Oriented Growth of Pollen Tubes in Strong Magnetic Fields

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It is well known that environmental factors such as light, gravitation, electric fields, and chemical gradients can stimulate oriented growth of individual cells [1, 2]. Oriented growth of whole plant organs in magnetic fields was first observed by Audus [3] and subsequently called "magnetotropism". He found that if cress roots or oat shoots were placed in an inhomogeneous magnetic field these organs grew toward the regions of decreasing field strength. He also demonstrated [4] that this oriented growth could not simply result from the diamagnetic force acting on the plant organ in a magnetic field gradient. No alternative explanation of this effect was given, and at present the physiological processes causing the phenomenon of magnetotropism of multicellular systems are still unknown. As indicated by Audus, cell divisions might be a prerequisite for the magnetotropism of plant organs. In order to find out whether cell divisions are necessary for magnetotropism, we tested the effect of magnetic fields on the elongation growth of individual cells – the pollen tubes of *Lilium longiflorum*. Their growth physiology and their ultrastructure are rather well known. For example, a number of investigations in the past few years have shown that the growing lily pollen tubes accumulate ^{45}Ca at their tips [5, 6] and that they generate a transcellular electric current that seems necessary for the elongation of the tube [7]. In electric fields of 5–6 V/cm most of the tubes germinate at the anodal side of the grains and afterward grow parallel to the applied field [8].

Pollen grains of *L. longiflorum* were sputtered with a fine brush onto the bottom of dishes of Plexiglass ($25 \times 25 \times 5$ mm). Then growth medium of 33 °C, containing 300 m*M* mannitol, 1.65 m*M* $CaCl_2 \cdot 2H_2O$, 1.0 m*M* KNO_3, 0.13 m*M* H_3BO_3, pH 5.1–5.3 [9], and 2% agar was carefully poured over the grains. Most of the grains adhered to the bottom of the chamber and were fixed in place by the hardening agar. The dishes were then placed immediately in the 50-mm-wide bore of a horizontal Bitter magnet at various positions along the axis. The dish at the center of the bore was exposed to a strong homogeneous field of 14 Tesla with a maximum deviation of 0.5% along the dish. The other dishes placed on either side of the center were exposed to a field gradient (e.g. 1.4 T/cm) and to lower field strengths (e.g. 9.7 T). The air temperature within the bore was kept at 30 ± 1 °C during all experiments. At the start of an experiment the field was increased linearly within 3 min to its maximum of 14 T and was kept constant at this value for 3 h. Immediately after the field was turned off the dishes were removed and the growing pollen tubes were photographed. The direction of growth of the first 200–300 µm of the tip of every tube was later measured from the slides thus obtained. As controls, pollen tubes were treated in exactly the same way as described above, but without turning the field on.

Figure 1a shows a batch of pollen tubes after a growth period of 3 h in a *homogeneous* magnetic field of 14 T. Figure 2a shows the control kept at zero field.

