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THE INFLUENCE OF DEFORMATION OF TRANSFORMED ERYTHROCYTES DURING FLOW ON THE RATE OF OXYGEN RELEASE

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SUMMARY

1. The deoxygenation rates of transformed erythrocytes were compared with those of normal discocytes by both stopped-flow and continuous-flow methods. Echinocytic and spherostomatocytic transformations were induced by various anionic and cationic drugs, respectively, without altering the oxygen affinity of haemoglobin, the cell volume or the membrane fluidity.

2. The echinocytic transformation reduced the deoxygenation rate at slow-flow velocities (50 cm/sec), as detected by the continuous-flow method. However, at higher flow velocities (150 cm/sec) the rate was similar to that seen in normal discocytes. A close correlation between the degree of echinocytosis, the retardation of deoxygenation rate and the increase of suspension viscosity were observed.

3. Microscopic observation of flowing erythrocytes revealed that the echinocytes scarcely deformed at the slower flow velocity, but clearly deformed at the higher flow velocity to various shapes resembling the flowing discocytes.

4. Transformation to spherostomatocytes had no effect on the deoxygenation rate, which was comparable with that of the discocytes, and even the higher flow force did not induce any deformation.

5. The retarded deoxygenation and the increased viscosity of echinocytes was probably due to an augmented stagnant layer around the cells (i.e. an increase of the hydrodynamic effective volume); this layer was reduced when the echinocytes were deformed with increasing flow force.

INTRODUCTION

It has been known since Roughton's pioneering study (1932), that the velocity of oxygen uptake by erythrocytes depends on the rate of association of oxygen to haemoglobin and on the nature of the oxygen diffusion barrier (Forster, 1964). The latter may be affected by erythrocyte deformation during flow. Therefore, it is physiologically important to study the relation between the velocity of oxygen transfer and the rheological properties of erythrocytes and to analyse the factors influencing the stagnant solvent layer surrounding the flowing erythrocytes.

As a first trial, our effort has been directed to find out the relations between the

rheological properties, the shapes of deformed cells and the rate of deoxygenation of flowing human erythrocytes. At the moment, only extreme cases can be studied *in vitro* due to the limited accuracy of the experimental techniques, i.e. the comparison between the normal discocytes and the drug-induced, transformed erythrocytes. The importance of erythrocyte morphology and deformability during flow is generally recognized (Chien, 1975; Oka, 1981). Morphological alteration of erythrocytes may occur in various diseases (Bessis, 1973), in metabolic ATP depletion (Nakao, Nakao & Yamazoe, 1960), in chemical injuries by various oxidants (Larkin, Kimzey & Siler, 1978; Verweij & Van Steveninck, 1980) or even in *in vivo* ageing (Canham, 1969; Shiga, Maeda, Suda, Kon & Sekiya, 1979*a*) and in *in vitro* blood storage (Rapoport, 1947; Maeda, Kon, Sekiya & Shiga, 1980). Erythrocytes thus impaired, not only decrease the blood flow due to their poor deformability, but are also sometimes poor oxygen transporters.

This paper considers the deoxygenation kinetics (by stopped-flow and continuous-flow methods), the rheological properties (by viscometry) and the deformation during flow (by flash microphotography) of drug-induced echinocytes and spherostomatocytes. The functional impairment of the echinocytes will be discussed in detail with emphasis on the stagnant layer.

METHODS

Preparation of erythrocytes

Fresh human blood was obtained from the cubital vein and was heparinized. After removal of plasma and the buffy coat, the erythrocytes were washed three times with phosphate-buffered saline: 99·4 mM-NaCl, 37·8 mM-Na_2HPO_4, 7·2 mM-NaH_2PO_4, 5·0 mM-KCl and 5·5 mM-D-glucose, pH 7·42.

Preparation of echinocytes and spherostomatocytes

The following chemicals were used for inducing echinocytosis (i.e. the morphological changes from a biconcave disk to a sphere covered with crenations or spicules, as defined by Bessis, 1973): trichlorobenzene sulphonate (TCBS; Tokyo Kasei Co.), dehydroepiandrosterone sulphate (DHAS; Sigma Chemical Co.), 4-acetamido-4′-isothiocyano-2,2′-disulphonic stilbene (SITS; ICN Pharm. Inc.) and 4,4′-diisothiocyano-2,2′-disulphonic stilbene (DIDS; Pierce Chemical Co.). These were used in concentrations of less than 6·0, 0·26, 0·20 and 0·20 mM, respectively. The washed cells were suspended in phosphate-buffered saline containing various concentrations of drugs at final haematocrit values of 0·7 or 1·8%. The mixture was incubated for 10 min at 37 °C.

