E-13

Bull Sperm

Fall 1952

Consideration of emulsion as medium in which to freeze sperm.

Conversation with Dr. Charles Fuchs (Emulsol).
1. General unpredictability of emulsions.

Request 1. Emulsion readily produced
low in water (5-10%)
Stable down to -20°
non-toxic

Consider Mayonnaise formula -
low water - water - mineral oil -
egg yolk —

Crude emulsion - lecithin ~ 5%
water ~ 10%
mineral oil ~ 85%
emulsion settled out over-night
to about 20% of total volume —
microscopically - variable drops.
that seemed to stabilize on
freezing (liq N2).

Coacervate — consider water poor layer as a medium for freezing sperm — or make coacervate of sperm as one of the constituents. eg sperm gelatine gum acacia.

Freezing in liq N2 of non-aq phase gives small-needle-like xstals — ~ 5-10 μ long & same size as human sperm — probably somewhat smaller than bull-sperm.

Preliminary experiments on mammalian sperm

approx. 2½ cc mammalian sperm obtained from young donor in fair health. about ½ were non-motile ½ hr after sample take. Considerable debris.

Exp 1. a smear of .01 cc of seminal fluid on a coverslip was placed over a H.O slide sealed with silicone grease. This was plunged into liq N_2. then thawed rapidly at 40-45°C. about 10% sperm motile. Seems like increased debris.

Exp. 2 Repeat # 1 with small amt glycerol. a very small fraction motile, sluggish and motility decays in few minutes to ~ zero

3. Coacervate made from eq. vol 2% gelatin and gum arabic mixed with 10% sperm quick frozen, thawed rm. temp. No motility don't mix.

4 Remains of sample were frozen down in liq N_2 placed in small dewar 24 hrs later the N_2 was evap.; all sample

were non-motile. Crystalline deposits (long needles were present).

Nov 3, 1952.

Viscosity & crystallization of water-glycerol mixtures

67% glycerol liquid — 19°C but begins to get viscous. likewise −23.5°C

Water crystallizes out of 10% glycerol soln at −5°C but remains soft (liquid phase down to −8 to 10°C

An 80% glycerol in H_2O is very viscous at −24°C & almost pourable at −14°C

Micrographs of Motile Sperm

Exposure # 1 = overexposure
spd : 1/5
objective : 44 X
lamp volt : 75 volts

mv

v

2
spd : 1/125
75 volts
obj 44 X

several sperm moving at once

Our 2, 3, 4 used only 1 shutter not exposed

3 Double exposure :
spd 125 - 5 sec apart
100 volt : lamp
44 X

4 double exposure

spd 125 (2 1/2 sec apart)
100 volts
44 X

5 ~~1/5 44 X 2 seconds apart~~

Start over: over expose one

#5

exposure: $\frac{1}{2}$ second
obj: 44 times
lamp volts 90

m

#6

exp $\frac{1}{125}$
obj: 44 X
vol 100 volts

#7 exp $\frac{1}{125}$

obj 44 times
volt = 100 volts

m (burned 2 frames)

#8 exp $\frac{1}{125}$ + $\frac{1}{125}$ $\frac{1}{2}$ see tube

obj 44 X
volts

[Results: Only marker exposure came out; ie, $\frac{1}{2}$" exp.]

Roll #1

Sat. Morning

No motile sperm in supernante
Sample was shaken; motility observed

Exposures:
#10
time: ½ sec., overexposure to ocular marks
lamp: 100 volts
objective: 44X
remarks: 1 or 2 sluggish sperm in field

#11
time: 1/125 single exp.
lamp: 110 volts
obj: 44X
remarks: one sperm moving in middle of fg.

#12 Double Exposure ½ sec each
time 1/25 sec.
lamp 110 volts
obj 44X
remarks: "great deal of swirling sperm

#13.

single
~~double~~
Time : $\frac{1}{50}$ sec
obj : 44 times
lamp : 95 volts
remarks : oscillatory motion only

#14 time $\frac{1}{50}$ sec double exposure $\frac{1}{2}$ sec apt
obj : 44 X
lamp : 95 volts
remarks : oscillatory motion

#15 Time $\frac{1}{50}$ sec + $\frac{1}{50}$ sec ($\frac{1}{2}$ sec apt)
obj = 44 times
lamp : 95 volts
remarks : one sperm moving in st. line

#16 time $\frac{1}{25}$ sec + $\frac{1}{25}$ sec ($\frac{1}{2}$ sec apt)
obj : 44 times
lamp : 95 volts
remarks : many sperm in left ½ field

Roll #2.

Microphotos: Yeast cells in H_2O drop
Kodak XX film

Exposure #1[1,2]
speed: 1/50 sec
objective 10X
Remarks: not bright in center, condenser up
resolution + contrast musically good

Exposure #2[2]
speed: 1/50 sec
objective 10X
Remarks: a very little

Exposure #3[3]
speed ~~1/50~~ sec
objective 10X
Remarks: bright

Exposure #4[4]
speed 1/25
objective: 10X
Remarks bright

Exposure # 5 [5]

objective : 10 X
lamp 110 volts
time : 1/25 sec
very bright

Exposure # 6 [6]
1/25 second
objective : 10 X
lamp 95 volts
Remarks condenser all down

Exposure # 7 [7]
1/25 second
objective : 10 time
lamp 95 volts
Remarks : condenser up

Exposure # 8 [8]
objective 20 X
1/25 second
lamp 95
Remarks : condenser at. for visual definition

Exposure # 9 [9]
objective 20X
speed 1/25 sec
condenser up slt. from best visual definition

Exposure # 10 [10]
objective 20X
speed 1/25 sec
condenser up sl. from best visual definition

Exposure # 11 [11]
objective 20X
speed 1/50 sec
condenser slt. for best visual

Exposure # 12 [12]
20X
~~Cond.~~ up to very bright all way
1/50 sec.

Exp # 13 [13]
20X
Condenser up all way
1/25 sec.

Exp #14
10X
time 1/25
condenser up

#15
10X
1/10 sec condenser up

#16
10X
1/5 sec
condenser up to give good visual focus

#17
10X
1/2 sec
condenser to good visual def

#18
10 time
condenser up stop down for best vis def
1/10 sec

#19

10 Tenies

1/125 sec.

condenser up too far

#20

10 Tenies

1/50 sec ~~Too~~

and up too far for mind

#21

10 tenies

Film didn't touch

in

cells somewhat out of focus; Babies.

