J.R. Derrick, Red blood cells change in shape in capillaries, Science 142 (1963) 1319–1321.
[100] R. Skalak, P.L. Branemark, Deformation of red blood cells in capillaries, Science 164 (1969) 717–719.
[101] H. Basu, A.K. Dharmadhikari, J.A. Dharmadhikari, S. Sharma, D. Mathur, Tank treading of optically trapped red blood cells in shear flow, Biophys. J. 101 (2011) 1604–1612.
[102] F.E. Weaver, Normal band 3-cytoskeletal interactions are maintained on tank treading erythrocytes, Biophys J 58 (1990) 1427–1436.
[103] G.M. Della Torre, Nuove Osservazioni Microscopiche, C. R. Somasco, Napoli, Italy, 1776.
[104] M. Bessis, Living Blood Cells and Their Ultrastructure, Springer-Verlag, New York, 1973.
[105] M.H. Knisely, E.H. Bloch, T.S. Eliot, L. Warner, Sludged blood, Science 106 (1947) 431–440.
[106] R. Fahraeus, The influence of the rouleau formation of the erythrocytes on the rheology of the blood, Acta Med. Scand. 161 (1958) 151–165.
[107] S.S. Norris, D.D. Allen, T.P. Neff, S.L. Wilkinson, Evaluation of 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid in the inhibition of rouleaux formation, Transfusion 36 (1996) 109–112.
[108] S. Chien, K. Jan, Ultrastructural basis of the mechanism of rouleaux formation, Microvasc. Res. 5 (1973) 155–166.
[109] G. Barshtein, I. Tamir, S. Yedgar, Red blood cell rouleaux formation in dextran solution: dependence on polymer conformation, Eur. Biophys. J. 27 (1998) 177–181.
[110] J. Derganc, B. Božič, S. Svetina, B. Žekš, Equilibrium shapes of erythrocytes in rouleau formation, Biophys. J. 84 (2003) 1486–1492.
[111] J.F. Hoffman, S. Inoue, Directly observed reversible shape changes and hemoglobin stratification during centrifugation of human and Amphiuma red blood cells, Proc. Natl. Acad. Sci. 103 (2006) 2971–2976.
[112] D.E. McMillan, N.G. Utterback, M.M. Lee, Red cells slide as they form doublets and deform in rouleaux, Biorheology 26 (1989) 899–906.
[113] K. Lee, M. Kinnunen, M.D. Khokhlova, E.V. Lyubin, A.V. Priezzhev, I. Meglinski, A.A. Fedyanin, Optical tweezers study of red blood cell aggregation and disaggregation in plasma and protein solutions, J. Biomed. Opt. 21 (2016) 1–10, 10.
[114] J. Spector, G.C. Kodippili, K. Ritchie, P.S. Low, Single molecule studies of the diffusion of band 3 in sickle cell erythrocytes, PLoS One 11 (2016) 1–11.
[115] K. Giger, I. Habib, K. Ritchie, P.S. Low, Diffusion of glycophorin A in human erythrocytes, Biochim. Biophys. Acta 1858 (2016) 2839–2845.
[116] J.S. O'Neill, A.B. Reddy, Circadian clocks in human red blood cells, Nature (London) 469 (2011) 498–503.
[117] A.K. Saha, B.R. Schmidt, J. Wilhelmy, V. Nguyen, A. Abugherir, J.K. Do, M. Nemat-Gorgani, R.W. Davis, A.K. Ramasubramanian, Red blood cell deformability is diminished in patients with chronic fatigue syndrome, Clin. Hemorheol. Microcirc. 71 (2019) 113–116.
[118] P.W. Kuchel, D. Shishmarev, Accelerating metabolism and transmembrane cation flux by distorting red blood cells, Sci. Adv. 3 (2017) 1–10.
[119] S. Svetina, T. Švelc Kebe, B. Božič, A model of Piezo1-based regulation of red blood cell volume, Biophys. J. 116 (2019) 151–164.
[120] G. Kollmann, B. Shapiro, D. Martin, The effects of lactose on human erythrocytes, J. Cell. Physiol. 77 (1971) 195–200.
[121] S.C. Derrick, G.M. Ihler, Deformin, a substance found in Bartonella bacilliformis culture supernatants, is a small, hydrophobic molecule with an affinity for albumin, Blood Cells Mol. Dis. 27 (2001) 1013–1019.
[122] R. Jevning, A.F. Wilson, H. Pirkle, J.P. O'Halloran, R.N. Walsh, Metabolic control in a state of decreased activation: modulation of red cell metabolism, Am. J. Phys. 245 (1983) C457–C461.
[123] I.C. Potter, E.S. Robinson, I.D. Brown, Studies on the erythrocytes of larval and adult lampreys (*Lampetra fluviatilis*), Acta Zool. 55 (1974) 173–177.