Jan 5

Micrococcus cryophilus

NA · 3 hr old – from old broth culture

1 Sch fixation Gierson 10, 12 hydrol underhydrolized
2 Sch fix Gierson 15, 18 hydrol slightly underhydrol.
3 Sch fix Gierson 18, 20 hydrol ok, nuclei
4 OsO_4, ~~Sch~~ 3 min, Sch fix Gierson 18, 20 hydrol little different than without OsO_4

Jan 6. M. cryophilus

old broth culture on NSA 3 hr incubation
Sch fix - Gierson new acid

5 10, 20 min hydrol 60° 10 little over, 20 over
7 0, 3 " " all look alike
8 5, 10 " "

[redacted] seems to give better slides

old broth culture on D-O 3 hr incubation
Sch fix - Gierson new acid

6 10, 20 min hydrol 60° 10 little over, 20 over
9 0, 3 " " all look alike
10 5, 10 " "

Jan 7 nothing on slide

M cryophilus

11 on NSA - little growth
hydrol 0, 5 min at 63°

12 on NSA little growth
hydrol 10, 20 min at 63°

13 on Yeast little growth
hydrol 0, 5 min 63°

14 on Yeast - little growth
hydrol 10, 20 min 63°

Jan 8

M. cryophilus
NSA - gives better slides

on NSA little growth - at 25° for 15 hrs
11 hydrol 0, 5 min at 64°
12 hydrol 10, 20 min at 64°
plates then at 30° for 1 hr
15 10, 12
16 14, 16
17 4, 6
18 8, 10

on Yeast little growth - at 25° for 15 hrs
13 hydrol 0, 5 min at 64°
14 hydrol 10, 20 min at 64°
plate then at 30° for

Jan 12 E coli on NSA

uncentrifuged cells incubated

19	0 hrs	hydrol	0,3 min	
20	0 hrs	"	5,10 min	ng
23	½ hr	"	0,3	ng v duty
24	½ hr	"	5,10	
27	1 hr	"	0,3	
28	1 hr	"	5,10	
31	1½ hr	"	0,3	
32	1½ hr	"	5,10	
35	2	"	0,3	
36	2	"	5,10	
~~37~~39	2½	"	0,3	
~~37~~40	2½	"	5,10	

centrifuged cells incubated:

21	0 hrs	hydrol	0,3 min	} stain n.g.
22	0 hrs	"	5,10 min	
25	½ hr	"	0,3	Stain
26	½ hr	"	5,10	n.g.
~~29~~	1 hr	"	0,3	
30	1 hr	"	5,10	
33	1½	"	0,3	
34	1½	"	5,10	
37	2	"	0,3	
38	2	"	5,10	

(1/10) cells mixed 12^{30} to 2^{10}. Centrifuging took 10 mins. (to concentrate)

1:1:10 4:1

Plate on NSA. Inc. Stain.

(Abopon OK again!

19 0 S! cells very dark metach. N

20 2 G, (N).

5 G, N. Gray background.

10 Nuclei well stained but fuzzy fixation?

H344.

Hyd. 0

21-23 21. (Cross.) — S, N-negative no bridges noted

Hyd 10

N, but moderately light. No bridges cells rather small.

out

as above.

22. cells scattered. two types evident. No bridges. Almost homogeneous stain.

Somewhat sharper but would not be adequate for bridges.

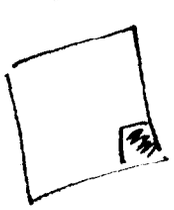
out

23 cells sparse dense red globules in many (P1) cells (nuclei?) but background also deep blue.

better dispersion of cells.

Nuclei clear, cytoplasm is not. No bridges visible

Saul



Need better counterstain (or longer in Eremia?)

0-hyd. from NSA is useless exc. for S.

2/3/54

H344
19 hydrolysed 0 and 2 minutes | clusters of v. large and intermediate cells.
20 hydrolysed 5 and 10 minutes

~~2/5/54~~
~~from NSA~~
~~H244~~

(P1 x P2) young cells. Plate + fix at intervals

2/4/54

21 both spread on plate and allowed to dry
hydrolysed 0, 10 minutes
22 incubated 30 minutes, 0,10 minutes
hydrolysis
23 incubated 45 minutes; 0;10 minutes
hydrolysis – stain OK. fix?