Bodal plus X
Roll #3

Exposure #1
objective 10 times
1/125 sec
Remarks condenser high for visual definition

#2
10 X
1/50 sec
condenser at for visual definition

#3
10 X
1/50 sec
condenser high

#4
10 times
1/25 sec
condenser high

#5
10 X
1/25 sec
condenser at. for visual

#6

10X

$\frac{1}{10}$ sec

condenser at visual

#7

10 X

$\frac{1}{10}$ sec

condenser high

#8

10 X

$\frac{1}{5}$ sec

condenser high

#9

10 X

$\frac{1}{2}$ sec

condenser at visually

#10

20 X

$\frac{1}{2}$ sec

condenser at visually

#11

20 X

$\frac{1}{2}$ sec

condenser high

#12

20 X
$\frac{1}{5}$ sec
condenser high

#13

20 X
$\frac{1}{5}$ sec
condenser set for visual def.

#14

~~20 X~~
~~$\frac{1}{10}$ sec~~
~~condenser set for visual def.~~

#15

20 X
$\frac{1}{10}$ sec
cond. too high

#15

20 X
$\frac{1}{10}$ sec
condenser set vis.

16

$\frac{1}{25}$ sec

20 X

condenser ~~very~~ ~~dry~~ high

17

$\frac{1}{25}$ sec

20 X

condenser ~~very~~ dry

18

$\frac{1}{50}$ sec

20 X

cond. high (very)

19

$\frac{1}{125}$ sec

20 X

condenser high

20

$\frac{1}{125}$ sec

20 X

condenser high

21

20 X
$\frac{1}{50}$ sec
condenser high

WW
Iodub ~~XX~~ put in
will work exclusively at 10 X objective
approx $\frac{1}{25}$ or $\frac{1}{50}$
5×10^{6} / cc.
Double exposures
Roll # 4

$\left(\frac{4}{3}\pi\right)\left(4 \times 10^{-4}\right)^{3}(1.25)$ = wt. one yeast cell.

=

$(5 \times 10^{6})\left(\frac{4}{3}\pi\right)(320)(10^{-6})\ 1.25$

$\frac{20}{\pi}\pi\ 10^{-4}$: 6 mg rw ~ .6 mg

250 10^{-12} 2×10^{-10} $1\ 10^{11}$

8
8
10
4
11
7
9

volume $= \left(\frac{1}{200}\right)\left(\frac{1}{200}\right)\left(\frac{1}{100}\right) = \frac{1}{4 \times 10^6}$ cc

$125 \times 10^{-6} = 2.5 \times 10^{-7}$ cc

there are ≈ 8 in 2.5×10^{-7} cc

In 1 cc $\frac{8}{2.5\ 10^{-7}} = 3 \times 10^7$

$$1.5 \overline{)2.5} \quad 1.6$$

$$\underline{15}$$

10

1.6 cm

$25 \quad 25x = 1.6$

$\frac{1.6}{25}$

$25 \overline{)160} \quad .06$

150

$\approx 3 \times 10^7$

Nov 11, 1952

Yeast cells in buffer (hanging drop

film: Kodak XX
objective: 40 times
light: 95 volts
Roll # 5

Exposure # 1 ___ marker

time = $\frac{1}{2}$ sec

remarks: visual definition good

Exp. # 2 #2, 5

time: $\frac{1}{125}$ sec
remarks: lines of grid sharp, clear

Exp. # 3
time: $\frac{1}{125}$ second + $\frac{1}{125}$ = $\frac{1}{62.5}$ sec

Exp # 4

time $\frac{1}{50}$ second + $\frac{1}{50}$ sec = $\frac{1}{25}$ second

Exposure # 5 ✓

time: $\frac{1}{50}$ second
lenses clear, sharp.

Exposure # 6 ✓

time $\frac{1}{25}$ second

Exp. # 7 triple
$\frac{1}{125} + \frac{1}{125} + \frac{1}{125}$ sec
changed field

Exp # 8

time $\frac{1}{50} + \frac{1}{50}$

Exposure # 9 : $\frac{1}{2}$ sec marker

Exposure # 10
$\frac{1}{50}$ second
focussed up slightly from # 9. couldn't tell visually which was better

11
$\frac{1}{50}$ second
down slightly (halo barely disappearing

Exp. # 12

$\frac{1}{50}$ second.

camera at focus – 3.0

Exp # 13

$\frac{1}{50}$ sec

camera at – 3.5

Exp # 14

camera focus at – 2.0

Exp # 15

$\frac{1}{50}$ sec ... condenser down, let sl. desire

Exp #16 Condenser up sl.

$\frac{1}{50}$ second

Exp # 17 Cond. up sl.

$\frac{1}{50}$ sec + $\frac{1}{50}$ second

Exp # 18 marker $\frac{1}{2}$ second

Exp # 19 $\frac{1}{125}$ second

Exp # 20

$\frac{1}{125} + \frac{1}{125}$

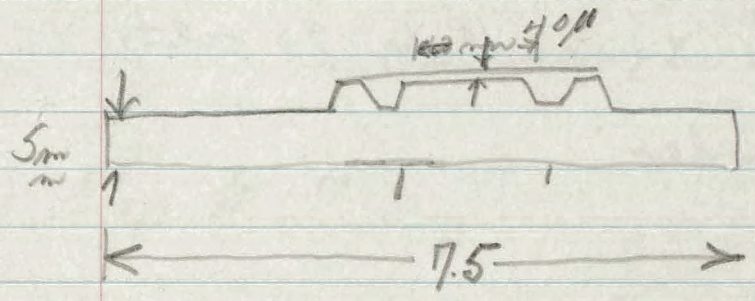
counting chamber:

