(Agrobacterium)

Cultures

CG-1	A. tumefaciens	A6 - virulent	} Ribier
CG-2	A. tumefaciens	A6-6 - attenuated	} Ribier
	" "	A6 PEN	penicillin resist. } Klemmer (see pA1a)
	" "	A6 P5a	polygynin resid. } Klemmer (see pA1a)
	" "	A6 C5	chloramphenicol resist. } Klemmer (see pA1a)
CG-3	" "	A6-S2	} partly identical } pA1-S ff
CG-4	" "	A6-S2	} partly identical } pA1-S ff
CG-5	" "	A6-6-S2	} partly identical } pA1-S ff
CG-6	" "	A6-6-S2	} partly identical } pA1-S ff
	A. radiobacter	1001	} Ribier
	" "	1005	} Ribier
	" "	1007	} Ribier
CG-7	A. tumefaciens	} identical to strains from CG-2	
CG-8	A. tumefaciens	} resist. against (?). Both identical	
CG-9	"	identical from CG-1-S. UV. grown on YNA	
CG-10-15	A. tumefaciens	from CG-1-S, UV. [illegible] cultures, not run down	
CG-16	A. Tumefaciens	" " " Ribier -	

H. W. Klemmer

Antibiotic resistant cultures of Agrobacterium tumefaciens

Resistant cultures were developed to five antibiotics from a sub-culture (A6K/b) of a single-celled culture of the virulent A6 strain of *A. tumefaciens*. Three of these cultures are still in stock (on antibiotic-free yeast extract-mannitol (Medium 79) slants), the others have been lost. Those remaining have not been tested for resistance for at least 2 months. All five cultures are listed below, along with the level of resistance obtained and the % loss of virulence as compared with the parent A6 strain.

The parent A6 strain has been assayed against several antibiotics by de Ropp [Phytopath., 39,(1949)] using a 100% inhibition in broth, 24 hour endpoint, and by Klemmer [method of Joslyn & Stubbraitis, J. Bact., 62 (1950)], using a 50% inhibition in broth, approx. 8 hour endpoint. The 50% inhibition levels (IO_{50}'s) of the A6 strain and are known, the 100% levels are given below in the table

Culture resistant to	Level of resistance γ/ml	% loss of virulence	Parent A6 culture inhibited at (γ/ml) 50%	100%
Penicillin A6PEN	1000	80	8.0	1000
Streptomycin lost	800	20	3.5	50
Polymyxin A6P5a	150	80-90	5.6	
Chloromycetin A6C540	50	50	.24	2
Terramycin lost	15	20-30	.04	

The cultures still in stock; resistant to penicillin [A6(Pen.)], to polymyxin [A6(P5a)] and chloramphenicol [A6(C5)]

If you want more information phone 2310 for Howard Klemmer

Media

I. To maintain stock cultures. Adaptation of "Medium 79" of Fred and Waksman

AGI

Medium 79

Mannitol	5.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g (anhyd. 0.1 g)
K_2HPO_4	0.2 g
$CaCO_3$	1.0 g
2% starch-free yeast extract	100 ml
Agar	15 g
Water to	1000 ml

pH 6.8 (adjust)

Modification I (DCG)

Sucrose	5.0 g
$MgSO_4 \cdot 7H_2O$	0.2
K_2HPO_4	0.2
$CaCO_3$	1.0
Difco YE	5.0
Agar	16.0
Water	1000 ml

pH 6.8 (adjust)

In making mix, $CaCO_3$ + agar could be omitted. Then medium $\bar{5}$ agar or $CaCO_3$ could be used as complete broth; $\bar{c}$ agar but $\bar{5}$ $CaCO_3$ as complete medium for plating. Also omit sugar from mix (Sucrose ok for crown gall org. according to Dr. Riker; mannitol used for Rhizobium.)

II. Mineral medium used in glycerol attenuation experiments (Cohen-Bazire, Baldwin, & Fraser, J. Bact. 63, 715-721 (1952)).

III

SM 1
ST 1
Aniline blue 1

AG II [redacted] 1

Mannitol	5.0 g	
KNO_3	5.0 g	According to Dr. Riker,
$CaCl_2$	0.19 reduce to 0.1	ammonia can be substituted
NaCl	0.2 see p 33	for [illegible] in media for
K_2HPO_4	0.2	Agrobacterium
KH_2PO_4	0.1	
$MgSO_4 \cdot 7H_2O$	0.2	Sterilize sugars separately?

& distilled water to 1 l.
Adjust pH to 6.8.

McIntire, Riker, and Peterson (1941) found traces of Mn. Fe+++ + Zn helpful in a synthetic medium (Mn as sulfate at 0.1 µg/ml Zn as sulfate at 0.5 µg/ml; Fe+++ as ferric citrate at 5.0 µg/ml.)

3-23-54

Made up mix for complete medium (Modification I of Medium 79 = AGI). Made up 200 ml for slants. Tested pH of mix + 0.5% sucrose (before adding $CaCO_3$ or agar). pH = 6.78 ≅ adjustment.

Possible approaches:—

1. Glycine
   A. Repeat glycine attenuation using A6. This would at least serve to give a second avirulent strain.
   B. Plate on glycine to get glycine-resistant col. (Could this be used as a marker?). Now Lanning, Baldwin, & Silver (1952, p 717) indicate that glycine-resistant ones are of normal virulence — This argues against "glycine attenuation" being a selection of cells which are glycine-resistant and avirulent. [Ed on typhoid paper — ~~v~~ virulent more sensitive than avirulent]
2. Markers other than avirulence
   A. Antibiotic resistance. Cultures or hard resistant to penicillin, chloramphenicol, and polymyxin. Try to get an S^R; also check others.
   B. Nutritional — Penicillin nuns?

3. Actual repetition of Klein's experiments, especially the U-tube experiments.
4. If markers are obtained, test for recombination of markers in mixture and U-tube experiments.

3-24-54

Transfer all Agrobacterium cultures to fresh AGI medium.

Try A6 & A6-6 on some routine E. coli media to see whether they can be used at all.

Penicillin method as used for E. coli

1. Grow up culture
2. Inoc. ca 1 ml grown culture into complete medium (Penassay for coli.)
   Add antifoam, aerate 3-3½ hrs.
3. Cfg 10 minutes, wash & saline.
   Resuspend in 10 ml saline, cfg 20 min.
   Wash pellet, resuspend in 10 ml saline
4. Add 0.1 ml suspension to each of 2 DO tubes
   Add 0.3 ml penicillin soln to one tube (other is control)
5. Incubate at least 4 hours.
6. Spread on complete plates (0.1 ml culture and 0.1 ml of a 1:10 dilution). Replicate to minimal.

1/2 [illegible] permissive growing survival of 10^{-6} or more (Milk Meal Pro)

Variables studied:
Permissive
Age of inoculum
Inc. time in penicillin containing
Complete medium
Minimal medium
Temp; aeration?

3-26-54

Test of growth on coli media -

A6 inoculum evidently pure - no growth in any medium tested.

A6-6 - growth in all liquid media tested (D-0, NSB, penassay)

Very scanty growth on EMB media and on D-0; more on EMS.

A6-6 growing well in stab; A6, little or no growth. (Re-inoculate A6 stab from fresh slant)

3-29-54

A6 & A6-6 well-grown in NSA & Penassay - growth scanty in D-0. Good growth on EMB media;

pour on D-O.

Try one of the regular Gp viscid materials
Growth more complete in NSB than in Penassay.
More clumping in latter.

Penicillin assay

1. Inoc. fresh Penassays from those inoc. 5-24.
(1 ml inoc / 10 ml Penassay tube). (Using
Penassay rather than NSB because it is a more
complete medium).

Streptomycin

Spread 2 drops Penassay culture on Blood agar plates
to look for S.R.'s

Sealed slabs of A6 & A6-C

Made up 20x sects for AG II. (minimal)

3-30-54

Made up AG II minimal liquid

Regulated [illegible] 213 to 26° and mixed streptomycin plates [illegible]

Pen runs

2. Penassay tubes incc. 3-29 fairly [illegible], aerated at low temp (from [illegible] 9:00 AM) to accel. [illegible] growth.

Try again at broth for next pen runs (AG I liquid)

Make up AG I and AG II plates.

Inoculated broth cultures of A6 + A6-6 into 10 ml tubes AG I. Incu. at 26°

Pen runs

3. 1:30 pm. [illegible] cultures, though [illegible] not as well grown as [illegible] corresponding slip. A6 fairly uniformly turbid; A6-6 strongly turbid.

3-31-54

Pen runs: Broth tubes barely turbid, penicillin tubes clear. (Used AG II as minimal). Plate out at ca 48 hrs?

How get heaviest & most rapid growth in complete medium?

Try: 1) AG-I broth at 26°
2) AG I broth aerated at room temp.
3) Renassay at 26°

Inoculate each tube ē 0.1 ml old Renassay culture. Inoc. 10:00 AM 3-31.

Streptomycin

Plates inoc a broth culture 3-29:

papillae, probably SR, against background of very scanty growth. Number per plate ranges from ca 6 to ca 20. [illegible] 50-200 per plate

Picked 12 papillae each of A6 & A6-6, streaked from small colonies onto E and SM.

If these appear to be SR, pick single colonies, streak one complete ē SM; inoculate to SM medium.

4-1-54

Parallel runs:

both parallel and control tubes turbid. Inoculum plate at 24 hours shows if growth in control tube is very scanty.

Comparison of complete media - 24 hr growth
Penassay at 26° - poor
AGI at 26° - a little better than Penassay
AGI aerobic at room temp - very good growth, especially of A6. Considerably more turbid than AGI tubes incubated at 26° I section for the day

Use aerated tubes to start second parallel run

4-2-54

Second parallel run

Good growth in control 24 hr tubes in 24 hrs.

1) For next run, use at least 1000 U per ml
2) Spread dilutions from old penassay tubes directly on AGI plates & replicate to AGI. These old tubes have accumulated many S^R cells and probably many penicillinase resistant, perhaps true mucoid mutants which could be picked up directly.

0.1 to 10 mls
↓ 0.1
10 mls - 10^{-4} ← 1 ml ← 10^{-5} 0.1 spread → 10^{-6}
↓ 0.1
10 mls - 10^{-6} 0.1 spread → 10^{-7}

On basis of 10^8 - 10^9 cells per ml plate 10^{-6} & 10^{-7} dilutions.

4-3-54

Replicate spread plates to AG II.
(May have to wait until Sunday.)

Pick 5 SN colonies, streak on AG I 5 SM.

Spread plates – wait for results of streaking until Sun or Mon. – no trouble with about 4 hrs.

Streptomycin –
A6 - Streaks from all 12 wells showed new well growth on 2 oral SM.
A6-6 Smears of 12 well grows; 4 mixed (3 shapes) heavy; 2 others scanty growth in streaked part of streak (SN resistants?)

Pick colonies from 2 streaks from A1 - to 6, streak on AG I plate 5 SM.

4-5-54 [illegible]

Streptomycin

Replicated S^R cultures streaked on AGI to AGISM.

Spread plates from old Hayward cultures (2-4 × 10⁻⁷)

A6 - Only 15-20 col per each 10^{-6} plate. Plates unruly. Picked all individual colonies, efforts on AGI & AGII. (Found latter too long to efforts complete, then replica'd to minimal - 2-3 days required at each step)

A6-6 Replicate to minimal (~ 15 col/plate in 10^{-7} dilution; 100-150/plate in 10^{-6})

Penicillin runs

Inoculate AGI broths from old Hayward cultures. Also inoculate broths from streaks from "clubs".

Note: Replica plating may not be very satisfactory for this bug - Colonies either don't stick to the velvet at all, or those are lifted off the plate completely.

How number any mutant strains? Using first A would lead to duplication. ∴ Will use CG for crown gall. A6 = CG1; A6-6 = CG2. Will not

send CG numbers to Klemmer's cultures until four markers are checked.

4-6-54

Spread plates –

A6 : spots are minimal not much enough grown to read

A6-6 : replicas minimal : Only 1 small colony on complete not grown, one more. Looks as though it was not picked up by velvet; however, pick & test to be sure. Spot one more of complete plates of A6 cultures being tested

Streptomycin –

Saved 2 5ml each of A6 & A6-6

CG3, CG4 CG5, CG6

AGI slants & nutrient stab.

Pen. run

Used 1000 U/ml

4-7-54

Penicillin resist.

A6 - seed growth in penicillin tube (no growth!)
A6-6 - pen. Tube clear. Plate not org. 9/10 dil.

A6 - Streaked & try to get penicillin sensitive colony. (Replica to plate spread ± penicillin ?). Then try pen. resist again.

4-8-54

(A6-6)
Pen. resist org. - Undiluted from pen. tube too crowded; 1:10 dilution OK for replication (ca 200 col/plate)

Started broths for pen. resist on CG 3 and CG 5.
Amphigha in 5% culture on old penicillin SRP type experiments.

4-9-54

A6-6 penicillin resist NOTE: A second type of colony has appeared on the AG-I plates spread from pen. resist. The colonies which were small and white at 24 hrs are now (48 hrs) yellowish, considerably larger, and gummy. Now some small white colonies have appeared which were not visible at all 24 hours

720. They are about 5 times as numerous as the earlier colonies. There is not a gradation in size of colonies at 48 hours, but a sharp, clear-cut difference.

