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W. P. MARSHALL, PRESIDENT

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SYMBOLS

DL=Day Letter
NL=Night Letter
LT=Int'l Letter Telegram
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NA184 PD=UG CHICAGO ILL 7 1035A=

DR LEO SZILARD, CARE A N SPANEL=

=DLR INTL LATEX CORP 350 FIFTH AVE=

SIXTY FIVE PERCENT MOTILE SAMPLE FROZEN GLYCEROL CONCENTRATIONS ZERO 21/2 5 71/2 TEN ASSAY RESPECTIVELY ZERO TWO FOUR TEN ELEVEN PERCENT MOTILITY=

EARL WILSON=NTO SZILARD

21/2 5 71/2=

THE COMPANY WILL APPRECIATE SUGGESTIONS FROM ITS PATRONS CONCERNING ITS SERVICE

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MA167 =

1955 MAY 20 PM 3 22

M.VSG050 PD=UG CHICAGO ILL 20 311P=

DR LEO SZILARD=

DELIVER UNIVERSITY OF COLORADO MEDICAL SCHOOL=DVR=

ALIQUOTS OF 25% MOTILE SAMPLE EXTENDED EGG WHITE FROZEN BY IMMERSION MINUS ONE. SIXTY MINUS EIGHTY MINUS TWENTY THREE SHOW EIGHT PERCENT FIFTY ONE PERCENT TWENTY TWO PERCENT RECOVERY RESPECTIVELY. SPEED SAMPLES AND CONTROL TWENTY NINE TO THIRTY MICRONS PER SECOND=

WILSON=

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MC158

1933 MAY 28 PM 4 24

M VSG035 LONG DL PD=UG CHICAGO ILL 28 257P=

LEO SZILARD, CARE A N SPANEL=

INTERNATIONAL ATEX CORP=350 FIFTH AVE NYK=

SAMPLE FORTY SEVEN PERCENT MOTILE SPEED TWENTY FOUR MICRONS PER SECOND. ALIQUOTS COOLED SLOWLY TO FIVE DEGREES AND IMMERSED IN COOLING BATHS MINUS ONE SIXTY MINUS EIGHTY MINUS FIFTY MINUS TWENTY THREE SHOWED RECOVERIES SEVEN EIGHTEEN TWENTY TWO THIRTEEN PERCENT RESPECTIVELY WHEN THAWED AT TWENTY TWO DEGREES. ALIQUOTS AT TWENTY TWO DEGREES PLUNGED INTO SAME ORDER OF BATHS SHOWED SIX TWENTY THREE TWENTY FIVE FIFTEEN PERCENT RECOVERY. MOTILITIES THAWED SAMPLES TWENTY ONE TWENTY TWO MICRONS PER SECOND=

WILSON=

NTO SZILARD=

at 25°C
20% weight
1/5 of sample every 5 min
1/2 hour label
1/2 hour centrifugation

THE UNIVERSITY OF CHICAGO
CHICAGO 37 · ILLINOIS
INSTITUTE OF RADIOBIOLOGY AND BIOPHYSICS

Dear Dr. Szilard,

I have repeated my earlier experiments on the variation of post-thawing motility of human semen samples frozen at different rates. In all cases the surviving fraction of samples frozen by plunging ampoules into a -55° C bath is 3 to 9 times as great as when the samples are plunged into a -160° C bath. This pattern is shown when samples are extended in phosphate-bicarbonate buffer alone, 25% egg white, bovine amniotic fluid, 90% egg white. The ampoules have stood in the baths for 10, 20, 30 minutes with the same pattern of post-thawing motilities.

The procedure when the usual 25% egg white is used is as follows:

The semen sample is extended with four times its volume of an extender made by homogenizing fresh egg white in a phosphate-bicarbonate buffer, PH^{+} = ~7.4-7.6.

The extended sample is then slowly diluted with a soln. of 20% glycerol in a buffer, PH^{+}=7.3-7.5. The glycerol buffer is added stepwise over a period of ½hour until the volume is doubled. Then the sample is dispensed into 16mm ID test tubes plugged with small Wasserman tubes to provide a sample thickness of 1.5-2.5 mm. These are sealed and after a total equilibration time of ½ hour after the last glycerol addition are frozen by immersion in the proper bath. After a period of 10-30 minutes the ampoules are rapidly transferred to a storage bath of CO_2-ETOH slush (-80° C).

With this procedure the following results are typical:

Immersion Bath	%Recovery	Original Motility
-160	8	25%
-80	51	"
-55	--	--
-23	22	25%
-160	2	60%
-80	13	"
-55	16	"
-23	5	"
-160	3	55%
-80	20	"
-55	27	"
-23	7	"

change in speed may be 0 10/0 lower but

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II. Substitution of lactate and citrate ions for chloride.

Extenders were made substituting lactate and citrate ions for chloride ion in the buffer used routinely. The solutions were made equi-ionic, assuming complete ionization. Freezing points were -.5°C- -.6°C. The same ratio of bicarbonate to phosphate was used, so that the PH of the lactate and citrate solutions were ~0.1 unit higher than the usual 7.3-7.4

Extender	Immersion bath	%recovery	Orig. motil. (same sample)
25% egg white in usual phos.-bicar. buffer	-55°C	25%	50%
25% egg white in lactate buffer	-55°C	21%	50%
25% egg white in citrate buffer	-55°C	15%	50%

III. The trial of an almost non-ionic extender (egg white-egg albumin in sucrose)

In substituting sucrose for an ionic extender the PH would be 8.5-8.7 upon addition of egg-white. So I have "Titrated" 15% egg white in 11.2% sucrose soln. with egg albumin to adjust the PH to 7.4 and used this soln. both as an extender and glycerol carrier.

