

Fluidity Properties and Lipid Composition of Erythrocyte Membranes in Chediak-Higashi Syndrome

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ABSTRACT We have earlier shown through electron spin resonance (ESR) studies of leukocytes that membranes of cells from both Chediak-Higashi syndrome (CHS) mice and humans have abnormally high fluidity. We have extended our studies to erythrocytes. Erythrocytes were labeled with the nitroxide-substituted analogue of stearic acid, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxy, and ESR spectra were obtained. Order parameter, S , at 23°C, was 0.661 and 0.653 for erythrocytes of normal and CHS mice ($P < 0.001$). S was 0.684 for normal human erythrocytes and 0.675 ($P < 0.001$) for CHS erythrocytes at 25°C. Because S varies inversely to fluidity, these results indicate that CHS erythrocytes tend to have higher fluidity than normal. In vitro treatment of both mice and human CHS erythrocytes with 10 mM ascorbate returned their membrane fluidity to normal. We prepared erythrocyte ghosts and extracted them with $\text{CHCl}_3:\text{CH}_3\text{OH}$ (2:1). Gas-liquid chromatography analysis showed a greater number of unsaturated fatty acids for CHS. The average number of double bonds detected in fatty acids for mice on a standard diet was 1.77 for normal and 2.02 for CHS ($P < 0.04$); comparison of human erythrocytes from one normal control and one CHS patient showed a similar trend. Our results suggest that an increased proportion of unsaturated fatty acids may contribute to increased fluidity of CHS erythrocytes. Our observation that both leukocytes and erythrocytes of CHS have abnormal fluidity indicates that CHS pathophysiology may relate to a general membrane disorder.

The Chediak-Higashi syndrome (CHS) is an autosomal recessive genetic disease that has been reported in man and mice as well as a variety of other mammalian species (3, 32). Characteristic of this disorder is the presence of enlarged granules in many cells, particularly the melanocytes and the polymorphonuclear leukocytes (PMN). Some phenotypic characteristics of the syndrome are reflections of these abnormal granules: a partial oculocutaneous albinism resulting from aggregation of melanin granules and an increased susceptibility to bacterial infection partly resulting from defective degranulation in PMN after phagocytosis.

The giant granules of the PMN appear to arise from the abnormal fusion of enlarged primary granules with normal secondary granules (34, 36), suggesting that membrane alterations may be a fundamental defect of CHS. This hypothesis is supported by observations that CHS PMN are impaired not only in degranulation but also in plasma membrane-mediated events of central importance in phagocytic function: chemo-

taxis (13) and adherence (5). Further, unlike normal PMN membrane, concanavalin A (Con A) receptors of the CHS PMN membrane undergo spontaneous capping when the cells are incubated with the lectin (30), suggesting a greater than normal mobility of membrane glycoproteins. Recently, we examined the membrane fluidity characteristics of PMN from both CHS and normal mice and humans through use of electron spin resonance (ESR) techniques. We showed that spin-label analogues of stearic acid appeared to have greater freedom of molecular movement in membranes of CHS cells than in membranes of normal PMN (21).

We wished to evaluate the possibility that abnormal lipid composition might underlie the increased fluidity of CHS PMN membrane. Techniques are available for preparation of PMN membrane fractions enriched for plasma membrane markers (41), but a fraction truly representative of plasma membrane is not readily obtainable (44). Because the membranes of erythrocytes are readily prepared, we turned our attention to these

blood cells. Abnormal erythrocyte fluidity has been reported for other pathological conditions, including spur cell anemia (14) and both myotonic muscular dystrophy and congenital myotonia (6, 8). In the former instance, the fluidity change is secondary to increased levels of cholesterol in the serum (14). Unusual lipid serum levels have been reported for CHS (25) as well as other lipid-related metabolic abnormalities (24, 33). With these considerations in mind, we elected to study erythrocytes from normal subjects as well as from those suffering from CHS. We have determined the fluidity characteristics of intact erythrocytes and the lipid composition of erythrocyte ghosts.

MATERIALS AND METHODS

Animals

Normal (C576J +/-) mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). CHS mice (C576J bg/bg) were originally obtained from the same supplier and were maintained in colony at Indiana University School of Medicine Animal Facility. Adult male animals were used for all experiments. They were fed Wayne Lab Blox (Allied Mills, Inc., Castleton, Ind.). Weights were 25–27 g for C57 animals and 22–27 g for CHS mice.

