nuity of red cell membrane ANS fluorescence. In contrast, the extracted membrane lipids are not influenced in the same way by the reagent. Since the discontinuity in this temperature region is due to the membrane lipids[10,17-20], it must be concluded that the reagent interfered with lipid-protein interaction. Diethylpyrocarbonate will react with histidine, tyrosine and lysine in proteins[2]. SH groups are also possible candidates for the reaction, depending on their reactivity. We did not find, however, any alteration in inhibition of glucose transport by diethylpyrocarbonate when 5 mM cysteine was present during incubation (not shown). Also, membrane SH groups were considered to be probably not essential for glucose transport activity[7]. Since there was also no difference detected between the controls and modified membrane when spectrophotometric measurements were carried out above 270 nm, extensive reaction of tyrosine rests may be excluded (not shown). The remaining rests are histidine-imidazole and lysine. On the one hand, both have already been implied to be probably essential for glucose transport[6,7]. On the other hand, basic amino acids have been considered for a while to be possible candidates in polar lipid-protein interaction in plasma membranes[21]. The polar head of lipid phosphate may interact electrostatically with the basic rest of the amino acid. It is known, furthermore, that lipid transition is dependent on liberty of motion of the polar head groups and also on an increase in mobility of the fatty alkyl chains. Thus, a change in mobility of the head groups induced by the reagent's interaction with basic amino acid residues may decrease activation energy below and increase above transition temperature (figure 1). Below the transition temperature, there is an increase of disorder of the system in the presence of diethylpyrocarbonate. Above the transition temperature, where we already observe an increase of disorder, the effect of the reagent is opposite.

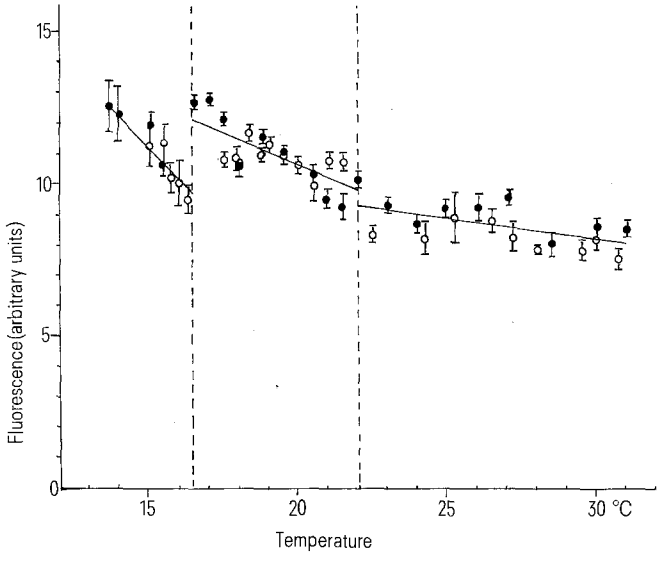

Fig. 3. *b* ANS fluorescence of extracted red cell membrane lipids over a temperature range. 0.78 mg of total lipid (30% cholesterol) was suspended with 2.4 ml 0.9% NaCl and sodium phosphate buffer, pH 7.0 to a final concentration of 0.01 M was added. The ANS concentration was 5.6 µM. Incubation with diethylpyrocarbonate similar to figure 3, a. The points±SD represent means of 10 recordings (see figure 3, a). ○, Control; ●, 5 mM diethylpyrocarbonate. The lines were calculated by the method of least squares.

