EMMA CRYOPRESERVATION WORKSHOP, MADRID, MAY 7, 2012

The Cryobiology of Mammalian Oocytes and Embryos– Past, Present, and Future

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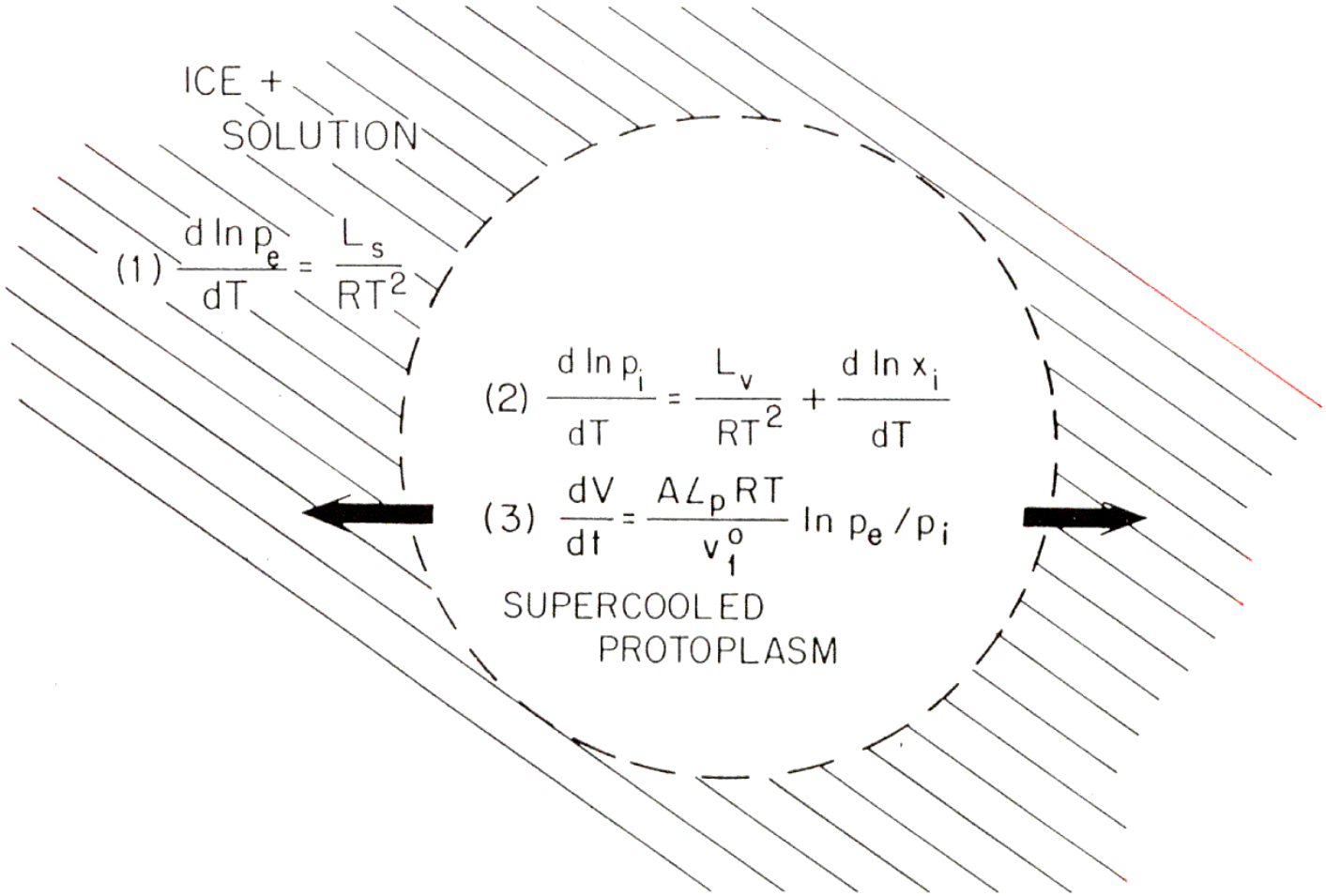
Research supported by NIH grant R01-RR018470


ICE +
SOLUTION
(1) $\frac{d \ln p_e}{dT} = \frac{L_s}{RT^2}$
(2) $\frac{d \ln p_i}{dT} = \frac{L_v}{RT^2} + \frac{d \ln x_i}{dT}$
(3) $\frac{dV}{dt} = \frac{A L_p RT}{v_1^o} \ln p_e / p_i$
SUPERCOOLED
PROTOPLASM

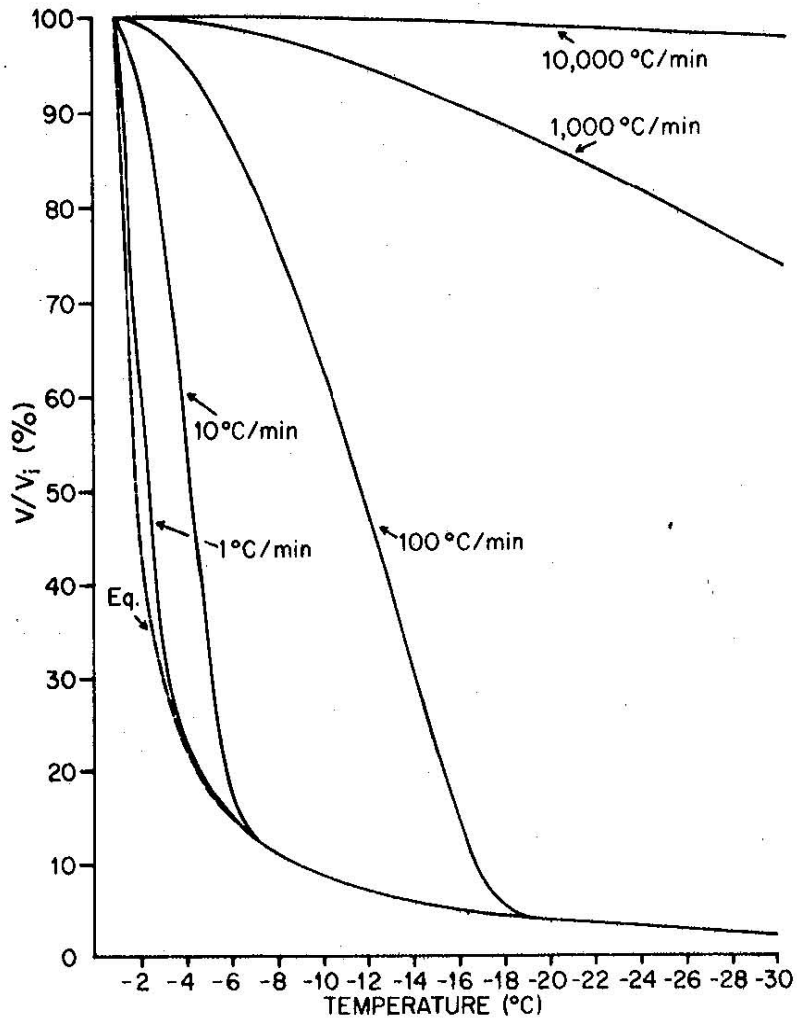
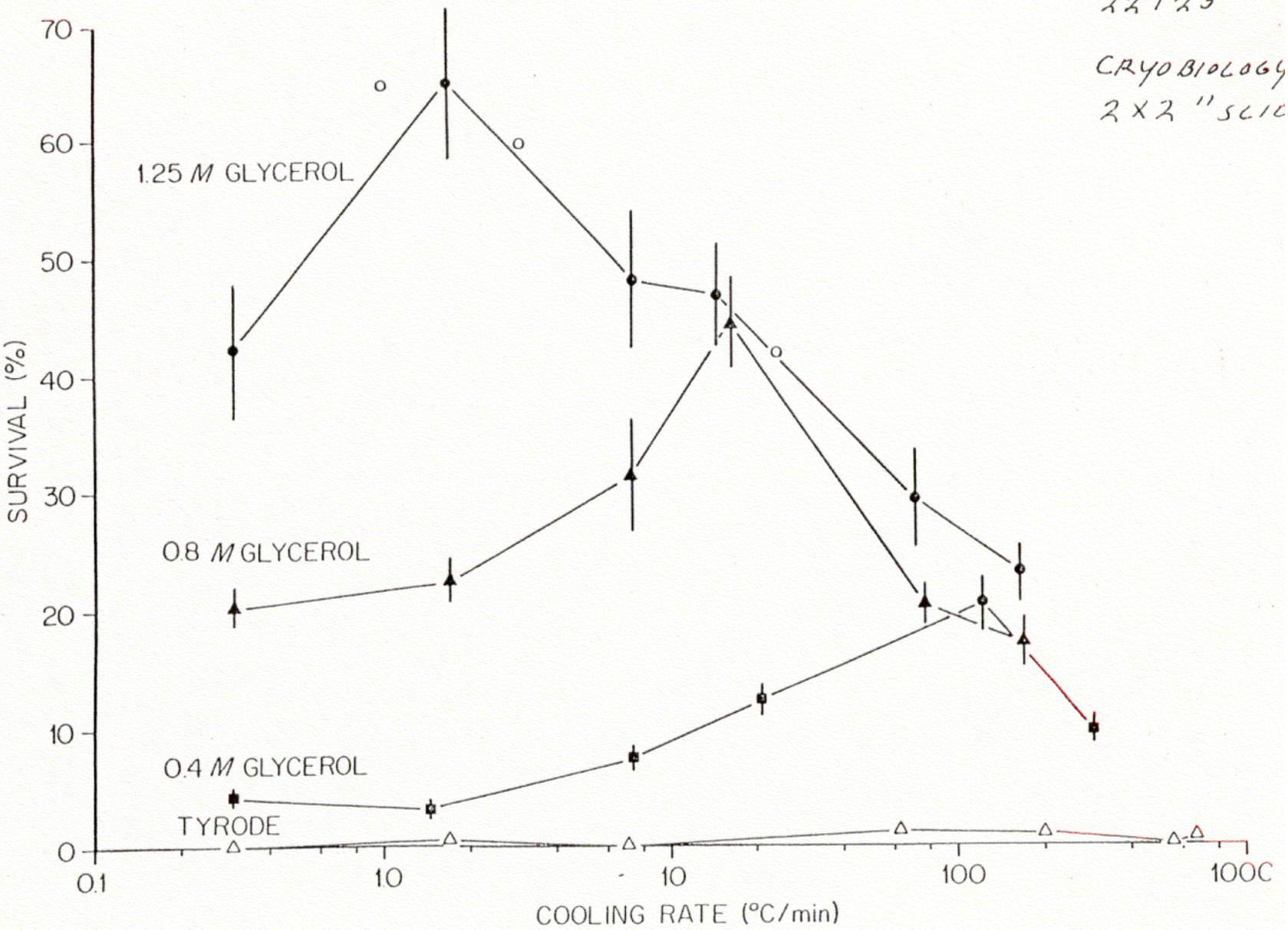