Although these later colonies were not visible when the plates were replicated to minimal, they have now come to the surface as well as are minimal as on complete, while the colonies which were quite well grown on complete when replicated, are now barely visible on minimal. This latter behavior rather slow growth on minimal after replication from complete, is more like that observed when plates were spread ± diluted culture ± penicillin treatment. (See pp 47 ff). However, in streptomycin experiment, many colonies appeared late on SM plates, after colonies had already been picked which were saved on 5 or 6 strokes. Will penicillin ± CG-5 yield only the cells which grow up fairly on complete? Why should a penicillin mass give a large yield of colonies which grow as rapidly on min. as on complete, when the mass culture used as inoculum apparently consisted mainly of cells which grow more readily

Tentatively, the old broth culture used to start this run has been discarded for broth more 4-5.

an complete time one more?)

Take the following steps:

1) Streak out a colony of each type on AG I and AG II, also with a mass culture of AG-6. Might as well include AG 6 also for comparison.
2) Test penicillin resistance of both types.

Tentatively,

Early growth on complete, slow on min = a

Late on complete, not on min = b

3) Save both types

4) Use all 3 in a single run with 1 as a control

5th pen run: Use 2d both tube from broths of CG 3 & CG 5 which have been aerated 24 hrs.

Pick a large no. of the type a colonies & spot directly (no suspensions) on AG I plates. They may all be auxotrophs, and in addition 24 hours the type b colonies will be so small as to that the plates will be not crowded for picking.

4-10-54

MAKE UP AG-I plates ✓

Penicillin runs on AG-6

All "type a" col. seem ~~are~~ satisfactory - may ~~be~~ ~~are~~ ~~not~~ ~~all~~ ~~at~~ 48 hrs.

Make up random new plates; test the colonies picked yesterday (on 20 col) Save ~~the~~ plates.

Do random, ~~also~~ using AA Strep, YE and ~~staphylococcus~~ aureus.

"Type b" ~~still~~ small and white at 72 hours

Streaks ~~are~~ ~~not~~ & complete; (about 20 hrs)

complete AG - very scanty - single ~~colonies~~ barely visible

AG-6 - slightly better ~~than~~ than AG

(these were streaked from old (wks)

"Type a" - Growth good, ~~but~~ - Individual colonies large enough so they could easily be picked.

"Type b" - growth very scanty - comparable with AG

Meningitis

A6 - very scanty

A6-6 slightly better than A6, but very scanty

"Type a" - no growth

"Type b" - barely visible

Plated per rum & CG 3 & CG 5. Used 1000 U/ml

A6 streaked for test on penicillin - not many isolated colonies - also one plate streaked for whole heart pus. Pick several colonies to small Kornavage & test coherence on rabbit.

Save a "Type b" A6-6 colony on slant

4-11-54

CG 3 OK for plating - CG 5 [illegible] per tube. Plated 0.1 and 1:10 & 1:100 CG 3

4-12-54

CG 3 plating - no growth [illegible] Try plating direct from pig's tube. (Use AGI, no AGIS14 available)

No turbidity in pig tube at 48 hrs.

Streaks on mammal & complete - 3 days.

Complete - AG and AG6 - both have 2 colony types; one small & dense; other larger and more translucent. Pick and save both types.

"Type a" from pandemic - Lumpy, large, margins, both more likely to translucent [illegible] dense type in AG6 [illegible]. Centers dense & pitted, more translucent.

"Type b" - Pick: smaller, less dense, (gentle dense) margins more likely in AG6 [illegible]

Mammal - AG & AG6-4; 2 colony types, more moderate size & translucent second dense set. very small - hardly [illegible] pencil size.

"Type a" - like first type of [illegible]

"Type b" - virtually no growth [illegible] margins, [illegible] dense [illegible] glucose; [illegible] dense [illegible] but they are translucent, not visible on less [illegible] just a pit.

Auxotrophs replicated to rundowns: No growth on AA's except for 4 prototrophs on above spores. Scanty growth on YEX and duplicating of cases. Try penicillin and UV. Try basic Medium's effluent - also try reduced sucrose.

4-13-54

Tuesday - Make up 2X AG-II - mid. some in tubes.

Pen. run on CG3 - Clones present at 48 hrs. Run off counts - these est. appear just 48 hrs in presence pen expt. Ca 50/plate from 1:100 dilution. This corresponds to ~5000 in AG-6 expt. Replicate to numerical. This is 5σ deviation of 16.

AG-6 run: choose 1 auxotroph from YNA, V175, & control AG II tube.

4-14-54

A6-6 auxotroph : no growth at 30 hours on minimal + YNA or minimal + vits. Incubate at least one more day.

To summarize:

Poor growth on hydrolyzed casein
Trace of growth on yeast extract
No growth on any AA group
" " (24 hrs) on YNA or vits.

Check whether present minimal medium favors growth of rough (small) colony type over mucoid (large).

CG 3 pneum -

Colonies on mucin not large enough to score. No further colonies are complete beyond 48 hrs. These colonies all are the small "type b" (or rough?)

It seems likely that each culture as received (A6 + A6-6) is a mixture of a "rough," small colony type, and a very gummy, mucoid type.

To be verified:

1) The "rough" grows more readily than the gummy on the present minimal medium (AG-II).

2) Despite this, the "rough" is selected for ... a peripheral ...
3) All "mucoid" survivors of a peripheral ... were either amputeeflue or very slow in their growth and mucoid.
4) C63 (A65m) is "rough"

Streak out all sticks on complete. Examine every type. Where mixed, save both (or all) components.

Also: streak A; 46-6 both types, on AGIM. Compare C63 + C65 ... daily streaks.

4-12-54

C63 pen ... three possible amoeboph... Inc. tube ... mucoid + complete.

46-6 amoeboph ... nits on YNA at 4 hrs.
Try single crossings to AA8 ... pos.

4-16-54

C63 possible amoeboph ... at 30 hours, growth in complete, none in mucoid, Inc. obl.; continue incubation of hours.

A6-6 auxo (ch. 1 = 1) - AA single omissions of groups
Growth in -AA+ ; also in hydrolysate
Test c; HC + AA+
AA 1, 2, 3, 5 + the individual components
of AA+.

also - to find actual requirements, test all combinations
of AA groups, excluding 4.

A1 + A2 ~~A2 + A3~~
A1 + A3 ~~A2 + A5~~
A1 + A5 ~~A3 + A5~~
A1 + A2 + A5
A1 + A3 + A5
A1 + A2 + A3

Just need to do single omissions -

1 + 2 + 3 1 + 3 + 5
1 + 2 + 5 2 + 3 + 5

Check of colony types of strains on complete medium

A6 original (CG-1) ; Predominantly large, dense, yellowish.
A few colonies smaller, white, translucent
A6-6 original (CG-2) ; Moderate size, dense, yellowish
centers. Seems homogeneous

A6 "R" : Small, white; mass growth looks rough
A6 "M" : Large, ~~c~~ yellowish centers, gummy.
A6-6 R : Like A6 R
A6-6 "M" : Like A6 "M"

Small (proteolytic) from A6-6 passages; Small but not rough. Centers yellowish,
Large (auxotrophic) from A6-6 passages: Colonies not very large, but more yellow & gummy than A6-6 "M".

A6 PEN (Klemmer) ; } Much like A6 "M"
A6 P[illegible]
A6C5 "

CG3 (A6 S[illegible]) - Some intermediate between A6 "R" & A6 "M"; colonies somewhat yellowish, but mass growth looks slightly rough
CG4 (A6S[illegible]) - Both large & small colonies; large have yellowish centers, Mass growth smooth, quite gummy
CG5 (A6-6S[illegible]) - Like CG-3.
CG6 (A6-6S[illegible]) - Like A6-6 "R"

Streptomycin resistance; A6 R+M and 96-6
"R" & "M" failed to grow at all on AGI SM in 48 hrs.
CG 3 - Grows on AGI 5 SM except colonies are smaller. This could be because medium is rather old.
CG 5 - Looks rough on SM medium, colonies very small.

Cross-feeding & penicillin (test of 10,000 units each); Pens. once generally unsuitable for any definite effect.
Break of A6-6 auxotroph from parent was to make cells compound & other cultures that it seemed wise to try to make sure it really is
A. Transferents.

4-17-54

CG-3 possible auxotroph; At 2 days, growth on minimal. So far (24 hrs) no growth on medium or incomplete - ie at same time (concentration probably very small). Streak from 48 hr complete onto complete plate & try several individual colonies

before discarding as no good.

AG-6 auxotroph:

Original single-omission Tubes (run 2 days):
Good growth in −AA 4; poor growth in hydrolyzed casein; no slight growth in −AA 5. Still no growth in other single omissions or in minimal.

Histidine 4-16 (24 hrs)

HC	+	complete	++
HC + A4	+	AG II-O	−
1+2+3	−	AA-4 +H	+
1+2+5	−	AA-4 +T	±
1+3+5	±	AA-4+G	+
2+3+5	−	AA-4+P	+
		AA-4+A	+

!!

4-19-54

CG-3 possible auxotroph: run duplicate, 3 days: Slight growth in all tubes, but good growth only in AA 5. Will pick & test several col. from plate streaked 4-17 in min, complete, & AA 5.

A6·6 auxo; original single omission tubes, incu 4 days; Slight growth in all tubes except minimal and −AA1 (includes milk + −b). Sulfide ?,?

Second set drop test (incu 3 days);

HC	++++	complete	++++
HC+A4	++++	minimal	—
1+2+3	+	AA−4+H	+++
1+2+5	+++	−4+T	+++
1+3+5	+++	−4+G	+++
2+3+5	—	−4+P	+++
		−4+A	+++

Try AA1 group + individual acids of group; incu 48 hrs

4-20-54

CG·3 picked 5 small separate colonies

At 24 hrs, no growth in any tube; incu +AS; still turbidity and no plate. Continue incubation

A6·6 single omissions Slight growth in A1 (Very small thin type on plate?) but not in individual AA of the group. Try single omissions Slight growth in all at higher conc.

4-21-54

A6-6 amotiph At ca 24 hrs, no growth in A1 single crossings in A1. ~~(Control number all mixed with [illegible] current label of [illegible] and acid)~~

[illegible] A1 (2 days) A1 group-quite good growth. Shall [illegible] turbidity, no mucin, [illegible] Negative. [illegible] at same day.

CG-3 "amotiph" from single colony growth vigorously and in mixed culture + A5. T. O.

4-23-54

A6-6 amotiph; A1 3 days, moderately good growth in AA1 - organisms + - hyphae. Poor growth in AA1 capsule group. No growth in AA1 - mucin - capsule. Kill. Typical organisms.

4-26-54

J.L. reports that Kleb. results were due to mixed culture & cannot be reported. However, these cases may be less explosive and [illegible] - [illegible] can be considered just as other organisms.

Try [illegible] use AG only, since it is not necessary to have ammonium with the AG broth. Use AG "R" & AG "M" to see if any difference in balance in pairings.

Inoculate to 5 ml of the [illegible]

AG "R" } as previously mixed
AG "M" }

A. radiobacter 1001
1005
1007 } to [illegible]

4-27-54

Bro the mic. 4-26: Noticeably good growth in all. The "rough" AG has a flocculent growth of A. radiobacter in this medium (AG-I) very similar to "smooth" in "mucoid" A. tumefaciens.

Streak each radiobacter culture on AG-I, see whether more than one colony type.

4-29-54

Strains of A. radiobacter (48 hrs):

1001 : 2 types of colonies : ① small
② large + spreading.

1003 : [illegible]

1004 : [illegible], same as 1001.

Pick strains of both types as before.
Also pick strains 1 & 5 to have
strain variation fresh overnight.

The large type is thought to be a [illegible] variant
because the mass growth has a mottled
appearance in small colonies.

Inoculate bottles for passage on A6 'A' + 'M'.
Acetic.

4-30-54

Started passage on A6 'A' + 'M'. 1000 U/ml penicillin

5-1-54

Both grew in pen tubes.

Henderson, A.A., Baldwin, T.L., and Fiske, A.S. 1934 Studies on certain physiological characters of Euplotes harpa, E. rheyanus and Euplotes adhaerens. Part II. J. Exp. Zool. 67, 247

Absorption of radiant energy as a measure

5-3-54

Start broth (see 4-6) of AG M for irradiation.

5-4-54

Pump got turned off, so culture was not aerated over night. Had been aerated for last 6-7 of the 24 hours it was grown

Plate 10^{-5} + 10^{-6} dil. of mix culture (cfa, washed, resusp. in 10 ml saline)

Irradiate 40 sec in saline in big lamp. Plate 10^{-3}, 10^{-4} & 10^{-5} dil of irradiated culture.

(Lo Fatt, Sinnott & Taylor made Nov 1 irradiation)

5-6-54

Irradiation expt: 40 sec. UV gave near lethal killing. 20 plates after irradiation to be counted to estimate fraction of 10^{-6} dilution giving a survivor count of 3.2 per plate, i.e. 3.3×10^{8}. Had estimated 10^{6}.

Five plates of irradiated culture (i.e. one showing which appeared at 24 hrs and is orange, yellow, + glowing like previous survivors. Stuck

cul + test.

5-7-54

Stock from cul picked from UV plate grow
well on plate in 24 hrs. Take cul to
incub + on plate both.

5-8-54

at about 48 hrs, good growth of cocci on
plate, none on incubated

Have cultures ready for plate incubation by
end of next week.

"R" and "M" cultures of A6 + A6-6
S^{IV} from A6

Also - grow A6-6 S^{IV} with A6 "M", recover
S^{IV}'s + test. Do residual plating of A6 "M" to
check rate of mutation to S^{IV}.

CG-1-M

Incubated together A6 "M" and CG-5 from water
suspension of seeds.

5-10-54

CG-1-M

Use A6-1 broth to A6 "M" & CG-5 respectively

UV ampoule [illegible] 3 days [illegible] A6 UV-1

Cultures to use for plant inoculation:

1) S^{IV} from mixture 2pl
2) CG-1 M
3) CG-1 R
4) CG-3
5) A6 UV-1
6) CG-2 M
7) CG-2 R
8) CG-4
~~9) A6 UV-1~~ 9) CG-5

Streaked mixed culture on AG I & AG I SM
[All but [illegible] streaked for light [illegible] 48 hours on rich media at room temperature with aeration.]

