"CYTOLOGY"

1952 - 1954

was in heavy cloth gray-blue binder

Rebound

SEP 12 1983

OsO_4 2 mins HCl 60° 12-14 min Giemsa 30 m. Noteworthy

5/9/52. 1. 1895 x 1956 } fresh culture Nutrient agar
2. 1956 } to Giemsa ca 2 h. several hours.
3. 1895

5/15/52 a 1895 x 1956 " 10^{-3} ml 6 h. Nutr. agar } ca 1%
b " x 10^{-4} ml 3½ h. " } lac + S^R m

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

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

5/20/52 x 3 mins. fixation 2 hrs. growth on agar
a 1895 def. blebs some areas. from 3 hr mix culture
b 1956 no blebs overstained! poor contrast (under hydrolysed ??)
restain but do. Also Tol. Blue s HCl!

5/21. x overnight 10 7 min osmic
a grow. 10 mix 1^30 blebs! fair stain
b plated 6 PM fix cells one small no blebs.

5/22 x 2 3 } min OsO_4 ± (no post fix $HgCl_2$). bleb
a 2 understained blebs
b. 3 esp = v. rare!

Sept 22 - 1 9-drops Giesma - understained, underhydrolyzed

Sept 24 2,3 12 drops Giesma hot HCl - 10 min at 58°, overhydrolyzed?, cultures 18 25 old

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

5 fix 2 min with OsO_4 "

6 fix 1 min OsO_4, 8 min at 65°. "

7 8 min at 60°

9/28/53. C2

1. W1895 ca 4/[illegible] from broth [illegible] (see 1075)
fix OsO4 2m. HCl 8 - 10 - 12 ... [illegible] 1:50
Restain 15 m. in fresh Giemsa (1:20 + .01% MB)
8, 11m HCl slides. Both show blebs.

2. W1895 (A) and X (B) — A. cells jumbled, numerous small protuberances but slide also dirty and understained.
B. cells small, jumbled. Blebs?? poorly developed

3. X from heavy mixture grown in broth, on NA at room temp!
1. Os - [struck] - no HCl — no [illegible], followed by MB
2. Os - Hg - no HCl — Giemsa — not saved, no record
3 - [struck] " 10m " " 30.

1. No stain in eosin. Solid MB, no blebs
2. ~~cells~~
3-4. (30m) (40min stain). no prominent blebs.

fix 2 min over OsO_4

$HgCl_2$ 1 min, ALC | H_2O | 1 m cold HCl | 8-10 min 60° HCl

1/20 Giemsa - 30-40 min | rinse, buffer, dry on

Sept 29 | 55° HCl

1 ——— | ~~no contrast~~

2 5 min | ~~little contrast~~

3 8 min | washed out

4 11 min | " "

W1895 .001 ml 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 Giem 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.
Blobs quite pronounced in 1.

Why Giemsa n.g?

10/6. E. W1177 [illegible] hydrolyzed, overstaining (80 min.)
1. Toluidine Blue 1%
* 2 Giemsa 1:10 in KP M/200! (Probably o.k. if [illegible] time)

CYE2: W1177. Blebs permanent
(onctamid)!

"Vital Staining"

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

Add various dyes to ca .3% Incubate 2 1/2 hours.
in K P buffer 7.5 M/15.

Centrifuge:

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

~~Other~~ Color in sup't: Resazurin
5 ~~Br~~ phenol
6 Congo Red
7 Trypan blue.

} Resuspend. } pellet almost colorless
light blue
distinct orange
distinct blue

Mici: 3: Various ~~ce~~ cells differently stained, but uniform 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 mici color.

6 No mici color.

7 V. faint blue, doubtful whether can be used

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

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

9/28/53.
-30.

add sterilized dye solutions to dense broths 1 hour.

W1895–1177.

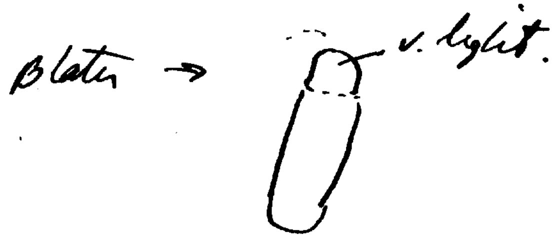
Janus Green C

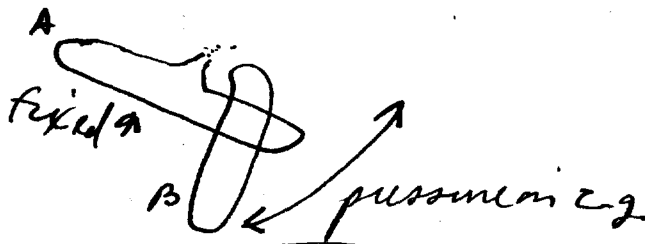
Acridine Orange A

Brilliant Cresyl Blue B — rapidly decolorized; restored on aeration.

Micr. indistinct

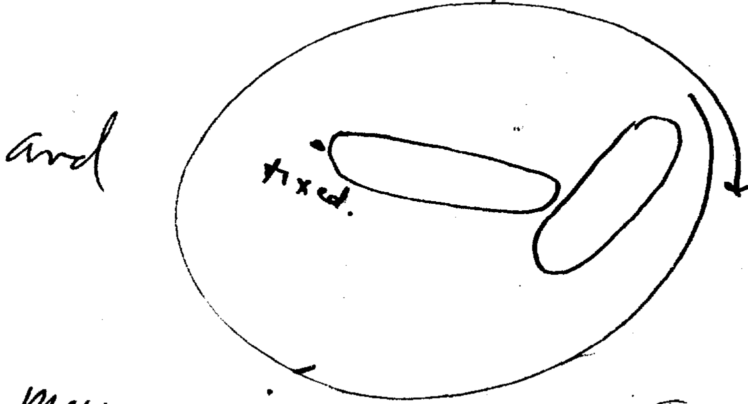
other cells also begin to "plasmolyse"?



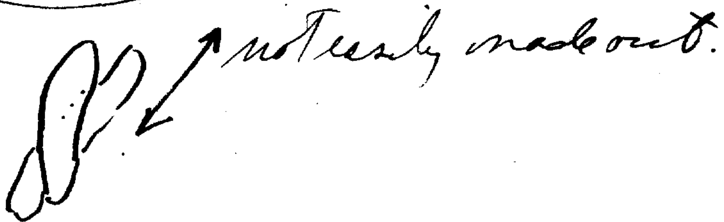


W1895 + W1177 2a shows

and



and many pairs



Giemsa.

7 min Os. 12 min N/1 HCl 60°. 30 min Giemsa in pH 7.5 buffer.

9/17/53. poor contrast. overhydrolysed??

Agar plate probably too thick. (10^{30}/– 3^{30} – 4.PM)

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

Try Fe-Hem. 30 m. 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 15 m. ✓

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

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. S [illegible] 12 minute hydrolyzed were not much different than the 8. Acid weak? All took the stain well. New batch of Giemsa stock solution

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

Living labelled cells + crosses.

Standard system. Made W1177 in .005% TZ.
Mix .1ml c 1ml W1895 in Penassay (7ml)
Examine at stated interval. No aeration.
Assay on EMB lac m.

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

10/17 See 1072. W1177 culture n.s. (T.O.).

