CYTOLOGY"

1952 - 1954

vols in heavy cloth gray-blue boards

Rebound
SEP 12 1983

Cytology. 950

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 } to Parnassay ca 2 h. dense several hours.
3. 1895

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

5/16-17/52 c ✓✓ " EMB lac 11^{30} - 5 PM. 10^{-4} ml
d " 10^{-3} ml

✓✓ nuclear definition and lateral protub.

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

5/20/52 x 3 min. fixation 2 hr. growth on agar } ~~no~~ ~~bleb~~
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 & HCl.

5/21. x overnight ~~mix~~ 10 2 min O_2 mix
a grow. 10 mix 1^{30} blebs ! fair stain cells are small
b ~~plated~~ 6 PM fix no blebs.

5/22 x 2 3 } min OsO_4
a 2
b. 3

≡ (no post fix $AgCl_2$). blebs
understained blebs
esp = ~~blebs~~ blebs !
v. rare

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 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

(see 1075)

1. W1895 ca 4 hours from both to plate
fix OsO4 2m. HCl 8-10-12 ... Gr... 1:50
Retain 15 m. in fresh Gr... (1:20 + .01% MB)
8, 11m HCl slides. Both show blebs.

2. W1895 (A) and X (B)
A. cells jumbled, numerous surf projections but slide also dirty and undertained.
B. cells small, jumbled. Blebs??

overhydrolysed Φ

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 Gr... not saved no record
3 - ~~[illegible]~~ " 10m " " 30.

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

C 3

fix 2 min over OsO_4

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

1/20 Giesma - 30-40 min rinse, buffer, adopon

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% in buf pH 7!)
Light Gum 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 Gum. 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 & further growth. (weak Giemsa!)

10/4. D. W1895 3 hours smear on 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 omhydrolysed, overstained (80 min.)
1. Toluidine Blue 1%
* 2 Giemsa 1:10 in KP M/200! (Probably OK if used right time)

CYE2: W1177. Blebs prominent
(onestamid)!

"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 2½ hours.
in KPhthalate 7.5 M/10.

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. } Reaction. } pellet almost colorless; light blue; distinct orange; distinct blue

Mica: 3: Various cells diffusibly stained, but uniform in any cell. Some cells may show marginal density or wall staining.

4. Cells distinctly orange–yellow. (added by distinct orange
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 ~~cog~~ cresyl blue
Janus Green (cells strongly agglutinated)
Acridine Orange

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

9/29/53. Add sterilized dye solutions to dense brothes 1 hour.
-30.

W1895—1177.

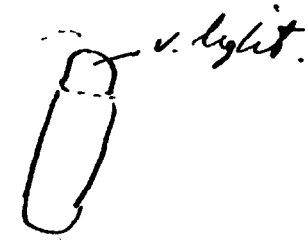
James Green ~~A~~ C

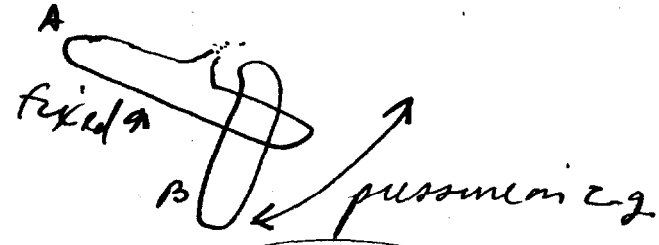
Acridine Orange A

Brilliant Cresyl Blue B — rapidly decolorized, restored on aeration.
Micr. indistinct

other cells also began to "plasmolyse?

