Use of Dielectric Heating (Shortwave Diathermy) in Thawing Frozen Suspensions of Tissue Culture Cells.* (28939)

RUTH K. SILVER, HERNDON B. LEHR, ALAN SUMMERS, ARTHUR E. GREENE AND LEWIS L. CORIELL (Introduced by I. S. Ravdin)

South Jersey Medical Research Foundation, Camden, N. J. and Harrison Department of Surgical Research, University of Pennsylvania, Philadelphia

The study of the use of shortwave diathermy for thawing frozen mammalian tissue culture cells was undertaken in this laboratory as part of a project of wider scope, the aim of which is the viable preservation of organs by freezing. In efforts to preserve mammalian cells and tissues by freezing, many workers(1,2) have reported consistently better results when the frozen material was thawed rapidly rather than slowly. One of the main problems encountered in the preservation of organs by freezing was that of obtaining a sufficiently fast thaw rate when large masses of tissue were involved. For thawing small masses of tissue (such as frozen skin), a sufficiently fast rate is obtained by conduction of heat resulting from agitation of the material in a 37°C water bath. For larger masses (such as dog kidneys), thawing by conduction does not yield a sufficiently fast rate. To increase penetration of heat, two methods were explored, the use of 3 kilomegacycle microwave (radar) and the use of 27 megacycle shortwave diathermy. The use of radar proved infeasible as uneven penetration of heat resulted in severe overheating of the outer portions of the kidney, while the inner portions were still frozen. In preliminary studies, uniform penetration of heat through a dog kidney was found to be possible with shortwave diathermy(3). Therefore, it was considered worthwhile to study the effect of shortwave diathermy on the viability of frozen mammalian tissue culture cells since data obtained in quantitative tissue culture studies might provide technics for preservation of organs. This is a report of the tissue culture studies.

Materials and methods. Five cell lines were investigated including 3 stable lines, HeLa S.J., L-cell 929 and Hull monkey kidney plus 2 primary lines — monkey kidney and calf kidney. All cell lines were grown as monolayers on glass, using Eagle's medium (4) and Earle's balanced salt solution(5) with 10% added calf serum as the growth medium. The monolayers of the primary lines were grown from trypsinized cell suspensions from the respective kidneys. The cells were allowed to form a uniform sheet and were fed the day before freezing. The cells were removed with trypsin, suspended in growth medium at a concentration of approximately 1×10^6 cells per ml. Either 5% dimethysulfoxide (DMS) or 5% glycerol was included and the additives were compared for their protective effect.

The cell suspension was dispensed in ampules adapted for thawing by shortwave diathermy (Fig. 1). The ampule was broken at the neck. A 5 mm glass rod covered with platinum foil with a platinum lead was placed in the ampule. Cell suspension (0.8 ml) was dispensed into the ampule which was then stoppered with a cork, the lead extending beyond the ampule. The ampules were frozen in a Linde BF1 apparatus at a rate of 1-2°C/min to —50°C and stored in liquid nitrogen vapor at —170°C to —190°C.

For thawing by 27 megacycle diathermy,† the ampule was placed in an aluminum foil holder (Fig. 2). One electrode was connected to the platinum foil lead extending from the ampule and the other electrode was attached to the metal foil holder, producing a cylindrical arrangement of electrodes.

The diathermy plate current was held between 240 and 280 milliamperes during the

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† The diathermy apparatus used was a Master Fischertherm model No. 1200 from R. A. Fischer & Co., Glendale, Calif.

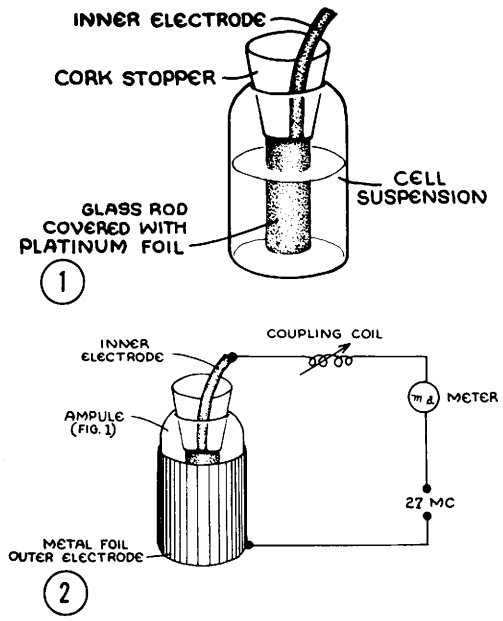

FIG. 1. Ampule adapted for thawing suspensions of cells by shortwave diathermy.

FIG. 2. Schematic diagram demonstrating thawing suspensions of cells in diathermy apparatus. ma = milliampere; Mc = megacycle.

thawing of the ampule. This corresponds to an input of about 200 watts. When the power input exceeded this for more than a fraction of a second, slight overheating or sparking resulted. At 27 megacycles the dipolar molecules rotate, energy is released in the form of heat, and thawing takes place. With this configuration, the contents of the ampule thawed in from 15 to 20 seconds. Control ampules thawed by agitation in a 37°C water bath were melted in 60 to 80 seconds. Following thawing, the cell suspensions were diluted with growth medium and checked for viability. The criteria for determining viability were: first, the dye exclusion test, using trypan blue, and second, the plating efficiency procedure.