$\frac{20}{50 / 1000}$

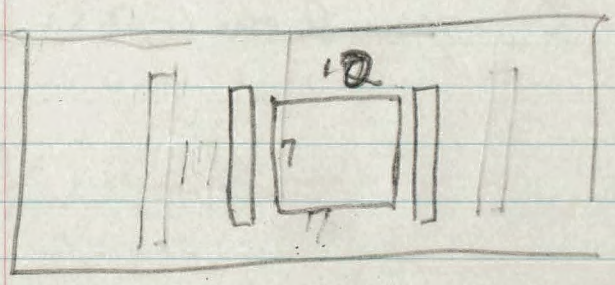
20 . PH

100 . H.C

40 . MAKE



cover slip 25X20



Locke's fluid : Human sperm

H_2O : 1000 cc
$CaCl_2$: 0.24 gm .335 / $CaCl_2 \cdot 2H_2O$
KCl : 0.42 gm
$NaHCO_3$: 0.10 gm
$NaCl$: 9 gm

$CaCl_2 = 110.99$
$2H_2O = 147.03$
$6H_2O = 219.09$
$\frac{147}{111}$ .24

Roll # m 6
Human Sperm Micro photographs Nov 10, 1952
x 3 cc sperm from donor diluted in Locke soln. Small fraction motile.

Exposure # 1 10 X, 98 volts

$\frac{1}{5}$ second Marker :

Exposure # 2
$\frac{1}{125} + \frac{1}{125}$ $\frac{1}{2}$ second later

Exposure # 3
$\frac{1}{125} + \frac{1}{125}$ $\frac{2}{3}$ second later

Exposure # 4

$\frac{1}{120}$ + $\frac{1}{125}$ second

Exposure # 5 $\frac{1}{3}$ second entered

$\frac{1}{50}$ + $\frac{1}{50}$

Expr. #6

$\frac{1}{50}$ + $\frac{1}{50}$

— new sample —

Expr # 7

$\frac{1}{125}$ + $\frac{1}{125}$ $\frac{1}{3}$ sec apart

Exposure # 8

$\frac{1}{50}$ + $\frac{1}{50}$

Exposure # 9

$\frac{1}{125} + \frac{1}{125}$

Exposure #10

$\frac{1}{125} + \frac{1}{125}$ 1 second apart.

Exp. # 11

$\frac{1}{125} + \frac{1}{125} + \frac{1}{125}$ fast as possible

Exp #12

$\frac{1}{125} + \frac{1}{125}$ 2 second apart

Exp # 13

$\frac{1}{125} \frac{1}{125}$ fast as possible

shutter is jammed

14 & 15 $\frac{1}{5}$ second marker

#16

$\frac{1}{125} + \frac{1}{125}$ $\frac{1}{2}$ second

jammed

#17

$\frac{1}{125} + \frac{1}{125}$ jammed.

18 manually operated

$\frac{1}{125} + \frac{1}{125}$

jammed

19

$\frac{1}{125} + \frac{1}{125}$

Nov. 18, 1952 — M. XX
Roll #7 — 10X objective

Exposure #1
$\frac{1}{5}$ second marker

#2
$\frac{1}{125} + \frac{1}{125}$ — $\frac{1}{5}$ - no good

#3.
$\frac{1}{125} + \frac{1}{125}$ — $\frac{1}{5}$ second

#4
$\frac{1}{50} + \frac{1}{50}$ — $\frac{1}{5}$ sec

#5
$\frac{1}{125} + \frac{1}{125}$ — $\frac{1}{5}$ second

#6 $\frac{1}{125} + \frac{1}{125}$ $\frac{1}{5}$ second exp

Remarks Condenser almost up to limit −1.0
Eyepiece + objective focused sharply
and diaphragm open wide

#7 $\frac{1}{50} + \frac{1}{50}$

same as #6

#8 $\frac{1}{125} + \frac{1}{125}$ +−2.3

#9 $\frac{1}{125} + \frac{1}{125}$ Condenser all up

New sample formed

#10 $\frac{1}{125} + \frac{1}{125}$ $\frac{1}{5}$ second

#11 $\frac{1}{50} + \frac{1}{50}$ $\frac{1}{5}$ second apart

new field

#12 $\frac{1}{50} + \frac{1}{50}$

#13 $\frac{1}{125} + \frac{1}{125}$

#14 $\frac{1}{125} + \frac{1}{125}$ − 3.2

#15 $\frac{2}{125} + \frac{1}{125}$ − 2.5

#16 $\frac{1}{125} + \frac{1}{125}$ − 2.6

#17 $\frac{1}{125} + \frac{1}{125}$ − 1.5

#18 $\frac{1}{125} + \frac{1}{125}$ − 1.0

19

$\frac{1}{50} + \frac{1}{50}$ second

20

$\frac{1}{50} + \frac{1}{50}$ second

Nov. 20, 1952 Roll # 8

Single + double exp. 35 Kodak - XX, 16mm (10X objective); 2nd $\frac{1}{125}$ second, room temp sample. 95 volts, Sr Blooms source

Donor 19 yrs old, excellent health

Exposure 1-6 incl. Drosophila $\frac{1}{25}$, alt. $\frac{1}{10}$, $\frac{1}{25}$, etc

7.

Time $\frac{1}{25}$, single, sharply focussed

8 $\frac{1}{125} + \frac{1}{125}$ $\frac{1}{5}$ second

9 $\frac{1}{125}$ single

10 double

#11 changed field
single 1/125

#12 double ✓

#13 single

#14 double

#15 single changefield

#16 double

changed field
#17 single

#18 double

#19 Hanging drop of original (candid) scene
single

20 double Hang. drop orig.

Points good; several moving could be seen

Dec. 3, 1952 Roll #9

Donor: Howe

objective 6X with or without mask

ocular: 10X

Triple exposure $\frac{1}{5}$ second apt each $\frac{1}{125}$

Exposure #1

upper lens condenser off; mask obj. on

#2 repeat after refocusing carefully

#3 mask off objective

#4 " " " refocused = 50 or from

#5 repeat (shutter cable release pull free)

✓# 6 top lens of con. [illegible] on object. free of mask
Cond. almost up fully — too faint no detail

7 duplicate

✓ # 8 ~~condenser down to fill field of eyepiece~~ $\frac{3}{4}$

✓✓ 9 Repeat except widened source stop, ~~raised~~ somewhat condenser

✓✓ # 10 masked objective; same as 9

11 masked objective
Lowered condenser to cover $\frac{3}{4}$ eyepiece field

12 40 μ counting chamber
5X objective, masked — condenser same as # 11

13 40 μ counting chr.
5 x objective, masked

14 same without mask

#15 40w chamber unmasked

~~5~~ 8X obj.
condenser down to cover all field of eyepiece

#16 same as 15 but masked

#17 Condenser lowered to cover $\frac{3}{4}$ field of eyepiece

Donor: H.; Sample 2 hrs old; 1:20 dilution in Lake

1. Visually: depth of focus < 100μ with 5X obj and naked aperture.

2. Camera jarred by shuttering mechanism.

3. Prints were blurred; poor definition

Dec. 4, 1952

To do!

