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BIOPHYSICAL CONSIDERATIONS CONCERNING GRAVITY RECEPTORS AND EFFECTORS

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TOBIAS C.A., RISIUS J., and YANG C.-H. (Donner Laboratory, Lawrence Berkeley Laboratory, Berkeley, California, USA)
Biophysical Considerations Concerning Gravity Receptors and Effectors.

The physical action of gravitational and inertial forces on graviceptors is considered. The motion of graviceptors as influenced by physical dimension, density, electric charge, composition of the suspending medium and flow variables is demonstrated.

Some aspects of the responses of various organisms to gravity are under genetic control. Mutants of Phycomyces blakesleeanus exhibit strikingly different rates of geotropic responses. It is shown that phycomyces grown in the dark lack normal geotropic responses: pre-exposure to light is necessay for the synthesis of structures responsible for geotropism.

A physical model is presented that may account for some of the geotropic phenomena observed in phycomyces.

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BIOPHYSICAL CONSIDERATIONS CONCERNING GRAVITY RECEPTORS AND EFFECTORS

by

Cornelius A. Tobias, Jack Risius and Chui-hsu Yang
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All life known to us has apparently evolved within gravitational and inertial fields. The idea of a gravitational field evokes the idea of directional, vector properties. It is difficult for a physicist to imagine that living objects other than those with perfectly spherical or multigonal symmetry might have evolved in the absence of vector, or of higher order, fields. In what manner living organisms have acquired directional shape and orientation is not presently fully comprehended according to the known laws of physics. Living objects consist of particles--particles which move and interact, responding simultaneously to several classes of vector fields (electrical and thermal) and to scalar forces such as chemical affinity.

The forces of gravity have a special property with regard to living objects in the sense that gravitational forces, compared to other forces between particles, are weak at the

molecular level of interaction and are only slightly more pronounced at microscopic dimensions. Differences in gravitational potential grow, however, with the dimensions of the object and, on the surface of the earth, for multicellular organisms at 1-g, these potential differences become of paramount importance to the organization, shape and energy exchange of the object. These questions remain: at what dimension do gravitational potentials become important? what are graviceptors and how do they exert an influence on vector properties of the whole organism?

We shall discuss only one class of gravity dependent biological phenomena: the ability of certain organisms to sense the direction and strength of the gravity-inertial vector field and to alter the rate and direction of growth as well as the morphology of the organisms to develop in a definite pattern with respect to this field. When the trend of the direction of growth is generally opposite to the gravity inertial vector, as in plants growing "upwards", this is termed "negative geotropism", whereas, when the trend of growth is parallel to the gravity inertial vector, it is known as "positive geotropism".

There is a generally held belief that there are certain high-density particles that act as gravity receptors. These, perhaps due to their sedimentation properties, not only "sense"

the direction of the force, but also modify in some way the microscopic pattern of growth in plant cells. The initial part of this paper is based on a recent discussion by E. Pollard (1).

Sedimentation of particles and related phenomena

One paradox on the part of physicists when discussing geotropism is their assertion that particles must have significantly different densities from the cellular interior and be several micrometers in size before they will sediment in a 1-g field rapidly enough to account for certain chronological phenomena of geotropism (1). In apparent contradiction to this assertion is the fact that certain small plant hormones low in molecular weight (e.g., auxin, which is known to stimulate growth) are known to quickly respond to a gravitational field by developing concentration gradients within the plant (2). In other studies, starch granules of microscopic size have been found to sediment in response to gravitational force and redistribute themselves evenly throughout the plant cells under conditions of weightlessness (the statolith hypothesis) (3, 4).

The following arguments are valid either for sedimentation (when $\rho > \rho_s$) or for flotation (when $\rho < \rho_s$). Using Professor Pollard's analysis as a starting point, the force $\vec{F}_g$ on a single spherical particle of radius r and density ρ , immersed in an isotropic liquid medium of density ρ_s with

acceleration $\vec{g}$ is equal to:

$$\vec{F}_g = \frac{4\pi}{3} r^3 (\rho - \rho_s) \vec{g} \qquad [1]$$

Where the particle is moving with average velocity v and the viscosity is η, the Stokes shearing force, $\vec{F}_{sh}$, that will retard the free fall of this particle is:

$$\vec{F}_{sh} = 6\pi \eta r \vec{v} \qquad [2]$$

Equating the two, we obtain for the steady state sedimentation velocity:

$$\vec{v} = \frac{2}{9} \frac{r^2 (\rho - \rho_s)}{\eta} \vec{g} \qquad [3]$$

When $\rho \leq 2\rho_s$ and in aqueous milieu, such as the interior of living cells, [3] gives a slow sedimentation velocity for particles smaller than 1 micrometer in size. The absolute value of sedimentation velocity is much smaller than the mean thermal velocity, v_{th}, calculated from the kinetic theory of gases:

$$v_{th} = \sqrt{\frac{9}{4\pi} \frac{\kappa T}{\rho r^3}} \qquad [4]$$

Here, T is the absolute temperature and κ is Boltzmann's constant.

Figure 1 shows these velocities as the function of particle radius and density. It is somewhat misleading, however, to compare thermal velocity with sedimentation, since thermal motion leads to diffusion which is much slower.

The diffusion constant D for a particle with radius r may be expressed as:

$$D = \frac{\kappa T}{6\pi r \eta} \qquad [5]$$

[1]

[1] See, for example, R.D. Present, Kinetic theory of gases, McGraw Hill (1958).

The diffusion constant derived in this manner is not always applicable to liquids: There is a better, quantum mechanical approach (the cage theory). However, Equation [5] is valid in a homogeneous liquid medium if r is much greater than the dimensions of the liquid molecules. Diffusion and sedimentation do not really compete; rather, diffusion helps the rearrangement of sedimenting particles so that, after an appropriate time, the particle distribution corresponds to sedimentation equilibrium. However, in order to assess the relative importance of diffusion, we shall calculate the time it takes for a particle to cross a distance, $\bar{x}$.

Since diffusion of a certain particle depends on a sequence of random, collisions, and the net distance moved for a given time t, exhibits a statistical distribution. We shall give only the mean linear distance traveled, $\bar{x}$, as intially calculated by Einstein:

$$\bar{x} = (2Dt)^{1/2} = \left(\frac{\kappa T t}{3\pi r \eta}\right)^{1/2} \qquad [6]$$

In Figure 2, we have compared the time it might take a particle to diffuse across 20μm or 100μm distances. Twenty micrometers is comparable to the size of many plant cells; 100μm is near the radial dimension of the sporangiophore stalk in Phycomyces. Note that, at a density value of 2, all particles with $r \geq 0.2\mu m$ sediment faster across the specified dimension than would be possible by diffusion alone.

Sedimentation equilibrium

What is the optimum distribution of an ideal gravity sensor in response to gravitational-inertial vector fields across the growth zone of a plant? We shall answer this for the example of a unicellular fungus, Phycomyces blakesleeanus, which will be considered in some detail below. The diameter of the growth zone of the sporangiophore stalk is 100μm, and 1 g across the stalk may produce a curvature of 0.4 cm radius. In order to achieve this curvature, the structural components of the "bottom" parts of the cell growth zone must grow or elongate at a rate 1% greater than the rate of growth of the top part. We may then hypothesize that a graviceptor concentration difference of 1% between top and bottom might induce such a difference.

When given sufficient time, the concentration c(h) of a set of uncharged graviceptors of radius r will distribute as a function of height h from the cell base according to the well known barometric altitude formula, borrowed from the kinetic theory of gases.

$$c(h) = c(0)\, e^{\frac{-h}{H}} \qquad [6]$$

H represents the mean layer thickness.

$$H = \frac{3kT}{4\pi r^3 (\rho - \rho_s) g} \qquad [7]$$

In Figure 3, sedimentation equilibria have been plotted for a hypothetical living cell of 20μm dimension ($\rho = 2$), for various graviceptor radii. It may be easily seen that a 1% concentration

difference between top and bottom is established by any particle of radius larger than about 0.04μm at 1 g. It is known, however, that geotropism in plants occurs even at 0.01 g; at this low gravitational force, a particle radius of 0.4μm or larger would be required.