5-11-54

Three renditions of A6 UV-1 (tubes)

Started work of A6 for UV testing curve

In mixture experiment, should have spread cultures & S SM E after dilution, rather than shaking, to get estimate of proportion of S^{IV} to S^{V}. Start an identical mixture, to be plated at ca 48 hrs. Also plate separate cultures on complete & incomplete + SM at 48 hrs.

5-12-54

AG UV-1 at 24 hrs slight growth only on complete and YNA.

Mixed culture – streaked on complete ♂ + ♀ SM –
On complete only, 2 colony types; large, gummy ("M" type) and small translucent ("R" type). The virulent sensitive culture used is an "M" type, and the avirulent, resistant, an "R" type.
On complete + SM; the "R" type only. Not yet large enough to pick readily.

UV killing curve;
Irradiated 10^{-4} dilution (in saline) of overnight, aerated, broth (AG-I) culture of AG (M). Plate as control final dilution from original culture of 10^{-7} and 10^{-8}. Irradiate 30, 60, 90, 120, 180 seconds.
Through 60 sec, same dilutions as control
90-180 sec, 10^{-6} and 10^{-7}

Spread AG-I + AG I SM plates ♂ 10^{-5} dilution of 2 day non aerated broth cultures of CG-1-M and CG-♀.

5-13-54

(48 hrs)

AG UV-1 sundowns: fair growth in min. + YNA, none
c other additions, but slight turbidity in minimal;
Recheck. Also check parents & progenitors (as 2 reps)

Mixture streaked 5-10 on complete c & s S14:

Complete; on S14: Colonies of dominately "M" type;
some "R" type. Pick a number of both
types, test for S character. ~~Have one~~
few of the large, opaque colonies which
would always turned out to be auxotrophs
~~Pick + test.~~ Don't know whether M-M or S

Complete + S14: Great majority of colonies are
the "R" type, like the S^R culture used. There
are now a few "M" type colonies appearing.
Pick a number of the "R" colonies and as
many as possible of the "M" type. Test both
in secondary test. The "R" type could be
pooled, but the "M" type should be streaked
individually.

Plant inoculations to be done 5-14. Will not be
able to pick & grow up separate colonies from
mixture plate (except for a few "M" type).

(SM plate)
Scrape colonies off agar from areas where there are no "M" type colonies; mix mass into both inoculate slant & this mixture.

These are the cultures to be used;

CG-1-M	1-M+5 (M1)	"M" type from mixture streaked on SM (Each 1 colony)
CG-1-R	1-M+5 (M2)	
CG-2-M	1-M+5 (M3)	
CG-2-R	1-M+5 (M4)	
AG UV-1	1-M+5 (R1)	"R" type from mix. on SM; each pooled colonies
CG-3	1-M+5 (R2)	
CG-4		
CG-5		

AG-1 SM
Spread AG-1 plate & 10^{-5} dil. of 1-M+5 mixture mix into both 5-11.

5-14-54

AG-UV 1 numerous; Still no growth (3 days) except in YNA, and few colonies, mucoid.
Sub up Mucoid, YNA, penicillin, pyrimidines

Plates of CG-1-M and CG-5 since 5-12;
(Spread of ~0.1 ml 10^{-4} dilution of 2 day, unaerated broth)

CG-1-M; [illegible] 616 col
[illegible] no colonies.

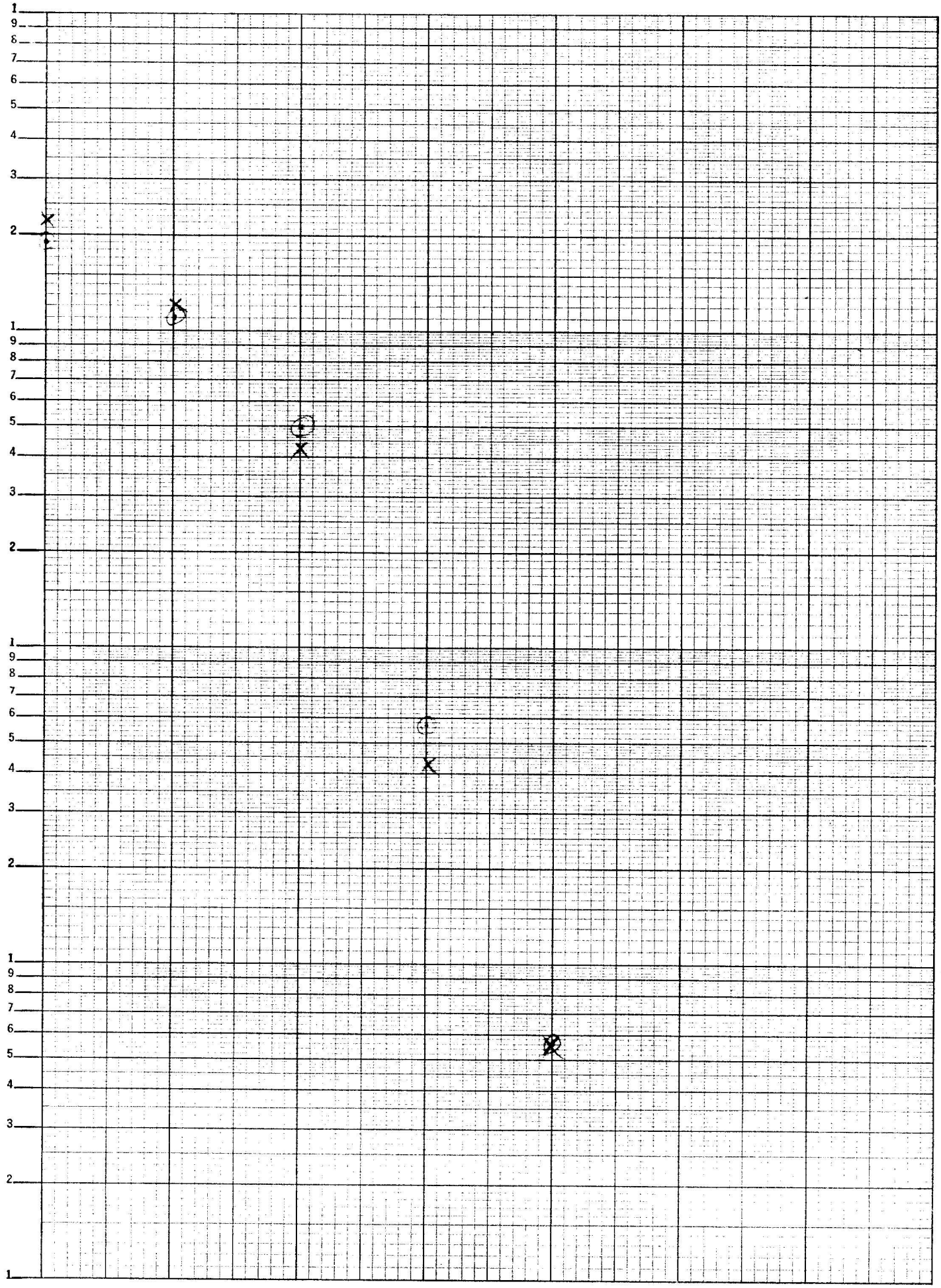
CG-5 [illegible] 997 col.
[illegible] much smaller. Looks like [illegible]
may be too high for this.

Colony types seem to be quite stable; on each AG-1 plate there is one colony which looks enough like the typical colony type (1 "A" on 1-17 plate; 1 "A" on 5 plate)

UV experiment

Exposure	10^{-6}	10^{-7}	10^{-8}	Exposure	10^{-6}	10^{-7}	10^{-8}
0	—	1465	219	120	54 0	2 0	—
30	—	1025	116	180	0	0	—
60	—	417	58				
90	432 165	70 20	—				

[illegible]


KEUFFEL & ESSER CO., N. Y. NO. 358-81
Semi-Logarithmic, 4 Cycles X 10 to the Inch, 5th lines accented

Plates after < 2 days' incubation. The colonies on the 90 sec plates are very tiny, can hardly be counted. Continue incubation of these and the 120 & 180 sec. plates.

On the 90 sec 10^{-7} plate there are three very large glossy colonies, one of which is regular and flower, circle O, one looks as though 4 areas of 4 cells stuck together E3, and one which has a fringe and the typical sector ⊗. Pick a & streak them.

After one more day, refrigerate plates & appropriate ones to be removed to check for autofluorescence.

Pick as many possible sectors from 1-M + 5⁻ plate streaked 5/10. Look for mycotrophy also whether S^a to determine [illegible].

Blood inoculations: Growth of the following very poor, [illegible] medium small: 1-R 3 5⁻ XM2 XM4
[illegible] pt to [illegible] 2-R 4 XM1 XM3 XR1 XR2

5-15-54

UV killing curve diff.

More colonies have come up (3 days) on 90 sec plates, and some more present on 120 sec plates. Dilutions:

	10^{-6}	10^{-7}	
90	432	70	Counts on 0-60 sec
120	56	2	plates individually
180	0	1	enlarged.

Streaks from large col on 90 sec plate

One type of colony from the large, translucent - gummy, glossy

Unlined col. 2 types, one as above, the other small & translucent. (2)

Irregular col. all individual col gummy & opaque; but many growth does not look uniform.

Some all these, test col types for auxotrophy

Possible auxotrophs from multiple plate: very scanty growth in 24 hrs, unlike previous ones on plate.

Spread plate of 1+M+5 mixture M-type seems predominant; difficult to tell the two types apart. Continue incubation.
Spread plate All col. very small; moderately dense mat in non-Sel. plate. (281)

5-17-54

90 sec. irradiation; streaks from large colonies; (more large)
All except sectored (#2) show only one type of colony: usual large gummy type.
Streak from sectored col. - 3 types
1) large, gummy
2) smaller, translucent
3) bright yellow, very rough, wrinkled (contaminant?)

Many of the large, gummy colonies have narrow sectors of translucent growth.
Pick, streak several of each type.

Possible auxotrophs from mixture plate. Pick, spot on complete for replication to minimal.

5-18-54

Possible auxotrophs from mixture expt: Replicate from AG-1 to AG-24 AG-1-SM to check for auxotrophy and to tell whether CG-1-M or CG-5.

AG UV-1; Attempt to run down specific growth requirement. At 24 hrs, grew thickly on YNA, not in penic mix nor peptone mix.

Cultures from selected colony, AG UV plate

1) "normal": Quite good growth for 24 hrs, no sectoring visible as yet; colonies appear uniform.
2) yellow; 2 col. were picked; one looks same as "normal"; the other shows very scanty growth at 24 hrs. No color. [EML had yellow colonies on one EMS plate which looked similar to this yellow culture at 3-4 days]
3) translucent: Had growth in 24 hours. Colonies appear uniform. Very similar to "normal" but slightly less opaque.

Plates saved from UV irrad 5/12: Replicated to AG-2.

Shaked broths (AG-1) 3:30 pm of all A. radiobacter cultures for microscopic examination. Inc. at room temp 5 aeration.

5-19-54

AGUV-1 medium: At 48 hrs, growth only in YNA, with no pronounced change in color. Before going into identity of organism, subcult on AA's + on Na2S.

Also subcult AG-6 mutants on AA's, YNA, and Na2S. Am adding Na2S to steril medium, very dainty fine steril stock soln and also diluted 1:10 and 1:100.

AGUV, culture from sorted colony

1) "Normal". Quite a number of colonies (about a quarter of the well separated ones examined) were shown small sectors of translucent growth. There is one "pure" translucent colony and several colonies consisting of a sectored mix of this & the normal form. ← dense translucent

Pick: (1) translucent
(2) sectored (several)
(3) "pure" opaque; streak out.

2) Translucent: Colonies are generally similar in size
from the opaque "normal". Pts. [illegible]
However, in one of the three strains, many small
translucent colonies [illegible]
"R" type have since died and (48 hrs)
From this stock, pick 4 streak cultures
and "R" type.
The above clones [illegible] normal
"M" type [illegible] associated [illegible] with
rapid growth, forming colonies 1-2 mm in
diameter in 24 hrs.

3) Yellow: The yellow [illegible] is a [illegible]
contrast to 1 - Zinc (Pale) [illegible]
a peculiar smooth [illegible] the [illegible]
[illegible] appearing on plate (24 hrs). The
colonies grown at 24 hrs (on S/C) [illegible]
1/2, many are noted as described under D.

Streak CG-1 M-R for [illegible]

Possible mixes: [illegible] after [illegible]
picked from one plate & streaked [illegible] on plate, 4
[illegible] were picked from each of the 4 strains. Of
these, 2 from #1 and 1 from #4 are S^n. [illegible]

[illegible] [illegible] [illegible] oriented [illegible] CG-5. [illegible] [illegible] whether any are [illegible] so far it appears [illegible] [illegible] [illegible] [illegible] [illegible] [illegible]

[illegible] [illegible] seems very satisfactory, especially [illegible] [illegible] liquid

Try: 1) [illegible] acetone

2) Addition of ferric [illegible] [illegible] [illegible] (Macdonald, [illegible] & Peterson, 1941)

Fe^{+++} 5 γ/ml

Mn^{++} 0.1 γ/ml

[illegible] whether these conc. refer to the [illegible] [illegible] salt ([illegible] [illegible] and $MnSO_4$)

Made up $MnCl_2 \cdot 4H_2O$ [illegible] 1.24 mg/ml [illegible] [illegible] 1:200

Fe_2 [illegible] stock 12.0 mg/ml ([illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible])

5-20-54 [illegible]

A6 IV-1 [illegible] [illegible] 3 days Still [illegible] only [illegible] YNA.

