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Effects of high magnetic field on *Euglena gracilis*

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Abstract. The effects of high magnetic field on *Euglena gracilis Z* were examined. When a horizontal magnetic field gradient (ca. 400 T^2m^{-1}) was applied, living *E. gracilis* moved to a higher field (positive magnetotaxis), whereas dead one gathered in a lower field. Magnetotaxis was not observed in a uniform magnetic field of 8 T. *E. gracilis* was oriented almost perpendicularly to the magnetic field regardless of life and death. Magnetotaxis of *E. gracilis* would be explained by taking into account inhomogeneous magnetic forces on and magnetic orientation of *E. gracilis*.

1. Introduction

Recently, effects of high magnetic field (>1 T) on chemical, physical, and biological systems have been studied very extensively. However, little is still known about the magnetic field effects (MFEs) on biological systems. One of the authors has investigated MFEs on various chemical and physical processes over 20 years [1,2]. On the basis of the mechanisms established in these studies, we have researched the MFEs on biological systems. In a previous paper [3], we examined the effect of a 45–200 mT magnetic field on the X-ray and ultraviolet-induced killing rates of cultured marmalian cells. However, no MFE was detected. The MFEs on biological systems were considered not to be very significant, if any. Therefore, in order to detect MFEs, it is indispensable to use techniques by which one can detect small and/or transient changes induced by the external magnetic field. Based on the above-mentioned argument, we decided to expose living things as a whole in a high magnetic field to observe their behavior. Indeed, we succeeded to see that *Caenorhabditis elegans* undergoes transient unusual behavior after a short-time exposure to a strong AC magnetic field (1.7 T, 60 Hz) [4].

In this paper, the effects of high static magnetic fields on the behavior of *E. gracilis* were examined. It was chosen since it was small enough to expose its whole body to a strong magnetic field in a bore tube of our magnet (50 mm in diameter). A dish containing a solution dispersed with *E. gracilis* was placed in a gradient magnetic field (ca. 400 T^2 m^{-1}). *E. gracilis* moved to the higher magnetic field (positive magnetotaxis), whereas dead one moved to the lower magnetic field. The mechanism of magnetotaxis for *E. gracilis* was discussed.

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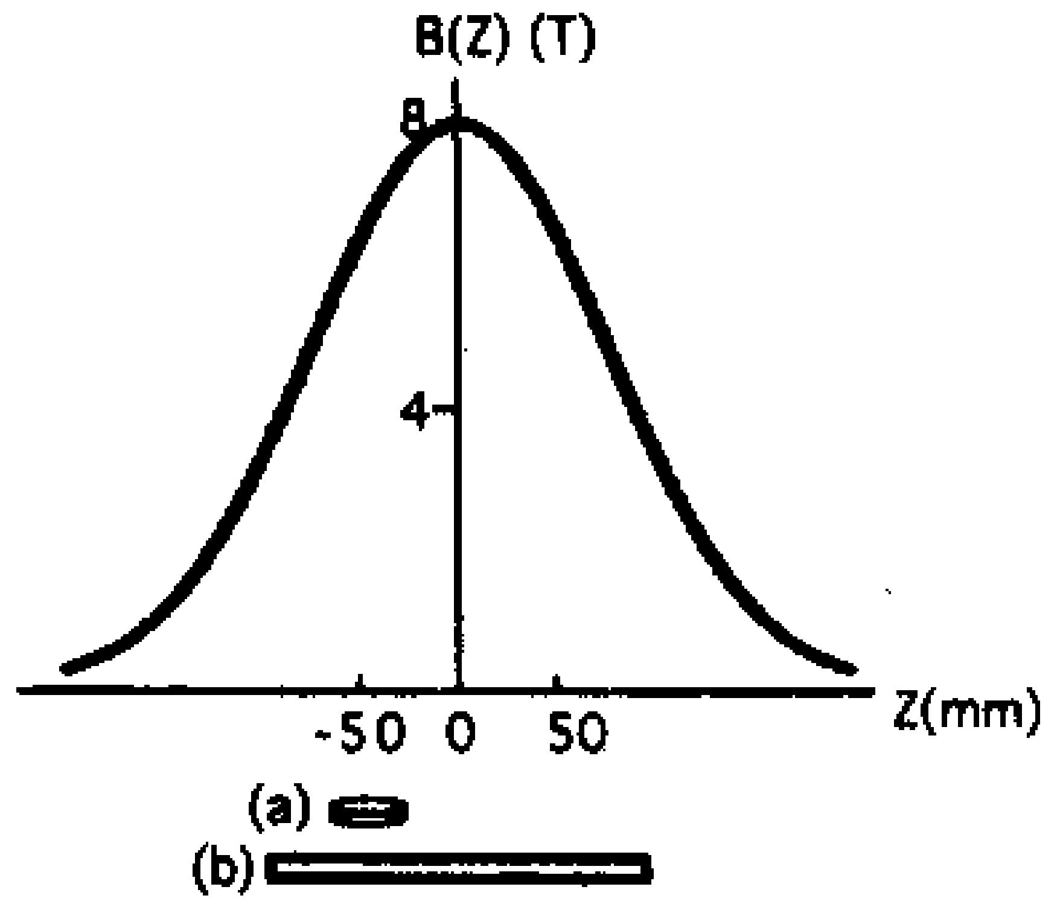