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2 A. Mühlrad, G. Hegyi and G. Toth, Acta biochim. biophys., Acad. Sci. hung. *2*, 19 (1967).
3 C. Huc, A. Olomucki, D.B.P. Le-thi-Lan and N. van Thoai, Eur. J. Biochem. *21*, 161 (1971).
4 J.J. Holbrook and V.A. Ingram, Biochem. J. *131*, 729 (1973).
5 Y. Burstein, K.A. Walsh and H. Neurath, Biochemistry *13*, 205 (1974).
6 L. Lacko, B. Wittke and P. Geck, J. Cell Physiol. *80*, 73 (1972).
7 R. Bloch, J. biol. Chem. *249*, 1814 (1974).
8 L. Lacko, B. Wittke and H. Kromphardt, Eur. J. Biochem. *25*, 447 (1972).
9 J.T. Dodge, C. Mitchell and D. Hanahan, Archs Biochem. Biophys. *100*, 119 (1963).
10 G. Zimmer and H. Schirmer, Biochim. biophys. Acta *345*, 314 (1974).
11 G. Zimmer, H. Schirmer and P. Bastian, Biochim. biophys. Acta *401*, 244 (1975).
12 R.M.C. Dawson, N. Hemington and D.B. Lindsay, Biochem. J. *77*, 226 (1960).
13 N. Zöllner and K. Kirsch, Z. ges. exp. Med. *135*, 545 (1962).
14 D. Watson, Clin. chim. Acta *5*, 637 (1960).
15 L. Lacko and B. Wittke, Experientia *33*, 191 (1977).
16 G. Zimmer, W. Gross, U. Mehler and D. Dorn-Zachertz, Arzneimittel-Forsch., in press.
17 M.H. Gottlieb and E.D. Eanes, Biochim. biophys. Acta *373*, 519 (1974).
18 P.R. Cullis, FEBS Lett. *68*, 173 (1976).
19 S.P. Verma and D.F.H. Wallach, Biochim. biophys. Acta *436*, 307 (1976).
20 P.R. Cullis and C. Grathwohl, Biochim. biophys. Acta *471*, 213 (1977).
21 D.F.H. Wallach and P.H. Zahler, Biochim, biophys. Acta *150*, 186 (1968).

Adhesion of human red blood cells to polystyrene. Influence of sodium chloride concentration and of neuraminidase treatment

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Summary. The adhesion on polystyrene of glutaraldehyde-fixed human red blood cells was found to increase with NaCl concentration. Half of the maximum of irreversible adhesion was obtained in 2.2 mM NaCl for neuraminidase-treated cells and in 5.5 mM NaCl for the untreated cells.

As Curtis[1] pointed out, the classical DLVO theory of double layer interaction developed by Derjaguin and Landau[2] and by Verwey and Overbeek[3] predicts that cell adhesion can occur in 2 minima of the potential energy of interaction. At some finite distance, where the surface does not come into molecular contact, an equilibrium is reached between electro-dynamic attractive and electrostatic repulsive forces (secondary minimum). At smaller distance there is a net energy barrier. Once overcome, the theory predicts another minimum (primary minimum). Both the height of

the barrier and the secondary minimum depend on ionic strength and electrostatic charge. Evidence has recently been presented to show that red blood cells (RBC) can adhere to a lead electrode and to an oil-water interface with a behavior characteristic of primary (irreversible adhesion) and/or secondary energy minima (reversible adhesion)[4,5]. The reversible adhesion of glutaraldehyde-fixed human RBC to a flat hexadecane-saline interface having a negative potential was found to decrease below 1 mM NaCl[5].

This report presents some observations on the adhesion of human RBC to polystyrene as a function of NaCl concentration in 2 different systems. Polystyrene was chosen because it is widely used in laboratory plastic ware. It has a negative surface potential whose value decreases as salt concentration increases[6]. RBC were fixed in 3.3% glutaraldehyde to prevent cell deformability and leak of cell components which would contaminate the interface. This type of fixation increases the net surface negative charge of human RBC by only 14%[7].

Blood from a human donor 0 Rh^+ was collected in citrated TBS (0.145 M NaCl-0.025 M Tris, pH 7.4) and washed in TBS (4×). The cells were fixed for 18 h[4,5] in 3.3% glutaraldehyde (adjusted to pH 7.4 with 1 N NaOH) at a cell density of 10^7 cells/ml[7]. Finally the cells were washed with water containing 1 mM EDTA (3×). In all experiments only water double distilled on quartz was used.