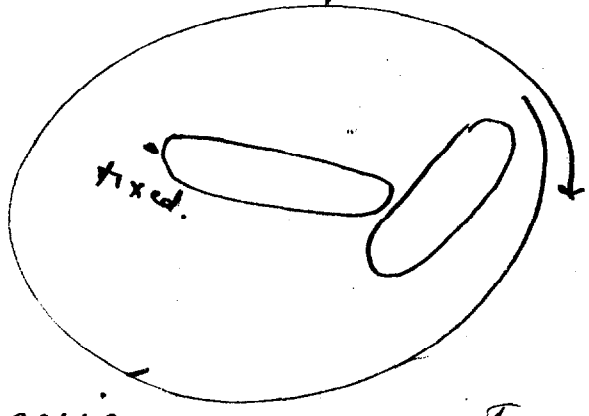
Blater →



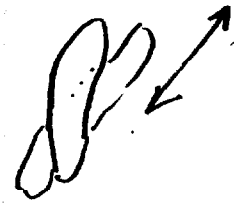


W1895 +
W1177
ca 2 hours

and



and many pairs



not easily made out.

Giemsa.

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

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

Agar plate probably too thick. (10³⁶/- 3³⁰ - 4.A17

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

Try Fe-Herex. 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.

C5

10/6
W 1177

1	3 min	O_5O_4	8 min	HCl at 59°	no contrast
2		"	10	59	"
3			12	59	"

all showed little contrast. She 12 minute hydrolyzed weren't 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
½ ml 7.5 buffer

Living labelled cells + crosses.

Standard system. Mark W1177 in .005% T2.
Mix .1ml & 1ml W1895 in Penassay (7ml)
Examine at stated interval. No aeration.
Assay on EMB loc rm.

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

10/77 See 1072. W1177 culture n.g. (T.O.).

Giemsa troubles.

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 HeOH. 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* Pinassay (to ~~0/0~~) 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	" "	+ T. Blue .1%
5	6 M/100	" 1:20	
6	5 M/100	" 1:10	

Most TZ in poor stain cells or extracell.

1: medium contrast, TZ readily observable. State of cells? fix?
2: cytoplasm overstained. TZ not easily scored
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* 7 hours NA. O_s-Hg. left in H_2O 3 hours.

pH5

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

HCl 10.

Delicately hydrol.

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

E W1895* 34. NA O_s-Hg. in H_2O 3:40 -

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

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

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

of 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_3 - $HgCl_2$. 10 mm 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 mils further trial.

No O_3 $HgCl_2$ only { 5 Giemsa 1:10 A pH 5. B pH 4 $\boxed{\text{Settle on Giemsa at pH 5.}}$

No obvious advantage to lower pH for any material.

B. W1895 11:50 - 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. ~~B~~ stain

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

1, 3, 5, 7, 9

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

TZ* 2 1/2 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. hyds.

4 Numerous polar granules.

6 Blebs very distinct. Rare polar granules.

8 similar to

10 blebs also noted. B. Most Brilliant nuclear stain

12 " " strongest nuclear contrast in B, weaker in A.

Main problem now: fixation.

[cnuarities not noted in C7A but dye at pH 6.
RNAse slide not very sharp]

10/20/53. Fix 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. (3-4) (10, 20 min staining).
(stain? see below).

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, 8C4).
Queries: viability of TZ-stained cells; do granules fall out?, relation to polar grains. cf also C7E1.

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

(1B) (cf. A3.) Most cells rather short, have TZ granule occ. cells c 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 uni nucleate; long cells 4-nucleate & TZ

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

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

3A. About 5 plump cells. plump : 1 short. Some of cells have TZ. 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 TZ.]

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

(overstained 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 O control to detect concavities).

A (15 min.) definitely superior to B., somewhat overstained

3: distinct polar bodies

but sl overstain | 6. occ. polar bodies } blebs also.
10

C. W1895* overnight. NA 11AM to: ① 1:30 PM ② (Moc 1/100) 2:45

③ (Moc 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 P21. (10, 15 min.)

	Hyd.
A	0
B	3½
C	6
D	10

Intermediate development grains + nuclei but slide dirty.

opt. ca 4½-5 hours.

10/21/53

Grow W1177 in TZ overnight. A. (B) 9^45 moc TZ Both c̄ A., and (C) c̄ unstained W1177.

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

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

2PM. C (live) shows ca 90% TZ - fairly long cells Moc C1 .001 ml per plate NA. 3:40 visible A2 microcolonies. F\x = C (cf E) 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 12^20 - 2^10.

