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Preserving tardigrades under pressure

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When an animal is exposed to high hydrostatic pressure, its cellular membranes[1,2], proteins[3–5] and DNA[6] are damaged. At pressures of around 30 megapascals (MPa), proliferation and metabolism in microorganisms stops; at 300 MPa, most bacteria and multicellular organisms die. But here we show that, in perfluorocarbon at pressures as high as 600 MPa, small terrestrial animals known as tardigrades can survive in a dehydrated state.

Terrestrial tardigrades become immobile and shrink into a form known as the 'tun' state when the humidity decreases. In this state, they can survive extreme temperatures, as low as −253 °C or as high as 151 °C, as well as exposure to a vacuum or to X-rays [7–10]. We have now tested the ability of the tardigrades *Macrobiotus occidentalis* (order Eutardigrada) and *Echiniscus japonicus* (Heterotardigrada) to survive under extraordinarily high hydrostatic pressures.

We sealed *M. occidentalis* tardigrades in a small plastic container (6 ml) placed inside a pressure capsule (R7K-3-10, Yamamoto Suiatu Kougyousho) and compressed using water as the pressure medium. The outside temperature was 21 °C and the water temperature inside the capsule was 25 °C; stepped hydrostatic pressures were applied for 20 minutes at a time (100, 200, 300, 400, 500 and 600 MPa). Pressure was increased by 100 MPa per minute and then decreased at the same rate. After decompression, *M. occidentalis* was removed from the capsule and examined under a light microscope, which revealed that all organisms died at pressures over 200 Mpa (Fig. 1).

We then investigated whether tardigrades could acquire pressure resistance in the tun state (a process known as anhydrobiosis) by dehydrating them before applying pressure. *M. occidentalis* and *E. japonicus* were dehydrated on filter paper in Petri dishes for more than 24 hours, when the relative humidity in the dishes dropped from 70–80% to 10–30%. To prevent the tardigrades from rehydrating during compression, we used an inert solvent, perfluorocarbon (C_8F_{18} Fluorinate PC77, Sumitomo 3M), as the pressure medium instead of water. Tardigrades were then removed from the pressure capsule and soaked in water to rinse off the perfluorocarbon. One hour later, we confirmed that they had changed from the tun state to the active state.

To test whether the perfluorocarbon increased the survival of tardigrades exposed to high hydrostatic pressure, we subjected tardigrades in perfluorocarbon, which were still in the active state, to the same hydrostatic pressure changes. All active-state tardigrades were dead at pressures above 200 MPa.

We evaluated the data at 600 MPa for the group ($n = 20$) in the tun state in perfluorocarbon and in the active state in water and perfluorocarbon. The survival rate of *M. occidentalis* was 95% at 600 MPa (Fig.1), and there was a difference between active-state and tun-state animals ($P < 0.01$; chi-squared). The survival rate of *E. japonicus* was 80%, as some animals had died and their fluid had leaked onto the filter paper, which we attributed to inadequate dehydration before the experiment.

Tardigrades are composed of about 40,000 cells, which survive not only high-speed compression under a hydrostatic pressure of 600 MPa (equivalent to six times the pressure of sea water at a depth of 10,000 metres), but also being maintained at this pressure, and high-speed decompression as well. With perfluorocarbon as the pressure medium, we have demonstrated the viability of tardigrades after keeping them in an anhydrobiotic state. This viability is influenced not just by pressure but by the absolute amount of water in the organism's body, enabling us to exploit its dehydration/water-absorption mechanism for preservation purposes.

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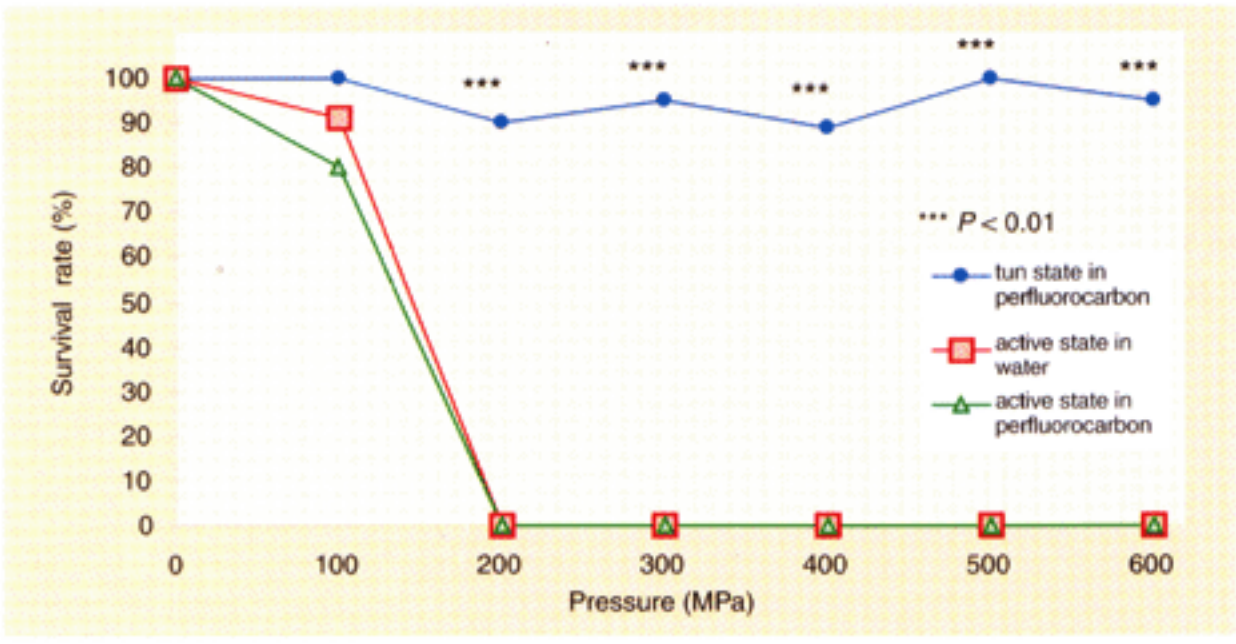

Figure 1 Survival rate of *Macrobiotus occidentalis* after exposure to high hydrostatic pressure. Before compression (100 MPa min^{-1}), tardigrades were either in the tun state (dehydrated) in perfluorocarbon, or in the active state in water or perfluorocarbon. Either water or perfluorocarbon was used as pressure medium. An hour after high hydrostatic pressure was maintained for 20 minutes and decompression to −100 MPa min^{-1}, the animals were soaked in distilled water for an hour, and those in the active state were examined with an optical microscope (magnification, × 40). When animals in the tun state were exposed to 100–600 MPa in perfluorocarbon, a high survival rate was obtained. The survival rate of active-state animals in perfluorocarbon is 80% at 100 MPa and 0% at pressures greater than 200 MPa. This survival rate at 100 MPa was lower than the 91% obtained using water at 100 MPa, so perfluorocarbon did not increase the survival rate.