[illegible] [illegible] A6 IV-1 [illegible] [illegible] [illegible] no growth [illegible] 20 hrs except in complete.

[illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible]!

A6 [illegible] [illegible] ? [illegible] [illegible] [illegible] [illegible] [illegible]

Streaked [illegible] 5/19 [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] (24 hrs).

From "transect" streaked on 5/17 plate, which I called "A" type was probably accidentally the yellow contaminant.

CG-1-R&M streaked [illegible]
No visible growth on AS-1 in 24 hrs.
All the dominants from the original plate (except the contaminant) grew up on this AS-1 [illegible]

Replication of UV plates (Exp. 2) [illegible]
Find possible duplicates and by eye
Six marked ones; 3 are the large
colonies with yellowish-white centers.
1.) Circle all dense, [illegible] seeing
all are ours. Pick up other 2 ml.
Other possible ones were small [illegible]
being of about normal appearance & small,
less dense than "normal"

Numbering:
2UV-1 (= 1 from 2nd irradiation)
Upper, more central from original.
2UV-2 } from 2 large red on 90 sec plate
2UV-3 }
2UV-4 } small ones from 90 sec plate
-5
-6 }

[illegible] A. radicicola with plant roots; [illegible] d, diluted 1:20, [illegible]

1 1001 S 1R 5 7R

[illegible] formation. Specific for 1007R, [illegible] 1; 1007R-def [illegible] Nutrilite ppt.

100 U/ml

Supplement-resistant strains were obtained in 1947; exact [illegible] number. Also prototroph, 1008/mL Inj-sitive, SM, ST resistant and A6-6 auxos; A6 UV-1; new auxos (2 UV series)

Try cross breeding 1008 to see whether it can be secured readily.

5/21/54

Numbers of A6 [illegible] and A6 UV-1: No growth on any supplement (48 hrs). [illegible]

2 UV 1 - 2 UV 6: No growth on only a Fam can mineral. 1, 2, & 3 will grow at 24 hrs on complete, 4, 5, & 6 scanty.

Derivatives from restreaked colony (= 2 UV-1);
Restreaked each on streaks done 5/19, both from restreaked and one unstreaked colony. This culture is being saved as 2 UV-1, so can be checked again later for mutagenic stability if necessary.

Inoculated soft agar tubes of Difco Bacto 1007S, and 1007R to see if any mutants (rough); if so, can get some highly unstable cultures.

Basic stock numbers to find each strain
A6-6 (1) = CG-7 } unstable?
A6-6 (2) = CG-8 }
A6UV-1 = CG-9 (YNA)

5/22/54

Readings: (4 days; 1 day when Mm & Pa added)
CG-7 & CG-8: faint growth on YNA, probably on highest Na₂S conc.
CG-9: good growth on YNA; possibly slight on highest Na₂S conc.

5/24

Revertants (6 days on ii Mn & Fe added)
CG-7 ++ fair growth in AA-1; ++ 11 in YNA; slight in Na, S neg. even and 1:10.
CG-9 Possibly slight growth in YNA [illegible]
(The Mn was added before autoclaving, & after autoclaving both plates 1 ppt.)

5/28

Plant inoculation (now 2 weeks):
Shells on all plants except CG-1-M, CG-1-R, CG-3, CG-4. 5th leaf on AG shows bleaching & loss of variegation.

Spotted CG 7, 8, & 9 and 2UV-1 through 6 on AG-1 for replication to suppl. plates.
(Note: Minimal plates now contain Mn & Fe)

Started aerated bottles of CG-7 & CG-9 to be spread on SM & ST media.

5-29-54

Spotted CG 7, 8, 9 & 2UV1-6 to supplemented plates

Spread aerated overnight cultures of CG 7 & 9 on Spread ST plates (SM 1/4 the conc. mixed with 0.05 M/ml Ca++; ST 100 U/ml.)

5/31/54

Numbers of cells (peaks)

	O	A1	A2	A3	A4	A5	HC	VITS	YNA	Solid
CG 7	−	−	−	−	−	−	−	−	−	−
X	−	−	−	−	−	−	−	−	−	−
1	+	+	+	+	±	+	−	−	+	+
2UV 1	+	+	+	±	±	+	+	−	+	+
2	−	−	−	+	+	±	+	−	−	−
3	+	+	+	+	+	+	+	−	+	+
4	−	−	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−

all [illegible] replications [illegible] complete.

Can tell [illegible] in spite of these results by assuming [illegible] for 11 plates from one original.

Further incubation. For 2UV 4, 5, 6 try AA [illegible] AA + citric against extract.

Recheck CG 7 and 2UV 1&3 in liquid [illegible] may [illegible] being [illegible] in [illegible] in numerical [illegible]

ST and SA1 expts:

CG 7: [illegible] too few; background growth quite heavy; no [illegible] suitable media.

ST: Almost confluent growth of very small colonies; perhaps enough ST^R present so inoculum should have been smaller.

CG 9: SN: Light background growth + ca 800-1000 resistant papillae per plate. Papillae are of both the smooth & rough (or dense and translucent) colony types.

ST: Very light background; no papillae as yet.

6/1/54

Rundown plates. Only change over night; response of 2 UV2 more clear cut

A3 +

A4 +

HC ++

All others —

Would have expected CG 7 & 8 to respond to A1 as they do in liquid media.

CG 9 S^R: All 4 col. picked as possible S^R growing well on AG2SM.

Other antibiotic plates (now 3 days)

CG 9 ST: No change; no papillae - background very light

CG 7 SM: Growth so heavy, suggests this culture may already be S^r. Check slant

CG 7 ST: Papillae almost confluent; picked a few of largest papillae & streaked on St medium. However, if this represents normal rate of mutation to St^r, this marker won't be very useful. Check slant - streak out & test individual colonies; if St^s, grow in broth & spread plates.

6/2/54

Rundown plates: CG 7+8 now well grown on H.C.; faint growth of these & of 2 UV 3, 4, & 5 on YNA.

CG 9 S^r: Pick 1 col.; streak on AG-1 $\bar{5}$ SM; pick & Test several of resulting col. for S character; save 1 pure S^r.

Need testing SM & St resistance of all auxotrophs by cross. Crushing on AG 1 $\bar{5}$ SM & St.

CG 7	2 UV 1
8	2
9	3
	4
	5
	6

5/3/54

Final readings on swendrow plates (5 days)

	O	A1	A2	A3	A4	A5	HC	VITS	YNA	Na_2S
CG 7	−	−	−	−	−	−	+	−	−	−
8	−	−	−	−	−	−	+	−	−	−
9	+	+	+	+	+	±	(−)	(−)	+	+
2UV1	+	+	+	+	+	+	+	(−)	+	+
2	−	−	−	±	±	+	+	−	−	−
3	+	+	+	+	+	±	+	(−)	+	+
4	−	−	−	−	−	−	±	−	−	−
5	−	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−

6/4/54

Tests of auxos for S & St sensitivity by cross-breaking

	S	St
CG 7	R	R
8	R	R
9	R	S

	S	St
2UV1	S	S
2	S	R
3	R	S & R
4	R	R
5	R	R
6	R	R

However, spread plates indicate that CG7 is not uniformly St R nor CG9 uniformly SR. CG7 did appear to be uniformly SR. CG7 [illegible] picked out on AS-1; pick & test at least

10 col. for St reaction.

6/5/54

Picked col. of CG7, cross streaked ē St.

CG9; 2UV1 and 3; Check for auxotrophy.
1) spotting on minimal
2) growing in minimal tubes,
ē & s̄ aeration.

Checked motility of auxotrophs (microscopically from rather old plate). Under these circumstances only CG7 and 8 motile. All should be checked in soft agar.

6/7/54

CG7 single colonies/St; all 10 St^R.

CG9, 2UV1 & 3; Failed to grow in aerated or non-aerated mini. tubes (2 days). On mini. plate, growth very scanty. Unfortunately failed to spot w.t. as control on plate.

6/8/54

Made rundrew plates - single omission of AA groups - to check 2UV3, 4, & 5. Will also include other

auxos, since none of the requirements is completely determined
Spotted auxos on AG 1 for repl. to rundrums

6/9/54

Auxos on AG-1 (24 hrs); Good growth of CG 7, 8 + 9; 2 UV 1, 2, + 3; fair growth of 4, 5, & 6.
Replicated to AA grasp single omission rundrums

6/10/54
Streaked all CG and radiobacter cultures on AG-1 + aniline blue added (1:10,000).

6/11/54

Examined galls (4 weeks). See results on next page. Saved plants for recovery of organisms from galls.
In plants in 1-14+5 series, where one +, for example is indicated, there were usually one or 2 good galls and very little hypertrophy at the other inoculation sites. In other cases (purified cultures - A6 derivatives) galls were quite uniform at all sites.

Doubtful auxotrophs spotted on AG-2 June 5th. Moderate growth (CG 9, 2UV1 & 3) Should have used prototroph control. Still no growth in minimal tubes inoculated at the same time.

Pseudorevs replicated 6/9

	0	-A1	-A2	-A3	-A4	-A5	AA+	HC	Y.EX	complete
CG 7	−	±	+	+	+	+	+	+	+	+++
8	−	±	+	+	+	+	+	+	+	+++
9	±	±	+	+	+	+	+	−	++	+++
2UV1	±	−	+	+	+	+	±	+	+	+++
2	−	+	+	+	+	+	±	+	+	+++
3	±	±	+	+	+	±	+	+	+	+++
4	−	−	±	±	±	±	−	−	+	++
5	−	−	±	±	±	±	−	−	+	++
6	−	−	±	±	±	±	−	−	+	++

Note: this is the second time CG 9 has shown anywhere from a trace to good growth on minimal and a variety of supplements, but no growth on minimal + HC.

Attempt isolations from (excised) plants. Macerate seeds in sterile H_2O, streak heavily on B glucose AG-1 + aa. pl.

Plant readings 6/11/54 #29

Control

CG5 +
" +
" ±

CG2M ±
" ±
" ±

CG4 +++
+++
+++

CG3 +++
+++
++

CG1-R + (pen south of plan
++
++

CG1M +++
+++
+++

AGUV-1 0
±
0

1M+5R1 ± (one good gall)
1M+5R1 +
" R4 +
" M3 +
M3 +
M3 ++
M2 +
M1 +
M1 ++
M2 ±
M2 +
M1 ±

1M+5R2 +
R2 ±
R2 +
M4 ±
M4 ±
M4 ±

When through 5 plants, cut tops off & throw in garbage can; did in large cans near door.

6/12/54

At 2 days, no absorption of aniline blue ☜ any of cultures used.

6/14/54

Made attempts to recover bacteria from galls, as listed on previous page. Macerated slices of gall tissue in 2-ml saline, streaked on ½ BC-1 at

Rundown - final reading (repeated 6/9)

	0	-A1	-A2	-A3	-A4	-A5	-A1+	HC	YEX	Complete
CG 7	−	±	++	+++	+++	++	++	++	+	++++
8	−	±	++	+++	+++	++	++	++	+	++++
9	±	+	+	++	+	+	+	−	+	+++
2UV1	±	±	++	++	+	+	+	+	+	+++
2	−	++	++	++	+	+	++	+	+	+++
3	±	++	++	++	+	+	+	+	+	+++
4	−	−	+	+	+	+	+	+	+	++
5	−	−	+	+	+	+	+	±	+	++
6	−	−	+	+	+	+	+	−	+	++

Being fed at one edge by mold colony

Note that most cultures grew best in the absence of A3, and CG 748, in the absence of A4.

2UV 4, 5, + 6 Type A1 alone ☜ various other complex, mainly. Also try this ☜ CG 748. In this expt.,

CG 9 & 2 UV 173 grew little if any and unsupplemented, in contrast to previous experiment.

6/16/54

Isolations from galls: After 2 days, all plates have colonies predominating that look like Agrobacterium. Some col. from galls and CG 4 + CG 5) absorb aniline blue strongly

Pick at least 10 col. each. Test for pentotrophy/auxotrophy and Say. Save for tests of virulence. CG-1-R as received is still smooth

Save actinomycete on 1-M+5 (173) plate for J.L.

6/17/54

At 3 days, the col. from CG-4 that absorbed aniline blue are very large, gummy, and yellow. Test one of these and one ordinary, colorless colony for SM resistance and auxotrophy. The blue-absorbing col. from CG 4 did not become yellowish or very gummy.

Now on 3rd day, more contaminants are showing up on streaks; most are highly pigmented, yellow, orange, and pink.

R2 from mixture appeared came out smooth, unlike CG-1-R, which was rough when received. This probably indicates that the organisms causing the gall were from the CG-1-M used in the mixture, carried over as a contaminant when SR-grown [redacted] picked, or perhaps a mutation to SR in CG-1-M

For consistency, CG-1-M and CG-2-M should be called smooth, rather than mixed, because they are not unusually gummy for this organism. As of now, CG-1-M = CG-1-S; CG-2-M = CG-2-S.

Made up a batch of AG-1 agar using 0.5% mannitol instead of 0.5% sucrose to see whether a different carbohydrate would make any difference in uridine blue absorption.

Made up 20x salts solution for AG-2, incorporating Fe & Mn in salts (from core stock [illegible]).

6/18/54

Some streaks have gall isolations not yet grown up enough to pick. Continue inc. for another day.

6/19/54

Spotted all aupos on complete for replication to AA's + AA1. Stored supplemented plates in frig.

Picked 1 col from each streak from 6/15/ plates of gall isolates.

CG-1-S: some streaks have a few R-type col.

CG-1-R; all R type col, except 1 streak mixed S & R.

