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Hypergravity promotes cell proliferation

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Summary. When HeLa cells, chicken embryo fibroblasts, sarcoma Galliera cells, Friend leukemia virus transformed cells and human lymphocytes are cultured in a hypergravitational field (e.g. $10 \times g$) proliferation rate is increased by 20–30%, whereas glucose consumption per cell is lower than at $1 \times g$. Tracking of cell movements on gold-coated substrates reveals that cell migration is hindered at high-g. These findings suggest that under gravitational stress the cell is either capable of shifting to other metabolic pathways and/or consumes less energy at high-g than at $1 \times g$. This work describes ground-based investigations related to experiments to be performed on future Spacelab missions.

Introduction

The success of the Space Shuttle Columbia which will carry the Spacelab in orbit opens new opportunities to biological research in space. One of the most attractive aspects is the exposure of living organisms to weightlessness, a new environment never experienced during evolution. We are presently preparing three experiments with cells in culture to be flown on Spacelab missions. The technological and scientific relevance of biological experiments in space have recently been discussed by us[2]. In preparation for our investigations in space we performed a ground based study on the effect of high-g on cells in vitro. Human lymphocytes exposed to the mitogen concanavalin A will be the objects of our experiments on the Spacelab. These cells offer a good model for the study of cell differentiation as well as of the cellular aspects of the immune system. Analysis of lymphocytes from crew members of Soviet and U.S. spaceships show a significant reduction of their reactivity after flight[3,4] (see for a summary, Cogoli[5]). This finding was confirmed after the first flight of the Space Shuttle[6].

We reported earlier on the effect of high-g and low-g (the latter simulated in a fast rotating clinostat) on lymphocytes[7,8]. The results lead us to suggest the hypothesis that moderate hypergravity has a stimulatory effect on the activation of lymphocytes by mitogens, whereas microgravity has a depressing effect. For a better understanding of the role of gravity on the cell proliferation and in order to test the general validity of our hypothesis we extended the investigations to other cell types. In addition we tracked cell migration on a gold coated substrate and determined the glucose consumption at high-g.

The first report on the effect of hypergravity on living systems appeared in 1806 when the role of gravity on plant growth was investigated[9]. Later on other objects like frog and sea urchin eggs, unicellular organisms and, particularly, plants were investigated under high-g conditions[10] (reviewed by Montgomery et al.[11]). Several biological experiments under microgravity conditions were performed on U.S. (summarized by Anderson[12]) and Soviet (summarized by Buderer[13]) space missions. However, biological payloads consisted mainly of simple packages most of which were designed only for studying the effect of cosmic radiation. An interesting experiment was performed on board of Skylab in 1973 with a sophisticated apparatus consisting of a fully automated unit for incubation of cells providing medium exchange, photographic and cine recording and daily fixation[11]. There, WI-38 embryonic lung cells were cultured for 28 days. No spectacular effects of weightlessness have been reported so far. However, conclusive results on the adaptation of animal cells to microgravity are still missing. Since a comprehensive study on the effect of gravity on cells cannot be limited to experiments in weightlessness, we performed experiments at high-g.

Materials and methods

Cells and media

Lymphocytes were purified from human peripheral blood (O+) by the Ficoll/Isopaque method of Boyum[14]. The cells were resuspended at a concentration of 1×10^6 cells/ml in RPMI-1640 medium supplemented with 20% human serum (O+), 50 µg/ml Gentamycin, 40 mM HEPES and 5 mM sodium bicarbonate. This medium has been found optimum for culturing lymphocytes in sealed culture flasks, i.e. without additional CO_2, as required by the layout of the experiments on Spacelab. Lymphocytes were activated with concanavalin A, 15 µg/ml.

HeLa cells, chicken embryo fibroblasts (CEF), SGS-3 cells (sarcoma Galliera strain-explant 3)[15] were cultured in Dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal calf serum and 50 µg/ml Gentamycin in sealed flasks.

Friend leukemia virus transformed cells, line FBU-3b, a clone provided by Dr F. Conscience, Basel[16], were cultured in DMEM as above, containing 4.5 g/l glucose.

High-g centrifugation

A table top centrifuge (MSE) has been modified in order to be loaded either with 24 cell culture flasks (Falcon 25 cm^2) or with 2 cell culture plates (Nunc 24 wells). The centrifuge was kept in a room at 37 °C, cell culture flasks and plates were kept sealed during the experiment. Periodical measurements during the centrifugation inside the culture flasks show that the temperature remained constant. A constant $10 \times g$ level was achieved by controlling the rotor speed at 230 rpm by means of a resistor.