Droplet sedimentation

The sedimentation formulas used in most earlier studies of sedimenting particles in relation to geotropism (1) have a validity for monodisperse uncharged spherical particles in very dilute suspension, but do not consider the possibility of particle interaction in dense suspensions. It has been established, however, that sedimentation coefficients in some cases are dependent upon the concentration of the sedimenting molecules and can also be influenced by additional factors. The Van der Waals forces, for example, can lead to temporary or permanent aggregation in certain macromolecules.

There are a variety of ways in which sols of colloidal particles may form coacervate droplets of larger diameter. The electrical charge of the particles, the molecular composition of the suspending medium and its ionic composition all have an influence.[2] Since the aggregation of droplets depends so much on their milieu, alterations in the state of aggregation may occur as they sediment. Co-operative phenomena may predominate, and an entire small fluid region containing a number of these

[2]See, for example, Colloid Science, V. 1, ed. by H.R. Kruyt, Amsterdam, Elsevier Publ. Co. (1952).

particles may sediment as a droplet.

Dr. Howard Mel has demonstrated how a somewhat different kind of droplet sedimentation can develop and proceed down across a flowing interface, in addition to how this process can be used in the presence of gravity, to "switch on" a biochemical reaction (5). The basic transport phenomenon may be thought of as a diffusion-driven, controlled microconvection, and its origin lies in the much faster (upward) diffusion of a small molecular constituent as opposed to the slower (downward) diffusion of a macromolecular constituent. The resulting interfacial instability (sedimenting droplets) can arise in as little as 30 seconds after formation of the fresh, initially density-stable, interface.

Figure 4 displays the plausibility of droplet sedimentation: the surface of shear is assumed to be at the outer envelope of the droplet, rather than at the individual particle surface. Quantitation of this phenomenon is dependent on structural details of the particle surfaces and on the nature of intermolecular forces. It is clear, however, that sedimentation rate in a droplet with radius R is greater than that of an individual particle with radius r , when R > r. It is also apparent that sedimentation rate is slower than in a rigid particle with radius R having appropriate equivalent density:

$$\frac{2}{9}\,\frac{r^2(\rho-\rho_s)}{\eta}\,g \leq v_{\text{droplet}} \leq \frac{2}{9}\,\frac{R^2(\rho_{drop}-\rho_s)}{\eta}\,g$$

$$\rho_{\text{droplet}} = \rho_s + \frac{r^3}{R^3}\,n\,(\rho-\rho_s) \qquad [8]$$

n is the number of particles in each drop.

Electrical properties of sedimenting particles

The above discussion concerns sedimentation or flotation of neutral particles only. This is an important point for our understanding of the manner in which statoliths are able to convey information which affects growth patterns.

In aqueous milieu, neutral particles, or those particles nearing their isoelectric point, tend to flocculate, coagulate and settle in response to gravitational forces, if they have external lyophobic surfaces. On the other hand, neutral particles with lyophilic surfaces can remain relatively stable in aqueous suspension. These particles tend to adsorb lipids and may interact with and enter into lipid membraneous structures. There are recent macromolecular studies which reveal that helical macromolecules with external hydrophilic groups can interact with membranes and modify their chemical and electrical properties accordingly (6, 7). For example, EIM, Alamycithin and Monazomycin impart electrical conductance properties to artificial lipid bilayers; the antibiotic Nystatin makes mitochondrial membranes permeable to potassium.

If sedimenting particles have a net electrical charge, diffuse ionic double layers form around each particle within the intracellular milieu. In the course of sedimentation, the effect of gravitational fields brings about an electrical potential gradient which acts to retard the sedimentation rate and to modify sedimentation equilibrium. This wellknown phenomenon

is the Dorn effect. It would appear from photomicrographs of starch statoliths in wheat coleoptiles that sedimenting starch granules may belong to this class of particle (3).

The electrical field $\vec{E}$ with a gravitational force field $\vec{g}$ induced by acceleration in an aqueous suspension with specific electrical conductivity κ and dielectric constant ϵ is:[3]

$$\vec{E} = \frac{\zeta \cdot \epsilon \cdot (\rho - \rho_s)\vec{g}}{3\pi\eta\kappa} \qquad [9]$$

Here, ζ is the potential of the Gouy-Chapman boundary layer and depends on net electrical charge of the sedimenting particle and on concentrations of electrical charges of the various ionic species contained in the cytoplasm. Using Formula [9], with appropriate constants, it appears that sedimenting particles of 1μm radius at 1 g may readily develop an electrical potential of ten millivolts across a 100μm distance. Geoelectric effects are well known: for example, a recent report (8) indicates that rearrangement of auxins during geotropism may be responsible for the geoelectric effects.

Charged sedimenting particles do not necessarily follow gravitational gradients alone: they are responsive to electrical fields in general (electrophoresis) and can also be dragged along by streaming ionic solutions (electroosmosis). In this case, electrophoretic velocity $\vec{v}_{el}$ of a particle in an electrical field $\vec{E}_{el}$ is:

$$\vec{v}_{el} = \frac{\vec{E}_{el}\,\epsilon\,\zeta}{4\pi\eta} \qquad [10]$$

[3]The induced electrical field acts in a direction opposite the direction of the gravitational force. However, the signs were neglected in this report. The sign of the field also depends on the sign of the charge of the particles.

Gravitational forces and electrical forces both represent vector fields. The material flow $\vec{J}_g$ and electrical currents produced $\vec{J}_e$ are coupled and must follow the laws of irreversible thermodynamics. In steady state flow systems, an adequate expression for this coupling can be found in Onsager's equations:

$$\vec{J}_g = L_{11} \Delta \mu_g + L_{12} \Delta \mu_e$$
$$\vec{J}_e = L_{21} \Delta \mu_g + L_{22} \Delta \mu_e \qquad [11]$$

where μ_g and μ_e represent gravitational and electrical potentials respectively. Equation [11] is of special interest here. Near equilibrium, or at steady state, the coefficients L_{12} and L_{21} should be equal. Using the relationship given above in Equations [3] [9] [10] all of which were derived for steady state conditions, we can write the Onsager equations for a suspension of homogeneous particles of concentration c, assuming coupled gravitational and electrical flows as:

$$\vec{J}_g = \frac{8\pi r^5 (\rho - \rho_s)^2 c}{27\eta} \vec{g} + \frac{\zeta \epsilon c r^3 (\rho - \rho_s)}{3\eta} \vec{E}$$
$$\vec{J}_e = \frac{\zeta \epsilon c r^3 (\rho - \rho_s)}{3\eta} \vec{g} + \kappa \vec{E} \qquad [12]$$

In these equations, the flows represent flux densities of mass and of electrical charge per unit area and unit time. They do satisfy the conditions $L_{12} = L_{21}$. However, their validity in a geotropic growth application for a living organism remains to be verified. If experiment should show that, actually, $L_{12} \neq L_{21}$, circular flows and nonlinearity of reaction and flow rates would be indicated. The development of structural organization (negative

entropy) and of biochemical periodicity is thought to occur in nonlinear systems. It would be of fundamental interest, therefore, to observe and quantitate the L coefficients in a controlled geotropic experiment. This might be accomplished by measuring sedimentation rates of the supposed graviceptors and recording voltage, current, pressure and temperature changes simultaneously, thus obtaining the required L coefficients.

Theoretical analysis of the geotropic phenomena in an entire organism is still more complicated than indicated from the above discussion of the sedimentation of graviceptors. Actually, what is involved is the synthesis of graviceptors and other structural precursors, the transport of such components to the growth zones, recognition of the force fields present, synthesis and deformation and finally fixing of the newly formed structures. Recently, an important development took place in our laboratory with respect to analysis of such complex systems. G. Oster, A. Perelson and A. Katchalsky have described a new approach called network thermodynamics (9) (10). This approach recognizes that complex systems can often be broken down for purposes of analysis into a network of interconnected channels where various flows and interactions occur. The conservation laws governing these channels (originating from Kirchhoff's laws for electrical networks and generalized as Tellegen's theorem) are applicable to the irreversible thermodynamics of biological phenomena, and Oster et al have now given us prescriptions for their application.

