examined without interference from the intraocular hemorrhage.

In conclusion, vitreous liquefaction and intraocular hemorrhage may be accurately assessed by proton NMR imaging. This approach may have important clinical applications, particularly in evaluating proliferative diabetic retinopathy, the most common cause of blindness in the United States for people under the age of 60 (*12*). Early detection of liquefaction and detailed studies of sequelae, including hemorrhage, appear feasible. Similar studies of the natural history of other ocular diseases may also be possible.

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Plasmodium falciparum Maturation Abolishes Physiologic Red Cell Deformability

Abstract. *Normal red cells deform markedly as they pass through the spleen and the peripheral capillaries. In these studies, the effects of* Plasmodium falciparum *infection and maturation on the deformability of parasitized red cells exposed to fluid shear stress in vitro were examined by means of a rheoscope. Red cells containing the early (ring) erythrocytic stage of the parasite have impaired deformability at physiologic shear stresses, and recover their normal shape more slowly. Red cells containing more mature parasites (trophozoites or schizonts) exhibit no deformation under the same conditions. These results provide a mechanism to explain the ability of the spleen to remove parasitized red cells from the circulation of both immune and nonimmune hosts.*

The ability of the normal red cell to deform like a liquid droplet when subjected to shear stress in vitro is well documented (*1*). The normal red cell (disk diameter 7 to 8 μm) also deforms markedly in vivo in order to pass through the interendothelial slits and basement membrane fenestrations of the spleen (*2*) and through peripheral capillaries with luminal diameters of 3 to 4 μm. The rotation of the red cell membrane around its cytoplasmic contents in a motion suggestive of a tank tread is particularly significant (*3*), and recent reports have shown that tank-treading occurs in capillaries both in vitro (*4*) and in vivo (*5*). This tank-treading motion reduces the hydraulic resistance to red cell passage through the capillary (*6*), and may also facilitate oxygen exchange between the red cell and the capillary endothelium. This report identifies a disease state (*Plasmodium falciparum* infection) characterized by a loss of red cell deformability and tank-treading in which the loss of red cell deformability presumably facilitates the splenic removal of parasitized red cells from the circulation.

Miller and his colleagues suggested previously that parasitized red cells are less deformable than normal red cells (*7*). However, at the time those studies were performed a technique for the continuous culture of malaria parasites in vitro (*8*) was not available; the investigators used mixtures of parasitized and unparasitized cells to perform measurements of macroscopic viscosity and resistance to passage through a filter. In these studies, we used the rheoscope (*1*) to define the effects of graded levels of shear stress on the deformability of *P. falciparum*–infected red cells. This was accomplished by measuring the elongation (length to width ratio) of infected and control (uninfected) red cells in the same microscopic fields, by determining the prevalence of tank-treading among infected and control cells in the same fields, and by measuring the time course of the recovery of cell shape after the abrupt release of the shear stress.

Plasmodium falciparum parasites were cultured in an environment with 3 percent oxygen and 3 percent carbon dioxide (*9*) according to the system devised by Trager and Jensen (*8*). Most studies were performed with the chloroquine-resistant Indochina I/CDC strain, although several experiments were also performed with the chloroquine-susceptible Honduras I/CDC strain (*10*). A knobless clone of the FCR-3 strain from The Gambia (clone D-4) (*10, 11*) was used to determine whether the observed changes in the deformability of parasitized red cells were associated with knob formation (*12*).

To produce parasitemias 15 percent or more for examination in the rheoscope without exhausting the ability of the culture medium to support parasite growth, we maintained heavily parasitized red cell suspensions at hematocrits near 1 percent and provided them with fresh medium two to three times daily. Red cells with trophozoites or schizonts from

Table 1. Red cell recovery time and *P. falciparum* infection. The characteristic shape-recovery time (t_c) is defined by a simple exponential fit of the time course of the recovery of cell length immediately after the release of a shear stress of 20 dyne/cm^2 (*17, 18*) as described by the equation:

$$\frac{L - L_f}{L_i - L_f} = e^{-t/t_c}$$

where L, L_i, and L_f are the instantaneous, initial, and final cell lengths, respectively, and t is the time after release of the shear stress. According to this formula, the elongation of a cell ($L - L_f$) is 36.8 percent of its initial value ($L_i - L_f$) at time t_c. The recovery times observed for red cells with rings were significantly longer than those of normal (uninfected) red cells ($t = 2.896$, $P < 0.005$).