2/5/54 – no cells – too light

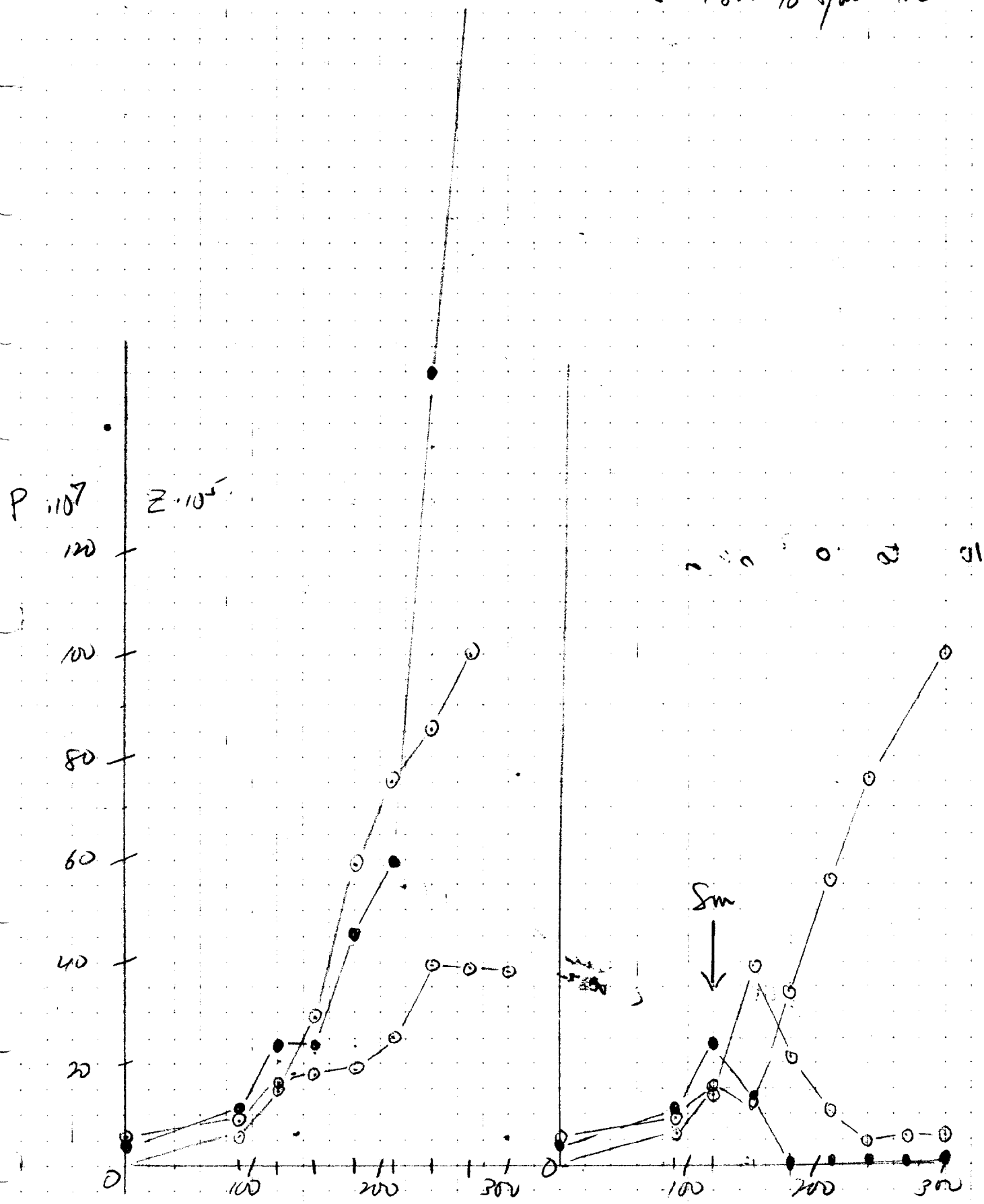
X 24 H244 – 0,10 minutes hydrolysis – from NSA
25 H245 – 0,10 minutes hydrolysis – from NSA

from D (lac) to NSA at 12 noon, fixed 2:30 PM

Si Nmet. light will disp. large cells

Insuff. hydrolysis ?

