The Membrane Change in En(a–) Human Erythrocytes

ABSENCE OF THE MAJOR ERYTHROCYTE SIALOGLYCOPROTEIN

By MICHAEL J. A. TANNER and DAVID J. ANSTEE

*Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K., and
South West Regional Blood Transfusion Centre, Southmead, Bristol BS10 5ND, U.K.*

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1. We investigated the membrane of En(a–) human erythrocytes as part of a study of the structure and biochemical function of the surface glycoproteins of the mammalian cell.
2. En(a–) erythrocytes were selected because they have more extensive changes at the cell surface than any other known erythrocyte variant.
3. Our results show that in En(a–) erythrocytes: (a) the major membrane sialoglycoprotein is lacking; (b) the other major membrane-penetrating glycoprotein (band 3) has an altered electrophoretic mobility.
4. The apparent clinical normality of En(a–) cells suggests that the change in band 3 may compensate for the loss of the membrane sialoglycoprotein. It is clear that a viable erythrocyte can exist despite the absence of one of its major surface components.

The study of genetic variants has long been a powerful approach towards understanding the relationship between the structure and function of cell components. Little is known about the structure and function of the glycoproteins which are always present at the surface of the mammalian cell and a similar approach should aid our understanding of this problem. The blood-group antigens form a well known class of genetic variants which result in changes at the erythrocyte surface. Although many of these antigen systems appear to result from the absence or altered specificity of a glycosyltransferase and cause only slight changes in the structures at the erythrocyte surface, one rare human erythrocyte type [En(a–)] is associated with more extensive changes at the surface of the erythrocyte than any other known variant. Hence the detailed study of the surface glycoproteins of this cell provides a particularly interesting system with which to investigate the role of cell surface glycoproteins.

The En^a antigen is an almost ubiquitous human erythrocyte antigen. Rare individuals whose erythrocytes lack this antigen are known and their cells are denoted as En(a–) cells. Individuals from three families have been reported to lack the En^a antigen. Two of these families have been found in Finland, and the other in the U.K. Their cells are characterized by a markedly lowered sialic acid content, very weak M,N blood-group antigens and a lowered electrophoretic mobility (Darnborough *et al.*, 1969; Furuhejm *et al.*, 1969, 1973).

We have studied the membranes of erythrocytes from two of these families. In both cases the polypeptide chain and carbohydrate moieties of the major erythrocyte sialoglycoprotein (PAS-1) are completely absent. The other major penetrating membrane glycoprotein, band 3 (Steck, 1974), the

erythrocyte anion-transport protein (Cabantchik & Rothstein, 1974), has an altered electrophoretic mobility which probably reflects a changed carbohydrate composition. These two proteins are the major carriers of carbohydrate on the surface of the human erythrocyte, the sialoglycoprotein alone contributing 30–45% of the total cell-surface carbohydrate. Despite the drastic alteration which must occur at the surface of these cells no obvious associated clinical abnormality has been reported in these individuals. Our results imply that the erythrocyte sialoglycoprotein itself is not required for the function of the normal erythrocyte. In En(a–) cells the loss of the sialoglycoprotein is probably overcome by a compensatory change in the carbohydrate composition of the other major surface glycoprotein, band 3.

Materials and Methods

The methods for the preparation of erythrocyte 'ghosts', sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, trypsin treatment of erythrocyte 'ghosts', radioiodination of erythrocytes with lactoperoxidase, isolation of labelled proteins and preparation of peptide 'maps' have been described (Boxer *et al.*, 1974; Jenkins & Tanner, 1975). Sialic acid was measured by the method of Warren (1959). Whole blood from En(a–) donors was provided by Dr. U. Furuhejm and Dr. J. Leikola, Finnish Red Cross Blood Transfusion Service, Helsinki, Finland, and by Dr. W. Wagstaff, Regional Blood Transfusion Centre, Sheffield, U.K. Anti-En^a was prepared from the plasma of En(a–) donors by addition of Fibrindex (Ortho) as described by Macpherson & Marson (1965). Rabbit anti-M and anti-N sera were from the Blood Group Reference Laboratory, London SW1

W8QJ, U.K. All other antisera and cells were available at the Regional Transfusion Centre, Bristol, U.K. Standard serological techniques were used (Boorman & Dodd, 1970).