Using egg albumin to adjust the acidity of 90% egg white, an extender of the following composition has been made:

166 ml egg white
23 gm egg albumin
20 ml phosphate-bicarbonate buffer } = high protein extender

Extender	Glycerol carrier	%recovery	immersion bath	orig. motil. (same sample)
25% egg white in usual buff.	usual phosph. bicarb buff.	23%	-55°C	30%
15% egg wht. in 11% sucrose; acidity adj. with egg albumin	25% egg white in 11% sucrose	19%	-55°C	30%
High protein extender	High protein extender	27%	-55°C	30%

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IV. Comparison of the 25% egg white extender versus 2% egg albumin in beef amniotic fluid.

Extender	Glycerol carrier	immers. bath	% recov.	orig. motil.
25% egg wht. in bicarb-phosph. buffer	usual buffer	-55°C	25%	45%
2% egg album. in bovine amniotic fluid	same as extender	-55°C	28%	45%

V. I have had difficulty in getting good photographs with the new microscope, the Zeiss-Winkel. The image was not in the plane of the film over part of the area. We now have a sleeve around the microscope tube which makes a very tight fit for the Zeiss attachment. I hope to get some good pictures tonight and have the whole set-up shipped tomorrow or the next day.

VI. My next experiment will be to get an optimum concentration of glycerol for the freezing rate I'm using now. I have done one experiment using a poor sample (20% motility) and it look s like I can get some survival with no glycerol. However the absolute motilities were too low (lesss than 5%) even with the usual 10% equilibrium concentration.

Sincerely,

Earl A. Wilson Jr.

Earl A wilson, jr.

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Memo to Dr. Szilard:

Earlier experiments have indicated that there exists an optimum rate of cooling for maximum post-thawing motility. Recoveries up to ~50 % have been obtained by plunging Wasserman test-tubes (11 mm. I.D.), containing 1 ml of semen extended with 25% egg white in tyrodes buffer into dry-ice alcohol cooling baths (-79°C). These high recoveries were obtained with samples of fairly low initial motility 25-30 %.

In order to provide for a more uniform cooling, the extended semen in the following experiment was placed in 15 mm I.D. test tubes, and the Wasserman tubes were used as plugs. This provided an annular ring of ~~2~~ 2.5 mm ~~thickness~~, and thus the great bulk of the cells were exposed to the same cooling rate.

Recovery of Motility after Freezing and Thawing at Various Rates

A good semen sample, 47% motile speed 24μ/sec was extended with 25% egg white in tyrodes buffer P_H t = 7.6 1 part of semen to 4 parts of extender.

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This was slowly diluted (1/5 vol. extended sample / 6 min.) with 20% glycerol in the same buffer. An equilibration period of 30 min. was allowed.

During this period the extended semen was dispensed into the plugged ampoules, and sealed at 22°C. Ten ampoules were cooled after the 30 min. equilibration period to 5°C at rate of ~1°C/min. Two or more of the ampoules at 22°C were plunged into each of the following cooling baths:

−160°C −	liquid - solid isopentane
− 79°C −	CO_2 - Et OH
− 50°C −	Eutectic Et OH - H_2O
− 23°C −	liquid-solid CCl_4

The ampoules remained in the baths for 5-10 minutes, 5 min in the −160°C bath and the −80°C bath and 10 min. in the warmer ones. After this period all ampoules were transferred to the −80°C storage bath, where they remained for 24 hours before assay.

The ten ampoules chilled to 5°C at rate of 1°/min were treated in the same way. One half of each series was thawed at 22°C, the other at 37°C, the ampoules being removed from the 37° thawing bath just before the last ice crystals disappeared.

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The assays were as follows:

Original sample: 47% motility - speed, 24 μ/second - in 12.5% eggwhite, 10% glycerol

24 hours at −80°C
Post thawing Motilities expressed as % of Original

Frozen from 5°C.			Frozen from 22°C		
Freezing bath temp.	Thawing temp. 22°C	37°C	Freezing bath temp	Thawing temp. 22°C	37°C
−160°C	7%	8%	−160°C	6%	7%
−80°C	18%	22%	−80°C	23%	17%
−50°C	22%	23%	−50°C	25%	23%
−23°C	13%	13%	−23°C	15%	10%

Speed of samples thawed at 22°C = 21-22 μ/sec.
" " " " at 37°C = not measured

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Experiments to be done in next few days

1. Determine % recoveries at one ~~of~~ 2 rates of freezing (−80°C bath and −50°C bath) for a sample of low original motility < 30% and a sample of > 40% motility.

2. Determine effect of substitution of Cl^- ion by citrate or lactate.

3. Measure percent of recovery as function of time after thawing.

4. Try an extender with a very high concentration of colloidal protein, say egg-albumin.

5. Try an extender with small concentration of gelatine to promote smaller ice crystals during freezing.

Earl Wilson