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Development of a Centrifuge for Acceleration Research in Cell and Developmental Biology.

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Introduction

The impact of gravity upon living systems was probably first recognized in the seventeenth century by Galileo Galilei, who enlightened the nonlinear relation between the rigidity of the skeleton and body mass.(1)

Much later, in 1806, the plants' positive and negative geotropism of roots and shoots, respectively, was pointed out by Knight, who used a modified water wheel functioning as a centrifuge, this was probably the first centrifuge used for scientific studies.(2) The constant force of gravity has always been present during the development of life on earth therefore it is interesting to see how living systems are influenced by gravity, utilize gravity, or how depending they are on it for proper development.

As stated before, it is known for plants to respond to gravity. This is partially due to dense particles, statoliths, in specialized cells, statocytes, in the root tip.(3) Plants need this information for proper growth. Also during the development of the fertilized amphibian egg to assure a normal development of the embryo, gravity is used to establish the dorsal ventral axes, due to the differences in specific gravity of oocyte components.(4) For most tissues or cells, however, it is not clear what the gravireceptor or mechanosensor, if present, is.

Due to the ongoing microgravity research programs in several countries, the centrifuge has gained more attention to compare experimental results obtained under microgravity and hypergravity condition, Relatively large centrifuges have been used to accommodate small mammals like rats,(5-10) hamsters(10,11) and mice(8,10) or chicken.(13,14) Even several generations of fowl have been raised at higher g-levels to investigate the tolerance of the progeny to this hypergravity environment. The response of the whole body to the increased body weight has been evaluated, Phenomena like motion sickness, cardiovascular and pulmonary functions, bone and muscle metabolism can be studied.

Hypogravity vs. Hypergravity

Although early. pre-spaceflight, mathematical calculations predicted little direct effects on small single cells in the absence of gravity,(16) more and more evidence is emerging that cells do sense gravity. The

effects of microgravity are more pinpointed to even sub-cellular levels such as the cytoskeleton[17,18] and signal transduction pathways.[19]

The relative effects, compared with the normal one g situation, under hypergravity conditions are, in general, opposite to microgravity effects. It is known from astronauts[20,21] and rats[22,23] that there is a reduced bone mass when returning from orbit. When rats are grown in a centrifuge (2.76×g) there is, however, an increase in bone mineral density compared to the l×g controls.[5,24] Also skeletal explants of fetal mouse middle foot long bones show a reduced mineralization under weightlessness conditions,[personal communication: van Loon/Veldhuijzen] whereas when cultured at 2.2-3.2×g there is an increase in calcification of the bone.[25]

Plant roots grow in various directions in simulated[26] or real[27] microgravity directions, compared to a directed gravitational field. The proliferation (thymidine incorporation) of lymphocytes in whole blood samples, activated with Concanavalin-A, was strongly reduced (>90%) in a weightlessness environment[28] while the same cells responded with an increased proliferation under l0×g hypergravity conditions.[29] An increased thymidine incorporation was also found in HeLa cells cultured at 18, 35 and 70×g,[30] in the mouse osteoblast like cells MC3T3-E1 cultured at 5×g[31] and in articular chondrocytes cultured at 1.3 to 27×g.[32] In human A43l cells, the epidermal growth factor (EGF) stimulated induction of the c-fos proto-oncogene, is reduced under real[33] and simulated[19] microgravity. In contrast, c-fos production is increased when cultured in a 10×g centrifuge.[19] Also the expression of another oncogene, c-myc was enhanced in hypergravity cultures of HeLa cells compared to unit gravity cultures.[30]

All previous studies indicate that hypergravity generates, probably dose dependent, opposite effects compared to microgravity. Tissues and cells reacting to hypergravity are more likely responding to microgravity. By using a centrifuge one can find some indications on what biological material to select, and which parameters to investigate during an actual spaceflight. Moreover, as has been often experienced in physics and chemistry, in order to fully understand observed phenomena and to arrive at a good theory for an adequate description, it can be very useful to perform additional measurements with parameter values extending a much larger interval.

