CYTOLOGY"

1952 - 1954

vols in heavy cloth gray-blue bindg

OsO$_4$ 2 mins HCl 60° 12-14 min Giemsa 30 m. Noteworthy

5/9/52. 1. 1895 x 1952 } fresh culture Nutrient agar
2. 1952
3. 1895 } to Penassay ca 2 h. dense several hours.

5/15/52 a 1895 x 1956 " 10^{-3} ml 6 h. Nutr agar } ca 1%
b " x 10^{-4} ml 3 1/2 h. " } lac^{+} S^{R} m

5/16-17/52 c // " EMB lac 11^{30} – 5 PM. 10^{-4} ml } moi to agar.
d " 10^{-3} ml } ← nuclear definition and lateral protub.

5/18/52 a x — to DeLamater x. 11^{30} 6^{30} 10^{-4} no bleb ~~[illegible]~~
b 1895 EMB. " condensed nuclei ~~[illegible]~~
c 1956 " granulocont n = (2), 3
d x cells small stain v. T. O. ~~[illegible]~~ in broth 11^{30} – 7^{30}. Prompt fix agar.

5/20/52 x 3 mins. fixation 2 hrs. growth on agar } ~~[illegible]~~
a 1895 def. blebs some areas. from 3 hr mix culture }
b 1956 no blebs overstained! poor contrast (underhydrolysed ??)
restain out do. Also Tol. Blue c̄ HCl!

5/21. x overnight ~~[illegible]~~ 10 7 min Osmic blebs! fair stain cells are small
a grow. 10 mix 1^{30} no blebs.
b plated 6 PM fix

x 2 3 ±(no post fix HgCl$_2$). bleb
a 2 } [illegible] OsO$_4$ understained [illegible]
5/22 b. 3 esp = v. rare [illegible]!

Sept 22 - 1 9 drops Giesma - under stained, underhydrolyzed

Sept 24 2,3 12 drops Giesma Lot HCl - 10 min at 55° overhydrolyzed?, cultures 1895 old

Sept 25 4 fix 1½ min mix OsO_4, 8 min at 65° overhydrolyzed

5 fix 2 min mix OsO_4

6 fix 1 min OsO_4, 8 min at 65°

7 8 min at 60°

9/28/53.

Of

1. W1895 ca 4/ [illegible] from broth to plate (see 1075)

fix OsO4 2m. HCl 8-10-12... Giemsa 1:50

Restain 15 m. in fresh Giemsa (1:20 + .01% MB) n.s.

8, 11m HCl slides. Both show blebs.

2. W1895 (A) and X (B) < A. cells jumbled, numerous nuclei positive but slide also dirty and under-stained.
B. cells small, jumbled. Blebs??

poorly developed ⌽

3. X from heavy mixture grown in broth, on NA at room temp!

1. Os-~~[illegible]~~ - no HCl in eosin, followed by MB
2. Os-Hg - no HCl Giemsa (not saved, no record)
3- ~~[illegible]~~ " 10m " " 30.

1. No st. in eosin. Solid MB, no blebs

2. ~~cells~~

3-4. (30m) (40min stain). no prominent blebs.

C 3

fix 2 min over OsO_4

$HgCl_2$ 1 mi, ALC H_2O in cold HCl 8-10 min 60° HCl

1/20 Giemsa - 30-40 min and, buffer dip

Sept 29 55° HCl

1	~~no contrast~~	
2	5 min	little contrast
3	8 min	washed out
4	11 min	" "

W185 .001 on nutrient agar at 10:00 AM slightly heavy
fixed at 2:20

H.

9/30. A. W1895. fix in Os, $HgCl_2$ ... Attempt stain c (1% osmium pH 7!) Light Green No st.
1 Pyronin Faint stain washed out
2 Methyl Green (stained as (probably) Me Violet — No diff.
Acid Fuchsin. No stain.

B. W1177 (?) Os-$HgCl_2$ - HCl 10 min. Giemsa to 1 hour.
1. poor diff.
2. ... HCl, Me Green. Poor diff.

droplets ??

10/2. same slides — in H_2O 4 h. v. poor Giemsa stain

10/2. C. W1177 + W1895 in broth 4 hours. Spread and smear s further growth. (weak Giemsa!)

10/4. D. W1895 3 hours smear agar OsO_4 - $HgCl_2$ - HCl - Giemsa.
1. Old Giemsa (1952) 1:20 in M/15 KP buffer + MB
2. New " 7/53 " "
3 " " Fresh dilution from stock.

Still n.g. (for nuclei, cytoplasmic staining fairly sharp in 1.
Blebs quite pronounced in 1.

Why Giemsa n.g?

10/6. E. W1177 overhydrolyzed, overstaining (80 min.)
1. Toluidine Blue 1%
* 2 Giemsa 1:10 in KP M/200! (Probably OK if used right there)

CYE2: W1177. Blebs prominent
(oncotarmid)!

"Vital Staining"

9/27/53. On hand W-1895 susp. (ca 3× from bottle) in H_2O, 24 hours

Add various dyes to ca .3% Incubate 2½ hours.
in KPhoffer 7.5 pH/15.

Centrifuge:

Color in pellet: 1 neutral red
2 brilliant cresyl blue
3 Janus Green
4. Acridine Orange

~~Color~~ Color in sup't: Resazurin, 5 Bromphenol, 6 Congo Red, 7 Trypan blue. } Incontin. } pellet almost colorless; light blue; distinct orange; distinct blue

Mica: 3: Various ~~etc~~ cells diffusely stained, but unformed in any cell. Some cells may show marginal density or wall staining.

4. Cells distinctly orange–yellow. (reddish by direct transp.
1. Uniform coloration rather faint
2. definite blue coloration.

5 No mica color.

6 No mica color.

7 V. faint blue, doubtful whether can be used

Best prospects are brilliant ~~cyp~~ cresyl blue
Janus Green (cells strongly agglutinated)
Acridine Orange

Try on Hfr XX. later decided n.g. for mica scoring

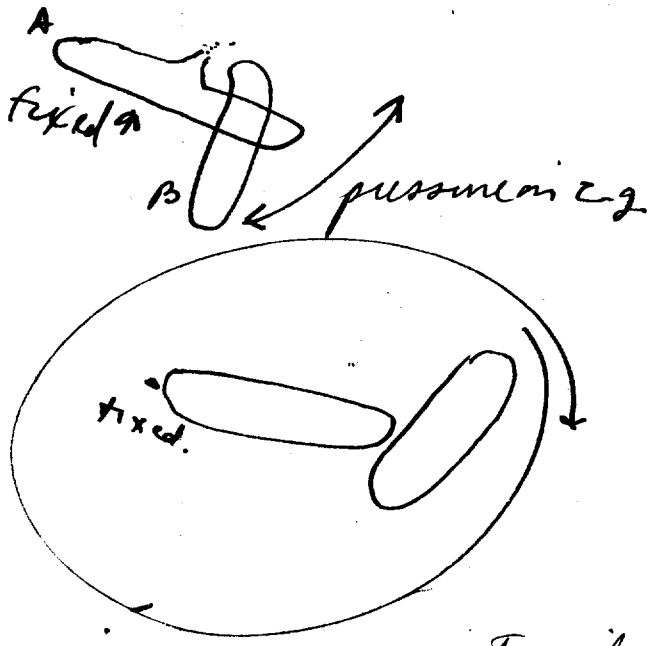
9/28/53. Add sterilized dye solutions to dilute bottles 1 hour.
-30.

W1895–1177.

James Green C
Acridine Orange A
Brilliant Cresyl Blue B — rapidly decolorized, restored on aeration.
Micr. underunit

other cells also began to "haemolyse"?

Blasts → v. light.



and

W1895 + W1177 za shows

and many pairs not clearly made out.

Feulgen.

2 min Os. 12 min N/1 HCl 60°. 30 min Feulgen in pH 7.5 buffer.

9/27/53. poor contrast. over hydrolyzed??

Agar plate probably too thick. (10%/- 3[30] - 4.[07])

stain at 60° (35 mn.) same as at RT (20-25°)

Try Fe-Heux. 30m. and overnight

↓

no differentiation

W1895.

8

9

10

11

12

stain probably too weak. 8-10 m. hydrolysis probably OK.

Try 1:20 (rather than "5 drops/10 ml").

Restain 11 and 8 in stronger stain 15m.

Both clearly show blebs stained red. Nuclei purple not too dextrant.

CS

10/6
W 1177

1	3 min O_sO_4	8 min	HCl at 59°	no contrast
2	"	10	59	"
3		12	59	"

all showed little contrast. Is 12 minute hydrolyzed were not much diff. ut than the 8. Acid weak? All took the stain well. New batch of Giemsa stock solution

Giemsa stain
1 ml Giemsa stock
9 ml water
½ ml 7.5 buffer

Living labelled cells + viruses.

Standard system. Made W1177 in .005% T→.
Mix .1ml & 1ml W1895 in Penassay (7ml)
Examine at stated intervals. No secretion.
Assay in EMB loc cm.

10/16. Cells > 24h old. 11:25 AM -

10/17 See 1072. W1177 cultures 4-5. (T-0).

10/16-17. Stain hitherto in dilute KP buffer ca 7.5 This is probably too alkaline. Slides overstained (40-50 mins.)

A). responded nicely to decolorization in dilute HcOH. Check further as opt. pH.

B. 10/17. W1895. nutrient agar 3h. Test series of pH, KP buffers at M/200. Giemsa 1.10 30 mins.

pH 6
6.5
7
7.5.

↓ progressive overstaining of cytoplasm.

pH 6 shows most promise
also try decol. 7.0, 7.5 in M/100 buffer pH 5 1 minute. did not remedy overst. cytopl.

C. 10/18 W1895* Pen assay (to D/O) overnight. pH 6 ... staining

*TZ.

	Buffer		Stain		
1	6	M/20	.9% Tol. Blue		Most TZ in poorly stain cells or extracell.
2	6	M/100	Giemsa	1:10	
3	6	M/10	"	"	
4	6	M/100	"	"	+ T.Blue .1%
5	6	M/100	"	1:20	
6	5	M/100	"	1:10	

1: medium contrast, TZ readily observable. State of cells? fix?
2: cytoplasm overstained. TZ not easily seen
3: slide acc. wiped, but better contrast?
4. badly overstained
5. Sharpest nuclear stain of all. TZ also clear!!

