Drift of an erythrocyte flow line due to the magnetic field

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Summary. Drifts of erythrocyte-flow lines due to inhomogeneous magnetic field in a laminar flow in a buffer solution are shown for the first time, and are interpreted as being due to the paramagnetism of hemoglobins included in the erythrocytes. The drifts were dependent on the hematocrit of the flowing erythrocyte suspension.
Key words. Erythrocyte flow; effect of magnetic field; inhomogeneous magnetic field; paramagnetism.

The biological effects of the magnetic field have attracted the attention of many researchers in recent years[1]. The effects of the magnetic field on chemical and biological systems are usually so small that not only the mechanisms but the effects themselves are not always clear. Since deoxygenated hemoglobin in the venous blood is paramagnetic[2,3], the effect of the magnetic field on the blood circulation might be one of the possible mechanisms by which the magnetic field exerts effects on humans and animals. In the present study, we show that an inhomogeneous magnetic field affects the erythrocyte flow distinctly. An erythrocyte-flow line (diameter = 80–120 μm) was made in a rectangular observing cell (0.4 mm × 4 mm × 150 mm), and the drift and the broadening caused by the magnetic field were observed under a microscope. This drift of the erythrocyte-flow line was dependent on the strength (and the gradient) of the magnetic field, on the electronic state of the hemoglobin, and on the hematocrit value of the erythrocyte suspension.

Human erythrocytes collected from the fresh venous blood of healthy donors were treated in the standard manner[4] to obtain erythrocytes containing hemoglobin of different electronic states, i.e. oxygenated state [Fe(II), S = 0], deoxygenated state [Fe(II), S = 2], oxidized state (methemoglobin) of high spin configuration [Fe(III), S = 5/2], and oxidized state with cyanide ion as a ligand (cyanomethemoglobin) [Fe(III), S = 1/2]. Figure 1 shows the drift and the broadening of the flow line of erythrocytes containing deoxygenated hemoglobin, due to interaction with the inhomogeneous magnetic field. The drift increased upon increasing the product of the magnetic flux density (B_z) and its gradient (dB_z/dz). The dependence of the drift on the electronic state of hemoglobin is given in the table, and it is approximately proportional to the square of the magnetic moment of the hemoglobin monomer.

The force (F_{mag}) which acts on the paramagnetic material of a volume (V) in an inhomogeneous magnetic field is represented below for the present experimental setup[5].

$$F_{mag} = \chi V \mu_w^{-1} B_z \, dB_z/dz, \quad (1)$$

where χ is the paramagnetic susceptibility of the material and μ_w is the magnetic permeability of water. Because the diamagnetic susceptibility of erythrocytes is almost equal to that of water, the contribution of diamagnetism can be neglected. According to Curie-Langevin's law, χ is proportional to the square of the magnetic moment(M) of the molecule constituting the paramagnetic material. Because the dependence of the drift of the erythrocyte-flow line on both $B_z \times dB_z/dz$ and M are the same as those predicted from Eq. 1 and from Curie-Langevin's law, the drift can be attributed to the interaction of the paramagnetism of hemoglobin with the inhomogeneous magnetic field. Although the Lorentz force also acts on flowing erythrocytes in a magnetic field, the effect calculated (1.0×10^{-3} μm drift at the observing point; -6.4×10^{-16} C for the charge per erythrocyte[6] was used) is much smaller than that due to the above mechanism, and the direction of the force should be tilted by 90° from the detected drift. Moreover the effect due to the Lorentz force should not depend on the electronic state nor on the magnetic field gradient, contrary to the experimental results.