Lectins

Pure concanavalin A (Pharmacia, Uppsala, Sweden) and purified phytohaemagglutinin (Difco, Detroit, Mich., U.S.A.; type P) were used at 1 mg/ml in phosphate-buffered saline, pH 7.4 (Tanner & Anstee, 1976). Crude *Lens culinaris* lectin was prepared as described by Howard *et al.* (1971). *Achatina granulata* (freeze-dried albumin glands) was from Dr. S. Khalap, Metropolitan Police Forensic Science Laboratory, London SE1 7JH, U.K.; 100 mg was dissolved in 1 ml of phosphate-buffered saline. The preparation of crude extracts of other lectins used has been described (Anstee & Pardoe, 1973). The lectins from *Maclura aurantiaca*, *Helix pomatia* and *Triticum vulgaris* (wheat-germ agglutinin) were used after purification by absorption and elution from formaldehyde-treated human erythrocytes. The binding of ^{125}I -labelled lectins to membrane components separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out as described by Tanner & Anstee (1976).

Neuraminidase treatment of normal erythrocytes

Packed washed normal human group-A erythrocytes (0.8 ml) were washed with a mixture of 9 vol. of 0.15 M-NaCl and 1 vol. of 0.2 M-sodium phosphate buffer, pH 6.5, which was made 1 mM in CaCl_2 . The cells were made up to 2 ml in the same buffer. Neuraminidase (0.2 ml; 500 units/ml from *Vibrio cholerae*; Hoechst Pharmaceuticals, Brentford, Middx., U.K.) was added and the suspension incubated at 30°C for 30 min. The cells were washed with 3×10 ml of 0.15 M-NaCl. One-half of these cells was retained and radioiodinated with lactoperoxidase (Boxer *et al.*, 1975). The remaining cells were treated with trypsin (Jenkins & Tanner, 1975) and then radioiodinated with lactoperoxidase.

Results and Discussion

The two samples of En(a-) cells that we have studied are from donor G. W. [first described by Furuhielm *et al.* (1973)] and from donor M. E. [first described as M.P. by Darnborough *et al.* (1969)]. Both are homozygous En(a-) cells. The blood-group antigens of both cell types are normal except for their M,N and Pr antigens. 'Ghosts' from the erythrocytes of donor G.W. contained 1.2% dry wt. of sialic acid. Comparable values from 'ghosts' from normal, trypsin-treated and neuraminidase-treated erythrocytes were 1.7, 1.2 and 0.2% dry wt. of sialic acid

respectively. These results confirm the low sialic acid content of En(a-) cells reported by Furuhielm *et al.* (1969) for a different En(a-) donor.

Polyacrylamide-gel electrophoresis

'Ghosts' were prepared from erythrocytes of donors G.W., M.E. and a 'normal' human and sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out on these samples. Electrophoresis was performed on gels containing 5, 8 and 10% (w/v) polyacrylamide, and the gels were stained for protein and for carbohydrate. Plate 1 shows the results obtained with the 5 and 8% gels. The major erythrocyte sialoglycoprotein (PAS 1) and component PAS 4 could not be detected with the periodic acid-Schiff's-base stain on either of the En(a-) samples. Scans of the periodic acid-Schiff's-base-stained gels are shown in Fig. 1. These show that bands corresponding to components PAS 2 and PAS 3 are present in both En(a-) cells. Bands PAS 2 and PAS 3 take up approximately the same amount of stain in the En(a-) samples whereas in samples from normal cells band PAS 2 is much more heavily stained than band PAS 3. To confirm that this represents a decrease in band PAS 2 and not an increase in band PAS 3, the area of the bands in the PAS 2 and PAS 3 region was measured from known amounts of the En(a-) and normal erythrocyte 'ghosts'. The data in Table 1 show that less than

Fig. 1. Periodic acid-Schiff's-base stain of polyacrylamide gels of 'ghosts' from En(a-) and normal human erythrocytes

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed in gels containing 10% polyacrylamide, and the gels were scanned at 500 nm. —, 'Ghosts' from normal human erythrocytes; ----, 'ghosts' from En(a-) erythrocytes (M.E.); - · - ·, 'ghosts' from En(a-) erythrocytes (G.W.)