(a) Fix spring on shuttering mechanism to soften thrust

(b) Fix snaps attachment to camera

(c) " rigid camera mount

Dec 5, 1952

Effect AgI on freezing of 10% glycerol soln

Several 1cc samples 10% glycerol dispensed into small test tubes. Half of them

Dec. 6, 1952

Roof #10

close to scope.
8 v vol Burton lamp
6 X ob marked

1 Frame #2 $\frac{1}{100}$ sec.

3 $\frac{1}{100} + \frac{1}{100}$

4 $\frac{1}{100} + \frac{1}{100} + \frac{1}{100}$ "

5 $\frac{1}{50}$

6 $\frac{1}{50} + \frac{1}{50}$

7 $\frac{1}{50} + \frac{1}{50} + \frac{1}{50}$

8 $\frac{1}{25}$

9 $\frac{1}{25} + \frac{1}{25}$

10 $\frac{1}{25} + \frac{1}{25} + \frac{1}{25}$

Lyiland

Microscope 12/8/52

ocular mag $M = \frac{250}{foc}$

Obj $M = \frac{\Delta}{f_{obj}}$ ~~$\frac{250}{\Delta} = M$~~

Δ = "tube length"

Total focal length = $\frac{f_1 f_2}{f_1 + f_2} = \frac{f_1 f_2}{\Delta}$

c = dis

c 16 mm obj

$$f = \frac{16 \times 25}{160} = 2.5 \text{ mm.}$$

$$M \text{ (microscope)} = \frac{250}{foc} \times \frac{\Delta}{f_{obj}}$$

$$= \frac{250}{2.5} = 100$$

Total magn

Experiment to compare methods of shuttering
Roll #11

Mechanical solenoid shutter:
1. between source + mike 3 times
2 between mike + camera 3 also at 1/5 sec

Method 2: Source at 140 volts, no filter

Exposure #1

objective 10 times no mesh

3 X 1/100 sec 10 X

2 3 X 1/50 sec 10 X

3 3X 1/25 sec.
print enlarger all up; f 8. 8 min. } duplicate x x
✓

4 3 X 1/25 sec

B = 6.3 7.T
6 = 12 6 12.6 T etc
1.2 25

#5 Photo shutter source ↔ mike

3 X $\frac{1}{50}$ 10 X object in chamber sperm now

#6 3 X $\frac{1}{25}$ 10 X object

#7 1 X $\frac{1}{2}$ time exp (photo shutter) filter .3 = 6.3 T

#8 1 X $\frac{1}{2}$ time exp photo shutter +6.6 12.6

6 X objective in source → 90 volts

#9 3 X ~~$\frac{1}{200}$~~ ($3\ \frac{1}{25}$) at 90

Replaced sperm suspension new field

#10 3 X $\frac{1}{50}$ ← printed

~~#11~~ #11 at 110 volts 3 X $\frac{1}{50}$ 25% T filter

110

~~#12~~ 4

#12 Photo shutter 10 X object in

~~3 X $\frac{1}{50}$~~ 3 X $\frac{1}{25}$

~~3 X $\frac{1}{50}$~~

3 X $\frac{1}{50}$

#13

Time exposures

#13 $\frac{1}{2}$ second filter .6 10X 110 volts

#14 $\frac{1}{5}$ second filter 1.2 10X

New Spec in

#15

[Spec shutter jaws cause so that Hemoglobin line are blurred]

$\frac{1}{5} \quad x = \frac{3}{50}$

Parent # 2, 10

↑ photo

Frame #	obj	eyp
1	10 X	3 X $\frac{1}{25}$
2	10 X	3 X $\frac{1}{25}$
7	6 X	3 X $\frac{1}{25}$
8	6 X	3 X $\frac{1}{50}$
9	6 X	3X $\frac{1}{50}$ (25% filter
10		
11		

50% transmission $\frac{1}{50}$ 3X

Bill Jones arrived 7 am Dec 16, 1952

Dec 11, 1952

Roll # 2 Bull semen in egg yolk - citrate was diluted 1:4 in Tocko's Saline. Appearance of material: Terrible specimen are efficiently camoflaged. Can barely trace progression.

Frame # 2

objective = 10 X Shuttering mechanism: Photo.
exposure: 3/50 second
chamber: Hemacytometer
light: 115 volts,
filter none

Comments: Undiluted, is orig 1:20 in egg yold - citrate

Dead Human Sperm in all chambers

Frame # 4 = (No good)

objective = 10 times condenser: up
exposure = 1/50 second when aperature = open
chamber = Hemacyt.
light = 115 volts
shutter = photo

\# 2 OK, # 3 little too dark   6 OK to dark
4 " "little" too light 7 OK too dark
\# 5 OK   # 8 OK too light
~~3~~ 2 - 4 same as $\frac{3}{50}$, # 2 OK   #9 OK too dark
~~2~~ 3 - " " # 2 but $\frac{3}{5}$ # 10 OK
1 little too dark   # 11 too lt.
\# 12 too lt
\# ~~5~~ 4   (little too dark)   # 13 too dark

✓ obj : 10X
epp : $\frac{3}{25}$
chbr : Henneyt.
shutter = photo
light : 115 volts

✓ # ~~6~~ 5   (OK)   Ibsa
obj = 10 x
epp = $\frac{3}{50}$
chamber = Henneyt
shutter : Ibso
light = 115 volts,

✓ # ~~7~~ 6 obj = 10 X   OK slt. too dark
epp = $\frac{3}{25}$
chamber : Henneyt.
shutter : Ibso
lt = 115 volts

\# ~~8~~ 7 repeat # 6 (OK too dark)

8

objective : 10 times (OK slt too lt)
shuttering : Disc
exposure : 3/50
Chamber : 40 μ slit
Light : 115 volts, 25% T (.6)

#9 - ½ record marker ✓ (too dark

#10
format #8 - 50% T filter : #0.3
OK

#11 #8 with 0.2 F T : 6.3%
too lt.

