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Decreased Rotational Diffusion of Band 3 in Melanesian Ovalocytes from Papua, New Guinea

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Summary. Melanesian ovalocytes from Papua New Guinea have an N-terminal extension of the band 3 polypeptide (Jones, G.L., Edmunson, H.M., Wesche, D., Saul, A. 1990. *Biochim. Biophys. Acta* **1096**:33–40). The ovalocytes showed a threefold increase in shear elastic modulus as determined by micropipette aspiration measurements of membrane rigidity. Time-resolved phosphorescence anisotropy has been used to study the rotational freedom of band 3 in membranes prepared from ovalocytes. The ovalocytic polymorphism was found to be associated with a marked decrease in the rotational mobility of band 3. This may indicate participation of band 3 in large homoaggregates or in complexes with other proteins at the cytoplasmic surface. There was no morphological clustering of band 3 detectable by immunofluorescence microscopy.

Key Words band 3 · rotational diffusion · ovalocytosis · erythrocyte membrane · membrane rigidity

Introduction

Melanesian ovalocytosis occurs in more than 20% of the population in some parts of the malarious coastal regions of Papua New Guinea (Castelino et al., 1981). These ovalocytes have been shown to be resistant to in vitro invasion by the malaria parasites, *Plasmodium falciparum* and *Plasmodium knowlesi* (Kidson et al., 1981; Hadley et al., 1983). Since these two malaria species have different receptor requirements (Miller et al., 1977), the mechanism for blocking entry of the merozoite is assumed to operate via an alteration in the physical properties of the ovalocyte membranes rather than via a defective receptor (Hadley et al., 1983).

Saul et al. (1984) studied the membrane deformability of ovalocytes during aspiration into 0.6-μm polycarbonate sieves. The ability of ovalocytes to undergo localized deformation was greatly reduced compared with normocytes. Furthermore, they found that ovalocytes are refractory to crenating drugs and unperturbed by contact with glass or alkaline pH.

Jones (1984) offered the first suggestion for the molecular basis for these altered membrane properties. Band 3 in ovalocytes was found to incorporate two to threefold more $^{32}P_i$ during metabolic phosphorylation of intact cells or during autophosphorylation of isolated ghosts than was the case for control normocytes. Recently, Jones et al. (1990) have described a structural difference in the cytoplasmic domain of band 3, in the form of an N-terminal extension of up to 30 mainly acidic amino acid residues.

The N-terminal region of normal human erythrocyte band 3 is extremely well conserved and very acidic (Lux et al., 1989). The first 11 residues comprise a domain which binds haemoglobin, hemichromes and glyceraldehyde 3-phosphate dehydrogenase (G3PD) (Walder et al., 1984; Low, 1986). More distal sites have been proposed for aldolase and phosphofructokinase (Higashi, Richards & Uyeda, 1979; Tsai, Prasanna-Murthy & Steck, 1982). The ankyrin binding site is thought to involve a complex folded domain which includes part of the acidic N-terminus (Willardson et al., 1989). Other parts of the cytoplasmic domain are responsible for binding to 4.1, 4.2 and for dimerization (Marchesi, 1985; Pasternak et al., 1985).

In normal human erythrocyte membranes, band 3 molecules show a somewhat restricted rotational diffusion which is taken as evidence for their participation in large homoaggregates or in complexes with other integral membrane proteins, such as glycophorin and/or peripheral membrane proteins that bind to the cytoplasmic domain of band 3, namely ankyrin, proteins 4.1 and 4.2 and the glycolytic enzymes, aldolase and G3PD (Clague, Harrison & Cherry,

1989). Alterations in the N-terminal sequence which affect the self-association state of band 3 or its binding to other proteins might be expected to alter its rotational freedom.

In this study, we have compared the rotational diffusion of band 3 in membranes prepared from normocytes and Melanesian ovalocytes. We find that the membrane organization in ovalocytes is altered such that band 3 mobility is markedly restricted. This immobilization of band 3 does not alter the light microscope appearance of fixed cells labeled with a fluorescent anti-band 3 monoclonal antibody, but it is associated with a dramatic increase in the rigidity of the membranes.