The drift velocity (u) of a single erythrocyte due to an external force (F_{ext}) is given by the Stokes Equation, thus:

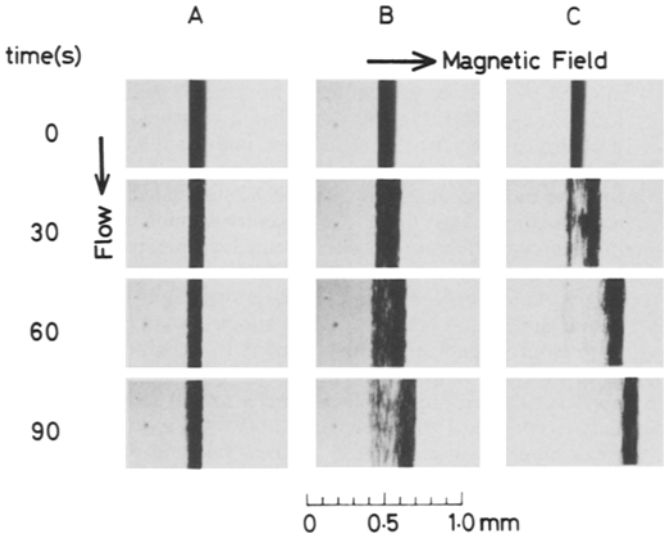

Figure 1. Drift of the flow line in the inhomogeneous magnetic field. The flow line is a stream of erythrocytes containing deoxygenated hemoglobin in a colorless buffer under laminar flow conditions. The directions of the flow and the magnetic field were vertical (downward) and horizontal as indicated in the figure. The product of the strength and the gradient of the magnetic field ($B_z \times dB_z/dz$) for each experiment is; (A): 1.5 T^2/m; (B): 15 T^2/m; (C): 29 T^2/m. Those were the averaged values along the path of the flowing erythrocytes. The flow velocity was 0.7 mm/s, the hematocrit value of the flowing erythrocyte suspension was 6.0%, and the diameter of the flow line was about 100 μm.

The details of the experiments are as follows: A narrow erythrocyte flow was made in a magnetic field in a relatively wide rectangular glass cell (0.4 mm × 4 mm × 150 mm). The erythrocyte suspension was ejected out from a narrow needle and ran initially at a high speed (150 mm/s) in a laminar flow of the isotonic buffered saline. The flow was decelerated at time zero to a desired speed and the flow line was recorded on photographs. The drift at time zero was neglected because at such a high speed the drift was not detectable. The erythrocyte suspension in the reservoir was stirred and circulated in a loop continuously to prevent sedimentation, a small part of which was taken into the needle. The photographs were taken under a microscope (Olympus SZ-TR-I, Japan). Drifts and the broadenings of the erythrocyte flow line were measured on the photographs.

The inhomogeneous magnetic field was made using a magnet of a Varian E-3 EPR spectrometer (∅ = 10 cm, gap = 4 cm) and an iron block, one side of which tapered by about 53°.

$$u = F_{ext}/6\pi\eta \, R, \quad (2)$$

where η is the viscosity of the medium and R is the hydrodynamic radius of the erythrocytes. This equation, combined with Eq.1, gives only the drift of 0.12 μm/s [$\chi = 0.26 \times 4\pi \times 10^{-6}$, $R = 3.0$ μm, $\eta = 1.0 \times 10^{-3}$ Ns/m^2, $V = 9.0 \times 10^{-11}$ cm^3, $B_z \times dB_z/dz = 29$ T^2/m], which is much smaller than the observed value of about 2.6 μm/s for erythrocytes containing high-spin methemoglobin.

To resolve this discrepancy the dependence of the drift on the hematocrit value of the flowing erythrocyte suspension was investigated. As shown in figure 2, the approximate proportional-

Dependence of the drift of the erythrocyte-flow line on the electronic state of hemoglobin[a]

	Oxy	CN-met	Deoxy	Met
Drift (μm/s)	0	0.38	2.0	2.6
M (μ_B)	0	2.2	5.3	5.8
M^2	0	4.8	28.1	33.6