Human Subjects

One white female patient, age 4 yr, was studied. The diagnosis of CHS had been established by clinical history and examination and by the presence of giant lysosomes in PMN (5). 8 wk before the present studies, ascorbic acid treatment had been discontinued as a check on the continued efficacy of this therapeutic approach (5). The patient's chief complaint was photophobia secondary to optic atrophy. Her leukocyte count was 300–500 cells/mm³ and she was free from infection. No indication of hepatosplenomegaly was observed. Cells from three normal white adult females were used as controls.

Erythrocyte and Ghost Preparation

For ESR studies, heparinized venous blood was obtained from human subjects; heparinized blood was obtained by cardiac puncture from mice. Blood samples from 10–15 animals were pooled before subsequent steps in erythrocyte preparation were taken. After centrifugation (2,500 g, 10 min, 4°C), the serum and buffy coat were aspirated. The pellet was washed three times in phosphate-buffered saline (PBS), pH 7.4. The supernate as well as the upper 3 mm of the erythrocyte pellet were aspirated with each wash to reduce the number of contaminating leukocytes. The final erythrocyte pellet was suspended at 50% hematocrit in PBS and held on ice. In some experiments, the RBC were incubated with 0.01 M ascorbate for 15 min at 25°C, washed in 0.9% NaCl, and suspended in PBS.

For ghost preparation, heparinized human venous blood was used. Heparinized blood was obtained from mice by cardiac puncture. Blood samples from 40–50 animals were pooled before subsequent steps in erythrocyte ghost preparation were carried out. Erythrocytes were washed three times in 20 mM NaPO₄, pH 7.4, containing 125 mM NaCl as described above. Then ice-cold 20 mM phosphate buffer, pH 7.4, was added to the washed erythrocyte pellet and centrifugation was carried out at 17,000 g, 20 min, 4°C. The supernate was aspirated and the tube rotated at an angle to allow the erythrocyte ghost pellet to slide away from the underlying smaller pellet of leukocytes which was also aspirated. Washes in 20 mM phosphate were repeated until the ghost pellet was white. Then three washes in 20 mM NaPO₄, pH 7.4, containing 125 mM NaCl were performed. Samples of erythrocyte ghosts were held on ice for later protein analysis by the method of Lowry (27). The remaining material was immediately extracted for lipid analysis.

Lipid Analysis

Lipids were extracted from erythrocyte ghosts (1.0–1.5 mg of protein) (18) with CHCl₃:CH₃OH (2:1 vol/vol) containing 0.0005% butylated hydroxytoluene. The Folch-washed (18) CHCl₃ extract was analyzed for total cholesterol, total lipid phosphorus, fatty acid composition, and phospholipid head group composition. Analysis of cholesterol content was performed by the enzymatic method based on cholesterol oxidase and cholesterol ester hydrolase (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) (35). Total lipid phosphorus was determined as described (11).

Analysis of fatty acid composition was performed by gas-liquid chromatog-

raphy. A sample of lipid extract was subjected to alkaline hydrolysis with 1.2 N KOH in 80% ethanol for 60 min at 57°C (1). Fatty acids in the saponifiable fraction were methylated (29) and the methyl esters separated, using a Hewlett-Packard 5710A gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with a 1.8 m 10% SP-2340 on 100–200 mesh chromosorb column. Peaks were identified by comparison of retention times to those of standard fatty acids (Supelco, Inc., Bellefonte, Pa.). Classes of phospholipids were separated by thin-layer chromatography (TLC) using plates prewashed in ethyl acetate and developed in CHCl₃:CH₃OH:CH₃COOH:H₂O (100:50:142:6) (39). Spots corresponding to location of known standards were scraped and analyzed for phosphorus content. The Student's *t* test was used to determine the statistical significance of differences detected in the lipid content of normal and CHS erythrocytes.

