endoplasmic reticulum and lipid droplets. Moreover, the turnover of these cellular organelles in relation to the cycle of interstitial, luteal and thecal cells of rat ovary should also be considered.

The close association of microperoxisomes with membranes of smooth endoplasmic reticulum and lipid droplets, which in active steroidogenic cells are considered respectively as sites of steroid enzymes and material precursor of the hormones[26], might suggest a probable role of these organelles in the synthesis of steroid products. Further the close association between microperoxisomes and partially empty lipids in some cases might be related with the anabolic phase of the cell, more than with the secretion of steroid material[27].

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Effects of low temperatures on survival of frozen-thawed mouse embryos

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Summary. Almost no damage to mouse morulae was observed between 0 and −40 °C, and freezing damage to embryos in DMSO, glycerol or ethylene glycol occurred after exposure to −60, −50 or −50 °C, respectively. Cooling embryos in DMSO or glycerol to temperatures below −50 to −60 °C increased freezing damage. To the contrary, in the presence of ethylene glycol, no more damage occurred after exposure to temperatures below −50 °C.

Although mouse embryos stored for 200 days or longer at −88 °C or −196 °C develop into viable fetuses, embryos stored at −44 °C are not viable after 3 days in storage[1]. Storage of embryos at −88 °C or lower is, therefore, necessary for practical application. A major cause of freezing damage is the formation of intracellular ice crystals during cooling and their growth by recrystallization during thawing[2]. Intracellular freezing of unfertilized mouse ova cooled in the presence of 1 M dimethyl sulphoxide (DMSO) generally occurs at about −40 to −45 °C[3]. The survival and fertilizability of unfertilized mouse ova after freezing and thawing are dependent upon the temperature to which they are exposed[4]. This report examines the resistance to cooling of mouse embryos to various low temperatures in the presence of different cryoprotectants.

Materials and methods. Female ICR mice were induced to superovulate and mated[5]. Morulae were flushed from the reproductive tracts with a modified Dulbecco's phosphate-buffered salt solution (PBS)[5] at 78–82 h after an injection of HCG. 10–20 embryos were transferred to each test-tube containing 0.1 ml PBS. The tubes, suspended in ethanol in a Dewar flask, were then cooled to 0 °C and the cryoprotectant in 0.05 ml PBS kept at 0 °C was added to samples in 3 increments at 10-min intervals. The cryoprotectants used were 1.2 M DMSO, 1 M glycerol and 1.2 M ethylene glycol. All samples were equilibrated for 10 min at 0 °C after the addition of the cryoprotectant and seeded with an ice crystal at −5 °C. They were cooled to −79 °C at 0.5 °C/min by adding dry ice to ethanol in a Dewar flask and then a Dewar flask containing samples was placed into liquid nitrogen to be cooled from −79 to −120 °C at 1–2 °C/min. After the temperature of samples was lowered to −120 °C, samples were transferred into liquid nitrogen. They were recovered for survival estimation from a Dewar flask or

Survival of mouse morulae after freezing to various low temperatures in the presence of DMSO, glycerol or ethylene glycol

Freezing temperature (°C)	Percentage of morulae developing to expanded blastocysts after culturing for 36 h Cryoprotectant		
	DMSO (1.2 M)	Glycerol (1 M)	Ethylene glycol (1.2 M)
0	96 (77)	100 (79)	96 (74)
− 10	100 (84)	100 (83)	100 (80)
− 20	94 (82)	97 (73)	95 (91)
− 30	91 (93)	93 (89)	92 (77)
− 40	93 (88)	91 (77)	93 (88)
− 50	91 (80)	79 (86)	86 (95)
− 60	86 (90)	74 (92)	84 (90)
− 70	87 (95)	68 (79)	86 (77)
− 79	87 (77)	70 (88)	88 (81)
−100	83 (80)	60 (75)	87 (79)
−120	78 (96)	53 (95)	85 (96)
−196	67 (95)	43 (81)	88 (91)

Figures in parentheses represent the number of thawed embryos recovered.

liquid nitrogen at the following temperatures during freezing: 0, −10, −20, −30, −40, −50, −60, −70, −79, −100, −120 and −196 °C. For thawing, samples were put in air at room temperature and then embryos were washed with culture medium. Embryos were cultured by the microdrop method in a modified Krebs-Ringer bicarbonate medium[5] at 37 °C for 36 h in 5% CO_2 in air. Since development of frozen-thawed mouse embryos in vitro was highly correlated with the normal embryonic development in vivo after transfer[6], survival was assessed by the ability of frozen-thawed morulae to develop into expanded blastocysts during culture. Experiments were replicated 4–5 times and statistical significance was determined by the χ^2 test.