EXPLANATION OF PLATE I

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of 'ghosts' from normal and En(a-) human erythrocytes

(a)–(c) Electrophoresis in 5% polyacrylamide gels; Coomassie Blue stain. (a) 'Ghosts' from normal erythrocytes; (b) 'ghosts' from En(a-) erythrocytes (M.E.); (c) 'ghosts' from En(a-) erythrocytes (G.W.). (d)–(f) Electrophoresis in 8% polyacrylamide gels. (d) 'Ghosts' from normal erythrocytes; (e) 'ghosts' from En(a-) erythrocytes (M.E.); (f) 'ghosts' from En(a-) erythrocytes (G.W.).

EXPLANATION OF PLATE 2

Polyacrylamide-gel electrophoresis of 'ghosts' from En(a-), normal and enzyme-treated erythrocytes radioiodinated with lactoperoxidase

Radioautographs of dried slices of 8% polyacrylamide gel. Gels (a)–(d) and (c)–(f) were run on separate occasions. SP, sialoglycoprotein; SP_N, desialylated sialoglycoprotein; SP_{NT}, tryptic fragment of desialylated sialoglycoprotein. (a) 'Ghosts' from radioiodinated normal erythrocytes; (b) 'ghosts' from radioiodinated En(a-) erythrocytes (G.W.); (c) 'ghosts' from radioiodinated normal erythrocytes; (d) 'ghosts' from radioiodinated En(a-) erythrocytes (M.E.); (e) 'ghosts' from radioiodinated neuraminidase-treated normal erythrocytes; (f) 'ghosts' from radioiodinated neuraminidase-treated normal erythrocytes that had been treated with trypsin before lysis of the erythrocytes.

Table 1. *Quantification of PAS 2 and PAS 3 bands in normal and En(a-) human erythrocyte 'ghosts'*

Parallel gels containing known amounts of sample were stained for protein and carbohydrate. Protein was estimated from the area of band 4.2. This value was used to normalize the areas of PAS 2 and PAS 3 peaks to the same unit of concentration of band 4.2.

Sample	PAS 2 peak per unit band 4.2 (arbitrary units)	PAS 3 peak per unit band 4.2 (arbitrary units)
En(a-) 'ghosts' G.W.	0.18	0.09
En(a-) 'ghosts' M.E.	0.21	0.13
Normal 'ghosts' (3 samples)	0.69	0.18
	0.73	0.15
	0.77	0.18

Table 2. *Estimated molecular weights of band 3 on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis*

The values for the molecular weights of the erythrocyte 'ghost' proteins reported by Steck (1974) were used to determine the molecular weight of band 3. The values for the molecular weight of the leading and trailing edge of band 3 are given in each case.

Gel concentration (%)	10 ⁻³ × Molecular weight		
	Normal cells	G.W. cells	M.E. cells
5	86-106	98-120	96-122
8	86-101	90-106	91-106
10	83-97	87-101	86-101

one-third of the normal erythrocyte component PAS 2 is found in En(a-) erythrocytes. There is evidence that some of the material found in band PAS 2 is an interconvertible form of component PAS 1 (Tuech & Morrison, 1974). It is also suggested that an additional component is present in band PAS 2 (Mueller & Morrison, 1974). Therefore the decrease in staining for component PAS 2 in En(a-) cells probably reflects the absence of component PAS 1 from these cells. This result provides strong evidence for the heterogeneity of component PAS 2 in normal erythrocytes.