ESR

The doxyl-substituted analogue of stearic acid, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyl-oxyl (5DS), was obtained from Syva (Palo Alto, Calif.). A stock solution at 5 mM was made in ethanol and stored at 4°C. 3 μl of 5DS stock solution was added to the bottom of a glass tube (13 × 100 mm). The solvent was evaporated with dry nitrogen gas. A 100-μl sample of erythrocyte suspension was added and vortexed briefly. After 5 min at 25°C the labeled erythrocytes were transferred to a 100-μl glass capillary. One end of the capillary was heat-sealed and the erythrocytes were centrifuged into this end (1,000 g, 2 min, 25°C). For experiments in which erythrocytes were treated with ascorbate (10 mM final concentration), the erythrocyte suspension was diluted 10-fold in 0.9% sodium chloride and centrifuged before spin-labeling of the erythrocyte pellet. ESR signal intensity of these samples indicated no significant reduction of spin label by the ascorbate.

ESR spectra were obtained on a standard balanced-bridge spectrometer with diode detection operating at 9.1 GHz at 23° or 25°C. In our hands, the change in order parameter as a function of temperature is ~ 0.007/°C. First derivative absorption spectra were recorded with a 100-G field sweep, a scan time of 10 min, a peak-to-peak modulation amplitude of 1.5 G and a time constant of 0.5 s. Other instrument settings and data analyses were done as previously described (21). In brief, the order parameter (*S*), a measurement of spin-label order and motion, was calculated (20) from the extrema of the ESR spectrum of 5DS-labeled erythrocytes (Fig. 1).

$$S = \frac{T_{II} - T_{I'} + C}{T_{II} + 2T_{I'} + 2C} \times 1.66,$$

where $2T_{II}$ and $2T_{I'}$ are the distances between outer and inner extrema, respectively, and $C = 1.4 - 0.053(T_{II} - T_{I'})$. As flexibility of the hydrocarbon chain of the spin label decreases, the order parameter increases. This implies that as the environment surrounding the nitroxide group of the label becomes more ordered (i.e., less fluid), the order parameter increases. The theoretical limits of *S* are *S* = 0 for a completely fluid, isotropic system and *S* = 1 for a completely rigid or ordered environment. Typical values for spin-labeled membranes are from 0.5 to 0.7. For experiments in which identical samples were run on the same day, the

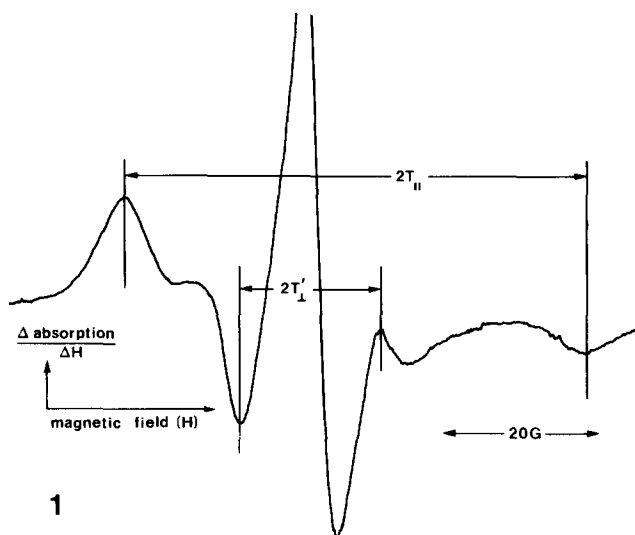


FIGURE 1 First derivative ESR spectrum at 25°C of 5DS-labeled erythrocytes from a CHS patient. The hyperfine splitting parameters $2T_{II}$ and $2T_{I'}$ are the separation between outer and inner extrema, respectively, and are used in the calculation of order parameter.

mean and standard error (SE) were calculated. For experiments in which several samples were measured and data from several days were available, a two-way analysis of variance was carried out. All *P* values given herein are for the probability that a null hypothesis is true.

RESULTS

Membrane Fluidity of Erythrocytes

The order parameter (*S*), a measure of membrane fluidity, was obtained from the ESR spectra of erythrocytes labeled with 5DS. Erythrocytes obtained from normal and CHS mice were compared at 23°C (Fig. 2). *S* for the CHS cells (0.653) was lower than for normal ones (0.661). Analysis of variance showed that the difference was highly significant (*P* < 0.001).

A similar comparison was done at 25°C for human erythrocytes (Fig. 3). A difference was again detected between normal and CHS erythrocytes: *S* was 0.684 for normals but for the CHS patient it was 0.675 (*P* < 0.001). Thus, in both mice and humans, CHS erythrocytes have greater membrane fluidity in the bilayer region probed by 5DS.