Fig. 1. Distribution of magnetic field intensities in a bore tube of a superconducting magnet. Locations of a dish (ϕ 30 mm) and an NMR tube (ϕ5 mm × 180 mm) in the bore tube are also depicted in the figure.

2. Experimental

Horizontal magnetic fields were generated using a superconducting magnet (Oxford Instruments, SM-1000, ϕ 50 mm × 375 mm bore tube). The magnetic intensities in the tube is depicted in Fig. 1. The maximum magnetic field $B(z)$ and the product of the magnetic field and magnetic field gradient $B(z)dB(z)/dz$ were 8 T ($z = 0$ mm) and ca. 400 T^2m^{-1} ($z = \pm$ 65 mm), respectively, z being the distance from the center of the magnetic field.

E. gracilis Z (wild type) was cultured as described elsewhere [5]. When needed, *E. gracilis* was killed by adding ethylenediaminetetraacetic acid (EDTA) or potassium cyanide. A plastic dish (ϕ 30 mm) containing a 3 ml solution of *E. gracilis* or an NMR tube (ϕ 5 mm × 180 mm) full-filled with the solution was placed in the bore tube (ca. 400 T^{2-1}) for 12 h. After taking them out from the bore tube, the distributions of *E. gracilis* in dishes or NMR tubes were recorded with a camera on photographs. The photographs of dishes were scanned into a personal computer and the green color-density profiles of *E. gracilis* were further analyzed with an image-processing program.

For in situ observation, a quartz cell (2 mm × 12.5 mm × 45 mm) which was full-filled with a solution containing *E. gracilis* was placed in the bore tube. The behavior and orientation of *E. gracilis* were observed and recorded using an optical fiber-microscope-video camera system.

3. Results

A dish containing a solution dispersed with *E. gracilis* was placed in the absence and presence of a horizontal gradient magnetic field for 12 h. The results are shown in Fig. 2 (a), (b). The green color-density along the $l - l'$ line indicated in the photograph, is also depicted at the right hand side of each photograph. *E. gracilis* moved to a higher field, whereas it was dispersed uniformly at zero field. The inhomogeneous dispersion of *E. gracilis* was observed after about 1 h exposure to the magnetic field gradient above ca. 50 T^2m^{-1}. When *E. gracilis* was placed in an uniform magnetic field of 8 T near the center of the tube, no magnetotaxis was observed. Furthermore, the distribution of the dead *E. gracilis*, killed by adding EDTA or potassium cyanide, inclined to the left hand side of the dish (lower field), as shown in Fig. 2(c). This means that living *E. gracilis* moves to the higher field, even though its tissue is diamagnetic.

Analogous magnetotaxis of living *E. gracilis* was observed when an NMR tube was used as a vessel. Figure 3 shows the effects of horizontal magnetic field on *E. gracilis*. Magnetic fields are 8 T at the center of the tube and ca. 3.6 T at two ends of the tube, as depicted in Fig. 1. The location of the maximum