Cell proliferation measurement

Cell counting was performed in a Neubauer hemocytometer after releasing adherent HeLa cells with 0.25% trypsin.

Thymidine incorporation into DNA was measured after a 2-h pulse of 2 µCi/ml ^{3}H-thymidine (sp. act. 40–60 Ci/mmole) in lymphocytes, CEF, HeLa and SGS-3 cells.

Tracking of cell movements

Glass cover slides (5×5 mm) cleaned with ethyl ether/ethanol (1:1) were coated with colloidal gold according to the method of Albrecht-Buehler and Lancaster[17]. The slides were put on the bottom of the wells in Nunc cell culture plates. Each well was inoculated with 1×10^4 cells and the cells were allowed to adhere for 12 h at 37 °C before centrifugation. After the experiment the cells were fixed with 3% glutaraldehyde in phosphate buffered saline and treated with Giemsa stain. The slides were removed from the wells, dehydrated in ethanol 90% and embedded in Euparal (Chroma, Germany) for preservation. Photographs were taken on a Wild-Leitz Diavert microscope with dark-field illumination.

Glucose determination

Glucose remaining in the medium after culture was determined with the glucose-dehydrogenase method (Gluc-DH UV Method, Merck) according to Banauch et al.[18].

Interference reflection microscopy

HeLa cells grown in Nunc cell culture plates were fixed with 3% glutaraldehyde in phosphate buffered saline for 50 min and observed in a Wild-Leitz Diavert microscope equipped with an interference reflection device according to Izzard and Lochner[19].

Proliferation under hydrostatic pressure

1 ml of culture containing 1×10^6 lymphocytes was cultured in Falcon tubes (17×100 mm). The medium (having a thickness of 0.9 cm) was overlayed with either 4.0 or 8.0 cm of liquid paraffin (Merck, DAB7) in order to generate a hydrostatic pressure of 4.4 and 7.9 cm water respectively. To guarantee analogous conditions the control was overlayed with 2 mm of paraffin. The sealed tubes were incubated at 37 °C for the given times. Thereafter the paraffin was cautiously removed leaving a 2-mm layer. Incorporation of ^{3}H-thymidine into DNA was measured as described above.

Results and discussion

Cell proliferation

The experiments described here were performed with: a) HeLa cells, a common and well known line of transformed cell; b) chicken embryo fibroblasts as an example of non-transformed cells; c) SGS-3 cells from a sarcoma of the Galliera rat[15]; d) Friend leukemia virus transformed cells, line FBU 3b[16], as an example of slow growing, nonadherent single cells; e) human lymphocytes. A high-g environment was generated in a table top centrifuge. Cell proliferation is the most important measurement in our experimental approach to the study of hypergravity effects. This can be done essentially in two ways, either by determining the cell number directly in a hemocytometer, or indirectly by determining DNA synthesis by incorporation of tritiated thymidine into trichloro acetic acid-precipitable material. Depending on the properties of the cells investigated either one or both methods can be used.

Counting under the microscope is not reliable with cells forming aggregates like lymphocytes exposed to concanavalin A, very difficult and time consuming with cells adhering to the substrate like CEF, HeLa and SGS-3 cells. Therefore we used this method only with non adherent FBU 3B cells and, for a comparison of the two methods, with adherent HeLa cells after release with trypsin. The incorporation of

radioactive thymidine into DNA for measuring cell proliferation is a widely used method. Advantages, disadvantages and possible artifacts are discussed by Adams[20]. We used this method with lymphocytes, CEF, SGS-3 and HeLa cells, and not with FBU 3b cells since these are dividing at a too low rate for accurate measurements.

When incubated with the lectin concanavalin A, resting T-lymphocytes are triggered to proliferate and to produce soluble factors like interleukines and interferon. The extent of this activation, which is maximum on day 3 of culture, is generally measured as rate of proliferation by incorporation of labeled thymidine into DNA. Our experimental conditions should give reliable results as discussed by Ling and Kay[21]. We applied the same method for measuring the proliferation of HeLa cells and we found that the results correlate fairly well with the cell counts in the hemocytometer as shown in table 1. Since SGS-3 and CEF cells have a division rate similar to that of HeLa cells we assume that the thymidine method is not affected by uncontrolled artifacts.