Giemsa troubles.

10/16-17. Stain lithuito [illegible] in dilute KP buffer ca 7.5 This is
A). probably too alkaline. Slides overstained (40-50 mins.)
responded nicely to decolorization in dilute HcOH. Check
further on 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.

pH6 shows most promise
also try decol. 7.0, 7.5 in M/100 buffer pH5 1 minute. did not remedy overst. cytopl.

C. 10/18 W1895* Pure assay (to D/O) overnight. pH6...staining

*TZ.

	Buffer		Stain	
1	6	M/20	.9% Tol. Blue	
2	6	M/100	Giemsa 1:10	
3	6	M/10	"	"
4	6	M/100	"	"
5	6	M/100	"	1:20
6	5	M/100	"	1:10

Most TZ in poorly stained cells or extracell.

+ T. Blue .1%

1: medium contrast, TZ readily observable. State of cells? fix?
2: cytoplasm overstained. TZ not easily seen
3: slide acc. ruined, 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. " "
5. " 1:50 M/10 " " "
4. " 1:10 M/100 overstained!
6 " 1:50 M/100. Deeply overstained !! (incomplete 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. pH 6 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. O_s – $HgCl_2$. 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 Os Hydroly { 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 Serra 6 alc : 3 form : 1 AcOH Stain v. light 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!)~~]

TE* 2½ hours NA.

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

Note: TE 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.

[concavities not noted in C 7A but dye at pH 6.
RNAse slide not very clear]

10/20/53. F. x Schaudinn ca 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 stain. (Compare with polar granules in D3, 8&4).
Issues: 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 + .025% TZ) 9³⁰AM-1³⁰PM. Distinctly colored culture.
1. Direct smear as above.
2. Incubate on NA to 2⁴⁰ (70 mins).
3. 3⁴⁰
4. 4²⁰.

A = hydr. B = unhydr. 8m.

(1B) (cf. A3.) Most cells rather short, have TZ granule. Occ. cells c̄ polar concavities. Some cells have 1 TZ + 1 polar conc. 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 latter have TZ. Plump cells as above. Nuclear stain metachromatic. Occasional concanthris.

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

---

4 B. (A unf. lost) Somewhat crowded. Similar to 2, 3. Occasional concanthris. (overstained in 10 mins; fresh!)

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

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

A. (15 min.) definitely superior to B., somewhat overstained
3: distinct polar bodies
but slow-acting: 6. occ. polar bodies } blebs also.
10

C. W1895* overnight. NA 11AM to: (1) 1:30 PM (2) (Hoc 1/100) 2:45
(3) (Hoc 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% 30 sec
B. Toluidine Blue .05% 30 sec (?
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.

opt. ca 4½-5 bonus.

10/21/53

Grow W1177 in TZ overnight. A. (B) 9^{45} inoc TZ Broth c A., and (C) c 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. microcolonies. Fix = C (E/E)

3:40 visible (A2) under phase n = ca 10^3. Most microcolonies have residual TZ granule in a more or less empty, shorter cell. 6 hours, too long.

D W1177 plain 12^{30} - 2^{10}.

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

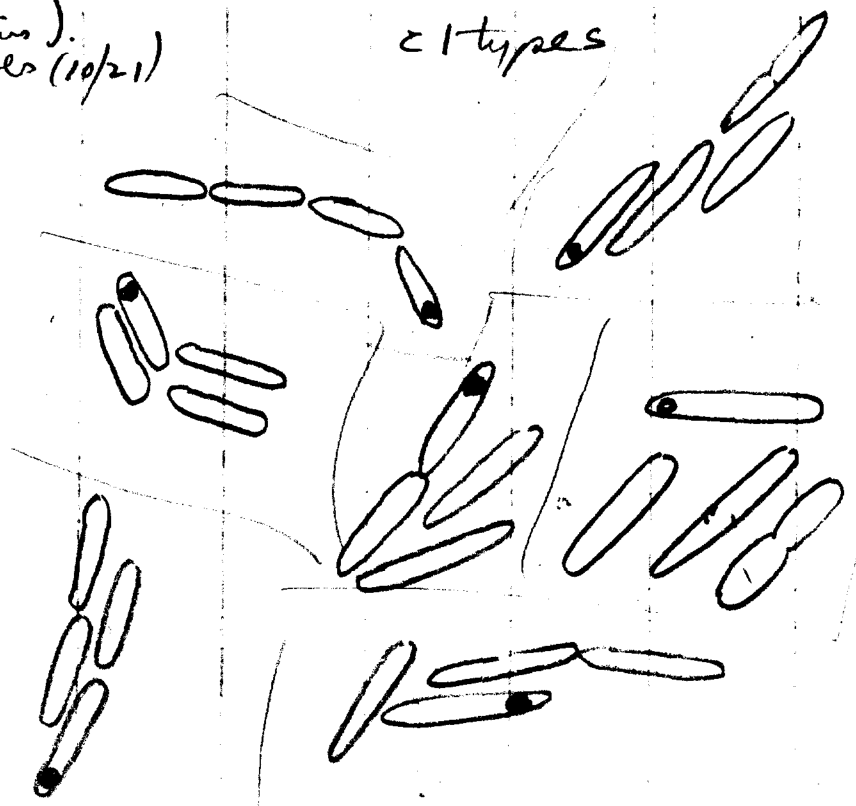
C1. 2PM - 4^{45} C2 2PM - 6:00 C3 2PM - 7:00.

Refrigerate overnight of three individually (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	

single cell unit? 2

C1 types



Granule never divided (could it be?)

Rexamine plates C1 - C3   9:15 AM 10/22.

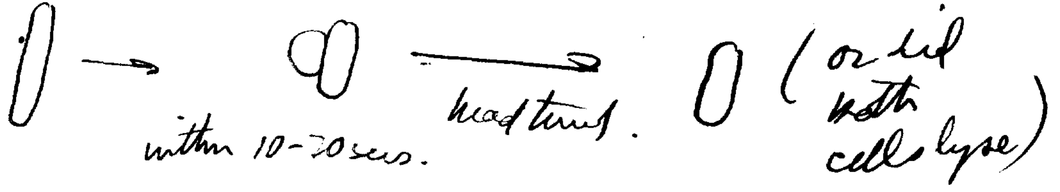
(C1)

+ = long cell almost double but counted as 1

N	T2
3+	1
2	1
2+2gh.	0
4	0
4	0
7	0
7	0
1	1
1	0
1	1
1	1
25	2
7	1
4	1
5	0
4	1
11	0
1	1
4	0
4	1

small but full (for 1 1 rows; " " for second)

noticed a more or less isolated cell originally



Blebs observable on same cells.

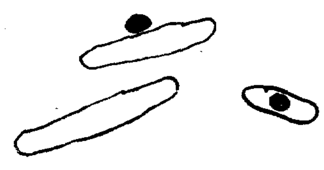
T2 cells in microcolonies not distinguishable

T2 never motile. Occ. cell have empty vacuole

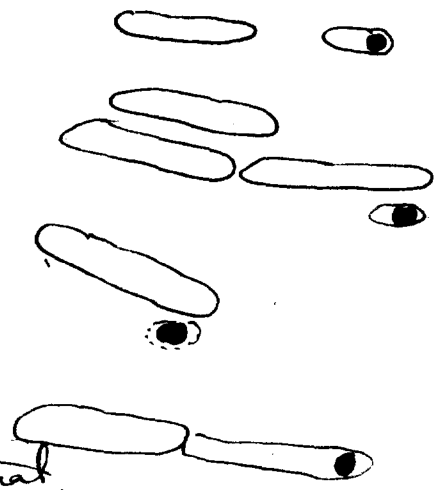
C3. 9:45 (915-445 at Room temp.)