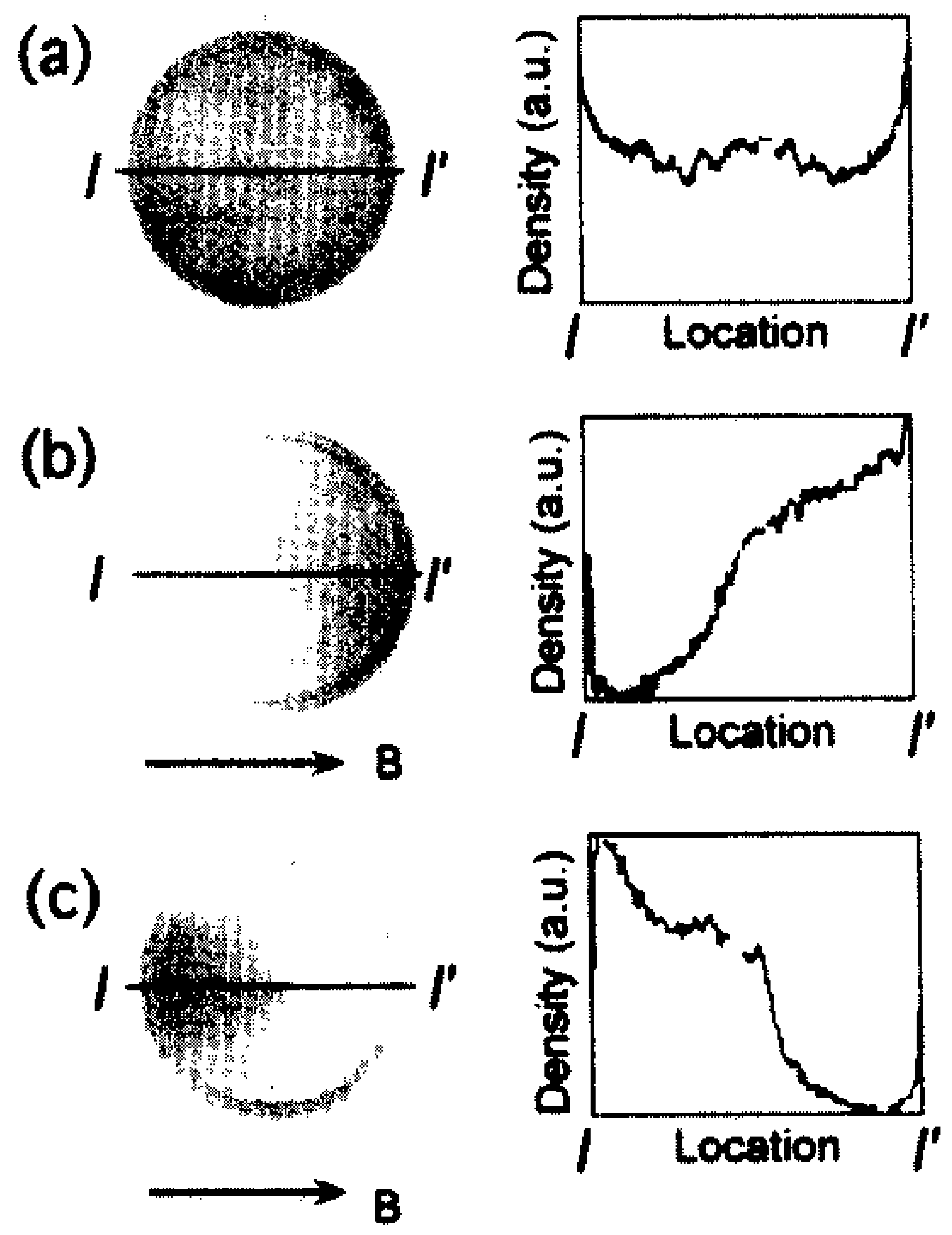

Fig. 2. Distribution of *E. gracilis* in a dish (ϕ 30 mm). (a) living E. gracilis at zero field. (b) living *E. gracilis* in a magnetic field gradient of ca. 400 T^2m^{-1}. (c) dead *E. gracilis* in a magnetic field gradient of ca. 400 T^2m^{-1}. The green color-density along the line $l - l'$ across the dish is also shown at the right hand side of each photograph. An arrow indicates the direction of the magnetic field.