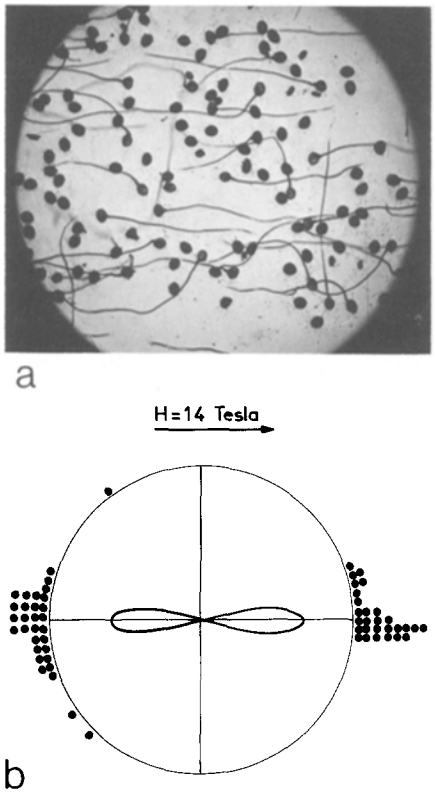

Fig. 1. (a) Oriented growth of pollen tubes of *Lilium longiflorum* in a *homogeneous* magnetic field of 14 T. Most of the tubes grow nearly parallel or antiparallel to the magnetic field. The photo was taken after a growth period of 3 h in agar medium of 30 °C inside a horizontal Bitter magnet. (b) Polar representation of the distribution function of the tips of many tubes. The distribution is bipolar and symmetric for homogeneous magnetic fields. The radial distance of the points to the origin is proportional to the number of tubes found with a direction of their tip growing within 10° intervals

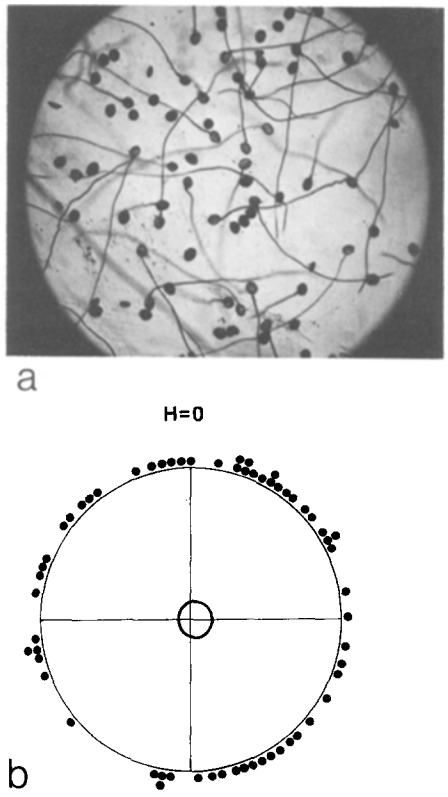

Fig. 2. Control experiment at zero field strength after a growth period of 3 h (a) and orientational distribution function of the growing tips (b)

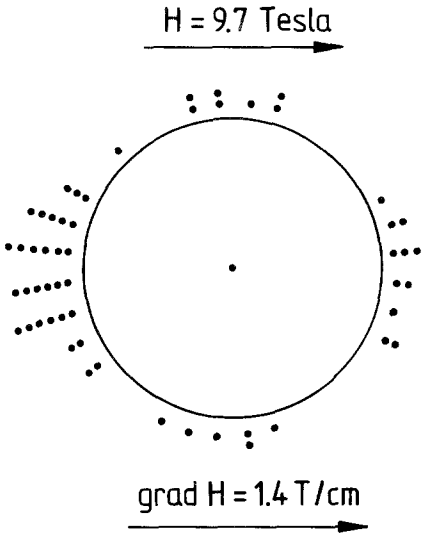

Fig. 3. Oriented growth of pollen tubes in an *inhomogeneous* magnetic field of $H=9.7$ T and grad $H=1.4$ T/cm. The samples were placed along the axis of a horizontal Bitter coil in the region of highest field gradient. The tubes grow preferentially toward the region of decreasing field strength

The tubes apparently grow strongly oriented and almost parallel to the magnetic field, with equal tendency to grow toward the north or toward the south pole. When the orientation of the growing tips of many tubes is plotted according to their growth angle their angular distribution shows up to be bipolar and symmetric (Fig. 1b). Similar experiments at lower fields reveal that the magnetotropism of growing pollen tubes becomes weaker with decreasing field strengths, oriented growth being still clearly observable at 7 T but no more at 3 T. (A more detailed investigation of the correlation between field strength and the degree of pollen tube orientation is in progress.) In *inhomogeneous* fields (Fig. 3) the pollen tubes grow preferentially toward the region of decreasing field strength. The corresponding angular distribution of pollen tube growth is asymmetric.