CG-4: 3 of the ten streaks are yellowish, with intense blue color in the medium. The remainder are ordinary white S colonies.

CG-5: One streak - no growth; of remaining 9, one has blue colonies & intense blue coloring in medium. Blue colonies are small and not gummy.

CG-9: White, S-type col. Some streaks have a very few R.

XM3: 1 yellow, very gummy; 2 moderately gummy remainder less gumming and rather translucent.

XR2: All colonies moderately smooth; not so smooth as CG-1-S

Spotted each suspension on complete for replication to minimal + SM.

6/21/54

used half the conc. used for E. coli (0.1 mg/ml)

Replicated gal re-isolates to AG1, AG1 (SM) + AG2. (3 of the isolates from CG4 & 2 from X173 are very yellow & "runny"; check identity)

Replicated auxotrophs to complete, mini, and A1 + other AA groups accordingly.

6/22/54

Put on stabs:

CG-1-S
CG-1-R
CG-2-S
CG-2-R
} Have stabs only of A6 and A6-6 as received (mixed S & R)

CG-7	2UV-1	2UV-4
CG-8	2	5
CG 9	3	6

In plant experiment, inocula taken from mixtures were put on slants as well as inoculated into plants. Strut out R2 + M3, pick 10 or more colonies, test for purity & respect to SM.

6/23/54

Gill isolates

	Type of growth	SM		Type of growth	SM
CG-1-S	10 S	$10 S^S$ (one now)	CG 9	10 S	$10 S^R$
CG-1 R	2 S, 8 R	$10 S^S$	X 1 M 3	10 S	$2 S^S$, $8 S^R$
CG-4	3 S yellow, 7 S white	$10 S^R$	X R 2	10 S	$10 S^R$
CG-5	8 S, 1 R	$9 S^R$			

S^S more gummy than S^R though all are smooth type of growth.

CG-1-S ; all S^S. But all spots have a few S^R colonies (2 days). CG-1-R ; no growth at all on SM.

All isolates grew very well on minimal.

The three yellow isolates from CG 4 are bright yellow and extremely gummy ; check microscopically

Rundown since 6/21

	0	A1	A1+2	A1+3	A1+4	A1+5	A1+HC
CG 7	−	−	−	−	−	−	+
8	−	−	−	−	−	−	+
9	−	−	−	−	−	±	±
2 UV-1	±	±	±	±	±	±	+
2	±	±	±	±	+	±	+
3	±	±	±	±	±	±	+
4	−	−	−	−	−	−	±
5	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−

Might be able to try some SRP (or St^R P) experiments, using the following cultures. All are apparently stable auxotrophs, although their requirements are elusive.

	S	St	Source	Arrangement on test plates	
CG 7	R	R	CG-2		
CG 8	R	R	CG-2	1 S	2UV 1
				1 R	3
2UV-2	S	R	CG-1	2 S	4
2UV-4	R	R	"	2 R	5
2UV-5	R	R	"	7	6
2UV-6	R	R	"	8	

Check these and CG-1 S & R and CG-2 S & R by cross brushing $\bar{c}$ S/R & St. If the prototroph used is $S^S St^S$, one of these markers could be used, possibly along $\bar{c}$ virulence, as an unselected marker.

In testing S/R & St status, include 2UV1, previously recorded as $S^S St^S$, as control.

Stabs - CG 7 stab appears to be contaminated (yellow). Use new stab from slant.

6/24/54

Pick colonies from streaks of XR2 and XM3 from slants; restreak.

Readings made 6/21:

	0	Al	Al+2	Al+3	Al+4	Al+5	Al+HC
CG 7	±	±	±	±	±	±	++
8	±	±	±	±	±	±	++
9	−	−	−	−	−	−	(+)
2 UV 1	±	±	±	±	±	±	+
2	±	±	±	±	+	±	++
3	±	±	±	±	±	±	+
4	−	−	−	−	−	−	±
5	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−

CG 9 has always failed to show even a faint trace of growth on HC alone.

Needed a better minimal medium.

Try medium based on Hodgson, Peterson, & Riker, 1951

$(NH_4)_2SO_4$	1.0
NaCl	0.2
$CaCl_2 \cdot 2H_2O$	0.1
$MgSO_4 \cdot 7H_2O$	0.2
$ZnSO_4 \cdot 7H_2O$	8.8 mg
ferric alum	43 mg
$MnSO_4 \cdot 4H_2O$	0.8 mg

Use K_2HPO_4 0.2 g/l
KH_2PO_4 0.1
per liter
+ sucrose 20 g/l for liquid
5 g/l for agar
+ 40 ml pH 7.0 phosphate buffer per liter

Use Mn + Fe solns as made up - Use 1:200

Make up $ZnSO_4$ soln to be used 1:1000

0.176 g / 20 ml acetic soln.

This differs from present medium chiefly in N source ($(NH_4)_2SO_4$ instead of KNO_3), also has less $CaCl_2$ and includes yeast.

Transferred to slants one example of each kind of isolate from galls - 1 per gall where all appeared identical; one example of each type when there were 2 or more types from one gall.

6/25/54

6/21 rundowns.

	0	A1	1+2	1+3	1+4	1+5	1+HC
CG 7	±	±	±	+	+	±	++
8	±	±	±	+	+	±	++
9	±	−	−	−	±	−	±
2UV 1	±	±	±	±	±	±	+
2	±	±	mold	±	+	±	+
3	±	±	±	±	±	±	+
4	−	−	−	−	±	±	+
5	−	−	−	−	±	±	±
6	−	−	−	−	±	±	−

Checks of streptomycin and streptothricin resistance:

	S	St		S	St
CG-1-S	S	R	2UV1	S	S
1-R	S	R	3 (?)	S	R
2-S	S	R	4	S	R
2-R	S	R	5	R	R
7	R	R	6	R	R
8	R	R			

Hence St not useful to distinguish between proto. & auxos. {2UV-4 had been scored S^R on previous test}

2UV6 looks like a good auxo to try in SRP test. Preliminary: How to make sure it is really A. tumefaciens?

1) Microscopic
2) Growth in complete broth, aerated and unaerated.
3) Growth at room temp + at 37°

(For (2) & (3), use CG-1-S & R for comparison).

4) - Eventually - plant inoculation.

6/26/54

2UV6, CG1-S, & CG-1-R; Aerated; Pump was turned off sometime during evening, so don't know how long cultures were actually aerated. CG-1-S aerated in

very much heavier than uncerated; slight difference between 1-R aerated and aerated; and little or no difference between 2UV6 aerated and unaerated. (ca 20 hrs)
Growth at 37°: CG1 S&R - slight growth; 2UV6 very little growth. Growth of all unaerated cultures at room temps much better than at 37°.

Yesterday made up 5 plates AG1 c 1:10,000 aniline blue (Coleman-Bell). Have been using Natural Aniline, same conc. Natl Aniline → pale greenish blue medium; Coleman-Bell → grayish purple.
Streaked CG1 S&R on plate made c Coleman Bell dye.

Motility check on 2UV6: Motility about the same as CG-1-R; higher proportion of motile cells than CG-1-S. (Observations on 20-22 hr cultures, AG-1 broth, room temp.

Inoc. both cultures of 2UV6 and CG-2-S, together and separately, to be inc. at room temp c +5 aeration

XM3 & XR2 from slants to ((1-S)+5 mycetism agar.)
Picked 1 col from each streak (12 from each culture)
spotted on AG-1 for replication to SM.

6/28/54

Tiel's Brown-Cell analysis Blue; Neither CG 1-5 or 16 colored on AG-1 with dye 1:10,000.

2UV-6 identity. Have reservations about this culture being Cryptococcus because:

1) cells appear slightly smaller than CG-1
2) CG-1 grows slightly at 37°; 2UV-6 not at all
3) CG-1 grows poorly in AG-1 broth S aeration 2UV-6 grows almost as well S as C aeration (Final opacity of culture about the same, but requires 48, rather than 24 hrs. CG-1 never does grow very well in unaerated broth).

XM3 & XR2 from slants Replicate to AG-1-SM.

Plated CG2-S / 2UV-6 crosses on AG2-SM.

Grown together - aerated — Grown separately - aerated
Grown together - not aerated — Grown separately - not aerated

2 UV-6 - In further check on identity, streak on EMB sugar media, see if it ferments any sugars.

6/29/54

XR2 & XM3 from slants; all 12 colonies tested from streak of each are S^R - no evidence of mixture. (Though of course S^R could come from mutation in CG-1-S, rather than from CG-5.

SRP plates (now only ca 20 hrs)

Controls: 2 UV6 aerated & unaerated, no growth.

CG-2-S unaerated: moderate background film, a few papillae ("S^R mutants")

CG-2-S aerated: moderately heavy background growth, no S^R papillae.

"Cross" plates

1) Grown together, aerated: Moderately heavy background film, possibly some papillae

2) Grown together, not aerated: Less heavy background film, many papillae.

3) Grown separately, aerated: Moderately heavy background, no papillae

4) Grown separately, not aerated: moderate background, many fine papillae.

Also papillae better grown, quite test for autotrophy. Probably autotrophs being fed by background of CG-1-S. If prototrophic - !!!

Too early to try reading any conclusions. Results observed so far could be explained by feeding of 2 UV6 by background growth of CG-2-S; in aerated cultures, the background growth of CG-2- may be heavy enough to obscure papillae. This growth of CG-2-S seems to be due to too low SM conc, rather than confluent growth of S^R mutants, since papillae are readily seen on plates spread ē unaerated cultures. SM conc critical; at twice the conc. used, growth of S^R cultures is inhibited.

Next - Try "cross" using S^R auxo from CG-2, so a "normal" CG-1 can be used, and virulence of any resulting colonies can be tested. (Quite likely that S^R auxo from CG-1 may have lost its virulence - this apparently happened ē CG-9).

Use CG7 (from CG-2) and CG-1-S.

Grow as before, mixed & separately, aerated & unaerated. Also one extra mixture tube, one ē aeration for several days, rather than only 4 & hrs.

(Again - identity of auxo. as Agrobacterium is not definite.)

1:30 pm 6/29

Picked 4 col. each 2-S / 2UV6 "crosses" grown together & grown separately & plated together. Streaked on AG 2 S M.

6/30/54

SRP plates:

Controls:

2UV6 aerated and unaerated: no growth

CG-2-S aerated; Moderate background growth; one large colony; many small papillae (thousands).

CG-2S unaerated: Heavy background growth (confluent growth corresponding to small papillae on plate from aerated culture?) ca 100 large papillae, of which 10 are as large as normal colonies.

"Cross" plates

1) Grown together, aerated: Rather heavy confluent growth. About 100 large papillae per plate (4 plates). About 10 per plate are the size of normal colonies. On 2 of the plates the background is not quite so heavy as on the other two, and there are thousands of small papillae.

One plate of the 4 has what appear to be

three plaques in the area of heaviest growth. Plaques are present only in the portion of the plate near two large mold contaminants. (Molds supplying factor needed for phage growth??)

2) Grown together, not aerated; Moderately heavy confluent background growth. Many more large & moderate-sized papillae than on plates from aerated culture. ("Colony-size" papilla on 4 plates 25, 30, 60, & 70).

3) Grown separately, aerated: Light to moderate background; thousands of tiny papillae; on 4 plates, total of only 3 large papillae.

4) Grown separately, not aerated: Moderate background, a few tiny papillae; rather large number of medium and large papillae ("Colony-size" papillae on 4 plates: 44; 29; 12; 58).

Colonies picked from "cross" plates & streaked on AG2SM no growth in 24 hrs. Continue incubation. Also: check SM level of new batch of AG2SM by streaks of CG2S and CG5 (or CG-2-S & CG3 or 4)

Pick more papillae from "cross" plates, streak on AG1 (no SM). Pick colonies from streaks, spot on complete, repl. to minimal & SM.

Phage: Scrape up growth and plaques, add to AG1 broth. Also add to cultures of CG-2-S and 2UV6 in broth. 10:10 AM. Started aerating cultures of 2UV6 and CG-2-S in AG1; also growth + plaques scraped from plate. 1:15: added 0.1 ml broth from culture from plate to each of other two tubes.

Cultures for CG-1-S / CG7 experiment: CG-1-S growing rather poorly, both in aerated and unaerated culture. However, should be OK to use at 48 hrs.

7/1/54

2S / 2UV6 SR Exptl.: papillae which were tiny yesterday now full-size colonies; evidently SR mutants of CG2S, since now are about the same on "cross" plates & CG2S control plates. About 200-300 per plate from unaerated cultures; several thousand from aerated cultures, which gave decent suspensions for plating.

Inoculated plates. (Most were moldy)

Phage

1) Aerated cultures: a) inoc direct from plate; b) 2UV6 + inoc from plate; c) CG2S + inoc from plate. None show evidence of lysis. Cross-streak each on complete med. ← 2UV6 & CG2S.

2) E-MB plates streaked ← 2UV6 (6/28); 2 kinds

Centers, are very translucent, are more dense. Could this difference be due to phage? Pick, streak out (on EMB), save 1 col. of each type.

3) Save mold from plate on which plaques were found, in case cannot get plaques on a dilution; complete medium + mold present.

4) Spread plates of complete medium + mixture of CG2S and 2UV6.

CG-1-S/CG7 SRP.exp: 4g, wash, plate. SRP's picked from previous SRP experiment are growing rather poorly on present batch of AG2SM; probably had the usual E. coli on it of SM added.

7/2/54

Colonies picked from 2S/2UV6 SRP plates: Streaked on complete → 2 kinds of colonies; Small translucent, a larger opaque. Check both for auxotrophy & S resistance (Pick to complete; replica to min + SM).