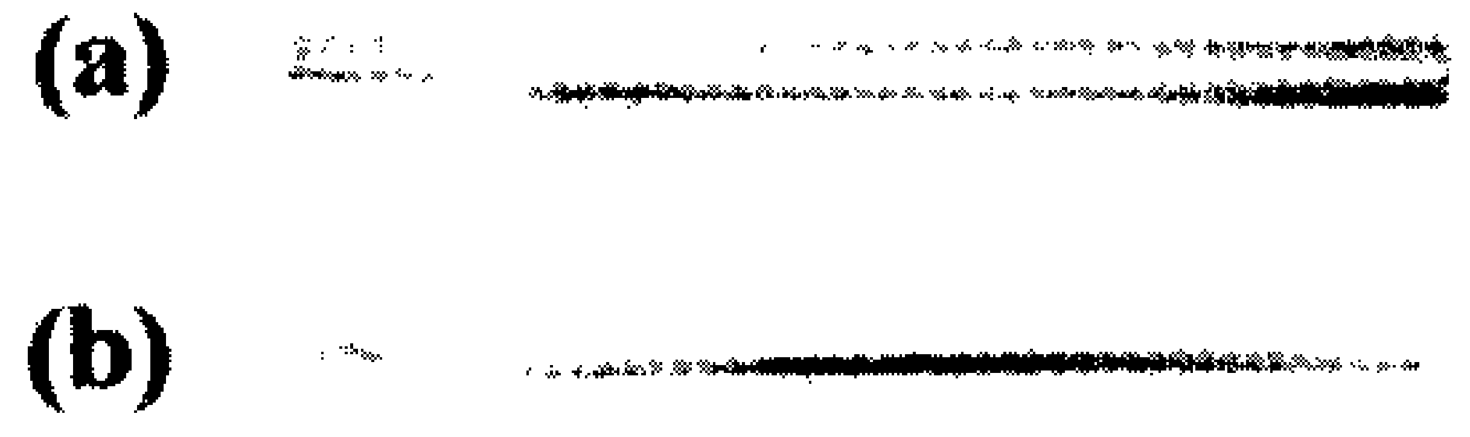

Fig. 3. Magnetic field effects on living *E. gracilis* in an NMR tube (ϕ5 mm × 180 mm). (a) at zero field. (b) in a magnetic field. Magnetic fields are 8 T at the center of the tube and ca. 3.6 T at two ends of the tube, as depicted in Fig. 1. The location of the maximum field gradient (ca. 400 T^2m^{-1}) is about ± 65 mm apart from the center of the tube.

field gradient (ca. 400 $T^2\ m^{-1}$) is about ± 65 mm apart from the center of the tube. Living *E. gracilis* moves to the center of the tube where the magnetic field intensity is maximum. On the other hand, dead *E. gracilis* does not move but remains at the same place, though in a dish it moves to the lower field. This difference arises from the shape of containers used. In a dish of 30 mm in diameter, the surface of the solution is uncovered and the solution has a space to move freely. Therefore, the solution in the dish can undergo convection in the magnetic field gradient. In the case of an NMR tube full-filled by the solution, however, there is no empty space to undergo convection of solution. Therefore dead *E. gracilis* can not move to the lower field, though living one can move to the higher field by *Euglena* motion.

Movements of *E. gracilis* in a horizontal magnetic field were observed using an optical fiber-microscope-video camera system. Figure 4 shows the effect of the magnetic field gradient on *E. gracilis*. *E. gracilis* (about 50 μm in length) appears as white traces in the photographs. Almost all living *E. gracilis* were oriented and moved perpendicularly to the magnetic field, whereas they moved randomly to all directions at zero field. A large number of *E. gracilis* were oriented in the uniform magnetic field of ca. 0.2 T, though no magnetotaxis was observed. Furthermore, dead *E. gracilis* was

