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Cryopreservation of Cestode, *Mesocestoides corti* Tetrathyridia

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Cryopreservation has been attempted for microfilariae of *Brugia pahangi* (Ogunba, 1969), *Onchocerca gutturosa* (Ham, James and Bianco, 1978), and *Dirofilaria immitis* (Lok, Mika-Grieve and Grieve, 1983). Cryopreservation of the second- (Ramp, Eckert and Gottstein, 1978) and third-stage larvae of nematodes has also been reported. The third-stage larvae of nematodes required artificial exsheathment with hypochloride before cryopreservation (Campbell and Thomson, 1973; Kelly and Campbell, 1974; Kelly, Campbell and Whitelock, 1976; James and Peacock, 1986). This has also been applied to other species such as *Schistosoma mansoni* schistosomules (Stirewalt and Lewis, 1979) and *Echinococcus multilocularis* metacestodes (Eckert and Ramp, 1985), and produced good results. However, little has been described of cryopreservation of trematodes and cestodes. This must reflect difficulty in cryopreservation of these flatworms. In the present study, cryopreservation of a cestode, *Mesocestoides corti* tetrathyridia was examined using a simple and rapid method.

Tetrathyridia proliferated in the peritoneal cavity of infested mice were collected and washed by repeated suspension and sedimentation in PBS (0.01 M, pH 7.2). Aliquots (0.1 ml) of deposited tetrathyridia (654 ± 75.8 larvae) were transfered into glass test tubes (12 × 90 mm), and supernatant was removed after allowing the larvae to sediment. One ml of a medium composed of 0–20% dimethylsulfoxide (DMSO) or glycerol as cryoprotectants, 80-60% Eagle minimum essential medium (EMEM), 10% calf serum and 10% tryptose phosphate broth was added into each test tube. The larvae were equilibrated for 1 or 3 hours in an ice-cold condition to induce infiltration of cryoprotectants into internal tissues of larvae. This was followed by direct transfer into a −80°C ultrafreezer. After storing for 4 weeks, the larvae were rapidly thawed, washed with EMEM and incubated at 37°C for 3 hours to restore viability. The influence of cryopreservation was assessed on the basis of 2 indicators; the motile rate of larvae and the lesion score of mouse livers. The motility rate was determined by counting the number of active larvae in 100 larvae. For further evaluation of larval viability, all the larvae were injected into the peritoneal cavity of mice (Std: ddy, ♂, 5 week age). Unfrozen larvae were also treated by the identical method. After 4 months of maintenance, the mice were sacrificed under anesthesia and autopsied to examine the liver damages by larval migration. The scores of the liver lesions was categorized in accordance with the indexes as noted below.

0: Normal liver
1: Presence of 1 or 2 necrotic maculae
2: Presence of many scattered necrotic maculae
3: Presence of many scattered necrotic maculae and their confluence
4: Presence of severe cirrhosis and hypertrophy

The results are summarized in Table 1. The motility rate of the larvae increased in proportion to the concentration of DMSO. Rates exceeding 90% were obtained with 10 and 20% DMSO. These concentrations, however, resulted in disrupture of larvae. This was confirmed from the damage and the existance of tissue cells discharged from the cavity of the larvae. On the other hand, on examining the lesion scores, the highest score 4 was obtained under two conditions; 2.5 to 5% DMSO and 3 hours of equilibration time. These concentrations of DMSO with 1 hour of equilibration time led to low lesion scores below 2.0. A high concentration (20%) of

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Table 1. Viability of *M.corti* tetrathyridia after cryopreservation

Protectant	Concentration of protectant in medium	Equilibration time	Motile rate after thawing	Lesion score of mouse livers after infection (Mean)
	0 (%)	3 (Hour)	4 (%)	0, 1, 1 (0.7)
DMSO	2.5	1	11	1, 1, 1 (1.0)
		3	38	4, 4, 4 (4.0)
	5	1	19	–, 1, 3 (2.0)
		3	81	4, 4, 4 (4.0)
	10	1	91	3, 3, 3 (3.0)
		3	98	3, 4, 4 (3.7)
	20	1	96	1, 2, 2 (1.7)
		3	95	–, 0, 0 (0.0)
Glycerol	10	3	0	0, 1, 1 (0.7)
	20	3	20	1, 1, 1 (1.0)
Infection control (Unfrozen)				4, 4, 4 (4.0)
Uninfection control				0, 0, 0 (0.0)

(–: Death of mouse)

DMSO produced minor lesions. From these results, the following 2 conclusions may be derived; (1) A 1-hour equilibration time with 2.5 and 5% DMSO is a inadequate for infiltration of DMSO into the larval tissues and (2) the motility rate does not reflect the true viability of larvae. The latter has been described by Ham (1982). It appears that 20% DMSO caused disrupture of the larvae and that the larvae were eventually digested in the mice. The other additive, glycerol was found unsuitable as a protectant for cryopreservation, as suggested by the low values of the two indexes.

The present paper demonstrated that *M.corti* tetrathyridia could be cryopreserved by the simple and rapid method without requiring strictly-prescribed cooling procedure in the process of freezing.

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