"Large" This may not be a phage at all. The plates spread with mixture of CG2S + 2UV6 have what look like large plaques with a few large members, but the plate

streaked the previous day with material scraped directly from "plaque" area on original plate has Two kinds of colonies:

1) Rather large, opaque (These were visible yesterday).

2) Very transparent. These little, water-clear colonies are not overgrown by the larger colonies, but when surrounded by a developing opaque colony, show up as round clear areas which are easily interpret as plaques were it not for the other small transparent colonies on the plate.

Make further attempts to run down requirements of auxotrophs. Find out smallest conc. of YE which will permit good growth when added to minimal tubes; then check for inhibition by AA groups, nuts, OC, etc.

Try some "crosses" of various auxotrophs to see whether they will grow on minimal either by recombination to form prototrophs or by syntrophism.

1 stock 5% inoculant to 10 ml tubes of AG 2, 0.1, 0.2, 0.4 & 1.0 ml per tube. Incl. E CG 9 and 2 UV 1 [illegible] because it has shown a better

[illegible] relative to YE and YNA, and latter because it is S^s, the only one among the auxotrophs, and thus has an additional marker for crosses.

[illegible] inoculated AG1 halves of CG9 and 2UV1 together a separate, fine crossing attempt. To be plated on minimal. Did not start aerated cultures this time.

7/3/5-4

CG-1-S / CG-7 SRP: (2 days)

Controls:

7, not aerated: Thin film, no papillae

7, aerated:

1-S, not aerated: fairly heavy background, large 16 papillae

1-S, aerated: Heavy background, no papillae

Grown together, not aerated: fairly heavy background, ca 50 - 100 papillae per plate. Very clear areas, either plaques or transparent colonies.

Grown together, aerated: Heavy background, few papillae no "plaques"

[illegible] not aerated: Moderate background, [illegible] papillae per plate

[illegible] aerated: Moderately heavy background,

only 2 - 4 positive per plate.

On checking back to plate on which "plaque" appeared - the "plaques" on that plate do not look like the effect produced by the small transparent colonies. They have ragged edges and turbid centers. Save plate, try again to get a phage from it.

7/5/54

Plate spotted ± two cul types from 25/2UV4 (7/2) ~~cif~~ Moldy; spot on fresh AG1 plate for replication in mini & 5/14.

~~Original "phage" plate; "plaques" still visible;~~
~~Try picking from individual clear areas, streaken~~
→ ~~small, clear colonies; also one in both~~
~~2UV4 + CG25 are if any phage~~

Took new to CG-1-5 similar to plate - see above

CG9 + 2UV1 in mini + yeast extract:

CG9: Good growth in 10 ml mini + 0.1 ml 5% YEX.
(Low readings. This assay was recorded as responding to YIVA).

2UV1: Some growth ± 0.1, 0.2, + 0.4 ml YEX; with good growth ± 1.0 ml, but still not as

good as mi complete. (c CG 9, growth ≥ 0.1 m YEX is about as good as mi complete). Growth mi mem + 0.4 ml YEX probably OK for testing for inhibition.

Add regular conc of vits & AA grps to mem + necessary amt of YEX.

CG-1-S / CG7 "SRP" expt: (plated 7/1)

Controls: 7 aerated & unaerated: several hundred colonies: evidently this single assay not too satisfactory for "crossing" effects.

1-S, unaerated: moderated background which has "plagued" appearance - apparently due, again, to small, transparent colonies. Also ca 100 large papillae.

1-S, aerated: moderate background, several hundred papillae.

"Cross." plates: very similar to CG1S controls.

2WI / CG7: CG, wash, plate, mem (source treatment for 2 hrs. ...). Also plate mem mixture of CG1S and CG7 incubated 5 days. Not too promising

in view of behavior of controls.

1-5/7 Result about the same as those incubated only 2 days, [illegible] + some and large papillae

7/6/54

Cultures from 2S/2UV6 expt. Replicated to AG2 + AG1S17.

CG9 + 2UV1 - in min + YE + rendimus (24 hrs)

	CG9 48 hrs	2UV1		CG9 48 hrs	2UV1
0	− −	± −	YE+AA4	− ±	± −
YE only	+ ++	+ ++	AA5	+ ++	+ ++
YE+AA1	+ ++	+ ++	HC	− −	± −
AA2	+ ++	± ++	VITS	− +	− −
AA3	+ ++	+ +	Na_2S	+ ++	++ +++

better growth in YE + Na_2S than in YE alone.

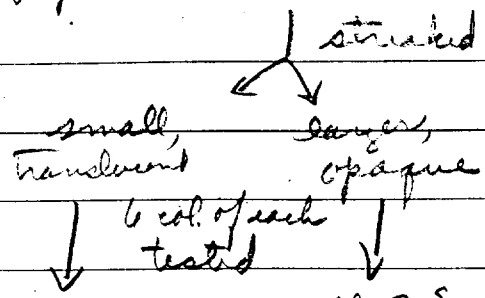
Incubate at least one more day, then try individual members of any AA groups which are inhibitory. Try vitamin mix for inhibition of prototrophic and other growth factors.

Agrobacterium

Start this type of experiment on other auxotrophs

7/7/54

Test of "papillae" from 2S/2UV6 SRP experiment. Papillae on minimal SM picked streaked on complete



2UV1/CG9 "cross"; No colonies on controls on cross plates. (2 days). Had to discard most of cross plates because of mold.

Check on inhibition of prototrophs by growth factor solution 24 hrs: growth poor; detectable only on complete, A A3, and sucrose.

Perhaps would be a good idea to add Na_2S routinely to minimal medium;

Also: try using $(NH_4)_2SO_4$ instead of KNO_3 as N source. See medium listed p A33

7/8/54

Make up 20x salts for minimal, using only 0.2 g $CaCl_2 \cdot 2H_2O$ per 100 ml 20x solu
(see p A33.)

Then make up 2 media

1) 0.5% sucrose, 0.5% KNO_3 ; Fe & Mn
(usual As 2, except for less $CaCl_2$)

2) 0.5% sucrose, 0.1% $(NH_4)_2SO_4$; Fe, Mn, Zn
(~ Hodgson, Peterson, & Riker 1951)

ADD Na_2S to portion of each

CG9 and 2UV1 in min. + YE + sucrose (3 days)

	CG9	2UV1		CG9	2UV1
0	—	—	YE + A4	+	—
YE only	++	++	A5	++	++
YE + A1	++	++	HC	—	—
A2	++	++	VITS	++	—
A3	++	+	Na_2S	++	++

CG1S and CG2S in min. + sucrose (2 days)

	CG1S 1:20		CG2S			CG1S		CG2S	
0	+	++	+	+++	A5	—	++	—	++
A1	+	++	+	+++	HC	—	—	—	—
A2	+	++	+	++	VITS	—	—	—	—
A3	++	+	++	++	Na_2S	+++	++	+++	+++
A4	—	—	—	—	complete	++	++	++	++

Inoculated CG1S into tubes as follows:
1 complete 3 A3 and components
2 Nutrimed 4 A4 and components
5 A5 and components

7/9/54

Discarded remaining plates of 2UV1/CG9 "crossing" experiment. 1 colony on 2UV1 control; 1 colony on a cross plate. Both near molds. No other colonies

CG1S in mM + AA groups & components:

	1 day	2 days	3 days		1 day	2 days	3 days
0	−	++	++++	glutamic	−	−	−
complete	++	+++	++++	aspart	−	−	contaminated mold, growth
A3	+	+++	++++	prol	+	++	++++*
tryptoph	±	++	++++	A5	−	−	++
tyros.	+	+++	++++*	alan	+	+++	++++
phal	+	+++	++++*	glyc	+	+++	++++
A4	−	−	−	serine	−	±	+++
hist	−	++	++++	OHprol	+	+++	++++
threo	±	+++	++++				

Tubes marked * there was very heavy growth in the upper 1/4 to 1/3 of the tube. Other tubes in which growth occurred were more uniform.

Prepared tubes of AG2 (low $CaCl_2$) $\bar{c}$ & $\bar{5}$ sulfide; also the $(NH_4)_2SO_4$ - Zn^{++} medium (AG-3) $\bar{c}$ & $\bar{5}$ sulfide.

Incc. $\bar{c}$ CG1S

Attempted crosses of auxotrophs

	CG7	8	9	2UV1	2	3	4	5	6
CG7		7/15/54 1 exp & control 1 on comp plate	7/15/54 0 7/15/54 0	7/26/54 ✓ 61; control 0					
8				7/9/54 0					
9									
2UV1									
2									
3									
4									
5									
6									

Start cultures of CG7, 8, 9, 7+8, 7+9, & 8+9 for crossing expt

Inoc. ē CG15 tubes of minimal + each vitamin available in solution.

7/~~10~~9/54

Test CG1R̄ ē individual acids of AA groups 1 and 2.
(Used CG1R by mistake — should not make any difference in eventual result.)

7/10/54

CG1S and CG2S in mini + growth factors.

	CG1S	CG2S		CG1S	CG2S
0	+++	+++	A5	+++	+++
A1	+++	+++	HC	—	—
A2	+++	+++	VITS	—	—
A3	+++	+++	Na_2S	++++	++++
A4	—	—	complete	+++	++++

Discard

See on page 43 results of adding individual AA's. Nicholini, Peterson, and Riker (1941) used glutamic acid at 2.5 gm per liter in their minimal medium!!! This was for the same strain (A6). Van Lanen, Baldwin, & Riker (1940) reported mono-amino-dicarboxylic acids inactive in attenuation (and presumably inhibition) up to M/20.

CG1R in groups AA1 & AA2 and components of those groups. No growth at 20 hrs in mini. + any supplement.

CG1S in Ag 2 & Ag 3 & 4S sulfide - no growth at 24 hrs.

CG1S in mini + individual vits - no growth 24 hrs.

7/11/54

CG1R in A1 + 2 + components:

	2 days	3 days		2 days	3 days
0	+	+++	A2	++ *	+++
A1	++ *	+++	leu	++ *	+++
lys	++ *	+++	isoleu	++ *	+++
arg	++ *	+++	val	++ *	+++
meth	++ *	+++			
cyst	+	+++	3rd day; all have heavier growth near top of tube.		

* Growth heavy in upper portion of tube (not a pellicle)

CG-1-S in AG2 + AG3 ē + s̄ sulfide

	2 days	3 days
AG2	−?	− (?)
2 + Na_2S	++ (uniform turbidity)	+++
AG3	−	−
3 + Na_2S	+ (bottom ½ of tube turbid)	+ (more uniform turbidity)

REPEAT

CG1S in individual vits.

	2 days	3 days		2 days	3 days
0	++	+++	Nic	++ *	+++
B6	++	+++	B_1	+++	+++
Choline	++	+++	B_2	++	+++
Mesoinose	++	+++	pABA	++ *	+++
Pant. Acid	++	+++	inositol	+++	+++
	+++ *	+++	Bio	+++	+++

7/12/54

F_3 ± plate crosses of CG 7, 8, & 9, pairs grown together & separately

Repeat of CG15 on AG2 + AG3 ± + S sulfide assn.
(May have been an error in conc. of AG 2 + 3 used above. If no growth in AG2 this time make up new 20x stock)

7/13/54

Repeat on AG 2 + AG 3 ± sulfide.

	1 day	2 days	3 days
2	−	−	−
2 + sulfide	−	−	−
3	−	−	−
3 + sulfide	+	++	++

Incr. AG 2 & AG 2 + folic acid (pteroylglutamic) ± CG15.

7, 8, & 9 crosses – Nothing at 24 hrs.

Try CG15 on min + glutamic & aspartic + sulfide.

7/14/54

CG 15 mi AG 2 ± PGA : nothing at 20 hrs.

Crosses involving 7, 8, & 9 : nothing at 2 days except good growth around molds. A few plates appear uniformly seeded (in the agar) with old friend yellow contaminant.

7/15/54

CG 15 mi AG 2 ± folic acid (PGA) : equally good growth ± & ± PGA.

Crosses involving 7, 8, 49: (3 days) - nothing definite. 1 possible prototroph on (7+8) plate. Plate contaminated ± yellow fuz, but this colony buff & looks like typical CG. Also one buff colony on CG 8 control plate, but aside from color it looks just like yellow contaminant.

Pick: 1) col from cross plate
2) buff col from control plate
3) yellow colony.

Streak out; test whether inhibited by glutamic acid.

7/16/54

Start repeat rundowns of all auxotrophs, avoiding any inhibitory supplements. (do vits individually).

Made up new 20x AG 2 salts; previous batch gave no growth ~~=~~ sulfide

CG1S ini AG2 ± glutamine ± Na_2S (2 days)

AG 2	−	AG 2 + Na_2S ++
AG 2 + glutamine	−	AG 2 + g.a. + Na_2S ++

Try CG2S ini mini. This was earlier batch than that which gave no growth in previous expt.

Try rundowns of auxos ± Na_2S added as detergent

For some reason – new batch of AG 2 salts ⇒ no ppt in 2x concentration

NOTE There is a large bottle of d-glutamic acid in the AA box, and much of it has been used. Could the present stock salts be d-glutamine? The auxo the most one lacks is dl, as possibly d some could explain inhibition by both glutamine and asparagine. Check

7/17/54

CG15 and 2UV6 in nutrients + sulfide

	CG25 1 day	CG25 2 days	CG25 3 days	2UV6 1 day	2UV6 2 days	2UV6 3 days
no Na_2S	−	+	++	−	−	−
Na_2S only	−	+	+++	−	−	−
A1	±	+	+++	−	−	−
A2	±	+	+++	−	−	−
A3	+	+	+++*	−	−	−
A4	+	±	+++*	−	−	−
A5	−	+	++	−	−	−
HC	++	++	+++	+	+	++
VITS	−	±	++	−	−	−
YEX	++	++	+++	+	++	+++

7/19/54

Prep'd slides from 7+8 cross ∘ & central plate. Both green very pearly on surface (plate rather old). Colonies look identical; the one from 8 central plate does not resemble the yellow contaminant. Put each into nutrient tube & nutr + AA4 or glutamic acid.