The other major penetrating membrane glycoprotein (band 3) shows an altered electrophoretic mobility when compared with that of normal erythrocytes. The apparent molecular weight of band 3 was estimated on 5, 8 and 10% gels by comparison with the known molecular weights of the other proteins of the erythrocyte 'ghost'. As the band given by band 3 is broad the molecular weights of both the leading and trailing edges were estimated. The results

are shown in Table 2 and demonstrate that band 3 from En(a-) cells shows a consistently higher apparent molecular weight than that of normal band 3, the difference being most pronounced on the 5% gels. The normal erythrocyte band 3 shows a similar apparent molecular weight on different concentrations of polyacrylamide gel whereas that of the two En(a-) cells shows a significant decrease in apparent molecular weight as the concentration of the gel increases. The normal erythrocyte major sialoglycoprotein shows a similar anomalous behaviour which has been attributed to the presence of large amounts of carbohydrate and to the abnormal binding of sodium dodecyl sulphate by the protein (Grefrath & Reynolds, 1974).

Band 3 of En(a-) erythrocytes shows a similar pattern of proteinase susceptibility to that of normal band 3. The protein is not degraded on trypsin treatment of the intact erythrocyte, but thermolysin treatment of En(a-) erythrocytes yields a fragment from band 3 of similar molecular weight to that obtained from normal band 3 (e; Boxer *et al.*, 1974).

Radioiodination of En(a-) cells

It was not possible to detect the major sialoglycoprotein in the membrane of En(a-) cells with the periodic acid-Schiff's-base reagent. This could be due to a lack of sialic acid in this protein as the stain depends mainly on the presence of sialic acid and En(a-) cells are known to be deficient in this sugar. It was therefore desirable to try to establish whether the polypeptide of the sialoglycoprotein was present in the membrane. The two En(a-) erythrocytes and control normal erythrocytes were radioiodinated with lactoperoxidase. 'Ghosts' were prepared and the proteins separated on sodium dodecyl sulphate/polyacrylamide gels. Radioautographs of these samples are shown on Plate 2. No band corresponding to the sialoglycoprotein (SP) could be detected in either of the En(a-) erythrocytes although it was clearly demonstrable in the control sample. Neuraminidase-treated normal cells labelled under the same conditions show the presence of a weak periodic acid-Schiff's-base-staining band of faster electrophoretic mobility than normal sialoglycoprotein. This band (SP_N) could be labelled by radioiodination with lactoperoxidase and is probably the desialized sialoglycoprotein (Plate 2). Thus the lack of sialic acid itself is insufficient to explain the observed lack of labelling of the sialoglycoprotein polypeptide in both En(a-) cells. The desialylated product of the sialoglycoprotein remains sensitive to trypsin. Normal erythrocytes which have been treated sequentially with neuraminidase and trypsin no longer show this band and give a new band (SP_{NT}, Plate 2). Trypsin treatment of En(a-) erythrocytes does not result in any alteration in the pattern of labelling, whereas

Table 3. *Agglutination reactions of lectins with En(a-) cells*

The method of neuraminidase treatment was as follows: 0.1 ml of washed packed erythrocytes was incubated with 10 μ l of neuraminidase (500 units/ml from *V. cholerae*; Hoechst) and 0.89 ml of sodium phosphate buffer, pH 7.4, containing 1 mM-CaCl₂ for 30 min at 37°C. The cells were washed with 3 $\times$ 10 ml of 0.15M-NaCl before use. The agglutination titre is the greatest dilution of lectin that still gives microscopic agglutination of the cells. n.d., Not determined.

