CYTOLOGY"

1952 - 1954

vols in heavy cloth gray-blue bindg

Cytology.

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

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

5/15/52 a 1895 x 1956 " 10^{-3} ml 6 h. Nutr agar
b " x 10^{-4} ml 3½ h. "

5/16-17/52 c " EMB lac 11^30 – 5 PM. 10^{-4} ml
d " 10^{-3} ml } ca 1%. lac+ S^R m moc to agar. — nuclear definition and lateral perturb.

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 " granulo cont n = (2), 3
d x cells small stain n. T.U. ~~growing~~ broth 11 30 – 7 30. Prompt fix agar.

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

5/21. x overnight ~~[illegible]~~ 10 2 min Osmic
a grow. 10 ~~mix~~ 1 30
b ~~plated~~ 6 PM fix blebs! fair stain cells one small no blebs.

x 2 3 ?
a 2 } min OsO4
5/22 b. 3 ± (no post fix $HgCl_2$). blebs
understained blebs
esp = ~~[illegible]~~ v. rare blebs!

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. C2

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

Jf fix OsO4 2m. HCl 8 - 10 - 12 ... Giemsa 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 puckered, numerous nuclei positive but slide also dirty and understained.
B. cells small, puckered. Blebs ??

poorly developed

Φ

3. X from heavy mixture grown in broth, on NA at room temp!
1. Os - [illegible] - no HCl on [illegible], followed by MB
2. Os - Hg - no HCl Giemsa
3 - " 10m " " 30.

not saved no record

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

9/30. A. W1895. fix in Os, $HgCl_2$ ... Attempt stain c Light Green No st. (1% osmium pH 7!0)
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 4th. 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 overnight stain)

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

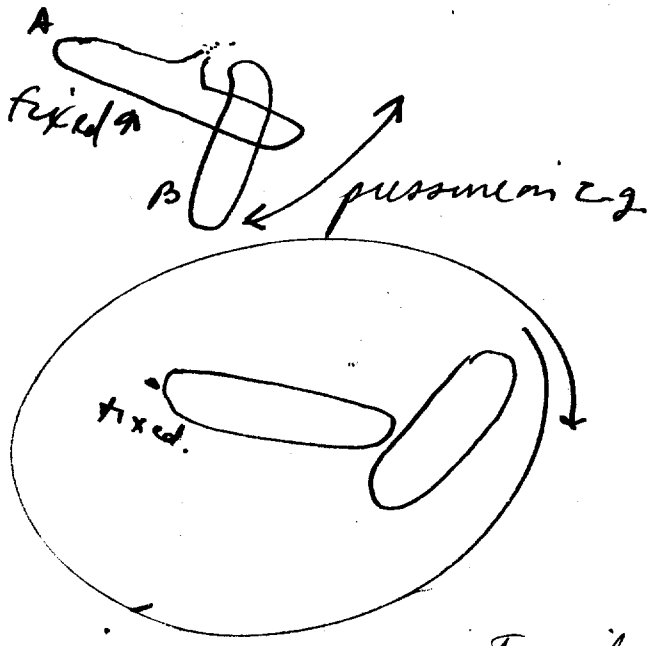
9/28/53. Add sterilized dye solutions to dilute bottles 1 hour.
-30.

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 OsO_4	8 min	HCl at 59°	no contrast
2	"	10	59	"
3		12	59	"

all showed little contrast. So the 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
1 ml Giemsa stock
9 ml water
1/2 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 without 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 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.

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* Penassay (to O/O) overnight. pH 6...staining

*TZ.

	Buffer		Stain		
1	6	M/20	.9% Tol. Blue		Most TZ in poorly stained 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. Gremsa 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 = Gremsa 1:10 10 mins B = Gremsa 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. Os – $HgCl_2$. 10min HCl

1 Giemsa 1:10 12m.	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 $HgCl_2$ only { 5 Giemsa 1:10	A pH 5. B pH 4		[Settle on 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. ~~Be~~ stain
5–10 Osm-Schaudinn not greatly diff. from 1.

1, 3, 5, 7, 9

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 stain. (Compare with polar granules in D3, 8&4).
Queries: viability of TZ stained cells; do granules fall out?, relation to polar grains. cf. also CTEI.

B. Fate of TZ. Move 1895 into TZ broth (= Penassay + .005% TZ) 9³⁰AM-1³⁰PM. Distinctly colored culture.
A = hydr. 8 m. B = unhydr.
1. Direct smear as above.
2. Incubate on NA to 2⁴⁰ (70 mins).
3. 3⁴⁰
4. 4²⁰.

(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, mg. 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 *overnight ~~old~~. 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. one. 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 Kept 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 c̄ A., and (C) c̄ 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 .001 ml per plate NA. 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 is too long.

microcolonies. Fix = C (E/E)

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.