W1895 & W1956 PEN aerated < A / B + Sm 10 γ/ml 120' #97

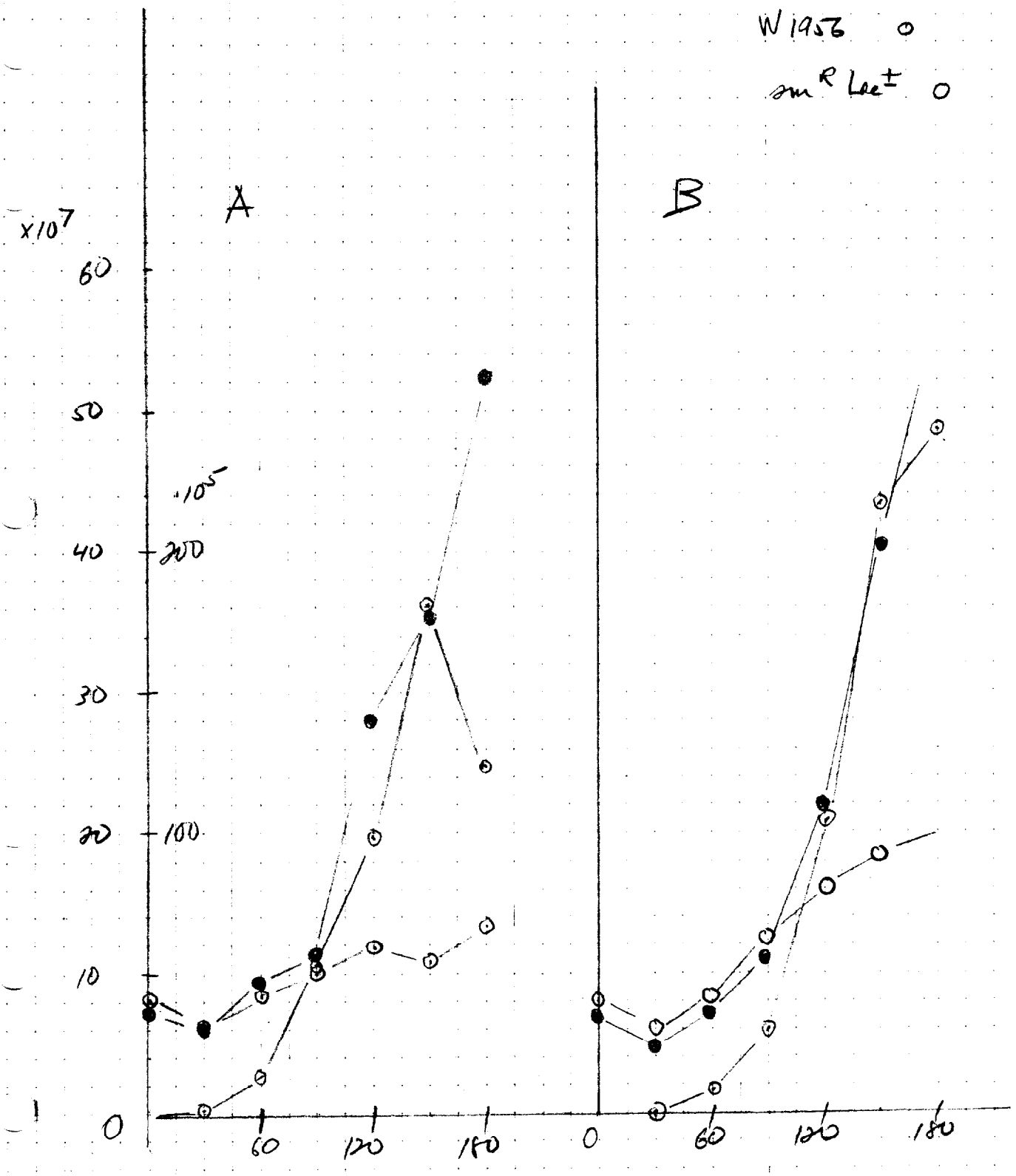


#74

W1895 × W1956

1.0 ml each → 10 ml < A PEN 10 ml } aerate
(12 hr stand PEN culture) < B PEN + DM 10 ml } 37°

