Chapter 6

The Fast Rotating Clinostat: A History of its Use in Gravitational Biology and a Comparison of Ground-Based and Flight Experiment Results

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ABSTRACT

The fast rotating clinostat has been used in gravitational biology since 1965 to investigate effects of simulated microgravity. Using a microscope, the behavior of cells and organelles within the cells under microgravity conditions can be directly observed during rotation. Experiments with several mammalian cells and unicellular organisms have shown that different cellular functions are affected in simulated microgravity. Almost no changes were noted in the area of developmental biology. A comparison of results from the fast rotating clinostat and flight experiments reveals that the clinostat is a valuable tool to evaluate an organism's sensitivity to gravity changes. Therefore, a biological object proposed for a flight experiment should first be investigated in the clinostat before it is selected. This is especially true in the use of single cells and unicellular organisms.

INTRODUCTION

Biological research in space began with the launch of the first satellites. Since then, the effect of microgravity on a broad spectrum of organisms, including humans, has been studied. Recently, all the experiments involving viruses, bacteriophage, unicellular organisms, lower fungi, animal and plant cells, and tissue cultures have been summarized (Dickson, 1991).

The results of investigations performed with cells and unicellular organisms clearly show that important cellular functions change in microgravity. A selection of the results is given in Table 1. The functions affected by a change of gravity in these cells are: energy consumption, biosynthesis, differentiation, gene-expression, mitosis, genetic transfer, membrane permeability, cell proliferation, and intracellular convection. It is not yet clear if the observed changes are due to a direct effect or an indirect effect of gravity. Nevertheless, these results demonstrate that gravity must be considered as an important environmental parameter, like temperature, light intensity, and atmospheric pressure, to which organisms may adapt. So, suddenly, new conditions have become available for biological investigation.

Since the possibilities to perform experiments in true microgravity have always been and still are very limited, devices for microgravity simulation, such as the fast rotating clinostat, were developed. The principle of the fast rotating clinostat and how it models the effect of microgravity is described by Briegleb in this issue of the ASGSB Bulletin (Briegleb, 1992). A more theoretical background of the technique of microgravity simulation with the fast rotating clinostat is presented by Schatz (1983).

HISTORICAL OVERVIEW

The study of gravitational biology, and with it the use of a clinostat, started prior to the onset of biological experimentation in space. In the early 19th century, plant physiologists already made use of the slow rotating clinostat. In the late 19th century, it was shown for the first time that the inner arrangement of the frog egg may be altered by gravity (Pflüger, 1883; Born, 1885). About 10 years later, Schultz (1894) observed a gravity-induced sensitivity of the frog germ cell caused by an incomplete rotation during the first cleavages of the egg. These early investigators used a principle which came very close to the fast rotating clinostat.

In 1958, Müller presented a clinostat for the investigation of the human statolith organ. According to his idea, a human test subject was fastened in a horizontal position within a cylinder and then rotated about the horizontal axis.

Based on this “human” clinostat and many theoretical considerations, Briegleb developed the fast rotating clinostat (Briegleb, 1965, 1967, 1968). In early experiments, the influence of simulated microgravity on the behavior of some microorganisms (Chlorella pyrenoidosa and the blue-green alga Phormidium autumnale) was studied. No changes in the metabolism of Chlorella or the orientation of Phormidium compared to a 1 g control were observed. Based on these results, Briegleb concluded that only cell functions in organisms, which have a special system for gravity perception, may be influenced by gravity changes. Therefore, he extended his investigations to the germ differentiation of the beetle Tribolium castaneum. In these studies, where the eggs were exposed during 80% of their development time to clinorotation, the embryonic development and the survival time of the hatched larvae were not changed com-
Table I.  *Alteration of Cellular Functions in Microgravity*