Many colonies uncountable, include smears, and spread out

N	T2	cnd. T
ca 100	0	—
"	0	
16	0	
10	0	
0	1	sl. smaller
2	0	v. small
0	1	
100	0	
> 100	1?	
> 100	1	



N	T2
60	0 ~
30	1
? spread.	
> 100	
?	?
2 or 100	



exceptional.

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

W1177
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

---

B1. Stored in cold water to Sunday 10/25.

1 HCl - 0 m   cells sparse [illegible]
2 - 3 m   Green 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 10⁻⁴ml 8:50AM) (2. 10⁻⁴ml)

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

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

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

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

A0 - stain
A1 8:50 - 12N Refr
A2 12 - 1:35 Refr.
A3 12N - 2PM stain
[10A2 ...]

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

A3 Staining brilliantly. TZ not prominent however:
A3-0 chromatin prominent.
-3 granules not press poles, but often lateral. Probably not hydrolysed long enough.
TZ now only in deg. cells
-6 missing   -10 sparse. gen staining

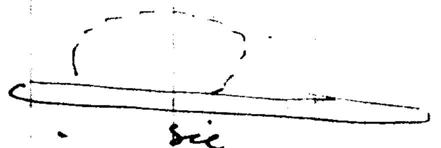
B - direct from scotch. (1) 8:45AM 8:45PM (2) 5:05PM - 8:45PM.

Neither gave nuclear stain. Why liquid medium unsatisfactory?

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

C2. (Stub of 10^{15}). possible blebs on some cells

n	TZ	
7	1	normal
4	0	
11	1	"
ca 40	0	" included a short pair.



Discrete microcolonies not very prevalent. TZ also infrequent. Ranges from typical to reduced, empty small cells. (Pre-critical stage)

survival may be correlated c̄ size of granule.

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

These cells showed slight medium differentiation

suggest lower conc of TZ for viable subclone cells?

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

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

exp. for TZ

C. Effect of refrigeration. 11:45 plates of TZ .002% ↑ .05ml!
1. Fix samples 2:45 10/23. Refrigerate remaining plates
2. Phase observations: 2/3 TZ cells are short, some empty 1/3 normal (generally those with least label). See A4. Comparison suggests a crowding effect. Stained 0-3½-5-6-8-10-12 m. hydrolysis; each stage from 3½m → shows distinct blue granules; fairly poor nuclear stain even at 12m HCl! slides not stored in HCl What is peculiar here? Overfixed? V. fresh stain? 0-3-10 intervals should be adequate for later tests. Go back to refr. plates for comparison.

A) – Examine 2, 4 ca. 3PM. (<5 hours) 2: Colonies 2 – 16 cells.
TZ. normal cell in microcolony 1/16 1/31 1/14 1/>30 1+1e/6 1/8 1/3
2) abnormal (2)5/30 1 single/4
alone. 1 ab s s 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 microc: 1/4 1/2 1/16 1/30 1/4 1/27 1/25
ab " " :
alone : 1n

ditto. of C!

cells spaces.

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

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

acid injs.?

✓ TZ cells no nuclei.

Repeat 10 m. (A1 A3) 10/25/53 A3 First-rate nuclear stg!

TN S & [illegible] 3m. granis beginning to appear [illegible] 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 /[illegible]

A	.02%	Growth ½ inhibited; cells 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.

	~~TI~~ −	+TZ	Free (or no detected cytoplasm)	Type.
A.	6	28	7	variable
B.	1,1	21,36	1,3	
C	8	53	1	ca 80+% st.
D	19, 11, ~~16~~ 14, 32, ~~30~~ 1, 2 (30:76)			about 2/3 stained

E. label rare (< 10%) Considerable free tetrazolium ? or poorly apparent underphase.

F. No label seen by phase. Occ. free TZ. No granularity

Opt conc. for label might be between D and C.

Compare B, D, G platings.

A: axial rods and coils usually free TZ

~~Most~~ the cells c̄ or s̄ TZ show ~~[illegible]~~ sl. refractile usually paired zones.

or.

axial TZ sometimes does not show up under phase

B. granules often doubled.

C. mostly fairly uniform. granules about cell diameter often doubled. aberrant crystals rare.

D mostly smaller granules. occas. forms % 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 on 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 10 m~~. 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 nodes?).
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 slight (? nuclei?) should be repeated with fresh material.
→ should have better Bism prep in this series.

CB. Fix 30 minutes. Store in H_2O ca 4 hours.
4: G very clear. Like 1 above.
10: clear nuclear stg. but axial 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; 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 [illegible]
22 Tol. Blue 1 min
23 Azure 3 mins. Clear stain — S (better [illegible])

[illegible]

Restain after hydrolysis

31 m+ Azure 3 m. G [illegible] (better than Tol Blue (or [illegible], CV)
Tol Bl 1 m. G
CV 1 m. overstained G not evident. almost uniform cyt. stain, possibly [illegible]!
→ CV 30s

22 Tol Blue ± HCl 3 m. S — G
21 Cr. Viol ± HCl Strong background stain
G not seen as such! ([illegible]?)

Oct. [illegible] 1953

W1177/TZ overnight. Mor plates 10:30 AM – Refr. 3:30

.05 ml

.01

.001

.0001

5 ml tubes c

.1.0

.1

.01 ml.

Examine N25, phase.

plate .0001 Nicely spaced. colonies from $10^2 - 10^3$ cells.
>1/2 have 1 TZ cell. These number:

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

normal – 0.

These plates should have been examined earlier prior to crowding.

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

.01 colonies semi confluent, large plane; some heaping

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

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

Fix .0001 ml plate

A Schaudinn
B Osmic · Schaudinn
C Osmic – alcohol.

done hastily. Save only <u>B.</u>

3m HCl – axial dist. heavy G?
10m " – axial shrinkage [illegible]?

Oct. 26, 1953.

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

~~B. [illegible] timessay 1/7. 12:30 [illegible] 1/20~~

C. W1637 – N.A. 9:30 – 12:00 (A) (B)

Stain each 10 m. 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 SchO3.

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 granules
3. Remarkable nuclear patterns. Probably chance this C3B.

W1637 might be useful as test of fixation!

A - Stained P26; stored in water through afternoon

14A.

1. (Sch.)

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

1. S definite but not crisp
Tz mostly extra[illegible]
N very faint.

2. G very prominent, very sharp contrast!

3. ~~No stain!~~ strong N but fuzzier ~~(less shrunken?)~~ than 1

2. Osmic 3m-Sch.

1. No S!
2. Mixed G and N, latter predominant and very dark.
"mitoses" blue
3. ~~[illegible]~~ V. sharp N (purple-red!)
"mitotic".