Prepared the following tubes of CG15:

nutr

nutr + l-glutamic

nutr + d-glutamic

{ nutr + d glut. + Na_2S
nutr + " + thiosulfate (1/10,000)

(to check on whether thiosulfate is as effective as Na_2S)

Started ~~aminos~~ + sulfide ~ CG7 & CG8

7/20/54

CG1S in l-glutamic + in d-glutamic alone + ~ Na_2S + thiosulfate : no growth at 24 hrs.

"Prototrophs" from 7x8 & 8 control : No growth at 24 hrs in min or min + glut.

Randoms on CG7 & 8 (black sulfide ppt in some tubes)

	CG7				CG8			
	1 day	2 days	3 days	4	1 day	2 days	3 days	
Na_2S only	−	−	±	±	−	−	−	
A1	−	++	++	+++	−	++	+++	+
A2	−	−	±	±	−	++	+++	+
A3	−	−	±	+	−	−	−	−
A4	−	−	+	+	−	−	++	++
A5	−	−	±	±	−	−	+	+
HC	+*	+++	++	+++	+*	+++	++	++
VITS	~~++~~ ?	?(-)	?(-)	−	~~+~~ ?	?(-)	?(-)	−
YEX	+*	++	+++	+++	+*	+++	+++	++

* ~~Turbidity~~ 7/21 : Turbidity in vitamin tubes apparently was ppt in medium ; has largely disappeared.

~~CG 1 in 1 ml~~

7/21/54

CG15 in d- & l-glut. acid (2 days)

Mold growing in 0, d-glut only, & l-glut only tubes.

	0	++	
l-glut	–	d-glut + Na_2S	+++
d-glut	–	d-glut + thiosulfate	–

CG7 & ~~CG8~~ wd rundowns + sulfide; Heavy ppt causing turbidity in tubes. This medium was made ∑ same salt mixture as that which gave little or no ppt in last batch of ★G2!

Started broths of CG7, 8, & 9 and 2UV1 for crossing attempts.
Also mic 2UV1 in broth ∑ 7, 8, & 9.

7/23/54

CG7 in wet rundowns; no growth (mic 7/20)

Plated crosses of CG7, 8, 9 ∑ 2UV1

7/24/54

Inoc CG7 into A1 rundowns; CG8 into A1 & A2

7/26/54

CG 7 & 8 rundowns

	CG7			CG8		
	2 days	3 days	4 days	2 days	3 days	4 days
0	−	−	−	−	±	±
A1	+	++	++	±	++	++
lys	−	−	+	−	−	±
arg	−	±	±	−	±	±
meth	−	−	−	−	−	±
cyst	−	±	+	−	±	+
complete	+++	++++	+++++	A2 +	+	+
				leuc −	−	−
				isol ±	±	+
				val −	−	−
				compl +++	++++	++++

This batch of minimal apparently not very satisfactory. Better growth in A2 at 2 days in previous test (A4 rundowns)

Crossing attempts of CG 7, 8, & 9 ± 2UV1

Controls: negative except CG8, which is quite heavily contaminated ± mold and a pink bacterial contaminant. A few col. present which look like Aerobacteriumi.

CG 8 & 9 +2UV1 cross plates: negative

CG 7 x 2UV1 : Grown together: 2 plates; negative

Grown separately: 1 plate; 61 colonies. Look like

Agrobacterium. Plate has some mold near edge, but bacterial col. evenly distributed on plate, do not seem to be stimulated by mold.

Pick colonies (at least 20); spot on complete, replicate to minimid. Repeat cross, this time grown together & separately, aerated & unaerated.

7/27/54

CG 7 & 8 Aa reminders: (See table on previous page)

CG 8 seems rather "leaky"; this has happened before. CG 7: Might try A 1 single omissions. Also, try individual vitamins and the vitamin mix used in yeast medium, since the sulfide does not counteract very well the inhibition by the vitamin mix.

Next reminder attempt - 2 UV 1

Repeat l- & d- glutamic eppt ± CG 1 S, since previous eppt. was lost because of mold.

Start cultures for repeat crossing attempt ± CG 7 & 2 UV 1. (together & sep; aerated & unaerated)

7/28/54

Col. picked from 7 x 2 UV1 plate & repl. from complete & minimal. 29 picked, all prototrophs.

CG1S on d & l glutamine; no growth in 24 hrs

7/29/54

Plated 7 x 2 UV1 crosses (grown together & separately, aerated & not aerated)

[Some cultures or non-aerated cultures that labels may have been interchanged on CG7 & CG7+2 UV1

CG1S / glutamine

	2 days	3	4
0	++	+++	
$Na_2S_2O_3$	+±	++	
d-glut	±	++ !	
d-glut + Na_2S	++	+++	
l-glut	++	+++	
l-glut + Na_2S	++	+++	

Checking reveals the "d-glutamine" is actually l(+) glutamine (Kodak). The other glutamine is l(+)glutam (GBI).

7/30/54

CG7 rundowns

	2 days	3 days		2 days	3 days
O (Na_2SO_4)	+	+	A1	++	+++
B2	+	+	" -lys	++	+++
Biotin	±	mold	" -arg	++	+++
B6	−	±	" -cyst	−	++
pantoth	−	−	" -meth	+	+++
K	−	±			
B1	−	±	complete	+++	+++
nic	++	mold			
B12	−	±			
inos	−	±			
chol	++	contamination? +++ (pellicle)			
paba	−	±			
pga	−	−			

Streak out CG7; check several colonies on anything giving 2+ or better.

7/31/54

CG7 x 2UV1 "cross": At 48 hrs, colonies on all three plates from cultures grown together, not aerated. None on other plates.

Inoc. rundowns (primary) of 2UV1

8/2/54

2UV1 primary rundowns;
At 2 days, growth only in YIVA.
Try individual vits; also p & p?

CG7: from individual colonies: Check each on: min (+ sulfide) +: O; biotin; nicotinic; choline; A1 + individuals of A1 group. Try first 2 plates; 2 or three on th

CG7 + 2UV "cross"; (4 days)
Colonies on all 3 plates grown together, not aerated. (Counted 1 plate: 115 col; other 2 similar). Thin film of background, so could be feeding. Pick a few col, streak, test for prototrophy. Col. are small. No real control on this tube; could be mutation to prototrophy.

Repeat cross again.

8/3/54
2UV1 primary rundowns; 3 days; still growth only in YIVA; possibly trace (±) on A1.

8/4/54
2UV1 primary rundowns 4 days

O	−	A3	−	HC	−	Try individual vits.
A1	±	A4	+	VITS	+	
A2	−	A5	±	VFX	±	

7×2UV1 "protos" (8/2): Very poor growth when streaked on minimal - very tiny colonies. Grew well on complete. Replicate from complete to mini.

Repeat 7×2UV1 cross: Use several cultures of each parent, plate control from each.

8/5/54

7×2UV1 "prototrophs" - poor growth when replicated to minimal (24 hrs)

C67 from single col. repl. to mini. agar + supplements - 48 hrs. No growth except on complete

(Suppl. used: Proteni, mei, choline, A1, & individuals of A1 group)

8/6/54

2UV1 in individual vits: no growth at 2 days.

Plated repeat of 7×2UV.1 cross.

8/9/54

Repeat of 7 × 2 UV1 cross

Grew up several tubes of 7 and of 2 UV1, plated control + cross from each (except 7-5 & 7-6, control only). Also grew 2 tubes of 7+2 UV1 together.

	CG7 control	2UV1 control	cross plate
1	ca 200 col	1 col.	ca 200
2	7 col (may be contaminants)	0	several very small
3	0	confluent	confluent
4	1 (may be contaminant)	0	3 + several very small
5	0	—	—
6	10 very small	—	—
7+2UV1 ①	—	—	confluent
7+2UV1 ②	—	—	0

8/10/54

Check whether filtered Ag 2 supports good growth of CG 15 (in order to get rid of confusing turbidity). Inoc. tube (= Na_2S) + water suspension.

Inoculum very small, probably no survivors on plate & 1 control tube

8/12/54

2 days growth in filtered medium only

8/13/54 3 days growth in both filtered & unfiltered

9/7/54

Made up 40 tubes Ag 2 (2x) + 40 tubes 1% sucrose. Ag 2 has no Fe, Mn, or Zn, also no sulfide. Filtered before tubing & autoclaving. Check all aspos

1) in smin
2) in smin + sulfide
3) ~~+ +~~ + ~~YE~~. Complete

before starting any expts.

9/13/54

Check of aspotrophs since into smin ± sulfide, & complete 9/11:

No growth in any smin or sulfide tubes.
Growth in all complete tubes except 2UV2.
Contam in.

9/14/54

Still no growth in any minimal; no growth in 2UV2 complete. Check again on viability of 2UV2 stab.

Transfer stabs to have one set as reserve.

9/15/54

Growth in CG7 in smin + sulfide. Could be contam, since another tube has mold.

1/11/55

Irradiation effected on WCG-1-S. Used overnight aerated culture of grown in Ag 1 broth, rm temp.
Irradiated 10^{-4} dilution; cfgd, washed, resuspended in saline, then diluted.
90 sec irradiation on big lamp.
Diluted further to give 10^{-7} and 5×10^{-7} dilutions of original culture on plates.
Inc. 25° incubator

1/13/55

9:00 AM: Irradiated culture plated 1/11: Dilutions used too high: 5×10^{-7} ⇒ about 20/plate. Colonies still very small; inc. another day before replicating.

Started culture for second irradiation: 0.1 ml Ag 1 broth culture (not aerated) → Ag 1 broth aerated, rm temp.

1/14/55

Replicated 5 plates of 5×10^{-7} dilution to mimeod. 6th plate contaminated, incl molds; picked colonies, spot to complete for later replication. Also picked the few colonies from 10^{-7} dilution to complete.

1/15/55

Expmtd plates refl. to mm from 1/11 irradiation: 9 possible auxotrophs from 245 colonies. Picked, spotted on complete. Some colonies very rough; use caution in identity. If possible, concentrate on isolated colonies.

Second UV run, WCG-1-S:

Culture: Aerated at rm temp overnight; stored s aeration at rm temp another 24 hrs.

Cfgd; pellet washed c saline; diluted to 10 ml in saline. 10^{-4} dilution irradiated 90 sec.

Plating: 0.1 ml and 0.2 ml 5×10^{-6} dilution.

1/17/55

Second UV run. Very few colonies visible at 48 hrs!

Continue incubation at 25°. By 2 30 pm colonies on [illegible] 5-12 per plate

"Autos" from first run: Of the 9 spotted on complete, three are gummy, look like typical Agrobacterium growth. Other six rough & rather leathery. Replicate all to minimal.

Of colo. picked from 1st run plates (plates which were contaminated or too dilute for replication) spotted, then repl to min, 1 of 66 appears to be an auxotroph. Test along c other 9.

Made up 2 minimal tubes. To check medium, inoc 1 drop water suspension from WCG 1-S stab into 1) complete, not aerated 2) min + $NaNO_2$ aerated 3) " " " not aerated

Used 1 drop 1% sterile $NaNO_2$/tube (10 ml)

1/18/55

"Autos" from first UV run: 4 of 10 grew well on min. in 24 hrs. These included all the 3 "typical" gummy growers. 2 of the rough type show poor

growth on ~~replication~~ replica plates on both to mini & complete at 24 hrs. Remaining 2 replicated show good growth on complete, poor growth on mini. The 10th "auxo", brushed on plates to which others were replicated, shows fair growth on compl at 24 hrs, no growth on mini. Continue incubation of these plates until early pm.

~~Several~~ Two of the "rough" spots ⇒ mixed growth when replicated to mini & complete; center of spot rough sparse growth; outer ring of heavier, gummy growth. Especially marked on complete. So it appears these rough colonies may be Lysobacter.

Colo picked from 2nd UV: Overnight growth poor; continue incubation until early pm before replicating to mini.

More colonies now visible (3 days) on 5×10^{-4} & on 10^{-7} plates from 2nd UV run. Pick; spot on complete for repl. to mini.

Check on Ag 2 minimal: No growth overnight in mini tubes 5 or 5 aeration. Unaerated Ag 1 tube (compl) slightly turbid. Room temp probably quite low during night.

1/19/55

Check of mineral liquid (see 1/17): At 2 days, very good growth of WC&-1-S in min (+ Na_2S) aerated. About the same turbidity in unaerated mineral as in unaerated complete.

Why Na_2S & aeration needed for best growth? Seems contradictory. Effect of aeration may be largely agitation rather than O_2 availability; but at pH slightly below 7 should think sulfide would be rapidly lost.

Set up: min + Na_2S } aerated
min + Na_2S }
min + Na_2S } unaerated
min + Na_2S }

1/20/55

Possible auxos from 1st UV run: The rough type grew up eventually on mineral. Check these on a couple of other sugars; could poor growth & lack of green be due to mutation involving utilization of sucrose?

#10 picked from first run appears to be auxotroph. Stab; prepare renderon tubes. Growth is rather scant even on complete. How be sure of identity ???

Second UV run: Of 53 col. picked, 10 are poorly-growing, rough type. If this is a contaminant, it certainly appears

consistently.

One possible aspo from 2nd run. Stab, streak on complete.

1/21/55

Effect of aeration & $5\ Na_2S$: (48 hrs)

Not aerated: Little difference & $5\ Na_2S$. Poor growth.

Aerated, no Na_2S: better growth than 5 aeration.