Source of lectin	Reciprocal of agglutination titre					
	Normal cells			En(a-) cells		
			G.W.			M.E.
	Untreated	Neuraminidase-treated		Untreated	Neuraminidase-treated	
<i>M. aurantiaca</i>	512	1024	128		64	
<i>L. culinaris</i>	32	256	128		128	
<i>Ricinus communis</i>	128	1024	2048		2048	
<i>H. pomatia</i>	256	1024	2048		nil	
<i>T. vulgaris</i>	nil	16	32		32	
<i>Canavalia ensiformis</i>	8	256	256		256	
<i>P. vulgaris</i>	16	128	128		256	
<i>Dolichos biflorus</i>	n.d.	128	256		nil	
<i>Ulex europeus</i>	16	32	n.d.		64	
<i>V. graminea</i>	8(MM)/32(NN)	32	32	32	nil	64
<i>A. granulata</i>	32	2/4	32	2	32	4
<i>Arachis hypogaea</i>	nil	512	nil	512	nil	512

in normal cells trypsin treatment clearly results in the removal of the erythrocyte sialoglycoprotein.

As the electrophoretic mobility of band 3 is clearly altered in En(a-) cells (Plate 1) it was decided to look for possible changes in the polypeptide chain of this protein. Band 3 from both En(a-) cells was labelled by radioiodination of the intact erythrocytes with lactoperoxidase. Band 3 was isolated and peptide 'maps' were prepared and compared with that of normal human band 3 labelled under the same conditions (Boxer *et al.*, 1974). No difference was found between the En(a-) and normal band 3, suggesting that the change in the electrophoretic mobility may reflect a change in the carbohydrate composition rather than the amino acid sequence of the protein. The similar patterns of trypsin resistance and thermolysin sensitivity of the protein in the intact erythrocyte are consistent with this premise.

Lectin receptors on En(a-) cells

The experiments described above do not give any evidence for the presence of the major erythrocyte sialoglycoprotein in En(a-) erythrocytes. This might, however, reflect the limitations of the techniques used; for example, the presence of incomplete oligosaccharide chains may result in an altered conformation of the protein causing decreased reactivity on radioiodination with lactoperoxidase. An alternative approach to the problem would be to examine En(a-) cells for the presence of lectin-binding sites

known to be associated with the sialoglycoprotein, since these should enable the detection of oligosaccharide side chains. It is known that En(a-) cells give enhanced agglutination reactions with a number of lectins (Bird & Wingham, 1973). Neuraminidase treatment of normal cells similarly enhanced the reactivity of lectins, suggesting that the loss of sialic acid and consequent diminution of surface charge is an important factor in these reactions (Table 3). The lectin from *M. aurantiaca* (syn. *Maclura pomifera*) is unusual in that it gives decreased reactions with En(a-) cells (Bird & Wingham, 1973). This lectin is thought to react with galactosyl groupings in the alkali-labile tetrasaccharide of the sialoglycoprotein (Dahr *et al.*, 1975). The lectin from *Phaseolus vulgaris* (phytohaemagglutinin) is believed to react with the alkali-stable oligosaccharide of the erythrocyte sialoglycoprotein (Kornfeld & Kornfeld, 1969). Since both these lectins agglutinate En(a-) cells, it was decided to define more closely the nature of the membrane components to which they bind. Erythrocyte membrane components separated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis can be fixed and the lectin-binding components made visible by incubation with radioactively labelled lectins (Tanner & Anstee, 1976). The results obtained for the lectins from *M. aurantiaca* and *P. vulgaris* with normal and En(a-) cells are shown in Figs. 2 and 3. It is clear that the band corresponding to the major erythrocyte sialoglycoprotein is absent from En(a-) cells and no new band appears. This provides further

Fig. 2. Binding of the radioiodinated lectin from *M. aurantiaca* to the components of 'ghosts' from En(a-) and normal erythrocytes

'Ghosts' from normal and En(a-) erythrocytes were separated on 8% polyacrylamide gels. Two flat central slices from each gel were each incubated with 0.5 ml of radioiodinated lectin from *M. aurantiaca* (agglutination titre 1:8) containing 3.5×10^4 c.p.m. of ^{125}I -D-Galactose (0.2 M) was also added to one of the pairs of gel slices as a control. The slices were washed, stained with Coomassie Blue and dried for radioautography. The scans of the radioautographs are shown. —, 'Ghosts' from normal human erythrocytes; ----, 'ghosts' from En(a-) human erythrocytes (M.E.); - · - ·, 'ghosts' from En(a-) human erythrocytes (G.W.). In each case, the binding of the lectin was completely abolished by the presence of 0.2 M-D-galactose. The side slices from the gels were stained with the periodic acid-Schiff's-base stain, and the positions of the bands PAS 1, PAS 2 and PAS 3 were determined from these gels.

