

MODEL EXPERIMENTS ON THE MOVEMENT OF STATOLITHS

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SUMMARY

Observations of the movements of particles in a viscous fluid contained in a model of the statocyst, rotated on a horizontal axis, lead to the following considerations in connection with the interpretation of klinostat experiments with plants.

1. An asymmetric distribution of the statoliths in the statocysts may be protracted, which might cause a continuation of stimulation, if plants are rotated at relatively high rates on a klinostat.
2. In oblong cells rotating with their longer axis at right angles to the axis of a horizontal klinostat, asymmetric distributions of the statoliths may disturb the so-called omnilateral stimulation postulated. This might be of significance in connection with the explanation of those reactions of roots on the klinostat, which are known in the literature as the "rotation curvatures of Zimmermann".

INTRODUCTION

The perception of the direction of gravity has been a point of discussion since the early days of plant physiology.

AUDUS (1962) has given an excellent review of the various theories proposed and according to his opinion the starch statolith theory is the most plausible one. This theory purports that the sedimentation of starch grains or of particles of the same order of magnitude and specific mass will function as the first link in the chain of reactions that lead to the correction of the position by means of asymmetric growth.

In many experiments the effect of a given geoinduction is studied by subsequent rotation of the plants on a klinostat. Little is known, however, about the movements of the statoliths in the statocysts of plants treated in this way. At fairly high rotation rates the starch grains in root-cap cells seem to get dispersed. At low speeds the starch grains are found on the wall that is nearest to the earth at the moment of the fixation of the cells (VON UBISCH 1928).

For a better understanding of the reactions of plants rotated at different rotation rates, knowledge of these movements seems of much value. Since it is very difficult to get these data from living cells, we tried to obtain the information desired, with an artificial system just as Audus did in 1964.

2. METHODS

To study the movements of particles in a situation simulating a statocyst we made two models, each representing a thin transsection through an oblong

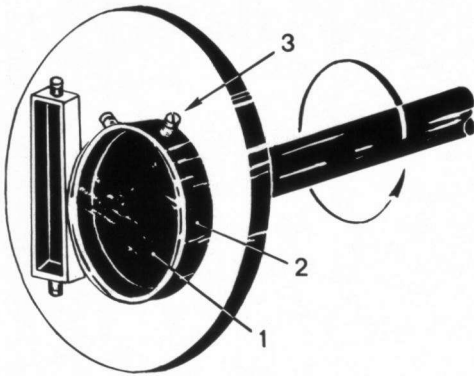


Fig. 1. The cells used in the experiments mounted on the klinostat.

1. Glass pane
2. Brass.
3. Opening for filling the cell.

cell of cylindrical shape. One of them was made to study the situation of a hypothetical cell oriented with its long axis parallel to the axis of the klinostat. It was of cylindrical shape, 8 cm in diameter and 1 cm thick. The other one was constructed to resemble the situation of an oblong cell orientated at right angles to the klinostat axis (as for instance endodermis cells would be if a plant were fastened at right angles to the axis of a klinostat).

It was 8 cm long, 1 cm wide and 1 cm thick.

Both cells were made of brass, and closed by a glass window as shown in *fig. 1*. In the cells particles were introduced to represent the statoliths. They were made of plasticine in which glass beads were kneaded to increase their specific weight slightly above that of a viscous solution of a detergent that was used to fill the cells. Measurements of the viscosity of the solution or of the specific weight of the particles were not made, since the combination of properties eventually obtained resulted in a sedimentation rate favourable for obtaining a set of different patterns of movements of the particles when the model cell was rotated at different rates on the klinostat. The used rotation rates were so low that no interference of centrifugal forces could occur. The lamps used to illuminate the cells were, in some experiments, connected to an electrical outlet via an interruptor driven by clockwork, so as to give one flash each second.

The records of the movements were made with a camera mounted on the klinostat. It could be connected to the model so that the camera and the model revolved together. Exposures resulted in records of the course of the particles relative to the cell wall. The camera could also be disconnected from the model, so that it remained stationary while the cell was revolving. In that case the photographs recorded the course of the particles in relation to the direction of gravity.