Red cell type	t_c (seconds)	
	Mean	Standard deviation
Normal red cells	0.121*	0.029
Red cells with rings	0.137*	0.036

*$N = 70$.

knob-positive strains (Indochina I and Honduras I) were also concentrated with the gelatin technique (*13*) to facilitate their examination in the rheoscope. These suspensions of parasitized red cells were washed twice in phosphate-buffered saline (PBS, 0.01*M*; *p*H 7.4) with dextrose (200 mg/dl), suspended at a hematocrit of 80 percent, and mixed 1:1 with PBS-dextrose containing 40 percent plasma. They were subsequently diluted 1:9 with PBS-dextrose containing 8 percent dextran (molecular weight 2×10^6) to produce an isotonic suspension (300 mos*M*) with a viscosity of 30 centipoise (25 times plasma viscosity) (*14*) and a 4 percent hematocrit for examination in the rheoscope. The effects of the different erythrocytic stages on the deformability and tank-treading of parasitized red cells were distinguished with the use of synchronized cultures (*15*).

Giemsa-stained smears were used to identify the erythrocytic stages present in culture material examined with the rheoscope. On the basis of the examination of such smears from synchronized cultures (*13, 15*), ring forms could be identified within biconcave disk-shaped red cells with careful focusing (Fig. 1A). In contrast, parasites containing pigment were readily visualized in the rheoscope and were found to represent trophozoites and schizonts (Fig. 1, B and C). Red cells containing trophozoites or schizonts were shrunken, irregular, and roughly spherical. With shear stresses $\geq$ 10 dyne/cm^2, uninfected (control) red cells elongated appreciably (Figs. 1 and 2). The deformability of these uninfected red cells was identical to that previously observed in the rheoscope with fresh red cells (*16*), although the red cells used in the culture system had been obtained from 2-week-old bank blood. Red cells containing ring forms of the parasite were less deformable than uninfected red cells, and red cells with trophozoites or schizonts did not deform (Figs. 1 and 2).

To calculate the mean steady-state length:width ratios of infected and uninfected red cells (that is, one measure of their deformability) at different shear stresses, we traced the outlines of cells on photomicrographs on a magnetic digitizing board coupled to a computer (Hewlett-Packard 1000). The computer was programmed to identify and exclude cells that were not tank-treading on the grounds that (i) they were not sufficiently elliptical, (ii) there was too large an angle ($> 7.5°$) between the principal axis of the cell profile and the direction of flow, or (iii) their length:width ratio was ≤ 1.

Videotapes taken at 60 fields per second were used to record the time course of cell-shape recovery after the abrupt release of the shear stress in rheoscope experiments. Field-by-field analyses of the recovery process of single cells (on these videotapes) yielded characteristic shape-recovery times (*17*) which can be interpreted as the ratio of membrane surface viscosity to the shear elastic modulus for deforming cells (*18*). The recovery times calculated for uninfected red cells were shorter than those of red cells with the ring form of the parasite (Table 1). Because recovery time is defined as membrane viscosity divided by membrane stiffness (*18*), these results suggest that the membranes of parasitized red cells have an increased viscosity relative to their shear elastic modulus. Measurement of the recovery time was not possible with trophozoite- or schizont-infected red cells because they did not deform.

Both direct observation (with a monitor) and review of the videotapes revealed normal tank-treading by uninfected red cells. Most red cells containing ring forms demonstrated tank-treading at shear stresses $\geq$ 40 dyne/cm^2, but not at 10 dyne/cm^2. In contrast, no red cells with trophozoites or schizonts exhibited tank-treading at shear stresses $\leq$ 80 dyne/cm^2 (Table 2). In another experiment, red cells were exposed to supraphysiologic shear stress (800 dyne/cm^2) in a PBS-dextrose medium with a viscosity of 100 centipoise. Approximately 20 percent of red cells with trophozoites