100
90
80
70
60
50
40
30
20
10
0
V/Vi (%)
10,000 °C/min
1,000 °C/min
10°C/min
1°C/min
100 °C/min
Eq.
-2 -4 -6 -8 -10 -12 -14 -16 -18 -20 -22 -24 -26 -28 -30
TEMPERATURE (°C)

FIG. 3

22125

CRYOBIOLOGY

2 X 2 "SCI



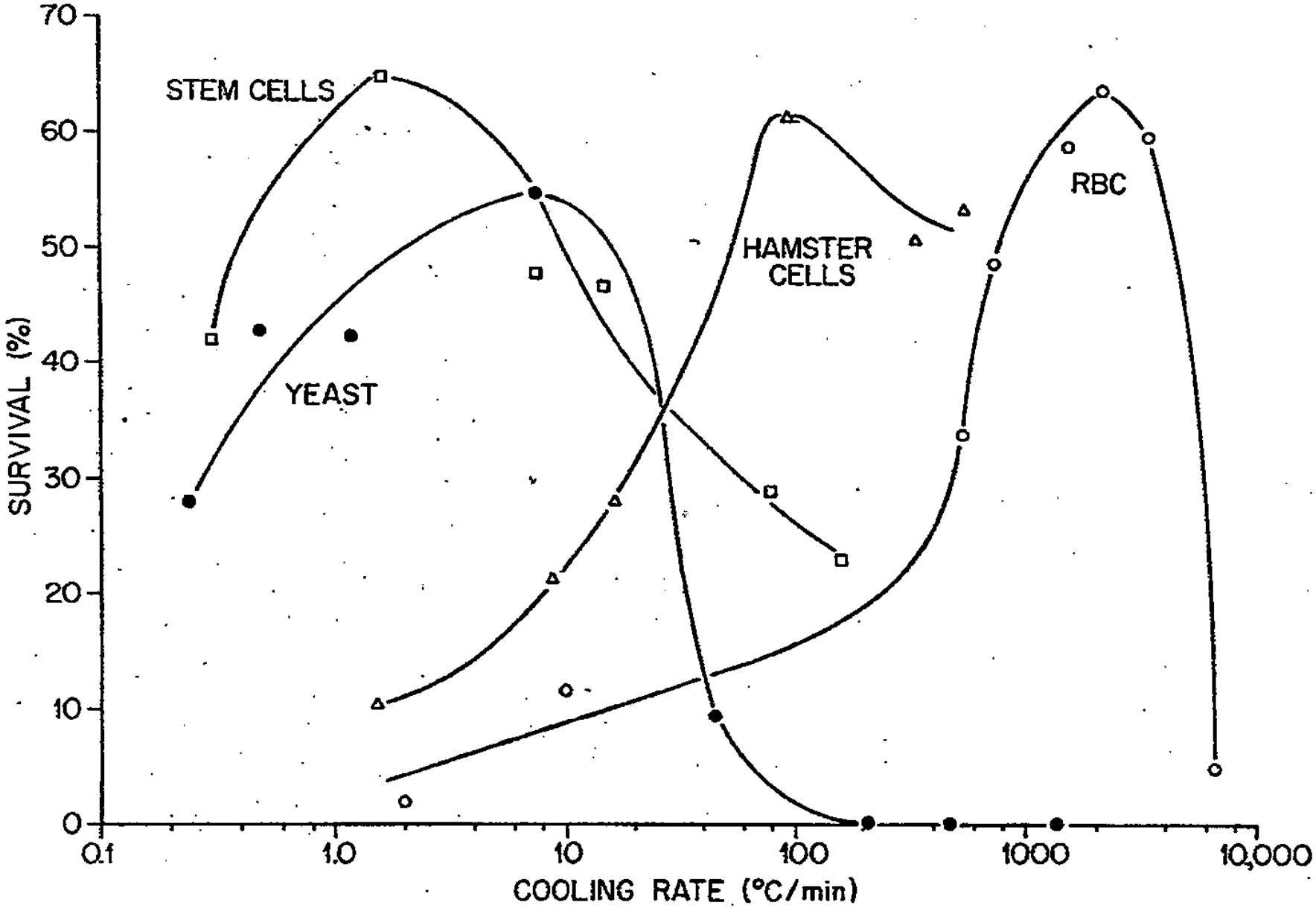
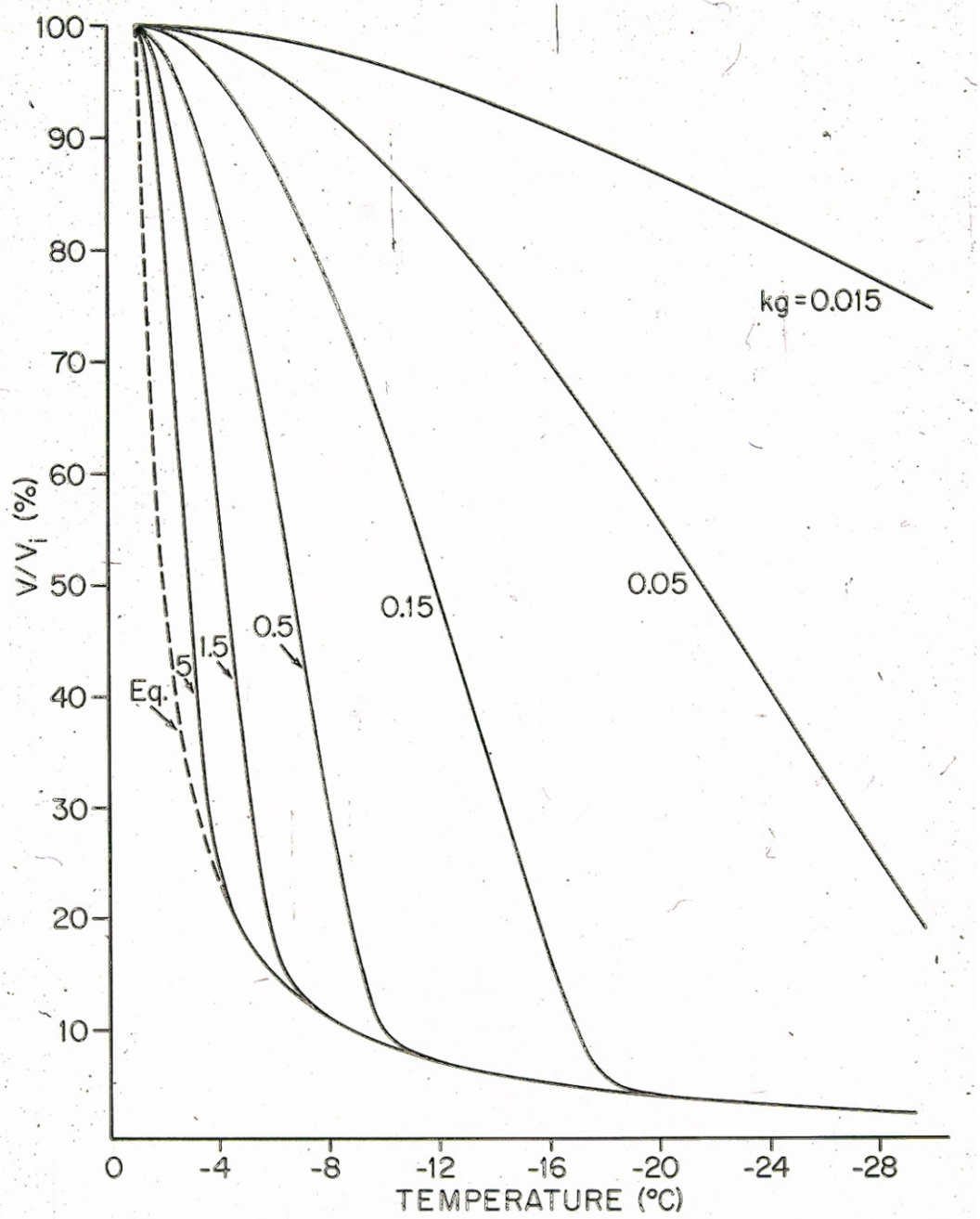