RESULTS

Figure 2 shows two series of photographs. The upper series was taken with the camera stationary. The lower series was taken with the camera fixed to the

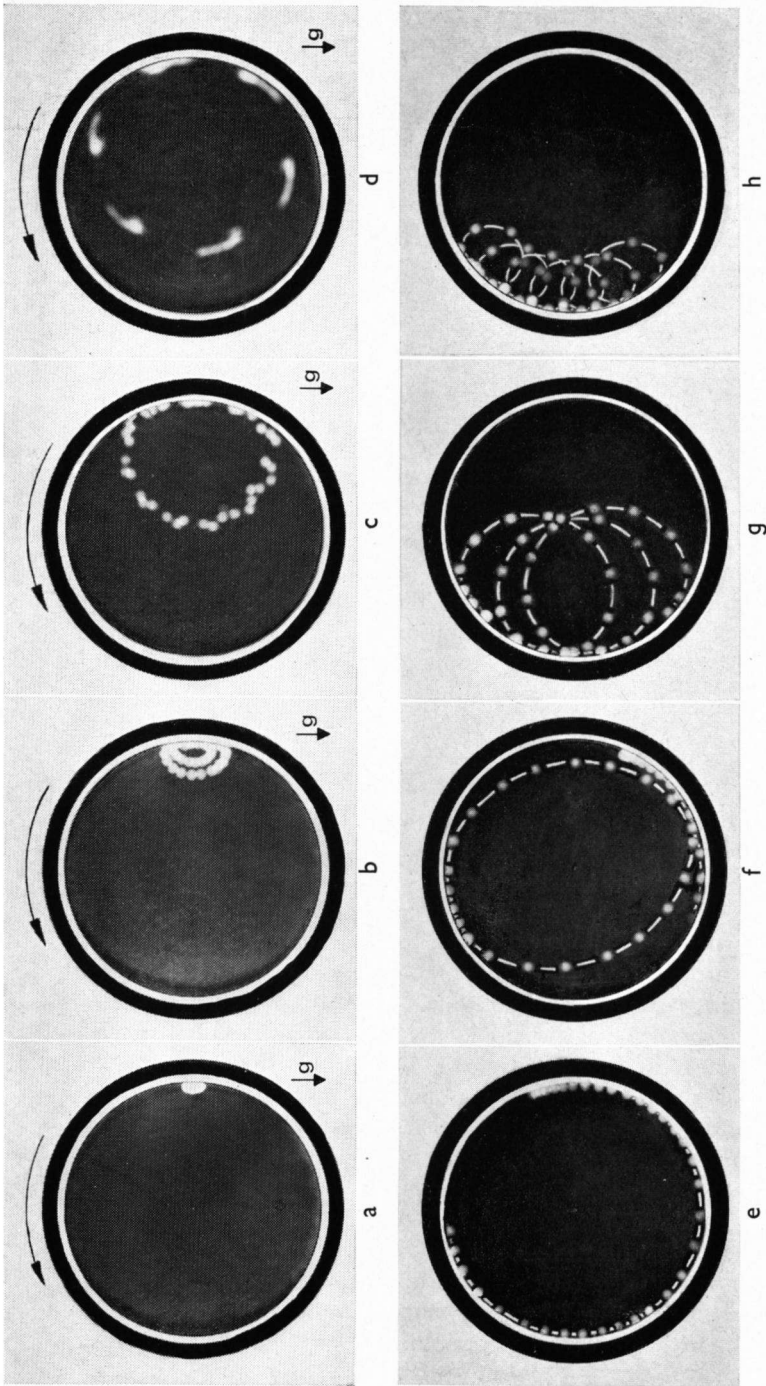


Fig. 2. Photographic records of the movement of a particle in the model cell. The upper row (a, b, c, d,) taken with the camera stationary. The lower row (e, f, g, h,) taken with the camera connected to the cell: movement of the particle in relation to the cell wall. Rotation rates: a and e 40 sec/rev. (1.5 rev/min) c and g 12 sec/rev. (5.0 rev/min) b and f 25 sec/rev. (2.4 rev/min) d and h 6 sec/rev. (10.0 rev/min) The sedimentation velocity of the particle in a non-rotating cell: 6 sec per diameter of the cell (8 cm). The arrows marked with *g* indicate the way gravity acts. The curved arrows indicate the direction of the rotation.

cell so that the movement of the particle was recorded in relation to the cell wall. The rotation rates of the upper and lower series were equal, so that *a* and *e*, *b* and *f*, *c* and *g*, *d* and *h* form pairs of records at the same rotation rate.

