Effect of very high pressure on life of plants and animals

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Abstract. We studied the tolerance of living organisms, such as a small animal (Milnesium tardigradum), a small crustacean (Artemia), non-vascular plants or moss (Ptichomitrium and Venturiella), and a vascular plant (Trifolium) to the extremely high hydrostatic pressure of 7.5 GPa. It turned out that most of the high pressure exposed seeds of white clover were alive. Those exposed for up to 1 hour and seeded on soil germinated stems and leaves. Considering the fact that proteins begins to unfold around 0.3 GPa, it seems difficult to understand that all the living samples which have been investigated can survive after exposure to 7.5 GPa.

1. Introduction
Effects of hydrostatic pressure on biological samples have mostly been investigated to the order of several hundred MPa. It was reported that proteins began to unfold around this pressure, where most bacteria and many other organisms die [1]. However, under the pressure of 0.6 GPa, which was two times higher than the pressure mentioned above, tardigrades (Milnesium tardigradum) were shown to survive [2]. Further extensions of the pressure to a few GPa order were made by Sharma et al. [3] and Margosch et al. [4] on some microbial activities.

On the other hand, using a technique of shock compression, survival of some microorganisms was reported after exposure to very high sock pressure up to several ten GPa for a very short time [5]. This result shows that the mechanism of unfolding of proteins is different from that under static high pressure, or the unfolding does not take place up to such a high pressure when applied for a very short time of the order of 10^{-6}s.

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Attempts to extend the investigations on mm-size animals and seeds of higher plants under static compression to an extremely high pressure range up to several GPa may produce a new research field, and introduce us some new or unknown phenomena and information. The present authors group have been extending the static pressure range to 7.5 GPa using a cubic anvil press.

2. Experimental Procedure
Specimens of living animal, tardigrades and spore cases of mosses, Ptychonitrium sinense and Venturiella sinensis have been collected from the field close to our university. Some other specimens, eggs of Artemia and seeds of Trifolium lepens L (white clover) have been purchased from companies. Before high pressure exposure, these living specimens were dehydrated by keeping them in a sealed desiccator of the size of 0.5x0.4x0.7 m³ with silicagel of about 0.5 kg in weight for more than one week. These specimens were put in a small teflon capsule with an inner diameter and length of 1.6 and 1.8 mm, respectively. As the liquid pressure medium, Fluorinate PC77 (perfluorocarbon, C₈F₁₈, Sumitomo 3M) was adopted and put into the teflon capsule together with the living specimens. It was proven that soaking in 100% fluorinate has no practical effect on the life of living specimens. The capsule was placed in the center of a cube which was made of pyrophyllite with an edge length of 6.0 mm.

To generate hydrostatic pressure up to 7.5 GPa a cubic anvil press was adopted. A pyrophyllite cube was placed at the centre of six tungsten-carbide anvils with a front edge length of 4.0 mm. These anvils were compressed by a 250-ton press. The pressure was determined by using a calibrated curve [6] of the relation between the press load and the actual pressure established before the experiment. The error of the intensity of pressure was smaller than 0.3 GPa. In the present apparatus, the press load was controlled automatically to keep the intensity of the pressure constant during the operation at the maximum pressure.

The pressure was increased from ambient to the maximum pressure of 7.5 GPa at a rate of 0.3 GPa/min⁻¹. From our former experiments observing temperature dependence of magnetic susceptibility on increasing and decreasing pressure using the same high pressure equipment [6], it was proven that the temperature of the sample had been kept constant within the relative error of about 1 K. The pressure was kept constant at the maximum pressure for various duration of time from 15 minutes to 6 days and then decreased down to the ambient pressure with the same rate as on increasing. Those high pressure experiments were made at room temperature. Immediately after the pressure was released, the specimens were brought out from the teflon capsule and they were cultivated or seeded on a humid media. Then, their survival, germination and growth rates were investigated using a microscope.