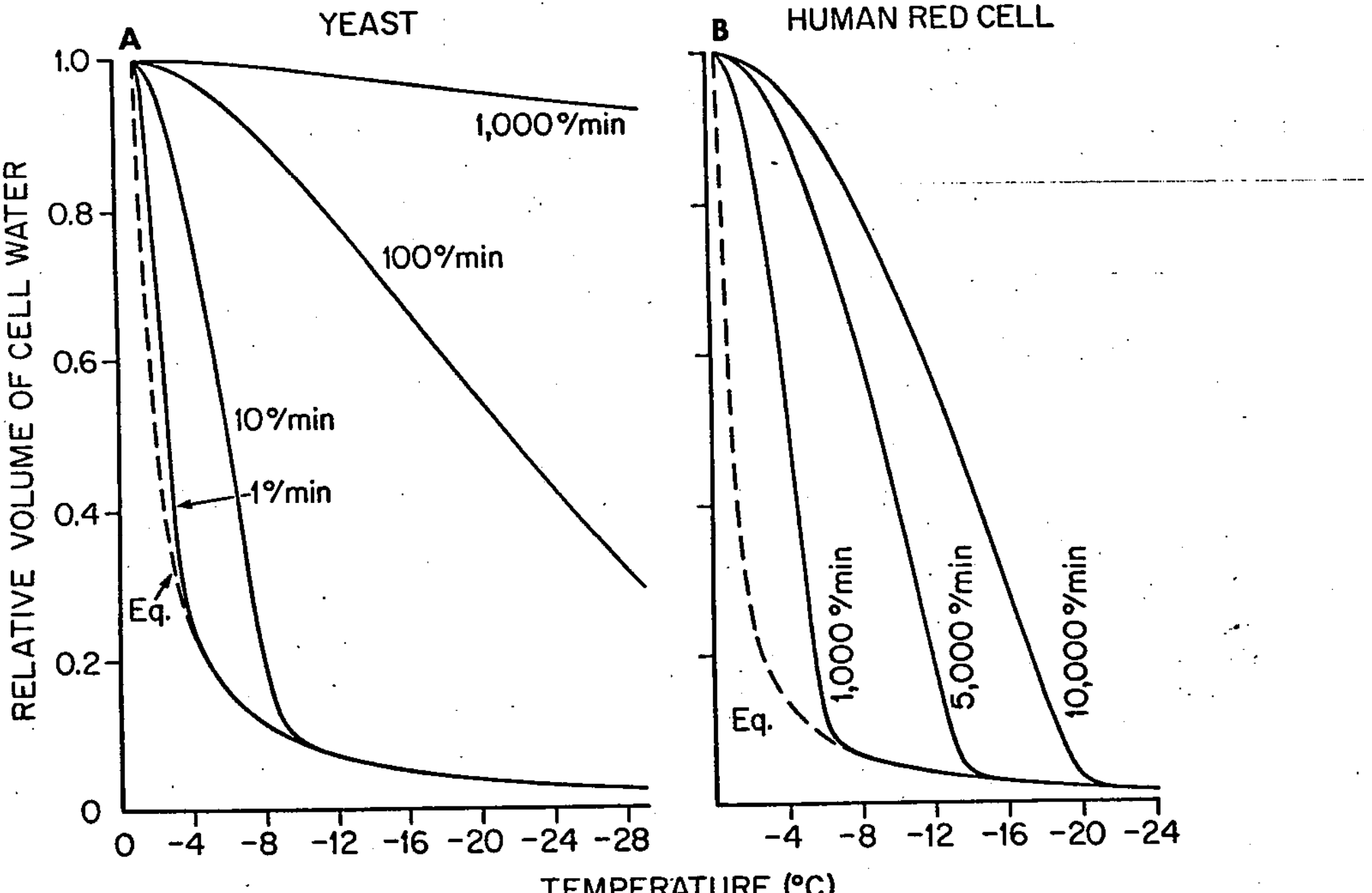

70
60
50
40
30
20
10
0
SURVIVAL (%)
STEM CELLS
YEAST
HAMSTER CELLS
RBC
0.1
1.0
10
100
1000
10,000
COOLING RATE (°C/min)

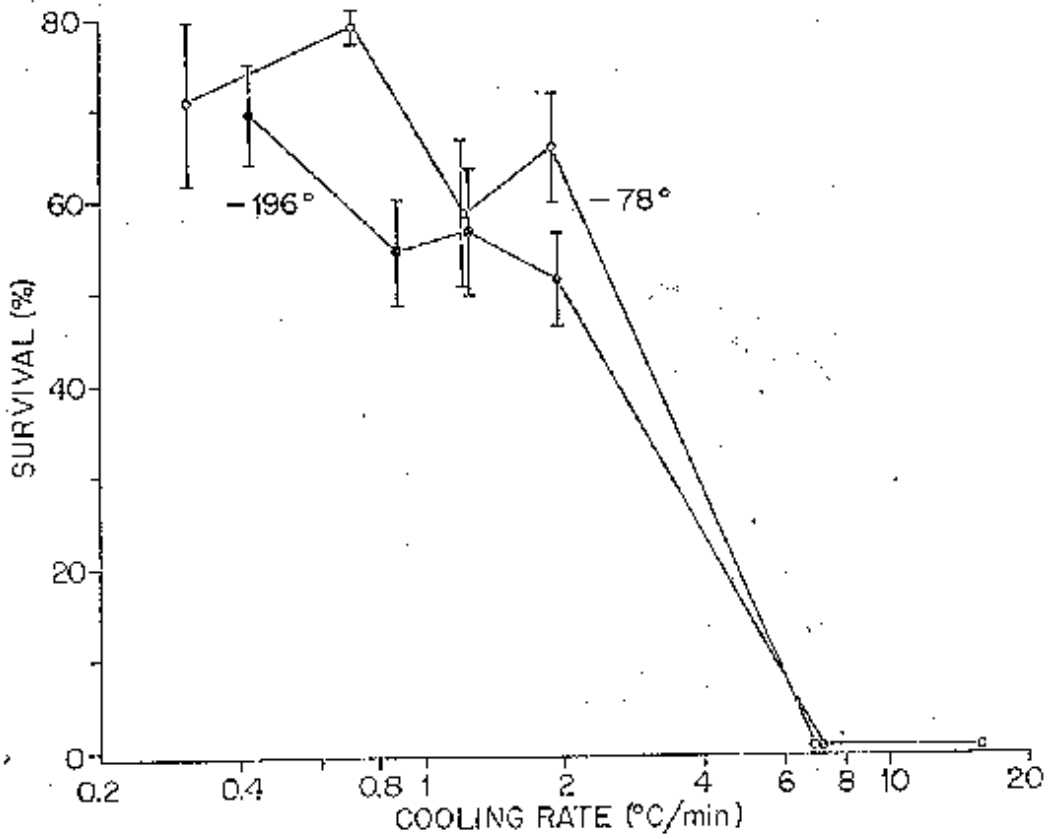
Fig. 2


100
90
80
70
60
50
40
30
20
10
V/Vi (%)
kg = 0.015
0.05
0.15
0.5
1.5
5
Eq.
0
-4
-8
-12
-16
-20
-24
-28
TEMPERATURE (°C)