At a rotation rate of 1.5 rev/min (40 sec/rev) the particle slipped along the cell wall at the same rate as the latter rotated (*fig. 2a*). The same situation occurred at lower rotation rates, but at higher rates the particle was lifted up and lost contact with the wall (*fig. 2b*). Then it moved downward in the rotating liquid and eventually made contact with the cell wall again. *Figures e* and *f* show these movements of the particle in relation to the cell wall at the same rotation rates as *a* and *b*. At increasing speeds (*fig. 2c* and *d*) the particle described loops of increasing size. *Figures 2g* and *h* present the photographs taken with the camera connected to the cell.

These records illustrate that with increasing rotation rate the movement of the particle relative to the wall changes. At low rotation rates the particle never loses contact with the cell wall. At higher speeds it loses contact with the wall periodically. The places of contact are closer together as the rotation rate increases.

In all cells of which the transverse section is circular, irrespective of their size or the absolute values of viscosity of the content or specific mass of the statoliths, the course taken by a statolith is mainly depending on the time the particles take to traverse the cell and the rotation rate. Differences in the friction of the statoliths with the cell wall will only modify the course of the statolith. An increase of the friction will reduce the speed of the statolith along the cell wall.

From the rotation rates and the number of contacts made on the circumferences of the cell we can calculate the rate of movement of the particle along the wall. The result is shown in *fig. 3*. In this graph the reciprocal of the rotation rate has been plotted on the abscissa in arbitrary units per revolution. The time the particle takes to traverse the non-rotating cells was chosen as the unit of time.

This unit was preferred to the traditional units, as it will enable us to compare the behaviour of particles with different sedimentation rates in cells of different dimensions. On the ordinate we have plotted the progression of the subsequent areas of contact of the particle per unit of time.

The rate of movement of the particle along the wall increased with increasing rotation rates and was the same as the velocity of the inner surface of the cell, as long as the particle slipped along the wall and did not loose contact. With increasing rotation rates, however, as the particle periodically lost contact with the wall, the rate of progression of the subsequent areas of contact slowed down. During the experiments the particle sometimes touched the front or the back wall (*i.e.* the walls in the plane of rotation) of the model, which influenced the movement and explains the scatter of the points in the graph. In other experiments a number of particles were enclosed in the model. At the start of the rotation the particles were grouped together, but when the revolutions were so fast that the contact with the wall was periodically lost they were dispersed through the cell.

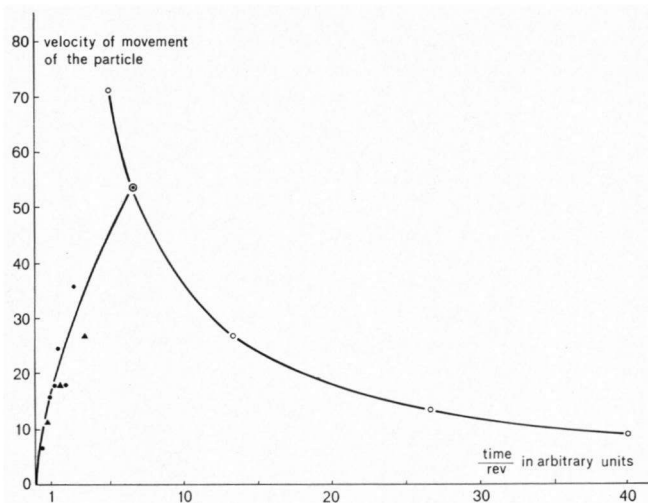


Fig. 3. Results of two experiments in which the particles have different sedimentation rates: \blacktriangle 0.2 cm/sec; \bullet 1.3 cm/sec.; \circ calculated from the rotation rate: particle slips along the cell wall. Abscissa: the reciprocal of the rotation rate in arbitrary units, one unit is the time the particle takes to traverse the cell (axis not rotated). Ordinate: the rate of movement of the contact places of the particle with the cell wall, in degrees per unit of time.