3. Osmic - Alc

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

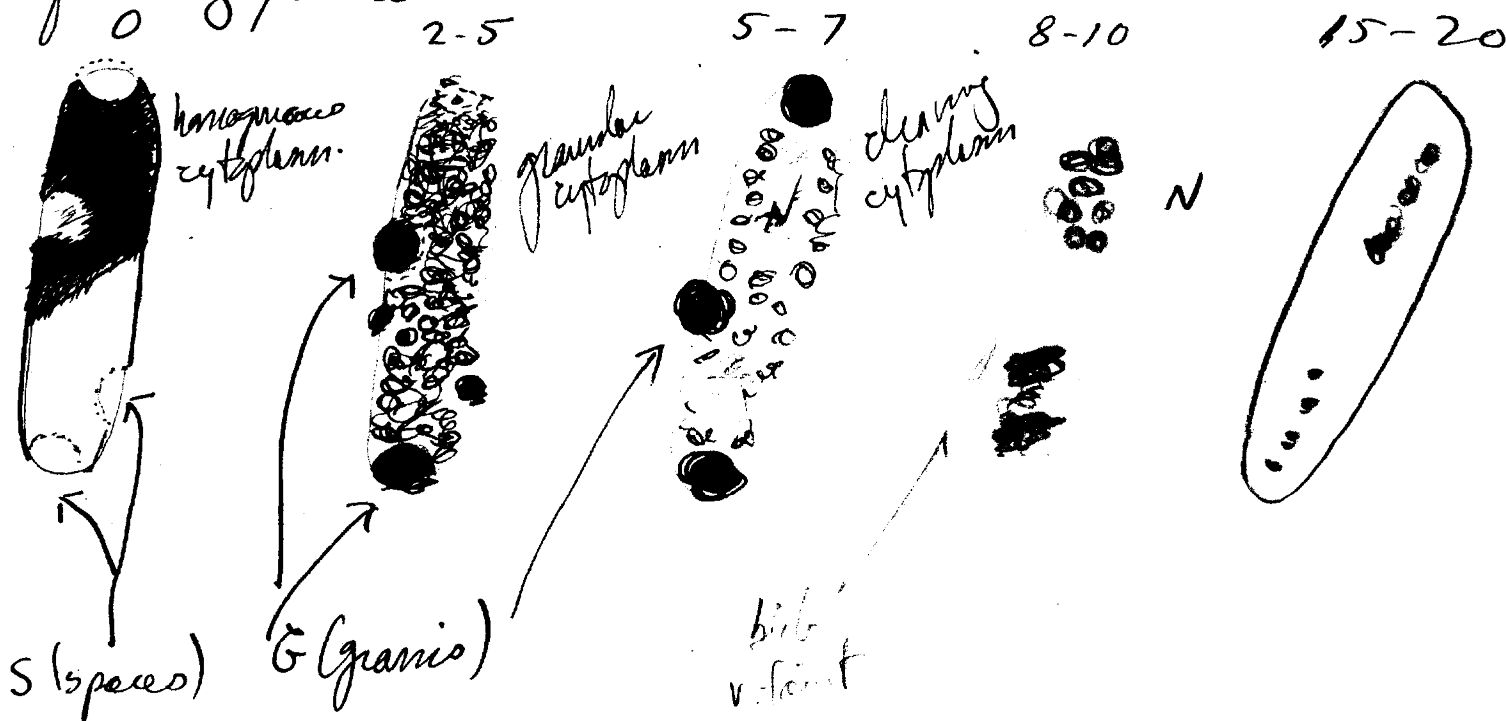
3 needs to be repeated!

2 and 3 are about equivalent.

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

10/26/53. Conidiospore Cytology.

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



In 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 A give picture very similar to Giemsa, but not so sharp or so dichromatic. Crystal violet gives a denser background color at all stages (protein?) so that S, G, N are not so well marked.

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

10-26-59

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

Tt. All cells probably viable, but Tt granule eventually gives a deteriorated cell in clone. Very small granules are consistent with normal appearance. (of 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" under phase.

Oct. 27, 1953.

Repeat 14A3 -2/3.

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

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

(acid must have been weak).

5" - 0
6" - 3m 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 v. faint + cytoplasm clear
~~B~~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. NSA. Fix Schaudinn
K K-12 }

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

i 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 - plasty; distortion ca = 1.
Note: S only in 3! (NSA, as in most expts). Compare cells grown in NSA, NA!

C W1637 11:40 – ~~4~~ 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 No S.
2-1 S
3-1 S ~~[illegible]~~ does not give

Chabaud does also give spaces; Os does not.
Granick - Stronomer
(Later: probably a matter of drying after fixation).

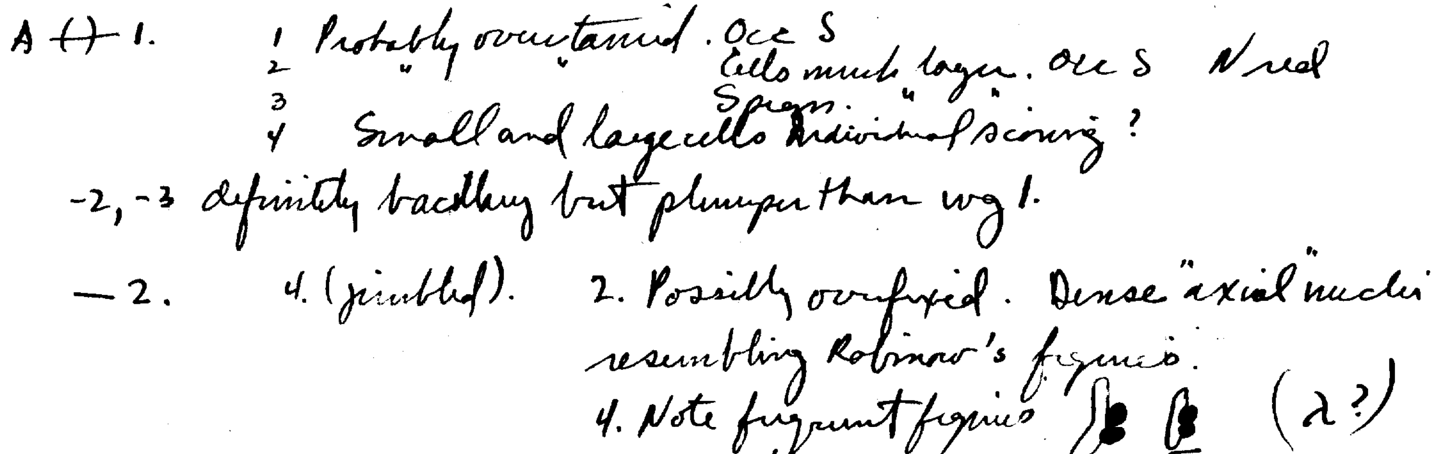
9/29/53

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 bacillary 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+ assay.

	Ref.	hours.	Condition.
	1	12 15	Moderate smear
$=10^{\ldots}$	2	1 35	Moderate smear
	3	4 20	Papillate confluence
	4	4 20	Separated to semi confluent colonies.
	5	4 20	well separated colonies ca. 1 mm.

Fix stain (G.D). A30. (Schaudinn) and streak for SR+

over: assay for recomb.

C. W1177 of NA (C2) and NSA (C1) .02 ml / 10 agar plates
~~[illegible]~~ - 0 12N - 2+ PM. Fix Schaudinn. (Many wpl of C1 for D...)