[a] M represents the magnetic moment of hemoglobin in units of Bohr magneton(μ_B)[2,3,7]. Erythrocytes containing oxygenated hemoglobin (Oxy) were obtained by washing and suspending the erythrocytes in a isotonic buffered saline (pH 7.7). Erythrocytes containing deoxygenated hemoglobin (Deoxy) were prepared by suspending the washed erythrocytes in the solution of 25 mM sodium hydrosulfite. In the experiment with deoxygenated erythrocytes, all the solutions in the flow apparatus were deaerated by bubbling nitrogen gas. The washed erythrocytes were treated with $NaNO_2$ (20 mM) to oxidize hemoglobin and washed five times and then suspended in 50 mM phosphate buffered saline of pH 5.7 to retain the electronic configuration in the high spin state (Met). Erythrocytes containing cyano-methemoglobin (CN-met) were prepared by incubating the washed erythrocytes containing methemoglobin, with KCN (10 equivalents of the total heme) and washed three times before suspending in the buffered saline of pH 7.4. The diameter of the flow line was about 80 μ.

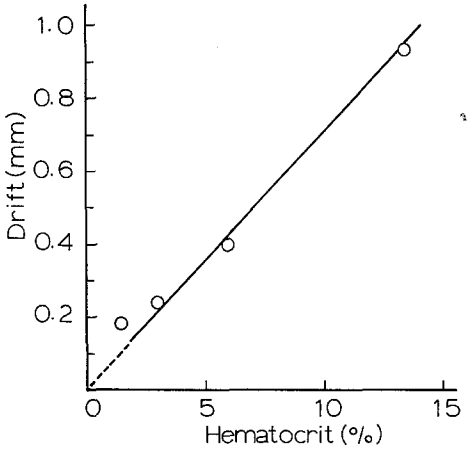

Figure 2. Dependence of the drift of the erythrocyte-flow line on the hematocrit value. The erythrocytes containing high spin methemoglobin was allowed to flow during 150 s in the magnetic field whose averaged strength and gradient were 0.6 T and 48 T/m, respectively. The hematocrit value of the flowing erythrocyte suspension was obtained as follows: (1) The erythrocyte suspension, which flowed out from the observing cell, was collected, and the density of erythrocytes was determined by counting the erythrocytes in it. The volume fraction of the erythrocyte flow in the bulk flow of the buffer was calculated using the diameter of the erythrocyte flow (circa 80 μm) and the theoretical velocity distribution in a rectangular cell (of infinite width).

ity of the drift to the hematocrit value indicates that the drift is very much amplified by the hydrodynamic interaction among the erythrocytes. This fact may imply that a certain volume of the erythrocyte suspension is attracted as a whole by the force represented by Eq. 1; in this case V and χ represent the volume and the magnetic susceptibility of the mass of the flowing erythrocyte suspension. This volume may be approximated as a sphere whose diameter is that of the erythrocyte-flow line. If 50 μm for R and 0.06 χ (the magnetic susceptibility of the erythrocyte) for the susceptibility are adopted for the suspension of 6% hematocrit, we obtain 2.0 μm/s for the drift velocity, which is in good agreement with the observed value.

In the actual flow line, there may be a little inhomogeneity in the distribution of erythrocytes. In addition, a flow like an eddy current of the flowing buffer, due to the drift of the erythrocyte flow, may occur. These factors may cause the broadening of the erythrocyte-flow line.

The physiological effects of this phenomenon, such as drift and broadening of the erythrocyte flow caused by the magnetic field, are not clear at the present stage. However, we would suggest that the rheological properties of erythrocytes in the venous system might be influenced by the inhomogeneous magnetic field.

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An antiviral factor from *Melia azedarach* L. prevents Tacaribe virus encephalitis in mice[1,2]

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Summary. Treatment of neonatal mice with an antiviral factor, (AVF), obtained from the leaves of *Melia azederach* L. protected them against lethal encephalitis caused by Tacaribe virus inoculation. The degree of protection obtained varied from 66% to 100% depending on the virus dose. Similarly, administration of AVF to nursing mothers protected their offspring from developing virus encephalitis. AVF does not directly inactivate Tacaribe virus; it inhibits an early step (s) in the replication process in cell cultures.
Key words. Antiviral activity; Tacaribe virus; *Melia azedarach* L.; arenavirus; viral encephalitis.