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Observations on geotropism in Phycomyces blakesleeanus

From the above, it is evident that the physical factors involved in sedimentation of graviceptors can be quite complex. Therefore, a qualitative check on the applicability of the ideas expressed here to gravitational bioexperiments seemed in order. The Donner Laboratory group participated earlier in a satellite flight for the purpose of testing synergistic effects of weightlessness (11). Subsequently, a one-dimensional clinostat equipped with a gamma-ray source was constructed, so that it became possible to carry out experiments observing the influence of gamma-rays on growth processes simultaneously with the effects of gravity compensation (12). Currently, response in two different organisms is under study: that of the flour beetle, Tribolium confusum and the unicellular fungus, Phycomyces blakesleeanus (13) (14).

Jack Risius and Chui-hsu Yang have initiated studies on geotropism and radiation sensitivity in the giantunicellular multinucleate fungus, Phycomyces blakesleeanus (14). The organism is well known for its usefulness in basic sensory and environmental studies (15). Geotropism in Phycomyces is also well documented in interesting studies done by D.S. Dennison (16). Radiation effects upon Phycomyces have recently been documented in the work of Arne Forssberg (17).

Although a number of gravity-dependent effects have been observed in Phycomyces, this paper is confined to the particular

phenomenon known as negative geotropism, observed at Stage IVb in the life cycle of this fungus. (See Figure No. 5 for designation of developmental stages.) The sporangiophore normally grows vertically, straight upward, reacting against the direction of the gravity vector (Figure 6). Growth takes place within a rather small zone a short distance below the sporangium and is accompanied by continual rotation of the sporangium above the growth zone, while lower parts of the stalk remain rigid. Some minutes after placing the axis of the sporangiophore at a finite angle to the gravity vector, however, bending of the sporangiophore can be observed within the growth zone. This bending continues until the angle of the axis through the growth zone becomes parallel to the gravity vector (Figure 7). Geotropism and phototropism interact and compete, with phototropism exhibiting the greatest influence.

Opportunities for additional studies in this direction developed when some mutants were isolated with special geotropic properties. We have been using the albino car-10 mutant, which differs from the wild type in its synthesis of beta carotene and its greater negative geotropic response (bending rate of car-10 is about three times greater than that of the wild type).[4]

At this writing, it has been generally assumed that graviceptors are sedimenting particles, although guesses as to the identity of these particles range from the large vacuole located near the stalk's central axis to the many small particles abundantly distributed throughout the cytoplasm. Without attempting to

[4] We are grateful to Prof. Max Delbrück of the California Institute of Technology for supplying our group with mutant and wild type strains.

give a detailed documentation, we would like to briefly describe some of the physical factors which may relate to sensing of gravitational forces and concurrent modification in growth responses.

Growth of the normal, vertical sporangium depends on maintenance of strong internal pressure--the turgor pressure--maintained by the cellular osmotic pressure and water transport from the mycellium. Turgor pressure keeps the entire sporangiophore inflated, while structural strength is carried by the chitin wall. This wall is rigid, with oriented structures below the growth zone, and is surrounded by a wax cuticle which helps to maintain turgor by preventing evaporation. The remaining top three millimeters of the sporangiophore contain a small growth zone and conical region above which is carried the sporangium.

Growth of the wall consists of depositing and stratifying new chitin (Acetylglucosamine) in the growth zone. It is believed that chemical precursors and energy-rich molecules for this wall synthesis originate chiefly in the cytoplasm of the mycellium, which is nearest to the source of nutrient. Apparently, these molecules then travel upward in the sporangiophore through microtubules by means of an active ATP-energized transport mechanism.[5] Upward cytoplasmic streaming is readily observable with the microscope. Near the top of the sporangiophore, the tubules apparently end, and the cytoplasmic flow turns downward,

[5]The physical evidence for the actual existence of microtubules is incomplete and motion might take place instead along long cytoplasmic strands (14).

carrying particulate matter with it. All this is schematically shown in Figure 8a. Part of the flow of water proceeds across the plasma membrane, moistens the wall, and then evaporates. When the sporangiophore is vertical, there is approximate symmetry of the outward flow of precursors through the cell membrane, and the synthetic process in the growth zone of the wall can proceed with circular symmetry. The assumed flow lines are displayed in Figure 8a.

When the sporangiophore is horizontal, the flow lines are distorted, due to the fact that the force of gravity acts perpendicular to the sporangiophore. An assumed flow distribution in this case is shown in Figure 8b. Though quantitative information is lacking, it seems plausible that the vacuole might shift upwards and additional return flow might occur in the lower part of the stem, allowing sedimentation of particles onto the lower plasma membrane. If some of these particles have properties causing the membrane to be more permeable to wall precursors and catalysts, more wall synthesis should occur in the lower part of the growth zone. Though relatively easy to describe, this process is apparently so complex that it has not been fully quantitated, and the identity of molecules that may sediment to increase membrane permeability is not known. However, the following physical flows and forces are certain to be present:

flotation;
sedimentation;
thermal flow;
evaporation;

cytoplasmic streaming;
streaming potential;
transmembrane potential;
sedimentation potential.

The transmembrane potential appears to be lower at the growth zone and higher near the sporangium and is further lowered during the bending process, which begins within one minute after shifting the direction of the g vector.

Evaporation of water at and near the growth zone is a substantial factor and probably results in radial and longitudinal thermal gradients.

Cell proliferation and growth are generally known to be associated with changes in membrane potentials. It has been shown in chick embryo fibroblast cultures that a drastically lowered membrane potential (20-30 millivolts) is associated with logarithmic growth and cell proliferation, whereas in the "plateau" phase, cells have greater membrane potential (70-80 millivolts) (18). The lowered membrane potential is thought to be associated with greater membrane permeability for inorganic salts and organic nutrients.

Geotropism is not the only tropic response that has been attributed to changes in transmembrane transport. There is evidence suggesting that positive phototropism in Phycomyces is the direct result of local light interaction at the membrane and wall of the growth zone (19). Tropic bending of the sporangiophore can also be achieved by placing a droplet of pure water or hypotonic salt solution on the growth zone.

Immediate local swelling occurs, and the sporangiophore bends away from the droplet. Isotonic droplets do not have this effect (14).

A chance observation led us to note that negative geotropism in Stage IVb of the car-10 mutants is very weak, or missing when the organism is grown in the dark and is exposed to changing gravitational forces in the dark. An example is seen in cultures of Phycomyces grown in diffused light compared to darkness (14). (Fig. 9) This phenomenon is being studied in detail (work in progress by Risius, Yang and Tobias). Unfortunately, the effects of light are very complex; these involve not only a wavelength dependence, but the level of light adaptation is an important parameter in all of the tropic responses. If it should turn out that light exposure is essential to the synthesis of a membrane-active gravity sensor, the use of radioactive tracers might lead us to the identification of some of the molecules involved.

Conclusion

Physical considerations show that, in living cells, complicated processes occur in response to gravitational forces coupled with electrical forces and cytoplasmic flow.

In plant cells of 20μm size or in Phycomyces blakesleeanus, cytoplasmic particles of 1μm diameter and a density of 2 gcm^{-3} could potentially fulfill physical requirements for graviceptors. Changes in gravitational-inertial fields not only cause alterations in graviceptor sedimentation, but also place structural stress

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on parts of the organisms and alter critical flow processes. In Phycomyces blakesleeanus, complete understanding of geotropic processes will probably require the use of appropriate mutants, the study of light activation, and further study on the synthesis of chitin walls.

Acknowledgments

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REFERENCES

1. E. C. POLLARD, Physical Determinants of Receptor Mechanisms, in: Gravity and the Organism (eds. S. A. Gordon and M. J. Cohen), University of Chicago Press (1971), p. 25.
2. M. B. WILKINS, Hormone Movement in Geotropism, in: Gravity and the Organism (eds. S. A. Gordon and M. J. Cohen), University of Chicago Press (1971), p. 107.
3. P. LARSEN, The Susception of Gravity by Higher Plants, in: Gravity and the Organism (eds. S. A. Gordon and M. J. Cohen), University of Chicago Press (1971), p.73.
4. S. A. GORDON and J. Shen-Miller, Simulated Weightlessness Studies by Compensation, in: Gravity and the Organism (eds. S. A. Gordon and M. J. Cohen), University of Chicago Press (1971), p. 415.
5. H. C. MEL, Chem. Eng. Sci. 19, Pergamon Press Ltd., Oxford (1964).
6. G. EHRENSTEIN, Excitability in Lipid Bilayer Membranes, in: Biophysics and Physiology of Excitable Membranes (ed. W. J. Adelman, Jr.), Van Nostrand-Reinhold (1971), p. 463.
7. L. ROTHFIELD, and A. FINKELSTEIN, Membrane Biochemistry, Am. Rev. Biochem. 3, 463 (1968).
8. L. GRAHM, A Vibrating-Reed Electrometer for the Measurement of Transverse and Longitudinal Potential Differences in Plants, in: Gravity and the Organism (S. A. Gordon and M. J. Cohen, eds.), University of Chicago Press, (1971), p. 159.

