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FROST-RESISTANCE OF THE ROTIFER IN ANTARCTIC REGION

By

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Through the kindness of Dr. HIROSHI Fukushima, Yokohama Municipal University, who joined the fourth Japanese Antarctic Research Expedition, 1959–1960, we could obtain the fresh material of fresh water algae which had been brought back in frozen state at about –5°C. We wish to express our thanks to Dr. H. Fukushima for the material.

In the frozen lump of the algae, when thawed, very active forms of rotifer, tardigrade, nematode and some kinds of protozoa, tecamoeba and ciliate were found, among which the rotifer is the most abundant. All these animals were thermosusceptible, especially tardigrade and nematode, becoming inactive at temperatures above 20°C and finally died. For this reason the observations were made always at 9–10°C. The higher the thawing temperature, the smaller the number of survivors is, as shown in table 1. Immediately after thawing even at a sutiable low temperature, some individuals died. A part of the frozen material, during the transport from Tokyo to Sendai, had suffered thawing followed by quick refreezing at ca. –30～–40°C owing to the careless treatment of the material. Since rapid freezing has very ill effect upon the animals, it is thinkable that some of them would be damaged by the rapid refreezing during the transport. This may be a cause for that the frozen lump of algae contained many dead animals. In the Antarctic region all of the animals living in the algae-lump would be capable of withstanding the severe cold of the long winter during which time these animals must be completely frozen, in other words, they would have high frost-resistance. This paper deals with the results of the preliminary experiments on the frost-

Table I
Living animals in the thawed lump of the fresh water algae

Thaw. Temp. (°C)	Number of the surviving : Number of the dead		
	Rotifer	Tardigrade	Nematode
28.0	335 : 81	5 : 30	0 : 11
9.6	369 : 35	42 : 23	11 : 7

resistance of the rotifer obtained from Ongul Island.

The rotifer used is *Philodina sp.* which is reddish orange in colour and ca. 700–800μ in length in the streched state.

Experiment I. A piece of the lump of algae which had been thawed was placed on the bottom of single or double glass tube and then the tube was immersed into the cooling bath at desired temperatures. After a definite duration of time the frozen piece was thawed in water at room temperature (ca. 18°C). After about one hour, the number of the individuals shaken off into water from the piece and those showing movement were counted. Further to measure the cooling rate, a thawed piece of the lump, into the center of which the tip of the thermojunction had been inserted, was put on a small glass plate hung in the protecting tube and cooled to -20°C. The cooling rate was regulated by changing the protecting tube, that is, single or double. The results shown in Table 2 clearly indicate the tendency of the rising of the survival rate according to the falling of the cooling rate.

Table II
Effect of refreezing on the rotifer in the lump of the algae

No.		Cool. Temp. (°C)	Cool. Time (hrs)	Protecting tube	Cool. Rate (°C/min.)	No. of the Surv. / No. of the Dead
A	1	−20	24	single	rapid	26 : 132
	2	−40	24	〃	〃	11 : 139
	3	−20	2	double	slightly slow	73 : 110
B	4	−20	2	single	4.3	15 : 20
	5	−20	2	doucle	1.3	37 : 21

Experiment II. The results of Experiment I reveal that the cooling rate is an important factor for the injury caused by feezing. To establish this point more exactly the next experiment was performed in which only the actively moving individuals were used. Ten to 20 individuals were picked out and removed to a small glass bowl with water of about 0.2 ml into which the thermojunction tip was immersed. The regulation of cooling rate was made both by change of the cooling temperature and of thickness of dead air space in the protecting tube.

Roughly speaking, the survival rate of the rotifer is strikingly influenced by the cooling rate. There exist a clear tendency that the number of the individuals that survived after thawing becomes large, when the cooling rate is slow. By slow cooling rate, the rate of ice formation after appearance of the freezing point should be slow. Therefore it must have an intimate correlation with the occurrence of the freezing injury. Then a comparison was made between the relative rates of ice formation which were indicated, for convenience, by the rate of temperature falling after the appearance of the freezing point, in other words, by the decrease of temperature per minute which was calculated from the declination grade of the plateau

on the freezing cruve (Table 3, Column 6). But these values are not exact in the strict sense, because the amount of the water in the glass bowl was not equal and the thermojunction tip was not situated always at the same level and the same place in the bowl. The results summarized in Table 3 show that the number of individuals not injured by freezing at the slow ice formation is greater than those by the rapid one. The data of the group B in Table 3 were obtained on the individuals which had been directly put on the bottom of a slender single protecting tube immersed directly in the cooling bath. In thses cases the cooling rate was very rapid and the survival rate very small. In almost all of the cases, an artificial inoculation of ice was not made, excepting No. 5 in Table 3 which was the only case in which a small ice crystal was thrown into the bowl at 0°C, accordingly supercooling did not occur and ice grew slowly. In this case all of the individuals were alive after thawing in spite of the cooling temperature of -80°C.

Table III
Forst-resistance of the isolated rotifer

No.		Cool. Temp. (°C)	Cool. Rate (°C/min)	Supercool. Point (°C)	Freez. Time (hrs)	Falling Rate of Temp. (°C/min)**	No. of the Surv. / No. of the Dead
A	2	−20	1.3	−6.8	2	0.037	9 : 1
	2	−20	1.4	−7.0	19	0.076	20 : 3
	3	−40	1.9	−7.2	1	0.08	12 : 5
	4	−80	2.6	−7.3	1	0.084	14 : 8
	5	−80		none*	1	0.064	19 : 0
	6	−20	4.5	−7.4	2	0.17	33 : 4
	7	−20	6.2	−7.5	2	0.51	13 : 5
	8	−80	26.8	indistinct	1	1.40	9 : 12
B	9	−20	rapid		2		2 : 21
	10	−20	〃		2		10 : 14
	11	−40	〃		1		5 : 16

* inoculated with ice crystal at ca. 0°C
** relative rate of ice formation

When the rate of ice formation is sufficiently low, the cooling temperature and the cooling time exert no effect upon the survival rate of the rotifer in the range of -20 and -80°C. For instance, after the keeping of 24 hours at -80°C, the lump of algae which had been stroed at -20°C for about eight months still contained many active rotifers. That the frost-injury is very light when the rate of ice formation is slow may be explained as follows. The slow ice formation results in slow extracellular freezing in the body of the rotifer, leading sufficient dehydration of the body cells. The cells thus desiccated becomes to endure further cooling even to -80°C. The prefreezing treatment at -30°C is very effective for withstanding freezing at very low temperatures as -180°C, for parenchyma cells of mulbery tree[3)4)] and for plant-parasitic nematode[2)] and slug moth caterpillar[1)]. In these cases it may be reasonable that there would exist no more easily freezable water in the cell-interior because of the sufficient desiccation by extracellular freezing during the early period of freezing or the prefreezing at –30°C. According to the meteorological record for 1957 in the vicinity of Showa base in Ongul Island in Antarctica, the highest rate of falling of air temperature in January is about 0.006°C per minute. The order of the cooling rate under the natural condition of Showa base is quite different from that under the experimental conditions, that is, the former is extremely slower than the latter. Accordingly in this place the rate of ice formation in the rotifer body would be very slow. Taking this fact and the experimental results mentioned above into consideration, it does not appear to be possible that the rotifer suffer frost-injury at low temperatures in the long winter season in the Antarctic region.

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