To study the effect of cell surface charge on adhesion, RBC were treated with 50 units/ml of neuraminidase (*Vibrio cholerae;* Calbiochem) at 37 °C for 1 h in TBS and at a density of 10^8 cells/ml. The cells (n-RBC) were then fixed with glutaraldehyde as described above. Neuraminidase reduces by 60% the net surface negative charge of RBC fixed afterwards in glutaraldehyde[7].

In the 1st system, the adhesion of RBC on polystyrene was studied in U-type microplates as a function of both NaCl concentration and cell density (figure 1). At a density of 1–1.5× 10^6 cells per well, adhesion of the RBC on the wall of the cone was total in 10–150 mM NaCl (no pellet formed at the bottom of the well). A loss of adhesion was noticed in 5 mM NaCl which became greater as the salt concentration decreased (as shown by the increasing quantity of cells collected at the bottom of the well). In pure water, a microscopical examination indicated that practically no cells adhered to the wall of the cone. At a cell density higher than 1.5× 10^6 cells per well, results were difficult to interprete since at all salt concentrations some cells collected at the bottom of the wells. At a density below 10^6 cells per well, much lower concentrations of NaCl were necessary to decrease adhesion (figure 1). With n-RBC, adhesion decreased below 0.7 mM NaCl at a density of 1.25× 10^6 cells per well.

Although these results do not constitute a definite proof, they are consistent with a duplex adhesive behavior characteristic of primary and secondary energy minima. At a low cell density, the cells appear to adhere in the primary minimum (the cells were shown to be irreversibly adsorbed). At a higher cell density where all cells cannot come into molecular contact with the polystyrene surface, these cells are prevented from sliding along the wall of the cone by adhesion in the secondary minimum. This implies a long-range attractive force between the polystyrene-saline interface and RBC. Evidence has been presented to show that adhesion of RBC to an hexadecane-saline interface is mediated by an attractive force acting at a distance apparently exceeding 100 nm[5]. Alternatively, at high cell density, one could postulate a weak cell to cell adhesion between RBC. However RBC are reputedly non-adherent. Since the height of the potential barrier depends not only on the salt concentration but also on the cell charge, the NaCl concentration necessary to decrease adhesion was lower for n-RBC (0.7 mM) than for RBC (5 mM) at a density of 1–1.5× 10^6 cells per well.

In the 2nd system, the irreversible adhesion of RBC to polystyrene was quantitatively determined under conditions where RBC, adhering possibly by weak forces, would be detached from the interface (figure 2). As found previously, the adhesion of RBC increased with NaCl concentration (50% of the maximum of adhesion was found in 5.5 mM NaCl). A similar curve displaced towards lower salt concentration was obtained with n-RBC (50% of the maximum of adhesion occurred in 2.2 mM NaCl). Adhesion was irreversible since adhesive cells could not be detached in the presence of water only.

The statistical adhesive behavior of RBC shown in figure 2 could reflect a distribution of surface properties of RBC and/or heterogeneity of the polystyrene surface roughness. Colloidal iron oxide particles adsorbed on n-RBC fixed with glutaraldehyde show a departure from the uniform

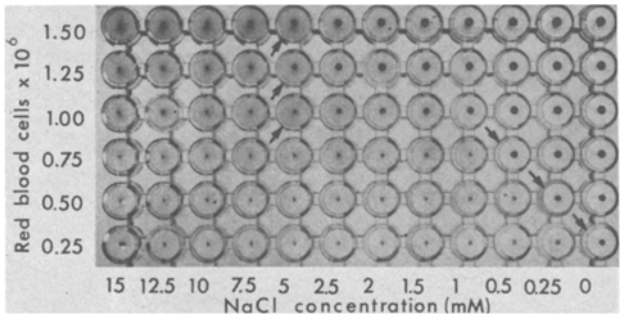

Fig. 1. Adhesion of human red blood cells to polystyrene microplates, as a function of NaCl concentration. Adhesion studies were performed in Takatzy microplates (Cooke Engineering Company, Alexandria, Va). The cells (0.25–1.5× 10^6 in 50 µl water) were mixed with NaCl solution (50 µl) in U-type plates. The plate was shaken for 5 sec (Cooke Microshaker). The cells were allowed to settle undisturbed for 2 h at room temperature (a longer time interval did not change the results). The arrow indicates the wells where adhesion of RBC started to decrease as seen by the cell pellet formed at the bottom of the well.