9. G. OSTER, A. PERELSON and A. KATCHALSKY, Nature 234, 393 (1971).

10. G. OSTER, A. Perelson and A. KATCHALSKY, Network Thermodynamics: Analysis of Biological Systems, Lawrence Berkeley Laboratory Report No. 588 (February, 1972).

11. B. BUCKHOLD, J. V. SLATER, I. L. SILVER, C.-H. YANG and C. A. TOBIAS, Some Effects of Spaceflight on the Flour Beetle, Tribolium Confusum, in: The Experiments of Biosatellite II (ed. J. F. Saunders), N.A.S.A., Washington, D. C. (1971), p.79.

12. I. L. SILVER, The Modified and Completed Clinostat Irradiation System, Lawrence Berkeley Laboratory Report No. 5111 (1971).

13. C.-H. YANG, B. D. HEINZE, I. L. SILVER and C. A. TOBIAS, The Combined Effect of Radiation and Compensated Gravity on the Wing Development in Tribolium Confusum, Lawrence Berkeley Laboratory Report No. 596 (January, 1972).

14. J. RISIUS, C.-H. YANG, J. LUCE, and C. A. TOBIAS, Studies on Geotropism and Radiation in Phycomyces blakesleeanus, Lawrence Berkeley Laboratory Report No. 596 (January 1972).

15. K. BERGMAN, et al., Bacteriol. Rev. 33, 99 (March, 1969).

16. D. S. DENNISON, Gravity Receptors in Phycomyces, in: Gravity and the Organism (eds. S. A. Gordon and M. J. Cohen), University of Chicago Press (1971), p. 65.

17. A. FORSSBERG, Int. J. Rad. Biol., Suppl. 2 (1960), p. 206.

18. P. O'LAGUE, An Electrophysiological Investigation of Intercellular Communication, Ph. D. Thesis, University of

California, Lawrence Radiation Laboratory Report No. 19445 (1969).

19. L. JAFFE, Exptl. Cell. Res. 15, 282 (1958).

Legends to figures

Fig. 1. The magnitude of sedimentation velocities, v_s, and of thermal velocities, v_{th}, with various particle densities. Normal gravitation of 1 g is assumed. The coefficient of viscosity used was $\eta = 0.01$ poise, $T = 300^{o}K$.

Fig. 2. Time to cross distances of 100 micrometer (———) and 20 micrometer (------) by diffusion and by sedimentation. $\eta = 0.01$ poise, $T = 300^{o}K$.

Fig. 3. The distributions of hypothetical graviceptor concentrations in sedimentation equilibria in a 20 micrometer deep cell. A temperature of $300^{o}K$ and a particle density of $\rho = 2$ was assumed.

A. On the surface of the earth, at 1 g.

B. At reduced gravitational-inertial force of 0.01 g.

If one assumes that a concentration difference of 1% between the top and the bottom of the cells is sufficient to cause differential growth, then at 1 g a radius $r \geq 0.04$ μm, and at 0.01 g, $r \geq 0.4$ μm, would be sufficient to cause geotropism.

Fig. 4. Several small particles may temporarily or permanently form a coacervate or droplet to sediment and thus increase their sedimentation velocity. Here it was assumed that several particles of density $\rho = 2$ formed a droplet of density $\rho = 1.5$.

Fig. 5. Developmental stages in the giant unicellular multinucleate Phycomyces Blakesleeanus. A single spore forms a mycellium on the horizontal surface of nutrient agar medium. When it has covered the surface, several vertical sporagiophores grow. At their upper end is the sporangium which contains spores. For the experiments described here only stage IVb has been used. In this stage the growth zone is 1-3 mm below the sporangium. The top of the sporangiophore, above the growth zone rotates slowly clockwise (when viewed from the top). The sporangiophore below the growth zone has fixed shape and does not rotate. (from LBL 596, p. 36)

Fig. 6. Normal negative geotropic growth of car-10 in diffuse light. Pictures were superimposed at 15 minute intervals. Ten scale divisions correspond approximately to 0.1 cm. (from LBL 596)

Fig. 7. Geotropic response of car-10 follow in horizontal placement in nonphototropic red light. Exposures are at 0, 15, 45, 75 minutes. (from LBL 596, p. 38)

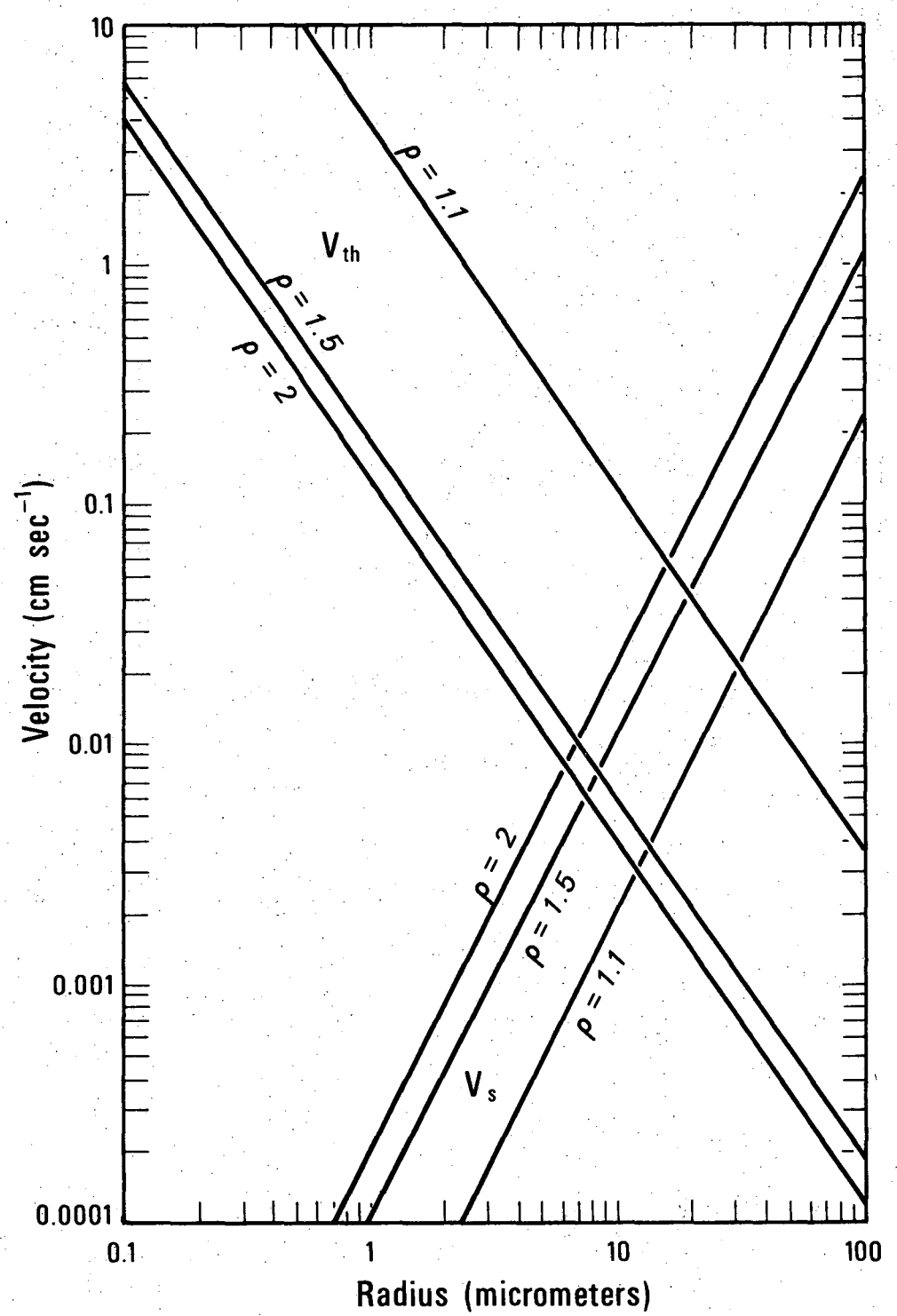
Fig. 8A. Schematic of a cross section of a Phycomyces sporangiophore in vertical growth phase, stage IVb. The entire sporangiophore is inflated under positive turgor pressure. Cytoplasmic streaming is seen upward, probably in microtubules as well as downward. Some of the water of the cytoplasmic sap evaporates

at the growth zones (see text).