Dig 22379


A
YEAST
B
HUMAN RED CELL
RELATIVE VOLUME OF CELL WATER
1.0
0.8
0.6
0.4
0.2
0
1,000%/min
100%/min
10%/min
1%/min
Eq.
0 -4 -8 -12 -16 -20 -24 -28
-4 -8 -12 -16 -20 -24
1,000%/min
5,000%/min
10,000%/min
Eq.
TEMPERATURE (°C)

Dig 26917-1
3¼ x 4
2 x 2



SCIENCE

27 October 1972

Vol. 178, No. 4059

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

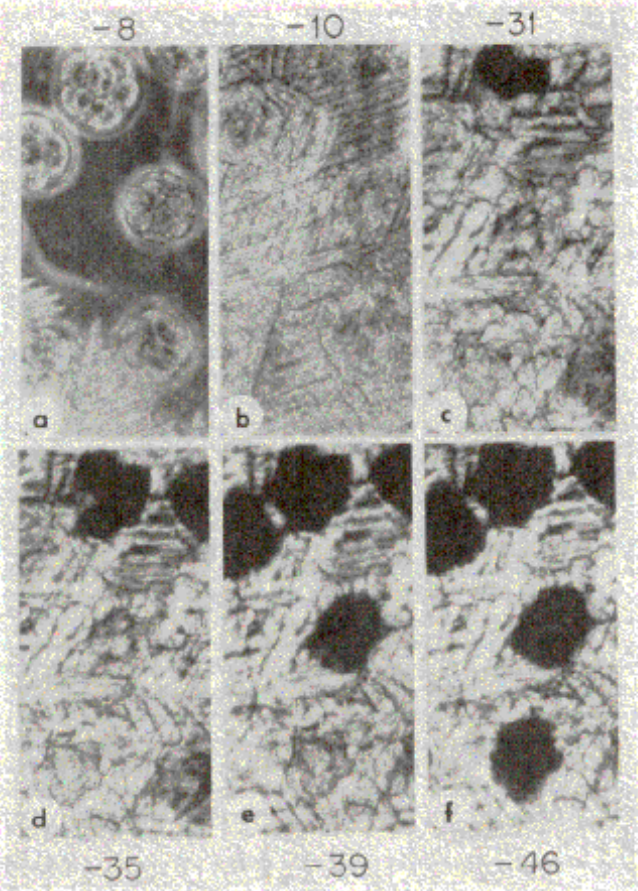
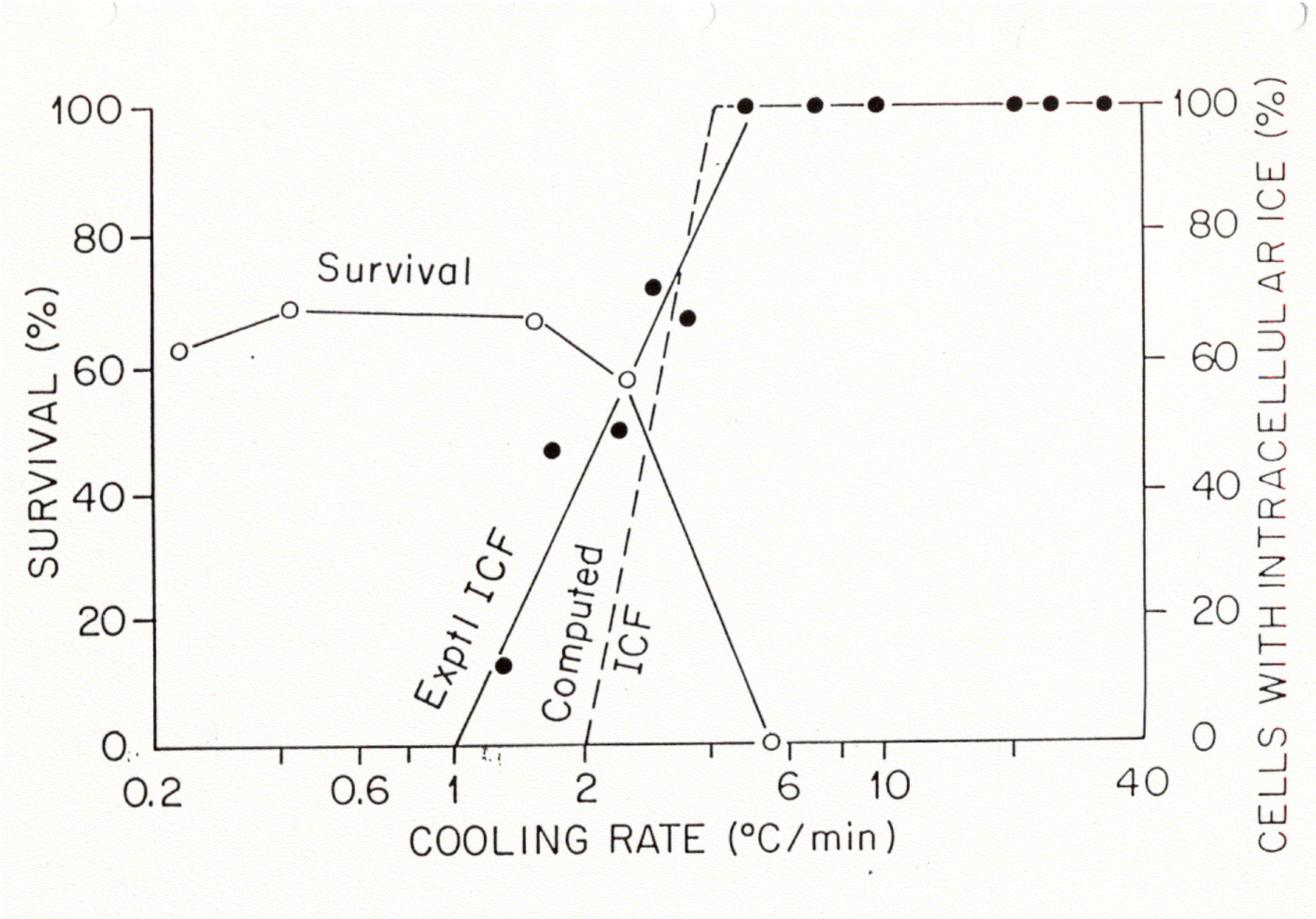
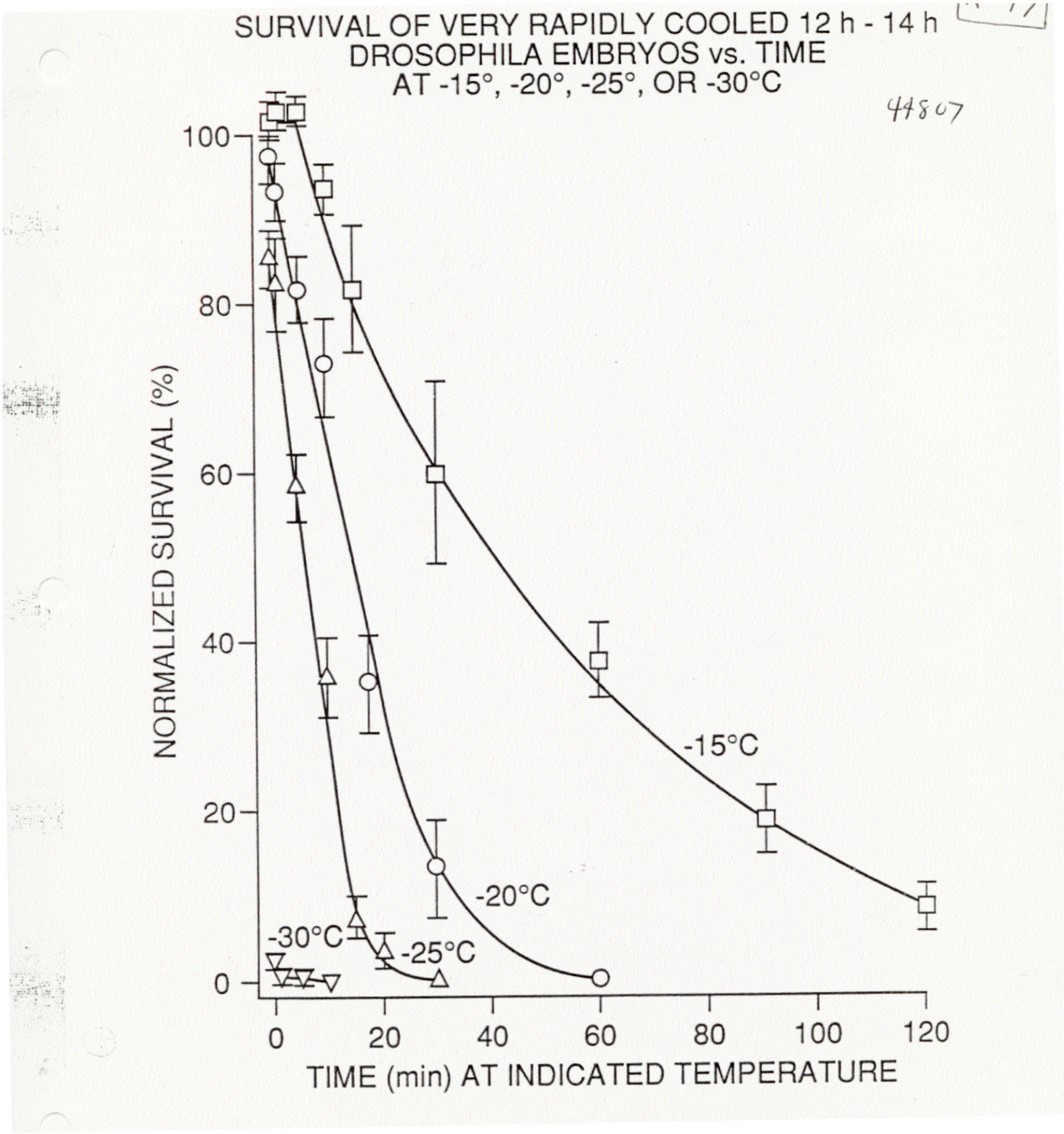
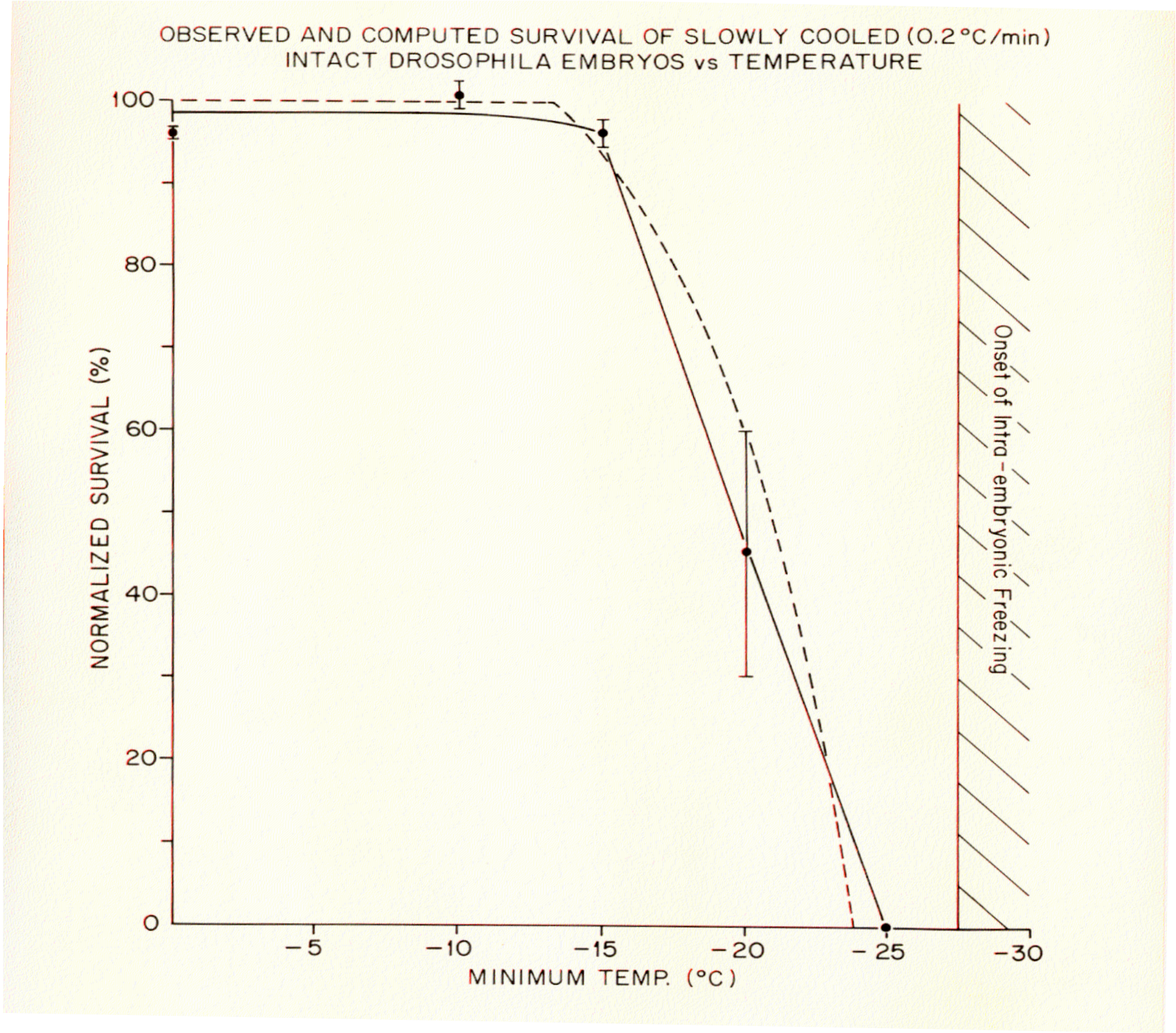