Table 1. The effect of hypergravity (10 × g) on ^{3}H-thymidine uptake, cell number and glucose consumption expressed as percent of the 1 × g control for HeLa cells

Incubation time	^{3}H-thymidine uptake	Cell number	Glucose consumption
24 h	114 ± 4	116 ± 9	101 ± 3
48 h	130 ± 10	130 ± 11	106 ± 4

^{3}H-thymidine uptake was measured after a 2-h pulse, cells were counted in a Neubauer hemocytometer, glucose remained in the medium was measured with the method of Banauch et al.[18]. Standard deviation from 4 experiments.

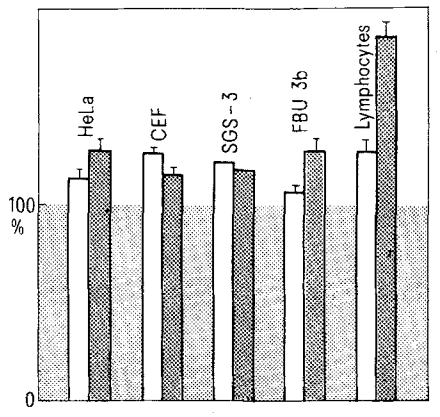

Figure 1. Effect of hypergravity at 10×g on cell proliferation. Results are expressed as percent of the corresponding 1×g control, indicated by the shadowed area. Cultures were centrifuged at 37 °C. Cell proliferation was measured by incorporation of ^{3}H-thymidine into DNA, except for FBU 3b cells which were counted in a Neubauer hemocytometer. In HeLa cells (11 experiments), chicken embryo fibroblasts, CEF (3 experiments), and SGS-3 cells (1 experiment) cell proliferation was measured after 24 h (void bars) and 48 h (hatched bars) incubation time respectively. FBU 3b cells (3 experiments) were counted on day 4 (void bar) and 5 (hatched bar) of incubation. Human lymphocytes (19 experiments) were activated by exposure to concanavalin A, cell activation was measured on day 2 (void bar) and 3 (hatched bar) of culture. Values and SD. The reduced proliferation observed after 48 h in CEF and SGS-3 cells reflects beginning of confluency.

Figure 1, in which the results obtained with the 5 types of cells investigated are summarized, delivers the essential message of this paper: cell proliferation is remarkably enhanced at 10×g.

When HeLa and Friend cells are cultured at varying g-levels between 10×g and 40×g as shown in figure 2, the g-effect is more apparent. However, the increase of cell division is rather low when compared to the 4-fold increase of the g-level. Lymphocytes do not survive a 3-day exposure above 20×g. Between 3×g and 20×g the response remains essentially the same.

It is important to mention what is known from experiments performed under simulated microgravity. When lymphocytes are cultured in a fast rotating clinostat, a device designed to change gravity from a vector to a scaler quantity, a depression of the activation by concanavalin A is observed[8]. Although the results gained with clinostats should be interpreted with great caution, we suggest the hypothesis that hypergravity has a stimulatory effects, whereas hypogravity has a depressing effect on lymphocyte reactivity.

When WI-38 embryonic lung cells were investigated on Skylab over a period of 4 weeks no major differences in cell division, ultrastructure and nuclear density between flight and ground samples were observed[11]. However, glucose consumption in the medium and the rate of cell migration were significantly reduced in the flight cells.

Effect of hydrostatic pressure

Cells cultured in vitro always sediment and deposit on the bottom of the culture flask, regardless wether they adhere or not to the substrate. Therefore cells in culture are subject to the hydrostatic pressure depending on the thickness of the medium and on the g-force applied to the flask.

One can argue that the faster proliferation rate observed in hypergravity might be attributed, at least in part, to the increase of hydrostatic pressure generated by centrifugation rather than to the high-g per se. In order to answer this question we cultured human

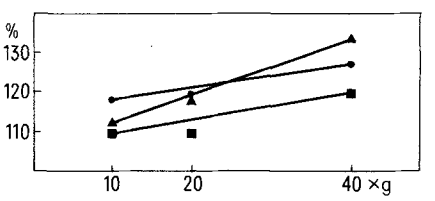

Figure 2. Effect of varying g-levels on cell proliferation. Cell proliferation at 10, 20 and 40×g is expressed as percent of the corresponding 1×g control and was measured as described in fig. 1.
■, HeLa cells after 24 h of culture;
▲, HeLa cells after 48 h of culture;
●, FBU 3b cells after 4 days of culture.