3. Results and Discussion
3.1 Tardigrades
A terrestrial tardigrade (Milnesium tardigradum) is a small animal with 0.6-0.7 mm in length. This animal is known to show very strong resistivity against extreme environmental conditions at cryptobiotic state, i.e., dehydrated state called “tun”. The first experimental search for life under extremely high hydrostatic pressures of several GPa order was made by the present authors’ group for this animal [7].

Figure 1. One of the 20 tardigrades exposed to 7.5 GPa for 3 hours and brought back to ambient pressure. All of them were found alive and moving around actively.
It was found that tardigrades could survive under 7.5 GPa for up to 13 hours. Figure 1 shows a tardigrade which was exposed to 7.5 GPa for 3 hours and brought back to ambient pressure. This tardigrade was moving around actively. Those tardigrades could live as long as control. Judging from their movements and the length of their life span after the exposure to high pressure, they had no remarkable damages in the body and the organs. It is a very striking result that an animal is still alive after being exposed to such a very high hydrostatic pressure.

3.2 Artemia eggs

*Artemia*, a kind of planktons and called brine shrimps, are found worldwide in inland saltwater lakes, but not in oceans. They have a biological life cycle of one year. Their eggs in a dried state, called cryptobiosis with a moisture level of below 10% are metabolically inactive and can remain alive for so many years as to keep the species without evolution since the Triassic period. They hatch into *Nauplii*. *Artemia* larvae several hours after soaked in seawater. The hatching rate of dried eggs of *Artemia* after exposed to 7.5 GPa for various durations up to 3 days was investigated by the present authors' group [8, 9].

The *Artemia* eggs exposed to 7.5 GPa for up to 48 hours began to hatch around 15-18 hours after soaked in seawater. The hatching rate reached maximum around 40-70 hours after soaked into seawater. Observed hatching ratio (hatched/total number of eggs) of 80-90% was as high as for control. Hatching to *Nauplius* was observed under a microscope, and no difference was confirmed between the high-pressure exposed group and control. After hatched into *Nauplius*, the high pressure exposed *Artemia* moved around as seen in figure 2 with no practical differences in speed and frequency with control.

![Figure 2. Artemia exposed to 7.5 GPa for 2 days. Some of the eggs hatched into Nauplii. The photograph was taken 24 hours after soaked in sea water.](image)

3.3 Ptychomitrium

*Ptychomitrium sinense* belongs to *Grimmiaceae*, and are mostly found on rocks at sunshiny places. In a dried atmosphere, the leaves of *Ptychomitrium* frizzle and their colour turns brown. On the other hand, when they get wet, they become straight and green, again. The spores of *Ptychomitrium* are spherical in shape with a diameter of about 20 µm. A spore case of *Ptychomitrium* contains tens of thousands of spores. Once spores are dried, they become resistant against extreme environmental conditions and remain alive for a long period. When they are seeded on a humid media, they begin to germinate within one week.

The investigation was extended to *Ptychomitrium* [10], and the germination ratio (the ratio of the number of germinated spores against the total number of the spores seeded on an agar) was investigated after exposing its spores to 7.5 GPa for various durations up to 6 days. Figure 3 shows *Ptychomitrium* spores exposed to 7.5 GPa for 6 days, and seeded on agar. In this photograph, germination of the spore is evident. However, no frizz in germinated protonema was seen in this figure. It is the characteristics of *Ptychomitrium* that their protonema frizzle when they are dried to the same extent as in the present case of being incubated on an agar for three weeks. After being exposed to 7.5 GPa for such a long time as 6 days, the mechanism of the characteristics of frizz has been distracted.
3.4 Venturiella

Venturiella sinensis belongs to Erpodiaceae, being one of the popular mosses seen in moderate climate areas such as Japan, Korea and China. The spores of Venturiella are spherical in shape with a diameter of about 20 µm. A spore case, being in the size of 0.5 mm in diameter and 1 mm in length contains tens of thousands of spores. Once spores are put in a dried atmosphere, they become resistant against extreme environmental conditions and remain alive for long years. When they are seeded on a humid media, they begin to germinate within one week.