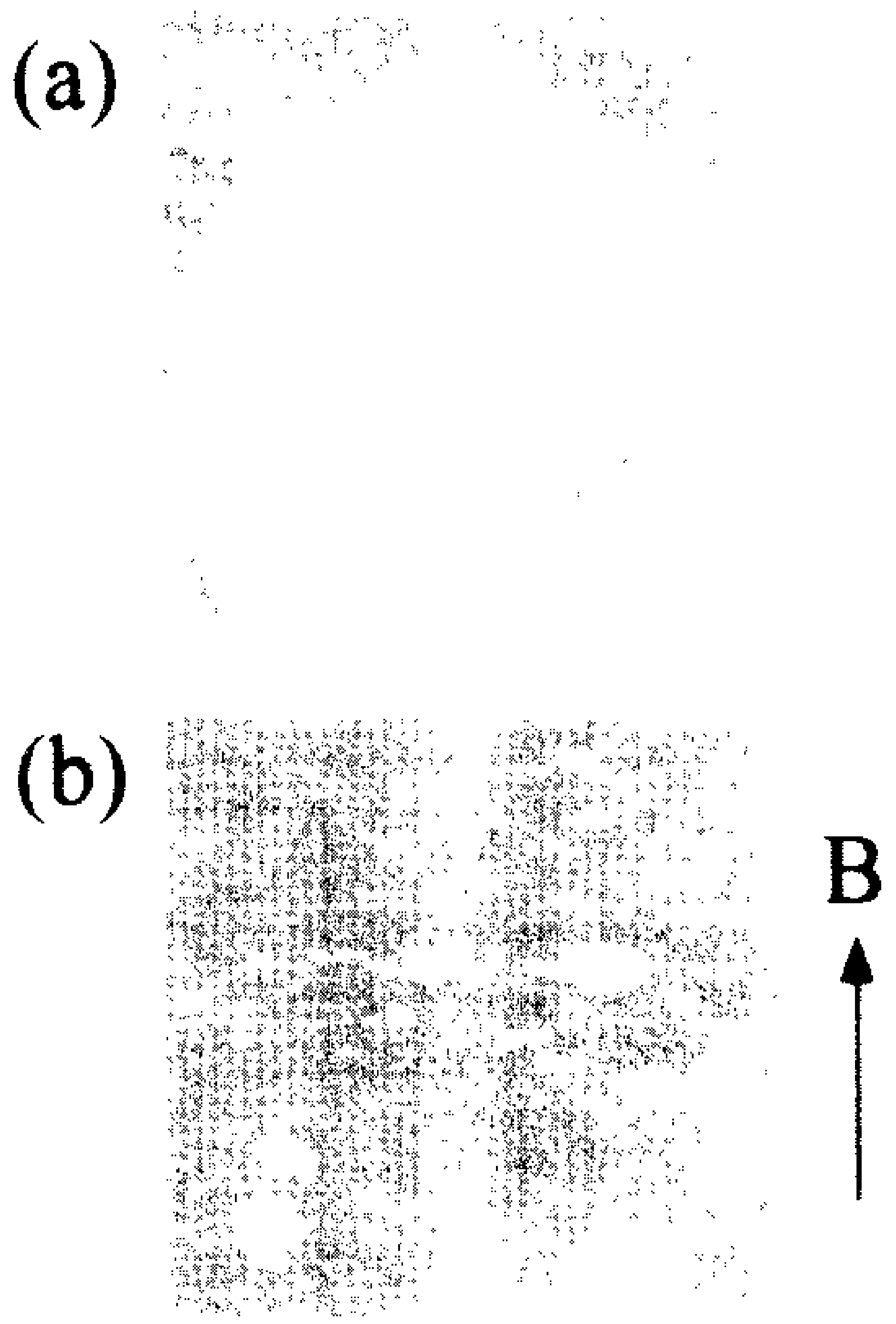

Fig. 4. In site microscopic observation of *E. gracilis* in magnetic fields. *E. gracilis* (about 50 μ m in length) appears as white traces in the photographs. (a) at zero field. (b) in a magnetic field gradient of ca. 400 T^2m^{-1}.

also oriented almost perpendicularly to the magnetic field. The magnetic orientation of *E. gracilis* occurs regardless of life and death. The magnetic orientation occurs as soon as the magnetic field was applied. The present observation is in parallel with the reports that fluorescence from whole cells of *E. gracilis*, suspended in an aqueous growth media, is polarized in magnetic fields of ca. 1 T because of their magnetic orientation [6].

It is very important to know that the picture taken in the magnetic field is distinct, though it is indistinct in the absence of magnetic field. This means that *E. gracilis* moves slowly in the magnetic field compared to the motion at zero field. The motion of *E. gracilis* is suppressed by the magnetic field. In other word, it receives mechanical forces in the magnetic field.

