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

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

C. Effect of refrigeration. 11:45 plates. of TZ .002% ↑ .05 ml!

1. Fix samples 2:45 10/23. Refrigerate remaining plates

2. Phase observations: 2/3 TZ cells are short, some empty 1/3 normal (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 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)s/30 1 smaller/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 1/15
ab " " :
alone : 1n

ditto. of C!

cells spaces.

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

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

acid hyg.?

✓ TZ cells not multi.

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

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

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

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

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

Underphase 10:25 AM.

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

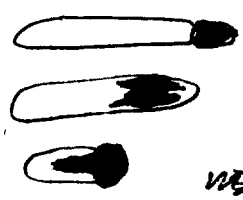
E. label rare (< 10%) ± recognizable fuc cytoplasm ? or poorly apparent underphase.

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

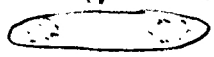
Opt conc for label might be between D and C.

Compare B, D, & platings.

A:



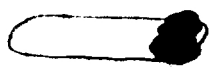
axial rods and coils usually fuc Tz cells i or s Tz show sl. reportable usually staining zones.



axial Tz sometimes does not show up underphase

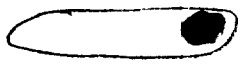
B. granules often doubled.

C. mostly fairly uniform.



granules about cell diameter often doubled. aberrant crystals rare.

D mostly



smaller granules.

scarce. fewer % label.

E.  to 

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

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

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

Repeat P24 on CA.

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

3 HCl (old) 5 mins G very prominent

4 HCl fresh 5 min. G less than 3.

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

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

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

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

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

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

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

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

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

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

16 HCl 10 m }

17 HCl 15 m } stg. progressively confined to

18 HCl 20 m. } small axial granules.

G 19 HCl 4 Giemsa 10, 20. 4 / 3 m) G. very clear; cytoplasm slight (numbers?) should be repeated with fresh material.

} Should have better 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 ; . Svariable, vague
some negative N.
v. slight G in places?

X 21 Unhyd. Bas. Fuch. 30 sec ... 3 min — S ... stain ...
S ... TZ about ½ ...
22 Tol. Blue 1 min
23 Azure 3 mins. Clear stain — S ... (...)

... ...

... after hydrolysis

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

22 Tol Blue ± HCl 3m. S - G ...

21 C. Viol ± HCl ... stain
G not seen as such! (... ?)

Oct. 14 1953

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

.05 ml

.01

.001

.0001

Sml tubes c

1.0

.1

.01 ml.

Examine N25, phase.

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

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

normal – 0.

These plates should have been examined earlier prior to recounting.

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

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

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

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

Fix .0001 ml plate

A Schaudinn

B Osmic - Schaudinn

C Osmic - alcohol.

done hastily. Save only B.

3m HCl - axial deuts heavy G?

10m " - axial shrinkage D?

Oct. 26, 1953.

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

~~B. Doc Tuesday 1/7. 12:30 ... 1/20~~

(A)

C. W1637 – N.A. 9:30 – 12:00 (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 Sch O3.

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

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

W1637 might be useful as test of fixation!

A - Stained P26; stood in water through afternoon

14A.

1. (Sch.)