The high pressure experiment was further extended to this moss, and germination ratio was investigated after exposing its spores to 7.5 GPa for various durations up to 6 days. One week after seeded, both the spores of high pressure exposed group up to three days and control began to germinate. The germination ratio of these high-pressure exposed groups were 70-90%, which is as high as control [11].

After being incubated for five months, those spores exposed to 7.5 GPa for up to 3 days grew up to have green leaves. On the other hand, those spores exposed to 7.5 GPa for 6 days, only 4 individuals in a hundred were germinated. To count the numbers of the germinated spores, we had seeded only about a hundred spores in tens of thousands in the spore case exposed to 7.5 GPa on an agar. One of the germinated spores is shown in figure 4. Considering that tardigrades can stand against 7.5 GPa for up to 13 hours, the pressure tolerance of moss is about one order stronger than that of tardigrades [7].

3.5 Trifolium lepens L

The experiment has been extended, further, to a vascular plant, white clover. White clover, Trifolium lepens L is distributed worldwide and often introduced as a pasture crop. Since white clovers contain proteins and are so abundant worldwide, they are considered to be a survival food. The high pressure study has been extended to this vascular plant.

A few seeds of white clover were exposed to 7.5 GPa for various durations from 30 minutes to 6 days, and then, seeded on agar or directly on sowing soil in flower pods. It was found that they were tolerable against 7.5 GPa for only one hour to germinate stems with trifoliate leaves [12]. However, they were tolerable for 1 day to germinate roots. Figure 5 (a) shows white clovers exposed to 7.5 GPa for 30 minutes and (b) for 1 hour. These photographs were taken six and a half months after seeded. Eight months after seeded, a flower came out from the group exposed to 7.5 GPa for 30 minutes as seen in figure 6.
Figure 5. (a) White clovers exposed to 7.5GPa for 30 minutes, and (b) for 1 hour and seeded on sowing soil. The photographs were taken six and a half months after seeded.

Figure 6. Eight months after seeded, a flower came out from the white clover seed exposed to 7.5 GPa for 30 minutes.

A close investigation was made under a microscope to examin damages in the cells of roots, stems and leafs after the exposure to the very high pressure. As seen in figure 7, no differences were observed between the high pressure exposed group and control. However, the average length of the roots of the clover exposed to 7.5 GPa for 1 hour was about a half of those exposed for 30 minutes.

Figure 7. Light-microscopy images of roots of white clover exposed to 7.5 GPa for 30 minutes (left) and for 1 hour (right) after treated to be transparent. No differences of the cells can be seen, except for the larger diameter and the shorter length for the high pressure exposed group for longer time (right).
The present investigation may give the possibility that very small creatures can travel through the space in a large meteorite, and reach the earth from other planet or galaxy. It may also give a support to the recent report on findings of some DNA building blocks in a meteorite by Callahan et al. [13].

4. Conclusion
Judging from the fact that all the living creatures we have examined, both animals (tardigrades and Artemia) and plants (Ptychomitrium, Venturiella and white clover) were alive after exposure to 7.5 GPa, it was suggested that most of the proteins of those creatures which unfolded at the early stage of the compression remain principally unchanged after exposure to the very high pressure of 7.5 GPa. It was also suggested that the unfolding of proteins was completely reversible up to 7.5 GPa.

The maximum hydrostatic pressure applied to all the living specimens investigated in the present experiments corresponds to that in the upper mantle, at the depth of 180 km from the surface of the earth. Considering only the magnitude of the pressure and imagining if there would be some way to escape from being heated up to such a high temperature as in the earth’s interior, it seems possible for a very small creature to travel through space in a large meteorite made of some sedimentary rock.

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6. References
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