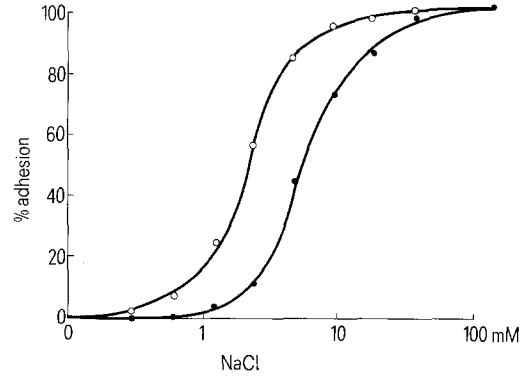

Fig. 2. Adhesion of human red blood cells to polystyrene tubes as a function of NaCl concentration. Adhesion studies were performed in triplicate in 4-ml polystyrene tubes (Milan Instruments S.A., Geneva, Switzerland). RBC (5× 10^7 cells suspended in 100 µl water) were mixed with NaCl solution (100 µl). The suspension was allowed to settle undisturbed at room temperature for 1 h (a longer time interval did not change the results). Water (1 ml) was added and the unattached cells were resuspended by hand shaking. The absorbance of the supernatant was immediately read at 650 nm. Results were not affected by increasing the cell density. RBC (●); neuraminidase-treated RBC (○).

distribution observed in RBC[7]. This would not explain both curves of figure 2 since they are so similar. Although it has been claimed that the oldest circulating RBC have electrophoretic mobilities up to 30% lower than the young cells[8,9], this was not confirmed recently[10]. However the extensive heterogeneity of cell surface carbohydrate of the circulating population of RBC[11] could influence RBC adhesive behavior to polystyrene.

In conclusion, n-RBC are more adhesive than RBC to a saline-polystyrene interface. Adhesion of both type of cells increases with salt concentration. This is consistent with the hypothesis that attractive electrodynamic and repulsive electrostatic forces underlie the behavior of biological cells[1,4,5]. Experiments are underway to assess to implication of these results on hemagglutination assays performed in polystyrene microplates and on the properties of polystyrene microbeads used as cytochemical markers for electron microscopy.

1 A.S.G. Curtis, Biol. Revs (Camb.) *37*, 82 (1972).
2 B. Derjaguin and L. Landau, Acta physicochim. URSS *14*, 633 (1941).
3 E.J.W. Verwey and J.T.G. Overbeek, in: Theory of the Stability of Lyophilic Colloids. Elsevier, Amsterdam 1948.
4 D. Gingell and J.A. Fornes, Biophys. J. *16*, 1131 (1976).
5 D. Gingell, I. Todd and V.A. Parsegian, Nature *268*, 767 (1977).
6 J. Visser, J. Colloid Interface Sci. *55*, 664 (1976).
7 L. Weiss, R. Zeigel, O.S. Jung and I.D.J. Bross, Expl Cell Res. *70*, 57 (1972).
8 D. Danon and Y. Marikovsky, C. r. Acad. Sci. *253*, 1271 (1961).
9 A. Yeari, Blood *33*, 159 (1969).
10 S.J. Luner, D. Szklarek, R.J. Knox, G.V.F. Seaman, J.Y. Josefowicz and B.R. Ware, Nature *269*, 720 (1977).
11 A. Baxter and J.G. Beeley, Biochem. biophys. Res. Commun. *83*, 466 (1978).

The asymmetry of the nucleotide bases and amino acids

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Summary. Due to the electric polarisation induced by the carbon and nitrogen atoms, the molecules of the nucleotide bases present a stereo-asymmetry. The DNA right-handed double helix is determined by the asymmetry of the nucleotide bases and it is concordant with the right-handed α-helix of the polypeptide molecules formed by L-amino acids.