Spherostomatocytosis (i.e. the shape changes to a sphere with irregular small hila, as defined by Bessis, 1973) was induced by isoxsuprine (Daiichi Seiyaku Co.). Washed cells were incubated with 3·6 mM-isoxsuprine for 30 min at 37 °C at a haematocrit of 1·8%.

The degree of transformation was controlled by the concentration of drugs in the incubation medium.

Measurement of the deoxygenation rate by the stopped-flow method

The deoxygenation rate of erythrocytes was measured at pH 7·42 at 26·5 °C by mixing an air-saturated erythrocyte suspension (haematocrit, 1·8%) and a deoxygenated hydrosulphite solution (25 mM), using a stopped-flow apparatus (Union Giken Co., RA 401) operated at 577 nm.

Measurement of the deoxygenation rate by the continuous-flow method

A continuous-flow apparatus was constructed and attached to a recording spectrophotometer (Union Giken Co., SM 401). Two plastic containers (60 ml. in volume) were connected to a mixing unit (double two-jet mixer, Union Giken Co., MX 7) by Teflon tubes (inner diameter, 1·5 mm). The top ends of the containers were connected to a N_2 gas reservoir which provided the driving pressure.

The outlet of the mixing unit was connected to a flat cell in the spectrophotometer by a Teflon tube (inner diameter, 2 mm). The dimension of the flat cell was 5 mm (width) × 10 mm (height) × 0·6 mm (depth, i.e. the light path) with a spindle-shaped inlet and outlet.

In order to measure the deoxygenation rate, the erythrocyte suspension (haematocrit, 0·7 %) in one of the containers and the deoxygenated hydrosulphite solution (25 mM) in the other were mixed and introduced into the flat cell at a constant-flow velocity controlled by the pressure of the N_2 gas. The degree of deoxygenation (percentage of deoxygenated haemoglobin) at various reaction times was obtained by adjusting the length of the tube (2–160 cm) connecting the mixing unit and the flat cell and was estimated from the difference in absorbance between 577 and 586 nm (the range of absorption spectra studied was 565 to 590 nm at a scan speed of 2 nm/sec). The erythrocyte suspension equilibrated with 1 atm of O_2 was used as a standard 100 % oxygenated sample.

The light scattering of the erythrocyte suspension was cancelled by dimming the reference beam as described previously (Kon, Maeda, Sekiya, Shiga & Suda, 1980). The reliability for determining the degree of deoxygenation was ascertained as follows. (i) The spectral changes accompanying deoxygenation of the erythrocyte suspension showed isosbestic points at 570 and 586 nm, in spite of the light scattering and the flattening effect (Duysens, 1956). Furthermore, the differences in absorbance between 577 nm and these isosbestic points for both deoxygenated and oxygenated samples were linearly related to the haemoglobin concentration (up to 23 μM-tetramer), though the molar absorption coefficients were slightly reduced (Kon *et al.* 1980). (ii) The spherostomatocytic transformation did not modify the absorption spectra in the above range. However, the difference in absorbance between 577 and 586 nm obtained with the echinocyte suspension was slightly lower (*ca.* 4 %) than that of the discocytes. This did not affect the estimation of percentage deoxygenation, because the isosbestic points were the same as those observed for discocytes, and the linearity between the difference in absorbance and the haemoglobin concentration was maintained.

Microscopic observation of flowing erythrocytes

In order to observe the shape of flowing erythrocytes, a microscope with a flash-photography facility was combined with the continuous-flow apparatus.

The flow system, mounted on the stage of the microscope (Olympus, type BH), consisted of the flow channel and the supporting base. The flow channel was moved in the opposite direction to the flow of the erythrocyte suspension, along the groove of the supporting base by the power of a spring. By exchanging the spring and by varying its expanded length, the speed of movement was adjusted to be the same as the flow velocity of the erythrocyte suspension; thus, the flowing erythrocytes were apparently fixed under the microscope. The dimension of the flow channel was 2 mm (width) × 20 cm (length) × 1·5 mm (depth). The distance between the mixing chamber and the flow channel was 40 cm. During flow, a light flash of 4 μsec duration (Stroboscope MS-203, Sugawara Manuf.) was triggered, and the photographs were taken by the auto-photographing apparatus (Olympus, model PM-10AD) using Kodak recording film (type 2475).