Materials and Methods

Materials

Glucose oxidase (type II) was obtained from Sigma. Eosin-5-maleimide was purchased from Molecular Probes. Anti-mouse Ig-FITC was from Boehringer-Mannheim.

Ovalocytes

Ovalocytes from donors in Pari, near Port Moresby in Papua New Guinea, were collected into acid/citrate/dextrose and stored at 4°C during transport to Australia or the UK. Experiments were performed within a week to 10 days from the collection date. In one case a fresh ovalocytic sample was available in the laboratory of G.B.N.

Membrane Rigidity

Blood was diluted 1 : 4 in culture medium (RPMI 1640) and incubated at 37°C for 2 hr to reverse echinocytosis found in stored samples. The incubated blood was diluted 1 : 10 in HEPES (20 mM) buffered saline (pH 7.4) plus 2% AB serum, and a portion was placed in an open-sided microscope chamber. Under microscope/video observation a micropipette attached to a hydrostatic pressure system was used to manipulate the red cells at room temperature. The membrane shear elastic modulus (μ, resistance to shear deformation at constant area) was determined by aspirating a membrane "tongue" from a flattened region of the red cell into a pipette with an internal diameter of 1 μm. The length (L) of the tongue was measured at 2 to 5 increasing pressures (P), and the elastic modulus calculated from dL/dP (Evans & La Celle, 1975). The same pipette was used for all blood samples. An approximate measure of membrane-bending rigidity was obtained by increasing P until the membrane buckled. This characteristic pressure is proportional to the bending modulus (Evans, 1983).

Immunofluorescence Microscopy

Thin smears of washed ovalocytes and normocytes were air-dried overnight, then defatted in 100% acetone at 4°C for 10 min. The smears were overlaid with a monoclonal antibody to the cytoplasmic domain of band 3 (Tilley et al., 1990), then washed and stained with fluorescein-conjugated anti-mouse IgG.

Labeling of Band 3

Fresh human blood was obtained from the Blood Transfusion Service, Melbourne, Australia. Band 3 was labeled in intact normocytes or ovalocytes by incubation with eosin-5-maleimide as described by Nigg and Cherry (1979), with the following modifications. Eosin-5-maleimide (0.4 ml $\times$ 0.5 mg/ml) was added to each 1 ml of packed erythrocytes, previously washed in 130 mM NaCl, 18 mM sodium phosphate, pH 8.0, and incubated for 1 hr at room temperature in the dark. Unreacted probe was removed by washing the cells three times with 160 mM NaCl, 5 mM sodium phosphate, pH 7.5. Ghosts were prepared by hypotonic lysis and washing in 5 mM sodium phosphate, pH 8.0.

The specificity of labeling of band 3 in membranes prepared from normal and ovalocytic erythrocytes was checked by viewing the labeled bands under UV light after SDS-PAGE. A labeling ratio of about 0.9 eosins per band 3 was determined spectrophotometrically for SDS-solubilized ghosts as described by Nigg and Cherry (1979).

Preparation of Samples for Spectroscopy

Eosin-labeled erythrocyte membranes were gently suspended at a concentration of about 0.8 mg/ml protein, which corresponded to an eosin concentration of approximately 2 μM. Deoxygenation was achieved enzymatically using 12 μg/ml glucose oxidase and 50 mM glucose, and this state was maintained by passing a gentle stream of argon over the sample during the measurement.

Instrumentation and Data Analysis

Time-resolved phosphorescence spectroscopy was carried out with an instrument described by Jovin et al. (1981) and Tilley et al. (1988). 512 decays of vertically and horizontally polarized components of the emission were collected. After subtraction of background signals, the decays were transferred to a PDP 11/23 computer, and the total intensity ($I(t)$) and the anisotropy ($r(t)$) curves generated according to the expressions

$$I(t) = I_{VV}(t) + 2I_{VH}(t) \quad (1)$$

$$r(t) = (I_{VV}(t) - I_{VH}(t)/(I_{VV}(t) + 2I_{VH}(t)) \quad (2)$$

where I_{VV} and I_{VH} are, respectively, the intensities of the phosphorescence emission polarized in the vertical and horizontal directions.