MidiCAR

In close cooperation with microgravity research biologists, physicists and representatives of industry a dedicated centrifuge facility for cell, tissue culture and developmental biology experiments has been defined (Figs. A.l, A.2 and A.3), the philosophy behind this medium sized Centrifuge for acceleration Research (MidiCAR) is that it should be suitable to perform experiments using standard laboratory hardware as well as existing hardware developed for previous and future space flights, the following set of requirements for such a facility was proposed as a baseline:

- Experiments on this centrifuge are to be carried out in a temperature

controlled environment between +l0 and +40 °C with an accuracy of ± 0.5 °C.

- Part of the biological experiments need to be carried out in an environment with l00% relative humidity at 5% CO_2 in air.
- The g-levels to be generated resulting in interesting biological responses, are in an interval varying from l×g to 100×g. Especially the lower range is considered to be of great interest to related microgravity experiments.
- In order to carry out simultaneous experiments at different g-levels during one experiment run it is necessary to provide a rotor with multiple arms and variable length.
- The static (l×g) control experiment needs to be placed under environmental conditions as close as possible to the hypergravity experiments.
- The facility is to be operated in a normal laboratory environment without the necessity for major changes in the available infrastructure. The possibility to use standard laboratory hardware (petri dishes, multiwell plates etc.) should be met.
- Electrical interfaces between the laboratory and experiments carried out on the rotating centrifuge are to be provided by the facility.
- Software controlled operation.
- The outer diameter of the centrifuge needs to be optimized with respect to g-level gradient, centrifuge mass and costs.
- For economical reasons in order to avoid duplication of the hardware a transportable multi user facility is preferred.

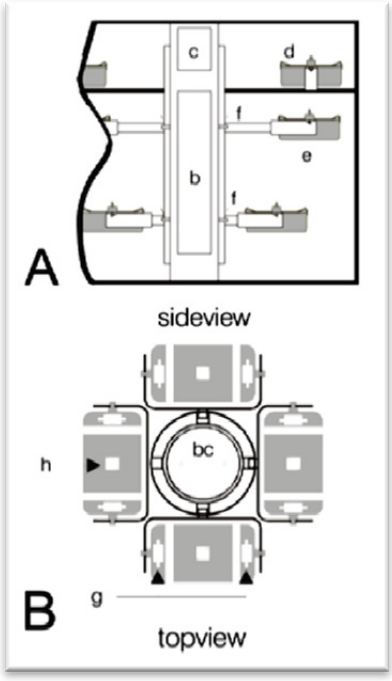

Fig. 1 Schematic layout of the MidiCAR centrifuge.
A: Side view: a: incubator, b: motor, c: slip ring set, d: static control cultures, e: experiment vessels, f: two levels of rotor arms. B: Top view: bc: core structure and housing slipring and motor, g: valves for flushing culture vessles, h:waterseal for reducing overpressure in vessles.

The specifications of the centrifuge which has been developed are summarized as follows:

- The radial acceleration of the experiments could be varied to values corresponding with g-levels between below 0.2 and 100 with an accuracy better than l%.
- The centrifuge speed controller can be programmed using a PC computer via the RS232 serial port, thus allowing the generation of various acceleration profiles.
- The centrifuge and the static reference experiments are accommodated in an incubator (outer dimensions 830×690×1100 mm [w×d×h] allowing to control and vary

the temperature from +5 to +50 °C, ± 0.3 °C.
- The experiments are housed in vessels which are hooked onto replaceable rotor arms. Because the culture vessels can swing out, the resulting g-vector remains perpendicular to the surface of the culture compartments as much as possible.
- The vessels body and lid are made of stainless steel. This facilitates the possibility of heat sterilization and possible radioactive contamination can be easily cleaned up.
- There are a maximum of 8 standard experiment vessels positions on the rotor, while 4 vessels can be accommodated on the static platter.
- Each experiment vessel can accommodate two standard laboratory tissue culture multiwell plates or two ESA Biorack Type-I/E containers.
- Gas mixture above the cultures can be conditioned to a 5% CO_2/air, 100% relative humidity atmosphere by using air-tight culture vessels provided with valves for flushing. A waterseal releases gas in case of over-pressure (Figs. A.lB and A.3).
- A 12 way slip ring assembly is used to provide an electrical interface with the experiments while the centrifuge is running.
- Rotor arms of various lengths are replaceable to vary the radius between 140 and 205 mm.
- Multi g-level experiments can be carried out simultaneously using different lengths rotor arms.