Oxygen dissociation curve

The oxygen dissociation curve of erythrocytes at pH 7·42 was recorded according to the method of Kon *et al.* (1980).

Electron paramagnetic resonance (e.p.r.) spectroscopy

Two kinds of fatty acid spin labels, 2-(10-carboxydecyl)-2-hexyl- and 2-(14-carboxydecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl (abbreviated as 12 NS and 16 NS, respectively; Syva Co.) were used. After spin labelling as described previously (Shiga, Suda & Maeda, 1977), the cells were incubated with drugs in the phosphate-buffered saline at pH 7·42 for 10 min at 37 °C. The e.p.r. spectra of packed cells in a capillary sample tube were measured by a Varian E-3 spectrometer.

Viscometry

The apparent viscosity of erythrocyte suspensions was measured by a cone-plate viscometer (Tokyo Keiki Co., model E) at 19·5 °C. The haematocrit of the erythrocyte suspension was adjusted to 37 %.

Scanning electron microscopy (s.e.m.)

Erythrocytes treated with various concentrations of drugs were immersed in phosphate-buffered saline containing 1 % (v/v) glutaraldehyde for 1 hr at room temperature. After washing with buffer, the cells were further fixed with 1 g % (w/v) OsO_4 in the phosphate-buffered saline. After washing with water, the cells were dried by air, and coated with platinum. A scanning electron microscope (Hitachi, S-5000A) was used. The morphological index described by Fujii, Sato, Tamura, Watatsuki & Kanaho (1979) was adopted to express the degree of transformation (Kon, Maeda & Shiga, 1982), following cell counts on the s.e.m. photographs ($n > 50$).

RESULTS

Deoxygenation rate measured by the stopped-flow method

The deoxygenation rate was affected by the echinocytic transformation. As the degree of echinocytosis (expressed by the morphological index) increased, the deoxygenation rate (expressed by the half deoxygenation time, $t_{\frac{1}{2}}$) was retarded. Fig. 1*A* shows that $t_{\frac{1}{2}}$ was *ca.* 100 msec for normal discocytes, but prolonged to *ca.* 200 msec for the echinocytes. The rate was entirely dependent on the shape at rest but was independent of the kind of anionic drug used, although the drugs differed in their effectiveness to form echinocytes (Fig. 1*B*).

On the other hand, the deoxygenation rate was unaffected in the spherostomatocytes induced by 3·6 mM-isoxsuprine.

The oxygen equilibrium curves of these transformed erythrocytes were similar to those of normal discocytes, i.e. the oxygen affinity and the haem–haem interaction were the same. Also, no alterations of intracellular pH and 2,3-diphosphoglycerate concentrations were observed. In addition, the mean corpuscular volume and the mean corpuscular haemoglobin concentration were unchanged by drug treatments.

Deoxygenation rate measured by the continous-flow method

In order to observe the early stage of oxygen release (i.e. 10–20 % deoxygenation) by the continuous-flow apparatus, the reaction rate needed to be reduced, thus the experiment was carried out at a lower temperature (19·5 °C) and at a slightly higher pH (pH 7·56).

The deoxygenation process of the discocytes occurred in an exponential manner. The semi-logarithmic plot gave a straight line (down to 40 % deoxygenation), consistent with the results of the stopped-flow experiment. The rate constant was not altered by the change of flow velocity from 50 to 150 cm/sec (Fig. 2*A*).

The results obtained with spherostomatocytes were identical to those obtained with discocytes (Fig. 2*B*).

The deoxygenation rate of the echinocytes was significantly retarded at the slow-flow velocity (50 cm/sec), compared with those rates measured at higher flow velocity (150 cm/sec) where the rate was similar to that of discocytes (Fig. 2*C*).