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Function Altered</th>
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<tbody>
<tr>
<td>Human embryonic lung cells</td>
<td>20% Reduction of glucose consumption (Montgomery et al., 1978)</td>
</tr>
<tr>
<td>Human T-lymphocytes &amp; monocytes in suspension</td>
<td>500% Increase in interferon-α biosynthesis (Talas et al., 1983; Bátkai et al., 1988); 90% Inhibition of mitogenic activation; 70% Inhibition of interleukin-1 production (Cogoli et al., 1984, 1988; Bechler et al., 1986, 1992)</td>
</tr>
<tr>
<td>Human T-lymphocytes &amp; monocytes attached to microcarriers</td>
<td>200% Increase of mitogenic activation; 350% Increase of interferon-γ production; Normal monocyte function (Bechler et al., 1992)</td>
</tr>
<tr>
<td>Jurkat cells</td>
<td>100% inhibition of IL-2 production (phorbolester, ionophor) (Limouse et al., 1991)</td>
</tr>
<tr>
<td>Monocytes (THP-1)</td>
<td>85% Inhibition of IL-1 production (phorbolester, ionophor) (Limouse et al., 1991)</td>
</tr>
<tr>
<td>Human epidermoid carcinoma cells</td>
<td>50% Reduction in c-fos expression (induced by EGF) (de Groot et al., 1990)</td>
</tr>
<tr>
<td><em>Paramecium aurelia</em></td>
<td>100% Increase of growth rate (Planel et al., 1982; Richoillez et al., 1988)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>40% Increase of conjugation (Ciferri et al., 1986, 1988); 300% Resistance to antibiotics (Tixador et al., 1984; Lachpche et al., 1988)</td>
</tr>
<tr>
<td><em>Chlamydomonas</em></td>
<td>Manyfold increase in biomass yield (Mergenhagen and Mergenhagen, 1987, 1988; Mennigmann and Mennigmann, 1986, 1988; Theimer et al., 1986)</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
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<tr>
<td><em>Anise cells</em></td>
<td></td>
</tr>
<tr>
<td><em>Physarum polycephalum</em></td>
<td>Increase of frequency and velocity of cytoplasmic movements (Briegleb et al., 1986; Block et al., 1988)</td>
</tr>
<tr>
<td><em>Paramecium caudatum</em></td>
<td>Same swimming behavior as at 1 g for 80 seconds, followed by random distribution (Hemmersbach-Krause et al., 1992)</td>
</tr>
</tbody>
</table>

pared to controls. Around 1970 researchers in the U.S. also started to use the fast rotating clinostat, especially in the field of developmental biology (Yang and Tobias, 1974).

Briegleb and his coworkers were not satisfied with the existing clinostat. They not only wanted to measure effects after having taken the samples from the clinostat; they also were interested in observing visually movements within a cell during rotation. Therefore, a microscope was combined with the fast rotating clinostat (Briegleb et al., 1973a; Briegleb et al., 1976). The first results obtained with this new instrument concerned the movements of intracellular free particles and the protoplasmic streaming in a leaf cell of *Elodea canadensis*, a water plant. An interesting observation was that particles with a size of about 1 μm were randomly distributed within the cell at a rotation speed of 100 rpm. Under this condition, their behavior was controlled by thermal agitation (Brownian movement), whereas at 1 g 90% of these particles sedi-

mented. These findings demonstrated that the fast rotating clinostat is a valuable tool to simulate microgravity.

In 1979, the first experiments with mammalian cells (human lymphocytes) were performed in a fast rotating clinostat by our group (Cogoli et al., 1980). Today, different research teams in the U.S., Japan, and Europe use the fast rotating clinostat mainly for investigations with mammalian cells and unicellular organisms, but also with plant cells and protoplasts. In the field of developmental biology, the fast as well as the slow rotating clinostat is used. In this review I will not consider the work with plant cells and protoplasts.

**RESULTS FROM CLINOSTAT EXPERIMENTS**

**Developmental Biology**

The evolutionary development of all the organisms on Earth has occurred over millions of years under a con-
Table II. *Developmental Biology on the Clinostat*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td><em>Rana temporaria</em></td>
<td>Normal differentiation of the frog germ (Briegleb et al., 1971); Normal development of vestibular organs of embryos and larvae, but: statolith sensory epithelium is vacuolized (Briegleb, 1974; Neubert and Briegleb, 1977; Neubert, 1981)</td>
</tr>
<tr>
<td><em>Tribolium confusum</em></td>
<td>Normal development from fertilized egg until late larvae. Malformations when beetles are rotating during 1.5 generations (Briegleb et al., 1973b, 1975; Yang and Tobias, 1974)</td>
</tr>
<tr>
<td>Sea urchin</td>
<td>Normal fertilization and early development (Schatten and Schatten, 1987)</td>
</tr>
<tr>
<td>Mouse</td>
<td>No abnormalities in the efficiency of fertilization or in the appearance of fertilized ova (Wolgemuth, 1987)</td>
</tr>
</tbody>
</table>

constant gravitational force. It is, thus, conceivable that a change of this environmental parameter might have dramatic influences on the normal development. Therefore, the study of developmental biology has been a research objective since the beginning of spaceflight. On Earth, the influence of simulated microgravity on the development of several organisms has been studied in the fast rotating clinostat. Some of the results are summarized in Table II.