lymphocytes at $1 \times g$ under varying hydrostatic pressure. This was accomplished by incubating 1 ml of culture containing 1×10^6 cells in tubes and overlaying the medium with a column of liquid paraffin producing a hydrostatic pressure equivalent to that of a 4.4 cm high water column in one series and of 7.9 cm water in an other series of tubes. Taking into account that the thickness of the medium was 0.9 cm, the pressure thus obtained corresponds to that generated if the tubes were centrifuged, without paraffin layer, at $4.8 \times g$ and $8.8 \times g$ respectively. A pressure of 7.9 cm water is well above that (5 cm water) obtained either in culture flasks or in plates centrifuged at $10 \times g$, where the thickness of the medium was 0.5 cm. The use of liquid paraffin, a biological substance which does not harm cells, instead of medium for increasing hydrostatic pressure guarantees the same cell-versus-nutrient ration in all cultures.

The results in table 2 clearly indicate that the stimulation of lymphocytes exposed to concanavalin A is independent from the hydrostatic pressure and therefore the increased growth at high-g is actually due to a g-effect.

Cell motility

Cellular motility and stability mechanisms have been recently reviewed by Gall et al.[22]. Allen[23] defines motility as the ability of the cell to exhibit and to perform mechanical work at the expenses of metabolic energy, whereas mobility is manifest in Brownian motion and is usually the result of thermal agitation. Conceivably a higher gravitational stress could impair cell motility in the medium. Therefore we decided to investigate cell migration at high-g with a technique described by Albrecht-Buehler[24] and by Albrecht-Buehler and Lancaster[17] which tracks movements of cells adhering to a substrate coated with colloidal gold particles. This approach proved particulary useful since cinematographic recording is costly and complicated in a centrifuge. HeLa cells offered rapid growth, good motility at $1 \times g$, and, because of their adhesive properties, left behind a clear trace on gold-coated