Figure 3. Intracellular freezing of 8-cell mouse embryos cooled at 20 °C/min in 2 M DMSO. The black "flashing" occurring in cells at –31 to –46 °C is characteristic of intracellular ice formation and probably reflects the scattering of light by many small highly branched ice crystals (modified from Rall et al., 1983).

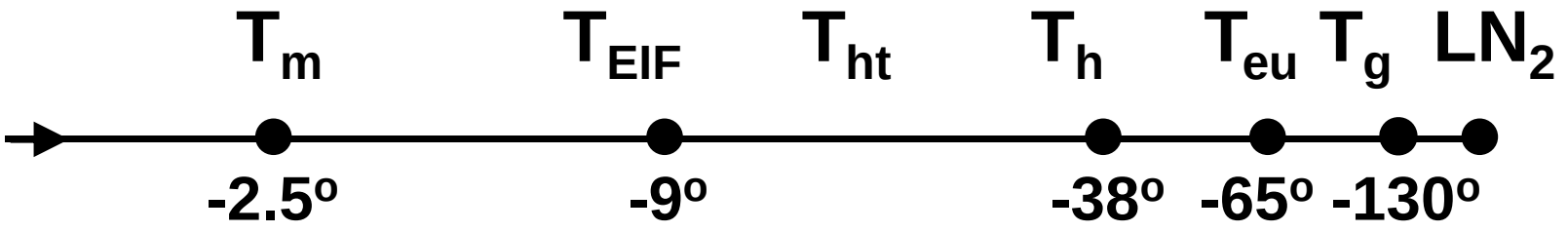

100
80
60
40
20
0
SURVIVAL (%)
Survival
Expt'l ICF
Computed ICF
0.2
0.6
1
2
6
10
40
COOLING RATE (°C/min)
100
80
60
40
20
0
CELLS WITH INTRACELLULAR ICE (%)


SURVIVAL OF VERY RAPIDLY COOLED 12 h - 14 h
DROSOPHILA EMBRYOS vs. TIME
AT -15°, -20°, -25°, OR -30°C
44807
NORMALIZED SURVIVAL (%)
100
80
60
40
20
0
-15°C
-20°C
-25°C
-30°C
0
20
40
60
80
100
120
TIME (min) AT INDICATED TEMPERATURE