The asymmetry of the nucleotide bases can be defined by 3 orthogonal axes, which form an asymmetric system: a) The (x→x′) axis goes from the external side of the nucleotide base molecule to its internal side, where the hydrogen bonds with the complementary nucleotide base are formed (figure 1, a). b) The (y→y′) axis goes from the ($-NH_2$), or ($-C=O$) groups (situated at the superior pole of the molecule) to the nitrogen atom, situated opposite in the ring of the purine or pyrimidine molecule (figure 1, a). c) The (z→z′) axis is perpendicular on the molecular plan, and passes through the intersection of the 2 other axes.

In the ring of the pyrimidine and purine molecules, there is an alternation of carbon and nitrogen atoms C(1)→N(2)→C(3)→N(4)→ (figure 1, a). The nitrogen atom is more electronegative than the carbon atom[1], with the result that in a covalent bond (C:N) the electronic cloud is deviated from the carbon to the nitrogen atom (C→N). The electric polarization is increased by the presence of the chemical group situated at the superior pole of the nucleotide base[2]. The electric polarization is propagated along the chain of atoms in the direction: $C^+(1)\rightarrow N^-(2)\rightarrow C^+(3)\rightarrow N^-(4)\rightarrow$ (figure 1, a). Due to the presence of the delocalized π-electrons, an electronic current arises in the molecular ring, in the presence of an outer magnetic field. Subsequently, a magnetic molecular moment is also produced[2]. Starting from these elements, we define: a) The superior face of the nucleotide base is the molecular surface at which an observer sees the rotation: $C^+(1)\rightarrow N^-(2)\rightarrow C^+(3)\rightarrow N^-(4)\rightarrow$, following a clockwise direction; b) the inferior face of a nucleotide base is the molecular surface at which an observer sees the above mentioned rotation following a counter clockwise direc-

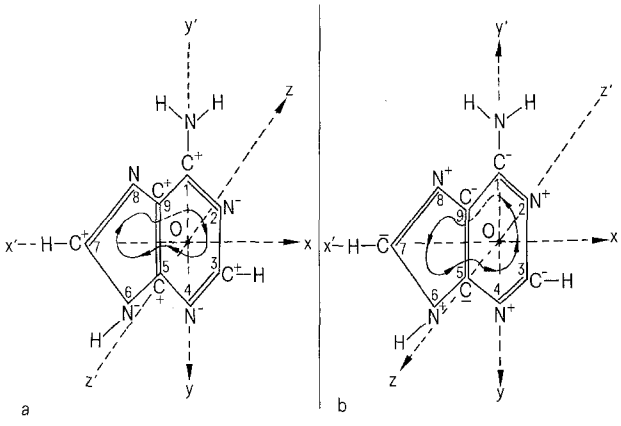

Fig. 1. Adenine (a) and antiadenine (b). (x→x′), (y→y′) and (z→z′), the axes of the molecular asymmetry.

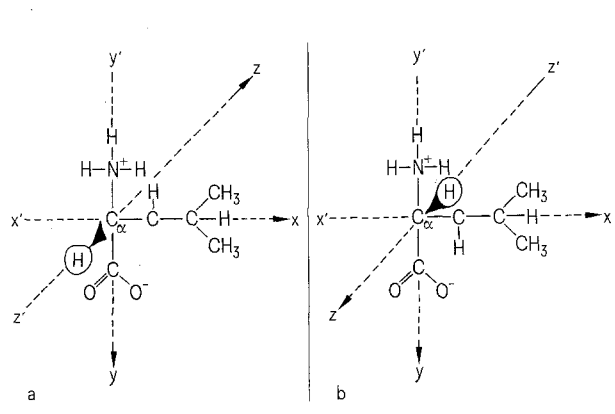

Fig. 2. The asymmetry of the amino acids: a) L-valine; b) D-valine.