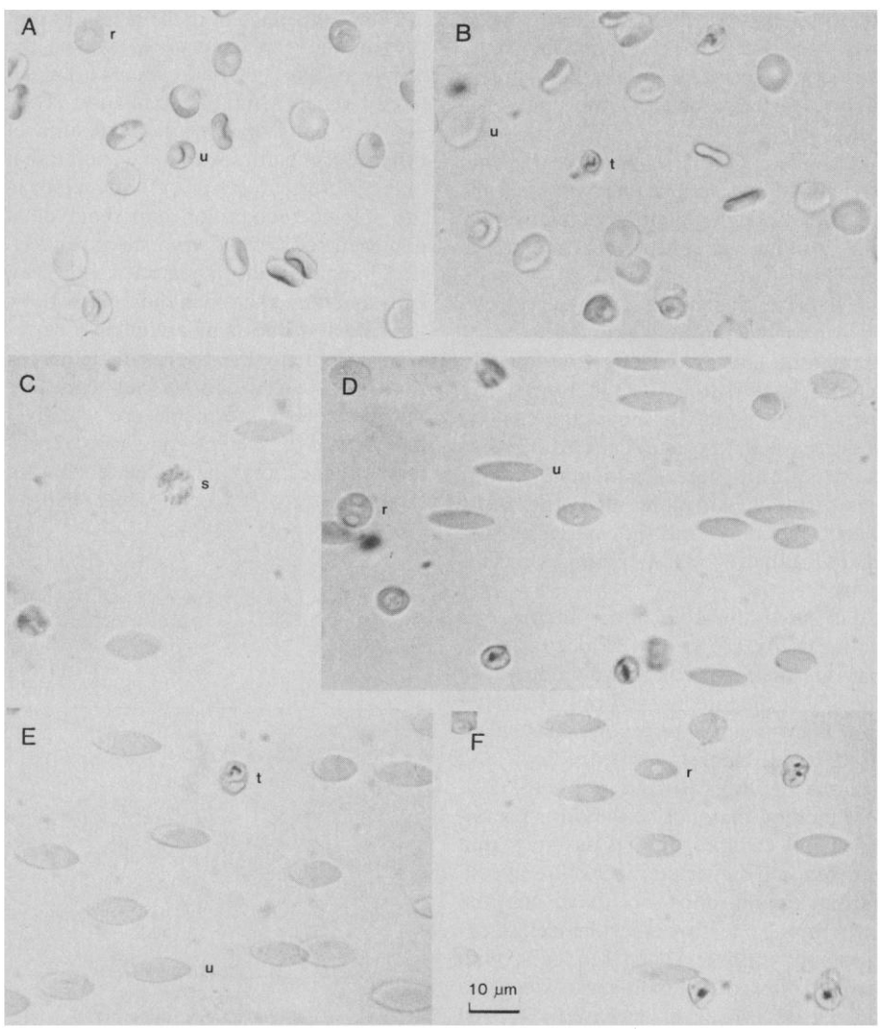

Fig. 1. At zero shear stress, uninfected red cells (*u*) were disk-shaped and oriented randomly in the rheoscope (A and B), as were red cells containing ring forms of the parasite (*r*) (A). Red cells with trophozoites (*t*) appeared smaller, more irregular, and spherical (B), as did schizonts (*s*), even at shear stresses of 40 dyne/cm^2 (C). With shear stresses of 10 (not shown), 40 (C and E), and 80 dyne/cm^2 (D and F), normal red cells deformed (elongated) in the direction of the shear stress (left to right in the photomicrographs), red cells with rings deformed less than normal red cells, and red cells with trophozoites or schizonts did not deform. The results were similar with the chloroquine-resistant Indochina I strain (A to D), the chloroquine-susceptible Honduras I strain (not shown), and the knobless clone D-4 derived from the FCR-3 strain of *P. falciparum* (E and F).

Table 2. Red cell tank-treading and *P. falciparum* infection. Results are expressed as the percentage of all cells examined in each category that were tank-treading. More than 100 cells of each cell type were examined at each shear stress. The differences observed between red cells with rings and uninfected red cells were significant at each shear stress by the χ^2 test ($P < 0.001$).

Red cell type	Shear stress (dyne/cm^2)		
	10	40	80
Normal (uninfected) red cells	80	92	92
Red cells with rings	38	72	72

(but none with schizonts) were visibly deformed at this shear stress.

Although most observations were made with the Indochina I strain (which has knobs and is chloroquine-resistant), similar observations were also made with the chloroquine-susceptible Honduras I strain and the knobless D-4 clone of the FCR-3 strain. Thus, the loss of deformability as the parasite matured from the ring to the trophozoite stage was not associated with either chloroquine resistance or the knob phenomenon.

The mechanisms responsible for this loss of red cell deformability remain undefined although quantitative microrheologic studies have shown that the deformability of the normal red cell depends on both its high surface area:volume ratio and the physical properties of its membrane and cytoplasm (*19*). Specific factors known to reduce deformability include: (i) increased sphericity, that is, a decrease in the surface area:volume ratio (S/V), (ii) increased membrane stiffness, that is, an increase in the shear elastic modulus, and (iii) increased viscosity of the red cell membrane or cytoplasm.

