A.) 1 Plate W1177/TZ .01% .01 ml NA 11:15 AM – 2PM
2 " " .01% .0101 "
3 " " .002% .01 " – 2PM.
4 " " .002% .0001 "

B. 1 = smears from both mor↑ Stain Giemsa 1/10 10 0, 10 m HCl.
exc. for TZ 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 observation: 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 3½ m → shows distinct blue granules; fairly poor nuclear stain even at 12 m HCl! What is peculiar here? Overfixed? v. fresh stain? slides not stored in HCl 0-3-10 intervals should be adequate for later tests. Go back to refr. plates for comparison.

A)- Examine 7, 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)s/30 1 snake/4
alone. 1 ab s s n se s

abnormal empty v. short = s or empty = e

∴ at this stage many cells are normal in phase appearance

4) normal TZ in microc: 1/4 1/2 1/16 1/30 1/4 1/27 1/25
ab " " :
alone : 1n

data. 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 charing!

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

acid ing.?

✓ TZ cells no nuclei.

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

TN
S G
[illegible]

3 m. granis 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 / Pinassay.

A .02% Growth ½ inhibited; cells at bottom

B .01 Deepest red

C .005 deep red

D .002 med red

E .001 barely pumpable red

F .0005 faintly darker than control

G —

Underphase 10:25 AM.

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

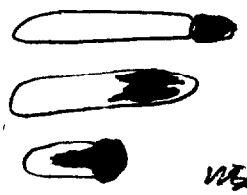
E. label rare (< 10%) [Insidiable free tetrazolium ? or poorly apparent underphase.

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.

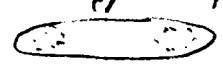
A:



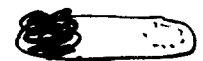
axial rods and coils usually free TZ

~~mostly~~ cells ¿ or ¿ TZ show ~~[illegible]~~ sl. refractile usually paired

zones.

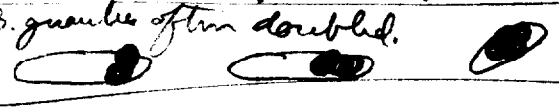


or

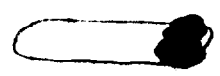


axial TZ. sometimes does not show up underphase

B. granules often doubled.

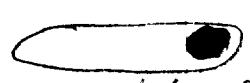


C. mostly fairly uniform.



granules about cell diameter often doubled. abnormal crystals rare.

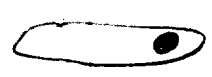
D mostly



smaller granules.

rare ab. 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 cf. 5 minutes M/10 buffer 5.1 Giemsa 10.

3 HCl (old) 5 min 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. TZ in empty.

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

Best S. 7 Repeat unhyd. S very clear

8 heat 5 m. pH 6 S very clear (less? than 7). cf 1/2 !!

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

New series at one time: 11 No hyd. S. clear w.c. where overstained

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) NoS. NoN.

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

16 HCl 10 m

17 HCl 15 m } stg. progressively confined to small axial granules.

18 HCl 20 m.

(G) 19 HCl 4 Giemsa 10, 20. 4) G. very clear; cytoplasm light (nucleus) should be repeated with fresh material.

Should have better 3 m prep in this series.

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

4: G very clear. Like 19 above.

10: clear nuclear stg but axial shrinkage

weaker acid w. 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 color

S ✓✓ TZ about ½ in [illegible]

22 Tol. Blue 1 min

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

[illegible]

Restain after hydrolysis

31 m + Azure 3 m. G ✓✓ background (lowering than Tol Blue ([illegible], CV)

Tol Bl 1 m. G ✓✓✓

CV 1 m. overstained. G not evident. almost uniform cyt. stain, possibly nuclei darker!

→ CV 30 s

---

22 Tol Blue ± HCl 3 m. S – G ✓✓

21 Cr. Viol ± HCl strong background stain

G not seen as such! ([illegible])

Oct. ~~14~~ 1953

W1177/TZ overnight. Moz plates 10:30 AM – Rifi. 3:30

.05 ml

.01

.001

.0001

5 ml 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 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 9.

.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 –
Differences less obvious in phase than in stained pups.

Fix .0001 ml plate

A Schaudinn

B Osmic - Schaudinn

C Osmic - alcohol.

done hastily. Save only B.