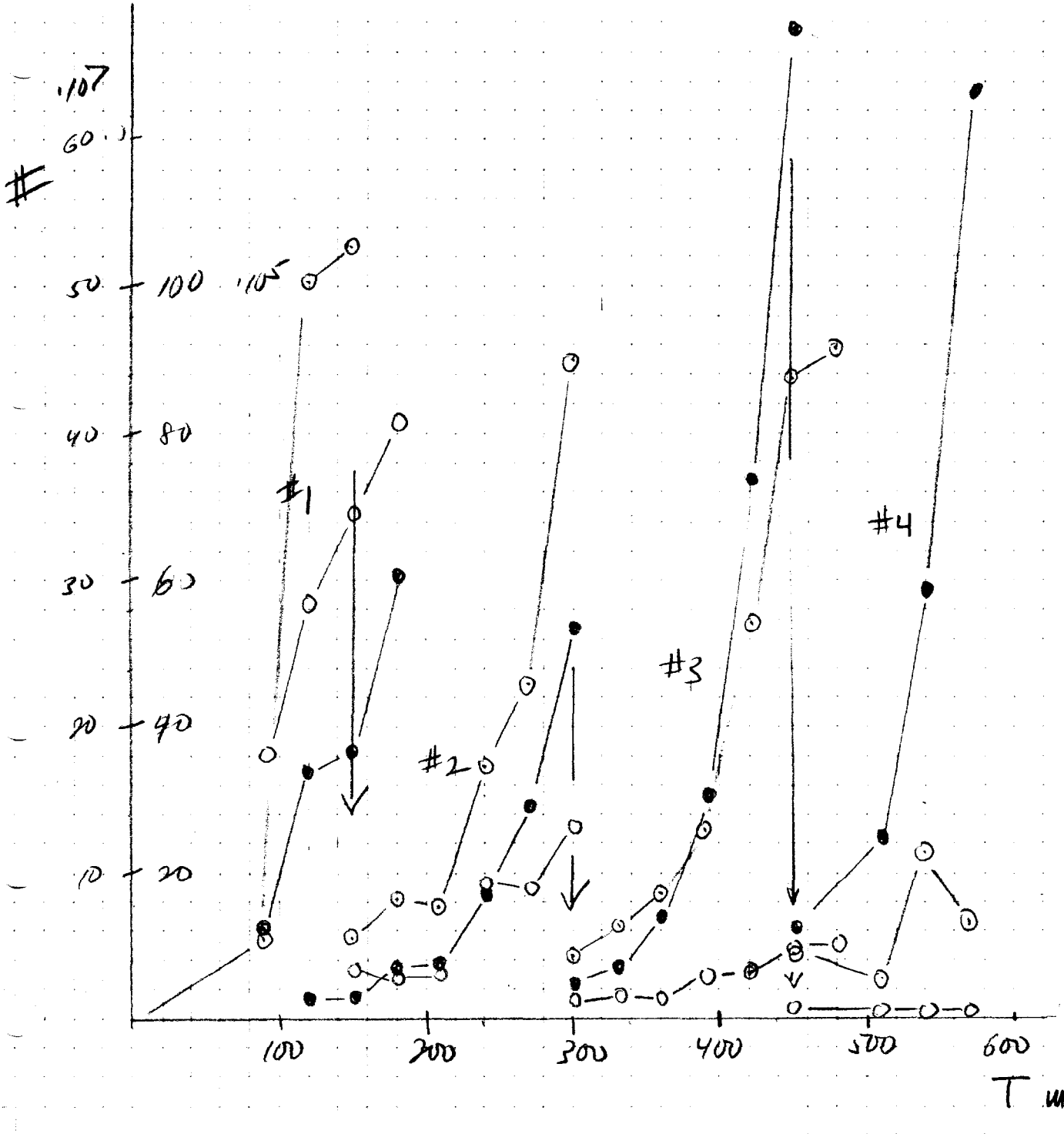
W1895 •
W1956 ○
sm^R Lac± ○



#79