To clarify the relation between the degree of echinocytosis and deoxygenation rate, the percentage of deoxygenation at a reaction time of 330 msec after mixing (at a flow velocity of 50 cm/sec) was determined (Fig. 3): with increasing degree of echinocytosis (by increasing TCBS concentrations), the deoxygenation rate decreased. Further, the percentages of deoxygenation at *ca.* 350 msec after mixing were

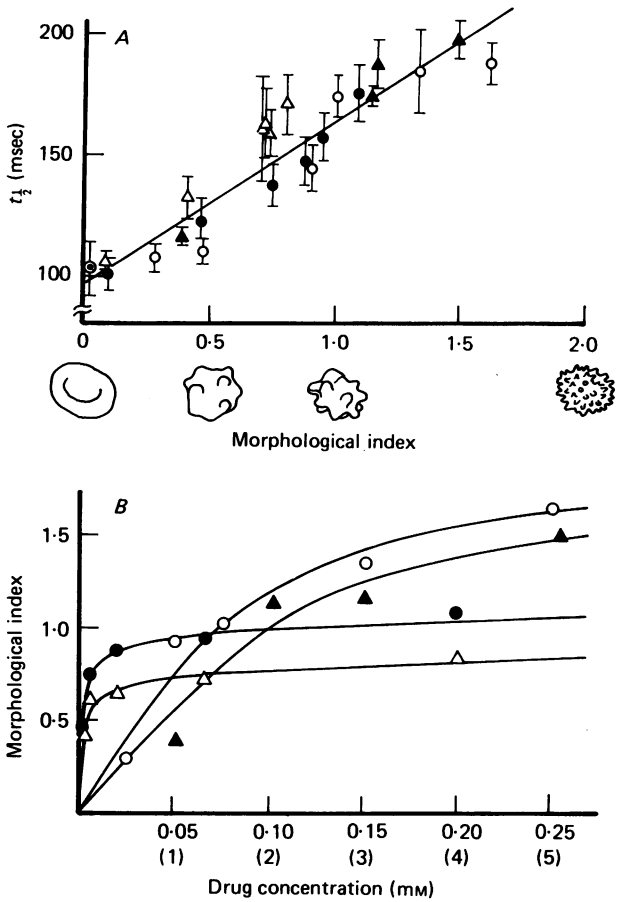

Fig. 1. Relation between echinocytic transformation and deoxygenation rate measured by the stopped-flow method. *A*, effect of echinocytosis on the deoxygenation rate. Echinocytosis was induced by various concentrations of anionic drugs: TCBS, (○); DIDS, (●); SITS, (△) and DHAS, (▲), as shown in *B*. Control discocytes (⊙). The degree of echinocytosis is expressed by a morphological index calculated on the basis of the shape as shown on the abscissa. The deoxygenation rate is expressed as the half time of deoxygenation (±S.D.) measured in phosphate-buffered saline (pH 7·42) at 26·5 °C. *B*, the effect of increasing concentrations of anionic drugs on the morphological index. Symbols as in *A*. The numbers in parenthesis on the abscissa indicate the concentrations of TCBS.

dependent on the flow velocity (Table 1): as the flow velocity decreased, the deoxygenation of the echinocytes was gradually retarded.

Microscopic observation of flowing erythrocytes

Erythrocytes were photographed in the continuous-flow channel attached to the microscope. Representative pictures of flowing discocytes, spherostomatocytes and echinocytes are shown in Pl. 1.

The discocytes were deformed to various shapes by flow force (such as parachute-like, slightly-folded ellipsoidal, flattened, etc.) even at the slow-flow velocity (50 cm/sec).

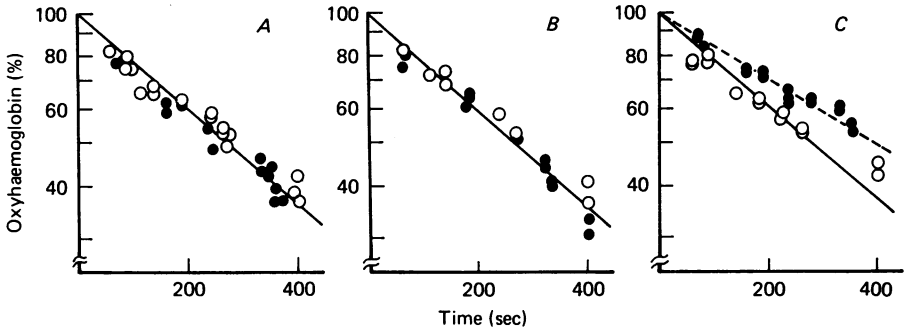

Fig. 2. Semi-logarithmic plots showing the rate of deoxygenation of *A*, discocytes; *B*, spherostomatocytes and *C*, echinocytes, as measured by the continuous-flow method. Spherostomatocytes and echinocytes were prepared by 3·6 mM-isoxsuprine and 6·0 mM-TCBS, respectively. The measurement was carried out at 19·5 °C in the phosphate-buffered saline (pH 7·56). The flow velocities were 50 cm/sec (●) and 150 cm/sec (○). The continuous line in *B* and *C* is that of discocytes, *A*.