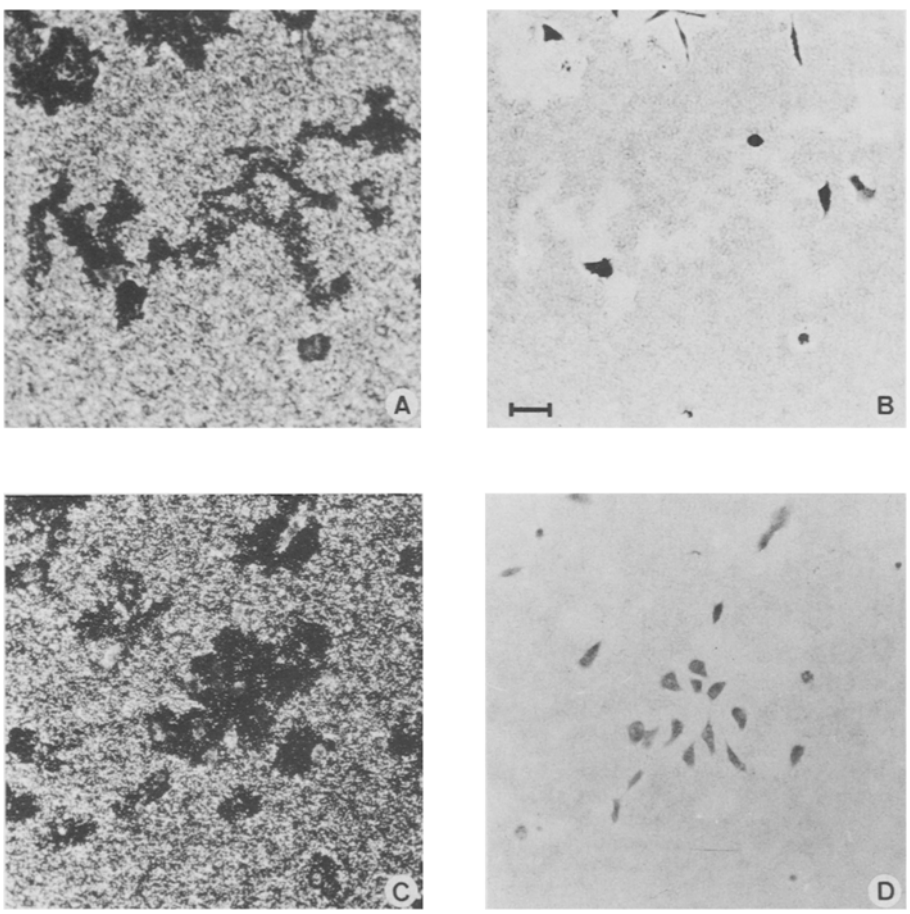

Figure 3. Movements of HeLa cells at $1 \times g$ (A,B) and at $10 \times g$ (C,D) during a period of 48 h. A and B show the same field in the microscope. Picture *A* was taken with dark-field illumination and tracks cell movements, *B* shows the stained cells. *C* (dark-field illumination) and *D* (stained cells) show the same field for cells cultured at $10 \times g$. Prior to centrifugation cells were allowed to adhere for 12 h at 37 °C (no movement observed during this time) on a substrate consisting of a glass cover slide coated with colloidal gold according to the method of Albrecht-Buehler and Lancaster[17] and laying on the bottom of a multiwell plate (Nunclon, Nunc). After the experiment cells were fixed with 3% glutaraldehyde in phosphate buffered saline and treated with Giemsa stain. Bar = 50 µm.

substrates. From results shown in figure 3 we can make two important conclusions: a) at 1×g (fig. 3A) cells show a normal pattern of migration which is very similar to that described by Albrecht-Buehler[24] for other cell types. Conversely, the cells do not change their position significantly at high-g (fig. 3C) during a incubation time of 48 h. b) At 1×g the cells (fig. 3B) are widely spread on the substrate, i.e. when one cell has undergone mitosis the 2 daughter cells go in different directions following symmetrical patterns[24] as visible in figure 3A. At 10×g, however, the cells remain almost motionless forming aggregates by successive divisions (fig. 3D). When we compared the focal contacts of the control and of the high-g samples in the surface interference microscope or when we measured the longest axis of the cell body with a Nikon profile projector[25] no significant differences were observed. The focal contacts, as described by Izzard and Lochner[19] and Ploem[26] are visible expression of the contacts between cell and substrate. Therefore our observations indicate that these contacts are not altered at high-g. Finally, inspection on the light microscope shows that the cells are intact and that their morphology is not changed by the g-stress. This is true for all cell types investigated.

Table 2. The effect of hydrostatic pressure on lymphocyte activation expressed as percent of the control

Pressure in cm of water	Incubation time 48 h	72 h
4.4 cm H_2O	103 ± 9	103 ± 8
7.9 cm H_2O	98 ± 2	105 ± 9

Standard deviation of independent triplicates. 1 ml of culture containing 1 × 10^6 lymphocytes was overlayered in tubes with liquid paraffin in order to generate the desired hydrostatic pressure. Activation was achieved by exposure to concanavalin A. Lymphocyte activation has been measured as rate of proliferation by incorporation of ^{3}H-thymidine into DNA as described in the methods' section.

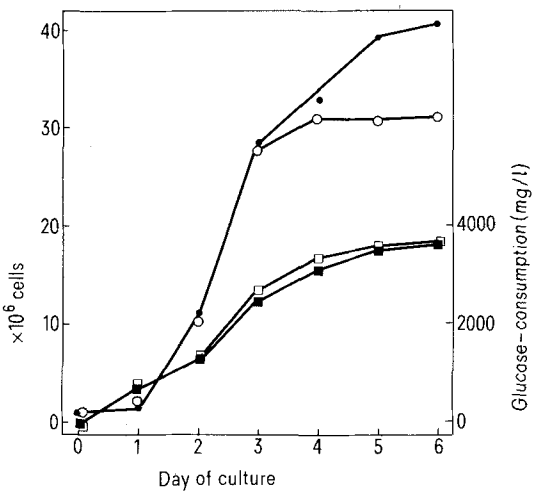

Figure 4. Growth and glucose consumption of Friend leukemia-virus transformed cells line FBU 3b cultured at 1×g and 10×g. Cells were counted at given times in a Neubauer hemocytometer. Glucose remained in the medium was measured with the method described by Banauch et al.[18].
○, Cell number at 1×g;
●, cell number at 10×g;
□, glucose consumption at 1×g;
■, glucose consumption at 10×g.

Glucose consumption

The experiment performed by Montgomery et al.[11] showed that the growth of WI-38 cells remained unchanged at 0×g while the consumption of glucose in the medium was reduced by approximately 20% compared to the ground control. In addition, the number of cell displacements was lower at 0×g than at 1×g. For this reason and since glucose is an important energy supplier in most culture media used[27], we decided to study the glucose balance in the medium of cells cultured under hypergravitational stress. Friend leukemia cells (fig. 4) and HeLa cells (see table 1) were cultured at 10×g. The glucose left in the medium was measured at given times and compared to cell division. We observe that the consumption of glucose at 10×g is approximately the same as at 1×g. This means that, since the cell growth is faster at high-g, the consumption per cell is higher at 1×g than at 10×g. However, from this experiment we cannot conclude that the cell requires less energy at high-g than at 1×g since glutamine also has been identified as an important energy supplier in culture media (see for a review McKeehan[28]).