Previous work has shown that the abnormally low *S* values for CHS leukocytes could be restored to normal by treatment with 10 mM ascorbate (21). Similar effects of ascorbate were found herein for erythrocytes. In vitro ascorbate treatment (10 mM) for 15 min before spin-labeling with 5DS gave *S* values for CHS erythrocytes that were not significantly different from those for normal cells in both humans (*P* > 0.99) and mice (*P* > 0.80) (Figs. 2 and 3).

Cholesterol, Phospholipid, and Protein Content of Normal and CHS Erythrocyte Ghosts

Table I gives molar ratios of cholesterol/phospholipid (chol/PL) for chloroform extracts of erythrocyte ghosts from mice

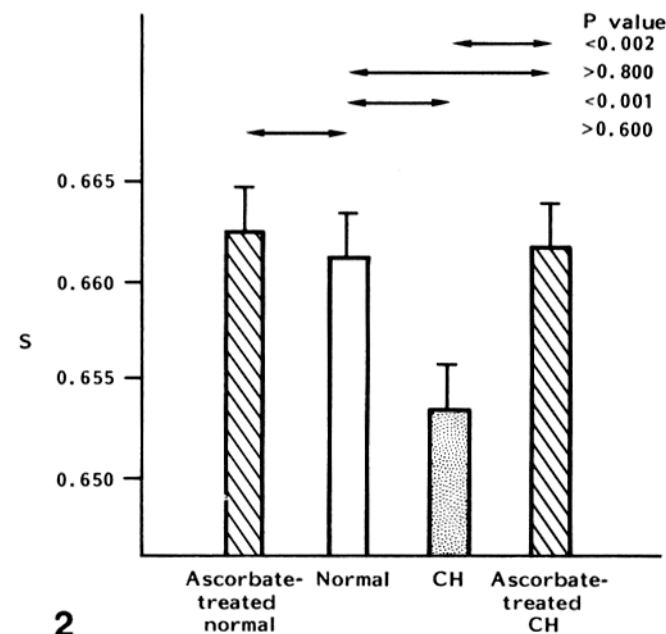


FIGURE 2 Order parameters at 23°C for 5DS-labeled erythrocytes from normal and CHS mice. Incubation and labeling conditions are described in Materials and Methods. Final concentration of ascorbate was 0.01 M. Bars indicate 2 SE above the mean. Values are from analysis of 12 or more samples from three separate experiments. *P* values are for the probability that a null hypothesis comparing the indicated values is true.

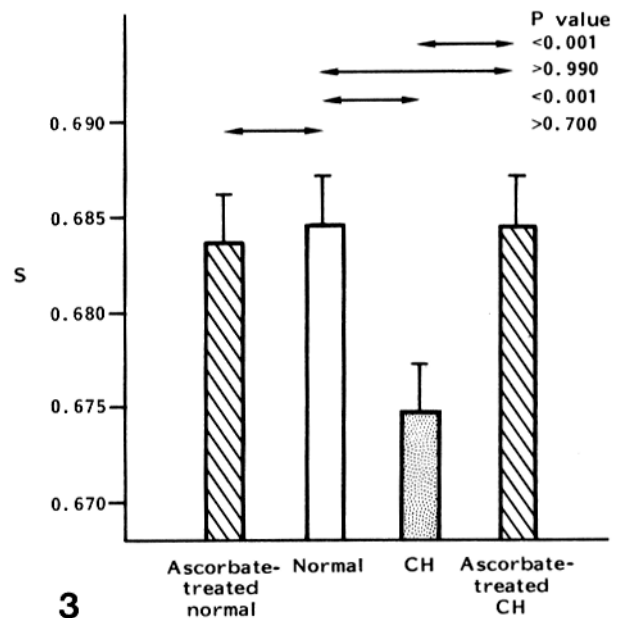


FIGURE 3 Order parameter at 25°C for 5DS-labeled erythrocytes from three normal humans and the CHS patient. Incubation and labeling conditions are described in Materials and Methods. Final concentration of ascorbate was 0.01 M. Bars indicate 2 SE above the mean. Values are from analysis of nine or more samples from three separate experiments. *P* values are for the probability that a null hypothesis comparing the indicated values is true.

and humans. The CHS cells of humans had a lower chol/PL than normals, whereas the reverse was true of erythrocytes from mice. The differences between CHS and control erythrocytes are statistically significant for mice (*P* < 0.03), but not for humans (*P* < 0.15).