Since Schultz's findings on a gravity induced sensitivity of the frog germ, the embryogenesis of amphibian eggs has been the subject of many investigations in different laboratories, and in true and simulated microgravity. Briegleb et al. studied the development of *Rana temporaria* on the fast rotating clinostat. They observed no changes in the phenotype of the frog if development from fertilization of the egg until the early larvae took place in the clinostat (Briegleb et al., 1971). Further investigations were dedicated to the influence of simulated microgravity on the development of the vestibular organ of frog embryos and larvae (Briegleb, 1974; Neubert and Briegleb, 1977). In these experiments, no abnormalities in the morphological structure were found in embryos and larvae grown in the clinostat until stage 15. There are similar results from two flight experiments, although the experimental conditions were very different (Vinnikov, 1974). In a later investigation, Neubert (1981) observed that the statolith sensory epithelium is vacuolized, an effect found to be reversible.

The flour beetle *Tribolium confusum* has been studied by different research groups on the fast rotating clinostat (Briegleb et al., 1973b; 1975; Yang and Tobias, 1974). There are two different stages in the life cycle of *Tribolium* during which the organism might be particularly sensitive to environmental changes: the early development of the fertilized egg into larvae and the metamorphosis from pupal to the adult beetle. In their investigations, both groups reached qualitatively the same conclusion—the first stage in the development was not influenced by simulated microgravity. It is interesting to note that, in nature, the eggs are deposited randomly in the medium by adults and are constantly tumbled by larvae and adults. Therefore, it might well be that the egg has become insensitive to gravity changes. When young larvae were subjected to clinorotation for a period of 30-40 days, i.e., more than one generation time, wing abnormalities were found. These results are in agreement with flight experiments (Parfenov, 1981). The combined effect of microgravity and radiation on the development of *Tribolium* pupae was studied in a flight experiment by Yang and Tobias (1974). More wing abnormalities were observed in pupae irradiated in orbit than on the ground. The same synergism was found in a clinostat experiment.

The effect of simulated microgravity on the development of sea urchins was studied by Schatten and Schatten (1987). At several rotational speeds ranging from 1/4 to 60 rpm, fertilization and early development occurred normally.

The influence of gravity changes on mammalian development and differentiation was investigated by Wolgemuth (1987). When mouse ova were rotated in a fast clinostat in the presence of sperm, no alterations in the efficiency of fertilization were observed. The resulting zygotes appeared normal.

In conclusion, most of the results in this research area are negative in the sense that the development of different organisms is not sensitive to simulated microgravity as modelled in the fast rotating clinostat. The few results showing some anomalies have to be evaluated carefully, since with organisms of the size of a few millimeters no complete gravity compensation will be reached in the clinostat, and several side effects are possible (Briegleb, 1983).
Table III. Experiments with Single Cells in Clinostats

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human T-lymphocytes in suspension</td>
<td>Activation by Con A reduced by 50–75% in cultures of purified cells as well as in whole blood cultures (Cogoli et al., 1980, 1990)</td>
</tr>
<tr>
<td>K-562 (human)</td>
<td>No effect on proliferation after exposure to hemin, but 10% decrease of glucose consumption and 50% decrease of hemoglobin production (Wiese et al., 1987)</td>
</tr>
<tr>
<td>Friend cells (murine)</td>
<td>Exposed to DMSO: increased proliferation, decreased glucose consumption and hemoglobin production (Lorenzi et al., 1988)</td>
</tr>
<tr>
<td>Myocytes (Xenopus)</td>
<td>Reduced maturation, morphological changes (Gruener, 1985)</td>
</tr>
<tr>
<td>Spinal neurons (Xenopus)</td>
<td>Reduced maturation, morphological changes (Gruener, 1985)</td>
</tr>
<tr>
<td>Spinal neurons/Myotomal myocytes</td>
<td>In cocultures: reduced synaptogenesis (Gruener and Hoeger, 1988, 1990)</td>
</tr>
<tr>
<td>Mouse oocytes</td>
<td>Changes in success of meiotic progression to metaphase II (Wolgemuth and Grills, 1984)</td>
</tr>
<tr>
<td>Physarum polycephalum</td>
<td>Changes in frequency of oscillations, 40% increase in cytoplasmic streaming velocity (Briegleb et al., 1986; Block et al., 1988)</td>
</tr>
<tr>
<td>Loxodes</td>
<td>Regular distribution (40-80 rpm) to positive gravitaxis (80-100 rpm) (Hemmersbach and Briegleb, 1987, 1992; Hemmersbach, 1988)</td>
</tr>
<tr>
<td>Paramecium caudatum</td>
<td>First, same swimming behavior as at 1 g, followed by random distribution (Hemmersbach and Briegleb, 1987, 1992; Hemmersbach, 1988)</td>
</tr>
</tbody>
</table>

Single Cells

According to theoretical considerations, single cells with a diameter of a few μm are the ideal objects to be studied in the fast rotating clinostat. Therefore, in the last decade the behavior of several types of mammalian cells and unicellular organisms in simulated microgravity has been studied. A summary of results is given in Table III.