The generated curves were fitted to functions

$$I(t) = \Sigma_i \alpha_i \exp(-t/\tau_i) + B \quad (3)$$

$$r(t) = \Sigma_j \beta_j \exp(-t/\phi_j) + r_\infty \quad (4)$$

where α_i represents the initial intensity of the decay component i having a lifetime τ_i, and β_j the partial anisotropy of component j associated with the rotational correlation time ϕ_j. For a hindered rotor, r_∞ is the limiting anisotropy, determined in part by the distribution function for the population of chromophores at times long relative to the correlation time. Both r_∞ and β_j are determined by the angles that the absorption and emission transition moments

Table 1. Membrane shear elastic modulus for melanesian ovalocytes and controls

	Shear elastic modulus (10^{-3} dyn/cm)
Ovalocytes	
Donor A	20.8 ± 9.7 (10)
B	22.2 ± 6.5 (18)[a]
C	24.1 ± 8.0 (10)
Normocytes	
Donor D	7.1 ± 1.7 (10)
E	7.7 ± 1.7 (8)
Laboratory control	
Donor F	7.2 ± 1.6 (18)[a]

Data are mean ± SD for n cells.
[a] Pooled data from measurements on two occasions.

make with the axis of rotation (for review, *see* Jovin et al., 1981). B is a fitting parameter that accounts for any offset in the total intensity decay and was close to zero in the experiments described below. These data were fitted with one to four exponentials using a Chesbyshev transformation procedure, contained within a data acquisition operating system (DAOS) supplied by Labsoft Associates, Melbourne, Australia. Goodness-of-fit was determined from values of the reduced chi-squared or from plots of the weighted residuals (Restall et al., 1984).

Tsuji et al. (1988) have suggested that the different correlation times for the rotation of band 3 correspond to populations of different aggregate size. Using a value of $r_\infty/r_0 = 0.18$ for band 3 in membranes stripped of all peripheral proteins, the relative amplitudes for the anisotropy decays can be expressed as fractions of the maximum possible decay amplitude

$$f_j = (\beta_j/r_0)/0.82 \quad (5)$$

$$f_{im} = (r_\infty/r_0 - 0.18)/0.82 \quad (6)$$

where r_0 is the initial anisotropy and f_j and f_{im} are, respectively, the mobile and immobile populations of band 3.

Results and Discussion

Deformability

The membrane rigidity of ovalocytes was measured by micropipette aspiration. The shear elastic modulus was raised threefold compared to normocytes from control whose blood was drawn at the same time (Table 1). These normocytes had equal rigidity to a freshly drawn laboratory control (G.B.N.) tested in parallel with the Melanesian samples. The pressure required to cause ovalocytes to visibly buckle was about twice that for controls, suggesting about a 100% increase in bending resistance (*data not shown*). These results are qualitatively similar to those obtained by Saul et al. (1984), using aspiration of membrane portions into microporous filters.

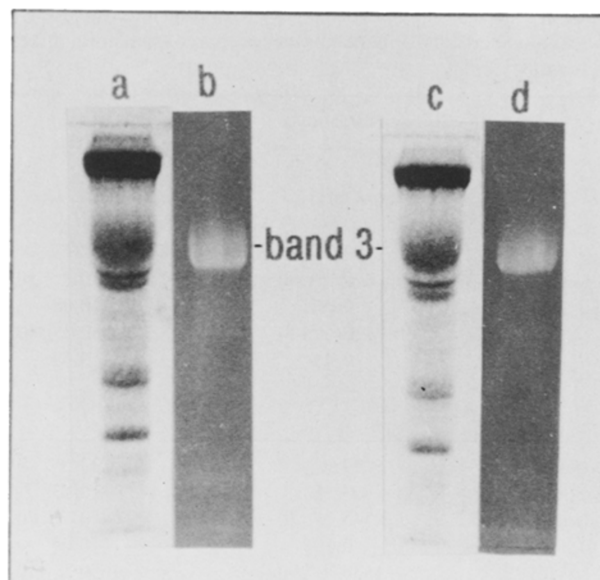

Fig. 1. SDS-polyacrylamide gels (7.5% acrylamide) of membranes prepared from normocytes (*a* and *b*) and ovalocytes (*c* and *d*). Eosin fluorescence is detected under UV light. The major labeled species is band 3, although a further faint fluorescent band can be detected at the dye front. Coomassie blue staining of the gels (*a* and *c*) reveals no obvious differences between membranes prepared from normocytes and ovalocytes, except that band 3 has a slightly higher apparent molecular weight in ovalocytes