1. No hydrolysis Giemsa 10. (Try fresh NA). No S seen.
1 S patchy but definite
2 Note numerous snakes in this batch
Are S shrinkage artifacts?

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

1 1m. — Progressive general decolor. No spec. stain
2 12m.
3 29m
4 58m ↓
5 8 30A 30. — Same G, v. faint.
6 5P30 — Weak overall stain. No N.
7 { 8P30. "Toluidine Blue 1 min.

B. Streak out sus[illegible] suspensions from agar
block on EMB lac ± sm.

	Lac	Lac sm
1	~~[illegible]~~ + >> −	~~[illegible]~~ 50% +
2	+ >> −	" (circled)
3	"	< 1%
4	"	< 1%
5	2+ only	——

some streaks obviously n.s.

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

6/30/53.

Schaudinns

A. W1177 ~~10~~ 5×10^{-2} ml./

1. NSA
2. NA
3. NA Suc.

12:10 / 2:20 ~~3~~ 1 — 3. large cells, beautifully stained ~~R~~ pink + violet. 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 4×10^{10} + 1 ml W2333) .02

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

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

2 1 Large plump cells. S in patchy ~~areas~~ areas only: Dying?
2. Like B1 character. Numerous blebs + extensions

3. 1, 3 2 cell types S patchy (covers both types i.e. 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 overnight Penassay culture to NSA 12¹⁵ – 2⁴⁵ Then refrigerated 1-2 hours before fixing.

BC 1x28. B. W2333 < 20s / 4 sub. C. W1895 + W2333 < 4/41 SR+.

Assay.

E W2333 + λ (1ml + .1ml 10^{10}) < ..

D. 1 .01 ml. Assay SR+/– 16/79
2 .02 19./86
3 .05 Stain s hydrolysis to judge density. 28/99

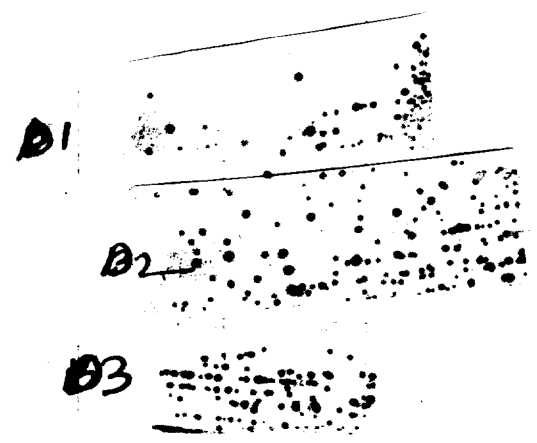
These yields are therefore all comparable and suitable for cytological analysis on agar

A. 1. Brilliant stain metachromatic N. (old) 2. Flat (spotting) (new Giemsa)
no S. (dried?)
but different in other end of smear.

repeat 1. flat n.g.

2. S— flat, dent. no N. 2. same S—

D. 1. moderate – 2 – 3 mapping.
stain as in A1



Seems to be an artifact of drying. However cells are possibly flattened out somewhat by drying.
Fixation hence: immediate. Do not dry.
old, new Giemsa are comparable

± Os probably interchanged

B 1, 2, 3 same N; (G) ~~[struck out]~~ after Os. 1, 2 N clear. no G, 3

C heaped up

(E) No obvious effects.

No [symbol] seen.

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

1 flat.

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

too dense!

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

also slide looks heterogeneous. probably E3' - dirty

A2 1, 2 weak G. Heavy background. No N. Same S 3 C.

Stains, HCl OK now

MA4 was probably artefact. (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. D. Also incubate broth.
C. .01 ml

Assay: C 4/ D 7/ E 11/ F 1/ — SR+ ; totals lost. ca 100 each. 200.

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

Note: some stained in 30% alc. after fix till staining

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

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 of cell sizes. S prominent. N met in places in 1.
Both types prominent. Some N(?)+ in 18 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).
~~E 1 Very few cells. (wiped?) appearance as in B1.~~

~~E. 1 No cells. F 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 c 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 ... ng 28

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

Note 2 shades [2 strains?]

11/4/53.

A. ~~Bs~~ Rotman pups. (W1317) Direct c.g. smears
1 Control — grown in .2% D(glu) Fix Schaudinn. Stain Giemsa
2 Benzene 10m. still log phase (4 hours...) (no HCl)
and washed.

1. S mostly very clear. Same N (red.) Dup, 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 pups.

10/6/53.

Schaudinn

A. Various wg. 1. wg 2 2. wg 31 (n.g.) 3. wg 47. (n.g.)

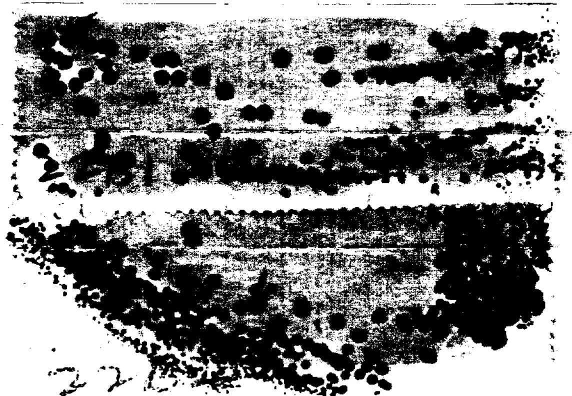
.01ml/NSA 12^{30} – ca. 2^{30} PM.

1 – 0 HCl
2 – 3 m. HCl
3 – 10 m. HCl

~~ss~~ 25.

B. W1895 + W2333 fermentassay. .02ml. Fix and assay.

1. 12^{40} – 2 2. 12^{40} – ca 3 PM.



not countable. 1 ≠ 1%
2 > 1.

C. W2333. .01ml 12^{35} – ca 3 PM.

1. /NSA prompt fix
2. " " Store in 30% alc 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:)

C. NSA dried
D. " " /alcohol

0 3m HCl (1, 2 to 6PM ca 3+ hours in alc)

A NoS. N faint neg., occ. mit. ~~Blue~~
B NoS. Coarse cytopl. network. Blue

-1. 6PM.

C S. (cells also dried out).
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
or
D see D1

A see B2
B "

6

C Cells mixed. Most have minute dark N granules on purple cyt. (like sepd?) Others have clear cyt.
D see D2

7

A N. granules not so sharp. Overstain?
B N granules v. sharp. Metaster? 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 Z 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! [illegible] N
9 ditto but sharper.
(cf 7 more deeply stained).

~~#~~ 11/10-11/53.