3m HCl - axial distr heavy G?

10m " - axial shrinkage D?

Oct. 26, 1953.

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

~~B. Inoc Tuesday 1/7. 12:30 [illegible] 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 (930-1730)/NA 1730-2:30 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 chaust in C3B.

W1637 might be useful as test of fixation!

A - Stained P26; stud in water through afternoon

14A.

1. (Sch.)

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

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

2. G very prominent, very sharp
   contrast!

3. ~~No stain~~! 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. ~~[illegible]~~ V. sharp N (purple-red!)
   "mitotic".

3. Osmic - Ale

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

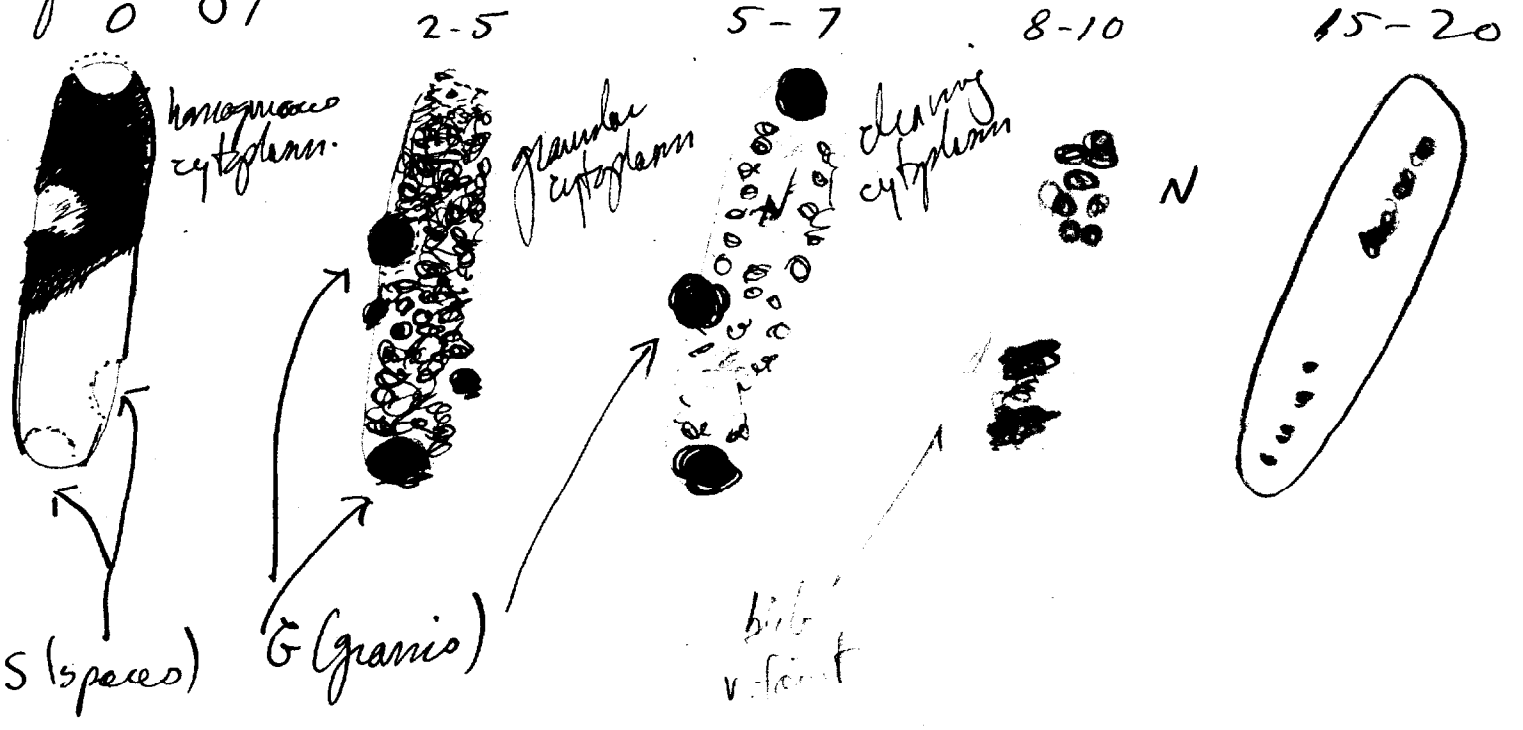
3 needs to be repeated!