The dispersal is due to the following causes:

- particles "push" each other toward the centre of the cell;
- they may show different sedimentation velocities.
- the particles may touch the front or back wall or the cell.

At high rotation rates the particles showed a tendency to stay together for a longer period than at relatively low rotation rates. If a particle was forced towards the centre of the cell, it would take a number of revolutions before the wall was touched again since its speed of movement was much slower than when it was forced along by friction with the wall. The contact with the front or back wall appeared to be the most effective cause of the dispersal. The sooner this contact took place the earlier the particles started on individual courses. The experiments with the oblong model were made to imitate the movements of particles in an oblong statocyst as for instance an endodermis cell of a plant fixed at right angles to a klinostat axis. The records are shown in *fig. 4*. The particle slipped along one of the long walls at the part of the cell that one might designate as the basal end. It then traversed the cell, slipped up and then down the opposite wall at a more apical part of the cell, traversed the cell again, slipped down and up the former wall etc. Thus describing a complicated "loop" the size of which varied with the rotation rate. In living cells such intermittent stimulations of the two opposing walls, at different distances from the ends of the cells, could induce a curvature in case of a difference in sensitivity of the apical and the basal end of the statocyst (*c. f.* HABERLANDT, 1928).

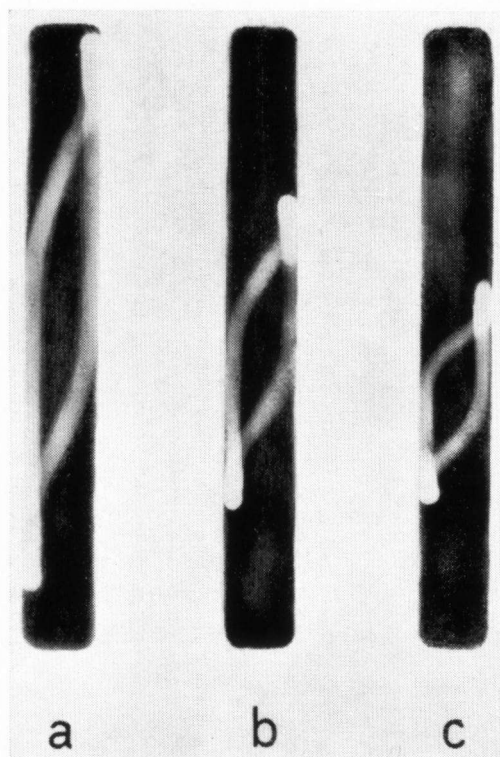


Fig. 4.

Records of the movement of a particle in relation to the cell wall, in an oblong cell. The camera was connected to the cell. a, b, c, increasing rotation rates.

4. DISCUSSION

To estimate the merits of the use of the klinostat in research on tropisms knowledge of the exact movements of the statoliths of a plant rotating on a klinostat will supply a valuable contribution. It is admitted that the movements of statoliths in living cells will not be exactly the same as the movements of the particles in the models (filled with a fluid that is homogenous, which the protoplasm evidently is not) but there might be some resemblance in the behaviour of the two systems.

The results of the experiments with the circular model show that at their relatively high rotation rates at which the particles periodically lose contact with the wall the sum total of the time of contact with the wall is less than at lower rotation rates.

According to the statolith theory this would mean that the total amount of geostimulus of the wall is less.

Moreover, at these rates of rotation the rate of progression of subsequent places of stimulation along the wall decreases with an increasing rate of rotation. From this it follows that a given progress of stimulations along the cell wall can be realised at two rotation rates. There are, however, two differences.