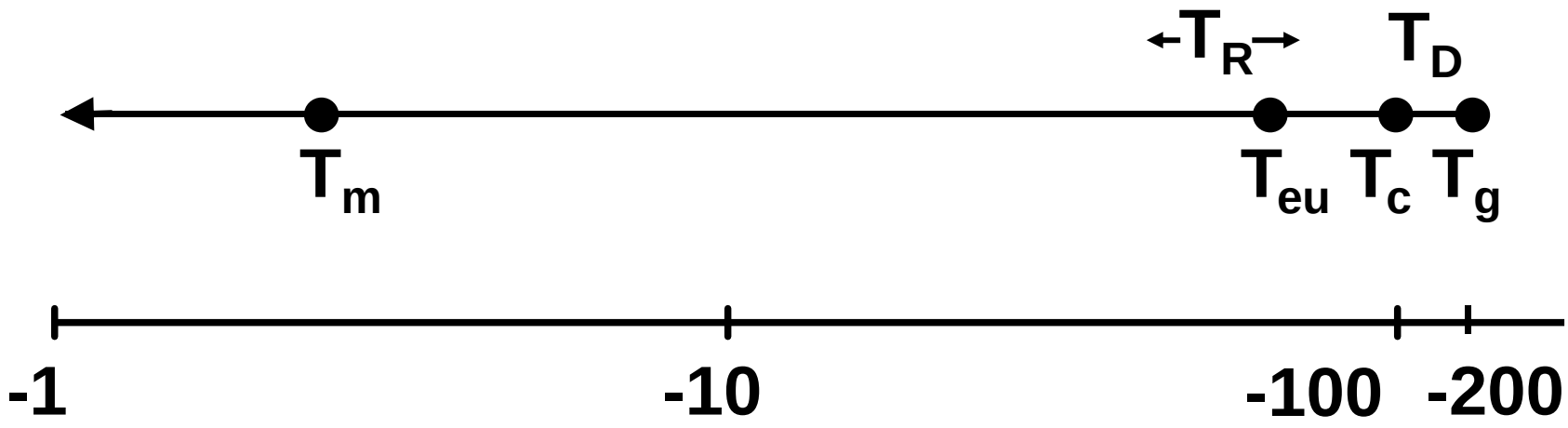

OBSERVED AND COMPUTED SURVIVAL OF SLOWLY COOLED (0.2 °C/min)
INTACT DROSOPHILA EMBRYOS vs TEMPERATURE
NORMALIZED SURVIVAL (%)
100
80
60
40
20
0
−5
−10
−15
−20
−25
−30
MINIMUM TEMP. (°C)
Onset of Intra-embryonic Freezing

Important temperatures in cryopreservation *

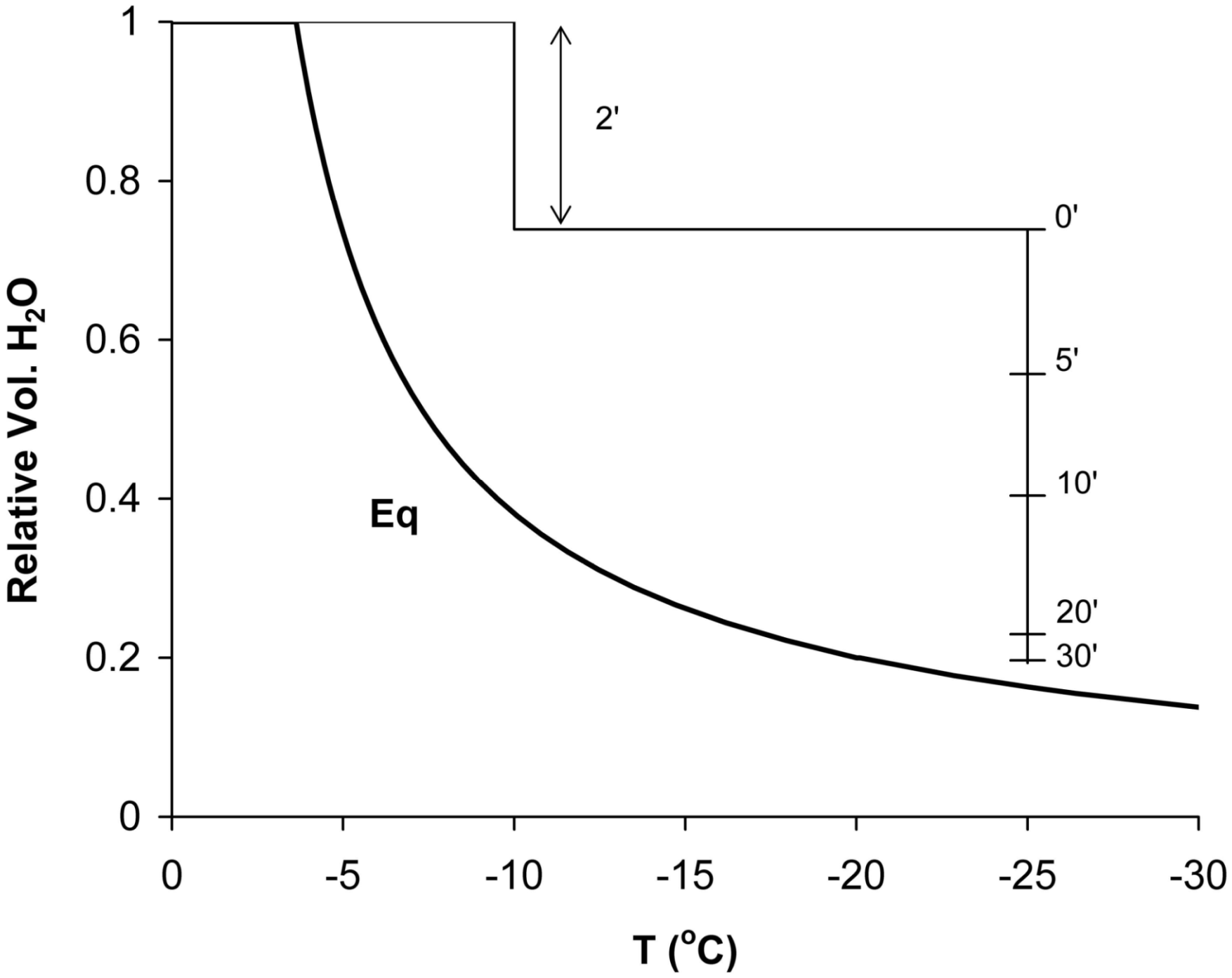
Cooling



Warming



(* Data for 1 M ethylene glycol)