Use pH 5.1!

10/18. D. W1895* 2 hours NA. Os-Hg. left in H_2O 3 hours.

(pH5)

1. Tol. Blue 1% M/10. Contrast good but low intensity.
2. Giemsa 1:10 M/10 Overstained, good contrast
3. " 1:20 M/10 Nuclear staining excellent. " "
4. " 1:50 M/10 " "
5. " 1:10 M/100 overstained!
6 " 1:50 M/100. Deeply overstained!! (increased hydrolysis??)

HCl 10.

Definitely hydrol.

try 2 - 10m = A
4 30m. = B

E W1895* 3h. NA Os-Hg. in H_2O 3:40 -

A + B.
1. pH6 M/10 4:– to 37°.
2 " " +RNAse 1:10
3 HCl 10 mins.

A = Giemsa 1:10 10 mins B = Giemsa 1:50 30 m.

4 RNAse 6:30 – 9:00. 5. RNAse 6:30 - 9:50

cf 1-2-3 RNAse does not seem to remove as much nuclear stg material as does acid. Note metachromasy also (in 1). Short staining seems OK. (cf A-B. Settle on 12 minutes for 1:10

note 4: nuclear material more diffuse than in 2. (swelling?)

10/19. A. W1895 9:30 – 1:30. OsO_4 – $HgCl_2$. 10 min HCl

1 Giemsa 1:10 12 m. pH 5 understain

2 " pH 4.5 "

3 " pH 4 (KHPhth.) no stain

4 Tol. Blue .9% pH 4 " ". fair (about like C7D1 needs further trial.

No OsO_4 $HgCl_2$ only { 5 Giemsa 1:10 A pH 5. B pH 4

[Little or Giemsa at pH 5.]

No obvious advantage to lower pH for any material.

B. W1895 11:30 – 3:30 Fix. comp. Giemsa 15, 25 m. A B.

1-2 Schaudinn A somewhat understained. Fair detail in B

2-4 Carnoy 6 alc : 3 chlor : 1 AcOH adherence, detail poor

3-6 Sera 6 alc : 3 form : 1 AcOH Stains lightly but detail may warrant further examin.

4-8 30% alcohol. Poor. ~~Bo~~ stain

5-10 Osm-Schaudinn not greatly diff. from 1.

Proceed c Schaudinn fixation for now. Prepare slides of W1895 for study. ~~Stock Giemsa~~ [~~1:10 seems weak. (ca 2.5 fold!)~~]

TZ* 2½ hours NA.

C. Time of hydrolysis. Giemsa 1:10 pH 5.1 Schaud fix. A stain 10 min, B " 18-20

Note: TZ mostly in ghosts! 4-6-8-10-12 m. hydr.

4 Numerous polar granules.

6 Blebs very distinct. Rare polar granules.

8 similar to

10 blebs also noted.

12 " " strongest nuclear contrast in B, weaker in A. *Most brilliant nuclear stain

Main problem now: fixation.

[concentrations not noted in CTA but dye at pH 6.
RNase did not enzymes]

10/20/53. Fix Schaudinn 2 min.

A. W1895* overnight, direct from broth. Liquid smears = (1) agar impressions (2,3,4). 1-2 hydrolyzed 3-4 not.
* 1-2 n.g. - T.O. (stain? see below). (3-4) (10, 20 min staining).

Note: TZ mostly in v. small or ghost cells. (Compare incidence with live observation). Also note holes, often polar in cell stains. (Compare with polar granules in D3, 8 & 4).
Queries: viability of TZ stained cells; do granules fall out?, relation to polar grains. cf also CTE1.

B. Fate of TZ. Move 1895 into TZ broth (= Penassay + .005% TZ) 9:30 AM - 1:30 PM. Distinctly colored culture.
1. Direct smear as above.
2. Incubate on NA to 2:40 (70 mins).
3. 3:40
4. 4:20.

A = hydr. 8 m. B = unhydr.

(1B) (cf. A3.) Most cells rather short, have TZ granule. Occ. cells c̄ polar concavities. Some cells have 1 TZ + 1 polar con. Neg. nuclear stain.
1A. Weak nuclear stain. Most cells have 1 TZ and 1 "nucleus".

(2) A. Types of cell: larger, deeply staining, TZ rare, nuclei reddish, occ. polar concav. and smaller, more empty, neg. nuclei and TZ more frequent. Slide dirty.
B. Not too clear; short cells (some TZ) usually uninucleate; large cells 4-nucleate c̄ TZ

(3) B. Poor nuclear stain. TZ almost all in occasional uni- or 2-nucleate short cells.

Until D-A, old diluted Giemsa was used above.

3A. About 5 plump cells. 1 ghost. Some of cells have TΞ. Plump cells as above. Nuclear stain metachromatic. Occasional concavities.

[Relation of concavities to blebs? to grains? Too frequent here to have anything to do with TΞ.]

4B. (A mf. host) Somewhat crowded. Similar to 2, 3. Occasional concavities.

(concentrated in 10 mins; fresh!)

D) W1177 *~~old~~ overnight. N.A. 12N–3PM. Undiluted (Rather dense)

Compare fresh (A) and previously, 48hr., diluted Giemsa (B). Hydrolysis 3, 6, 10 min. (Unfortunately no 0 control to detect concavities).

A. (15 mins.) definitely superior to B., somewhat overstained
3: distinct polar bodies
best slow ratio | (6. orc. polar bodies
10) } blebs also.

C. W1895* overnight. NA 11AM to: (1) 1:30 PM (2) (Moz 1/100) 2:45
(3) (Moz 1/1000 – discrete but perhaps too large colonies at 4:30PM).
(2) Used by G Davenport, 8m HCl, test various other dyes. Approximate best times: (in pH 5 buffer)
A. Crystal Violet .05% 30sec
B. Toluidine Blue .05% 30sec (?
C. Giemsa (old)! 15, 25 mins.
D. Safranine n.g.
E. Azure A 1% 1, 3 minutes
F. Basic Fuchsin .02%, .1% 1 min.

None especially advantageous over Giemsa. (F might be useful for comparing extra-nuclear granules).

(1) Hyd: stain n.g. Restain 10/21 Still n.g. (overhydrolysed?)

(3) Keep in water overnight. Stain 10/21. (10, 15 min.)

	Hyd.
A	0
B	3½
C	6
D	10

Intermediate development grains + nuclei but slide dirty.

bgt. ca 4½-5 hours.

10/21/53

Grow W1177 in TZ overnight. A. (B) 9^45 inoc TZ Broth c A., and (C) is unstained W1177.

A1 - B1 11^45 Fix from broth. (Live B shows a fraction of large TZ bacteria.)

(A2 Inoc NA 9^45. 10^-4/ml. At (1:35, microcolonies ca. 50-100 cells).

2PM. C (live) shows ca 90% TZ - fairly long cells. Inoc C1 .001ml per plate NA. 3:40 visible (A2) under phase n = ca 10^3 microcolonies. Fix = [redacted] C (D/E)

Most microcolonies have ~~residual~~ TZ granule in a more or less empty, shorter cell. 6 hours is too long

D W1177 plain 12^30 - 2^10.

E. C direct (from plain agar) 2^30.

C1. 2PM - 4^45 C2 2PM - ~~7:30~~ 6:00 C3 2PM - 7:00.

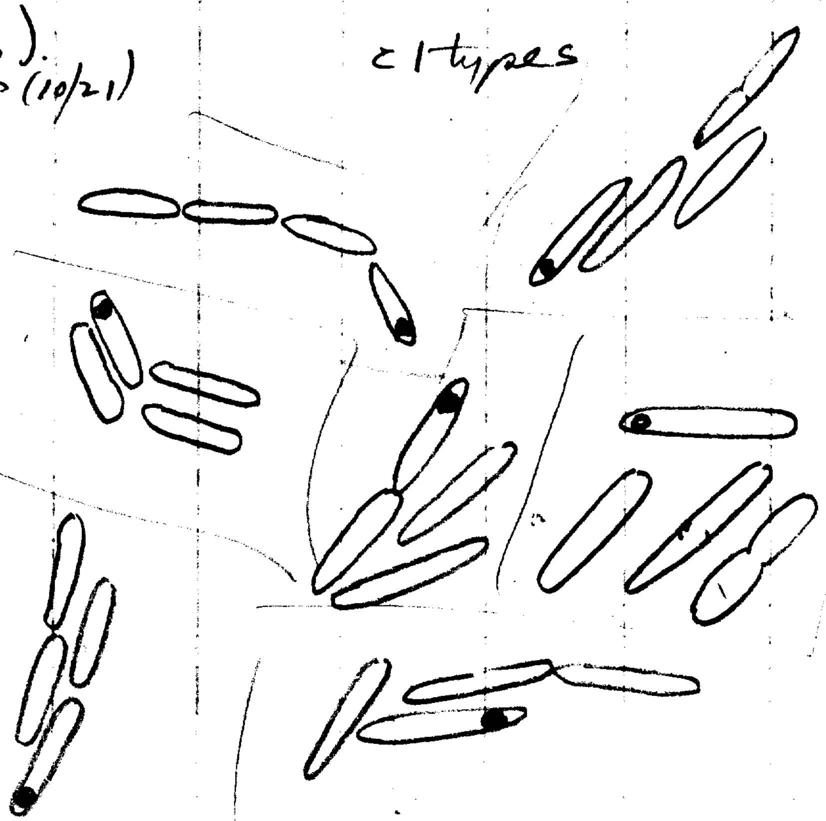
Refrigerate overnight of time industry

(4 and 8 cell microcolonies). Protocols (10/21) showed.

n	TZ
4	0
8	0
4	1
4	1
4	0
4	1
8	0:
3+	0
4	1
4	0
8	0
1	1
1	1
4	1
4	1
4	1
6	0
7	4..
8	1

single cell init? > 2

C1 types



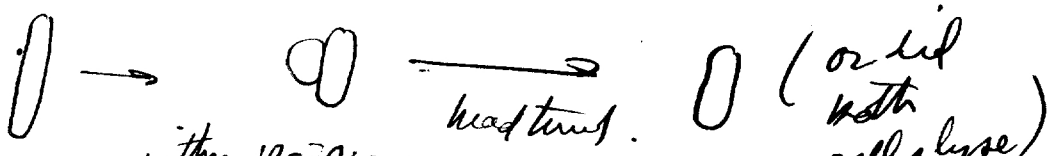
Granule never medial (could it be?)