support for the contention that En(a-) cells lack the major erythrocyte sialoglycoprotein. The binding of *P. vulgaris* lectin to band 3 and to material in the region of band PAS 2 is probably responsible for the strong agglutination observed with intact En(a-) erythrocytes. Similarly, the residual reaction of *M. aurantiaca* lectin can be attributed to the presence of binding sites on other membrane components, PAS 2 and PAS 3.

M,N antigen activity of En(a-) cells

As the M,N blood-group antigens are known to be located on the major erythrocyte sialoglycoprotein, lack of this protein would be expected to result in the absence of M,N antigens on En(a-) cells unless these antigens are also carried by other minor membrane components. The erythrocytes of G.W. were reported to have decreased M and N antigens (Furuhjelm *et al.*, 1973) and those of M.E. to have weak M antigen activity (Darnborough *et al.*, 1969). Although G.W. cells react with certain anti-M sera this reaction does not reflect the presence of M antigen on these cells. Absorption of anti-M sera by MM cells to remove

Fig. 3. Binding of the radioiodinated lectin from *P. vulgaris* to the components of 'ghosts' from normal and En(a-) erythrocytes

The method was as described in the legend to Fig. 2, except that each reaction mixture contained 0.25 mg of ^{125}I -labelled lectin from *P. vulgaris* (2.5×10^6 c.p.m.), and no simple-sugar inhibitor was used as a control. (a) Scans of protein stain (Coomassie Blue) of dried gels. —, 'Ghosts' from normal erythrocytes; ----, 'ghosts' from En(a-) erythrocytes (M.E.). The 'ghosts' from En(a-) erythrocytes (G.W.) gave a similar pattern to those from donor M.E. The apparent extra peak in the control normal 'ghosts' in the region between peaks PAS 1 and PAS 2 is an artifact which arose during the drying of the gels. (b) Scans of radioautographs of the dried gels shown in (a). —, 'Ghosts' from normal erythrocytes; ----, 'ghosts' from En(a-) erythrocytes (M.E.); - · - ·, 'ghosts' from En(a-) erythrocytes (G.W.).

the anti-M does not decrease the agglutination of G.W. cells by the anti-serum. In addition human anti-M cannot be absorbed on and eluted from G.W. cells. It was therefore necessary to define the presence of M and/or N antigens on En(a-) erythrocytes by absorption experiments rather than by direct agglutination (Table 4). Cells from M.E. had weak M activity but N antigen activity was completely absent. No N activity was detected with *Vicia graminea* lectin on these cells (Table 3). This complete absence of N antigen is very unusual (Race & Sanger, 1975). In contrast, G.W. cells have very weak N antigen (comparable with that found on normal MM cells) and a complete absence of M antigen.

The presence of small but definite M and N activity in En(a-) cells raises the question of whether these antigens are present on small amounts of the sialoglycoprotein not detected by the techniques described above or on other minor membrane components. The M,N antigen activity associated with the sialoglycoprotein is known to be removed from normal erythrocytes by trypsin treatment. The results of a single experiment indicate that the M activity

Table 4. *M,N status of En(a-) cells*

Anti-M and anti-N sera were each pools of four batches of rabbit antiserum selected to give strong and specific reactions with normal MM and NN erythrocytes; 0.1 ml of anti-M and anti-N serum was used in each case. For adsorption of anti-M serum 7.5×10^5 erythrocytes were added, and for anti-N serum 3×10^5 cells were used. Incubation was at room temperature (21°C) for 1 h. The mixture was then centrifuged at 900g for 5 min, and the supernatants were examined by haemagglutination titration against the appropriate cells.