Nevertheless, our results indicate that under g-stress the cell is capable of shifting to other metabolic pathways and/or consumes less energy than at 1×g. This aspect is presently under investigation in our laboratory.

Concluding remarks

The results presented in this paper clearly show that cells in culture are sensitive to the gravitational environment: Generally speaking, high-g enhances cell proliferation. This effect does not depend on hydrostatic forces which may be active at high-g, nor the morphology of the cells is altered.

This is in contrast to was has been recently claimed by Tairbekov and Parfyonov[29]. Based on theoretical consideration and on studies with microorganisms on board of space missions the authors maintain that unicellular free living organisms whose volume is below 1 mm^3 are gravity independent. Their conclusion also disagrees with the results of Montgomery et al.[11] (see above) and of Tixador et al.[30] obtained with *Paramecium aurelia* on board of the space laboratory Salyut 6. *Paramecium aurelia* grows markedly faster under space conditions. However, a possible interaction with cosmic radiation cannot be excluded.

A question arises: How does the cell sense gravity? A number of effects of gravity on cells in culture can be considered, e.g., gravity may modify the cell shape. In

this instance the microtrabecular lattice of the cell, described by Wolosiewick and Porter[31], would be distorted under the gravitational stress. Although the role of the microtrabecular lattice in interacellular and cellular movements is not yet clear, we can assume that the distribution of the organelles in the cell would be altered by gravity as a consequence of the change in cell shape. Folkman and Moscona[25] have shown that there is a relationship between shape and cell growth: When the cell has a spheroidal conformation the growth rate is considerably lower than when the cell is flattened by adhesion to a substrate. Membrane permeability can be altered at different g-levels as discussed by Pollard[32] due to a change of hydrostatic pressure. The experiments with cells under increased hydrostatic pressure make the hypothesis of Pollard unlikely. Another possibility is that cell proliferation is enhanced because migration inhibition at high-g favors the formation of colonies of cells. It is difficult, at present, to assess this hypothesis, however, while colonies may favor proliferation of cells, also the opposite effect can be considered. In fact hypergravity may generate a situation similar to confluency and consequently inhibit division even in transformed cells which, at high-g, cannot move freely above the monolayer.

Earlier studies have shown that gravity may interfere with other cell structures. Wohlfarth-Bottermann[33] found that in the slime mold *Physarum* more fibrils are needed for pumping the cytoplasm against gravity than when the flux is assisted by gravity. Although we cannot make a direct comparison with *Physarum,* the size of animal cells is compatible with cytoplasmic streaming. In fact we could record and measure movements of organelles in lymphocytes cultured at simulated low-g in the clinostat[8]. The structure of the mitotic spindle and of the chromosomes can be distorted by centrifugal forces as shown by Shimamura[10] long ago in *Lilium japonicum.* Schatz and Teuchert[34] reported that when eggs of the nematode *Ascaris suum* are exposed to accelerations up to $1000 \times g$ for 10–15 days, 70–80% of the eggs develop normally. Only at $1500 \times g$ normal development broke down. The authors suggest that a still unknown mechanism regulates normal development even at high-g.

In conclusion, we are convinced that the line of investigation described here, coupled with experiments in space, will contribute to demonstrate the important role of gravity in cell growth and motility and may lead to the identification of a gravity sensor within the cell.