Reexamine plates C1 – C3 9:15 AM 10/22.

(C1)

+ = big cell almost double but counted as 1

noticed a more or less isolated cell originally



N	T+	
3+	1	
2	1	
2+2gh.	0	
4	0	
4	0	
7	0	
7	0	
1	1	
1	0	
1	1	small but full
1	1	" "
25	2	
7	1	
4	1	
5	0	
4	1	
11	0	
1	1	
4	0	
4	1	

Blebs observable on same cells.

T+ cells in microcolonies not distinguishable

T+ never medial. Occ. cell shows empty vacuole

C3. 9:45 (915–945 at Room temp.)

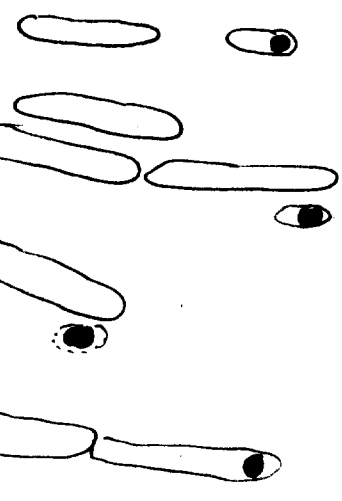
Many colonies uncountable, include cocci, and spread out

N	T+	
ca 100	0	—
11	0	
16	0	
10	0	
0	1	sl. small.
2	0	v. small
0	1	
100	0	
> 100	1?	
> 100	1	

60	0	—
30	1	
2 pul.		
> 100		
? ?		
2 or 100		

exceptional.

Occ 100 cell colonies have normal (small T+ granule) cells.



W117)
stain A1-A2 10/22 A1 fix from broth overnight A2
A2 microcolonies 5 hours?

1. -0 TZ granules, occ. concavities.
-3 } nonnuclear stain 10, 20 m. stain
-10 }

2. TZ generally absent. ρ
(-0) Prominent concavities. Heterochromatic nuclei
(-3) Prominent grains
-10 Mixed! (accuracy of hydrolysis??) < weak nuclear in parts / strong grain. in parts

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B1. Stored in cold water to Sunday 10/25.
1 HCl-0 m cells sparse [illegible]
2 -3 m G very clear; [illegible] nuclei in background
3. -10 m Clear nuclear st[illegible] But 1-2 nuclei predominate

Suggests log phase cells OK from liquid medium! (Try aeration)

E. (fresh TZ cells). 1-3
2 -10 Small cells. Staining nuclear, but not very distinct.
TZ cells random.

T.O.

10/22/53. W1177, TZ overnight (10c). Refr 12N

A. (1. Plate on NA 10^{-4}ml 8:50AM) (2. 10^{-4}ml)

8:50AM. 0. Direct observations ratio of TZ:- 21:14 various fields
30:18:4 free granule?
32:15:3

Under phase, cells c and s TZ were indistinguishable otherwise. No internal differentiation whatever; all cells have typically rounded (convex) ends.

0; staining (s hydrolysis) TZ cells substantially similar to
1. non TZ cells.

3. W1177 TZ overnight. .01 ml 12N.).

A0 - stain

A1 850 - 12N Refr.

A2 12 - 135 Refr.

A3 12N - 2PM stain

[10A2 ... ...]

A0. Refused to stain for nucleus (up to 40 mins.)
Keep slides with 0, 6, 10 min hydrolysis.

A3 Staining brilliantly. TZ not prominent however:

A3-0 clear spaces prominent.

-3 granules not prime poles, but often lateral. Probably not hydrolysed long enough.
TZ rare only in dry cells

-6 missing —10 sparse. general staining

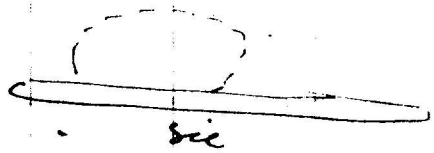
B - direct from scotch. (1) 845 AM 845 PM (2) 505 PM - 845 PM.

Neither gave nuclear stain. Why liquid medium unsatisfactory?

C3 suggests the deterioration of the TZ subclone by the 100 cell stage. Some disappearance of TZ?

C2. (Stuck at $10^{1.5}$). possible blebs in some cells

	TZ	
4		
7	1	normal
4	0	
"	1	"
ca 40	0	included a short pair.



Desiccate microcolonies not unprevalent. TZ also unfrequent.
Longforms typical to ordered, empty small cells. (Pre-critical stage)
survival may be correlated c size of granule.

24	1	normal	small granule
ca 75	1	v. short but dense large.	
6 (+40, 40?)	1	mid-length, empty.	
60	1	normal (small granule)	
22	1	short, med. dense	mid granule
3	1		
150	0	v. short, dense	
6	1	mid. short, dense	mid granule
13	1	empty mid length	large
3	1	short, half empty	large
80	3	two v. short, 1 mid empty	"
80	0		
80	0		
40	0		
20	0		
30	1	empty	
50	1	normal	
32	1	short	

These cells showed slight nuclear differentiation

suggest lower conc of TZ for viable subclone cells!

A.) 1 Plate W1177/Tz .01% .01ml NA 11:15 AM – 2PM
2 " " .01% .001 "
3 " " .002% .01 " – 2PM.
4 " " .002% .0001 "

B. 1 = smears from both mounts Stain Giemsa 1/10 10 0,10m HCl.
3 = " " " V. poor) nuclear stain. some cells have a blue granule nucleus?

C. Effect of refrigeration. 11:45 plates of Tz # .002% ↑ .05 ml
1. Fix samples 2:45 10/23. Refrigerate remaining plates
2. Phase observations: 2/3 Tz cells are short, some empty 1/3 normal (usually those with least label). See ~~B~~ A 4. Comparison suggests a crowding effect. Stained 0-3½-5-6-8-10-12 m. hydrolyses; each stage from 3½m → shows distinct blue granules; fairly poor nuclear stain even at 12m HCl! What is peculiar here? Overfixed? slides not stored in HCl
V. fresh stain? 0-3-10 intervals should be adequate for later tests. Go back to refr. plates for comparison.

A)- Examine 2, 4 eg. 3PM. (<5 hours) 2: Classes 2-16 cells.
Tz. normal cell in majority 1/16 1/31 1/14 1/30 1 file/6 1/3 1/8
2) abnormal (2) 5/30 1 single/4
alone. 1 abs e n se s

∴ at this stage many cells are normal in phase appearance

abnormal empty v. short = s or empty = e

4) normal Tz in majority: 1/4 1/2 1/16 1/30 1/4 1/27 1/15
ab " " :
alone : In

Data. of C!

cells spaces.

A1 HCl 0 TZ granules in poor cells. some spaces.
3 few cells. no granules or spaces.
10 holes + granules. no clearing!

A3 0 spaces v. clear. some TZ in normal cells., most degen.
3 as 0.
10. punc-nuclear staining but some residual granules.

acid hyg.?

✓ TZ cells not multi.

Repeat ~~A1A3~~ 10m. 10/25/53 A3 First-rate nuclear stg!

FN S (3m.) granules beginning to appear S. still noted.
cytoplasm very dark, granular.

[These cells seemed generally more resistant to acid than most. Why?]

10/23. Grow W1177 overnight in various conc. Tz / phenazine.

A .02% Growth ½ inhibited; cell at bottom
B .01 Deepest red
C .005 deep red
D .002 med red
E .001 barely perceptible red
F .0005 faintly darker than control
G —

Underphase 10:25 AM.

	−	+Tz	Fuc (or no detected cyto(a))	Type.
A.	6	28	7	variable
B.	1,1	21,36	1,3	
C	8	53	1	ca 80+% ++
D	19, 11	14, 32	1, 2	about ⅔ stained

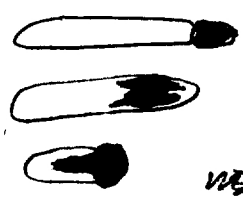
E. label rare (< 10%) ± recognizable fuc cytoplasm ? or poorly apparent underphase.

F. No label seen by phase. Occ. fuc Tz — No granularity

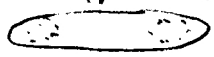
Opt conc for label might be between D and C.

Compare B, D, & platings.

A:



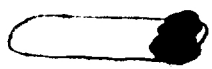
axial rods and coils usually fuc Tz cells i or s Tz show sl. reportable usually staining zones.



axial Tz sometimes does not show up underphase

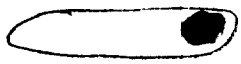
B. granules often doubled.

C. mostly fairly uniform.



granules about cell diameter often doubled. aberrant crystals rare.

D mostly



smaller granules.

scarce. fewer % label.

E.  to 

10/23 C1. W1177/TZ .002% ,05 ml [2:45] N.A. Fix.

10/24 CA. Plates refrigerated 24 hours. Fix (2-5 mins) P24

Excellent series in C1 in hydrolysis time but acid evidently weak.

Repeat P24 on CA.

1-2 no hydrolysis of. 5 minutes M/10 buffer 5.1 Giemsa 10.

3 HCl (old) 5 mins G very prominent

4 HCl fresh 5 min. G less than 3.

5 HCl old 10 m. Strong G weak nuclei } note blebs! G no longer terminal.

6 " new " . [accident in prep.] G less prom.

Best 5. 7 Repeat unhyd. S very clear [TZ in empty of 1/2 !!

8 heat 5 m. pH 6 S very clear (less? than 7).

9. Fresh HCl 10 m. N. (strong but somewhat fuzzy)

New series at one time: 11 No hyd. S. clear etc. where unstained

12 ~~pH 6 10m~~. HCl cold 10 m. S distinct. Similar to 11.

13 pH 6 60° 10 m. S greatly reduced (or absent?)

14 HCl 3 m. G (not too strongly dev) No S. No N.

15 HCl 6 m } nuclear stg. (possibly indist.

16 HCl 10 m }

17 HCl 15 m } stg. progressively confined to

18 HCl 20 m. } small axial granules.

19 (HCl 4 Giemsa 10, 20. 4) G. very clear; cytoplasm slightly [tinted?] should be repeated

→ should have better Bin prep in this series. with fresh material.