4. Discussion

The mechanisms of effects of a static magnetic field on chemical and physical processes can be classified into three groups, i.e., (1) quantum mechanical effect (mechanism 1), (2) thermodynamic effect (mechanism 2) and (3) mechanical effect (mechanism 3) [1,2]. In mechanism 1, electronic spin states of reaction intermediates are perturbed quantum-mechanically by a magnetic field. This type of effects has been widely observed in organic photoreactions where a radical pair takes important role as a short-lived reaction intermediate [8]. Mechanism 2 is further divided into two subgroups. When isotropic magnetic energies of reactants and products are very much different, a chemical equilibrium shifts to the direction where magnetic energies are minimum (mechanism 2a). Usually magnetic energies of diamagnetic and paramagnetic substances are very small compared with their enthalpies and, therefore,

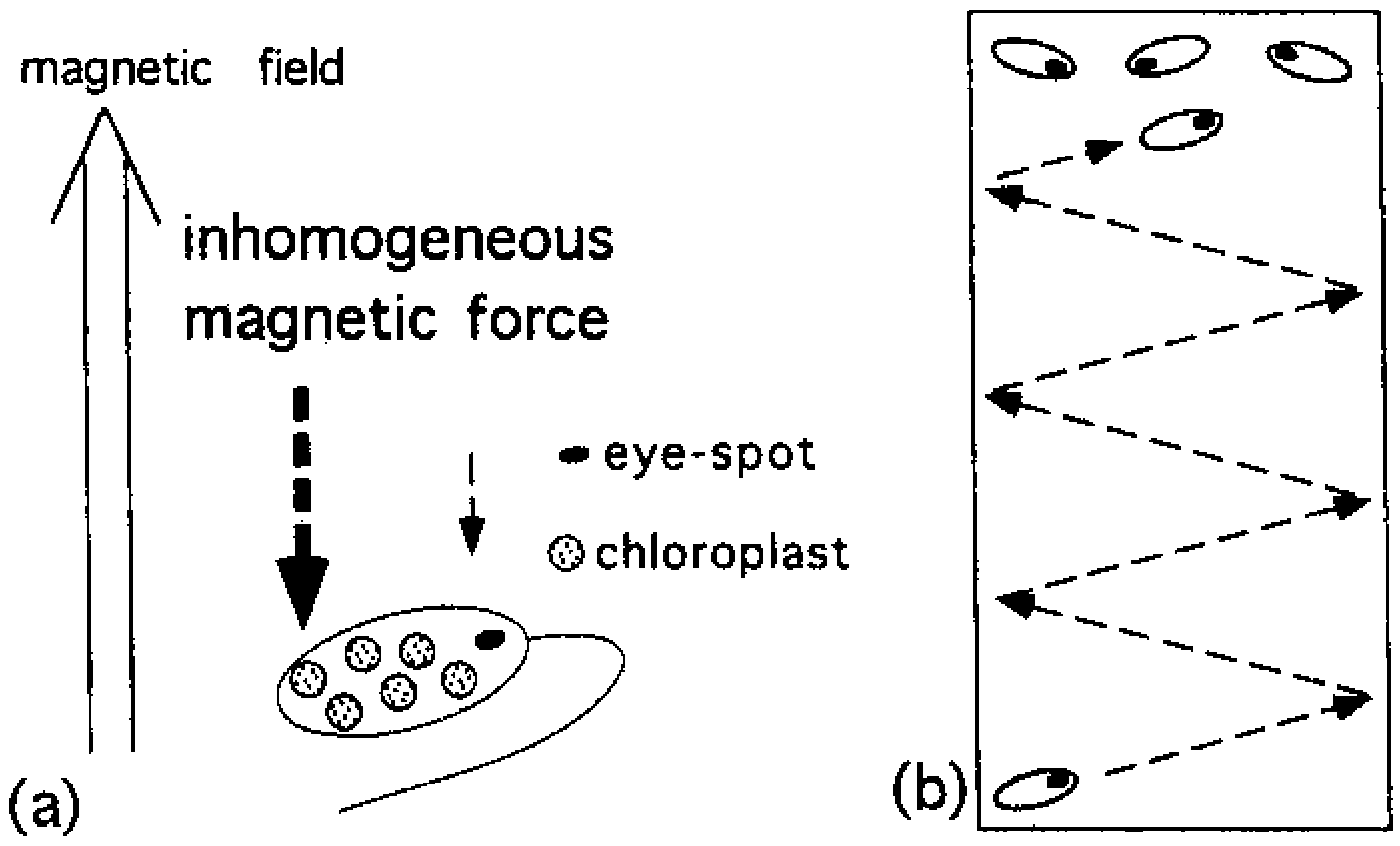

Fig. 5. A proposed mechanism of magnetotaxis of *E. gracilis* in a horizontal magnetic field. (a) magnetic forces on *E. gracilis*. (b) motion of *E. gracilis* in a vessel in a magnetic field gradient.

