Orientation of Erythrocytes in a Strong Static Magnetic Field

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The frequency of exposure to strong magnetic fields has increased as the magnetic-resonance image-diagnostic technique (MRI) and passenger transport systems based on the principle of magnetic levitation have come into wider use. Accordingly, it has become necessary to more systematically assess their influence on the body and set strict guidelines on acceptable limits of magnetism exposure. Therefore, we have assessed the influence of an uniform static magnetic field (8 T in maximum) on normal erythrocytes. The erythrocytes were oriented with their disk plane parallel to the magnetic field direction. These erythrocytes were influenced even by 1 T and almost 100% of them were oriented when exposed to 4 T. Furthermore, the degree of orientation was not influenced by the state of hemoglobin (oxy: diamagnetic, deoxy and met: paramagnetic). The dependence of the measured degree of orientation on the intensity of the magnetic field was in good agreement with the theoretical equation for the magnetic orientation of diamagnetic substances. As a result of a numerical analysis based on the equation, the anisotropic diamagnetic susceptibility of erythrocytes was found to be \( \Delta X = 8 \times 10^{-22} \text{ electromagnetic units/erythrocyte} \). It was almost in agreement with the calculated value \( \Delta X = 6 \times 10^{-22} \text{ emu/erythrocyte} \) estimated from the diamagnetism of the membrane constituents of erythrocyte.

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MAGNETIC FIELDS have long been assessed for their beneficial and adverse influence on the body and applied to various aspects of medical treatment. However, only a few attempts have been made to scientifically determine their effects or elucidate the mode of action. On the other hand, the frequency of exposure to strong magnetic fields has increased with the rapid advances in science and technology, such as magnetic-resonance image diagnosis (MRI) and passenger transport systems based on the principle of magnetic levitation. Therefore, it has become necessary to more systematically elucidate the influence of magnetic fields on the body. A number of excellent reports have in recent years been presented concerning their influence.

When the influence of a magnetic field on the body is to be assessed, it is necessary to clarify whether the magnetic field is alternating or static. If it is static, it must be clarified whether it is uniform or gradient in nature. It is also necessary to clarify the intensity of the magnetic field, duration of magnetic action, and reaction characteristics of the body to the magnetic field. These were somewhat obscure in many of the previous reports. The possibility cannot be ruled out that such obscurity has caused some confusion in the understanding of the effects of magnetic fields on the body. In addition, it has posed the problem to setting stricter guidelines on the acceptable limits of exposure to magnetic fields.

When the literature was reviewed only for the orientation of high molecular body components in static magnetic fields, reports on the orientation of fibrinogen, retinal cells, sickled cells, etc. were found. The orientation of fibrinogen and retinal cells is caused by the diamagnetic anisotropy retained by the protein \( \alpha \)-helix structure and lipid bilayer in the biologic membrane. On the other hand, elongated sickled cells after deoxygenation are oriented with their longitudinal axes at right angles to such magnetic fields. This phenomenon is ascribable to paramagnetic anisotropy retained by the heme of hemoglobin S that is polymerized in fiber by deoxygenation.

This paper deals with the orientation of normal erythrocytes in a static magnetic field. It is hoped that these results will be useful in elucidating the influence of magnetic fields on the body and as basic data for setting guidelines on acceptable limits of exposure to magnetic fields.

MATERIALS AND METHODS

Materials
Reagent-grade sodium citrate, sodium chloride, potassium chloride, glucose, sodium phosphate, sodium hydrosulfite, sodium nitrite, and gelatin supplied by Nakarai Tesque (Kyoto, Japan) were used.

Methods
Preparation of erythrocytes. The fresh blood collected from healthy donors was mixed with a 1/10 volume of 3.1% sodium citrate. After 5 minutes of centrifugation at 3,000 rpm, the plasma and buffy coat were removed. After washing with three portions of an isotonic phosphate-buffered saline (PBS) solution (90 mmol/L NaCl, 5 mmol/L KCl, 5.6 mmol/L glucose, 30 mmol/L Na-phosphate, pH 7.4, saturated with air), oxygenated erythrocytes containing diamagnetic oxyhemoglobin were obtained. These were added with sodium hydrosulfite (25 mmol/L), kept anaerobic in nitrogen gas, and used as deoxygenated erythrocytes (containing paramagnetic deoxyhemoglobin). In addition, the washed oxygenated erythrocytes were allowed to react with sodium nitrite (20 mmol/L) and washed five times. After adjusting the pH to 5.7 with an isotonic PBS solution, oxidized erythrocytes containing methemoglobin (high-spin state, paramagnetic) were obtained.