2 and 3 are about equivalent.

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

10/26/53. Comparative Cytology.

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



In some early slides, N stained metachromatically at 0.

Spaces, Granules and Blebs are probably same material, though this would be difficult to prove. W1177-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 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 nuclear fixation still too poor for careful comparison.

10-26-55

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. (opt 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 Pure N, weak
2 } { 10m } Orinase G and N (well marked G)
N blue. acid
weak or inconsis?

3 Fresh Osmic fix. 3 m N weak, purplish
4 W1177, 10^{-3} 5-6h. 10 m N very sharp, ~~bl~~ blue.

(acid must have been weak).

"5" - 0

"6" - 3m hydrolysis

A - Os as above
B alcohol
D Sura
S. Chabaud fix

} cells [illegible] than A

A5 NoS
A6 see above.
B5 NoS ~~eosinophil deposit~~
B6 G well stained N v. faint cytopl clear
C5 NoS
C6 Unstained
D5 S poorly developed (terminal)
D6 Unstained or faint blue granules

Throw out

Needs repeating!

10/28/53.

B E coli B } Plate .05 ml 9^{50}–12N. Fix Schaudinn
K K-12 } NSA.

1 0 HCl } B (K) both: very clear (S.)
2 3m HCl } K2: G – N faint cyt. dark.
3 10m HCl. } 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. Moz 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.
S well marked 3. Also distorted >1. 4 - polarity; distortion ca = 1.
Note: S only in 3! (NSA, as in most expts). Compare cells grown on NSA, NA!

C W1637 11:40 – ~~10~~ 2+PM. Fix in sch from 1-2 NA, 3-4 NSA, 5-6 NA Suc.
A - Okey dialysis. 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 Chaband. } – 3 10m. "

slides v. dirty

D1-1 NoS.
2-1 S
3-1 S ~~[illegible]~~ do not save

Chaband does also give spaces; Os does not.
Granis - ✓ tomorrow (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 overtanned. Occ S
2 " " Cells much larger. Occ S N red
3 Sporn. " "
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 frequent figures (λ?)

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

	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: assay for recomb.

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

D. Perchloric acid. Room Temp. from 4:40 PM. Slides fixed ↑. (C1) Stain 1-5 A30.
1 1m. Progressive general decolorizing.
2 12m. No spec. stain
3 29m
4 58m.
5 8 30 A 30. Some G, v. faint.
6 5 P 30 Weak overall stain. NON.
7 8 P 30.
" Toluidine Blue 1 min.

B. Streak out suspensions from agar block on EMB Lac & sm.

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

some streaks obviously n.c.

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. large cells, beautifully stained N pink + ingest. Walls sharp. No S!
2. Variable shrinkage (drying?) Cells long: some few snakes No S!
1. Sim to 3; slightly shrunken. Repeat i 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 - ~~4:10~~ 3:55 SR+: (ca 5%) Lact >> -

B1- 1 Sv. char cf. A1! (A1 std. by G.D. old Gurnisa?)
3 overhydrolysed! Poor stain N extrusions? Too hot?

2 1 Large phrys cells. S in patchy ~~area~~ areas only: Drying?
2. Like B1 character. Numerous blebs + extrusions

3. 1. 2 cell types S patchy (covers both types 2. not clonal)
3

Repeat 18A1-3.

No S in A1!
?

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 12^15 - 2^45 Then refrigerated 1-2 hours before fixing.

BC 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 19./86
3 .05 Stain & hydrolysis to judge density. 23/99

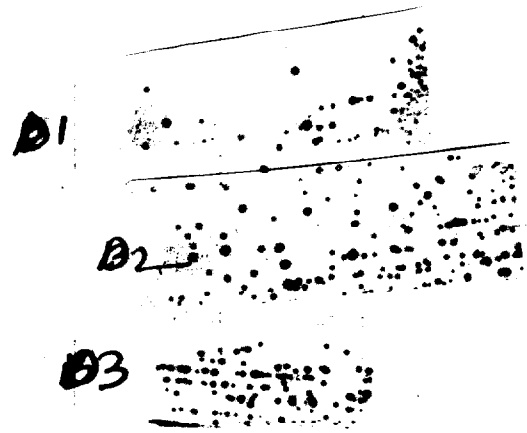
These yields are therefore all comparable and suitable for cytological analysis on agar

A. 1. Brilliant stain old metachromatic N. no S. 2. Flat new Giemsa (Spatulary) (dried ?)

repeat 1. but different in other end of smear. flat n.g.