Fig. 8B. Bending of Phycomyces sporangiophore when g force is applied transversely. Sedimentation of graviceptors transported in microtubules to the end of the sporangiophore could occur at the lower part of the growth zone. Such sedimentation might be sided by rearrangement of vacuole and of flows. (Drawings not to scale.)

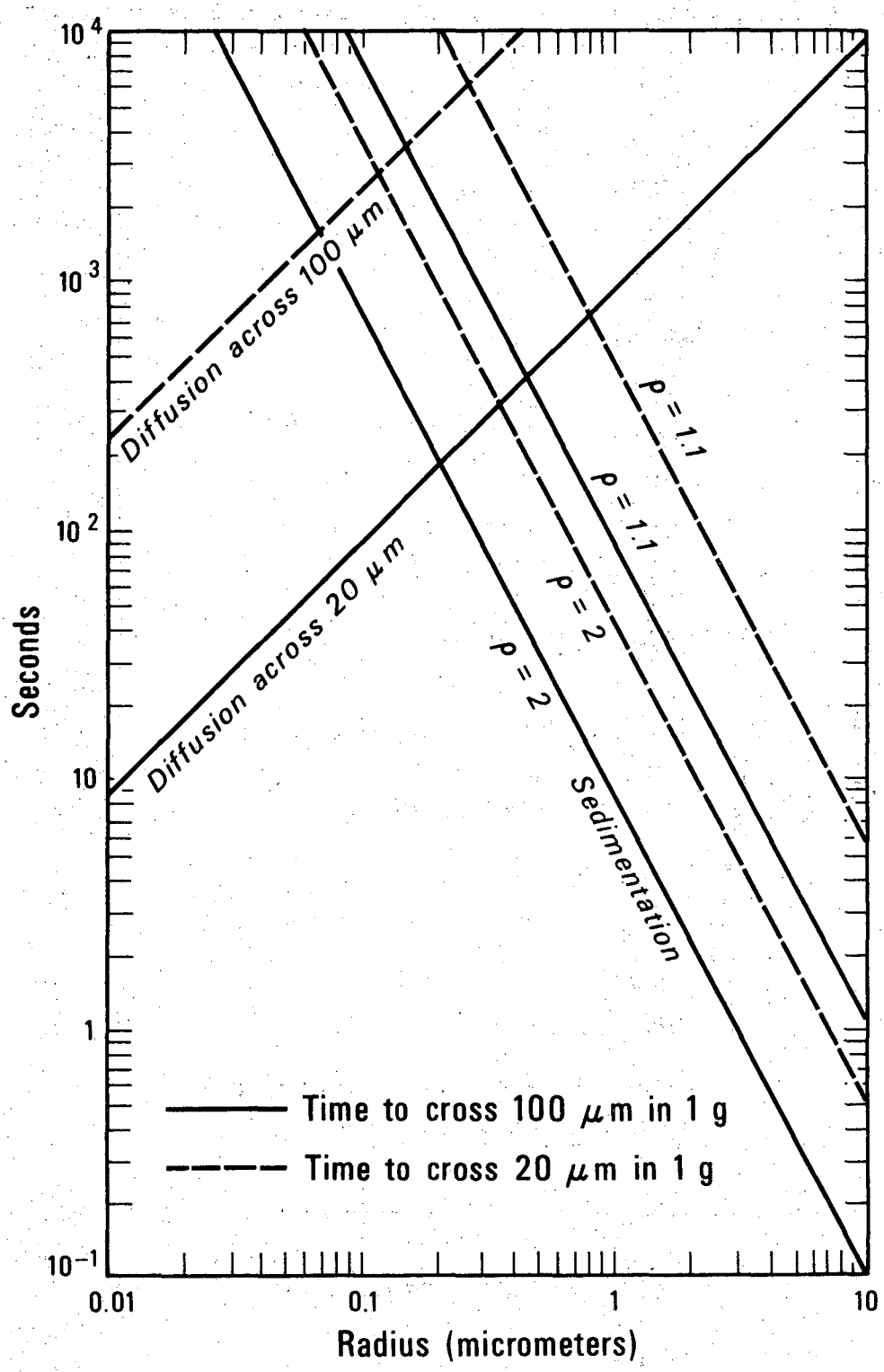
Fig. 9A. Left side. Sporangiophores grown from a single spore and mycellium in diffuse white light at 1 g (5th day at 23°C).

Fig. 9B. Right side. Sporangiophores grown from a single spore and mycellium at 1 g. The organisms were in total darkness from late mycellial stage until the picture was taken (5th day at 23°C). The sporangiophores show a comparatively weak gravity sensitivity, and branching of the sporangiophores, an abnormal process, can also be seen. Exposure to light of wavelength greater than 0.5μm is apparently necessary sometime during the development of the fungus in order to have normal negative geotropic response.