CB. Fix 30 minutes. Store in H_2O ca 4 hours.

4: G very clear. Like 1 above.

10; clear nuclear stg but axial S shrinkage

weaker acid ev. more favorable to G. Fixation should be improved for nuclear detail. Should one dry?

(over)

20≡ unhyd. Crystal Violet 30 sec.
Understained; ~~[illegible]~~. S variable, vague
some negative N.
v. slight G in places?

X 21 Unhyd. Bas. Fuch. 30 sec [illegible] 3 min – S [illegible] stain [illegible]
S [illegible] TZ about ½ [illegible]

22 Tol. Blue 1 min

23 Azure 3 mins. Clear stain – S [illegible] ([illegible])

[illegible]

[illegible] after hydrolysis

31 m + Azure 3 m. G [illegible] ([illegible] than Tol Blue ([illegible], CV)
Tol Bl 1 m. G [illegible]
CV 1 m. overstained G not evident. almost
uniform cyt. stain, possibly [illegible]!

→ CV 30 s

---

22 Tol Blue ± HCl 3 m. S – G [illegible]

21 Cr. Viol ± HCl [illegible] stain
G not [illegible] ([illegible]?)

Oct. 14 1953

W1177/T2 overnight. Moz plates 10:30 AM – Refr. 3:30

.05 ml

.01

.001

.0001

Sml tubes c

1.0

.1

.01 ml.

Examine N25, phase.

plate .001 Nicely spaced. colonies from 10^2 – 10^3 cells.
>1/2 have 1 T2 cell. These number:

T2 cell normal	abnormal (short, empty)	T2 cell single, abnorm.
8	24	9 (some might be related to neighboring colonies)

normal – 0.

These plates should have been examined earlier prior to recounting.

.001 colonies nearly confluent, still plane. Pattern similar to 9.

.01 colonies, semi confluent, large plane; some heaping

.05 confluent, moderate heaping, mostly close packing. T2 cells almost all empty.

tubes: .01 } rare cells; 2 with T2 seen ?
.1 } 1/2 cells T2 are short ca 1/2 more or less normal
1.0 }
Differences less obvious in phase than in stained preps

Fix .0001 ml plate

A Schaudinn

B Osmic - Schaudinn

C Osmic - alcohol.

done hastily. Save only B.

3m HCl - axial deuts heavy G?

10m " - axial shrinkage D?

Oct. 26, 1953.

A. W1177/TZ .005% overnight. 9:30AM. Incubate NA plates c̄ .01 ml each. to 11:45

~~B. Inc. tuesday 1/7. 12:30 ... 1/20~~

C. W1637 – N.A. 9:30 – 12:00 (A) (B)

stain each 10m. after 0-3-10 HCl.

A. 1. Fix Sch (6)
2. Fix Osm - Sch (6)
3. Osm-Alc (6).

C.
D W1637/TZ (930-1230)/NA 1230-2:30 1-4 Sch
5-6 Sch O3.

C1 HCl - 0
2 3
3 10
D 1
2 } cells sparse
3

1. Show distortions of cell form. About = in A, B.
2. Occasional grains
3. Remarkable nuclear patterns. Probably chromatin. C3B.

W1637 might be useful as test of fixation!

A - Stained P26; stand in water through afternoon

14A.

1. (Sch.)

1 - 0	
2 - 3m	HCl.
3 - 10m	

1. S definite but not crisp
T+ mostly extracellular
N very faint.

2. G very prominent, very sharp contrast!

3. ~~Nothing~~! Strong N but fuzzier (less shrunken?) than +

2. Osmic 3m-Sch.

1. No S!
2. Mixed G and N, latter predominant and very dark.
"mitoses" blue
3. ~~Not~~ V. sharp N (purple-red!)
"mitotic".

3. Osmic - Ale

1. No S. V definite (red)
2. Pure N (mitotic) blue
3. ~~Pure - rather faint.~~
~~basic pattern resembles 2.~~

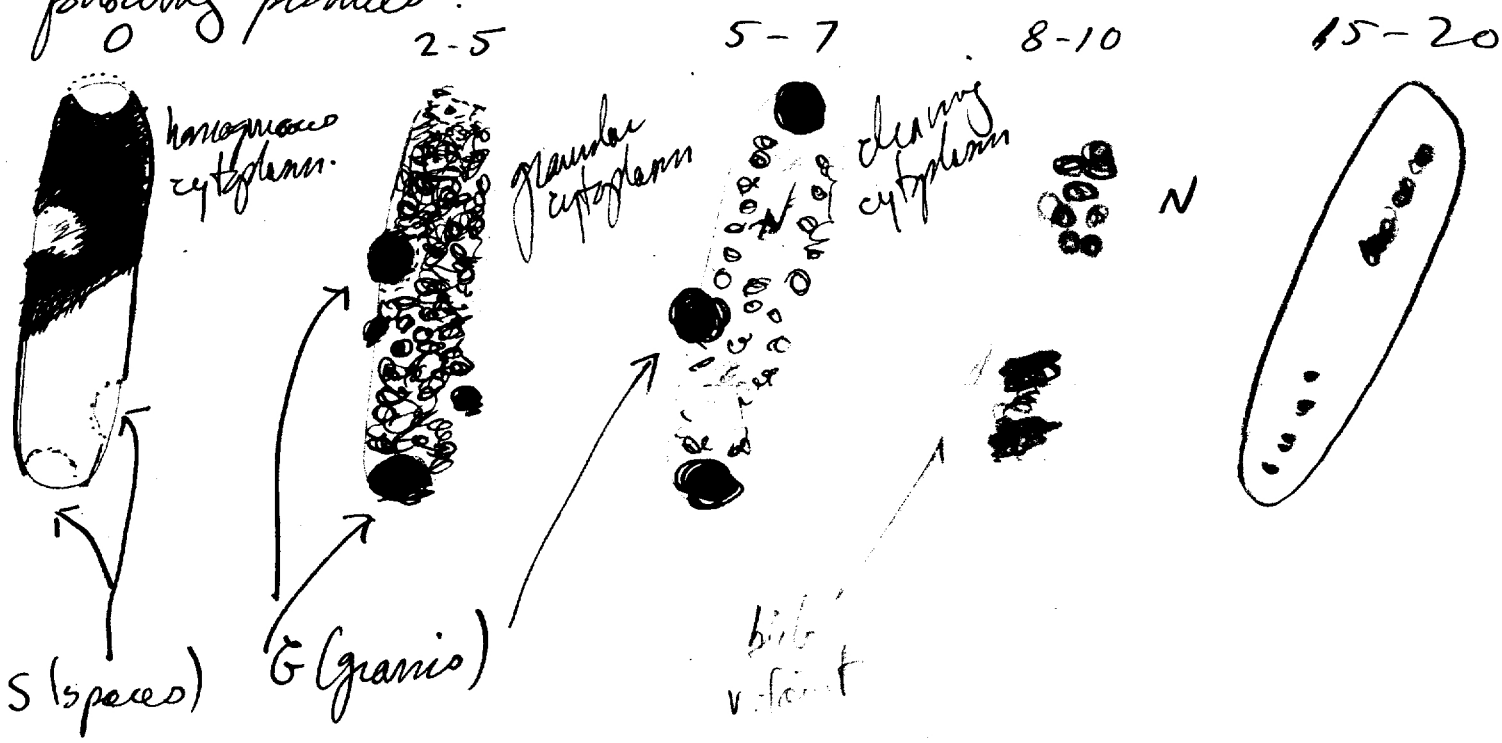
3 needs to be repeated!

2 and 3 are about equivalent.

Hence, Os = 3 mins followed by alcohol.
Sch = " " "

10/26/53. Conidiosacis Cytology.

After Schaudinn fixation, increasing hydrolysis times give, generally, following pictures:



* some early slides, N stained metachromatically at 0.

Spaces, Granis and Blebs are probably same material, though this would be difficult to prove. W1127-W1895 are similar. Not clear whether bridges would be simulated.

Toluidine Blue + Azure Azure picture very similar to Giemsa, but not so sharp or so dichromatic. Crystal violet gives a dense background color at all stages (protein?) so that S, G, N are not so well marked.

Refrigerated plates; coverglasses stored several days give some pictures but median fixation still too poor for careful comparisons.

10-16-53

Cultures from liquid medium have been badly distorted when plated directly. Impression smears are not tolerably distorted, but nuclear detail has not yet been very clear. (cf 10E, 12B). Thin, well sponged, aerated cultures.

I+. All cells probably viable, but I+ granule eventually gives a deteriorated cell its clone. Very small granules are consistent with normal appearance. (optimum concentration about .002-.005% for this purpose). May be dangerous as a marker. Crowding seems to be related to the deterioration. Noted as ① loss of basophilic cytoplasm ② loss of stainable nucleus ③ short, sometimes "empty" mitotic phase.

Oct. 27, 1953.

Repeat 14A3 – 2/3.

A. 1, 2 } 14A3 impressions { 3 m, 10 m HCl } 10 m orcinol — pure N, weak; G and N (well marked G); N blue.

3, 4 Fresh osmic fix. W1177, 10^{-3} 5-6h. — 3 m, 10 m — N weak, purplish; acid weak or incomplete? N very sharp, ~~but~~ blue.

(acid must have been weak).

" " 5 – 0

" 6 " – 3 m hydrolysis

A – Os as above
B alcohol
D Sera
S. Chabaud fix

} [illegible] than A [illegible]

A5 — No S
A6 see above.
B5 — No S ~~[illegible]~~
B6 — G well stained N [illegible] cytoplasm clear
C5 — No S
C6 — Unstained
D5 — Sparsely distributed (Feulgen)
D6 — Unstained or faint blue granules

Thrown out

Needs repeating!

10/28/53.

B E coli B { Plate .05 ml 9^30 – 12 N. Fix Schaudinn
K K-12 } NSA.

1 0 HCl } B (K) both: very clear (S).
2 3m HCl } K2: G – N faint cytoplasm dark.
3 10m HCl. } K3: Nasuenal B3N, very sharp

Study B2 further. G large, spherical (Nuclei?). N v. faint cyt. dark

A. W1177, W1895 grown in Penassay overnight. Mix each 1:20 Penassay, aerate 10^45 – 1^30. Fix from smears on (Schaudinn)

1 NA Suc 5% 2 NA 3 NSal A 4 agar 2½%.

Distortion in 2 > 1. Also more debris (too much in 1!) 2,3 ca = dist.
Swell marked 3. Also distorted > 1. 4 – plasticity; distortion ca = 1.
Note: S only in 3! (NSA, as in most expts). Compare cells grown on NSA, NA!