The in vitro activation of human lymphocytes by mitogens is a good and suitable model for the study of the mechanism of cell differentiation and of the immune response. When purified lymphocytes in suspension culture were exposed to the mitogen concanavalin A (Con A) in the fast rotating clinostat, the activation of the T-cells was reduced by 50-75% compared to a static control (Cogoli et al., 1980). The same degree of depression of the activation was observed in whole blood cultures incubated with Con A (Cogoli et al., 1990) on the clinostat. The results from an experiment performed on SLS-1 have shown that in suspension cultures, the function of monocytes, acting as accessory cells, and not that of the T-cells is depressed (Bechler et al., 1992). Therefore, we are at present investigating the monocyte function in the clinostat.

The K-562 cell line, a human erythroleukemic cell line, is another model for the study of cell differentiation and biosynthesis in vitro. In the presence of hemin in the culture medium the cells undergo a drastic change: cell proliferation is stopped after one or two divisions, while the onset of hemoglobin production is triggered. In simulated microgravity no effect on the proliferation after exposure to hemin was observed, but glucose consumption was decreased by 10% (Wiese et al., 1987). Hemoglobin production was decreased by 50%, although no change in the percentage of hemoglobin producing cells was noticed.

Another cell line which can be induced to synthesize hemoglobin is the Friend leukemia virus-transformed cell (Friend cells). These cells, when incubated in the clinostat in the presence of dimethylsulfoxide (DMSO), show increased proliferation and decreased glucose consumption and hemoglobin production. The number of hemoglobin producing cells is not changed (Lorenzi et al., 1988).
A series of interesting experiments was performed with nerve and muscle cells from *Xenopus laevis* embryos by Gruener and coworkers (Gruener, 1985; Gruener and Hoeger, 1988, 1990) in the slow rotating clinostat. Spinal neurons and myocytes in separate cultures showed reduced maturation and morphological changes. In co-cultures, synaptogenesis can be investigated by measuring the formation of nerve-associated acetylcholine receptor patches (NARPs). Synaptogenesis was observed to be gravity sensitive. Moreover, a window of sensitivity was found during which exposure to simulated microgravity resulted in a significant alteration in synaptogenesis. A marked inhibition of NARPs was observed only in cultures in which nerve-muscle contact took place during rotation, whereas in cultures in which this contact was established before the onset of clinorotation, NARPs were not affected.

The binding of the epidermal growth factor (EGF) to A431 human epidermoid carcinoma cells induces immediate cell rounding and activates a cascade of signal transduction events, resulting finally in cell proliferation. The induction of the proto-oncogene c-fos is one of the earliest detectable nuclear indications for a normal functioning of this system. To detect possible gravity effects on receptor-mediated signal transduction, the induction of c-fos after binding of EGF to A431 was studied by a Dutch research team under different gravity conditions. In simulated microgravity the expression of c-fos was found to be decreased, whereas EGF-induced cell rounding was enhanced (de Groot et al., 1990, 1991; Rijken et al., 1991).

The effect of simulated microgravity on meiotic maturation in mouse oocytes was studied by Wolgemuth and Grills (1984). Progression to the second meiotic metaphase in a timely sequence is a prerequisite for normal fertilization, as in most mammalian oocytes fertilization occurs with the oocyte arrested in this phase. In these experiments the efficiency of metaphase II formation was similar to controls when rotated below 100 rpm. At 100 rpm, however, a significant decrease in the number of oocytes reaching metaphase II was observed.

Freely moving unicellular organisms studied most extensively in simulated microgravity were investigated in the fast rotating clinostat microscope. The slime mold *Physarum polycephalum* can be regarded as a giant amoeba. The size of this organism can change between a few μm in diameter to a flat organism of several square meters called a macroplasmodium. In the clinostat, plasmodia with a size of 4-5 mm were used to study the pattern of the regular contractions of protoplasmic strands. In simulated microgravity, distinct changes in the kinetics of the contraction periods were found, and the velocity of the endoplasmic shuttle streaming increased (Briegleb et al., 1986; Block et al., 1988).