Table I also gives ratios on a weight basis for phospholipid/protein (PL/prot). In both species, the abnormal cells had relatively higher amounts of phospholipid than did the normal erythrocytes. However, these differences are probably not significant (*P* < 0.15).

Phospholipid Composition of Erythrocyte Ghosts

The phospholipid composition of erythrocyte ghosts from mice and humans is given in Table II. Statistical analysis suggested that only the differences in phosphatidylcholine (PC) were significant (*P* < 0.03 for mice, *P* < 0.04 for humans). We also compared the ratios of sphingomyelin (SP)/PC and PC/phosphatidylethanolamine (PE). For humans the SP/PC ratio is increased in the CHS (from 0.55 to 0.62, *P* < 0.06), whereas in mice this ratio is decreased (from 0.21 to 0.15, *P* < 0.049). Mice showed increased PC/PE ratios in the CHS extracts; however, this increase was not statistically significant.

Fatty Acid Composition of Erythrocyte Ghosts

The percents of different fatty acids and of monoenoic and polyenoic vs. saturated fatty acids are given in Table III. Among the differences detected was a relatively higher proportion of polyunsaturated fatty acids in CHS erythrocytes of both humans and mice as compared with the respective control values for each species. In human cells, polyenoic fatty acids account for 35.5% of total fatty acids in normal erythrocytes but are increased to 48.3% in CHS erythrocytes (*P* < 0.01).

Among the individual fatty acids that are increased in CHS are 20:4 (from 7.1 to 16.2%, $P < 0.001$) and 18:2 (from 7.4 to 12.5%, $P < 0.001$). Likewise in the mouse, polyenoic fatty acids increase from 39.4 to 47.4% ($P < 0.025$) for CHS erythrocytes. Again, increases are seen in 20:4 (10.7 to 14.8%, $P < 0.02$) and 18:2 (8.3 to 12.6%, $P < 0.001$). Mice also have a significant increase in 22:6 (from 4.1 to 8.4, $P < 0.005$). The increases in 18:2 and 20:4 for both mouse and human CHS subjects may reflect the relation between the two fatty acids; 18:2 is a precursor for 20:4. As would be expected from the above described fatty acid differences, the average number of double bonds is increased in CHS erythrocytes for both humans ($P < 0.03$) and mice ($P < 0.04$). Note that in the instance of human subjects there was a large difference in the unidentified (*other*) categories for normal and CHS. However, it is unlikely that the differences in percent polyenoic fatty acids are attributable to this large difference in "other" fatty acids, because most "other" fatty acids in the normal human subject were eluted from the column before any polyunsaturated fatty acids.

DISCUSSION

Our results show that, regardless of species, the spin-label 5DS has greater freedom of molecular movement in CHS erythrocyte membranes than in normal cells. We have previously

TABLE I
Comparison of Cholesterol, Phospholipid, and Protein
Content of Normal and CHS Erythrocyte Ghosts

Erythrocyte	Cholesterol/phospholipid (molar ratio)	Phospholipid/protein ($\mu\text{g}/\text{mg}$)
HUMAN		
Normal*	0.54 ± 0.04 (0.50-0.61)‡	696 ± 52 (635-799)
CHS§	0.49 (0.46-0.52)	789 (755-824)
MOUSE		
Normal	0.33 ± 0.03 (0.28-0.37)	878 ± 56 (772-960)
CHS	0.42 ± 0.01 (0.40-0.44)	948 ± 38 (891-1,019)

* Average and SEM of triplicate determinations on erythrocyte ghosts of one normal subject.

‡ Range of values.

§ Average of duplicate determinations on cells of one CHS patient.

|| Average and SEM of triplicate determinations carried out on erythrocyte ghosts prepared from pooled blood samples collected from 40-50 mice.

demonstrated a similar difference between normal and CHS PMN (21). The order parameter, which varies inversely with fluidity, is lower in CHS cells. The lipid analyses show differences in erythrocyte ghost composition between normal and CHS cells; some of these differences correlate with the observed differences in fluidity.