Detector for erythrocyte orientation in a strong magnetic field. Using a superconducting magnet (Low Temperature
Center, Osaka University), a uniform static magnetic field (8 T in maximum) was allowed to occur in a space measuring 60 (diameter) × 80 mm. The cylindrical sample portion measuring 50 (diameter) × 60 mm contained a spectroscopic cell holder for the samples, a temperature-controlling water circulator, etc. It could be smoothly introduced into the magnetic field and removed from it along the guide way. The main parts of the optical analyzer and constant-temperature water bath were installed apart from the magnet. He-Ne laser rays for measuring the intensity of transmittance (T%) were introduced into and removed from the sample portion using an optical fiber. In addition, the same equipment was installed outside the magnet and used to obtain the control values. During the experiment, the temperature within the cells was monitored using a temperature sensor and maintained at 24.0 ± 0.05°C in both sample and control cells (Fig 1).

**Spectroscopic measurement.** At first, the relationship between the concentration of erythrocyte suspension and the optical density (OD = 2 − log T%) was examined to determine the range where the change in OD was linear to it. The oxygenated normocytic erythrocytes in the isotonic PBS solution and the spherocytic erythrocytes in half the concentration of it in the range of 2,000 ~ 12,000 cells/μL were introduced into a spectroscopic cell, and their T% were measured without the magnetic field. The shape of spherocytic erythrocytes was microscopically observed. Next, the magnetic field effects on them were investigated at the concentration of 5,000, 6,450, and 8,000 cells/μL. The intensity of the magnetic field was increased from 0 to 8 T. The sample portion was introduced into and removed from the magnetic field at each magnetic intensity and differences in T% were determined. Furthermore, the deoxygenated and oxidized erythrocyte-suspending buffer solutions diluted to a concentration of 5,000 cells/μL were supplied to the same experiment.

Method for calculating the degree of orientation 〈m〉 and anisotropic diamagnetic susceptibility (ΔX) of erythrocytes. If the anisotropic diamagnetic susceptibility of erythrocytes placed within the magnetic field H is represented as Δχ, and the values of susceptibility perpendicular to and parallel with the disc surface are given as χa and χr, respectively, the field-induced energy U is given as:

\[ U = -\frac{H^2}{2} (2\chi_r + \Delta \chi \cos^2 \theta), \]

\[ \Delta \chi = \chi_a - \chi_r, \]

where θ is the angle between H and the erythrocyte disk plane. The order parameter 〈m〉 = (3 cos²θ − 1)/2, which is the mean degree of orientation of erythrocytes, is written as:

\[ 〈m〉 = \frac{1}{2} \int_0^\pi (3 \cos^2 \theta - 1) \exp(-U/\kappa T) \sin \theta d\theta \]

where κ is the thermal kinetic energy (κ, Boltzmann constant, 1.38 × 10⁻²³ erg/deg molecule; and T, absolute temperature, 297°F). Accordingly, Δχ can be obtained, if the intensity of the magnetic field H at given temperature is changed stepwise and 〈m〉 at each intensity of H is measured.*

〈m〉 is proportional to ΔI/Io when the concentration of erythrocyte suspension is sufficiently low and the change in OD is linear to the concentration of erythrocyte suspension, where ΔI = I − Io, I represents T% at each intensity of H and Io, that outside the magnetic field.†

**Morphological observation.** Three kinds of erythrocytes described under “Preparation of erythrocytes” (60,000 cells/μL) were suspended in a gelatin solution (15% v/v) that was made isotonic (290 mOsm) by adding NaCl (1 mol/L), and incubated at 37°C for 30 minutes to 2 hours within the 8 T magnetic field so that the erythrocytes could be sufficiently oriented. After cooling to 20°C, the samples gelled. The cut surface was observed under a light microscope (original magnification × 200 to 400) to confirm the orientation of the erythrocytes fixed in the gelatin gel.

**RESULTS**

**Morphologic Observation.**

Erythrocytes fixed in the gelatin gel were observed morphologically under a light microscope. As shown in Fig 2A, the erythrocytes were oriented with their disk plane parallel to the magnetic field direction, but they were at random in the control cell as shown in Fig 2B.

**Spectroscopic Measurement.**

As shown in Fig 3, the change in OD was linear to the concentration of both normocytic and spherocytic erythrocyte suspensions in the range of 0 ~ 8,000 cells/μL. The spherocytic erythrocytes in half the concentration of the isotonic PBS solution were observed as spherical forms by a microscope. The optical density of spherocytic erythrocyte suspension was 20% smaller than that of normocytic erythrocyte suspension at the same concentration. The dependence of T% on the intensity of the magnetic field was investigated in the range of 3,000 ~ 8,000 cells/μL, because the necessary and sufficient condition to analyze 〈m〉 and Δχ

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* 〈m〉 is obtained as the spatial mean value of m. If all erythrocytes are oriented with their disk plane parallel to the magnetic field direction, 〈m (θ = 0)〉 = 1. If all of them are suspended at random, 〈m (θ = Random)〉 = 0.