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

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

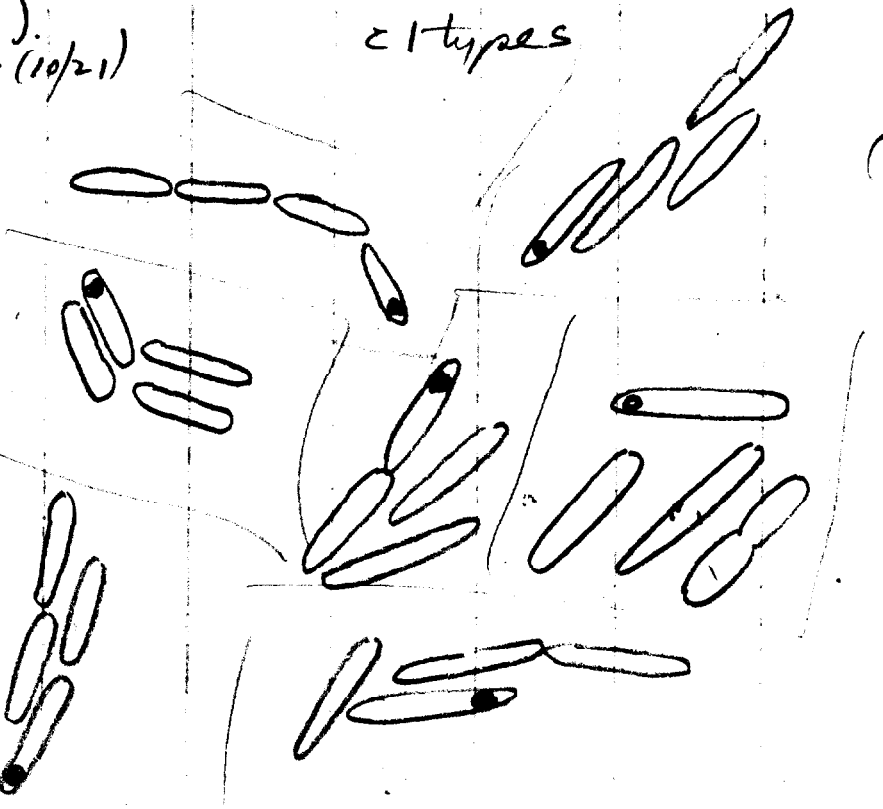
Refrigerate overnight at time indicated

(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
27	4..
8	1

single cell unit ?

c1 types



Granule never medial (could it be?)

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

C1

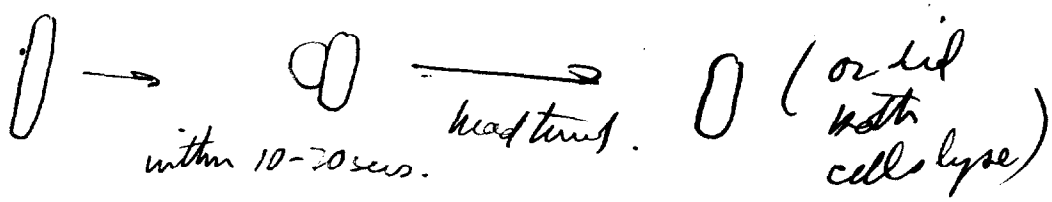
N	T2
3+	1
2	1
2+2gh.	0
4	0
4	0
7	0
7	0
1	1
1	0
1	1
1	1
25	2
7	1
4	1
5	0
4	1
11	0
1	1
4	0
4	1

+ = long cell almost double but counted as 1

small but full

" "

noticed a more or less isolated cell originally



Blebs observable on same cells.

T2 cells in microcolonies not distinguishable

T2 never medial. Occ. cell have empty vacuole

C3. 9:45 (915–945 at Room temp.)