C W1637 11:40 – 2+ PM. Fix in sch from 1-2 NA, 3-4 NSA, 5-6 NA Suc.
A – 0 hydrolysis. to test distortion.
1 > 3 > 2 flattening and distortion. Some v. large bodies seen.
Here some salt seems desirable!
Probably matter of prior shrinkage before flattening.

D. W1177 11^40 – 4 (10^-2 ml) NSA. 1 Os-alc. } –1 0 HCl
2 Schaudinn } –2 3m "
3 Chabaud. } –3 10m."

slides dirty

D1-1 N o S.
2-1 S
3-1 S [scribbled out] do not save

Chabaud does also give spaces; Os does not.

Granis – Stromonov

(Later: probably a matter of drying after fixation).

9/29/53

A. 1 W1895 | -1 0 HCl. 3m. OS-Sch fix.
2 W2333 | -2 6m } possibly interchanged }
3 W2049 | -3 10m }
4 W1895 + W2333.

A (-) 1. 1 Probably overstained. Occ S
2 " " Cells much larger. Occ S N red
3 Spores "
4 Small and large cells individual scoring?

-2, -3 definitely baccilary but plumper than wg 1.

—2. 4. (jumbled). 2. Possibly overfixed. Dense "axial" nuclei resembling Robinow's figures.
4. Note fragment figures  (λ?)

B. W1895 x W1177. mixed broths 9:30 + plate at $10^{-1} \ldots 10^{-5}$ NSA.
Refrigerate after growth for later fixation and SR+ essay.

	Ref.	hours.	Condition	Fix Stain (G.D.).
	1	12 15	Moderate Smear	A30. (Schaudinn)
= 10^...	2	1 35	Moderate Smear	and streak for SR+
	3	4 20	Papillate Confluence	
	4	4 20	Separated to semi confluent colonies.	
	5	4 20	well separated colonies ca. .1 mm.	

over: essay for recomb.

C. W1177 of NA (C2) and NSA (C1) .02 ml / 4 agar plates
~~[illegible]~~ - 12N - 2+ PM. Fix Schaudinn. (Many upl of
1. No hydrolysis Cremesa 10. | 1 S patchy but definite | C1 for D...)
(Try fresh NA). No S seen. | 2 Note numerous snakes in this batch
Are S shrinkage artifacts?

D. Perchloric acid. Room temp. from 4:40 PM. Slides fixed ↑. (C1) Stain 1-5 A30.

1	1m.	↓ Progressive general decoloring. No spec. stain
2	12m.	
3	29m	
4	58m.	
5	8 30 A 30.	Same G, v. faint.
6	5 P 30	Weak overall stain. No N.
7 {	8 P 30.	
	"Toluidine Blue 1 min.	

B. Streak out suspensions from agar blocks on EMB lac + sm.

	Lac	Lac sm
1	~~[illegible]~~ +?? −	~~5%~~ +
2	+?? −	"
3	"	<1%
4	"	<1%
5	2+ only	—

some streaks obviously 4-1.

10^{-1} to 10^{-2} OK on agar.

6/30/53.

Schaudinns

A. W1177 ~~10~~ 5x10^-2 ml./ 1. NSA
2 NA
3 NA Suc.

12:10 2:~~2~~ ~~3~~ 1 { 3. large cells, beautifully stained pink + turgid. Walls sharp. No S!
2. Variable shrinkage (dying ?) Cells long: some few snakes No S!
1. Sim to 3; slightly shrunken. Repeat c fresh stain No S!

B.1 W1895 .02 1:0 HCl 3:10 m Sch. fix

2 W2333 .02

3 W1895 + W2333 .02 assay for SR+ . (ca 5%)

4 W2333 + λ. (.01 ml 4x10^10 + 1 ml W2333) .02

5 W1895 · W1177 .05 1:30 - ~~4~~ 3:55 SR+: (ca 50%) lact+??-

B1- 1 Si. char of A1! (A1 std. by G.D. old Giemsa ?)
3 overly hydrolysed! Poor stain N extrusions ? Too hot ?

2 1 Large plump cells. S in patchy ~~...~~ areas only: Dying ?
2. Like B1 character. Numerous blebs + extrusions
3. 1. 3 2 cell types S patchy (covers both types - not clonal)

Repeat 18A1-3:

No Sir!!
?

11/2/53.

A. Effect of drying W1895

1. Fix smears immediately.
2. Dry 1-2 mins. Then fix.

All platings .05 ml covering lot Penassay culture to NSA 12.5 - 24.5 Then refrigerated 1-2 hours before fixing.

B C ~~[illegible]~~ 1x28. B. W2333 < 20s 4 sch. C. W1895 + W2333 < 4/41 SR+.

Assay.

E W2333 + λ (1ml + .1ml 10^{10}) <

D. 1 .01 ml. Assay SR+/- 16/79
2 .02
3 .05 Stain is hydrolysis to judge density. 19/86 19/99

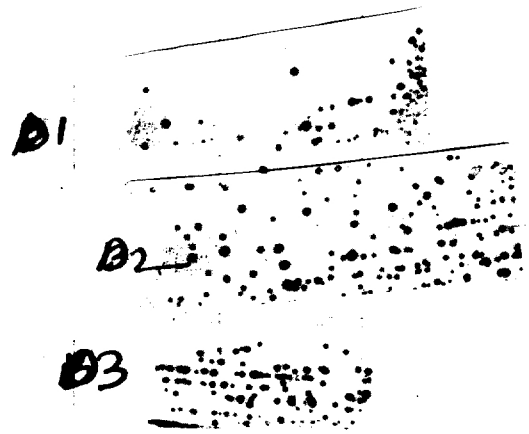
These yields are therefore all comparable and suitable for cytological analysis on agar

A. 1. Brilliant stain old metachromatic N. 2. Flat (Spatulate) new Guinea

repeat 1. but different in other end of assay. no S. (cloud ?) flat n.g.

2. S— flat, dent. no N. 2. same S—

D. 1. Moderate -2- 3 mapping. + stain as in A 1



Seems to be artifact of drying. However all are possibly flattened out somewhat by drying.

Fixation: hence: immediate. Do not dry. old, new Guinea are comparable

± Os probably interchanged

B 1 2 3 same N; (G) ~~heaped up~~

after Os. 1 2 3 N clear. no G

C heaped up

(E) No obvious effects.

No sem.

Brilliant nuclei in 3! G or N in 2 also visible.

1 flat.

2. N brilliant (like 3). occasional G (= "centriole"?)

"Os-2" G, N clear but poor impression

also side looks heterogeneous. probably E3' - dirty

too dense!

A2 1

2 weak G. Heavy background. No N. Same S C.

3

Stains, HCl OK now

MAY was probably artifact. (HCl ca 65°)

11/4/53.

A. W2333 (Effect of drying. Try to make best preps for nuclei. Also drying
1 no dry
2 dry 30 sec. in re B. Plate .01 ml at 9:50 — 12 N.

B. W1895 + W2333. Dilute 1+1 in 8 ml broth (= 1:5).
B. Plate .05 ml 9:50 AM.
C. .01 ml
D. Also incubate broth.

Assay:

	SR+	
C	4/	total bact.
D	7/	ca 100 each.
E	11/	200.
F	1/	

E. W1895 + W1117 as above.
E G.
F

Note: zone stood in 30% alc. after fix till stained

A 1/2 S unstained (>10 mins). Excellent smear impression
S very clear! N faint red.

Repeat 10 m. stain 1. Pos. nuclear stain! No S
2. " " Marked S.

S: again probably an effect of drying: This is not always avoided in handling 1.

B. 1. Good comparison / cell types. S prominent. N met in 1. in places.
Both types prominent. Some N(?)+ in vo. esp.
1' do. C1': scattered cells. V. clear prep. occ S. N(met) v. prominent.
E. Clear N(met.). Scattered S. F. ditto. Denser (E & F).
~~C + Many few cells. (wiped?) appearance as in B+.~~
~~E. + No cells. I do.~~

A – 1 stained 11/4

A–F – 2,3 " PM 11/5.

– 2 (3m HCl)

A1. No G. No S. Faint N granules against cytopl. background almost as dark as A1–1.

B. As above!

E. G prominent – dark blue / grayblue backgr. No N

F. ditto – but N visible also. (bluish)

–3

A1. Vesicular? nuclei c̄ understained spots.

note! dark spot in many cells. –4, –5 = 6m, 10m HCl 15m Giemsa.

4: note G ? [115/65] crushed cells elsewhere.

5: Sp. granules also seen.

B. As above almost peripheral ... ing 28

E. ~~G v. prominent~~ N – (granules) – rather light.

Note 2 shades [2 strains?]

11/4/53.

A. ~~B~~ Rotman preps. (W1317 ~~[illegible]~~) Direct c.g. smears
1 Control — grown in .2% D(glu) Fix Schaudinn. Stain Giemsa
2 Benzyme 10m. still logphase (??hours...) (no HCl)
and washed.

1. Smostly very clear. Same N (red.) Deep, full cytoplasm
(2 slides)
2. Cytoplasm washed out. Some cells shrunken. Others pale, with residual N chiefly in background. Not much detail observable but N seems more vesicular than in other preps.

10/6/53. Schaudinn

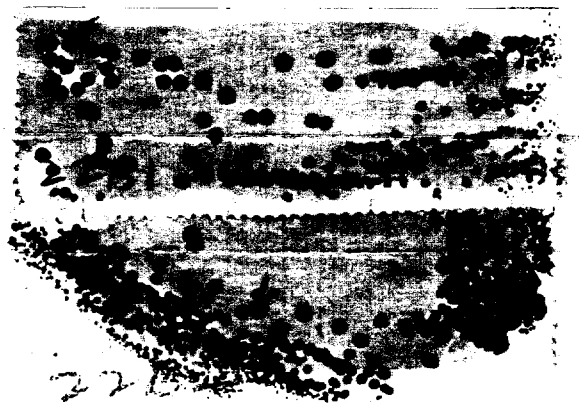
A. Various wg. 1. wg 2 2. wg 31 h.i. 3. wg 47 h.i.
.01 ml/NSA 12^{30} – ca. 2^{30} PM.