2. S-- flat, dest. no N. 2. same S--

D. 1. moderate -2-3 heaping. stain as in A1



S 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) ~~Heaped up~~

after Os. 1, 2, 3 N char. no G

C heaped up

E No obvious effects.

No ⚘ sem.

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

1 flat.

2. N brilliant (like 3).

occasional G (= "centriole?")

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

also slide looks heterogeneous. probably C.

too dense!

A2 1, 2, 3 weak G. Heavy background. E3'- dirty No N. Same S

MA4 was probably artifact. (HCl ca 65°)

Stains, HCl OK now

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

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

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

A 1 S very clear ! ~~No~~ 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).
~~C 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. NoG. NoS. Faint Ngranules against cytopl. background almost as dark as A1–1.

B. As above!

E. G prominent – dark blue / grayblue backgr. NoN

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

–3

A1. Vesicular? nuclei & understained spots.

note! dark spot in many cells!

–4, –5 = 6m, 10m HCl 15m Gumsa.

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

5: Sp. granules also. gen.

B. As above almost peripheral in wg 28

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

Note 2 shades [2 strains?]

11/4/53.

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

1. S mostly very clear. Some N(red.) Dup, full cytoplasm
(2 slides)
2. Cytoplasm washed out. Some cells shrunken. others pale, with residual N clearly in background. Not much detail observable but N seems more vesicular than in other pups.

10/6/53. Schaudinn

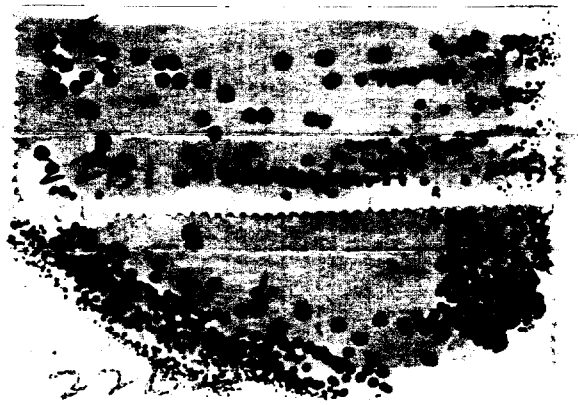
A. Various wg. 1. wg 2 2. wg 31 (n.g.) 3. wg 47 (n.g.)
.01 ml/NSA 12^{30} – ca. 2^{30} PM.

1 – 0 HCl
2 – 3 m. HCl
3 – 10 m. HCl

~~ca~~ 25.

B. W1895 + W2333 from Pinassay. .02 ml. Fix and assay.
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% 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 Sec prompt fix, Schaudinn, alc., water.
B. " " ; lift in alcohol
C. NSA dried
D. " " /alcohol (0 3m HCl 1, 2 to 6PM ca 3+ hours in alc)

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

A NoS. N faint neg., occ. mit. ~~Blue~~
B NoS. Coarse cytopl. network. Blue
-1. C S. (cells also dried out).
6P7. D S. very clear. Purplish cyt. homogeneous.
A Most cells uniform purple. Few show clear cyt and N+. NoG.
-2 B " " ".
C Some residual S! Begin G. Not very conspicuous. Dense not quite homogeneous purple cytoplasm.
D. Faint residual S. NoG.

5,6,7 stained 5P8 (B, D stood in alc.) 0, 3, 10m HCl. NoC-5.
8,9 = 5,7m. HCl.
A see 1.
5 B ~~[illegible]~~ see B2
~~[illegible]~~
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 sepia?)
D see D2

7 A N. granules not so sharp. Overstain?
B N granules v. sharp Mitotic? Many possible res. 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 G in any part!, despite S.

A 8 N only (like A7)
9 "

(what ED calls a good slide)

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

11/10-11/53.

W2333 ca. 2homes

A. NSA, dry, fix, — water.

B. NA sec — water

C. A sec — alcohol.

D. NSA — A, dry.

11/11. .02 ml 1:15 – 3:15

E. as A

F.

1,2 stained P10.

1,2A " P11.

series also stained 11/12 as marked.