† ΔI has been expressed as a function of the angle φ formed by the erythrocyte axis and optical axis of spectroscopy.Recently the theoretical equation has been experimentally proved by Lee and Tarassenko. As a result of our numerical analysis of ΔI as a function of φ and 〈m〉 in equation (2) as a function of θ, where φ = θ + constant, the both curves almost agree within 5% errors.
was not satisfied above 8,000 cells/μL and the signal/noise ratio was reduced under 3,000 cells/μL.

The experiment data of oxygenated normocytic erythrocyte suspension (5,000 cells/μL) was shown in Fig 4. T% was increased with good reproducibility when the suspension of erythrocytes was introduced into the magnetic field (15% in maximum at 8 T). The degree of orientation ⟨m⟩ at each intensity of the magnetic field was calculated from such dependence according to the theory described under Methods (Fig 5). ⟨m⟩ at the concentration of 6,450 and 8,000 cells/μL and in cases of the deoxygenated and oxidized erythrocytes were also plotted in the same figure.

Anisotropic Susceptibility of Erythrocytes (Δχ)

The solid line in Fig 5 represents the curve obtained by the numerical analysis based on the theoretical equation for the magnetic orientation of diamagnetic substances described under Methods. Measured values were in good agreement with the equation. As a result of curve fitting, the anisotropic susceptibility of oxygenated normocytic erythrocytes was independent from their concentration (Δχ = 8.0 × 10⁻²², 8.5 × 10⁻²² and 8.0 × 10⁻²² emu/erythrocyte at 5,000, 6,450, and 8,000 cells/μL, respectively). Furthermore, ⟨m⟩ of three kinds of erythrocytes was not influenced by the state of hemoglobin (oxy: diamagnetic, deoxy and met: high-spin state, paramagnetic), and their Δχ equaled 8 × 10⁻²² emu/erythrocyte. In case of the spherocytic erythrocytes in the hypotonic PBS solution, there was no change in T% and ΔI could not be read off when the sample portion was introduced into and removed from the magnetic fields in the range of 0 ~ 8 T, and Δχ could not be obtained (data not shown).

DISCUSSION

Influence of Hemoglobin on the Magnetic Field-Induced Orientation of Erythrocytes

As shown in Fig 2A, erythrocytes were found to be oriented with their disk plane parallel to the magnetic field direction. The erythrocytes were oriented within 5 minutes in the 15 vol% gelatin solution at 37°C. The fixation of sample that started 30 minutes to 2 hours after did not prevent their motion. The polymerized gelatins used for the erythrocyte suspension are also oriented with their long axis

![](image1.png)

Fig 2. Erythrocytes inside and outside the magnetic field. (A) Inside the magnetic field. (The magnetic field is normal to this paper in direction and 8 T in intensity. Erythrocytes are oriented with their disk plane parallel to the magnetic field direction, and are photographed on edge.) (B) Outside the magnetic field. (Erythrocytes show no particular orientation.)
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Fig 3. Linearity of OD to the concentration of oxygenated normocytic (C) and spherocytic (D) erythrocyte suspensions. The solid and broken lines are proportional straight lines for the normocytic and spherocytic erythrocytes, respectively. The dependence of T% on the intensity of the magnetic field was investigated at A (5,000 cells/μL), B (6,450 cells/μL), and C (8,000 cells/μL).

perpendicular to the magnetic field direction. But they do not influence to the erythrocyte orientation under our condition, because of their much smaller magnetic field-induced energy than that of the erythrocytes.

In this instance, T% of the suspension was increased as shown in Fig 4. The degree of erythrocyte orientation (m) can be obtained quantitatively from ΔI, if the concentration of suspension is low enough to permit a proportional relationship to exist between the concentration of erythrocyte suspension and OD as shown in Methods. The linearity of OD to the concentration of erythrocyte suspension was kept in the range of 0 to 8,000 cells/μL (Fig 3). It was independent from the shape of erythrocytes. But, OD of spherocytic erythrocyte suspension was 20% smaller than that of normocytic erythrocyte suspension at a same concentration. If the phenomenon is statistically simplified, one third of erythrocytes can be seen en face and two thirds on edge in the normocytic erythrocyte suspension as viewed from the side of the photo-receiver. In the spherocytic erythrocyte suspension, all erythrocytes can be seen as round cells. This change induced a 20% increase in T% (40% → 48%) at 5,000 cells/μL. On the other hand, one half of normocytic erythrocytes can be seen en face and the other on edge inside the magnetic field. As shown in Fig 4, a one-sixth increase in the ratio of erythrocytes seen en face induced a 15% increase in T% (40% → 46%). Though the quantitative comparison between them is difficult because of the difference in erythrocyte shape, the intensity of transmittance changes in the same direction.