1 – 0 HCl
2 – 3 m. HCl
3 – 10 m. HCl

~~25~~ 25.

B. W1895+W2333 fem pronuc assay. .02 ml. Fix and essay.
1. 12^{40} – 2 2. 12^{40} – ca 3 PM.

not countable. 1 ♂ 1%
2 > 1.



C. W2333. .01 ml 12^{35} – ca 3 PM.

1. NSA prompt fix
2. " " Store in 30% ale 3:30 – 8 PM
3. NSA dry ca 5 m before fix.
4. NA. A dry B prompt.
5. NA Suc

11/7/53. overnight broth
W2333 12N-2PM .02ml/.
A. NA suc prompt fix, Schaudinn, alc., water.
B. " " ; left in alcohol

(note same smears fixed briefly in alcohol < Schaudinn's)

C. NSA dried
D. " " /alcohol (1, 2 to 6PM ca 3+ hours in alc) 0 3m HCl

A NoS. N faint neg., occ. mit. ~~Blue~~
B NoS. Coarse cytopl. network. Blue
-1. C S. (cells also dried out).
6PM. D S. very clear. Purplish cyt. homogeneous.
A Most cells uniform purple. Few show clear cyt and N+. No G.
-2 B " " ".
C Some residual S! Begin G. Not very conspicuous. Dense not quite homogeneous purple cytoplasm.
D. Faint residual S. No G.

5, 6, 7 stained 5P8 (B, D stored in alc.) 0, 3, 10m HCl. No C-5.
8, 1 = 5, 7m. HCl.
A see 1.
5 B ~~[illegible]~~ see B2
C
D see D1

A see B2
B "
6 C Cells mixed. Most have minute dark N granules on purple Cyt. Others have clear cyt. (like expected?)
D see D2

7 A N. granules not so sharp. Overstain?
B N granules v. sharp. Mitoses? Many possible ves. nuclei
C cf ~~[illegible]~~ Many cells like 22C3-2 but reddish. (Possibly N. just beginning to differentiate)
D like C7. (Drying definitely impairs sharpness of N.

(over)

No effect of alcohol seen. But no [illegible] in any part!, despite S.

A 8 N only (like A7)
9 "

(what ED calls —
[illegible])

B 8 N only granules v. condensed & sharp but not connections! Obvious N
9 ditto but sharper.
(of 7 more deeply stained).

11/10 - 11/53.

W2333 ca. 2 hours

A. NSA, dry, fix, — water.
B. NA sec — water
C. A sec — alcohol.
D. NSA — A, dry.
11/11. .02 mil 1[15] - 3:15
E. as A
F.

1,2 stained P10.
1,2A " P11.

series also stained 11/12 as marked.

Jan 5

Micrococcus cryophilus

NA · 3 hr old – from old broth culture

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

Jan 6. M. cryophilus

old ~~broth~~ culture on NSA 3 hr incubation
Sch fix - Bienen 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-0 3 hr incubation
Sch fix - Bienen 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 slides

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

unconcentrated cells incubated

19	0 hrs	hydrol	0, 3 min
20	0 hrs	"	5, 10 min
23	½ hr	"	0, 3
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
39	2½	"	0, 3
40	2½	"	5, 10

ng Q duty

centrifuged cells incubated:

21	0 hrs	hydrol	0, 3 min
22	0 hrs	"	5, 10 min
25	½ hr	"	0, 3
26	½ hr	"	5, 10
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

Stain n.g.

Stain n.g.

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

1:1:10 4:1

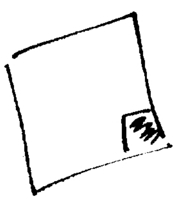
Plate on NSA. Inc. Stain.

H 344.

Abogen 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 ?

	Hyd. 0	Hyd 10
21-23 2/. (Cross.) —	S, N-negative no bridges noted	N but moderately light. No bridges. Cells rather small.
out		
22. out	cells scattering. two types evident. No bridges. Almost homogeneous stain.	as above. Somewhat sharper but would not be adequate for bridges.
23 save	cells sparse dense red globules in many (P) cells (nuclei ?) but background also deep blue.	better dispersion of cells. Nuclei clear, cytoplasm is not. No bridges visible



Need better counterstain ?
(or Amigen in Evans ?)
O-hyd. from NSA is useless
[illegible] for S.

2/3/54

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

~~2/6/54~~
~~from NSA~~
~~H244~~ (P1 x P2) grey cells. Plate + fix at intervals

2/4/54

21 both spread on plate and allowed to dry
hydrolyzed 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

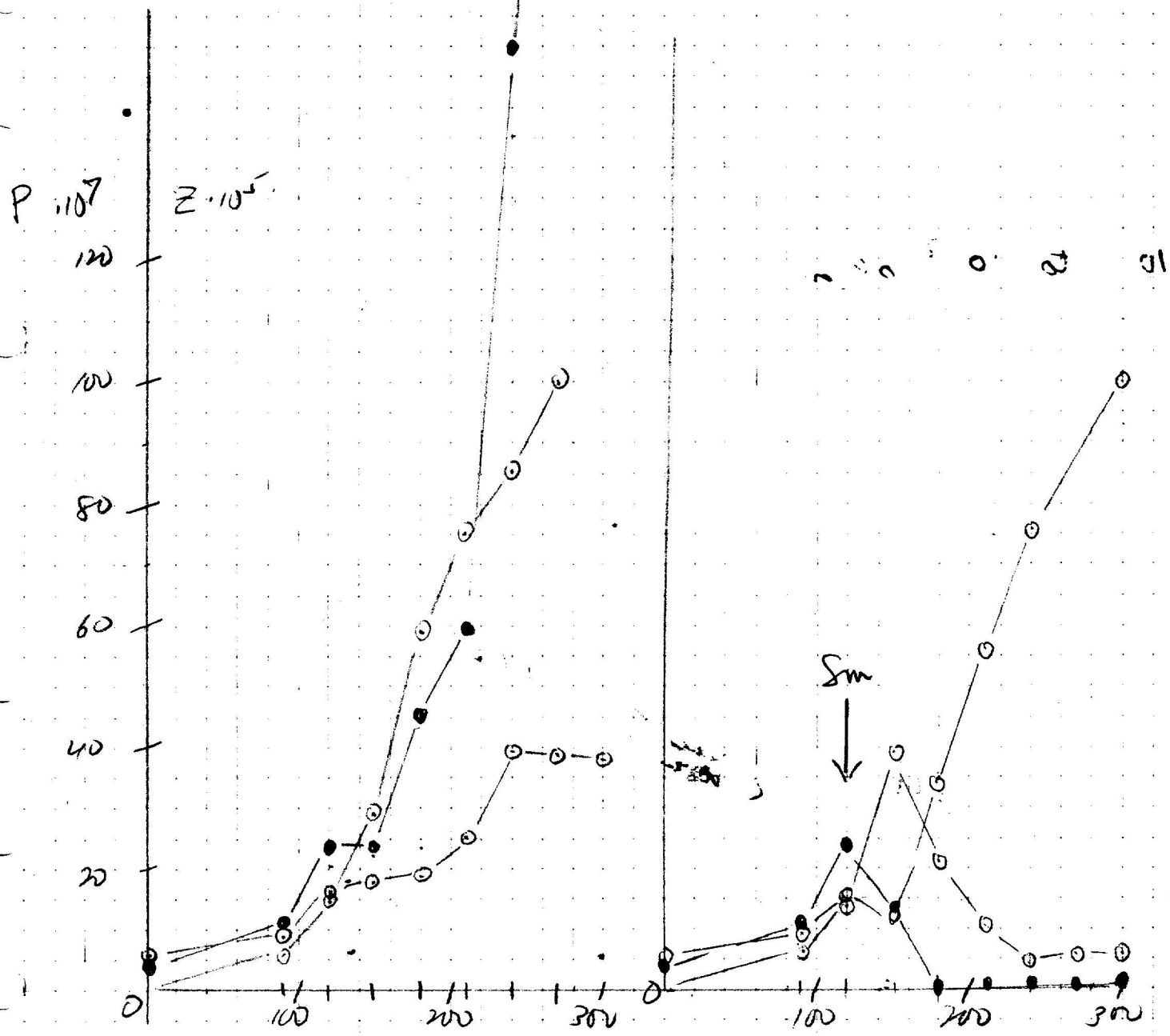
X24 H244 – 0, 10 minutes hydrolysis – from NSA
25 H245 – 0, 10 minutes hydrolysis – from NSA

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

5c Digest. by VT with disp. large cells

Insuff. hydrolysis ?