Table IV shows changes in lipid composition that would be expected to promote greater freedom of molecular motion (i.e., greater fluidity) in membranes. These changes include decreased chain length of fatty acids, increased degree of unsaturation of fatty acids, decreased amount of cholesterol relative to phospholipid (chol/PL), increased amount of phospholipid relative to protein (PL/prot), decreased amount of SP relative to PC, and increased amount of PC relative to PE. Thus, for example, on the basis of our lipid analyses one could have predicted that normal mouse erythrocyte membranes would be more fluid than those from humans. Compared to human erythrocytes, mouse erythrocytes have lower average fatty acid chain length, higher degree of unsaturation of fatty acids, decreased chol/PL, increased PL/prot, decreased SP/PC, and increased PC/PE (Tables I-III). All these differences are consistent with order parameters that are lower for mouse erythrocytes than for human; our ESR results verify that such a difference between species exists. For mouse cells $S = 0.647$ (23°C data corrected to 25°C), whereas for human cells $S = 0.684$ at 25°C. Such variations in membrane fluidity of similar cells from different species are expected. Small but significant differences in phospholipid distribution, fatty acid composition, and protein content among species have been demonstrated (42), and this difference contributes to the interspecies membrane fluidity variations.

In light of the above considerations, comparison of lipid composition in normal and CHS cells within a single species might yield valuable information about the contribution that specific lipid membrane constituents may be making to the abnormal fluidity characteristics of CHS cells. Mouse CHS erythrocytes have membranes more fluid than controls. Among the statistically significant variations in lipid composition that may contribute to this difference are: increased degree of fatty acid unsaturation ($P < 0.04$) and decreased SP/PC ($P < 0.049$). These factors evidently dominate the two that are inconsistent with a more fluid environment: increased average chain length ($P < 0.05$) and increased chol/PL ($P < 0.03$). Human CHS erythrocyte membranes also are more fluid than those of normal cells. The statistically significant difference in lipid composition consistent with this result is an increased degree of fatty acid unsaturation ($P < 0.03$). Inconsistent with the

TABLE II
Phospholipid Percentage Composition of Erythrocyte Ghosts

Erythrocyte	U*	LPC	SP	PC	PS + PI	PE
HUMAN						
Normal‡	$1.3\text{§} \pm 0.3$	2.7 ± 0.4	19.5 ± 0.3	35.5 ± 0.7	15.6 ± 1.4	25.4 ± 1.8
CHS	2.8	3.7	20.7	33.3	15.8	23.7
MOUSE						
Normal¶	0.7 ± 0.1	2.2 ± 0.2	9.2 ± 0.1	43.5 ± 1.1	16.5 ± 0.5	27.9 ± 1.2
CHS¶	1.4 ± 0.6	1.7 ± 0.6	7.1 ± 1.8	47.4 ± 0.8	15.0 ± 1.7	27.4 ± 1.0

* U, unidentified; LPC, lysophosphatidylcholine; SP, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.

‡ Average and SEM of triplicate determinations on erythrocyte ghosts of one normal subject.

§ Percentage of total recovered phospholipids.

|| Average of duplicate determination on cells of one CHS patient.

¶ Average and SEM of triplicate determinations on erythrocyte ghosts prepared from pooled blood samples collected from 40-50 mice.

TABLE III
Fatty Acids of Erythrocyte Ghosts

Erythrocyte	Fatty acids											
	12:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:2	20:3	
HUMAN												
Normal*	2.2 ± 0.7‡	3.2 ± 0.9	2.2 ± 0.1	13.4 ± 0.2	2.2 ± 0.1	8.9 ± 0.2	16.0 ± 0.3	7.4 ± 0.1	5.5 ± 0.3	3.7 ± 0.6	3.8 ± 0.2	
CHS§	0.2	0.4	0.3	14.7	0.2	15.1	17.1	12.5	2.4	1.3	3.5	
MOUSE												
Normal¶	0.4 ± 0.2	1.4 ± 0.4	1.1 ± 0.2	21.3 ± 0.2	2.0 ± 0.1	10.5 ± 0.4	17.8 ± 1.2	8.3 ± 0.2	3.9 ± 0.1	1.2 ± 0.1	3.5 ± 0.1	
CHS¶	0.2 ± 0.1	0.1 ± 0.1	0.6 ± 0.3	23.8 ± 1.3	0.6 ± 0.1	11.1 ± 0.6	12.8 ± 0.6	12.6 ± 0.1	2.5 ± 0.9	—	3.0 ± 0.7	

* Average and SEM of triplicate determinations on erythrocyte ghosts of one normal subject.