Figure 5 shows the dependence of (m) on the intensity of the magnetic field. Because reduced (Fe2+) heme is in low (s = 0)- and high (s = 2)-spin states when oxygenated and deoxygenated, respectively, the magnetic properties of hemoglobin molecules as a whole are diamagnetic and
strongly paramagnetic, respectively. On the other hand, as oxidized (Fe$^{3+}$) heme is in a high-spin state ($s = \frac{5}{2}$) when introduced in an acid solution, methemoglobin is strongly paramagnetic. But the state of hemoglobin produced no influence on the degree of erythrocyte orientation in the uniform magnetic field.

Hemoglobin makes no contribution to the orientation of erythrocytes for the following two reasons. (1) The anisotropic paramagnetic susceptibility is as low as $2 \times 10^{-27}$ emu/hemoglobin molecule even in deoxygenated hemoglobin.\textsuperscript{19} Briefly, because magnetic field-induced energy is lower than thermal energy, monomolecular hemoglobin is not oriented. (2) There is a report that hemoglobin may organize a crystal-like small group within the erythrocytes.\textsuperscript{20} This group of hemoglobin may show magnetic field-induced orientation, because magnetic field-induced energy becomes higher than thermal energy. However, their motion within a uniform magnetic field is a spinning one and makes no contribution to the orientation of the erythrocytes as a whole.

What needs to be answered is whether hemoglobin bound to the erythrocyte membrane is concerned with the orientation of erythrocytes in a magnetic field or not. The pocket between the $\beta$-chains of hemoglobin is introduced into the amino-terminal of Band I11 protein contained in the erythrocyte membrane and bound to it in 1% to 2% of hemoglobin.\textsuperscript{21,22} If the hemoglobin-binding amino-terminal of Band I11 protein is fixed firmly on the cell membrane, the hemoglobin must be concerned with the orientation of erythrocytes in a magnetic field. However, as shown in Fig 5, its influence could not be observed. This result suggests that hemoglobin moves freely at the amino-terminal of Band I11 protein as has been assumed previously.

In sickled cells, much of intracellular hemoglobin is deoxygenated and polymerized to cause cell deformation and integrated with the cell membrane. As described below, the lipid bilayer of the cell membrane acts so that erythrocytes within a magnetic field are oriented in parallel with the magnetic field. However, polymerized hemoglobin with strong paramagnetism overcomes the energy and orients erythrocytes perpendicular to the magnetic field.\textsuperscript{12,23} Unlike sickled cells, normal erythrocytes are oriented with their lipid bilayer parallel to the magnetic field direction primarily by the diamagnetism of membrane components alone.

Anisotropic Diamagnetic Susceptibility of Erythrocytes ($\Delta\chi$)

The solid line in Fig 5 is the theoretical curve indicating the magnetic field-induced orientation of diamagnetic substances.\textsuperscript{14,15} It was prepared by the numerical analysis of equation (2) and curve fitting to the experimental values. According to the analysis, the anisotropic diamagnetic susceptibility of the erythrocytes was $\Delta\chi = 8 \times 10^{-22}$ emu/erythrocyte.

In advance, the independence of the results from the concentration of erythrocyte suspension was examined. The same value of $\Delta\chi$ was obtained at the concentration of 5,000, 6,450, and 8,000 cells/$\mu$L as described in Results. The necessary and sufficient condition for the theory was not satisfied above 8,000 cells/$\mu$L (Fig 3), and the signal/noise ratio was reduced under 3,000 cells/$\mu$L. The effects of the magnetic fields on the erythrocytes different in shape was also examined. The spherocytic erythrocytes gave no change in T% even if the sample portion was introduced into and removed from the magnetic fields. This means that the change in T% was not induced except by the orientation of normocytic erythrocytes that were geometrically anisotropic particles.