#12
object 10 X too lt.
Shuttering : Disc
exp : 3/50
Chamber : 40 μ
Light : 115 volts 25% T #9

✓ # ~~14~~ 13 ~~Same as 13~~ too dark
obj 10X
eyp $\frac{3}{25}$
shutter Ibso — too dark
chamber 40 μ
Light 115 volts, 50% T

✓ # ~~15.~~ -1
14 Obj. 6X — OK little dark
eyp: $\frac{3}{25}$
chmbr Idemagt.
shutter: Ibso
Lt. 115 volts, 25% T 0.6

✓ # ~~16~~ 15
obj. = 6 X (too dark)
eyp = $\frac{3}{25}$
chmbr = Idemagt.
shutter = Ibso
Lt : 115 volts, 50% T, .3

~~17~~ $\frac{1}{2}$" timed exposure
16 obj : 10X — OK
eyp : $\frac{1}{2}$ second
chmbr : 2bso
Lt : 115 volts, # 1.2 F = 6.3% T

#18 17

obj = 10 X OK

e/p : 1/2 second

shutter : 2 lens

Chamber : Kenney Co.

Set : 115 volts, 0.9 F = 12.5% T

#19 18

obj 10 X

e/p : 1/5 second OK

shutter : 2 lens

chamber : Kenney Co.

Set : 115 volts. .6 F = 25% T

Human sperm! Donor A, 4 pm. Dec. 11, 1952

Roll #13

~~Semen dilute~~ Undiluted ~~[illegible]~~

Do live sperm 6X, 10X obj in Hemcyt. all photoshutter

Frame #1 + 2 print 1

objective: 10 times
exposure: $\frac{3}{50}$ second
light: 115 volts, no filter

Frame # 3-4

objective: 10 X Print 3
exposure: $\frac{3}{25}$
light: 115 volts, filter 50%, 0.3

Frame 5

objective 6X Print 5
exposure $\frac{3}{50}$
light 30% filter

Frame 6

objective 6X
exposure: 3/50, 50% F, 0.3

7

obj: 6X
exp: 3/25
light filter 25%, 0.6

8

exp ½ second
objective: 6X
filter: 0.9 12.5% T

9 exp ½ second
objective: 6X
filter 1.2 6.3% T

print tonic or flat

#10

exp. $\frac{1}{5}$ second
object 6x
25% T

#11

exp $\frac{1}{5}$ second
objective 10x
25% T

#2

obj 10x
exp $\frac{3}{50}$ second
no field

#13 same

Dec 12, 52 Note Condenser down & Bill took lens of obj

Roll #14 K. Panchromatic XX

Human Sperm: Donor A collected 3:15 pm Dec 12, 52. Kept at room temp all time measurements were made

diluted 1-2 in Tashes

light source at 115 volts, focussed as Roll 12, 13

Frame # 1

objective = 6 X

exposure = 3/5 sec

chamber = hemacy

light = 115 volts .0.9

Remarks:

Prints:

paper: A5-

exposure: F=

? enlargement = ~15

filter

slt dark

Print

not enough

Frame # 2 repeat.

objective =

exposure =

chamber =

light =

Remarks =

Prints paper: A5

exposure =

F =

slt dark

Frame # 3

objective =
exposure = 3/25
chamber =
light =
Remarks =

Print =
Paper = A5
exposure =
enlargement =
F =

Frame # 4

objective = 6X
exposure = 3/8 1/5 second
chamber =
light = 12.5%
Remarks =

Print =
paper =
exposure =
enlargement =
F =

Frame # 5

objective =
exposure = 3/2 1/5 second
chamber =
light = 6.3%
remarks

Print
paper =
exposure =
enlargement =
F =

Drone # 6

objective = 6X
exposure = 1/5 sec
chamber =
light = 12.5 %
Remarks =

Print
exposure =
F =
enlargement =

Drone # 7

objective = 6X
exposure = 1/5 sec.
chamber =
light = 6.3 %
Remarks

Print =
Exposure = 8.5
F = 8
enlargement = 15
Ok - print) Enlarger
sort to focus; too dark

Drone # 8

objective = 6X
exp = 1/2 second
chamber = penny
lt = 6.3 %

too dark

Frame #9 duplicate

#10

6X
3/50
heavy tonsils
.3 filter

too dark

#11

10X obj
3/50
heavy tonsils
no filter

X 15
eyp 8.5'
5 = 8
print OK focus sharp

#12 repeat #11

10 X obj
3/50
heavy tonsils
no filter

OK.

#13

10 x obj
3/50
heavy T.
50%. T.

OK.

#14. Repeat 13

#15 $\frac{1}{5}$ record
25% Trans.
10 X.

#16 $\frac{1}{5}$ record
12½ % trans
10 X

#17 $\frac{1}{5}$ record
10 X
12½ T.

Print 4pp enlrg. 15 X
6

#8 ½ record
12½ T
10 X

#19 ½ record
60% T
10 X

try to record

40 record
F-8
8 lines

#20 repeat #19

#21

10X

3/50

no filter

aerotometer chamber

1'48"

F-8

Leitz shutter

Print

December 13, 52

Roll # 15 K. Panchromactic XX

4:30 pm Dec 13

Human Sperm : Donor A Collected: 3:15 pm Dec 12, 52

room temperature throughout measurements.