Results and discussion. The results are summarized in the table. When embryos were cooled in the presence of DMSO, almost no decrease in survival occurred between 0 and −50 °C and a small decrease ($p < 0.05$) was observed after exposure to −60 °C. Compared with survival of embryos exposed to 0 °C, a large decrease ($p < 0.001$) in survival occurred after exposure to −120 and −196 °C. After cooling embryos in the presence of glycerol, almost no damage to embryos occurred at 0 to −40 °C. Considerable decrease ($p < 0.001$) in survival, however, occurred at −50 °C or lower temperatures, and especially a large damage ($p < 0.001$) was observed after exposure to −120 and −196 °C. When embryos were cooled in the presence of ethylene glycol, survival hardly decreased between 0 and −40 °C. Compared with survival of embryos exposed to 0 °C, a small decrease ($p < 0.05$) in survival was observed after exposure to −50 °C and no more damage occurred after exposure to temperatures below −50 °C in the presence of ethylene glycol.

These results show that when mouse morulae are cooled to −79 °C at 0.5 °C/min before cooling to −196 °C in the presence of DMSO, glycerol or ethylene glycol, damage to embryos occurs after exposure to −60, −50 or −50 °C, respectively and also show that particularly when DMSO or glycerol was used as the cryoprotectant, survival of morulae are dependent upon the low temperature to which they are exposed.

When unfertilized mouse ova in DMSO were cooled to −30, −45 and −75 °C, survival was 51, 56 and 18%, respectively[4]. Following the transfer of rat embryos in DMSO unfrozen or frozen to −50 and −79 °C into recipient rats, 100, 83 and 64% of recipients became pregnant, respectively[7]. After sheep embryos in DMSO were cooled to −25, −45 and −60 °C, survival was 100, 67 and 75%, respectively[8]. Results in the present study are similar to the earlier reports regarding unfertilized mouse ova[4], rat[7], sheep[8] embryos.

A major cause of freezing injury is intracellular ice formation[2] and intracellular freezing of unfertilized mouse ova in 1 M DMSO occurs at about −40 to −45 °C[3]. The present results may support the claim[3] that mouse embryos still contain water capable of freezing intracellularly at temperatures above −50 °C.

When mouse morulae were frozen to −196 °C in the presence of DMSO, glycerol and ethylene glycol, survival rate was 67, 43 and 88%, respectively. Ethylene glycol, therefore, afforded more ($p < 0.01$) effective protection to mouse morulae frozen to −196 °C than DMSO or glycerol, and DMSO better ($p < 0.01$) than glycerol. The present experiments also confirmed the report[5] that greater survival is obtained for mouse embryos frozen in the presence of DMSO than in glycerol.

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Cyclosporins: Immunosuppressive agents with antitumor activity[1]

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Summary. Initial screening of the 2 recently developed immunosuppressive agents, cyclosporin A and cyclosporin C, in 11 murine transplantable neoplasms revealed significant increase of lifespan with long-term survivors after i.p. injection to the ascites tumors, Taper liver, Sarcoma 180J and Ehrlich.

The biological activity of cyclosporins, a group of antifungal metabolites recently isolated from *Trichoderma polysporum* (Link ex Pers.) Rifai[3–7], consists in the inhibition of humoral immunity as evidenced by the reduction of plaque-forming cells and haemagglutinin titers in mice[8]. Suppression of cell-mediated immunity was demonstrated in test systems for skin graft rejection, graft-versus-host disease and experimental allergic encephalomyelitis in rodents[8]. The most studied representative is cyclosporin A, a neutral cycloendecapeptide with several N-methylated amino acids and 1 new amino acid. This new agent proved to be very active in chronic inflammatory reactions and produced only mild myelotoxicity[8]. It displayed inhibition of transplant rejections in patients receiving renal allografts[9] and graft-versus-host disease in man[10]. The drug delayed the hypersensitivity skin reaction to oxazolone in mice and to tuberculin in guinea-pigs. It did not suppress antibody synthesis to lipopolysaccharide antigens in nude mice, indicating a selective effect upon T-cells[11]. Further evidence for selectivity for T-cells was also provided in in vitro lymphocyte proliferation tests[12]. The present exploratory study was undertaken to test for possible antitumor activity of 2 representatives of this new group of compounds.

A series of solid and ascitic mouse tumors [mast cell tumor, ascites (P815), leukemia L1210 (L1210), Taper liver tumor, ascites (Taper), Sarcoma T241, solid (T241), adenocarcinoma EO771, solid (EO771), Sarcoma 180J, ascites and solid (S180J), T-cell tumor EL-4, ascites and intradermal (EL-4), leukemia L5178Y resistant to 1-β-D-arabinofuranosylcytosine, ascites (5178Y/CA-55), Ehrlich ascites (Ehrlich), Ridgeway osteogenic Sarcoma (ROS), Lewis lung carcino-