Apart from the microgravity condition, the main difference between actual spaceflight samples and ground controls, are the launch characteristics and cosmic radiation. Programmable centrifuges, like MidiCar, can be used to simulate launch accelerations for ground controls. Better control conditions can thus be obtained for actual spaceflight launches as well as for parabolic flight profiles. One can also consider qualification of hardware, containing biological material, going through launch characteristics of a particular flight in addition to, or in stead of the standard vibration tests.

There are several drawbacks on doing research in a microgravity environment. The most important being the infrequent flight opportunities. Secondly, the restriction of infrastructure available on the different platforms to use more advanced techniques for monitoring the different phenomena. Because microgravity research is a relatively young discipline, most experiments are still in a stage of ‘inventarization’: Are these cells or tissues responsive to microgravity?
There are also high costs related to space experiment, because of special, high demanding hardware and launch. With the use of a centrifuge it is possible to reduce most of the disadvantages described of space experiments as mentioned above.

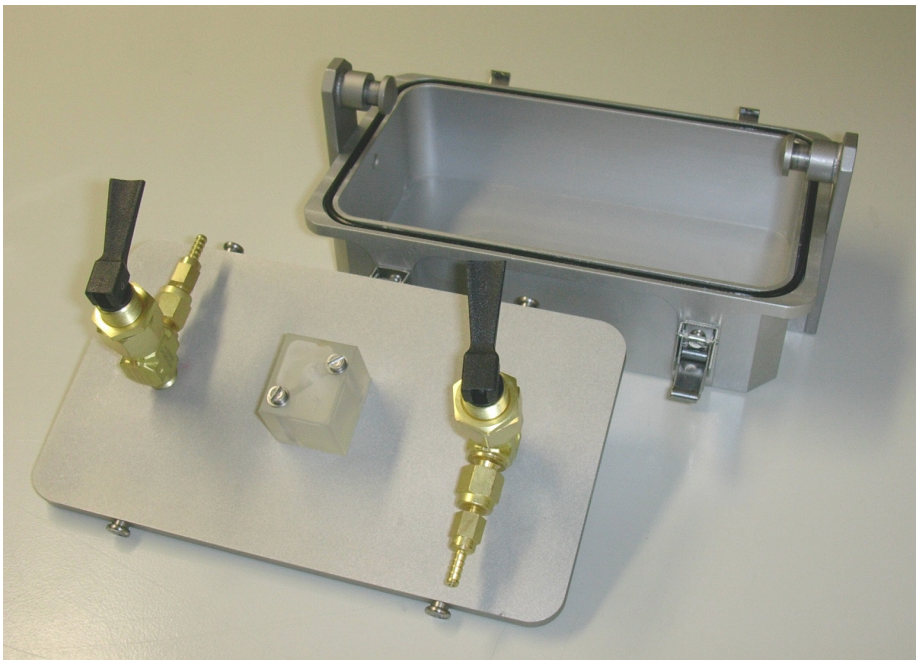

Figure 2: A: The MidiCAR centrifuge. B: Detail showing an opened culture vessel.

Conclusion

Hypergravity experiments can reveal the mechanisms involved in whole animals or plants, tissues and cells in response to gravity changes and thus also to microgravity. The presented centrifuge facility developed for this purpose will allow scientists to perform a range of experiments at relatively low cost and facilitates their spaceflight preparations. Hypothesis on mechanisms studied in microgravity experiments can be postulated upon results obtained from hypergravity studies, those hypothesis, however, still have to be tested under weightlessness conditions.

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