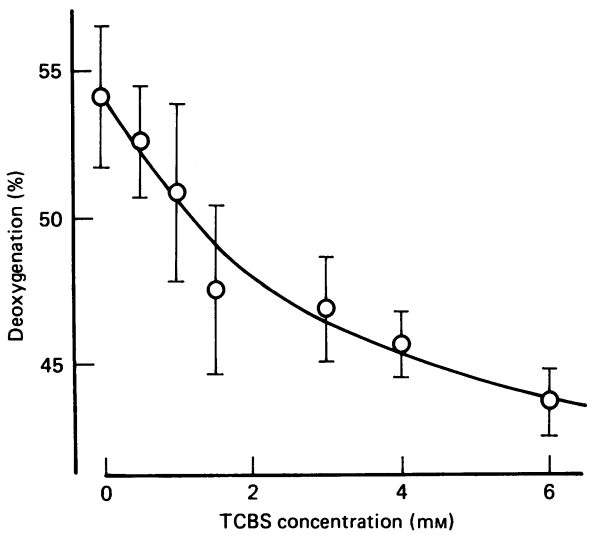

Fig. 3. Effect of increasing degrees of echinocytosis on the deoxygenation rate. The degree of deoxygenation (%) of echinocytes induced by various concentrations of TCBS was measured at a flow velocity of 50 cm/sec and at a reaction time of 330 msec after mixing with 25 mM-hydrosulphite solution. Other experimental conditions as in Fig. 2.

The spherostomatocytes were not deformed even at the high-flow velocity (150 cm/sec).

The echinocytes scarcely deformed and their spicules persisted at slow-flow velocity (50 cm/sec). However, at the higher flow velocity (150 cm/sec), they were deformed to shapes similar to (though variable) those observed for the discocytes, and most of the spicules were smoothed.

These deformations were reversible, i.e. after sudden cessation of flow, the shape of the erythrocytes was restored to that seen at rest; the relaxation time could not be determined by our apparatus.

TABLE 1. Effect of flow velocity on the deoxygenation of echinocytes

Flow velocity (cm/sec)	Reaction time (msec)	Deoxygenated haemoglobin (%)		
		Discocytes (*A*)	Echinocytes (*B*)	*B/A*
50	330	52·4 ± 1·2	40·8 ± 0·9	0·779
76	370	57·1 ± 1·1	51·3 ± 1·0	0·898
110	350	54·9 ± 1·3	47·3 ± 1·1	0·862
150	380	56·7 ± 1·2	54·3 ± 1·1	0·958

The degree of deoxygenation of (*A*) discocytes and (*B*) echinocytes (induced by 6·0 mM-TCBS) after mixing with deoxygenated hydrosulphite (25 mM) measured at various flow velocities but at approximately constant reaction time by the continuous-flow method. Mean ± S.D., $n = 4$.

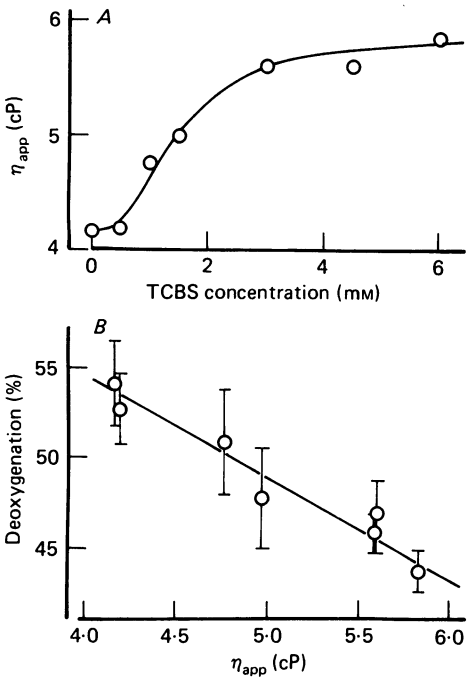

Fig. 4. Relation between the morphology of erythrocytes, the suspension viscosity and the deoxygenation rate. *A*, the suspension viscosity of echinocytes induced by various concentrations of TCBS (haematocrit, 37 %). The measurements were performed at a shear rate of 376/sec at 19·5 °C. *B*, relation between the deoxygenation rate and the suspension viscosity, on the basis of results in Fig. 3 and Fig. 4*A*.