As shown in Fig 5, the erythrocytes were influenced even by 1 T and almost 100% of them were oriented when exposed to 4 T. The thermal energy ($kT$) at 24°C is $4 \times 10^{-14}$ erg. On the other hand, the magnetic field-induced energy $U$ is given as equation (1) described in Methods. The term $(H^2/2)\Delta\chi \cos^2 \theta$ related to the rotation has a maximum, $H^2\Delta\chi/2$, when the angle $\theta$ is zero. It is $36 \times 10^{-14}$, $64 \times 10^{-14}$ and $100 \times 10^{-14}$ erg at 3, 4, and 5 T, respectively. In general, a particle can be perfectly oriented, if $H^2\Delta\chi/2$ is 16 $\sim$ 25 times higher than $kT$.\textsuperscript{24} Practically, $(H^2\Delta\chi/2)/(kT)$ becomes 16 at 4 T, where almost 100% of erythrocytes were oriented.

Contribution of Membrane Components to Magnetic Field-Induced Orientation

On the assumption that the orientation of normal erythrocytes is ascribable to the diamagnetism of biologic membrane components as described above, the contribution of the lipid bilayer and transmembrane proteins (Band I11 and glycoporphin) to anisotropic susceptibility is estimated below. The number of molecules calculated from the amount of total lipids composing the membrane of erythrocytes (about $5 \times 10^{13}$ g/erythrocyte) and average molecular weight of lipids (about 1,000) is about $3 \times 10^8$ molecules/erythrocyte. The lipids, including phospholipids (lecithin, etc), which account for 60% of them, form a self-sealing lipid bilayer.\textsuperscript{25} In a magnetic field, the lipid bilayer is oriented with the hydrocarbon chain perpendicular to the magnetic field (Fig 6, upper left). Furthermore, according to the value obtained for phospholipid molecules in a liquid crystal state similar to the condition of the biologic membrane, $\Delta\chi$ is about $1 \times 10^{-29}$ emu/phospholipid molecule.\textsuperscript{26,27}

On the other hand, the $\alpha$-helix portion of the transmembrane proteins (Band I11 and glycoporphin) is oriented with its longer axis in parallel with the magnetic field (Fig 6, lower left).\textsuperscript{28} In short, the transmembrane proteins and lipid bilayer counteract each other within the biologic membrane with regard to the orientation of erythrocyte membrane. The number of peptide groups in the $\alpha$-helix is about $2 \times 10^8$/erythrocyte and $\Delta\chi$ is about $7 \times 10^{-30}$ emu/particle group.\textsuperscript{29}

Taking the shape of erythrocytes, ie, biconcave discs, into consideration, their anisotropic susceptibility is calculated below (Fig 6). It is estimated to be nearly $\Delta\chi = 6 \times 10^{-22}$ emu/erythrocyte as calculated by subtracting the contribution of transmembrane proteins $\Delta\chi = 7 \times 10^{-22}$ emu/erythrocyte from that of lipid bilayer $\Delta\chi = 13 \times 10^{-22}$ emu/erythrocyte. It is almost the same in order as the value of $\Delta\chi$ obtained experimentally.

Incidentally, it is known that unlike the membrane of the erythrocytes, the silicoid membrane of the outer segment of
Fig 6. Schematic diagram showing the magnetic field-induced orientation of the erythrocyte membrane components and erythrocyte model for calculating anisotropic susceptibility. Upper left: lipid bilayer is oriented with the hydrocarbon chain perpendicular to the magnetic field. Lower left: the a-helix portion of transmembrane proteins (Band III and glycophorin) is oriented with its longer axis in parallel with the magnetic field. Accordingly, both components counteract each other in the orientation of erythrocyte membrane. Right: the difference ($S_1 - S_2$) in the area of membrane parallel with and perpendicular to the magnetic field calculated in consideration of the size of erythrocytes and concaves near the center accounted for 44% of the total area ($S_1 + S_2$). Because the portions of the erythrocyte membrane parallel with and perpendicular to a magnetic field counteract each other to induce orientation, one must multiply it by the calculated value for the whole membrane in the estimation of the anisotropic susceptibility of the erythrocytes as a whole. Refer to the Discussion for details.

As described above, hemoglobin contained in erythrocytes has little influence on the orientation of erythrocytes in a static magnetic field and their orientation may primarily be caused by the diamagnetism of the cell membrane components. The intensity of the magnetic field is 0.5 ~ 2 T in the case of practical MRI, and 4 T in the case of one under examination. It is very strong also in the working environments of nuclear fusion reactors and passenger transport systems based on the principle of magnetic levitation. In the future, attention will be focused on the orientation of high molecular substances of the body in a uniform magnetic field in the reexamination of safety standards of magnetism exposure. We will also conduct further detailed studies on the contribution of various cell components together with the magnetic field-induced orientation of other cells, such as platelets.

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