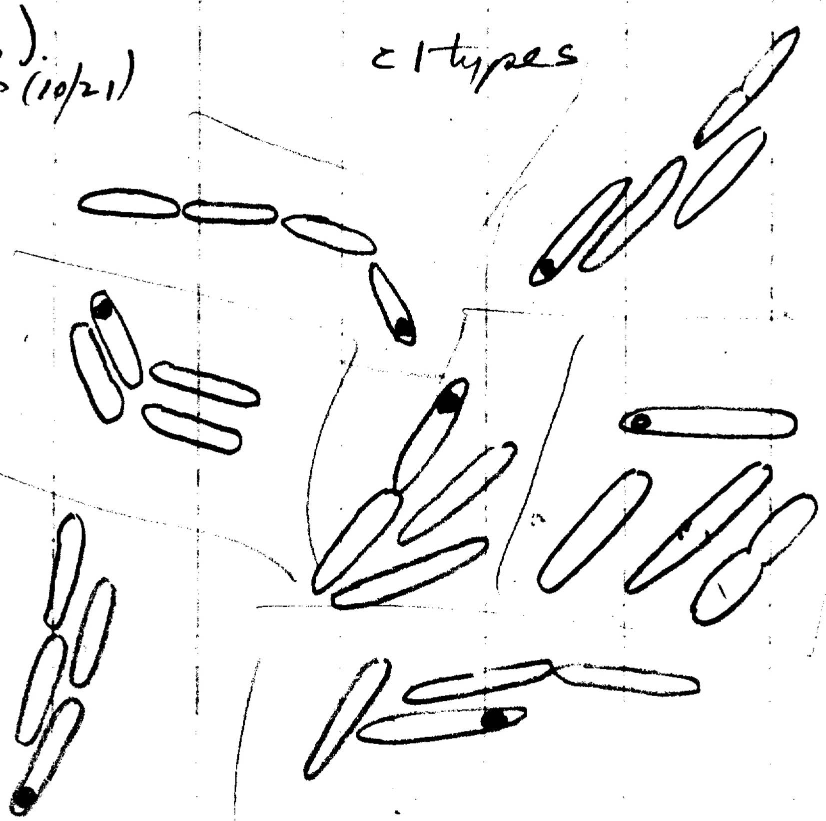
Refrigerate overnight of time indicated

(14 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
2 7	4..
8	

single cell init?

C1 types



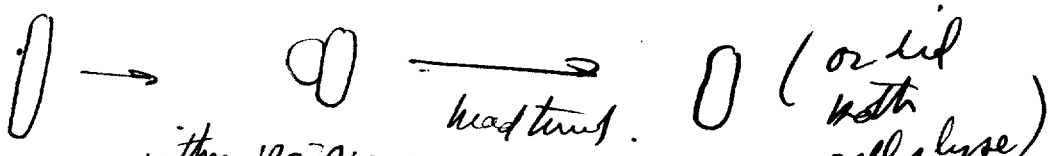
Granule never medial (could it be?)

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.)

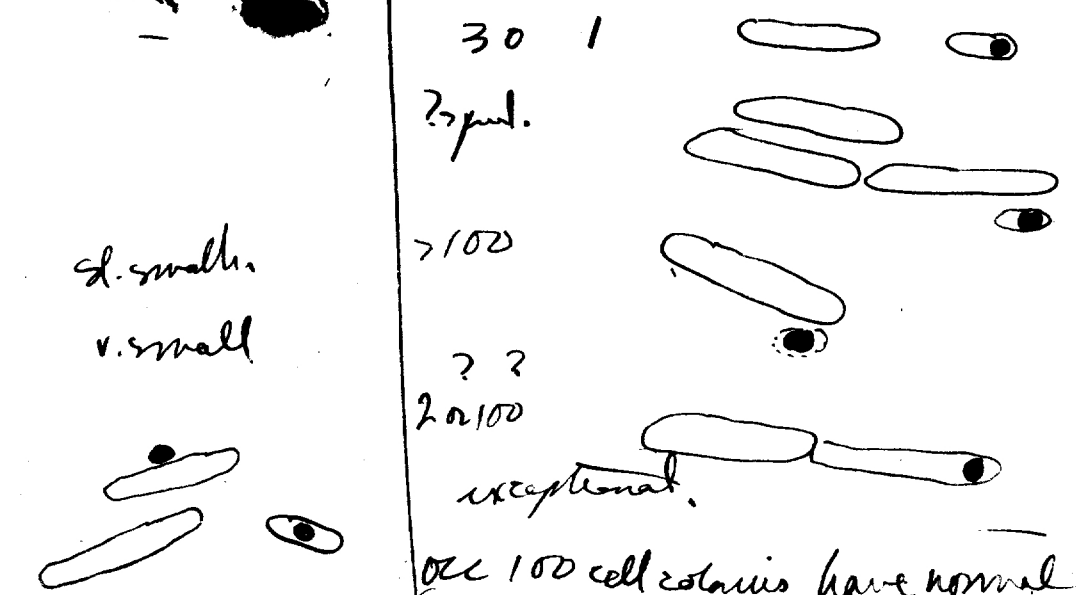
Many colonies uncountable, include cocci, and spread out

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 pml.		
> 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 gran. 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   Swollen 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 ; 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.)

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which refr?

AO - stain

TG A1 8:50 - 12N Refr.

A2 12 - 1:35 Refr.

A3 12N - 2PM stain

[10A2 no nuclear? ... nucleoid.]

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 prom. poles, but often lateral. Probably not hydrolysed long enough.

TZ more obvious dig. cells

-6 missing —10 sparse. green staining

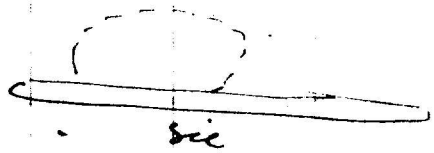
B - direct from scott. (1) 8:45AM - 8:45PM (2) 5:05PM - 8:45PM.

Neither gave nuclear stain. Why liquid medium unsatisfactory?

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