Suspension viscosity of the transformed erythrocytes

The viscosity of echinocytes in suspension is higher than that of discocytes (Meiselman, 1978; Suda, Shimizu, Maeda & Shiga, 1981; Kon, Maeda, Suda, Sekiya & Shiga, 1983; Suda, Maeda, Shimizu, Kamitsubo & Shiga, 1982). In order to examine the relation between the deoxygenation rate and the rheological behaviour, the change of viscosity following echinocytic transformation was also measured.

At increasing TCBS concentrations, the viscosity of the suspension increased (Fig. 4*A*). In addition, a remarkably good correlation was apparent between the

deoxygenation rate (Fig. 3) and the viscosity of the suspension of echinocytes (Fig. 4*A*), as shown in Fig. 4*B*.

In contrast to this, a decreased viscosity of suspensions of spherostomatocytes has been described by Suda *et al.* (1981, 1982).

Fluidity of the membrane lipid portion of the transformed erythrocytes

E.p.r. spectra of 16 NS and 12 NS, incorporated into the lipid portion of the erythrocyte membrane, were compared at various temperatures. No differences were observed between discocytes and echinocytes. Only for DHAS-induced echinocytes, a decreased correlation time of 16 NS at lower temperatures was found (see Kon *et al.* 1982).

Also, no alterations in e.p.r. spectra of the spin labels were observed for the isoxsuprine-induced spherostomatocytes (Suda *et al.* 1981).

DISCUSSION

The present results have demonstrated that functional impairments (of both rheology and oxygen transport) of echinocytes paralleled the degree of morphological alterations. However, no functional changes were found in spherostomatocytes. These findings may give an insight into the correlation between the deoxygenation rate and the flow properties on one side and the morphology of the erythrocytes on the other side.

Main factors affecting the deoxygenation rate measured by the stopped-flow method

Since Roughton (1932), it has been known that the factors affecting the velocity of oxygen release from erythrocytes are: (a) the chemical reaction between haemoglobin and oxygen; (b) the oxygen diffusion barrier: first, inside the erythrocytes, secondly in the erythrocyte membrane and thirdly outside the erythrocytes. In (a) the intracellular factors affecting the rate of haemoglobin–oxygen binding (the 2,3-diphosphoglycerate concentration and pH) were not altered by the drugs used. Consequently, the oxygen equilibrium curves of the transformed erythrocytes were quite similar to those of the normal discocytes.

First in (b), the cell volume and the intracellular haemoglobin concentration are known to modify the intracellular oxygen diffusion by altering the diffusion distance and the intracellular viscosity, respectively (Carlsen & Comroe, 1958; Sirs, 1963; Harrington, Elbaum, Bookchin, Wittenberg & Nagel, 1977; Kon *et al.* 1980). Both these factors were found to be similar in the transformed cells and in the normal discocytes.

Secondly, the alteration of membrane organization affects the deoxygenation rate, e.g. retarded deoxygenation in cholesterol-loaded erythrocytes (Shiga, Maeda, Suda, Kon, Sekiya & Oka, 1979*b*; Shiga, Maeda, Suda, Kon & Sekiya, 1982; Suda, Maeda & Shiga, 1980). In the present experiments, the membrane fluidity of the lipid portion was not modified by any of the drugs except DHAS, as observed in the e.p.r. spectra of fatty acid spin labels.

In addition, the echinocyte-inducing anionic drugs used here are known to bind with band 3 protein, an anion transport protein (Cabantchik, Knauf & Rothstein,

1978; Kon *et al.* 1982), but no correlation between inhibition of anion transport and retarded deoxygenation rate has been observed (Kon *et al.* 1982). The effect of DHAS on membrane fluidity and erythrocyte deoxygenation has been discussed in detail elsewhere (Kon *et al.* 1982).

