# BIOLOGY ON SOUNDING ROCKETS: HISTORY, REQUIREMENTS, RESULTS AND SCIENTIFIC INTERPRETATION

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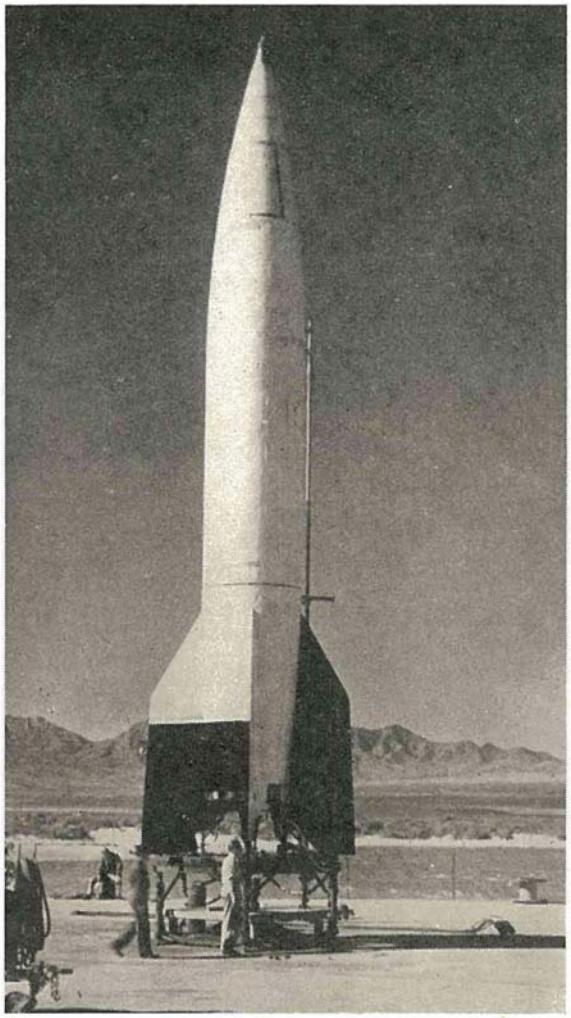
## ABSTRACT

Biology on sounding rockets began in Europe in 1985 to complement the longer-duration experiments on the Shuttle, later as an autonomous branch of research. Since then, sounding rockets have been playing an eminent role in investigations on gravisensing in plants, protists, *in vitro* cell cultures and sub-cellular systems. Biology on sounding rockets comes with biology-specific technical requirements which often include a reference centrifuge to provide 1*g* during the flight. The short duration of microgravity offered by the sounding rocket sets constraints on the experiment design but on the other hand helps to provide a correct interpretation of the biological effects that are observed. A significant bonus of the sounding rocket missions for biologists is the fast turn-around time: samples can be delivered just before the launch, to be returned to the scientists shortly after landing. This enables the investigators to expose freshly-prepared samples to microgravity and to start the post-flight analysis that same day.

## 1. HISTORY

On 17 December 1946 a V-2 rocket, carrying the spores of a fungus, was launched from White Sands USA (Fig. 1). An apogee of 187 km was reached. The scientific objective was to see whether the space flight would cause any unusual effects on growth and mutation. The spores were placed in five plastic cylinders, four being launched in space and one retained on the ground as a control sample. This must have been the first biological experiment on a sounding rocket and at the same time, the very first biological experiment in space. During follow-up V-2 flights fruit flies, mice and monkeys were taken aloft to investigate whether living organisms of increasing complexity were able to survive a rocket flight [1]. In this way, information was acquired about the feasibility of human space flight. During the next decades this trend was further pursued by the USA, the USSR and by France, using a wide variety of launch vehicles.

Interest in space biology in a stricter sense was aroused in 1985 by the ESA Biorack flight on Spacehab D-1 [2]. The many unexpected results that came to light from the thirteen Biorack experiments, focussed on eleven different small organisms, indicated that a variety of *g*-dependent phenomena was waiting to be uncovered in the biological field and furthermore, that some of those effects could be investigated by means of simple, short-duration experiments on sounding rockets. At that time, in Western Europe experience with biology on sounding rockets was quite limited: only one biological payload − with yeast cells − had been flown [3]. Still, as from 1977 the sounding rocket was already widely used for research in materials sciences and fluid physics, whereby six minutes of near-weightlessness was obtained [4]. This timeframe was considered



A captured German A-4 (V-2) rocket about to be fired at White Sands, New Mexico

*Figure 1. Biology on sounding rockets started with the V-2. Illustration from [1].*

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sufficiently long for testing gravitational effects on plants, protozoa and quickly-evolving cellular processes like fertilization, signal transduction and cell surface modulation. The short duration of the flight also offered an immediate advantage for biology: On long-duration orbital flights, biological samples are exposed in concert to microgravity and cosmic radiation; additional control experiments are often required to differentiate between the effects of one from the other. On a sounding rocket the impact of cosmic radiation was considered as negligible, thus facilitating the interpretation of the scientific results. As a consequence, after 1985 biological experiments were flown at an ever increasing frequency on European sounding rockets. Within ten years time more than 50 were completed on TEXUS, MASER and MAXUS missions [5].