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

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

2. G very prominent, very sharp contrast!

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

2. Osmic 3m-Sch.

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

3. Osmic - Ale

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

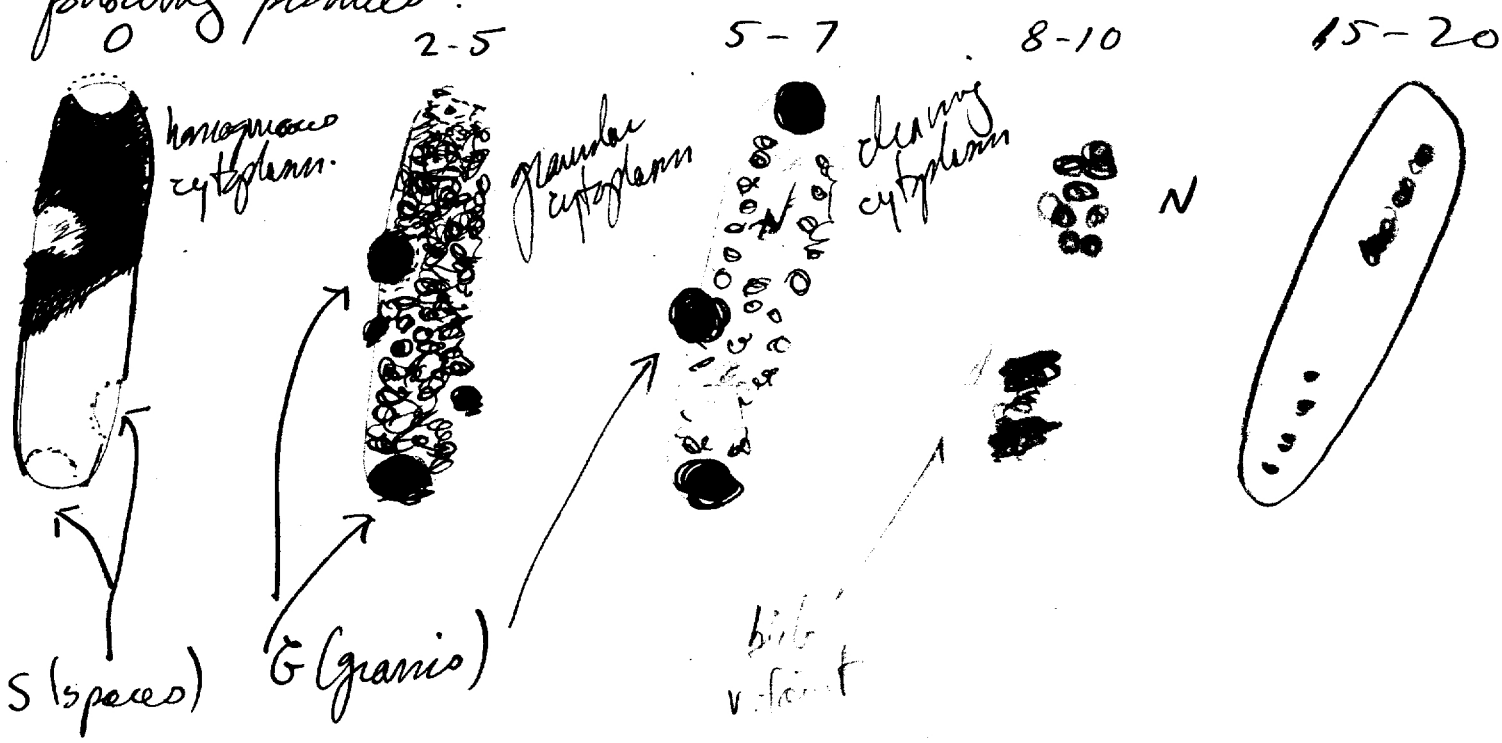
3 needs to be repeated!

2 and 3 are about equivalent.

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

10/26/53. Coccidiosis Cytology.

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



* some early slides, N stained metachromatically at 0.

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

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

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

10-16-53

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

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

Oct. 27, 1953.

Repeat 14A3 - 2/3.

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

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

(acid must have been weak).

" " 5 - 0
" 6" - 3 m hydrolysis

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

} [illegible] than A [illegible]

A 5 — No S
A 6 see above.
B 5 — No S ~~[illegible] deposit~~
B 6 — G well stained N [illegible] + [illegible] clear
C 5 — No S
C 6 — Unstained
D 5 — Sparsely distributed (+[illegible])
D 6 — Unstained or faint blue granules

Throw out

Needs repeating!

10/28/53.

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

1 0 HCl } B (K) both: very clear (S).
2 3 m HCl } K2: G – N faint cytoplasm dark.
3 10 m HCl. } K3: Nasmuel 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 on 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 3 m "
3 Chabaud. } – 3 10 m. "

slides dirty
D1-1 No S.
2-1 S
3-1 S ~~[illegible]~~ does not
gave

Chabaud does also give spaces; Os does not.
Granis - Simmons
(Later: probably a matter of dying 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 real
3 Spores. "
4 Small and large cells individual scoring?

-2, -3 definitely backing 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 $10^{-1} \dots 10^{-5}$ NSA.
Refrigerate after growth for later fixation and SR+ assay.

	Ref.	hours.	Condition	Fix Stain (G.D).
	1	12 15	Moderate Smear	A30. (Schaudinn)
$=10^{\dots}$	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 of NA (C2) and NSA (C1) .02 ml / plates
[struck out] - 0 12N - 2+ PM. Fix Schaudinn. (Many upl of C1 for D...)