W895 & W1956 PEN aerated < A. B +Sm 10 µg/ml 120' #97



W1895 × W1956

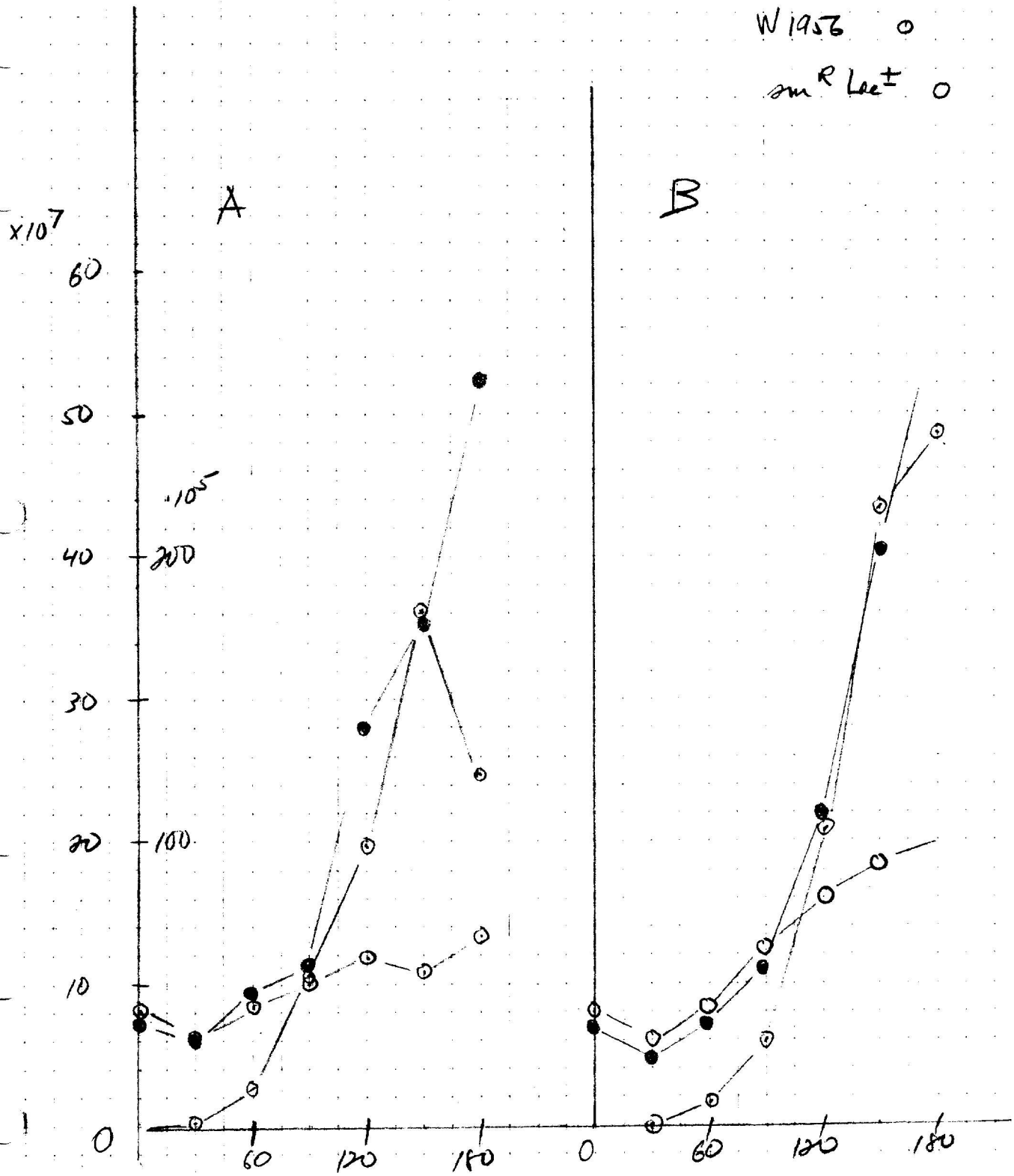
1.0 ml each → 10 ml { A PEN 10 ml, B PEN + DM 10 ml } aerate 37°

(12 hr stand PEN culture)

W1895 ●

W1956 ○

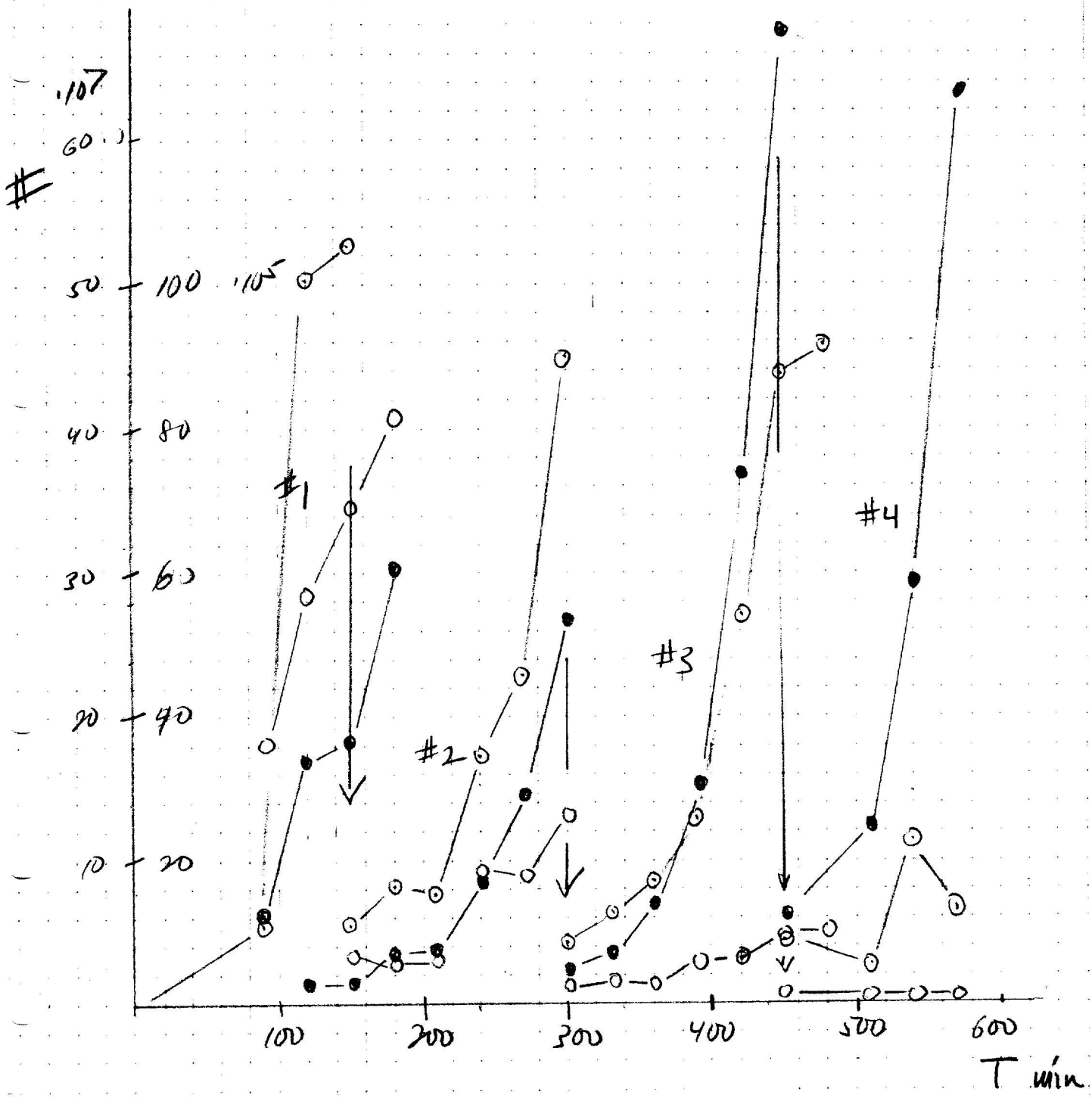
sm^R Lac± ○



#79

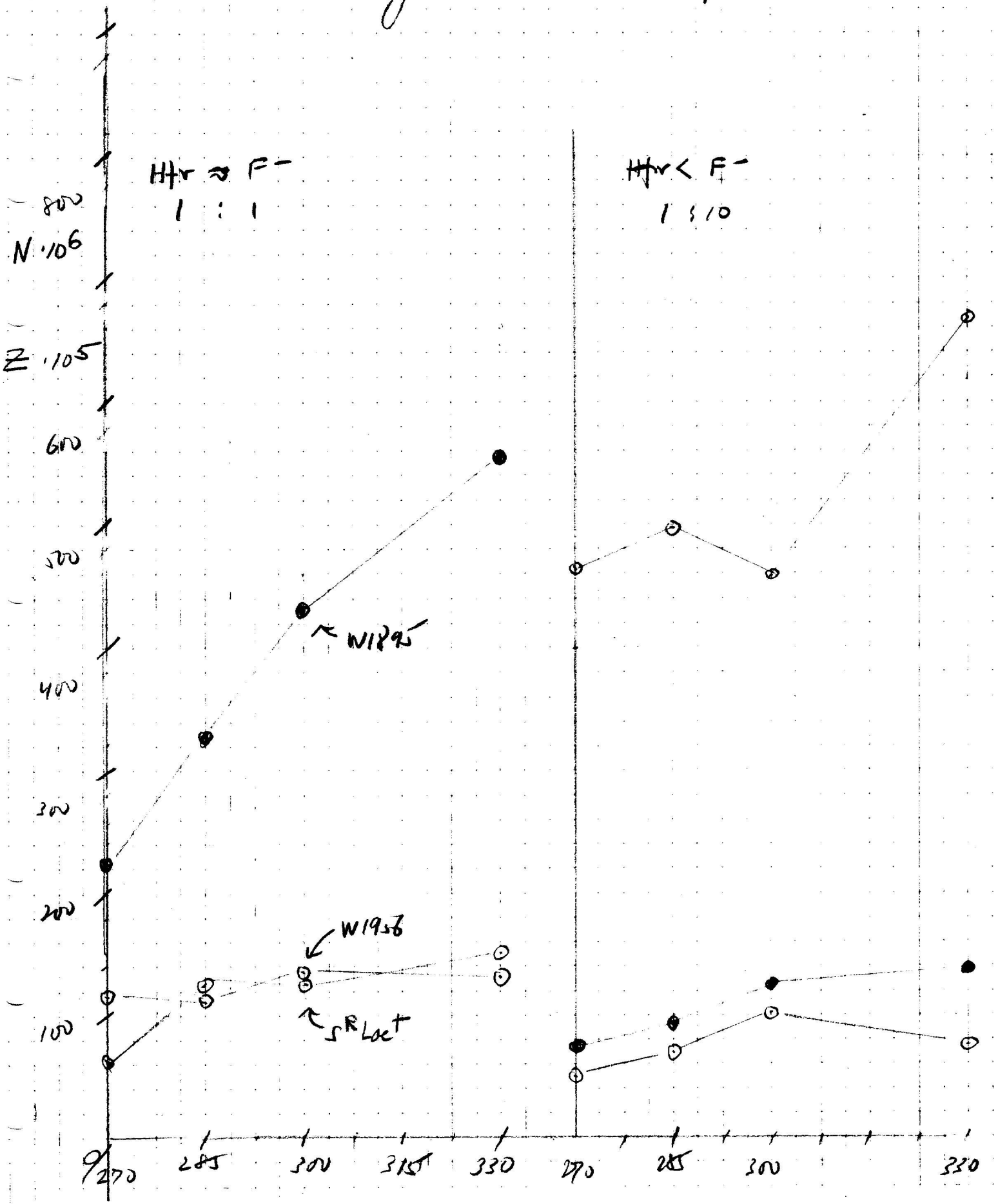
W1895 & W1956 turbidostat PEN+DM + Th 0.2 g/ml