Diluted No

1 : over exposed

Frame #2 ~~lt.~~ dark

objective: 10 X, masked

exposure: 3/50

Chamber: P.H. 20c

light : 115 volts, no filter

remarks: photo shutter, condenser = 2/0 " mirror

cen. disp = 17.5

Prints

paper:

exposure:

enlargement:

Frame # 3 same as # 2 (dark)

objective: 10 X masked

exposure: 3/50

chamber: PH

light: 115 volts

remarks: photo shutter condenser 3/8, no filter

C.D = 7.5

Prints:

paper:

exposure:

enlargement:

X 15

Frame # 4 (dark) print prints 3½', F8

objective 10 X paper:

exposure 3/25- exposure:

chamber P/+ enlargement:

light 115 V, 50 % T

remarks photo shutter, condenser down to 5/8 from previous

CD - 7.5

Frame # 5 Sl. light prints:

objective paper:

exposure 3/25 exposure:

chamber enlargement:

light , 50 % T

remarks

Frame # 6 checked all parameters prints: , too dark

objective 10 X paper:

exposure 3/25- exposure:

chamber P/+ enlargement:

light 115 V, no filter

remarks CD - 7.5-

60 | 160 2
120
40

Frame #7 slt. dark Prints:

objective: 10X masked
exposure: 3/50 r
chamber: PH
light: 115 volts no filter
remarks: C.D. = 7.5 ; C = 3/8"

paper:
exposure:
enlargement:

X-5 2'40"
6-8
too light

Frame # 8 density OK; too Different Prints:

objective: 6X masked
exposure: 3/50
chamber: PH
light: 115 volts blue F. blue
remarks: 4 = CD ; C = 3/8"

paper:
exposure:
enlargement:

Frame # 8 density OK, poor Prints:
dep.

objective: 6X
exposure: 3/25
chamber: PH
light: 115 volts blue Filter
remarks: 4 = CD ; C = 3/8" above mirror

paper:
exposure:
enlargement:

print

density too dark

Frame #19 marker : $\frac{1}{5}$" Prints:

Objektive:
exposure: $\frac{1}{2}$"
chamber:
light: 115 volts blue filter
remarks: CO : 4 ; C = $\frac{3}{0}$"

paper:
exposure:
enlargement:

Frame # 10 not exposed Prints.

objective: 6 X marked
exposure: $\frac{3}{50}$
chamber: P 14
light: 115 volts no filter
remarks: CO : 4 ; C = $\frac{3}{0}$"

paper:
exposure:
enlargement:

density of ~~100 ft~~ poor definition

Frame # 11 Done on 10, Prints:

objective:
exposure:
chamber:
light:
remarks: ok at dark

paper:
exposure:
enlargement:

two lt.

Frame # 12

Objective: 6X marked
exposure: 3/5
chamber: PH
light: 115 volts no filter
remarks: same as 11, 12

Prints:
paper:
exposure:
enlargement:
too dark

X15 - f-8.

Frame # 13

objective: 6X marked
exposure: 3/5
chamber: PH
light: (50% T)
remarks: same as 11, 12, 13

Prints: opp
paper: A-5
exposure: 31 12"
enlargement: X15
OK

Frame # 14 very dark

objective: 10X marked
exposure: 3/100
chamber: PH
light: 115 volt
remarks: Iris shutter
CD = 7.5 C = 3/0"

Prints:
paper:
exposure:
enlargement:

lt light

32 | 60 | 19 2 | 180

45 - 240

Frame # 15 very dark

objective: 10 x masked

exposure: 3 sec

chamber: P/+

light: 115 volts w/ filter

remarks: CD : 7.5 ; C = 3/5

also slt. lt

Prints:

paper: A-5,

exposure: 4" ; f-8

enlargement: X 15

Print

Frame # 16 very dark

objective:

exposure: 3/25 too dark

chamber:

light:

remarks:

also

Prints:

paper:

exposure:

enlargement:

slt dark

slt dark

Frame # 17 zone VI 6

objective:

exposure: 3/50 too dark

chamber:

light:

remarks: CD : 12.5

also

Prints:

paper:

exposure:

enlargement:

60 25 6 4 16
240 60
16

slt dark

Frame # 18

objective: 10 X
exposure: 3/25"
chamber: Hemacytometer
light: 115 volts no filter
remarks: CD = 7.5
C = 5/0"

Prints:

paper: A5
exposure: 4"/16", F-8
enlargement: x15
hard to focus
enlarger

slt dark repeat

Frame # 19

objective: 10 X
exposure: 3/25
chamber: Hemacyt.
light: 115 volts no filter
remarks: CD

Prints:

paper:
exposure:
enlargement:

Frame # 20

objective:
exposure:
chamber:
light:
remarks:

Prints:

paper:
exposure:
enlargement:

F-18 - #15 but with $\frac{3}{50}$" green filter

F-19 #18 but $\frac{3}{25}$" with green filter

F-20 repeat #19

Roll # 16 K Panchromatic XX

Human Sperm: Donor H
collected: 5:30 pm Wednesday Kept at room temp.
Ejaculate small

Frame #13
objective: 10X, masked
exposure: 3/50"
chamber: P.H. 20μ
light: 115 volts, no filter
shutter: Photo
condenser = 3/8" from mirror CD = 7.5

F-14 Repeat: #13

F-15 Frame # 2:
objective: 10X, masked
exposure: 3/50"
chamber: P.H. 20μ
light: 115 volts, 50%
shutter: Photo
condenser = 3/8" from mirror CD = 7.5

F-16 repeat-15 Ringed objectives, CD
Frame # 3
objective: 6X objective, masked

F-17 Repeat 15:- 9/50" filter
chamber: P.H.
light: 115 volts, 50% F
shutter: photo
condenser: CD:

Frame # 4
objective : 6 X
exposure : 3/50"
chamber : PH
light : 115 volts, 25 %
shutter :
condenser : CD 4.

Frame # 5
objective : 6 X
exposure : 3/50"
chamber : P.H.
light : 115 volts 25 %
shutter :
condenser CD 4.