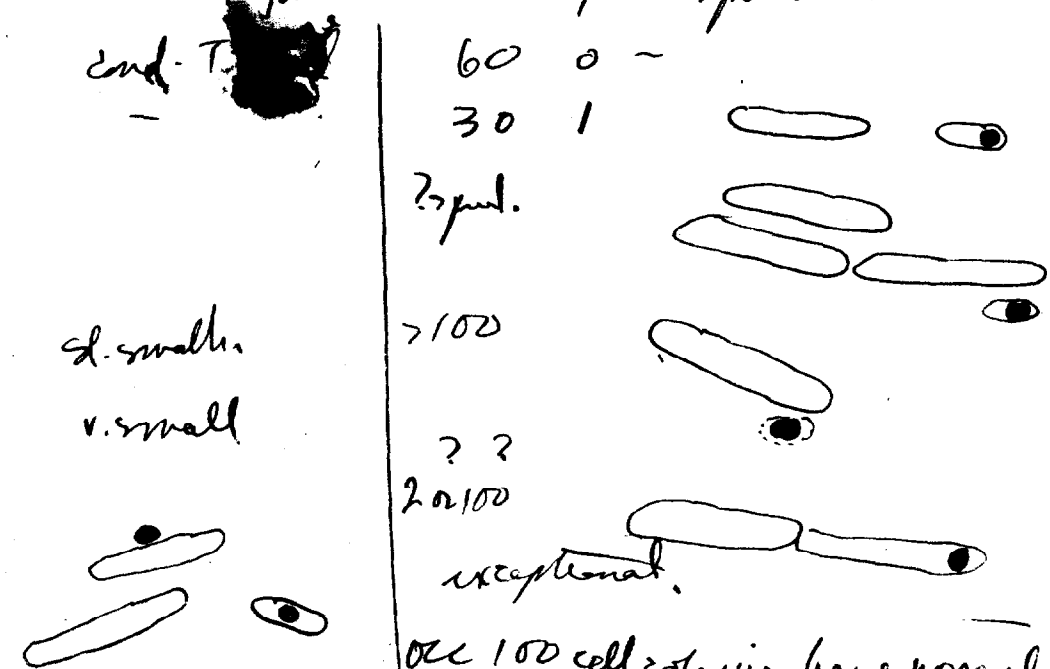
Many colonies uncountable, include centers, and spread out

N	T2	cond. T
ca 100	0	–
"	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	
? pmd.		
> 100		
? ?		
2 or 100		

exceptional.

Occ 100 cell colonies have normal (small T type mode) cells.



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

1. -0 Tr granules, occ. concavities.
-3 } no nuclear stain 10, 20 m. stain
-10 }

2. Tr granules absent - P
(-0) Prominent concavities. Heterochromatic nuclei
(-3) Prominent grains
-10 Mixed! (accuracy of hydrolysis??) ← weak nuclear in parts
← strong gran. in parts

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

1 HCl - 0 m cells sparse [redacted].
2 - 3 m Grey cells; nuclei in background
3 - 10 m Clear nuclei; [redacted] But 1-2 nuclei predominate

Suggests log phase cells OK from hydrid medium! (Trypsination)

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

T.O.

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

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

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

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

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

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

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what? [illegible]

AO - stain

A1 8:50 - 12N Refr

A2 12 - 1:35 Refr.

A3 12N - 2PM stain

[10A2 in [illegible] - microcol.]

AO. Refused to stain for nucleus (up to 40 mins.)

Keep slides with 0, 6, 10 min hydrolysis.

A3 Stained brilliantly. TZ not prominent however:

A3 - 0 char spaces prominent.

- 3 granules not prim. polar, but often lateral. Probably not hydrolysed long enough.

TZ rare only in deg. cells

- 6 missing - 10 sparse. gen staining

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

Neither gave nuclear stain. Why liquid medium unsatisfactory?

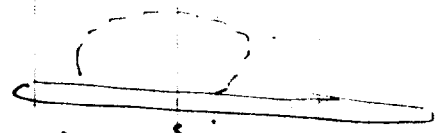
C3 suggests the deterioration of the TZ subclone by the 100 cell stage.

Some disappearance of TZ?

C2. (Studied 10^{15}).

possible blebs on some cells

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



sic

Desiccate 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 mucham diffinitiation

suggest lower conc of TZ for viable subclone cells?