The swimming behavior of two different protozoa—*Paramecium* and *Loxodes*—was studied in simulated microgravity by Hemmersbach (1988) and Hemmersbach and Briegeleb (1987, 1992). At 1 g *Loxodes* showed a positive gravitaxis. In the clinostat, the swimming behavior depended on the rotation speed. At 20-40 rpm the cells were randomly distributed, whereas at 80-100 rpm they were swimming in the direction of the onsetting vector. *Paramecium*, on the other hand, first showed negative gravitaxis (cells were swimming in the same direction as before the onset of rotation), followed by the expected random swimming.

In mammalian cells and unicellular organisms, such different cellular functions as differentiation, biosynthesis, energy consumption, gene-expression, meiosis, intracellular convection, and swimming behavior have been studied in the fast rotating clinostat; most were found to be sensitive to simulated microgravity.

**COMPARISON OF CLINOSTAT AND FLIGHT EXPERIMENT RESULTS**

For a valid comparison, only those results obtained with comparable experimental conditions, i.e., with an identical protocol, can be considered. So far, only a few cell systems, studied in simulated microgravity in the clinostat as well as in true microgravity in space, fulfill this requirement.

The cells investigated under both conditions include: human lymphocytes, A431 human epidermoid carcinoma cells, the slime mold *Physarum polycephalum*, and the protozoan *Paramecium caudatum*. This comparison is summarized in Table IV. It is interesting to note that the function affected in a distinct cell system in the clinostat always goes in the same direction as in true microgravity, but its magnitude is less pronounced. One of the reasons for this fact may be that in the clinostat the conditions simulating microgravity are only valid for cells at the axis of rotation, whereas cells further away are exposed to a g-level in the range of 10^{-2} g (this value is given for a tube with a radius of 10^{-1} cm rotating at a speed of 100 rpm). In space the microgravity levels are more uniform and depend on the type of carrier (manned or unmanned spacecraft).

**CONCLUSION**

The fast rotating clinostat is an efficient and inexpensive tool for the simulation of microgravity in Earth laboratories, assuming that certain conditions for a good simulation are observed. In combination with a microscope, the behavior of organisms or organelles within the cells under altered gravity conditions can be directly observed.

Comparison of results, obtained in simulated and true microgravity, demonstrates that the clinostat is a useful tool to evaluate whether organisms or cells may be sensitive to gravity changes. Therefore, a biological
Table IV. Comparison of Clinostat and Flight Experiment Results

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Clinostat</th>
<th>Flight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human T-lymphocytes &amp; monocytes in suspension</td>
<td>Activation by Con A reduced by 50-75% in cultures of purified cells as well as in whole blood cultures (Cogoli et al., 1980, 1990)*</td>
<td>90% reduction in activation by Con A; 70% Inhibition of interleukin-1 production (Cogoli et al., 1984, 1988; Bechler et al., 1986, 1992)</td>
</tr>
<tr>
<td>Human epidermoid carcinoma cells A431</td>
<td>20-25% depression of EGF-induced c-fos expression (de Groot et al., 1990, 1991)</td>
<td>50% reduction in c-fos expression (de Groot et al., 1990)</td>
</tr>
<tr>
<td>Physarum polycephalum</td>
<td>Changes in frequency of oscillations (first increase then decrease) Increase in velocity of cytoplasmic streaming (50%) Coincidence of maximum streaming velocities 50 min after the onset of μg and simulated μg (Briegleb et al., 1986; Block et al., 1988)</td>
<td>Frequency of oscillations comparable to clinostat 110% increase in velocity of cytoplasmic streaming</td>
</tr>
<tr>
<td>Paramecium caudatum</td>
<td>Same swimming behavior as at 1g for 120 sec, followed by random swimming (Hemmersbach and Briegleb, 1987, 1992; Hemmersbach, 1988)</td>
<td>Same swimming behavior as at 1g for 80 sec, followed by random swimming (Hemmersbach and Briegleb, 1992; Hemmersbach et al., 1992)</td>
</tr>
</tbody>
</table>

*Clinostat work to measure the monocyte function; i.e., the production of interleukin-1 in simulated microgravity is in progress.

object proposed for a flight experiment should first be investigated in the clinostat before it is selected. This is especially true for single cells, such as mammalian cells and unicellular organisms, as they best fulfill the conditions for the clinostat. As flight opportunities are rare, the clinostat must also be used to perform investigations in relation to flight experiments.

REFERENCES