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2 Cogoli, A., and Tschopp, A., Biotechnology in space laboratories. Adv. biochem. Engng *22* (1982) 1–50.
3 Konstantinova, I.V., Antropova, Ye.N., Legen'kov, V.I., and Zazhirey, V.D., Study of the reactivity of blood lymphoid cells in crew members of the Soyuz-6, Soyuz-7 and Soyuz-8 spaceships before and after flight. Space Biol. Med. *7* (1973) 48–55.
4 Kimzey, S.L., Hematology and immunology studies, in: Biomedical results from Skylab, pp. 249–282. Eds R.S. Johnston and L.F. Dietlein. NASA SP-377, NASA, Washington, D.C., 1977.
5 Cogoli, A., Hematological and immunological changes during space flight. Acta astronautica *8* (1981) 995–1002.
6 Taylor, G.R., Hematological and immunological analyses, in: STS-1 medical report, pp. 51–52. Eds S.L. Pool, P.C. Johnson, Jr, and J.A. Mason. Eds NASA Technical Memorandum 58240. NASA, Johnson Space Center, Houston 1981.
7 Cogoli, A., Valluchi-Morf, M., Boehringer, H.R., Vanni, M.R., and Müller, M., Effect of gravity on lymphocyte proliferation, in: Life sciences and space research, pp. 219–224. Ed. R. Holmquist. COSPAR, Pergamon Press, Oxford 1979.
8 Cogoli, A., Valluchi-Morf, M., Müller, M., and Briegleb, W., Effect of hypogravity on human lymphocyte activation. Aviat. Space environ. Med. *51* (1980) 29–34.
9 Knight, T.A., On the direction of the radical and germen during vegetation of seeds. Phil. Trans. R. Soc. London *96* (1806) 99–108.
10 Shimamura, T., Studies on the effect of the centrifugal force upon nuclear division. Cytologia *11* (1940) 186–216.
11 Montgomery, P.O'B. Jr, Cook, J.E., Reynolds, R.C., Paul, J.S., Hayflick, L., Stock, D., Shulz, W.W., Kimzey, S., Thirolf, R.G., Rogers, T., Campell, D., and Murrell, J., The response of single human cells to zero gravity, in: Biomedical results from Skylab, pp. 221–234. Eds R.S. Johnston and L.F. Dietlein. NASA SP-377, NASA, Washington, D.C. 1977.
12 Anderson, M., in: Biospex: Biological space experiments. A compendium of life sciences experiments carried on U.S. spacecraft. Eds J.A. Rummel and S. Deutsch. NASA Technical Memorandum 58217, 1979.
13 Buderer, M.D., in: Russian Biospex: Biological space experiments, a space life sciences bibliography. NASA, Johnson Space Center, Houston 1981. JSC-17072.
14 Boyum, A., Isolation of lymphocytes, granulocytes and macrophages. Scand. J. Immun. *5* (1976) Suppl. 5, 9–15.
15 Pippia, P., Cogoli, A., Gaias, M., Piras, G., and Ivaldi, G., Determination of adhesive rate constant in normal and neoplastic homogeneic cells. Experientia *37* (1981) 698–700.
16 Conscience, J.F., and Meier, W., Coordinate expression of erythroid marker enzymes during dimethylsulfoxide-induced differentiation of Friend erythroleukemia cells. Exp. Cell Res. *125* (1980) 111–119.
17 Albrecht-Buehler, G., and Lancaster, R.M., A quantitative description of the extension and retraction of surface protrusions in spreading 3T3 mouse fibroblasts. J. Cell Biol. *71* (1976) 370–382.
18 Banauch, D., Brümmer, W., Ebeling, W., Metz, H., Rindfrey, H., and Lang, H., Eine Glucose-Dehydrogenase für die Glucose-Bestimmung in Körperflüssigkeiten. Z. klin. Chem. klin. Biochem. *13* (1975) 101–107.
19 Izzard, C.S., and Lochner, L.R., Cell-to-substrate contacts in living fibroblasts: An interference reflexion study with an evaluation of technique. J. Cell Sci. *21* (1976) 129–159.
20 Adams, R.L.P., Use of radioactive isotopes in cell culture, in: Cell culture for biochemists, pp. 181–203. Elsevier/North-Holland Biomedical Press, Amsterdam, New York, Oxford 1980.
21 Ling, N.R., and Kay, J.E., DNA synthesis and the cell cycle, in: Lymphocyte stimulation, pp. 357–378. North-Holland/Elsevier Publishing Company, New York 1975.
22 Gall, J.G., Porter, K.R., and Siekevitz, P., eds, Motility and stability mechanisms, in: Discovery in cell biology. J. Cell Biol. *91* (1981) 107s–186s.
23 Allen, R.D., Motility, J. Cell Biol. *91* (1981) 148s–155s.
24 Albrecht-Buehler, G., Daughter 3T3 cells. Are they mirror images of each other? J. Cell Biol. *72* (1977) 595–603.
25 Folkman, J., and Moscona, A., Role of cell shape in growth control. Nature *273* (1978) 345–349.