Thirdly, the importance of the stagnant water layer surrounding the erythrocytes has been emphasized frequently. However, in a stopped-flow apparatus (Sirs, 1963; Holland & Forster, 1966; Coin & Olson, 1979; Rice, 1980), the size and form of the stagnant layer may undergo a time-dependent change. Also, conditions in the resting condition (Weingarden, Mizukami & Rice, 1982*a*; Weingarden, Mizukami & Rice, 1982*b*) may not be applicable for investigation of the relation between the rheological behaviour and the deoxygenation process of erythrocytes.

Suspension viscosity and the hydrodynamic effective volume of erythrocytes

The transformation to echinocytes increased the suspension viscosity (Fig. 4*A*), as was already known (Meiselman, 1978; Suda *et al.* 1981; Kon *et al.* 1983; Suda *et al.* 1982). In contrast, the suspension viscosity of the spherostomatocytes decreased slightly, as described elsewhere (Suda *et al.* 1981, 1982).

The viscosity of particles in suspension depends on the volume fraction (ϕ) of the particles, though the simple Einstein equation,

$$\eta_{\text{suspension}}/\eta_{\text{solvent}} = 1 + 2{\cdot}5\phi,$$

cannot be applied to the suspension of deformable erythrocytes due to the shear thinning (Oka, 1981). The increased suspension viscosity of the hardened erythrocytes has been explained by a disturbance of the stream-lines in the vicinity of the cells due to the increased hydrodynamic effective volume (Chien, 1975). Regarding shear forces, both discocytes and spherostomatocytes deform easily as observed by the rheoscope, whereas echinocytes hardly deform and their spicules are rarely smoothed, as previously reported (Suda *et al.* 1982).

In short, as the number and the size of spicules increased in the process of echinocytosis, the hydrodynamic effective volume of the cells increased, probably due to the augmentation of the stagnant layer, and consequently the suspension viscosity increased.

Relation between the shapes of flowing erythrocytes and the deoxygenation rate

The observed deformation of erythrocytes during flow confirmed the relation between cell shape and deoxygenation rate.

(*i*) *Discocytes.* The flowing discocytes showed various kinds of deformation, and the shapes resembled those observed in *in vivo* capillaries (Brånemark & Bagge, 1978; Bagge, Brånemark, Karlsson & Skalak, 1980), in spite of the differences in the geometry of the flow pathway, the flow velocity, the stream-lines, and so forth. The degree of deformation did not progress with further increases of flow velocity (Pl. 1) Miyamoto & Moll (1972) observed no deformation of discocytes flowing at 150 cm/sec. The discrepancy with our results may be due to geometrical differences of the flow channel and to the photographic resolution.

In the present experiments, the deoxygenation rate was not accelerated by increasing the flow velocity from 50 to 150 cm/sec (Fig. 2*A*). This finding is

contradictory to that of previous reports, in which the oxygenation rate was accelerated by increasing the flow velocity (Koyama & Mochizuki, 1969) and by increasing the Reynolds number (Rice, 1980). This discrepancy may arise from the different methods used; the deoxygenation rate was in our experiments determined using hydrosulphite, which reduces the thickness of the stagnant layer (Coin & Olson, 1979) and thus makes our method rather less sensitive than others.

No acceleration of oxygen release and no further shape changes were observed when the flow velocity was increased from 50 to 150 cm/sec in our experimental conditions.

(*ii*) *Spherostomatocytes.* These cells did not deform during flow. The deoxygenation rate agreed with that of normal discocytes and was independent of the flow velocity.

(*iii*) *Echinocytes.* The deoxygenation rate was clearly related to the degree of echinocytosis (Fig. 3) and to the flow velocity (Table 1). At slow flow, the cells were scarcely deformed and many spicules still persisted, while at the higher flow, the cells were deformed to various shapes resembling the flowing discocytes and the spicules tended to disappear (Pl. 1).

It appears that the retarded deoxygenation rate of the echinocytes results from the increased thickness of the stagnant layer at the slow-flow velocity, and that the stagnant layer is reduced at the higher-flow velocity when the cells are deformed. The close relation between the suspension viscosity and the deoxygenation rate observed for the echinocytes (Fig. 4*B*) may be explained by their larger effective volume caused by the development of spicules, which increases the thickness of the stagnant layer.