The relative magnitudes of the changes in shear and bending resistance are in fact reversed between the two studies, but this may be due to the different techniques and theoretical analyses. The data are also consistent with the findings of Chasis and Mohandas (1986) who studied ovalocytes from individuals with Malaysian ovalocytes using an ektacytometer. They found that ovalocytes required much greater shear stress to cause equivalent elongation in flow. This type of deformation cannot be simply related to membrane rigidity, depending also on cell viscosity and geometry. The molecular defect in Malaysian ovalocytosis has not been studied, but epidemiological studies suggest that Melanesian ovalocytosis was derived from the Malaysian polymorphism in the course of population migration (Holt, Hogan & Nurse, 1981).

Rotational Mobility

Band 3 was labeled in intact cells with eosin-5-maleimide. The specificity of labeling of band 3 in normocytes and ovalocytes was assessed by detection of the fluorescence of the labeled proteins on polyacrylamide gels (Nigg & Cherry, 1979). Gels are shown in Fig. 1. The major labeled component in both cases

Table 2. Fitted parameters using Eq. (3) for total intensity decays of eosin-labeled band 3 in membranes prepared from normocytes and ovalocytes[a]

	Normocytes	Ovalocytes
	(20°C)	
τ_1 (μsec)	37 ± 2	34 ± 6
α_1/I_o	0.21	0.23
τ_2 (μsec)	420 ± 60	470 ± 10
α_2/I_o	0.06	0.08
τ_3 (μsec)	2220 ± 140	1960 ± 360
α_3/I_o	0.73	0.69
	(37°C)	
τ_1 (μsec)	29 ± 2	33 ± 3
α_1/I_o	0.25	0.23
τ_2 (μsec)	345 ± 30	410 ± 70
α_2/I_o	0.10	0.09
τ_3 (μsec)	1530 ± 290	1650 ± 110
α_3/I_o	0.65	0.68

[a] α_i represents the initial intensity of the decay component i having a lifetime τ_i. I_o is the total amplitude of the phosphorescence decay.

Table 3. Analysis of the anisotropy decays of band 3 at 20°C in membranes prepared from normocytes and ovalocytes[a]

	Normocytes	Ovalocytes
ϕ_1 (μsec)	11 ± 3	14 ± 4
f_1	0.19 ± 0.05	0.24 ± 0.06
ϕ_2 (μsec)	170 ± 35	—
f_2	0.12 ± 0.01	—
ϕ_3 (μsec)	3170 ± 120	—
f_3 (μsec)	0.13 ± 0.01	—
$f_{(immobile)}$	0.56 ± 0.05	0.76 ± 0.06

[a] Decays shown in Fig. 2 were fitted to Eq. (4), and the amplitudes were analyzed using Eqs. (5) and (6). Data represent the average ± SD for at least three measurements.

corresponds to band 3 in the Coomassie blue stained gels. There is a small amount of label which runs at the dye front and presumably represents free or hydrolysed eosin reagent. The only apparent difference between the normocytes and ovalocytes is a small increase in the apparent molecular weight of band 3 in the ovalocyte membranes (as indicated by a slightly increased separation of bands 3 and 4.1). There is no evidence for increased binding of peripheral membrane proteins such as band 6 (G3PD) or hemoglobin.