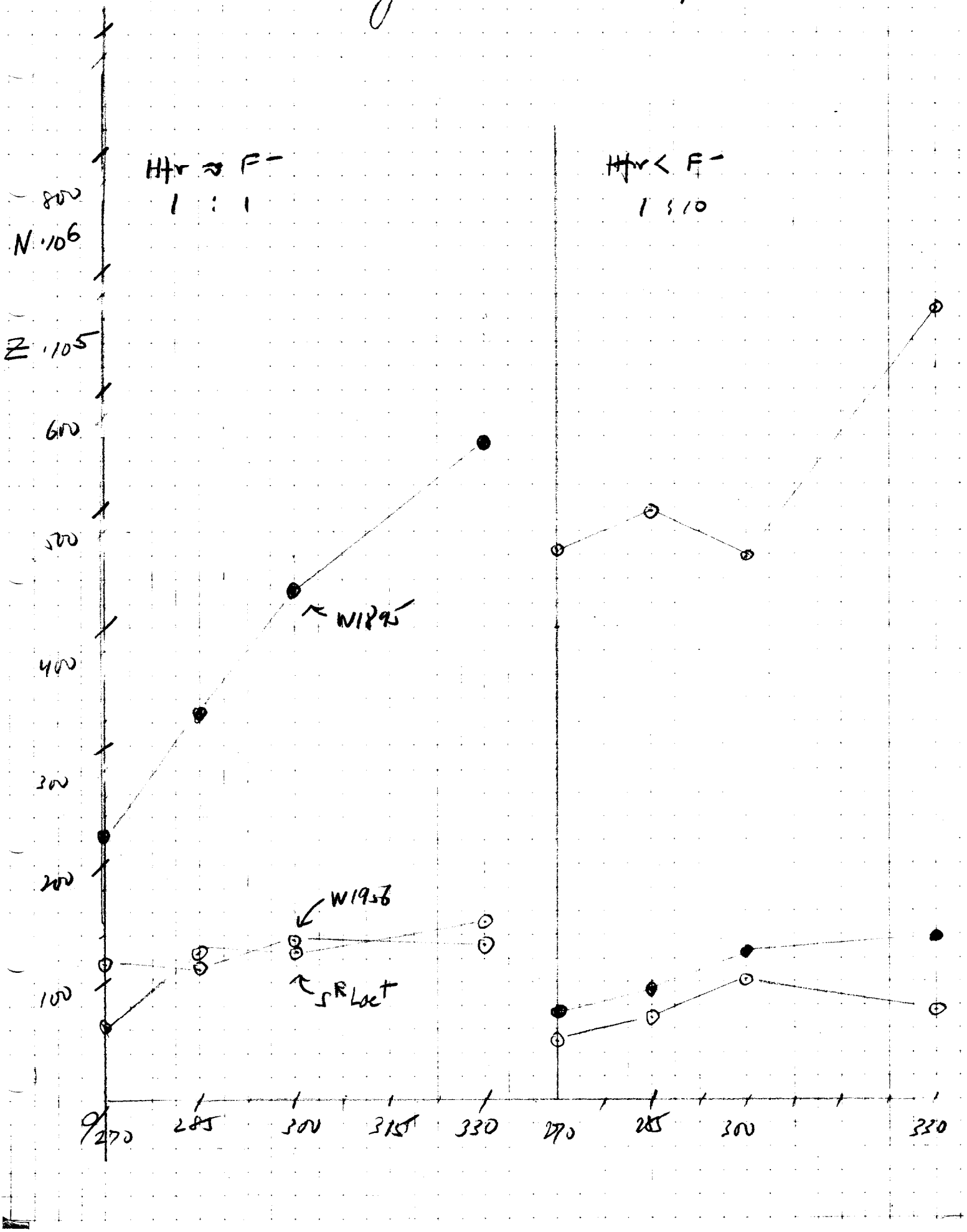
W1895 & W1956 turbidostat PEN+DM + Th 0.2 g/ml