DBL 725 5298

Fig. 1



DBL 725 5297

Fig. 2

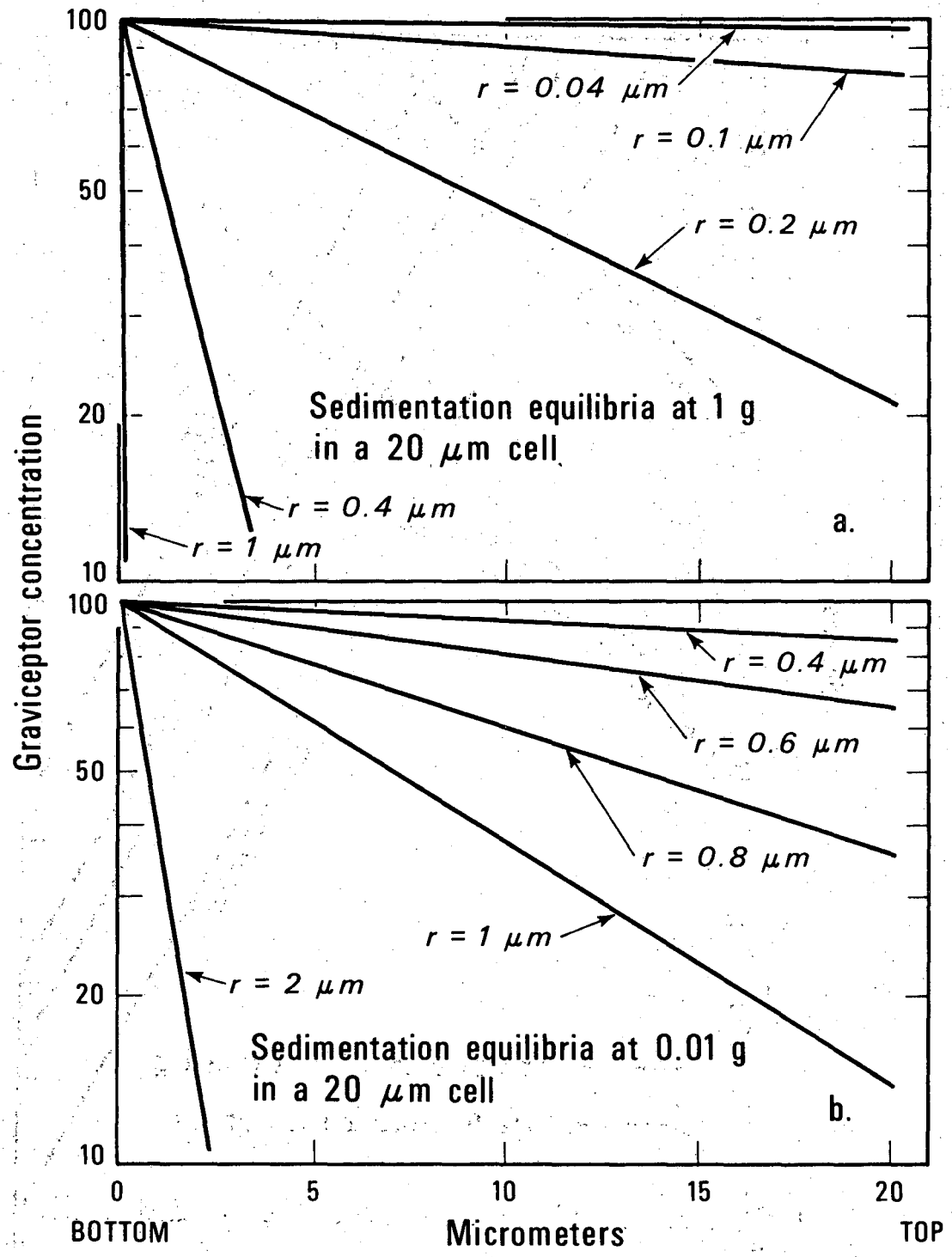


Fig. 3

DBL 727 5348

0 0 0 0 3 8 0 3 5 4 9

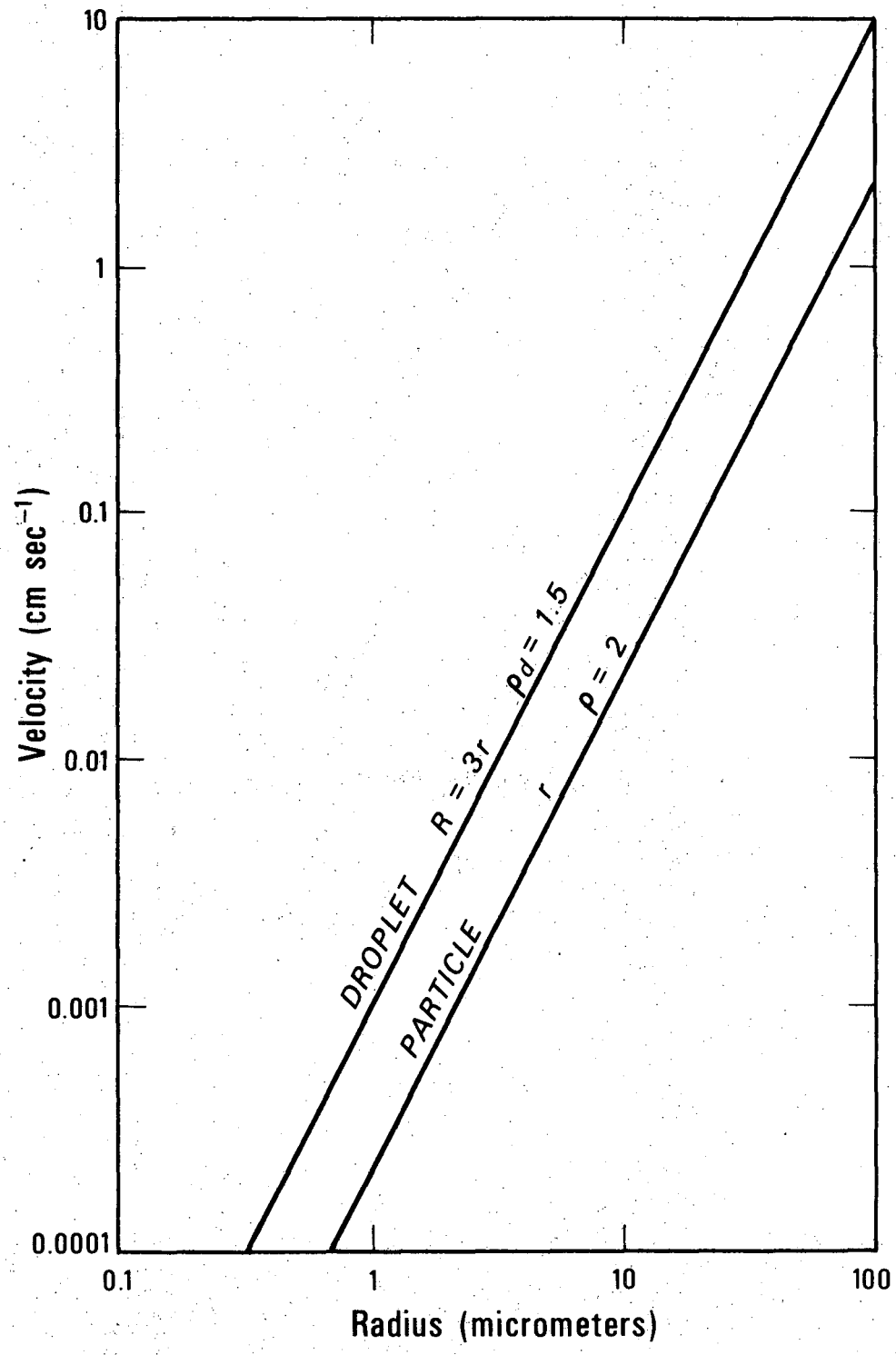


Fig. 4

DBL 725 5299

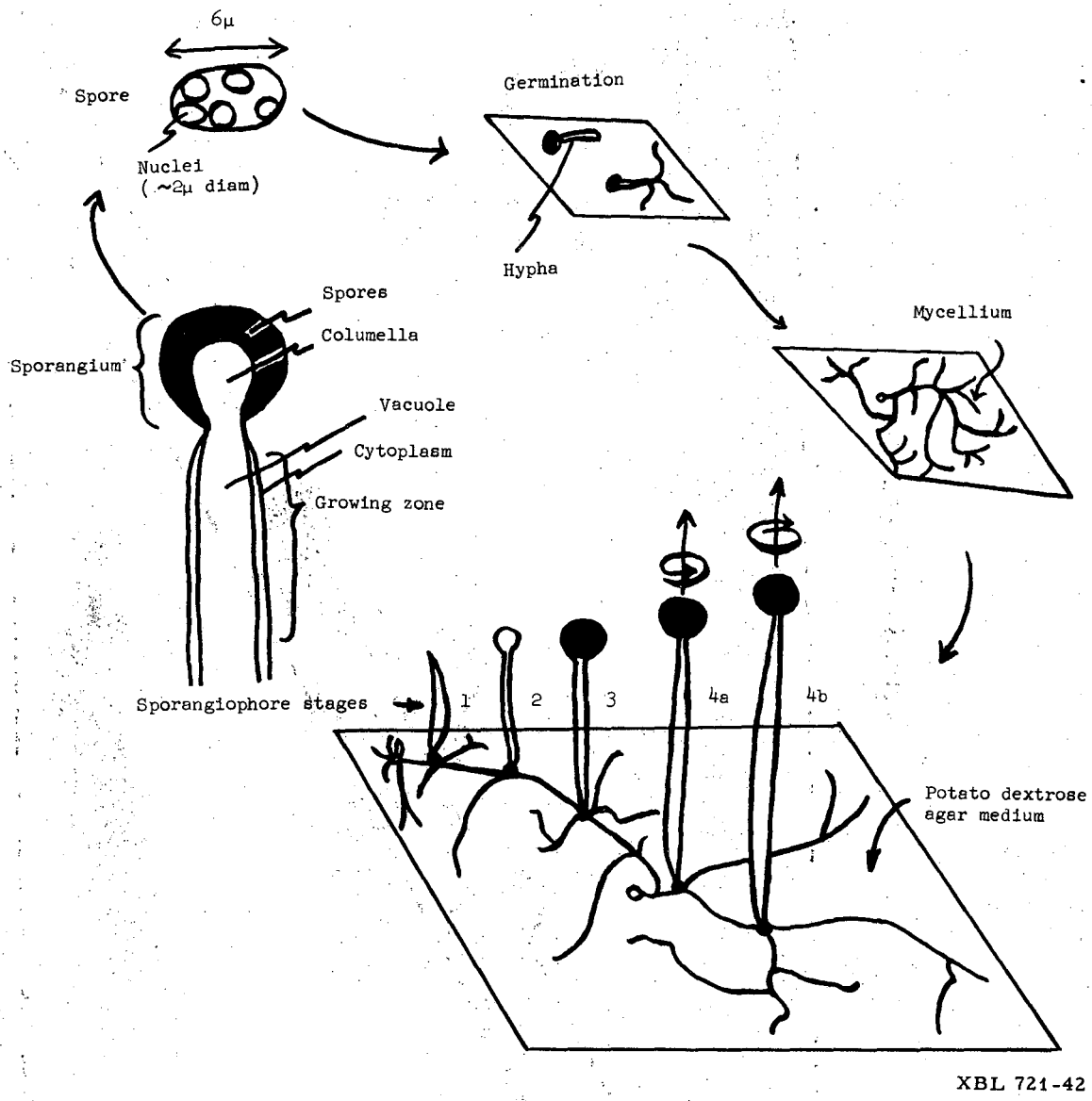