Parasite-induced changes in red cell permeability are known to occur as the parasite matures (*20*), and may decrease the S/V of the infected red cell. Although the micropipette technique has been used to measure the surface area and volume of unparasitized red cells (*21*), this method may not be valid for parasitized red cells because it assumes that the red cell being studied is freely deformable and does not have internal structure. To test whether the lack of deformability observed in red cells with trophozoites or schizonts was due to a decreased S/V, or an increased cytoplasmic viscosity, we attempted to perturb these properties osmotically (*22*). First, we suspended the cells in a hypertonic medium (400 mos*M*), expecting to decrease their volume and thus increase S/V. Next, we used hypotonic suspending media (100, 150, and 200 mos*M*), expecting to force water into the cells and thus decrease their cytoplasmic viscosity. Red cells with trophozoites or schizonts were nondeformable in all these media at shear stresses $\leq$ 80 dyne/cm^2. Assuming that parasitized red cells are osmotically active (*20*), these results suggest that neither a decreased S/V nor an increased cytoplasmic viscosity alone can satisfactorily explain the loss of deformability observed in parasitized red cells.

The significance of these results is that they define a mechanism to explain the ability of the spleen to remove parasitized red cells from the circulation. They are also consistent with the results of other investigators who have shown that loss of deformability is a critical factor in the splenic recognition and removal of damaged red cells in vivo. Sandza *et al.* (*23*) mechanically sheared rabbit red cells and then examined their removal by the spleen of the same animal in a perfusion system in vitro: the results indicated that the sheared cells (which appeared morphologically normal) were selectively removed by the spleen. Those experiments suggest that the spleen recognized (and removed) red cells which had altered physical properties as a result of the in vitro shearing procedure. Other studies have also suggested that the spleen removes red cells with reduced deformability (*24*); previous ultrastructural studies have demonstrated that parasitized red cells may be trapped within the interendothelial slits of spleen (*25*); and the studies of Quinn and Wyler (*26*) have shown that the spleen is responsible for the antibody-independent clearance of *P. berghei*–infected red cells in nonimmune rats.

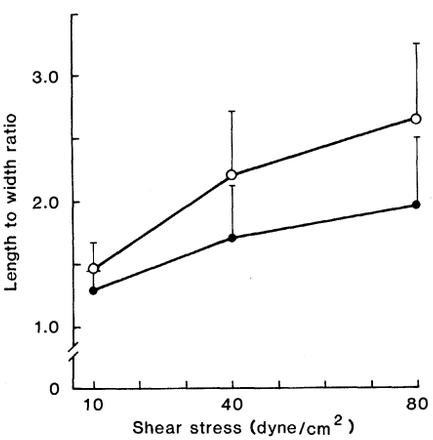

Fig. 2. The length to width ratios for normal red cells (○) and red cells with ring forms (●). Each data point represents the mean + 1 standard deviation for more than 100 cells. No data are shown for red cells with trophozoites or schizonts because none of these cells deformed (that is, they all had length to width ratios of 1.0). The differences observed were significant ($P < 0.01$) for shear stresses $\geq$10 dyne/cm^2.

Our results show that *P. falciparum* trophozoites and schizonts abolish physiologic red cell deformability. Because the spleen has been shown to trap nondeformable red cells, these results suggest that this loss of deformability is the critical factor in the splenic recognition and removal of red cells containing *P. falciparum* trophozoites and schizonts from the circulation. Although the loss of deformability we have observed with parasitized red cells does not explain the phenomenon of peripheral sequestration, recent studies by David *et al.* (*27*) suggest that the spleen modulates the adherence of parasitized cells to the vascular endothelium in vivo. Taken together, their results and ours suggest that the role of the spleen in *P. falciparum* infection includes both the removal of parasitized cells (with reduced or absent deformability) and the control of peripheral sequestration.