mechanism 2a is only observed in the reaction where ferromagnetic substances are involved in the reaction under very intense magnetic field [10]. On the other hand, when it has anisotropic magnetic energies, an aggregate with ordered structure undergoes magnetic orientation to minimize its energy (magnetic orientation, mechanism 2b). This type of effects has been widely observed in many aggregates such as biological organs [6,11], crystals [12], and films [14]. Mechanism 3 is composed of two subgroups. The first mechanism is called magnetohydrodynamic mechanism (mechanism 3a). When they move in a homogenous magnetic field, charged particles like electrons and ions receive the Lorentz force [15]. The second mechanism is associated to a magnetic field gradient. When they are placed in a magnetic field gradient, substances receive the magnetic forces (mechanism 3b). Thus displacement of paramagnetic and diamagnetic materials are affected by the magnetic force [17]. It is noteworthy that mechanism 3b is the effect of magnetic field gradient, whereas the mechanisms 1–3a are the effects of homogeneous magnetic field. Furthermore, mechanisms 2b, 3a, and 3b are the effects which are dependent on the relative orientation between magnetic field and substances placed.

Taking into account these mechanisms, the magnetotaxis of *E. gracilis* in a horizontal magnetic field could be explained as follows. As given in the previous section, magnetotaxis of *E. gracilis* is a phenomenon which depends on the direction of an applied magnetic field. Thus mechanisms 1 and 2a may not be applicable to explain the observed effects. Furthermore, mechanism 3a would not be the case, since *E. gracilis* has no electric charge. As shown in the previous section, living *E. gracilis* moves finally to the higher field. Even though the solution surrounding *E. gracilis* has electirc charges, the Lorentz force does not influence significantly the motion of *E. gracilis*. This is because its motion is parallel to the magnetic field. Magnetotoxis of *E. gracilis* would be explained by applying mechansims of 2b and 3b. Magnetic orientation of *E. gracilis* occurs regardless of death of life. Thus, magnetic orientation of *E. gracilis* arises from its anisotropic magnetic susceptibilities of its organs and tissue [6]. As observed by microscope, however, *E. gracilis* moves almost perpendicularly to the magnetic field in short time scale. If the direction of motion of *E. gracilis* is perpendicular, it does not move to the higher field. Even if the direction of motion of *E. gracilis* is not perpendicular, it would move to both higher and lower fields with equal probability. Consequently, it does not show magnetotaxis after all. We must add some other effect of magnetic field to explain the observed phenomena. Only when influence of inhomogeneous magnetic forces are taken into account, we can explain the observation depicted in Figs 2 and 3. A body of *E. gracilis* is constituted of various diamagnetic substances which are organized inhomogeneously. Since it is magnetically anisotropic, *E. gracilis* undergoes magnetic orientation. At the same time, it receives inhomogeneous repulsive magnetic forces in a magnetic field gradient as shown in Fig. 5. If the force at the front of the body is smaller than that at the rear, then the front of the body always tilts slightly to the higher field (Fig. 5a). These inhomogeneous forces make *E. gracilis* move to the higher field very slightly, while it moves almost perpendicularly to the magnetic field. As a result of repeated turning motion at two walls of the vessel, *E. gracilis* moves gradually to the higher magnetic field as shown in Fig. 5b. This is why magnetotaxis of *E. gracilis* takes long time (more than 1 h) to be observable, though magnetic orientation occurs instantaneously.

It should be mentioned whether gravity, the force of biological importance, influences magnetotaxis of *E. gracilis* or not. It is well known to affect behavior of many living things (i.e., gravitaxis). However, it has nothing to do with the present magnetotaxis of *E. gracilis* in a horizontal magnetic field, since the direction of the magnetic field is perpendicular to the gravity. Behavior of *E. gracilis* would be affected by both gravity and magnetic forces in a vertical magnetic field.

Living things are composed various diamagnetic and, sometimes, paramagnetic materials which are distributed inhomogeneously in their bodies. This means that almost all living things receive inhomogeneous magnetic forces when they are placed in a magnetic field of $> 100\ \mathrm{T}^2\mathrm{m}^{-1}$. Thus it is most plausible that magnetotaxis will be commonly observed when living things are placed in an inhomogeneous strong magnetic field, as demonstrated here. Researches to show importance of magnetic forces on living things are in progress and will be reported in near future.

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