The stimulation is periodical at the high rate, whereas at the low rate it is continuous. Moreover, at the high rate the particles will get sooner or later dispersed through the protoplasm. The above observations might necessitate to change the theory of klinostat action on a few points. One may doubt whether it is true that the asymmetrical stimulation received previous to klinostating, really ends at the moment the plant starts to rotate. In case the rotation rate is sufficiently high we must expect, therefore, that stimulation of the cell wall in the vicinity of the area occupied by the statoliths at the onset of rotation, will continue for some time after the beginning of the rotation. This idea of a prolonged stimulation on the klinostat will be applied below in an attempt to explain a few observations made by LARSEN (1965) with the roots of *Artemisia* and *Lepidium*. From the experiments of Larsen in which the experimental plants were treated in various ways, we discuss the type that is disturbing least the distribution of the statoliths. Larsen transferred normally oriented roots to the horizontal position for periods varying between 4 minutes and 1.5 minutes. The first set of the plants was then immediately rotated on the klinostat. The other set was returned to the normal, vertical position for 5 minutes and only then rotated. The plants that were rotated immediately after the stimulation curved more than those of the second set, a result that is against all expectation. At first sight one would expect no difference at all, but in case of a difference, one would predict a result just opposite to the one observed, because in the second set the statoliths might remain somewhat asymmetrically distributed during the period of vertical position preceding the klinostating. This unexpected result might be explained by the observation made with the model, that the "statolith" does not leave the side wall immediately. Though revolving, it continues to "stimulate" that part of the wall it is near to, provided the rotation rate is sufficiently high. In these experiments of Larsen the rotation rate was very high, i.e. 4 rev/min. Larsen determined the time the amyloplasts in the rootcaps took to traverse the cells. The rootcap cells are probably acting as statocysts in roots, (JUNIPER *c.s.* 1966, KONINGS to be published). From these data and the rotation rate of his klinostat we calculated a value in our arbitrary units of roughly about 0.1. The progression of the contacts with the cell wall we can estimate from our *fig. 3*. At the rotation rate mentioned it would take the statoliths several hours to move from a given position to the opposite side of the cell.

If plants are placed at right angles to a klinostat axis they may develop curvatures that are known as "rotation curvatures of Zimmermann". In his experiments ZIMMERMANN (1927) used roots of seedlings of *Lepidium*. He fixed them at right angles to the klinostat axis in four ways (see *fig. 5*). He observed that the roots did not remain straight as one might expect on account of a putative symmetric stimulation. The roots curved, however, and the direction of the curvature was determined by the direction of the rotation. It appeared that for the roots receiving antagonistic stimuli, the stimulus received in the position that is followed by the inverse position, was the more effective one. This position is marked by black in *fig. 5*.

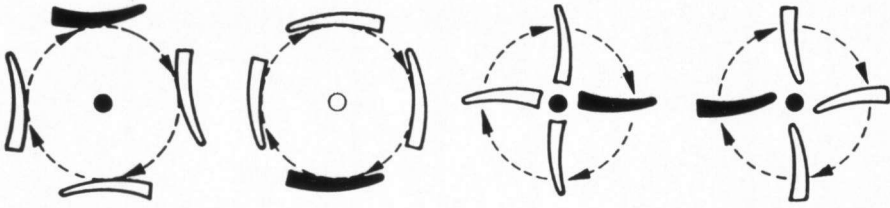


Fig. 5. Zimmermann's experiment. Four ways of fixing roots to the klinostat and the resulting curvatures.

To explain the occurrence of these curvatures we assume that differences exist within the statocyst that influence the effectiveness of the stimulation by the statoliths.

This assumption is backed by the idea of a physiological length polarity of the cell. If the statoliths in the statocysts behave in the same way as is shown by the "statolith" in the model (*fig. 4*), the apical end of the cell is stimulated at one side and the basal end at the opposite side. Because of differences in effect of the stimulation in the two ends of the cell, the seeming symmetry of the stimulation is in fact not present, since one of the antagonistic stimuli will be more effective than the other, which might be the cause of the root curvatures on the klinostat.

Comparison of the efficiency of continuous and intermittent geotropic stimulations revealed that intermittent stimulations are more effective. GÜNTHER MASSIAS (1928/29) showed moreover that the treatment of the experimental plants in the period between the stimulations is not without importance, because if the plants are rotated on a klinostat between the stimulations the effect of the stimulations is far greater than if the plants are placed in the vertical position during those periods. This difference might also be explained by the mechanism described above.

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