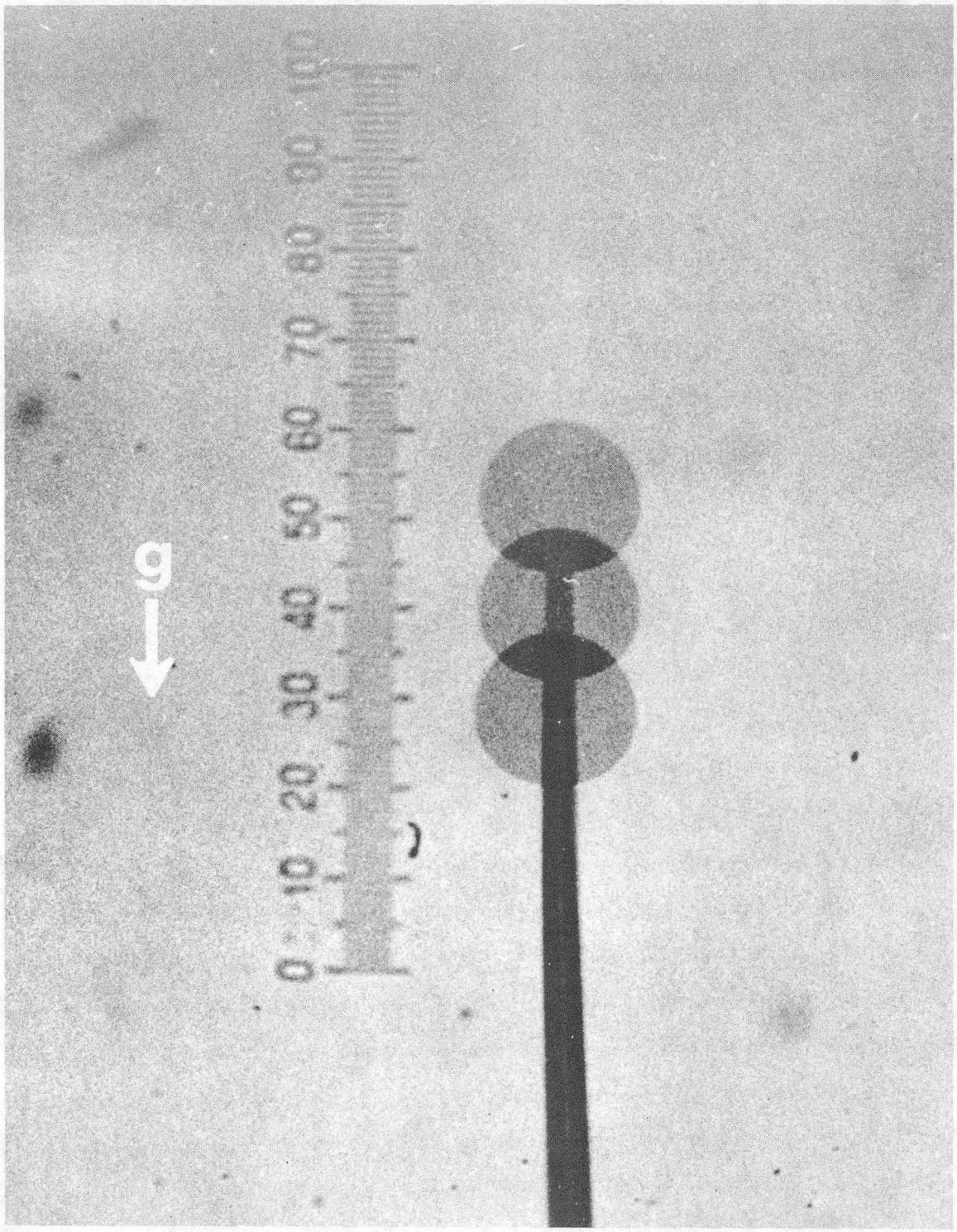

6μ
Spore
Nuclei
(~2μ diam)
Germination
Hypha
Mycellium
Spores
Columella
Sporangium
Vacuole
Cytoplasm
Growing zone
Sporangiophore stages
1
2
3
4a
4b
Potato dextrose
agar medium
XBL 721-42