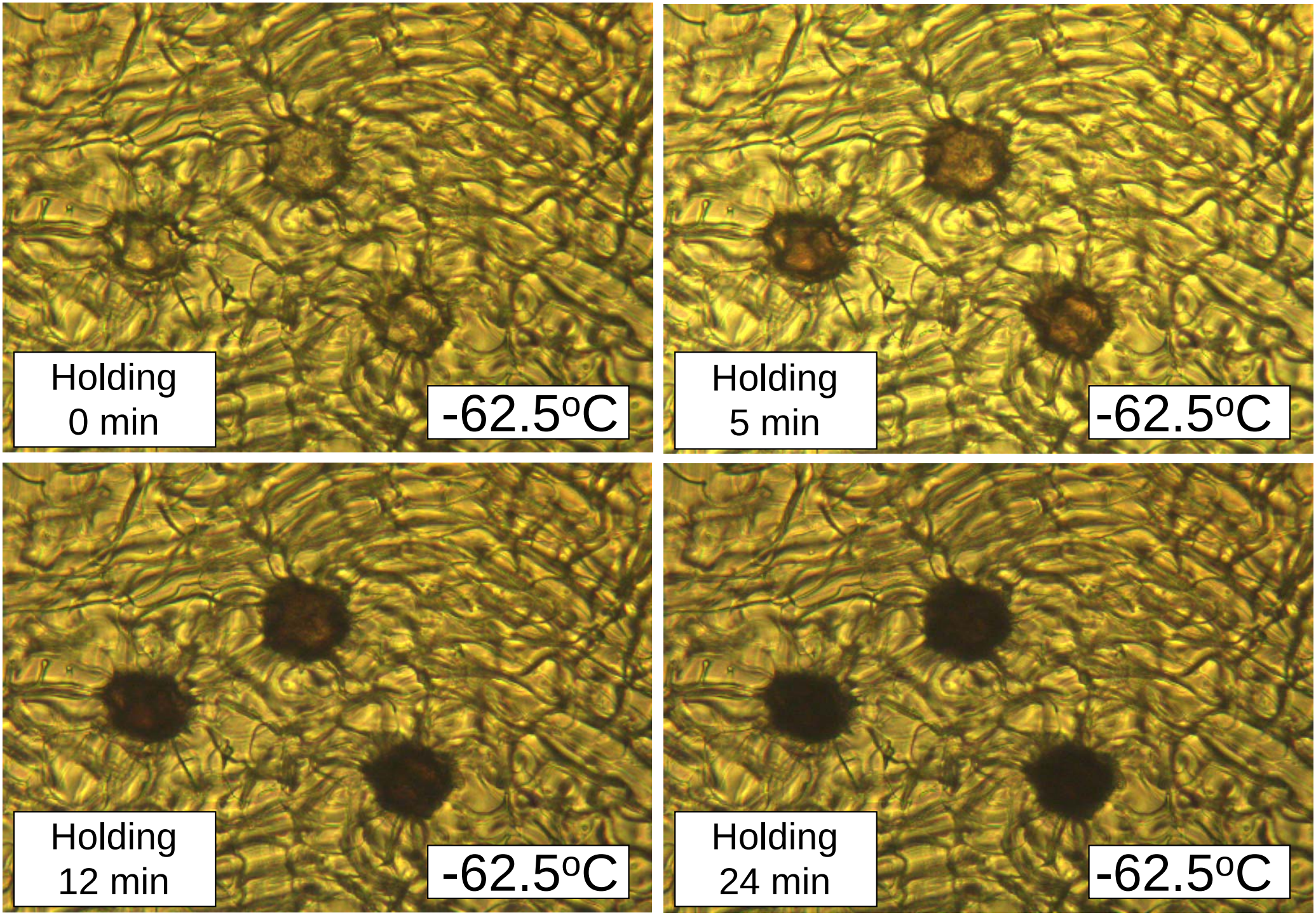

1
0.8
0.6
0.4
0.2
0
Relative Vol. H2O
2'
0'
5'
10'
20'
30'
Eq
0
-5
-10
-15
-20
-25
-30
T (°C)

Ice formation in mouse oocytes suspended in 1.5 M EG, rapid cool to EIF, 2-min hold after EIF, rapid cool to –25°C, variable hold, and rapid cool to –70°C, and warm at 10°C/min

	Hold temp.	Hold time)	# oocytes	% Flash during Ramp 4	% Blacken during warm	% Normal after thaw
	(°C)	(min)		**Class 2 & 3 (%)**	**Class 4 (%)**	**Class 5 (%)**
	–25	5	10	80	10	0
	–25	10	8	12	88	0
	–25	20	8	0	38	38
	–25	30	25	0	12	80
	–25	40	9	0	0	78

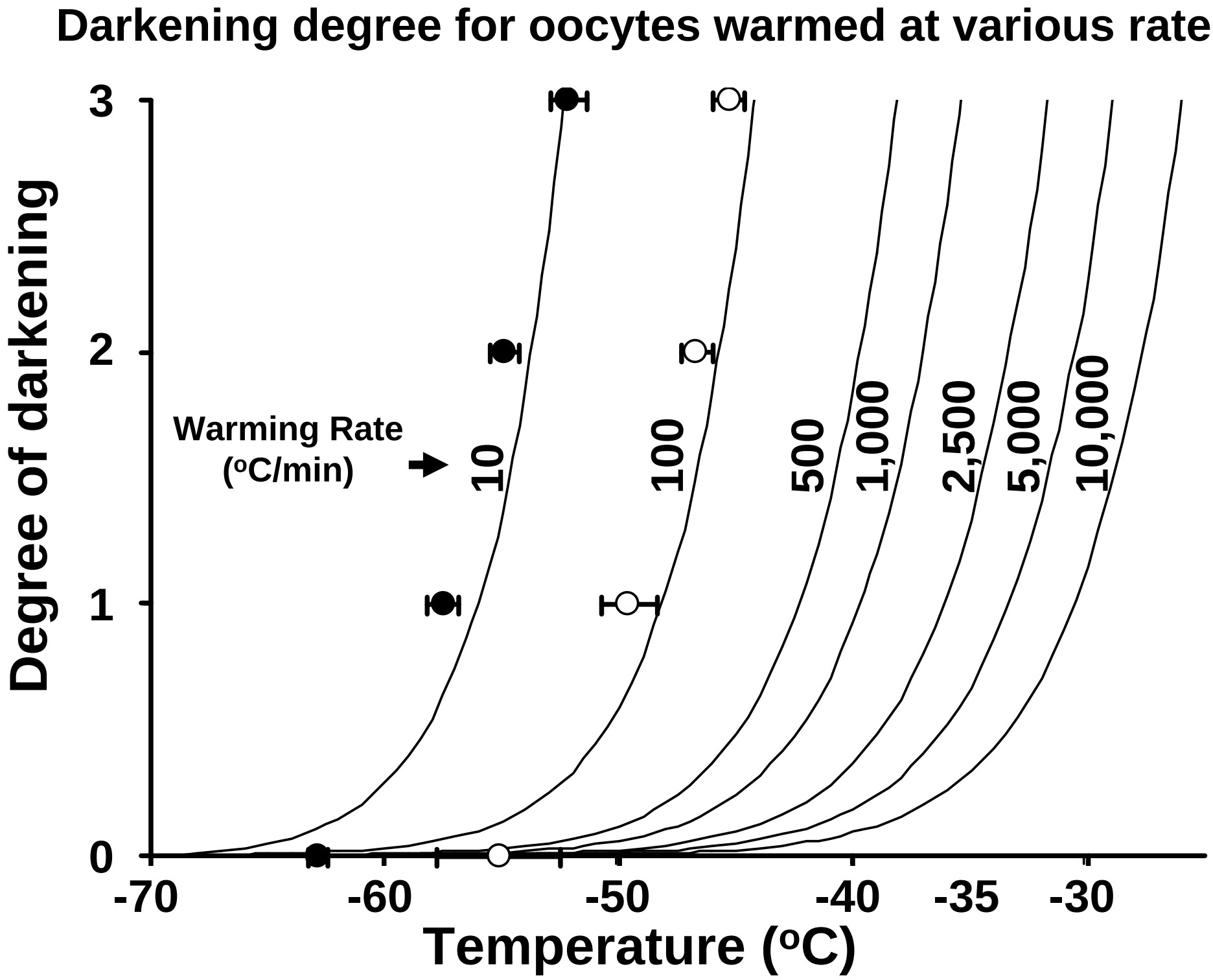
Ramp 4 is a 50°C/min cool from the hold temperature to –70°C

[Modified from Table 1,, P. Mazur et al, Cryobiology **55** (2007) 158-166


Holding
0 min
-62.5ºC
Holding
5 min
-62.5ºC
Holding
12 min
-62.5ºC
Holding
24 min
-62.5ºC