W2333 ca. 2 hours

A. NSA, dry, fix, — water.

B. NA suc — water

C. A suc — alcohol.

D. NSA — A, dry.

E. 11/11. .02 ml 1:15 – 3:15 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 Giemsa 10, 12 hydrol underhydrolized
2 Sch fix Giemsa 15, 18 hydrol slightly underhydro.
3 Sch fix Giemsa 18, 20 hydrol ok, mixed
4 OsO_4 ~~Sch~~ 3 min, Sch fix Giemsa 18, 20 hydrol
little different than without OsO_4

Jan 6. M. cryophilus
old broth culture on NSA 3 hr incubation
Sch fix - Giemsa
new acid
5 10, 20 min hydrol 60° 10 little over, 20 over
7 0, 3 " " all look alike
8 5, 10 " "

seems to give better slides

old broth culture on D-O 3 hr incubation
Sch fix - Giemsa
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
13 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	"	S, 10 min	n g
23	½ hr	"	0, 3	
24	½ hr	"	S, 10	
27	1 hr	"	0, 3	
28	1 hr	"	S, 10	
31	1½ hr	"	0, 3	
32	1½ hr	"	S, 10	
35	2	"	0, 3	
36	2	"	S, 10	
~~37~~ 39	2½	"	0, 3	
~~37~~ 40	2½	"	S, 10	

n g v dirty

centrifuged cells incubated:

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

Stain n.g.

(1/10) cells mixed 12^{30} to 2^{10}.

1:1:10

Plate on NSA. Inc. Stain.

Centrifuging took 10 mins (to concentrate)

4:1

Abopon OK again!

H344.

19 } 0 S! cells very dark metach. (N)
20 } 2 G, (N).
5 G, N. Gray background.
10 Nuclei well stained but fuzzy fixation?

21-23 24. (Cross.) — Hyd. 0: S, N-negative, no bridges noted. Hyd 10: N but moderately light. No bridges. Cells rather small.

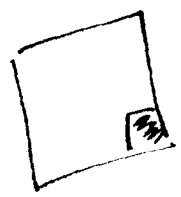
out

22. cells scattered. two types evident. No bridges. Almost homogeneous stain. — as above. Somewhat sharper but would not be adequate for bridges.

out

23 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

save



Need better counterstain ?)
(or Amgen in Gramis ?)
0-hydr. from NSA is useless
exc. for S.

2/3/54

H344
19 hydrolysed 0 ad 2 minutes
20 hydrolysed 5 and 10 minutes

clusters of v. large and intermediate cells.

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

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

2/4/54

21 broth spread on slate 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

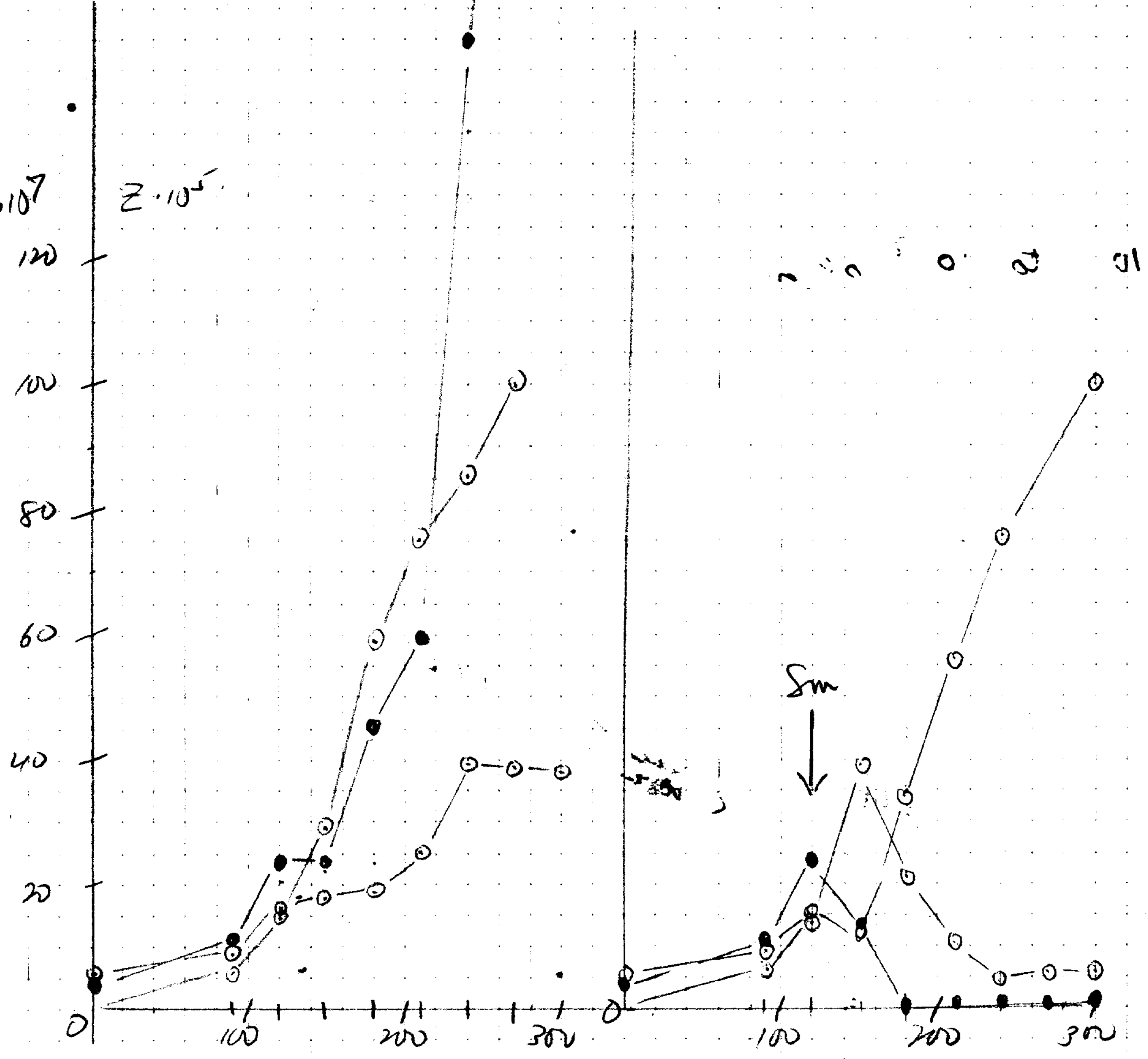
X 24 H244 – 0,10 minutes hydrolysis – from NSA
25 H245 – 0,10 minutes hydrolysis – from NSA

from B (lac) fr. NSA at 12 noon,
fixed 2:30 PM

5 – Neg. light
well disp.
large cells

Insuff. hydrolysis?