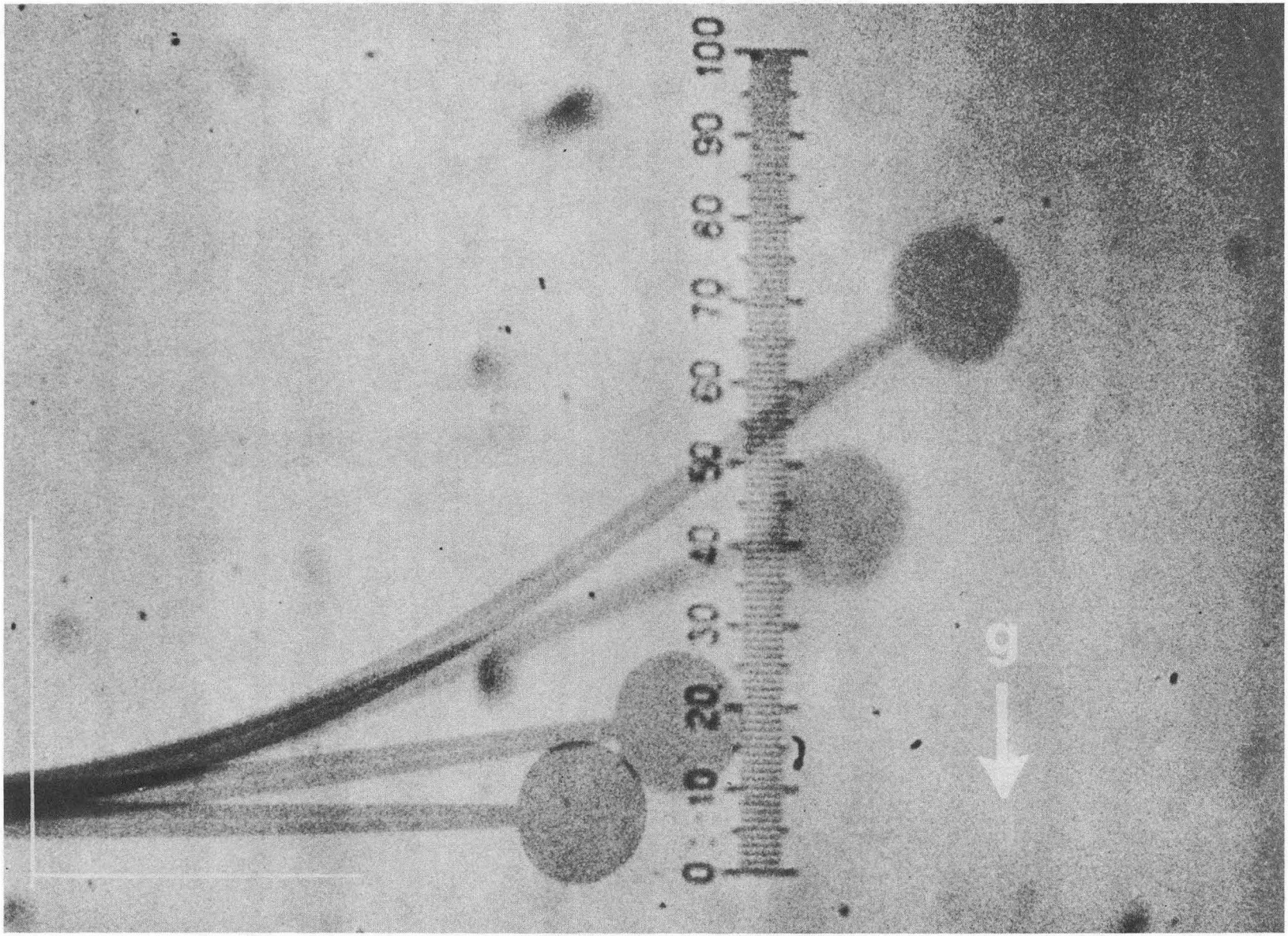
Fig. 5

0 0 0 0 3 8 0 3 6 5 0



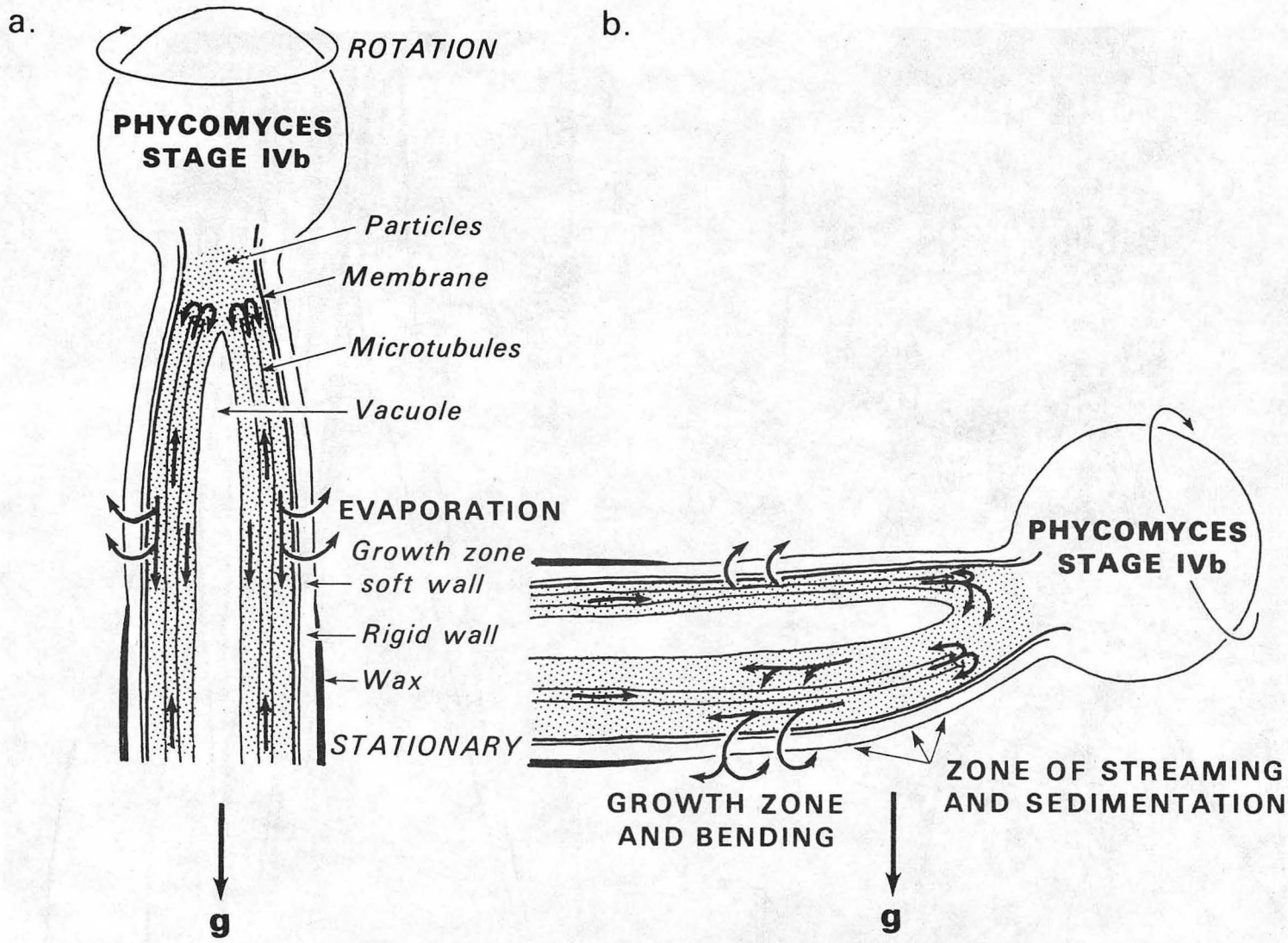
XBB 7110-5098

Fig. 6



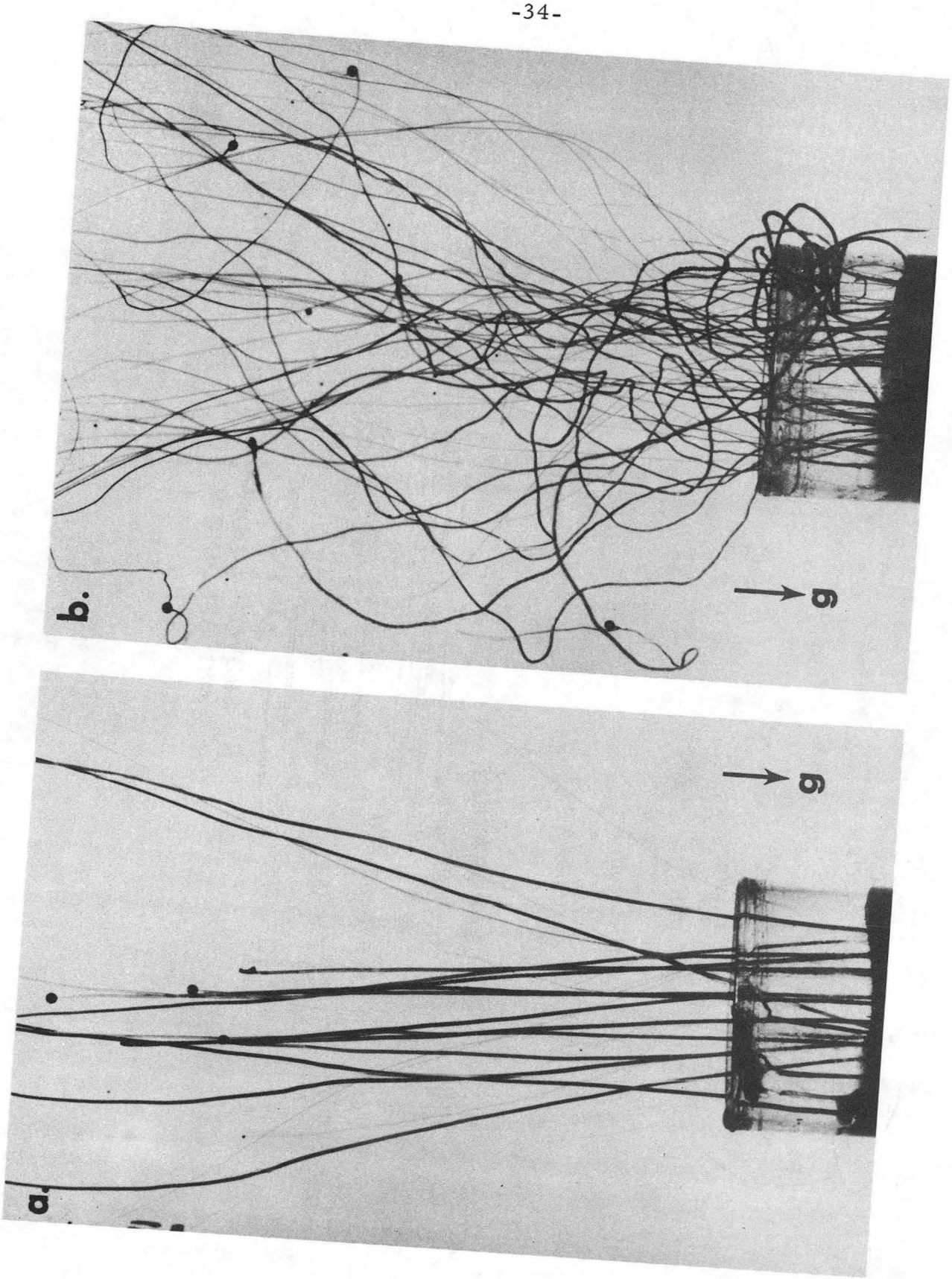
XBB 7110-5099

Fig. 7



DBL 727 5347

Fig. 8



XBB 727-3586

Fig. 9

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