## 2. BIOLOGY-SPECIFIC REQUIREMENTS

Biology differs from physics in one main aspect: the test samples are alive. This single feature profoundly affects the way the experiment is conducted, the design of the hardware, as well as the pre- and post-flight logistics. Biologists prefer to investigate fresh sample materials, carefully selected from stock cultures just before the experiment begins. When the flight is over the samples must be recovered as quickly as possible from the landing site for the post-flight analysis. To meet these requirements a fully-equipped biological laboratory was established at ESRANGE in 1990 (with equipment provided by DLR, SSC and ESA) allowing several teams of biological scientists to work in parallel.

To determine the effects of weightlessness, test samples exposed to micro-$g$ are accompanied by reference samples that are maintained at 1$g$. Since temporal fluctuations, biological rhythms and ageing are commonplace in living organisms, a reliable comparison can only be made if both components of the experiment are conducted in synchrony. It implies that a double set of hardware is required: one for flight, another for ground.

Another biology-specific requirement emerged in the early nineties when the outcome of the first experiments was screened. It turned out that biological samples are sometimes impacted by the launch environment (a mixture of vibrations and linear accelerations) and subsequently, on top of that, by the micro-$g$ conditions. This complicates the interpretation of the results. This issue was never relevant on the longer-duration Shuttle flights where biological samples would have plenty of time to recover from the launch before the experiment was started. In contrast, on sounding rockets a biological experiment begins within 2 minutes after lift-off, to be completed (depending on the type of rocket) 6 to 12 minutes later. To overcome this problem an on-board 1$g$ reference centrifuge was introduced. After flight, differences between the on-board micro-$g$ and 1$g$ test series can now solely be attributed to weightlessness because both sets undergo exactly the same launch environment. The launch effects themselves can be identified by comparing the 1$g$ series from ground with their 1$g$ counterparts from the on-board centrifuge. Note that on-board 1$g$ centrifuges are nowadays commonplace in space biology but for varying reasons: on sounding rockets the centrifuge is included to check for launch effects, whereas on orbital flights the 1$g$ centrifuge is mostly used to check for effects by cosmic radiation.

## 3. BIOLOGICAL QUESTIONS AND ANSWERS

Biology on sounding rockets roughly deals with five scientific themes: gravisensing in plants, gravisensing in animals, gravisensing in protists, gravisensing in cells and gravisensing in sub-cellular systems. The common goal is to understand **how** and **why** biological organisms react to conditions of (near)weightlessness.

Graviperception in plants was already studied, described and partly understood long before the advent of the sounding rocket. On Earth, plant roots grow downwards (gravitropism) due to the fact that specialized cells (statocytes) in the root tips are equipped with heavy particles (statoliths) which change their position according to the direction of the gravity vector. The repositioning of the statoliths is monitored by the statocytes and translated into directional growth of the root tip. How the statocytes are able to monitor the position of the statoliths and how the root tips are subsequently instructed to grow down are questions that nowadays are being resolved. Microgravity experiments play a significant role in this branch of research [6, 7, 8].

Animal research on sounding rockets has been so limited in Western Europe that it does not warrant a summary or review. A very recent experiment is described in [9].

Several motile unicellular organisms (protists) display directional swimming along or against the gravity vector (gravitaxis). How such is accomplished has been under debate for many years. Two different explanations were considered: A.) Passive: Like a buoy, these organisms are endowed with an off-set centre of mass, the swimming direction is the consequence of physical forces; B.) Active: These organisms do the steering by themselves, relying on some sort of biological gravity sensor. Over the years, in several species evidence has been collected against hypothesis A and in favour of hypothesis B. In some protists, intracellular structures involved in graviperception have even been identified. The sounding rocket fulfils a prominent role in this domain of biological research [10].