26 Ploem, J.S., Reflection-contrast microscopy as a tool for investigation of the attachment of living cells to a glass surface, in: Mononuclear phagocytes in immunity, infection and pathology, pp.405–421. Ed. R. van Furth. Blackwell Scientific Publications, Melbourne and London 1975.
27 Morgan, M.J., and Faik, P., Carbohydrate metabolism in cultured animal cells. Biosci. Reps *1* (1981) 669–686.
28 Mc Keehan, W.L., Glycolysis, glutaminolysis and cell proliferation. Cell Biol. int. Reps *6* (1982) 635–650.
29 Tairbekov, M.G., and Parfyonov, G.P., Cellular aspects of gravitational biology. Physiologist *24* (1981) S69–S72.
30 Tixador, R., Richoilley, G., Grechko, G., Nefedov, Y., and Planel, H., Multiplication de *Paramecium aurelia* a bord du vaisseau spatial Saliout-6 (Expérience Cytos). C.r. Acad. Sci. Paris *D287* (1978) 829–832.
31 Wolosewick, J.J., and Porter, K.R., Microtrabecular lattice of the cytoplasmic ground substance. Artifact or reality. J. Cell Biol. *82* (1979) 114–139.
32 Pollard, E.C., Physical determinants of receptor mechanism, in: Gravity and the organism, pp.25–34. Eds A. Gordon and M.J. Cohen. University of Chicago Press, Chicago 1971.
33 Wohlfarth-Bottermann, K.E., Differentiations of the ground cytoplasm and their significance for the generation of the motive force of amoeboid movement, in: Primitive motile systems in cell biology, pp.79–109. Eds R.D. Allen and N. Kamiya. Academic Press, New York and London 1964.
34 Schatz, A., and Teuchert, G., Effects of combined O-g simulation and hypergravity on eggs of the nematode *Ascaris suum*. Aerospace Medicine *43* (1972) 614–619.

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Control of corpus allatum activity in *Diploptera punctata:* roles of the pars intercerebralis and pars lateralis

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Summary. Control of the corpora allata (CA) of *Diploptera punctata* is maintained by at least 2 factors. The glands are directly inhibited by an allatostatin arriving at the CA via the nervi corporis cardiaci I (NCC I). Destruction of the putative source (median neurosecretory cells, MNC) of the allatostatin by radio-frequency (RF) cautery relieved the inhibition imposed on the CA of virgin females, and the glands became active. Similarly, destruction of the lateral neurosecretory cells (LNC) also relieved the inhibition. We propose that the LNC stimulated the MNC to release allatostatin. RF-cautery did not result in the activation of CA of pregnant or ovariectomized females. Activation of the CA may therefore require not only absence of the inhibitory factor but also the presence of a stimulatory one (perhaps from the ovary).

Introduction

In the adult female cockroach *Diploptera punctata*, the stimulus of mating enhances the biosynthesis of juvenile hormone (JH) by the corpora allata (CA) and results in a cycle of hormone production and subsequent oocyte growth. JH biosynthesis may be controlled by inhibitory substances (allatostatins), stimulatory substance(s) (allatotropins) or both. In most species studied, nervous connexions (i.e. nervi corporis cardiaci I, NCC I: nervi corporis cardiaci II, NCC II) from the brain to the CA appear to control CA activity. For example, nervous connexions appear to be stimulatory in *Schistocerca paranensis*[28,29], *S. gregaria*[35] and *Locusta migratoria*[11]. On the other hand, centers in the brain and their axonal extensions to the CA appear to be inhibitory in *Leucophaea maderae*[3], *Diploptera punctata*[4,25], *Gryllus domesticus*[13] and *Nauphoeta cinerea*[15,16] to name but a few examples.

Destruction of a stimulatory cerebral center can provide for an overall decrease in relative CA activity whereas destruction of an inhibitory center can result in an overall increase in activity. For example, cautery of the lateral neurosecretory cells (LNC) or severance of the NCC II prevents normal oocyte growth in *S. paranensis*[28,29]. Destruction of the axons from the medial neurosecretory cells (MNC) prevents the normal cycle of JH biosynthesis in *L. migratoria*[11]. Conversely, destruction of the axons from the MNC stimulates oocyte development in *L. maderae*[4].

Following denervation, the CA of *D. punctata* become more biosynthetically active and a normal cycle of JH biosynthesis ensues, resulting in oocyte development and oviposition[25,36]. Thus, innervation of the gland is not necessary for either the increase in JH biosynthesis at the beginning of the cycle or the decrease at the end. In addition to the inhibitory influence of the NCC I, it has also been demonstrated that the ovaries have a stimulatory effect on the CA and are necessary for a normal gonotrophic cycle[26,27]. Recent evidence indicates that the stimulatory effect of the ovary acts directly on the CA and not via the brain[21,22].

It is presently believed that JH biosynthesis by the CA of *D. punctata* may be controlled by direct neurohormonal inhibition or by neurohormonal or neural