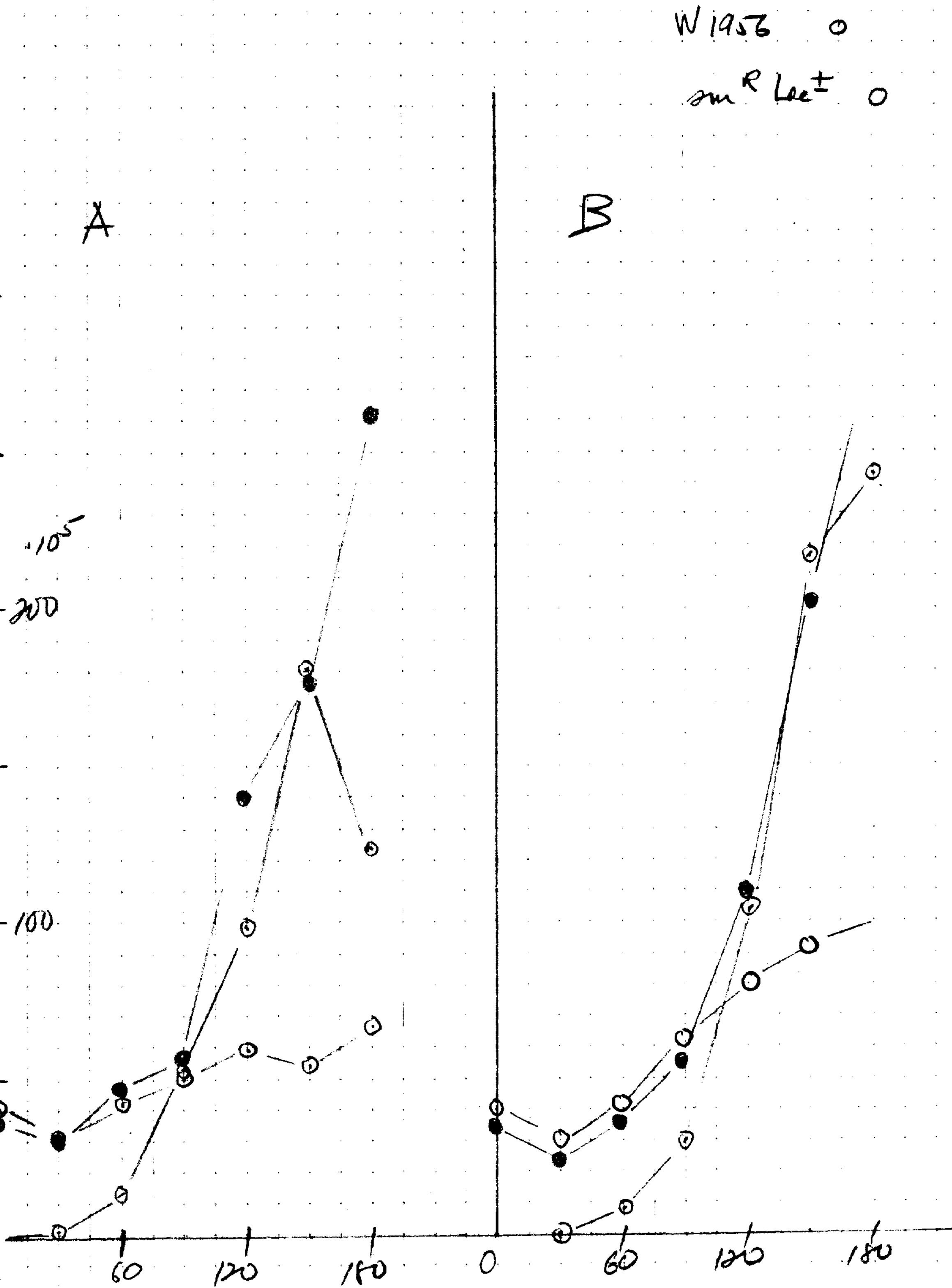
W1895 ♂ W1956 PEN aerated < A / B + Sm 10 γ/ml 120' #97



W1895 × W1956 #74

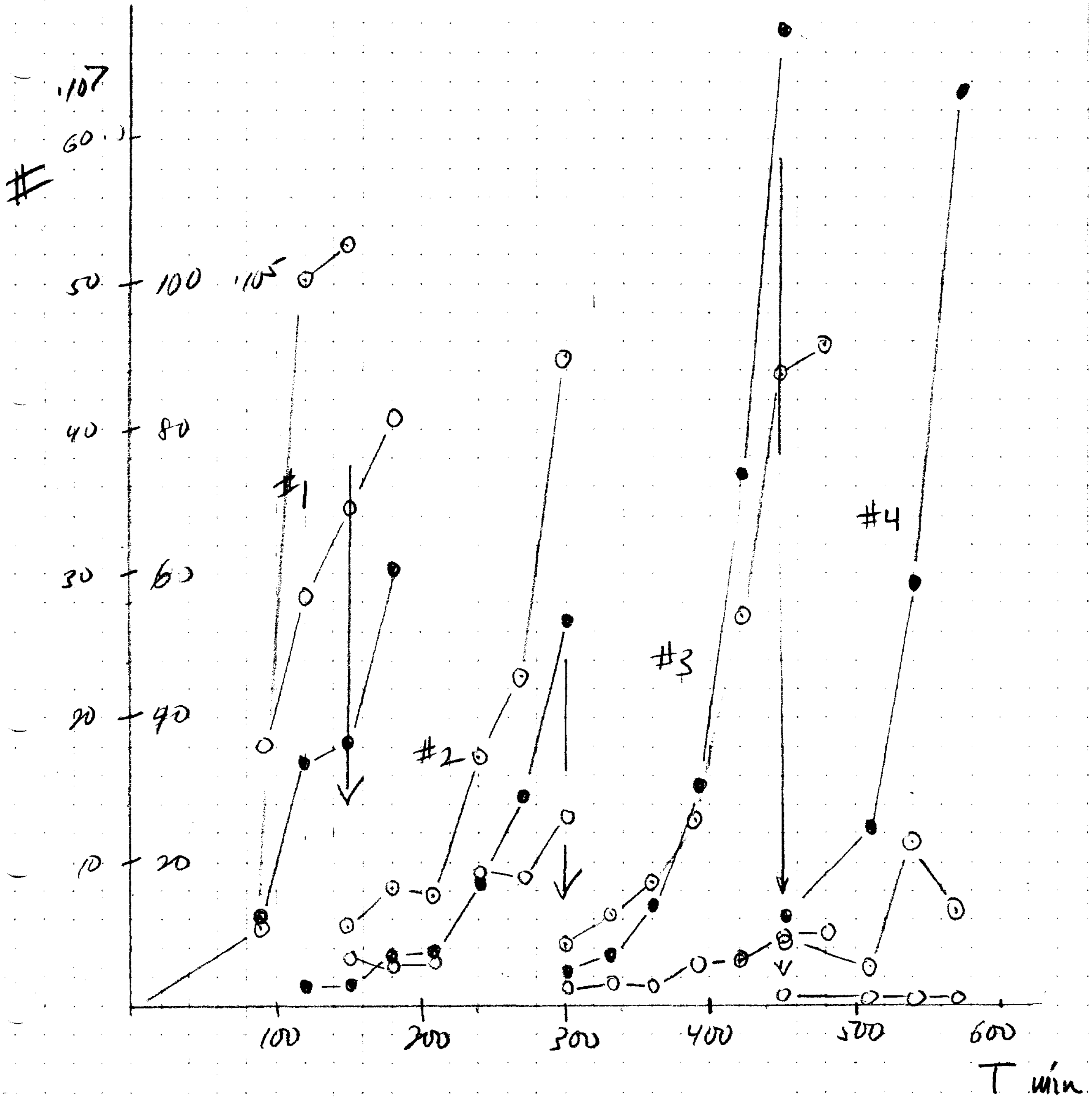
1.0 ml each → 10 ml < A PEN 10ml ; B PEN + DM 10ml } aerate 37°

(12 hr stand PEN culture)