Much less is understood about the way mammalian cells, in isolation from the body, respond to the absence

of weight – but they do. Since 1983 [11] *in-vitro* cultures (blood cells, skin cells, bone cells etc.) have been widely investigated on a multitude of microgravity platforms. Technically, such cells do not need to sense the direction of the gravity vector. Cells *in-vitro* can easily be cultured in an upside-down position (in contrast to plants) and never have to leave their position in search for nutrients or light (in contrasts to protists). In fact, in the early days the microgravity results obtained from *in-vitro* cell cultures [2] were put in doubt. Couldn't it be that the observed effects were only indirect, ascribable to the culturing fluid behaving differently in micro-*g*? Lack of convection could easily lead to a reduction of nutrient supply or to a deficiency in waste removal, with ensuing biological effects. This explanation, whereby the cells themselves were not directly impacted by micro-*g* but suffering from a change in their microenvironment, remained valid until the first mammalian *in vitro* cultures were flown on a sounding rocket [12]. How could a cell suffer from starvation, or be impaired by non-removal of waste products, if the surrounding culturing fluid was motionless for not more than six minutes? Still, microscopy in search for a specialized biological sensor inside blood cells, skin cells and bone cells never yielded a compelling result.

## 4. BIOPHYSICS

From a biophysical point of view, gravisensing by a single cell was for a long time considered impossible due to its small size. Within a cell, the forces generated by weight were considered too minute to produce any effect, unless supported by some intracellular amplifier or gravisensor [13]. However, this amplifier or gravisensor would not necessarily be recognizable as such under the microscope – it could in fact be represented by a macromolecular superstructure, spread out over the entire cell: the cytoskeleton (Fig. 2) which in its entirety could be tension-sensitive, and might generate signals in response to changes in the gravity environment. This model, known as 'tensegrity' [14, 15], would follow rules that have been demonstrated to occur in mechanical structures at a macroscopic level (Fig. 3). Still, there is no solid experimental proof available that tensegrity is really at work inside a biological cell.

Another concept that could possibly explain why cells react to micro-*g* is the lack of pattern formation by microtubules. Microtubules are rod-shaped polymers, abundant in almost every cell type, composed of many monomer proteins called tubulin. Under specific *in vitro* conditions on Earth, a solution of tubulin molecules can be triggered to assemble into microtubules, whereafter these microtubules spontaneously arrange themselves into macroscopic patterns. On sounding rockets, it has been demonstrated that such patterns do not appear in micro-*g* [16]. It shows that, remarkably, a very simple chemical system can function as a gravity receptor. The proposed explanation is that pattern formation of microtubules involves a non-linear reaction-diffusion process whereby the presence of gravity determines the ultimate result [17]. It remains to be seen however if these findings and models can readily be extrapolated to events that happen inside a living cell.

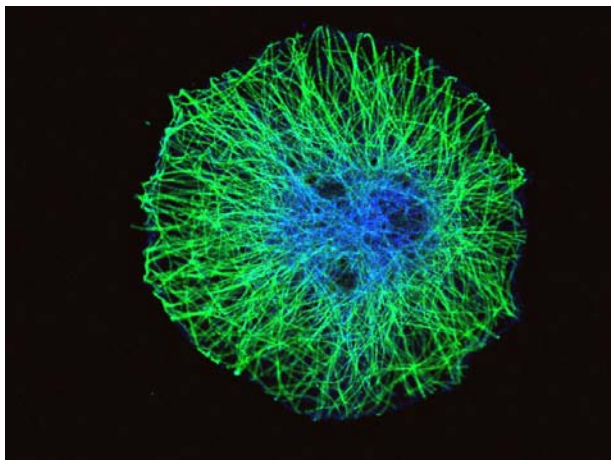

*Figure 2. According to the tensegrity model the complete cytoskeleton may act as a gravisensor.*

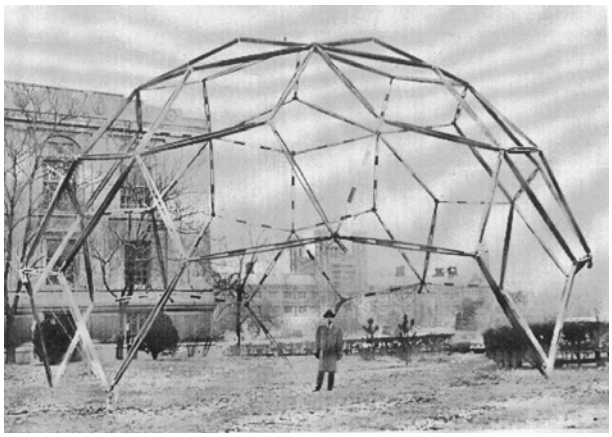

*Figure 3. Tensegrity follows rules that occur in Buckminster-Fuller structures.*

## 5. CONCLUSION

Over the years, the understanding of gravisensing in plants and protists has significantly increased by experiments on sounding rockets. Mammalian cell cultures were found to react to micro-*g* as well but in this case, no conclusive evidence is available about the underlying biological mechanism, neither about the biological meaning. Some biophysical concepts have been proposed, one of them derived from sounding rocket experiments showing that a simple chemical system could act as a gravisensor. Proof about the applicability of these models to cell biology is waiting to be delivered – perhaps by means of sounding rocket experiments.

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