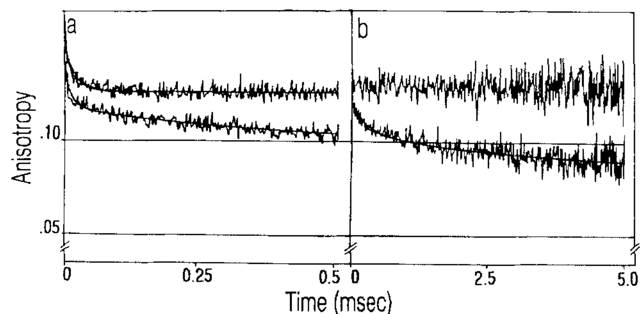

Fig. 2. Decay of phosphorescence anisotropy of eosin-5-maleimide labeled band 3 in ghosts prepared from normocytes (bottom curves) and ovalocytes (top curves) at 20°C. Data were collected in two different regimes of time resolution: *a*, 0–0.5 msec; *b*, 0–5.0 msec. Data are fitted to Eq. (4). The best fit parameters are summarized in Table 3.

The decay of phosphorescence intensity of eosin-labeled band 3 in erythrocyte membranes was measured over a 5-msec time period. The intensity of the phosphorescence emission shows a complicated decay with time, which requires a three exponential fit (*see* Table 2). There is little difference in intensity decays for band 3 in membranes prepared from normocytes and ovalocytes.

The time-resolved phosphorescence anisotropy decay of eosin-band 3 in membranes prepared from ovalocytes and normocytes is shown in Fig. 2. The decay of phosphorescence anisotropy was examined using two different time regimes so as to resolve both rapid and slow motions of the band 3 dimer (*see* Tilley et al., 1990 for discussion). Band 3 rotation in normocytes can be analyzed in terms of three mobile populations and an immobile component (Table 3). The immobile fraction accounts for about 55% of the total band 3 population in membranes from normal erythrocytes, at 20°C.

The rotational freedom of band 3 was found to be markedly restricted in the ovalocyte membranes (Fig. 2). The fitted data in Table 3 indicate that band 3 in ovalocyte membranes is 76% immobilized at 20°C compared with 56% for controls. Increasing the temperature to 37°C decreased the population of immobile band 3 to 33% in normocytes but had little effect on the mobility of band 3 in the ovalocyte membranes (Fig. 3, Table 4).

Cherry (1978) has calculated the expected rotational correlation times for a band 3 dimer, modeled as a cylinder in a planar bilayer. In this case, two correlation times, ϕ and $\phi/4$ result, where

$$\phi = \frac{\eta V}{kT} \tag{7}$$

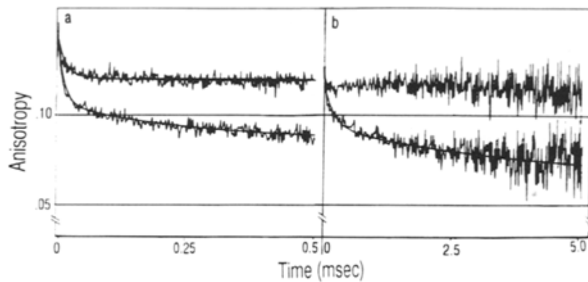

Fig. 3. Decay of phosphorescence anisotropy of eosin-5-maleimide labeled band 3 in ghosts prepared from normocytes (bottom curves) and ovalocytes (top curves) at 37°C. Data were collected in two different regimes of time resolution: *a*, 0–0.5 msec; *b*, 0–5.0 msec. Data are fitted to Eq. (4). The best fit parameters are summarized in Table 4.

Table 4. Analysis of the anisotropy decays of band 3 at 37°C in membranes prepared from normocytes and ovalocytes[a]

	Normocytes	Ovalocytes
ϕ_1 (μsec)	13 ± 1	24 ± 1
f_1	0.28 ± 0.04	0.23 ± 0.01
ϕ_2 (μsec)	230 ± 50	—
f_2	0.19 ± 0.02	—
ϕ_3 (μsec)	3540 ± 980	—
f_3 (μsec)	0.20 ± 0.05	—
$f_{(immobile)}$	0.33 ± 0.07	0.77 ± 0.01

[a] Data represent the average ± SD for at least three measurements.