In the trypan blue test, 0.1 ml of a 1% aqueous solution of the dye was added to 0.9 ml of the cell suspension. The stained and unstained cells were counted in a haemocytometer, the percent of unstained cells represents the percent viability by this test. In the test for plating efficiency, 50-100 single cells in 3 ml of growth medium were placed in a non-toxic plastic culture dish (65 × 15 mm) and incubated at 37°C under 5% CO_2 in air. The percentage of viable single cells as determined by the trypan blue test, which were capable of reproducing and forming colonies comprises the plating efficiency. The plating efficiency varies with the cell line and is characteristic for that line provided that the details of the specific test procedure are strictly reproduced.

Results. Table I shows the percent viability of frozen mammalian cells when thawed by agitation in a 37°C water bath or by dielectric heating. The cells were frozen at a rate of 1-2°C/min and 5% DMS was used as the protective additive. There is no significant difference in the results between the two methods of thawing. Table II shows results obtained when 5% glycerol was used as the protective additive. Again, no difference is noted between the two methods of thawing. However, this was true only when the thawing by shortwave diathermy was well controlled. Any slight overheating evidenced by sparking or milliampere input over 300 resulted in decreased viabilities. The viability of the HeLa cells following well-controlled heating averaged 88% by the trypan blue method, whereas viabilities of 33, 49, and 85% were obtained in overheated

TABLE I. Viability of Frozen Cells Thawed in 37°C Water Bath *vs* Shortwave Diathermy.*

Slow freeze: 1-2°C/min
Storage: −170 −190°C
Medium: Eagle's, Earle's 10% calf serum
Protective additive: Dimethylsulfoxide (5%)

Cell line	Freeze No.	Trypan blue test % viable 37°C W.B.	Trypan blue test % viable Dia-thermy	% plating efficiency 37°C W.B.	% plating efficiency Dia-thermy
HeLa	1	91†	88	41	30
Hull monkey kidney	1	81	74	21	14
	2	74	72	28	29
L cells	1	80	73	63	56
	2	76	77	45	51
Primary monkey kidney	1	72	67	39	34
Primary calf kidney	1	57	49	22	28

* Dielectric heating.
† No. represents avg of 3-8 ampules.

TABLE II. Viability of Frozen Cells Thawed in 37°C Water Bath *vs* Shortwave Diathermy.*

Slow freeze: 1-2°C/min
Storage: −170 −190°C
Medium: Eagle's, Earle's 10% calf serum
Protective additive: Glycerol 5%

Cell line	Freeze No.	Trypan blue test % viable 37°C W.B.	Trypan blue test % viable Dia-thermy	% plating efficiency 37°C W.B.	% plating efficiency Dia-thermy
HeLa	1	91†	86	40	37
Hull monkey kidney	1	78	80	16	11
	2	79	72	28	39
L cells	1	76	70	62	40
	2	76	74	45	55
Primary monkey kidney	1	57	59	25	21
Primary calf kidney	1	47	30	23	25

* Dielectric heating.
† No. represents avg of 3-8 ampules.

ampules. When plating efficiency was used to determine viability, the results averaged 48% with controlled heating vs 9, 18 and 4% with slight overheating. Extreme care must be taken, therefore, to avoid overheating when thawing.

In comparing the protective additives, it was evident that glycerol and dimethylsulfoxide were equally effective except in the primary monkey kidney freeze. In this instance, the use of dimethylsulfoxide resulted in better recoveries (Tables I, II). Viability by the trypan blue method when using DMS was 70%, with glycerol 58% and plating efficiency with DMS was 37% and with glycerol 23%.

Summary. 1. Thawing of frozen mammalian cells by well controlled shortwave diathermy is feasible. 2. Such thawing causes no apparent injury to the cells. 3. Shortwave diathermy offers no advantage over agitation in a 37°C water bath for thawing small masses of frozen tissues and cells. 4. The use of shortwave diathermy appears to be desirable for thawing large masses of tissue such as the dog or human kidney and the present studies indicate that this method can be safely used.

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Species Resistance to Histamine and Endotoxin: The Response of the Chicken.* (28940)

MARGARET M. JORDAN AND LERNER B. HINSHAW
Civil Aeromedical Research Institute, Environmental Physiology Branch, Federal Aviation Agency, and Department of Physiology, University of Oklahoma Medical School, Oklahoma City

Hemodynamic responses of the dog to a lethal injection of endotoxin have been well established(1). Recent studies in the monkey and observations in man have shown the pattern of endotoxin shock in the primate to differ from that in the dog(1-4). Other animals, such as the rabbit, also show unique responses to endotoxin (generalized Schwartzman reaction) not seen in most other species (5). The only consistent finding in all mammals studied (mice, rats, guinea pigs, rabbits, cats, dogs, monkeys, sheep, goats, horses, asses, and pigs) was that all succumbed to an injection of bacterial endotoxin(4,6). Conflicting evidence has been given regarding the resistance of the fowl to endotoxin and no report of hemodynamic changes has been found. Grasset *et al* have reported the mini-

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