‡ Percentage of total fatty acid.

§ Average of duplicate determinations on erythrocyte ghosts of one CHS patient.

¶ —None detected.

¶ Average and SEM of triplicate determinations on erythrocyte ghosts prepared from pooled blood samples collected from 40–50 mice.

greater fluidity is the increased SP/PC ($P < 0.06$).

It is not surprising that in the abnormal cells we find lipid differences that would tend to decrease fluidity along with other differences that would tend to have the opposite effect. Because membrane fluidity is apparently important in cell functions such as maintenance of permeability characteristics and activity of membrane-associated enzymes, it is likely that fluidity properties for individual species are maintained within relatively narrow limits. If the cell had fluidity properties outside this range it might be unable to survive. Recent studies of mutant Chinese hamster ovary cells with defective cholesterol biosynthesis support this view (40). Cholesterol accumulates in the membranes of these cells. This abnormality, which would tend to decrease fluidity, is offset by increases in oleic acid and in PC/PE, and these cells have order parameters similar to those of normal cells, despite the change in cholesterol composition (40).

In selecting the lipid differences that might account for the greater fluidity of CHS erythrocytes, we have made the following assumption: the important differences relative to CHS defects are presumably those present both in humans and in the mouse model system. The one difference that is consistent with greater membrane fluidity and that is found regardless of species is an increased degree of fatty acid unsaturation. This alteration in the average number of fatty acid double bonds in the lipids derived from erythrocytes of normal and CHS mice or humans can account for the observed differences in membrane fluidity. Gaffney (19) has shown that a 10% increase in the number of unsaturated fatty acid chains can result in a significant change in membrane fluidity. Her studies were carried out in a model bilayer system of synthetic dioleoyl and diarachidonoyl lecithins. An increase in the amount of diarachidonoyl lecithin increased the average number of double bonds by 0.3 and increased the bilayer fluidity (decreased the order parameter) by ~ 0.01 . In the present study, we observed increases in membrane fluidity (decreases in the order parameter) of 0.009 and 0.008, concomitant with increases in the average number of double bonds of 0.32 and 0.25 for humans and mice, respectively. Our results do not rule out the possibility that other factors, such as lipid-protein interactions or membrane-cytoskeletal interactions, may also contribute to the observed fluidity differences. However, it is clear that the detected change in degree of saturation can account for the observed fluidity change.

Fatty acid composition of membranes is profoundly influenced by diet (17, 45). Our lipid analysis of human erythrocytes, obtained by comparing one normal control to one CHS patient,

may be influenced, therefore, by dietary differences between the two subjects. Neither the control nor the patient was on a specialized diet at the time of the study. However, the normal and CHS mice received comparable diets and dietary contributions should be minimized in the animal data. Further, these data were obtained on pooled samples from 40–50 individual animals. Accordingly, we conclude that the increased degree of fatty acid unsaturation is a factor contributing to the more fluid membranes detected in CHS erythrocytes.

It appears from our data that the human erythrocyte is more rigid normally than is that of the mouse. We have previously shown that the same is true for the PMN from the two species (21). We do not see this as being incompatible with normal cell function in one or the other species. When a cell such as a PMN is called upon to perform, the absolute value of S may not be as important as the change in S value that occurs. The expected normal range of S values could be the same for both species although the absolute values encompassed by that range are different. A fluidity abnormality would probably be expressed as impaired function if that abnormality makes receptors unavailable for interaction with their specific ligands or if it prevents fluidity changes that normally accompany a cell's function. We suggest that one or more of these possibilities may be true for CHS PMN.