V is the volume of the membrane-embedded portion of band 3, experiencing a viscosity, η, of about 6 Poise at 20°C (Cooper, 1978). k is Boltzmann's constant and T is the temperature (in Kelvin). For a freely rotating dimer of band 3, two rotational correlation times are thus expected with values of about 20 and 80 μsec. In normocytes, three correlation times are required to fit the data. These differ from each other by a factor much greater than four, which suggest that they represent different populations of band 3 (*see* Clague et al., 1989, for discussion). The two populations with longer correlations are assumed to represent slowly rotating band 3 complexes. The shorter correlation time could represent $\phi/4$ for a population of free dimers or alternatively, some kind of internal flexibility within band 3. In normal membranes the population of the less mobile species of band 3 increases with decreasing temperature, due to temperature-dependent protein-protein interactions (*see* Nigg & Cherry, 1979, for discussion).

The only rotational process for band 3 in the ovalocyte membranes had a correlation time of about 17 μsec. This value is similar to $\phi/4$ for a freely rotating band 3 dimer; however, there is no corresponding correlation time four times greater. This suggests that it is more likely to represent segmental motion within band 3 or a more global conformational flexibility of band 3. The absence of an anisotropy decay at longer times and the greatly increased infinite time anisotropy indicate a substantial restriction of the mobility of band 3 in these ovalocyte membranes.

The ovalocytes used for the above experiments were obtained from a donor whose erythrocytes were about 70% ovalocytic as determined from light microscopic examination of glutaraldehyde-fixed specimens. Another donor with close to 100% ovalocytic cells was also studied. There was no significant difference in the degree of immobilization of band 3 for the two donors (*data not shown*). Jones et al. (1990) have reported that, in a study of 12 ovalocyte samples from the Pari region in Papua New Guinea, 11 were heterozygous by the criterion that they yielded both normal and aberrant proteolytic fragments of band 3. We have not determined whether the donors for these experiments were homozygous or heterozygous for the polymorphism.

Structural Implications and Physiological Consequences

Two possible mechanisms might explain the marked decrease in mobility of band 3 in ovalocytes: increased interaction of the band 3 cytoplasmic domain with one of the peripheral membrane proteins or clustering of band 3 into homoaggregates. A possible candidate for interaction with band 3 is the tetrameric enzyme, glyceraldehyde 3-phosphate dehydrogenase (G3PD). The addition of G3PD to ghosts has previously been shown to greatly reduce band 3 mobility (Matayoshi, 1983). Similarly, hemoglobin, and particularly denatured hemoglobin, bind strongly to erythrocyte membranes and can restrict band 3 mobility (Low et al., 1985; R.A. McPherson, W.H. Sawyer and L. Tilley, *unpublished results*). However, the ghosts prepared from ovalocytes do not retain more G3PD or hemoglobin than normocyte ghosts (Fig. 1), which argues against a stronger interaction of these components with band 3 in the ovalocyte membranes. Clague et al. (1989) have discussed several other possible aggregating agents, in particular, ankyrin and bands 4.1 and 4.2. The rotational mobility of band 3, in normal erythrocyte membranes has been shown to be largely indepen-

dent of its linkage, via ankyrin, through to the cytoskeleton (Clague et al., 1989). However, an altered interaction with these components in ovalocytes could conceivably restrict band 3 mobility. Similarly, the increased acidity of the N-terminal region may alter the steric or electrostatic interactions of band 3 molecules with neighboring membrane proteins. However, in fact, an increased electrostatic repulsion between band 3 molecules might be expected to decrease the population of larger aggregates.

Changes in band 3 mobility or structure *per se* would not be expected to alter membrane rigidity. However, interactions of band 3 with other membrane proteins might provide a basis for alterations in this property. Membrane rigidity is mainly conferred by the cytoskeletal proteins, particularly spectrin (Waugh & Agre, 1988), and a significant perturbation in their organization is expected to be necessary to cause the marked rigidification found in the ovalocytes.