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Salt Taste Transduction Occurs Through an Amiloride-Sensitive Sodium Transport Pathway

Abstract. *An important early event in mammalian gustatory transduction with respect to sodium chloride has been found to be the passage of sodium ions through specific transport pathways in the apical region of the taste bud. The inward current caused by sodium chloride placed on the mucosal surface of an in vitro preparation of rat dorsal lingual epithelium can be substantially reduced by the blocker of sodium ion transport, amiloride. The data show (i) that amiloride is a specific blocker of the chorda tympani response to sodium chloride, but not to potassium chloride, (ii) that the sodium and potassium gustatory systems are largely independent at the peripheral level, and (iii) that the classical ion taste "receptor" is actually a specific transport pathway permitting the cation to enter the taste-bud cell and thereby to spread depolarizing current.*

The classical interpretation of the events surrounding the excitation of mammalian taste-bud cells by salt is based on two fundamental assumptions:

1) The dorsal lingual epithelium, including the taste-bud cells, acts as an impermeable barrier against ions and other tastants.

2) The essential interaction between stimulus ions and taste buds is adsorption to an apical receptor.

From these assumptions follow models of salt taste transduction that have regarded penetration of the stimulus ions into the taste-bud cells as nonessential and in some cases impossible (*1*). However, the canine lingual epithelium actively transports ions, and the active transport system is particularly stimulated by NaCl concentrations spanning the gustatory range of 1 mM to 1M (*2, 3*). Furthermore, stimulation of the transport system by hyperosmotic NaCl can be blocked by amiloride (*3*). These results call into question the general validity of assumption 1, particularly regarding a barrier against ions. There remained the possibility, however, that assumption 1 might be valid for taste buds only. We now present evidence that the amiloride-sensitive sodium transport system includes the apical regions of the taste buds. The finding has important consequences for gustatory transduction. The conclusions are drawn from studies on the rat in which amiloride (i) inhibited the short-circuit current (I_{sc}) that results from placing hyperosmotic NaCl on the rat lingual epithelium in vitro and (ii) likewise inhibited the neural response to NaCl in vivo without effects on the KCl response.

Sprague-Dawley rats were used in both the in vitro and in vivo experiments. In the first case, rats were decapitated and the tongues removed. A section of the anterior dorsal epithelium was freed from the underlying skeletal muscle and placed between plexiglass chambers (*4*). Under symmetrical conditions in Krebs-Henseleit buffer (*5*), a steady open-circuit potential (V_{oc}) was achieved within 1 hour. The mean (± standard error) V_{oc} for 14 preparations was 13.4 ± 0.7 mV (inside positive). The I_{sc} was 8.2 ± 0.9 μA/cm^2, and the resistance was 1686 ± 147 ohm-cm^2. When the mucosal solution was replaced by a series of NaCl solutions ranging from 0.01M to 2M, I_{sc} increased in a graded manner (Fig. 1A). Like canine epithelium (*2, 3*), the rat epithelium responded to hyperosmotic NaCl with a two-component increase in V_{oc} when the adapting mucosal NaCl concentration of 0.001M was replaced by 1.5M NaCl (upper trace in Fig. 1B). This hyperosmotic NaCl response could be sharply attenuated by placing $10^{-4}M$ amiloride in the mucosal adapting solution. The lower trace in Fig. 1B is the response to 1.5M NaCl after a 5-minute exposure to $10^{-4}M$ amiloride in 0.001M NaCl. Both the rapid rise and the quasi-steady component were reduced. The corresponding reduction in I_{sc} extended over the entire hyperosmotic range (Fig. 1A). The effect was selective for NaCl over KCl (*3*).

If the amiloride-sensitive inward current extends to the taste-bud cells, and if it is coupled to early events in gustatory transduction and subsequent neural events, it should be possible to reversibly and specifically block the neural response to NaCl by rinsing the rat's tongue in amiloride. The entire NaCl

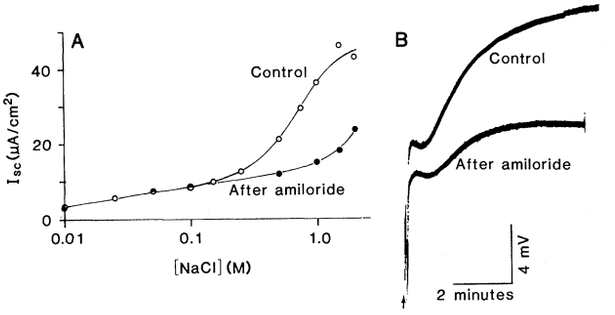

Fig. 1. (A) Short-circuit current (I_{sc}) resulting from NaCl solutions placed in the mucosal chamber. (B) Time course of the open-circuit potential after 0.001M NaCl in mucosal chamber was replaced with 1.5M NaCl.