Absorbing cell	Agglutination titre of supernatant after adsorption	
	Rabbit anti-M/MM cells	Rabbit anti-N/NN cells
MM	1	2
MN	2	1
NN	16	nil
En(a-) (G.W.)	16	1
En(a-) (M.E.)	4	4

of M.E. cells is not affected by treatment of the cells with trypsin, suggesting that this M activity is not present on small amounts of otherwise undetected sialoglycoprotein but on another membrane component.

The En^a antigen does not appear to be located on the sialoglycoprotein since the anti-En^a sera obtained from G.W. and M.E. could not be inhibited by the isolated glycoprotein and also react very strongly with trypsin-treated normal cells. Similar observations have been made with anti-En^a serum from the other Finnish family (Furuhjelm *et al.*, 1969).

All of the experimental data presented above are consistent with the absence of the major erythrocyte sialoglycoprotein from En(a-) cells. This protein (5×10^5 copies/cell) together with band 3 (9.4×10^5 copies/cell) comprise the major polypeptides accessible at the surface of the human erythrocyte (Steck, 1974). The sialoglycoprotein alone carries 30–45% of the total cell-surface carbohydrate. Clearly its loss must result in a substantial change in the surface of En(a-) erythrocytes yet, suprisingly, no associated clinical abnormalities have been reported in En(a-) individuals. This raises questions about the function of the sialoglycoprotein in the normal individual. As yet, no clear functional role has been defined for this molecule. It is generally assumed that the negative charge associated with the sialoglycoprotein serves to keep erythrocytes apart. En(a-) cells are known to have a decreased surface zeta potential and electrophoretic mobility and are more easily agglutinable than normal erythrocytes (for example see Table 3). However, the increased agglutinability caused by the loss of the sialoglycoprotein is apparently not great

enough to have clinical significance. Clearly other membrane components must also be involved in keeping cells apart since En(a-) cells do not spontaneously aggregate. Alternatively, Bretscher (1973) has suggested that the carbohydrate bound to the sialoglycoprotein may contribute to the rigidity of the cell membrane. If this is the case then one might expect En(a-) cells to be more fragile than normal cells. Little evidence is available on this point, although the work of Darnborough *et al.* (1969) suggests that the osmotic fragility of En(a-) cells is unimpaired.

The removal of terminal sialic acid residues from the surface of rabbit and rat erythrocytes results in a decreased survival time of the cells in the animal. This has been attributed to the exposure of subterminal galactose residues (Durocher *et al.*, 1975). A major source of subterminal galactose residues in normal human erythrocytes would be the large number of alkali-labile tetrasaccharides found bound to the sialoglycoprotein (Jackson *et al.*, 1973). If such galactose residues are important in the turnover of normal human erythrocytes then one might expect En(a-) cells to have an altered turnover rate. At present no data are available on this point, but it is clearly of importance to investigate the survival of En(a-) cells.

The viability and apparent normality of En(a-) cells seems likely to be the result of a compensatory change in the carbohydrate composition of the remaining major surface protein (band 3). The change in electrophoretic mobility of band 3 that we have observed may reflect such a compensatory change. It is unlikely that this change in band 3 is independent of the defect which results in the loss of the sialoglycoprotein. It may be that the change in band 3 is a consequence of the altered structure of the cell surface and the resulting differences in the accessibility of potential precursors of oligosaccharide chains of the normal and En(a-) cell. This situation may result in the addition of carbohydrate residues to band 3 which are not related to the oligosaccharide chains normally found on sialoglycoprotein. Our results imply that the major erythrocyte sialoglycoprotein is not indispensable to the normal functioning of the erythrocyte.

Band 3 is involved in erythrocyte anion transport (Cabantchik & Rothstein, 1974) and is probably a constituent of the membrane intercalating particles observed on freeze-fracturing of erythrocyte membranes (Pinto da Silva & Nicolson, 1974). It will clearly be of interest to establish whether the alterations in band 3 have an effect on the erythrocyte anion-transport system or the membrane morphology of En(a-) cells.

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