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

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

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

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

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

some streaks obviously 4-1.

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

6/30/53.

Schaudinns

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

12:10 / 2:20 — 3. Large cells, beautifully stained ~~R~~ pink + magenta. 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 4x10¹⁰ + 1 ml W2333) .02

5 W1895 · W1177 .05 1:30 – ~~4:10~~ 3:55 SR+: (lac+?? – ca 50%).

B1 – 1 Sim. char of A1! (A1 std. by GD. old Giemsa?)
3 overly hot fixed! Poor stain N extrusions? Too hot?

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

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

Repeat 18A1-3:

No Sir!!
?

11/2/53.

A. Effect of drying W1895

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

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

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

Assay.

E W2333 + λ (1ml + .1ml 10^{10}) <

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

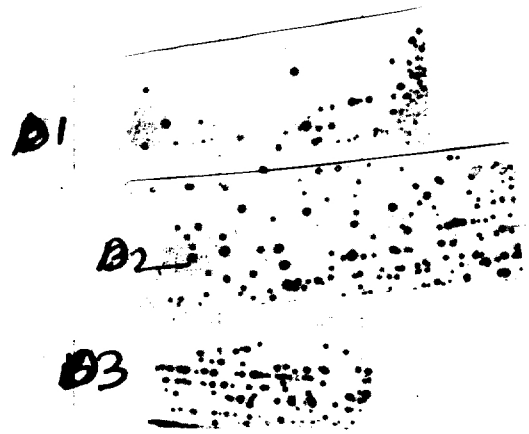
These yields are therefore all comparable and suitable for cytological analysis on agar

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

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

2. S— flat, dent. no N. 2. same S—

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



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

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

± Os probably interchanged

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

after Os. 1 2 3 N clear. no G

C heaped up

(E) No obvious effects.

No  sem.

too dense!

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

1 flat.

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

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

also side looks heterogeneous. probably E3' - dirty C.

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

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 B. Plate .01 ml at 9:50 — 12 N.

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

Assay:

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

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

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

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

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

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

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

A – 1 stained 11/4

A-F – 2,3 " PM 11/5.

– 2 (3m HCl)

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

B. As above!

B1. G prominent – dark blue/grey blue background. No N

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

–3

A1. Vesicles? nuclei & unidentified spots.

note! Indistinct 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 purplish c.f. sg 28

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

Note 2 shades [2 strains?]

Effects of Benzene (c̄ B.R.).

11/4/53.

A. ~~Bl~~ Rotman preps. (W1317) Direct c.g. smears

1 Control — grown in .2% D(glu) Fix Schaudinn. Stain Giemsa (no HCl)
2 Benzene 10m. still logphase (~~H~~ hours...)
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 preps.

10/6/53. Schaudinn

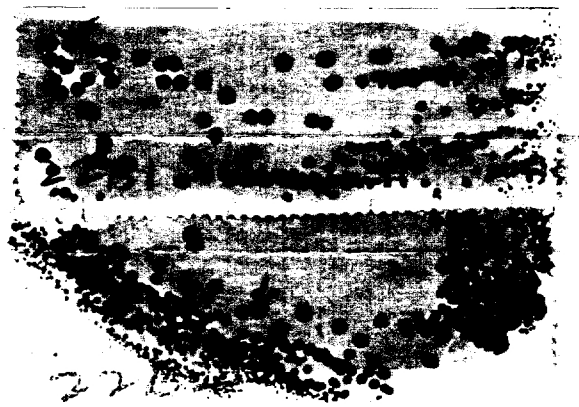
A. Various wg. 1. wg 2 2. wg 31 h.i. 3. wg 47 h.i.
.01 ml/NSA 12^{30} – ca. 2^{30} PM.

1 – 0 HCl
2 – 3 m. HCl
3 – 10 m. HCl

~~25~~ 25.

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

not countable. 1 ♂ 1%
2 > 1.



C. W2333. .01 ml 12^{35} – ca 3 PM.

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

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

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

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

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

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

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

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

(over)

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

A 8 N only (like A7)

9 "

(what ED calls = [illegible])

B 8 N only granules v. condensed & sharp but not connections! [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 sec — water
C. A sec — alcohol.
D. NSA — A, dry.
11/11. .02 mil 1[15] - 3:15
E. as A
F.

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

series also stained 11/12 as marked.