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

W1956 in initial X∅



\#96 *turbidostatic growth* PENASSAY

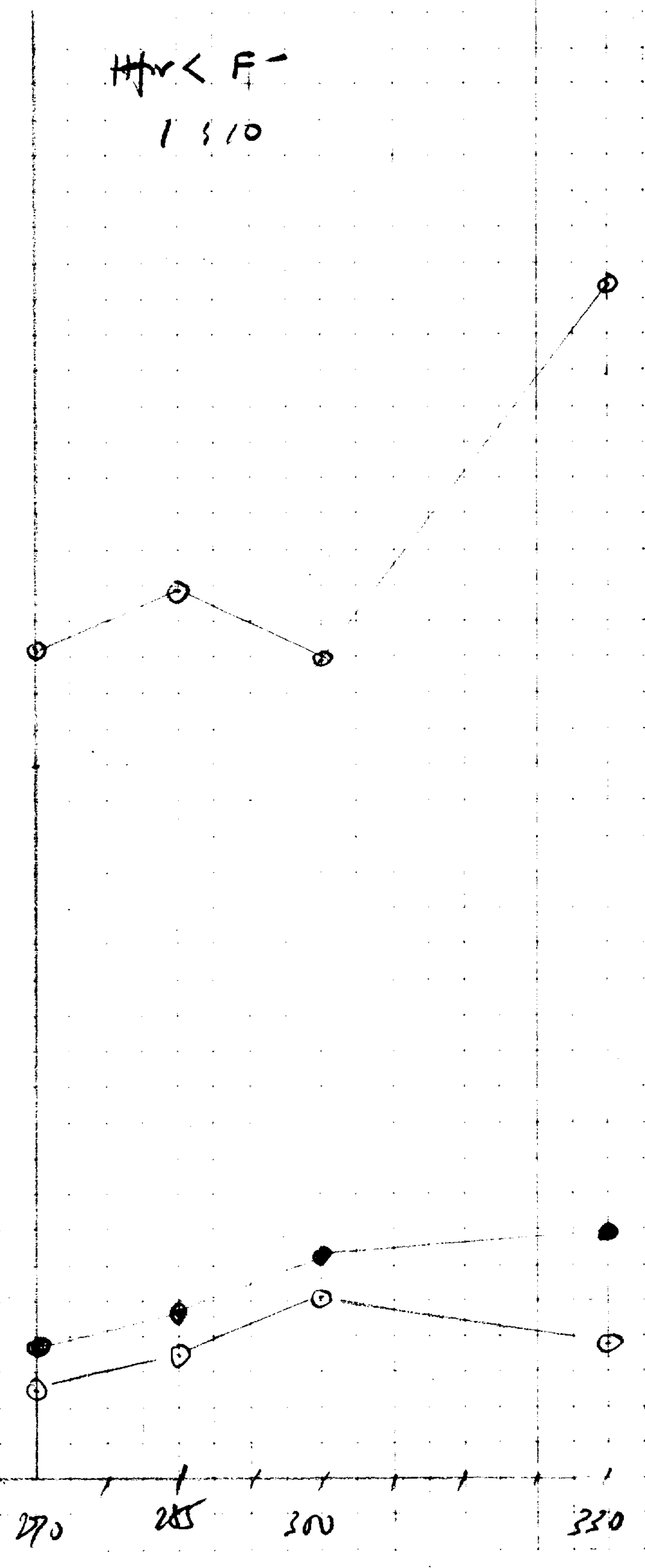
Hfr ≈ F⁻
1 : 1

N ·10^6
Z ·10^5

800
600
500
400
300
200
100

W1895
W1956
$S^R Lac^+$

9/270 285 300 315 330



Hfr > F-
10 : 1

800

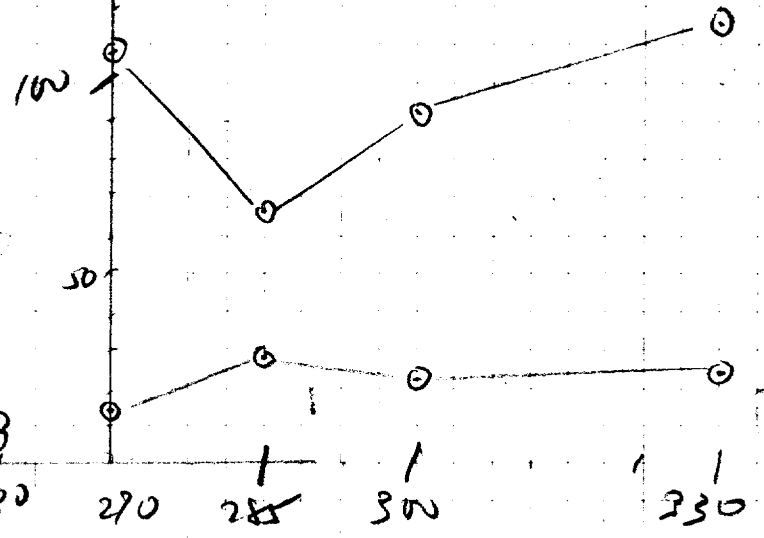
600

400

200

100

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 tetraoxide for 2 1/2 to 3 minutes. Do not dry cells until after fixation. 1 m.
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. 12-14
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.) 5 drops + 2 drops 1% M15/10 ml buffer.
9. Wash briefly in buffer.
10. Mount in water or Abopon (diluted 2 parts in 1 part water).

Chalaud'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 drying flattens cells out 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 Gumsa seems OK.

222 Series complete except C2.

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

Also. G frees unlevel from Series:

study!

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

C5 A — 2 — N v. clear. G'l. No cyt.

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

A2

-1

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

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

5A (4B) Cells sparse. but resemble

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

} no S.

suggests NaSCN > NSA to minimize dispersing.

C1. -1 No 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 at

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 C)?

11/10/53.

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

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

A. W1177 x W1895 from Perrassay, comparing NA ± Sal ± Succ.
Note S only in NSA.

Make nuclear preps - asbestos can

W1637. ~~W2333~~. other neg...

Fri W2333 HCl series.
Bz effects.
crosses.

Bz effect

Alcohol effect

data on Hfr x F- persistence of Hfr genome 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. agi comp. 123?

B1-1 too sparse. T.O.

B2-1 too dense in parts but usable.
stain up balance

3/26

Cm 0, 3, 5, 10 sec. 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 chromatin.

3/29/24 slides

c2-4

0: ✓✓ ~~[illegible]~~ N (negative + [illegible]) dep [illegible]

10: Gr but N v weak

---

c1-3 0 as above. Some S v weak staining

---

1 - C_M 1

ditto

2 - C_M 2 0 faint N ; some S. dep [illegible]

10 [illegible] ([illegible]) [illegible] - short [illegible] cells

some signs of transverse walls.

slides P4

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

acid or A too weak!

4 stalky rods
homog. purple

V. l. much detail:

5. 7m HCl.
Rounded cells
homogeneous purple
blebs! usually polar G8!

13m sl. washed out
but differentiation
of nuclei.

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

13. as 4. N may
be negative.

7/14/57 slides (GD)

Hg/ bremise

B/Kee 00 in HCl. N.v. dense blue cytopl., N neg.

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 and 2, so I did another pair

1 - 0, 2 minutes hydrolysis at 59°
Giemsa stain

2 5, 10 minutes hydrolysis - Giemsa

10

BSI Cu -

3 0, 2 minutes hydrolysis - Giemsa

4 5, 10 minutes hydrolysis - Giemsa

20

BSI Cu - rev

5 0, 2 minutes hydrolysis - Giemsa

6 5, 10 minutes hydrolysis Giemsa

30

40

50