Frame # 6
objective : 6 times
exposure : 1/5"
chamber : PH
light : 115 volts 12.0 %
shutter : Photo
condenser : 7.5 CD = 4

Frame # 7
objective : 6 X
exposure : 1/5"
chamber : PH
light : 115 volts 6.3%
shutter :
condenser : CD 4

changed objective CD 4 → 7.5

Frame # 8
objective = 10 X
exposure : 1/5"
light = PH
shutter = 25%, 25% T
condenser = CD

Frame # 9
objective = 10 X
exposure : 1/5"
chamber = PH
light = 115 volts 12%
shutter =
condenser = CD 7.5

Frame # 10
objective : 18X area exposure marker
exposure : 1/2"
chamber :
light : 115 volts no filter
shutter : photo
condenser : CD 7.5

Frame # 11 Frame # 1
objective : 10X
exposure : 1/2"
chamber : PH
light : 115 volts 12 %
shutter :
condenser : CD 7.5

Frame # 12
objective : 10X
exposure : 1/2"
chamber : PH
light : 115 volts 6 % T
shutter :
condenser : CD 7.5

changed shutters photo → Ilbso

Frame # 13

objective : 10X
exposure : 3/50
chamber : PH
light : 115 volts, no filter
shutter : Ilbso shutter photo open on time
condenser :

Frame # 14

objective : 10X
exposure : 3/50
chamber : PH
light : 115 volts, 50% T
shutter : Ilbso shutter
condenser :

changed chambers, shutter back to photo

Frame # 15

objective : 10X
exposure : 3/50
chamber : 33/11 (steel)
light : 115 volts no filter
shutter : photo
condenser : 3/0 11, CO = 7.5

Frame #16
objective = 10X
exposure = 3/5"
chamber = steel 33μ
light = 115 volts, 50% T
shutter = photo
condenser = CD: 7.5

Frame #17
objective = 6X
exposure = 3/5"
chamber = steel 33μ
light = 115 volts, 50% T
shutter = photo
condenser = CD = 4.5

Frame #18
objective = 6X
exposure = 3/5"
chamber = steel 33μ
light = 115 volts, 25% T
shutter = photo
condenser = CD = 4.5

Trans #19

objective :

exposure :

chamber :

light :

shutter :

condenser :

Trans #28

objective :

exposure :

chamber :

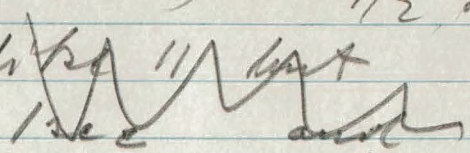
light :

shutter :

condenser :

4

No 1 ~~[illegible]~~ (~ 11) 1/2 sec, 12.5% Tr

No 2 
repeat

No 3 ~ 11 unit 6.3% Tr. and 1 sec

No 4 repeat

No 5 like (11)

green filter, 1/2 sec, 25% Tr

No 6 repeat like 11

No 7 green filter 1/2 sec 50% Tr

No 8 repeat

No 9 green filter 1 sec. 12.5% Tr

No 10 repeat

No 11 green filter 1 sec 25% Tr

No 12 repeat

F # 7
objective 10 x
exposure : 1/2 second
light 50% + green filter

F # 8 repeat 7

F # 9
objective 10 x
exposure : 1 second
light : 12.5% T + green filter

10 repeat # 9

11 objective 10 x
exposure : 1 sec
light : green filter + 25% T

12 repeat # 11

Frame # 1

objective : 10X
exposure : $\frac{1}{2}$"
chamber : P.H
light : 115 volts 12% T
shutter : photo
condenser : $\frac{3}{8}$" ; CD = 7.5

Frame # 2 repeat # 1

Frame # 3
objective : 10 X
exposure : 1 sec
chamber : PH
light : 115 volts 6.3% T
shutter hand , photo

Frame # 4 repeat # 3

Frame # 5 # 6 repeat 5
objective 10X
exposure : $\frac{1}{2}$ sec.
chamber : PH
light : 25% T , green filter

Roll # 17 K. Pan. XX
objective 10 X no diaphragm
Condenser disp = 4
condenser = $\frac{3}{8}$" above mirror
P.H. Number

Frame # 1	$\frac{3}{50}$"	no filter	100 %	good Printed

Frame # 2 Repeat # 1

Frame # 3 $\frac{3}{50}$" 50 % filter

Frame 4 Repeat # 3

Frame 5 $\frac{3}{50}$" 25 % T

" 6 repeat

Frame 7. $\frac{3}{50}$" 6.3 %

8 repeat.

Frame # 9 $\frac{3}{25}$" no filter

10 repeat

frame # 10 x objective

Replaced top lens of condenser

C.D = 22.5

Heat mirror rotated to give dark field

~~Frame # 11~~

12/18/52

Dr. Blum suggested using phase contrast stop for ×44 obj with 10× obj. gives dark field not phase contrast
C.D. all open
Roll # 18 -

Frame # 1
objective = 10X phase contrast stop for 44X
light : record type
exp. = 1/50

2 repeat

3 3X 1/50

4 repeat

5. 1/25"
6 repeat

7 3X 1/25
8 repeat
9 repeat # 7
10 1/2 record.

Print

5 good

#11 repeat
#12 1 "

#13 repeat

#14 ~~3 × 1/25~~ spoiled }

#15 3 × 1/25 2 rounds, total

#16 ~~3 repeat~~

#17 ~~3 × 1/10 fast~~ } rerun

#18 3 × 1/10 fast }

#19 ~~software~~ empty }

12/19/52

Roel #19

#1 3 6/25 —— fairly rapidly

#2. unprocessed

#3 — 3 X 1/25 — worked open

#4 — 3 X 1/25

#5 — 3 X 1/25

#6 — 3 or 4 X 1/25

#7 — 3 X 1/25

#8 — 3 X 1/25

#9 1/2 "

#10 1/2 "

#11 1/2 "

#12 1/2 "

#13 1/2"

#14 1/2 "

#15 1/2"

#16 3 × 1/10 "

#17 3 × 1/10 " (3 moving apertures)

#18 3 × 1/10 "

#19 3 × 1/25 "

#20 3 × 1/25 "

#21 blank

New Format

#22 1 " apt

#23 1 " apt

#24 1/2 " apt

#25 1/2 " apt

#26 3 × 1/25 "

#27 3 × 1/25

#28 3 × 1/25

#29 3 × 1/25

#30 3 × 1/25

#31 3 × 1/25

32 3 × 1/10

33 3 × 1/10

34 3 × 1/10

35 3 × 1/10

Sunday Dec 28, 1952

Roll #20

Donor: W. age 25 4:05 pm

human sperm

microscope set up same as #19; dark field, reversed light, condenser — CO open.

dilute 1:20 Locke at 4:35

Frame #1

objective 10X

exposure: 3/25] 1.5 sec.

light : reversed B+L. Eggs unexposed

chamber. : P.H.

remarks :

Frame #2

3/25 " tm 1.5

#3 3/25 " unexposed

in 1.5

#4 start (point)

3/25 in 1.5 sec.