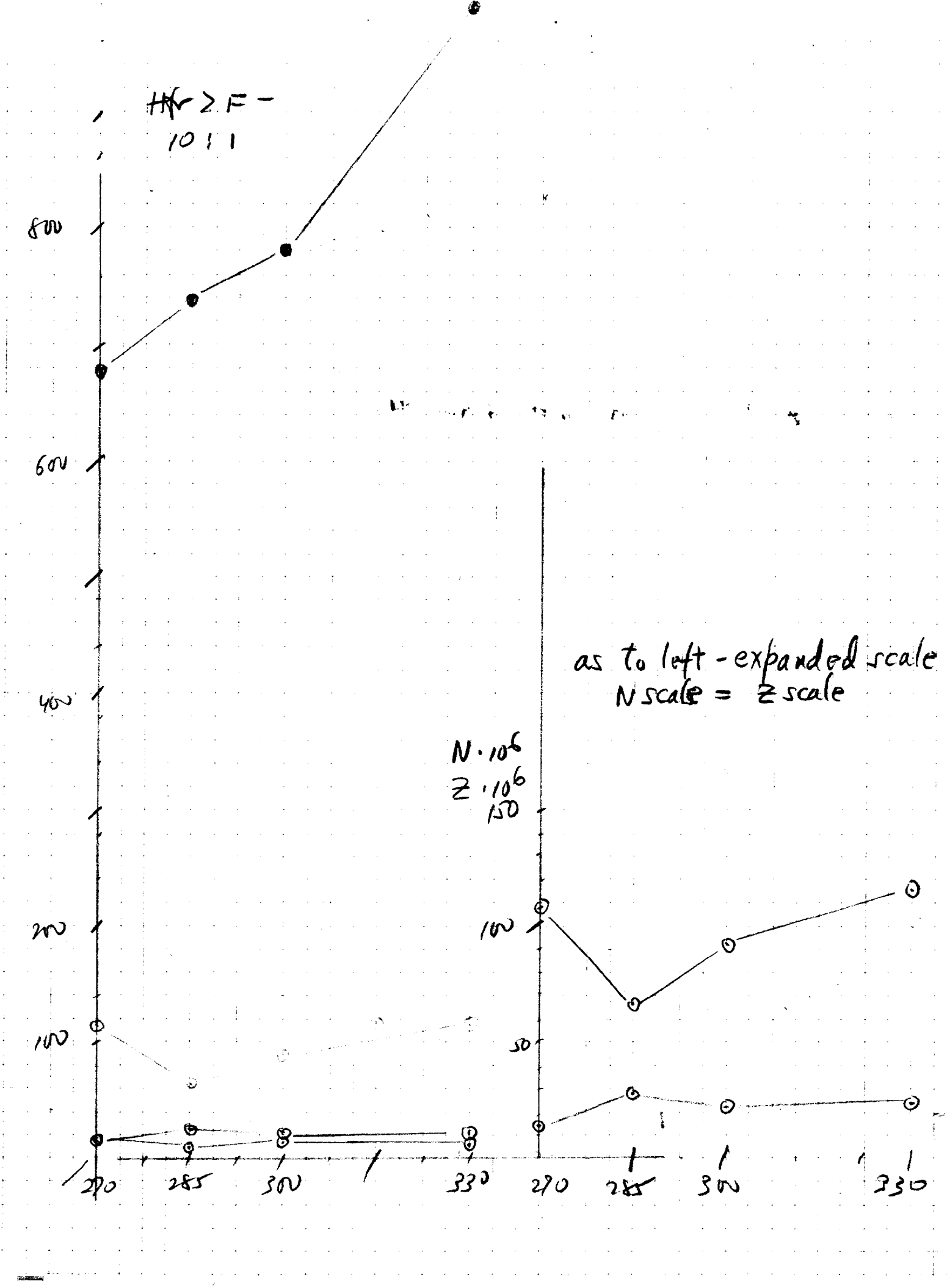
W1956 in initial X0



#96

turbidostatic growth PEN ASSAY




HF ≥ F −
10 : 1
800
600
400
200
100
as to left - expanded scale
N scale = Z scale
N · 10⁶
Z · 10⁶
150
100
50
270
285
300
330
270
285
300
330

Nuclear Staining Technic

Materials:

Buffer KH_2PO_4 M/15, 0.9078 gm./100 ml. use 4 ml. in about 200 ml.
Na_2HPO_4 M/15, 0.947 gm./100 ml. use 6 ml. distilled water

Abopon mounting material: Glyco Products Co., a water-soluble resin.

Saturated aqueous mercuric chloride (sublimate): use 2 parts in 1 part absolute ethyl alcohol.

1 ml. conc. HCl plus 9 ml. distilled water makes approx. N/1.

Method:

1. Fix bacteria (in hanging drop or on sector of agar) in 2% osmium tetroxide for 2 1/2 to 3 minutes. Do not dry cells until after fixation.
2. Spread on coverslips or make impression smear. Dry 10 seconds.
3. Robinow uses additional fixation for 1 1/2 min. in $HgCl_2$, followed by washing in several changes of 95% alcohol and water.
4. Place slide in cold N/HCl for 60 seconds.
5. Hot HCl (about 60°C.) for 10 minutes.
6. Cold HCl for about 20 seconds.
7. Wash several times in buffer, pH 7.
8. Stain for 30 minutes in Giemsa, diluted 1-100 or 1-20 with buffer. (Ordinary commercial Giemsa usually requires stronger soln.)
9. Wash briefly in buffer.
10. Mount in water or Abopon (diluted 2 parts in 1 part water).