Our observation that both PMN (21) and erythrocytes of CHS have abnormal fluidity indicates that CHS pathophysiology may relate to a general membrane abnormality. The giant granules of CHS cells may result from a tendency to membrane fusion that would be expected for membranes containing relatively greater amounts of unsaturated fatty acids (28). Furthermore, higher unsaturated fatty acid content may make these cells more susceptible to lipid peroxidation. The return to more normal fluidity values with ascorbate treatment may occur because of ascorbate-mediated peroxidation (16, 31) rather than from a "correction" of a basic membrane defect. That such normalization is important to the cell's function is suggested by the improved chemotaxis of CHS PMN treated with ascorbate (5). The greater fluidity of CHS membranes may also result in receptors for chemoattractants being relatively less exposed for combination with their specific ligands (2, 4). Unavailability of these receptors could account, at least in part, for poor chemotactic responsiveness. The ascorbate treatment, by rigidizing the membrane, could therefore bring about displacement of these receptors so that they are more exposed, and although ascorbate does not repair the underlying abnormality its net effect is therapeutic.

No erythrocyte abnormality has previously been reported

TABLE III, continued

Fatty acids							Percent of total					Average no. of C atoms	Average no. of C = C
20:4	24:0	20:5	24:1	22:4	22:5	22:6	Other	Saturated	Monoenoic	Polyenoic			
7.1 ± 0.4	3.2 ± 0.1	2.0 ± 0.1	2.6 ± 0.1	1.6 ± 0.1	3.8 ± 0.2	0.6 ± 0.1	10.5 ± 0.3	31.0 ± 1.4	20.8 ± 0.3	35.5 ± 0.7	18.4 ± 0.1	1.62 ± 0.06	
16.2	—	1.4	—	3.4	6.0	1.7	3.1	31.2	17.3	48.3	18.5	1.94	
10.7 ± 0.8	—	2.9 ± 0.5	—	1.3 ± 0.3	3.6 ± 0.3	4.1 ± 0.6	6.1 ± 1.4	34.3 ± 0.6	19.7 ± 1.3	39.4 ± 2.7	18.1 ± 0.1	1.77 ± 0.10	
14.8 ± 0.9	—	2.5 ± 0.9	—	0.9 ± 0.1	2.6 ± 0.8	8.4 ± 0.7	3.4 ± 1.6	35.6 ± 1.7	13.4 ± 0.6	47.7 ± 0.8	18.4 ± 0.1	2.02 ± 0.04	

TABLE IV

Membrane Lipid Composition Changes that Increase Lipid Fluidity and Decrease the Order Parameter

Composition change	References
Decreased chain length of fatty acids (avg. no. of C ↓)*	12
Increased degree of unsaturation of fatty acids (avg. no. of -C = C- ↑)	12, 14, 19, 38
Decreased amount of cholesterol relative to phospholipid (chol/PL ↓)	14, 26
Increased amount of phospholipid relative to protein (PL/prot ↑)	2, 23
Decreased amount of sphingomyelin relative to phosphatidylcholine (SP/PC ↓)	2, 15
Increased amount of phosphatidylcholine relative to phosphatidylethanolamine (PC/PE ↑)	22

* Material in parentheses denotes quantitative measure of composition change; ↑, increase; ↓, decrease.

for CHS. This does not, however, preclude the possible important contribution of increased fluidity to the pathology of the syndrome. The erythrocyte (unlike the PMN) possesses no granules, does not undergo rapid amoeboid movement, and is not a chemotactically responsive cell. Therefore, the erythrocyte may perform all of its functions normally despite the fluidity increase, whereas the PMN, because of its specialized role in host defense, is impaired in its physiologic responses.

The abnormal membrane fluidity detected in CHS PMN (21) and in CHS erythrocytes reported herein suggests that a generalized membrane abnormality may be associated with the disease. Precedents for this concept are known. In spur-cell anemia, an increased cholesterol content of erythrocyte and platelet membranes is reflected in a decreased membrane fluidity (14). Several inherited neurological disorders are also associated with abnormal erythrocyte membranes. An increased erythrocyte membrane fluidity was observed in myotonic muscular dystrophy and congenital myotonia (8). Abnormalities in the lateral diffusion of SDS (43), in a lipid-protein interaction (37), and in the physical state of membrane proteins (7) have been observed in RBC membranes in Duchenne muscular dystrophy. In Huntington's disease, alterations in the physical state of erythrocyte membrane proteins (9) and in erythrocyte deformability (10) were found. Thus, in these several examples, as well as in the CHS, the presence of the disease is associated with an alteration in erythrocyte membranes.

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