Aerated, $\bar{c}$ Na_2S: Very good growth; est. 10^9/ml.

1/22/55

Inoc Ag 2 roundrums $\bar{c}$ UV1-10, UV2-1, and WCG1S from water suspensions from slabs.

UV2-1 grows very poorly when streaked on complete.

"Rough" strains streaked on compl. $\bar{c}$ glucose & mannitol look the same as on sucrose.

1/24/55

Roundrums (2days)

	WCG1S	UV1-10	UV2-1
O	++	−	−
A1	++	−	−
A2	++	−	−
A3	++	−	−
A4	++	−	−
A5	++	−	−

	WCG1S	UV1-10	UV2-1
HC	++	+	−
YNA	+	±	−
VITS	++	−	−
compl	++	++	++

1/25/55

Lundrums (3 days)

	WCGIS	1-10	2-1		WCGIS	1-10	2-1
0	+++	−	−	AA5	+++	−	−
AA1	+++	−	−	HC	+++	+++	++
2	+++	−	−	YNA	++	−	+
3	+++	−	−	VITS	++++	−	−
4	+++	−	−	compl	++++	+++	+++

Try amino acid single omissions. [The HC used is contaminated, so growth in HC tubes not necessarily due to HC components].

UV runs (#3)

24 hr aerated culture (Ag 1, rm temp)

90 sec. . Pl. 0.1 & 0.2 ml 10^{-5} dil. (Irradiated 10^{-4})

1/28/55

UV 3: Replicated to minimal from plates c >10 col. Picked from plate c < 10 col & spotted on compl.

One yellow (or creamy) colony. Picked to stab, labelled UV 3-1. Colony was same size, shape, height, & texture as others on plate. Check motility; growth c & $\bar{c}$ sulfide, c & $\bar{c}$ aeration.

1/28/55

Rundowns, single omissions of AA groups (2 days)

	UV1-10	2-1		1-10	2-1	
0	0	0	-5	+	+	
-1	+	+	all	+	+	
-2	+	+	all + vits	+	+	
-3	+	+	HC	-	+	Fresh HC used (taken from freezer)
-4	+	+	compl	++	++	

Very similar to behavior of previous mutants. New vit mix not inhibitory. Try various carbon sources & N sources

1/29/55

Rundowns, single omissions, 3 days

	UV1-10	2-1		1-10	2-1
0	0	0	-5	+++	+
-1	++	+	all	+++	+
-2	++	+	all + vits	++++	++
-3	++	+	HC	±	+++
-4	++	+	compl	++++	+++

3rd UV run: Replicated to mini. col. picked to complete 1/28.
Direct replications: 2 possible auxos out of ca 80 col. One is the yellow colony.

1/31/55

UV3: Col. picked, repl. to mini 1/29: 2 possible auxos (both rough, so possibly just poor replication). [UV3-3 & UV3-4)

WCG

Screen as follows: 1) min. 2) min + HC. 3) min + all AA groups together. 4) As 3, + VITS. 5) YNA.
Also include UV 3-1 + 3-2 in tests.

2/2/55

Rundowns of UV3 auxos: 2 days

	HC	AA	AAV	YNA	Ag 1	0
3-1	++	++	++	+	+	−
2	+	++	+++	+++	+++	−
3	±	+	−	−	+++	±
4	−	−	−	−	+++	−

2/3/55

UV3 rundowns, 3 days.

	0	HC	AA	AAV	YNA	Ag 1
3-1	±	++++	++++	++++	++	++++
2	±	+++	+++	+++	+++	++++
3	±	++	++++	−	−	++++
4	−	−	−	−	−	+++

These were kept for additional more days, no further growth in remained tubes, no change in other tubes

2/8/55

Inoc. AA rundowns of UV3-2 & 3-3. (Strepto, single additions)

2/9/55

Set up rundowns of UV3-4 as follows: HC, HC+V, HC+YNA, YNA, YNA+V, V, HC+YNA+V, YX. Grew only in YX

WCG

2/10/55

Rendrums, UV 3-2 & 3-3 (2 days)

	3-2	3-3		3-2	3-3	
O	–	–	A4	–	–	
A1	–	–	A5	–	–	
A2	–	–	~~VITS~~	+	not included	
A3	–	–	compl + vits	not included	++	To check for Tyrosine
			compl.	++	+++	

If these results check out after another 24 hrs, try 3-2 ⊂ individual vits + 3-3 ⊂ AA single omissions

2/12/55

Set up rendrums ⊂ individual vits ⊂ UV 3-2. (B-12 may be contaminated).

2/13/55

Run UV 3-2 vit rendrums.

2/14/55 UV 3-2

Vit rendrums: At about 28 hrs, fair growth in vits mix, trace of growth in biotin.

Would pay to do individual vit ⊂ all amps; behavioral has been constant ⊂ vit requirement, but mix may be toxic to some.

Also: 3-3 ⊂ ~~Leu~~ AA samples.

2/15/55 Vits, UV 3-2: Fair growth in biotin; other vits O

WCG

UV3-3 Incr. rendrous, AA groups.

2/17/55

UV 3-3 no growth in AA groups or in all AA's (2days)

2/24/55

UV 1-10 & 2-1 in vits: no growth in individual vits or mix. (Incr. 2/18)

2/22/55

UV 1-10 No growth in individual vits, but some growth in vit. mix (4 days) May have double requirement, or conc. of requ. vit in individual tube may be too high, since some are higher than in mix. Also may be reversion - see UV 2-1

UV 2-1 Growth in the following tubes (very slight)

Riboflavin	para	
B-12	vit mix	Reversions?
Pantothenate		Dirty tubes?

2/23/55 (5 days)

UV 1-10 Growth in: Biotin, choline, nic, para, vit mix (Dirty tubes)

UV 2-1 " " : Trace in those listed previously. Very scant even in vit mix.

2/24/55 Set up min, min + glycine (1 mg/10 ml), min + Vits, & min, vits, & glycine to see whether glycine will speed growth in vits 5 itself permitting growth (1-10 & 2-1)

WCG Jr. 2/25

3/1/55

UV 1-10 & 2-1 in glycine, vits:

At 3days, 1-10 ± in glycine, glycine + vits.

4days 0 —

glyc ±

vits +

vits+gl ++

2-1 at 4days. 0 —

glyc +

vits +

vits+glyc — (!?!)

3/4/55

UV 3-3	(2days)		3days		
0	—	—	A4	—	—
A1	++	++	A5	—	++
A2	—	—	all AA's	—	±
A3	—	+	compl	+++	+++

3/6/55 (UV3-3)

A1 rundowns since 3/4. Possibly trace of growth in lysine

3/7/55

A1 rundowns - 3days (at room temp; cold at night)

0	—	Meth	—
Lys	+	A1	—
Arg	—	compl	++
Cyst	±		

WCG

Try ē & s̄ lysine ē aeration: Usually lys - showed some rich clear-cut results because of more rapid growth.

3/8/55

Start culture of WCG 1 (S) for another UV exp't (#4) to try to get a readily identifiable auxotroph.

Ag 1, room temp, aeration

TRANSFER STABS

3/10/55

Excellent growth of WCG-1-S for irradiation. Irradiated 90 sec. (10^{-4} dil) Plated 0.2 ml/plate of 2×10^{-5} dilution (Ag 1 medium)

UV3-3 in min, lyp, A1 aerated: In 3 days, faint growth in all tubes.

3/14/55

UV4: 6 possible auxotrophs - 1 is a light yellow similar to UV3-1.

3/15/55

UV4-1 (yellow) is an auxotroph; all others grew in min.

3/18/55

UV5: 48 (approx) hr culture, Ag 1 broth, aerated. Irradiated 10^{-4} dilution for 90 sec. Plated .1 ml/plate of 10^{-5} dilution. Inc. 25°.

WCG

3/19/55

UV 4-1 & UV 3-1 (yellow): Streaked on complete. 3-1 rough, 4-1 smooth. Appearance ≅ rough & smooth WCG 1 except for color. Smell like Aerobacterium.

Try running down requirement(s)

Spread 4-1 broth (5 days old) on minimal; look for possible "reversions" to prototrophy which should appear as papillae - If any, what color? (i.e. is yellow color result of a metabolic block.)

Also - compare growth in complete broth $\bar{c}$ & $\bar{5}$ aeration $\bar{c}$ WCG-1.

3/21/55

UV 3-1 & 4-1 Both show scanty growth in complete broth $\bar{5}$ aeration, good growth aerated (but not so heavy as WCG-1)

4-1 spread on minimal - No papillae at 2 days.

Rundowns:

UV 3-1	++ in A4, + in A5, ± in others	} 2 days
UV 4-1	0 in all rundown tubes.	

3/22/55

UV 3-1 & 4-1 rundowns

4-1 no growth except in complete

3-1	A1	0	A5	+++	1) [illegible]
	A2	0	Vits	++	2) [illegible]
	A3	0	0	0	4) [illegible]
	A4	+++	compl.	+++	5) [illegible]

WCG

3/24/55

UV 5: Of 7 possible aspos picked, all show some growth in mini at 2 days. Two have only very faint turbidity; keep these, check especially for vits. [illegible] 4 days UV 5-1 UV 5-2

(3/19)

UV 4-1 Spread on Ag 2 plate: 1 papilla, which appeared in about 3 days. Appears to be slightly buff-colored. Pick, streak on complete, check on mini for color & prototrophy.

3/26/55

UV 3-1 Neurospora - 3 days (rm temp - cold at night)

B1	0	pyri	0	A4	++	A5	±
ribo	0	pant	0	hist	0	alan	0
nic	0	chol	++	Thre	0	glyc	±
paba	0	vits mix	0	glut	0	ser	0
bio	0			pro	0	OHP	+++
PGA	0			aspart	++		
Inos	++					compl	+++
K	0						

Streak out; recheck individual colonies.

3/28/55

Papilla from UV4-1 - picked from mini, streaked on complete - Colonies yellow. Pick several, check for auxotrophy.

NHC

3/31/55

UV3-1 : Rundowns mic. from 2 individual col. (2 days)

	(1)		(2)			(1)		(2)	(3 days)
0	0	0	0	±	A4	0	0	++	+++
vits	0	+	++	+++	hist	0	0	0	+
dist.	0	0	0	+	thre.	0	0	0	0
mos	0	0	0	0	glutn.	0	0	0	++
A5	+	+++	0	±	pro.	0	0	+	+++
alan	±	++	0	+	aspart	0	0	++	+++
glyc	0	0	0	+++	camp.	+++		+++	
Ser	0	0	0	0					
OHP	0	0	0	+					

Indicates erratic results prob due to reversions ?

5-1 & 5-2 rundowns: grew in min. However, there are apparently some inhibitions.

	5-1	5-2
0	+++	++
A1	+	±
A2	0	+
A3	±	0
A4	+++	++++
A5	0	0
Vits	+++	++++

4/2/55

Cols. from papilla from UV4-1; autotrophic: However, 1 streak - faint growth after 5 days, ~ 1 large colony, many small. On min, large col appears volute. Pick, streak in complete.

4/5/55

Final comparison of sundrums on two col. from UV 3-1 (ende. 3/29)

	(1)	(2)		(1)	(2)
O	±	+	A4	+	+++
rib	±	+++	hist	++	+
chol	±	++	threo	0	±
inos	±	+++	gluta.	++	++
A5	++++	+++	prol	+++	+++
alan	++++	++++	aspart	++	++
glyc	+++	++++			
ser	0	+++			
OHP	±	+++			

UV 4-1 Picked from large col. on mm: Streak mixed, yellow col as before, also a white, rough col. type. Latter extremely rough - probably a contaminant.

Might try plating UV 4-1 or 3-1 together & WCG 16. Main purpose would be to look for colonies & yellow & white sectors. First check nutrition of WCG 16; also make sure UV 4-1 (or 3-1) does not respond to biotin.

4/12/55

Spread water suspensions (from slants) of WCG 16 & UV 4-1 on plates of mm & ± biotin: Also cross-brushed sa. & SM.

4/14/55

WCG 16 - confluent growth on mm & bio; none ± bio

UV 4-1 - no growth on either plate

WCG 16 S^S

UV 4-1 S^R

WCG

4/22/55

Results of attempted cross of WC616 & UV-4-1 (4 days 25°)

Controls: WC616 can grow ē SM at 200 µg/ml

" " no growth s̄ biotin.

UV 4-1 Some background s̄ SM, none ē SM, though culture appeared to be S^R

With mixtures, confluent growth on all media. Growth is white, i.e. WC616

/ min
min + SM
min + SM + biotin

3-3 AA groups

~~Plated~~ ~~Starter cultures of 1K × 33-48~~ In loop dilutions to be plated on mod-agar: 10.1 → 1.0 → .1 → 10 loop → 10 plate on

1-10 HC ± ; in AA single group omissions, grew well in all tubes except min control. AAs-only additions all − ; late growth (4 day) in some wells

2-1 HC + ; poor growth in all AA single omission tubes. Late growth (4 days) in some wells.

3-1 (yellow) HC + ; AA's together + ; poor in YNA

3-2 HC faint +, AA's together + better in AAV ; good YNA
BIOTIN

3-3 HC +, AA's together strong +, AAV − ; YNA −

3-4 Grows only in complete or min + YX
(Have tried HC, HCV, HCYNA, YNA, YNAV, V, HCYNAV

Possible schemes ~~of~~ using WC616 & UV4-1
See whether both were ~~or~~ less S^S.

I could obtain:

yellow S^R x^- B^+
white S^S x^+ B^-

Plate on	Mut could grow:	Unselected:
1) min	all x^+ B^+	color, S
2) min + M	only x^+ B^+ S^R	color
3) min + biotin + M	all x^+ S^R	color, B

Lysogeny Mutl Cycl