Chalund's Fixative

EtOH 80%	60 ml
Phenol	15 gm
Formalin	5 ml
Acetic acid	2 ml

"N/1 HCl" = 1 vol conc HCl + 9 vol H_2O.

For further examination

19: generally too dense

A/B suggest that during flattening cells not which may be advantageous for some purposes, but may distort shape and engender S? Relation to G is not clear.

Owing to confusion, comparisons of ± Os are not worthwhile.

However Summa seems OK.

22C Series complete except C2.

Need time (HCl) series on alcohol effect: are grains worsen.

Also. G from unbind from Sucr:

~~study:~~ (4m.)

C4 A. – 2 G not prom. (G'l?) Granular cytoplasm! (bluish + reddish (N?)

C5 A – 2 – N v. char. G'l No cyt.

		axial ratio	size K-12 = 1 123 = 2
A1 -1	N, S. occ	3-5	1+
-2	N! (some cells still have blue cytoplasm)	shrunken?	1 or <.
-3	N.		

A2

-1

C. 4A -1 Flattened, dist. No S. N faint metachr.

4B (5B) N met deep red. (overstained). Sl. shrinkage but not dist.

5A (4B) cells sparse. but resemble

5B. (5A) Sl. distortion but less than 1st.

no S.

suggests NASuc > NSA to minimize deprivj.

C1. -1 No S. Pt. N variable

-2 G distinct over most of slide // dark purple cyt v. faint N?

-3 Typ. N

C3 -1 prominent S N ✓✓ but only in denser accumulations (high salt?) beautiful terminal S at 111/25.

C2 1 —

2 acG blebs. No G. dark, hom. cytoplasm

3 = 20A1 app.

C3 2 V. Prom G. blue No N? Granular cyt. purple.

3 typ N. Note residual G)?

11/10/53.

15: Compare fixatives. But acid possibly weak.
G not seen after osmic., v. prominent in 15A2-3 !

16. Y. E coli B, K12. Fix Schaudinn S, G prominent in both +1, -2;
B, K

A. W1177 x W1895 from Pennsaw, comparing NA ± salt ± sucr.
Note S only in NSA.

Make nuclear preps - as best can

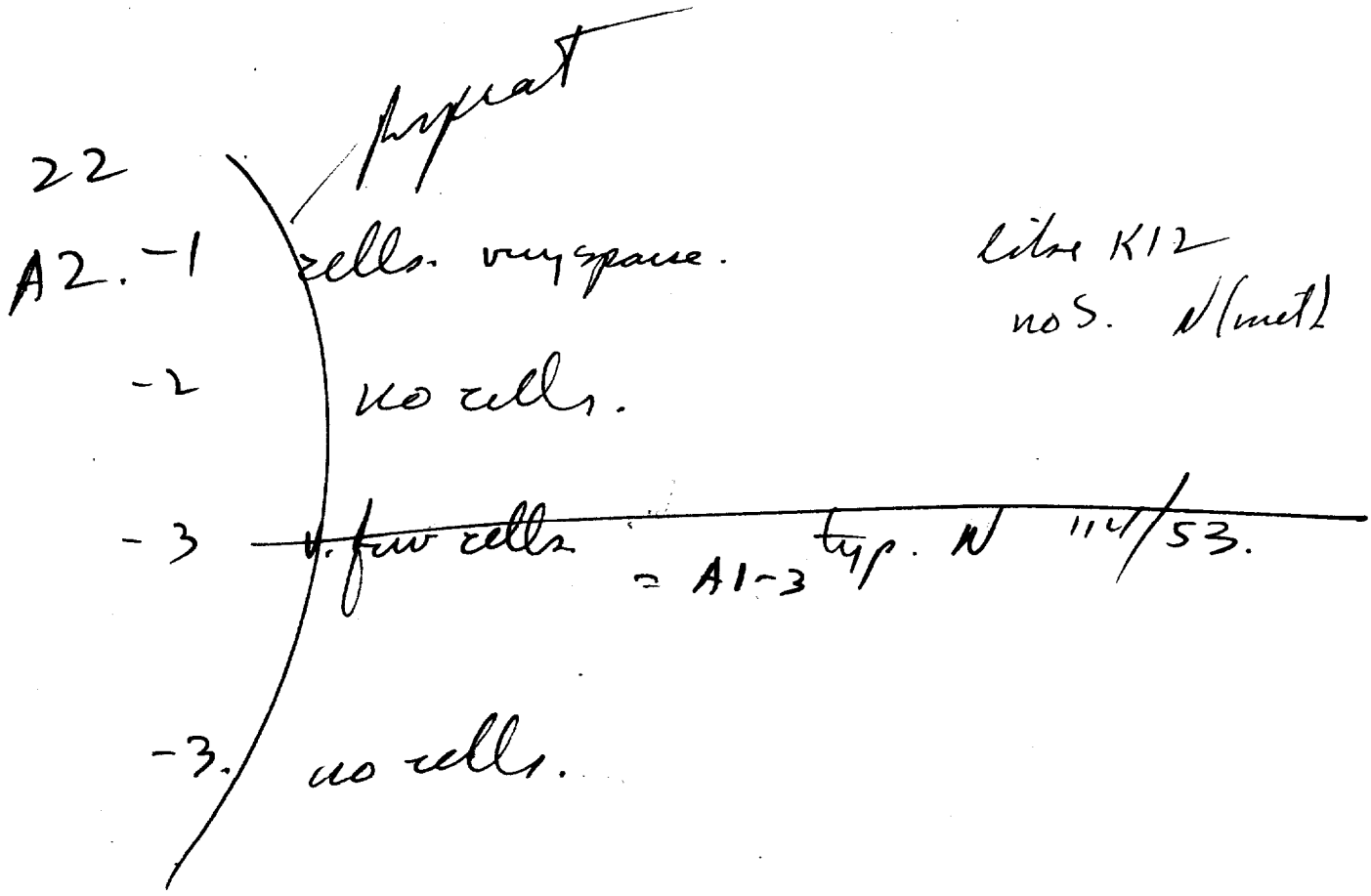
W1637. W2383. ✓ other wg...