Aggregation of band 3 has been implicated in the generation of the "senescence" antigen on erythrocytes. Auto-antibodies are envisaged as recognizing the external domain of aggregated band 3, thereby providing a signal for the phagocytosis of senescent erythrocytes by macrophages (Low et al., 1985). Schlüter and Drenckhahn (1986) examined the binding of fluorescently labeled anti-band 3 antibodies to erythrocytes that had been "aged" in vitro by treatment with phenylhydrazine. Band 3 aggregates were visible in the light microscope (Schlüter & Drenckhahn, 1986). We have also found that the band 3 mobility in membranes prepared from phenylhydrazine-treated erythrocytes is greatly restricted (R.A. McPherson, W.H. Sawyer and L. Tilley, *unpublished results*). However, when ovalocytes were permeabilized and labeled with fluorescent antibodies against band 3, a uniform staining of band 3 around the cells was observed; the complexes in which band 3 participates are therefore not large enough to be detected at the microscopic level. It is likely that the technique of time-resolved phosphorescence anisotropy provides a much more sensitive measure of band 3 aggregation than antibody binding.

None-the-less, it is possible that even a limited degree of aggregation of band 3 might be associated with some increase in auto-antibody binding and a consequent decrease in the lifespan of ovalocytes in the circulation. Similarly, the abnormal rigidity of ovalocyte membranes might be expected to promote removal of ovalocytes from the circulation during passage through the spleen. Carriers of this abnormal band 3 gene might therefore be expected to exhibit symptoms of anemia and to have enlarged spleens. Documented studies on the pathology of ovalcytosis are very limited; however, Booth et al. (1977) and Serjeantson et al. (1977) found no hematological abnormalities in individuals with ovalocytosis. Similarly, the donors of the cells used in this study presented no hematological symptoms, nor do they suffer from ischemic complications which might arise from impared red cell deformability.

One possible explanation for the apparent lack of pathology associated with Melanesian ovalocytosis is that the mode of aggregation of band 3 in ovalocyte membranes may be incompatible with increased auto-antibody binding. Alternatively, Kay et al. (1989) have suggested that red cell aging may involve proteolysis of band 3 as well as aggregation.

The relation between altered membrane rigidity and red cell function is unclear. The changes in rigidity do not markedly affect the red cell lifespan or blood supply. Saul et al. (1984) reported that red cell filterability through 4.5-μm pores was actually improved for ovalocytes compared to controls. On the other hand, Chasis and Mohandas (1986) found grossly impaired deformability of ovalocytes in shear flow. The filtration results may be explained by the relatively small mean cell volume of the ovalocytes tested (Saul et al., 1984). However, in recently studying a single case of Melanesian ovalocytosis with freshly available blood, we found that filterability through 5-μm pores was markedly impaired. Using a commercially available filtrometer and standard techniques (Dormandy et al., 1985), the red cell transit time and rate of pore blockage was approximately 50% above standard laboratory values. Overall, it would seem that membrane rigidification *per se* has little effect on in vivo circulation, so that rheological complications may only arise when even greater changes in rigidity occur (e.g., for sickled cells containing polymerized hemoglobin S (Nash, Johnson & Meiselman, 1986) or when geometric changes such as spherocytosis occur (Becker & Lux, 1985)).

The resistence of ovalocytes to malarial invasion might derive from the decreased mobility of band 3 in these cells. Dluzewski et al. (1983) proposed that an obligatory step in the invasion process is the formation of a protein-free patch on the erythrocyte membrane at the site of invagination. Both integral membrane proteins and cytoskeletal proteins are moved away from the site of initial contact along with the edges of the junction between the parasite and the erythrocyte. It is conceivable that participation of band 3 in large complexes may impede its movement and thereby interfere with the processes involved in junction formation. It is also possible that membrane rigidification could contribute, by acting as a physical barrier to parasite entry.

The polymorphism of the band 3 associated with ovalocytosis can be said to be particularly successful in that it confers some resistance to malaria without apparently adversely affecting red cell function and lifespan. Other band 3 extensions have been reported. Mueller and Morrison (1977) reported an asymptomatic variant of band 3 which occurs with a frequency of about 6% in the southern USA population. Ranney et al. (1990) reviewed several different band 3 extensions, some of which are associated with an alteration in erythrocyte shape; it would be interesting to examine band 3 mobility in these variant cells.

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