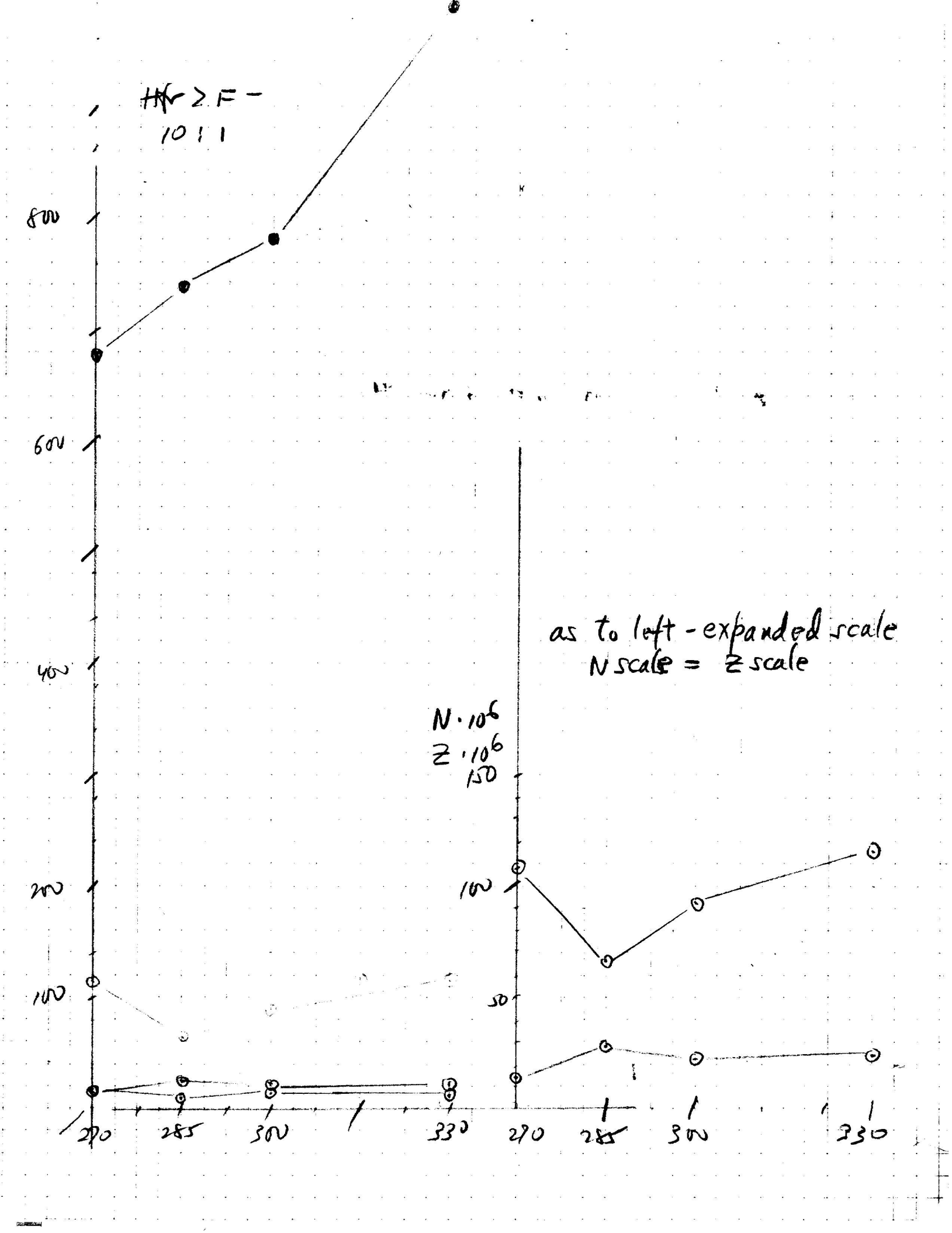
W1956 in initial Xo



#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).

Clafand'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.

222C Series complete except C2.

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

Also. G frass unbuid from Seen:

study!

C4 A. — 2 (4m.) G not pres. (G'l?) Granular cytoplasm! (bluish + reddish) (N?)

C5 A — 2 — N v. clear. G'l No cyt.

		axial ratio	scale 1-12 = 1, 12-3 = 2
A1 -1	N, S. occ	3-5	1+
-2	N! (some cells still have blue cytopl.) shrunken?		1 or 2.
-3	N.		

A2

-1

C. 4 A -1 Flattened, dest. No S. N faint metacher.

4B (5B) N not disrupted. (overstained). Sl. shrinkage but not dest.

5A (4B) Cells sparse. but resemble

5B. (5A) Sl. distortion but less than 1 & 4.

no S.

suggests NA Suc > NSA to minimize drying.

C1. -1 No S. ~~[illegible]~~ N variable

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

-3 Typ. N

C3-1 prominent S

N v v but only in dense accumulations (high salt?)

beautiful terminal S cat

VIII/26.

C2 1 ——

2 acG blobs. 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 - asbest can

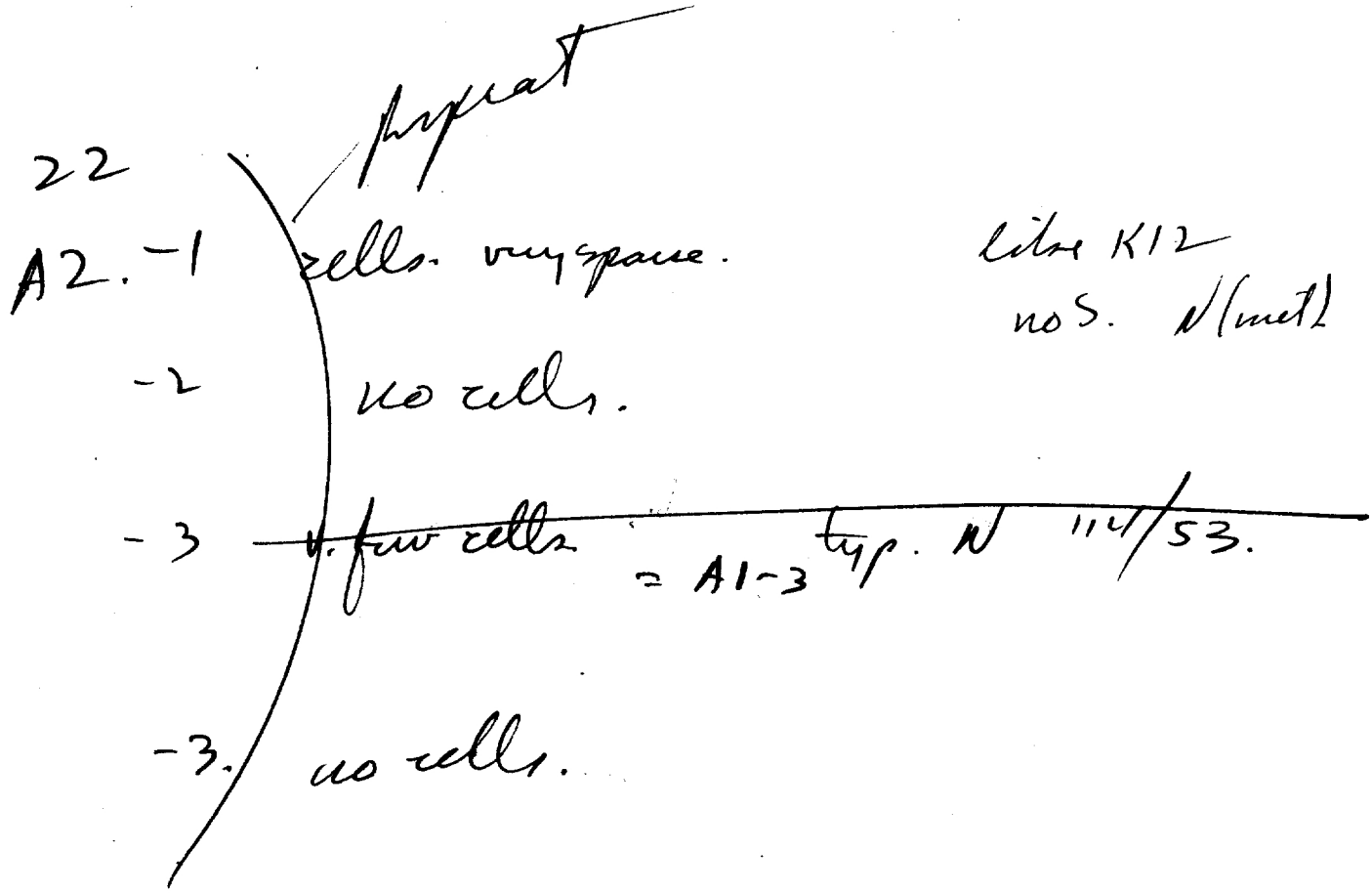
W1637. ~~W2333~~. ✓ other ug...

Fri W2333 HCl series.
Bz effects.
Crosses.

Bz effect

Alcohol effect

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



A3-1 Ax.R. ca 3-4. age 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 wk. series

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

3/31

E (W2049)

10 now OK. Quite distinct from Cm.

cells longer, transverse rather than peripheral elevation.

3/29/24 slides

c2-4
0 : ✓✓ ~~[illegible]~~ N (negative interior). dep ppt.

10 : G but N v weak

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

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1 - C_M 1 ditto

2 - C_M 2 0 faint N ; some S. dep cyt ppt

10 n (washed out) - short stubby cells
some signs of transverse walls.

slides 94

acid or A too much!

3/31/54 W2404
5 W2438
6. W2049

4 stellate cells
hazy. purple

V. l. much detail:

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

13m sl. washed out
but differentiation
of nuclei.

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

13. as 4. N may
be negative.

4/14/54 slides (G D)

B/Ku Hg/ breeing 0 in HCl. N.v. dense blue cytopl., N v.g.

2 deeper? no N detail

3-4 5 pale no detail

10 poor N detail, clear cyt.

(1) D cells red!!

DATE: 4/14/54 REF:

1 2 3 4 5 6 7 8 9 10

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 Co -

3 0, 2 minutes hydrolysis - Giemsa

4 5, 10 minutes hydrolysis - Giemsa

BSI Co - rev

5 0, 2 minutes hydrolysis - Giemsa

6 5, 10 minutes hydrolysis Giemsa