Fri W2333 HCl series.
Bz effects.
Crosses.

Bz effect

Alcohol effect

data on Hfr x F - presistance of Hfr gamete in 1 cell?



22 Repeat

A2. -1 cells. very sparse. like K12
no S. N(met)

-2 no cells.

-3 ~~v. few cells~~ = A1-3 typ. N 114/53.

-3. no cells.

A3-1 ax. R. ca 3-4. ggl comp. 123?

B1-1 too sparse. T.O.

B2-1 too dense in parts but useable.
Stain up balance

3/26

Cm 0, 3, 5, 10 inc. series

cells are more coccoidal than K (W2049) and may have more obvious vesiculate nucleus

3/31

C (W2049)

10 now OK. Quite distinct from Cm.

cells longer, transverse rather than peripheral chromatin.

3/29/24 slides

C2-4

0: ✓✓ ~~[illegible]~~ N (negative + interior) dup cyt.

10: G✓ but N ✓ weak

C1-3 0 as above. Some S, weak-stains

1-C_M 1

↓ little

2-C_M 2 0 faint N; some S. dup cyt pl

10 [illegible] (washed out) - short stubby cells

some signs of transverse walls.

slides P4

acid or A too weak!

3/31./54 4 W2404
5 W2438
6. W2049

4 stalky rods
homog. purple

v. l. much detail:

5. 7m HCl.
Rounded cells
homogeneous purple
blebs! usually polar. cf 8!

13m 8l. washed out
but v. l. differentiation
of nuclei.

6. as 4. Some weak blebs?
(weak acid ~~pr~~ hydrolysis → blebs?)

13. as 4. N may
be negative.

4/14/54 slides (GD)

Hg/ Gemisa

B/Cu 0 in HCl. N.v. dense blue cytopl., N mg.

2 duper? no N detail

3-4 5 pale no detail

10 poor N detail, clear cyt.

(1) 0 cells red!!

DATE: 4/14/54 REF:

from NSA

BSI I didn't think there was anything on the first 1 or 2, so I did another pair at 590

1 - 0, 2 minutes hydrolysis Giemsa stain

2 5, 10 minutes hydrolysis - Giemsa

BSI Cv -

3 0, 2 minutes hydrolysis - Giemsa

4 5, 10 minutes hydrolysis - Giemsa

BSI Cv - rev

5 0, 2 minutes hydrolysis - Giemsa

6 5, 10 minutes hydrolysis Giemsa