Our present experiments show for the first time that magnetotropism occurs in individual cells. They further show that magnetotropism is not necessarily related to ordered structures such as mitotic spindles or condensed chromosomes, occurring during mitosis, because the pollen tubes only elongate by tip growth and do not divide. Furthermore, our experiments demonstrate that magnetotropism occurs in homogeneous fields, i.e., in the absence of diamagnetic forces. In explanation of these phenomena, we speculate that magnetic fields may act on the plasma membrane like other stimuli which can affect local growth. In particular, magnetic fields might cause a redistribution and an accumulation of certain membrane proteins within the growing tip of the pollen tubes. This accumulation of proteins might then lead to localized fusion of vesicles into the membrane and/or affect the local flux of particular ions e.g., Ca^{2+} through the membrane.

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1. Jaffe, L.F., Nuccitelli, R.: Ann. Rev. Biophys. Bioeng. *6*, 445 (1977)
2. Weisenseel, M.H.: Encycl. Plant Physiol. *7*, 485 (1979)
3. Audus, L.J.: Nature *185*, 132 (1960)
4. Audus, L.J., Wish, J.C., in: Biological Effects of Magnetic Fields, Vol. 1, p. 170 (Barnothy, M.F., ed.). New York: Plenum 1964
5. Jaffe, L.A., et al.: J. Cell. Biol. *67*, 488 (1975)
6. Reiss, H.D., Herth, W.: Protoplasma *97*, 373 (1978)
7. Weisenseel, M.H., et al.: J. Cell. Biol. *66*, 556 (1975)
8. Sperber, D., Weisenseel, M.H.: unpublished
9. Weisenseel, M.H., Wenisch, H.: Z. Pflanzenphysiol. (in press)

Concept of Multiphasic Uptake in Plants Rejected

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In recent literature on uptake of solutes in plants considerable attention has been paid to the concept by which uptake isotherms are interpreted as a number of successive Michaelis-Menten phases separated by discontinuous transitions [1–6].

The concentration dependence of the uptake rate (uptake isotherm) of inorganic ions [7], sugars [8, 9], and amino acids [10] in plant cells or tissues typically yields a curvilinear, concave downward Lineweaver-Burk plot. Apparently these uptake isotherms do not conform the Michaelis-Menten equation. They may be described, however, by one of the following equations

$$v = Vm \cdot S/(Km+S) + k \cdot S \quad (1)$$

$$v = Vm_1 \cdot S/(Km_1+S) + Vm_2 \cdot S/(Km_2+S) \quad (2)$$

$$v = Vm_1 \cdot S/(Km_1+S) + Vm_2 \cdot S/(Km_2+S) + k \cdot S \quad (3)$$

where v is the uptake rate and S is the external concentration of the solute whose uptake is studied.

In Eqs (1)–(3) the uptake rate is a monotonically rising function of the solute concentration. There is, however, a widely held opinion among authors on ion uptake in plants that uptake isotherms display a number of inflections or discontinuous transitions, especially in the high concentration range [4, 6, 11]. Obviously this opinion has led to a quite different interpretation of uptake isotherms in plants: the multiphasic concept [1, 2, 8]. According to this concept uptake isotherms consist of several phases separated by discontinuous transitions, each phase obeying the Michaelis-Menten relationship. It is thought that the transitions reflect abrupt changes in the characteristics (Km and Vm) of the transport system at several discrete concentrations of the transportee [1–3].

I shall demonstrate here that the concept of multiphasic uptake is due to an artificial interpretation of uptake isotherms that yield non-linear Lineweaver-Burk plots. The demonstration will be limited to a dual isotherm, i.e., one which is described by Eq (2), but will apply to any isotherm that yields a concave downward Lineweaver-Burk plot.

Three sets of data that relate uptake rate (v) and external concentration (S) were computed according to Eq (2): a set of 'perfect', i.e. error-free data for v, and two 'experimental' sets in which v contained a 3% random error. The simulated iso-