Time for mouse oocytes to blacken to stage 1 as a function of temperature

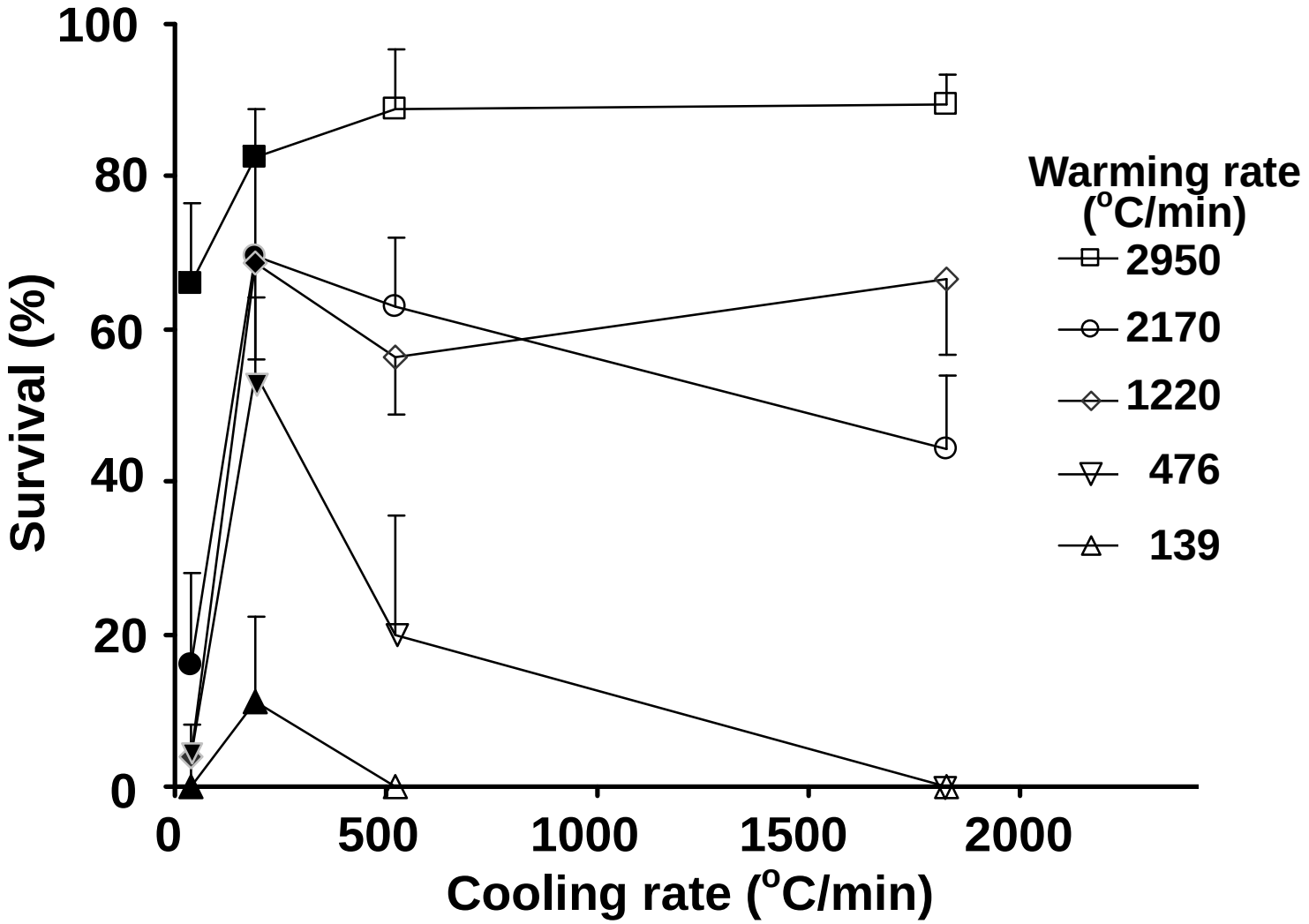
Temperature	1/T	k	Time to reach Black Stage 1	
(°C)	(K^{-1})	(1/min)	(min)	(sec)
-79	0.00515	0.002	620	37200
-70	0.00492	0.038	26.5	1590
-60	0.00469	0.918	1.090	65.4
-57.5	0.00464	1.946	0.514	30.8
-55	0.00458	4.055	0.247	14.8
-50	0.00448	16.770	0.060	3.580
-45	0.00438	65.140	0.015	0.921
-40	0.00429	238.8	0.004	0.251
-35	0.00420	828.6	0.001	0.072
-30	0.00411	2732	0.0004	0.022

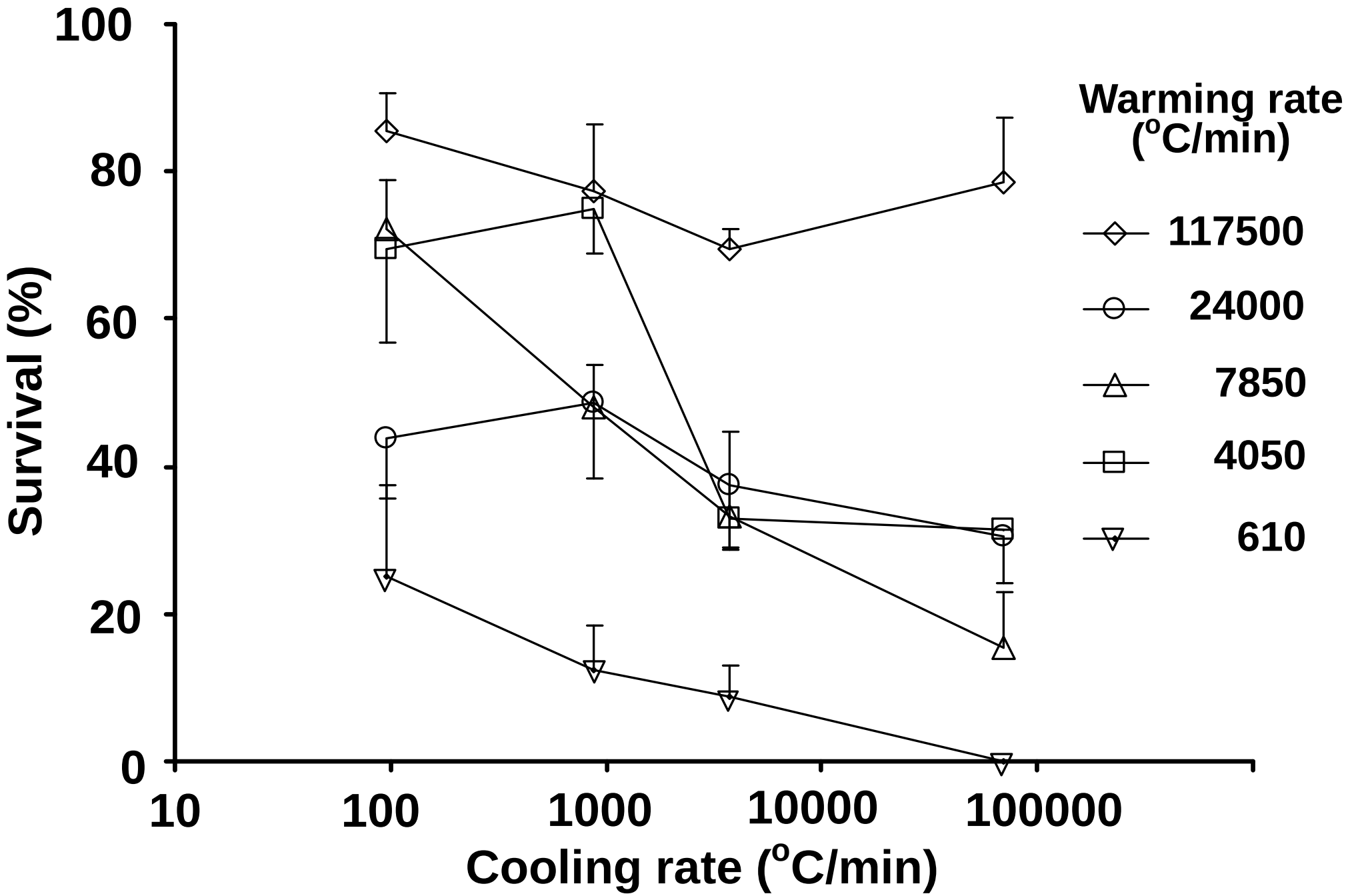

Darkening degree for oocytes warmed at various rates
Degree of darkening
3
2
1
0
Warming Rate
(°C/min)
10
100
500
1,000
2,500
5,000
10,000
-70
-60
-50
-40
-35
-30
Temperature (°C)

Survival of mouse oocytes in EAFS10/10 after being cooled to -196°C at each of four rates and warmed at each of five rates

Warming rate (°C/min)	Cooling rate (°C/min)			
	37	187	522	1827
139	0.0 ± 0.0	11.1 ± 11.1	0.0 ± 0.0	0.0 ± 0.0
476	4.0 ± 4.0	54.3 ± 9.9	20.0 ±15.5	0.0 ± 0.0
1220	4.0 ± 4.0	68.8 ± 12.8	56.4 ± 7.5	66.7 ± 9.9
2170	15.8 ± 12.3	69.4 ± 12.7	62.9 ± 9.2	30.0 ± 14.1
2950	65.8 ± 10.7	82.5 ± 6.3	88.9 ± 7.7	89.6 ± 3.7

[Modified from Table 3 of Seki and Mazur, Cryobiology **59** (2009) 75-82]


100
80
60
40
20
0
Survival (%)
0
500
1000
1500
2000
Cooling rate (°C/min)
Warming rate (°C/min)
2950
2170
1220
476
139


100
80
60
40
20
0
Survival (%)
10
100
1000
10000
100000
Cooling rate (°C/min)
Warming rate (°C/min)
117500
24000
7850
4050
610

ACKNOWLEDGMENTS

o Most of the newer experimental work has been conducted by Dr. Shinsuke Seki

o The research has been supported by the National Research Resources Center of the NIH under grant R01-RR 018474