#5 3/25 in 1.5 sec

#6 $\frac{3}{25}$ in 1.5 sec.

#7 $\frac{3}{25}$ in 1.5

#8 $\frac{3\times 1}{5}$ sec in <u>12</u> sec Print

#9 $3\times\frac{1}{5}$ sec " "
#10 $3\times\frac{1}{5}$ sec. " " [~~Print~~]

#11 $3\times\frac{1}{2}$ in ______
#12 $3\times\frac{1}{2}$" in ______ [Print]
#13 $3\times\frac{1}{2}$" in ______

#14 3 x 1 sec
15 3 x 1 sec ~~waited~~ long interval for all sound to cease
16 3 x 1 sec didn't wait

#17 3 sec ~~[illegible]~~ [print]
#18 1 sec.
#19 1 sec. [Print]
#20 1 sec [~~Print~~]
~~#2~~

#21 $\frac{1}{2}$ sec x 1 ~~Print~~
22 $\frac{1}{2}$ sec x 1 "
23 $\frac{1}{2}$ sec x 1 ~~Print~~

24 3 x 1 sec $\frac{1}{2}$ sec between each
25 3 x 1 sec $\frac{1}{2}$ sec between Print
26 3 x 1 sec $\frac{1}{2}$ sec "

27 1 X 1/100 sec.

28 1 x 1/50 sec

29. 3 x 1/10 " ~~(print)~~]

30 3 x 1/10 (Print)

31 3 x 1/10

32 same as 31 except total time greater

33 3 x 1/10 " (Print) " " "

~~34~~

34 1 sec X 1 (Print)

35 1/2 sec X 1 (Print)

Roll #21

frame #1 3 × ½ sec ½ sec interval
2- 3 × ½ sec " " "
3- 3 × ½ sec " " "
4 3 × ½ sec " " "
5 3 × ½ sec
6. 3 × ½ sec " " "
7 3 × ½ sec
8 3 × ½ sec " " " } good timing print #8
9 3 × ½ sec " " "

10 3 × ⅕ sec 2 ½ sec apart
11 3 × ⅕ sec ——— 2 sec total time
12 3 × ⅕ sec 2 sec total time
13 3 × ⅕ sec 2 sec. total

14 3 × ⅕ 1 sec apart
15 3 × ⅕ 2 " (print)
16 3 × ⅕ 2 " (print)

17 1 sec exposure "B" (print)
18 1 sec exp "B"
19 1 sec exp "
~~20~~
20 ½ sec exp. "B"
21 ½ sec exp " (print)
22 ½ sec exp "
23 ½ sec exp "
24 ½ sec. exp "

Continued

✓+

25 1X 2 sec. exp "B"
26 1X " " "
27. 1X " " "
~~28 1X 2 sec exp "B"~~

28 - 3 X 1/25 (2 sec total)
29 3 X 1/25 (1.7 total)
30 3 X 1/25 (1.5 sec)

31 3X 1/25 (1.5 sec total)
32 3X 1/25 (1 3/4 sec total)

33 1X 3 sec (B)
34 1X 3 sec "
35 1X 3 sec "
36 1X 1 sec "

✓✓✓✓✓✓

Madison -4-

old diluent: peptone
broiled ~~whole~~ milk a good or ~~yolk~~ for diluent

American ~~Foundation~~ Medical Study Genetics
Dr. Willett Box 5, mail Madison, Wis.
Office = ~~2267~~ 4-0748
Home = 9-2635

Mr. Bud Conrad, Mgr Wis dairy Breeders Assn
4-1756

Iprocedure Eng.
2nd 10% of 2 · C

~~Repeat AgI Eqs~~ I

Cover glass Hemocytometer 20 X 26 mm
.4 – .6 mm thick

Cenco No # 40261 .5 mm $5.00
Fisher Scientific Co: # 6-158 $.60 ea

None in Fisher .4 – .6 mm thick + circular

Warm stage $42 - $48 (+ thick)

Thermometer $10
Cenco Low temp - 200°C → 30°C in 1° div.
340 mm 7 mm diam, # 19365

19370 -100 → +50° in 10 div toluol filled $5.65

B. & L. Neutral density filters

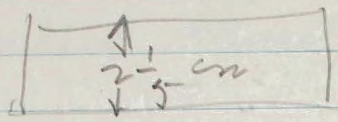
% Transmission	filter #
6.3 %	1.2
12.5 %	0.9
25 %	0.6
50 %	0.3

Enlargers high 1

HA-7.5580 -,

Q-B.4 995 } .5
B-3 150 }

2.15 cm



← 7.2 cm →

2 cm ht

[ ]

$$\begin{array}{r} 6.6 \\ 60 \overline{)400} \\ 360 \end{array}$$

Chicago Elect. + Surg. Co. (Heated Stage)

3 X 1 sec ½ sec int.

1 X 1 sec.

2 wheels
2 micro switches

filtered egg yolk
filtered boiled milk

B 30 oz

Buy P.H chamber, 1 or 2 pieces

Study samples photograph

Decay 11/1 human sperm

Hemoglobin

approx	objective 6X	10 line
3/50	50 % T	no filter
3/25	25 %	50 %
1/5	12.5 %	25 %
1/2	6.3 %	12.5 %

{ C.D. all open / condenser all up } lamp 115 volts

With condenser down; opp are too much cut by factor 2, or 4

$\frac{1}{2} X = \frac{3}{50}$

$\therefore$

$6X$

$\frac{1}{2}x = \frac{3}{50}$

Time opp $\frac{1}{5}$, $\frac{1}{2}$ with cover

$\frac{1}{5}x = \frac{3}{50}$

$\frac{3}{50}$ filter factored

with on opp, $10 \times \frac{1}{5}x = \frac{3}{5}$

$\frac{1}{2}(x) = \frac{3}{50}$

$\frac{3}{50}$ / $x =$

$\frac{1}{} \quad 6 \quad \frac{1}{5} = \frac{3}{50}$