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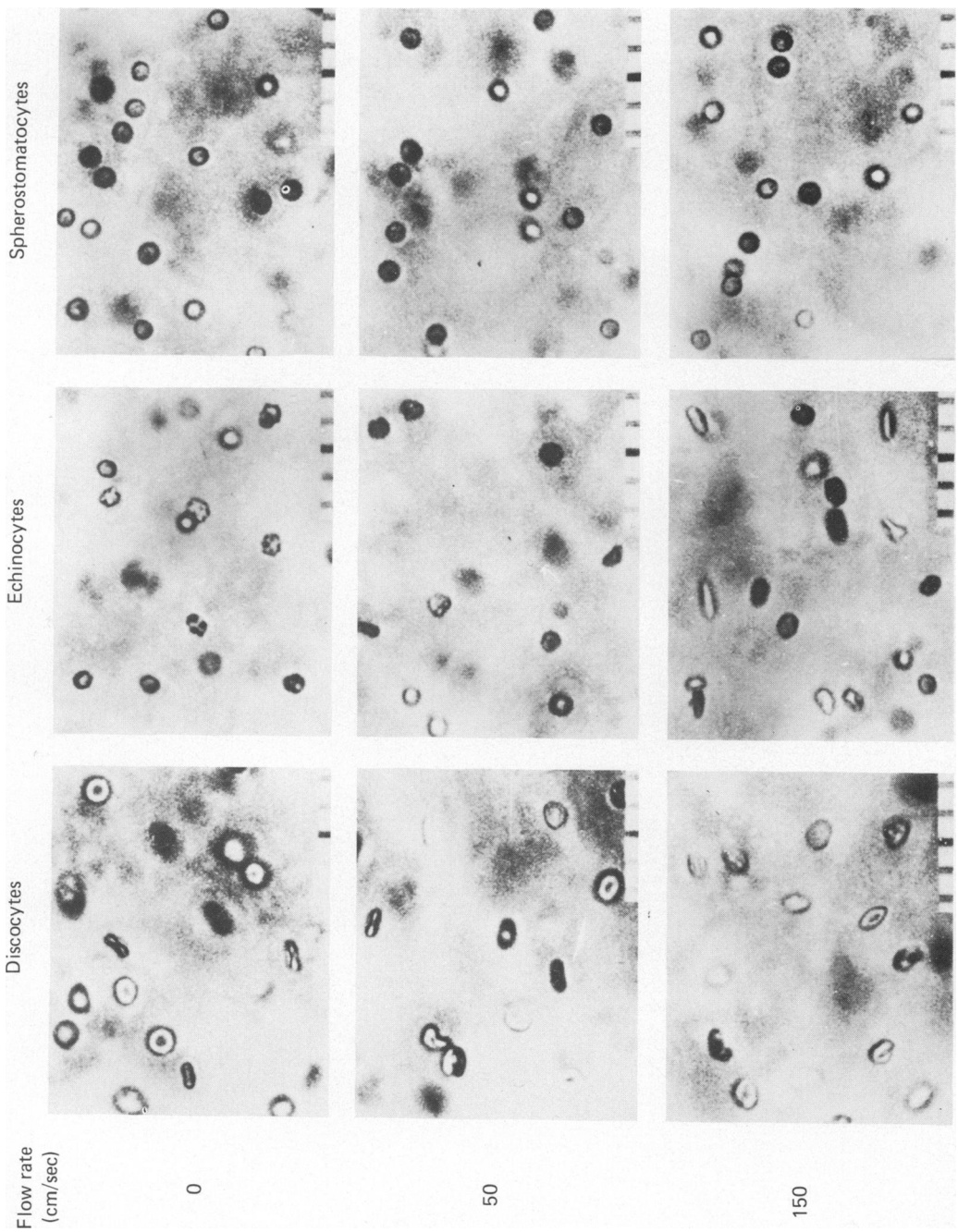
EXPLANATION OF PLATE

Microscopic observation of flowing erythrocytes. Observations were carried out at rest and at flow velocities of 50 and 150 cm/sec, as described in the text. The transformed cells were prepared as shown in Fig. 2. The direction of flow is from left to right in the photographs. Scale shown in the right-hand corner of each photograph is graduated in 10 μm.


Spherostomatocytes
Echinocytes
Discocytes
Flow rate (cm/sec)
0
50
150