E. D. [illegible]
[illegible]

Cultures

CG-1 A. tumefaciens A6 - virulent } Riker
CG-2 A. tumefaciens A6-6 · attenuated }
" " A6 PEN penicillin resist. }
" " A6 P5a polymyxin resist. } Klemmer (see pA1a)
" " A6C5 chloramphenicol resist. }
CG-3 " " A6-S^r } probably identical } pA5 ff
CG-4 " " A6-S^r }
CG-5 " " A6-6-S^r } probably identical
CG-6 " " A6-6-S^r }
A. radiobacter 1001 }
" " 1005 } Riker
" " 1007 }

CG-7 A. tumefaciens } [illegible] as auxotrophs from CG-2
CG-8 A. tumefaciens } [illegible] (?). Both identical
CG-9 " [illegible] from CG-1-S. UV. [illegible] YNA
CG-10-15 A. tumefaciens from CG-1-S, UV. ~~[illegible]~~ Auxos, not run down
CG-16 A. tumefaciens " " " Biotin -

H. W. Klemmer

Antibiotic resistant cultures of Agrobacterium tumefaciens

Resistant cultures were developed to five antibiotics from a sub-culture (A6K1a) 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 & Galbraith, J. Bact., 59 (1950)], using a 50% inhibition in broth, approx. 8 hour endpoint. The 50% inhibition levels (ID50's) of the A6 strain and, where known, the 100% levels are given below in the table

Culture resistant to		Level of resistance µg/ml	% loss of virulence	Parent A6 culture inhibited at (µg/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	A6C5	40	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 2318 for Howard Klemmer

Media

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

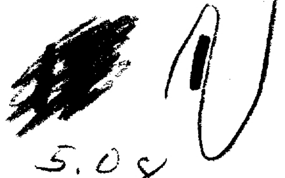
AGI

Medium 79		Modification I (DCG)	
Mannitol	5.0 g	Sucrose	5.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g (anhyd. 0.1 g)	$MgSO_4 \cdot 7H_2O$	0.2
K_2HPO_4	0.2 g	K_2HPO_4	0.2
$CaCO_3$	1.0 g	$CaCO_3$	1.0
2% starch-free yeast extract	100 ml	Difco YE	5.0
Agar	15 g	Agar	16.0
Water to 1000 ml		Water	1000 ml
pH 6.8 (adjust)		pH 6.8 (adjust)	

In making mix, $CaCO_3$ & agar could be omitted. Then medium $\bar{s}$ agar or $CaCO_3$ could be used as complete broth; $\bar{c}$ agar but $\bar{s}$ $CaCO_3$ 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. Minimal medium used in glycine attenuation experiments (van Lanen, Baldwin, & Riker, J. Bact. 63, 715-721 (1952)).

SM
ST
Aniline blue



AG II

Mannitol	5.0 g
KNO_3	5.0 g
$CaCl_2$	0.1 g (reduce to 0.1 see p 33)
NaCl	0.2
K_2HPO_4	0.2
KH_2PO_4	0.1
$MgSO_4 \cdot 7H_2O$	0.2

Distilled water to 1 l.

Adjust pH to 6.8.

According to Dr. Riker, sucrose can be substituted for mannitol in media for Agrobacterium.

Sterilize sugar separately?

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 alum 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 s̄ 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?). Van Lanen, Baldwin, & Riker (1952, p 717) indicate that glycine-resistant clones are of normal virulence — This argues against "glycine attenuation" being a selection of cells which are glycine-resistant and avirulent. [But see Tryptophane paper — & which more sensitive than avirulent]
2. Markers other than virulence
   A. Antibiotic resistance. Cultures on hand resistant to penicillin, chloramphenicol, and polymyxin. Try to get an S^r; also check others.
   B. Nutritional — Penicillin runs?

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

Transferred all *Agrobacterium* cultures to fresh AG-I 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 c̄ saline.
   Resuspend in 10 ml saline, cfg 20 min.
   Wash pellet, resuspend in 10 ml saline
4. Add 0.1 ml suspension to ea. 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.

JACS 70 4267 (1948)

For each run, use penicillin conc. giving survival of 10^{-6} or more (Meth Med Res)

Variables to check:

- Penicillin conc.
- Age of inoculum
- Inc. time in penicillin medium
- Complete medium
- Minimal medium
- Temp; aeration?

3-26-54

Test of growth on coli media –

A6 inoculum evidently poor – no growth in any medium tested.

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

Very scanty growth on EMB media and on D-O; none 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-O. Good growth on EMB media;

grow on D-O.

Try one of the regular [illegible] minimals

Growth more uniform in NSB than in Penassay. More clumping in latter.

<u>Penicillin Screen</u>

1. Inoc. fresh Penassays from those inoc. 3-24. (1 ml inoc/10 ml Penassay tube). (Using Penassay rather than NSB because it is a more complete medium).

<u>Streptomycin</u>

Spread 2 drops Penassay culture (A6 & A6-6) on B. lac. Sm plates to look for S^R's

Sealed stabs of A6 & A6-6

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

3-30-54

Made up AG II minimal liquid

Regulated small incubator in room 213 to 26° and moved streptomycin plates there.

Pen run

2. Penassay tubes inoc. 3-29 fairly well grown; aerated at room temp (beginning at 9:00 AM) to see if it would help growth.

Try yeast broth for next pen. run (AG I liquid)

Make up AG I and AG II plates.

(yeast)
Inoculated 1 ml old cultures of A6 + A6-6 into 10 ml tubes AG I. Inc. at 26°.

Pen run

3. 1:30 pm. Yeast cultures, though they are not as well grown as those on corresponding slip, A6 fairly uniformly turbid; A6-6 stringy & gummy.

3-31-54

Pen run: Control 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) AGI broth aerated at rm. temp.

3) Penassay at 26°

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

Streptomycin

Plates inoc c̄ broth culture 3-29:

papillae, probably Sr, against background of very scanty growth. Number per plate ranges from ca 6 to ca 20. (many more after further inc. 50-200 per plate)

Picked 12 papillae each of A6 & A6-4, streaked from small centers onto B and SM.

If these appear to be Sr, pick single colonies, streak on complete c̄ SM; inoculate to SM medium.

4-1-54

Penicillin run

Both penicillin and control tubes turbid. ∴ Infer replicate plate at 24 hours even 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 aerated at room temp - very good growth, especially of A6. Considerably more turbid than AGI tubes incubated at 26° ̄s aeration for 48 hrs

Use aerated tubes to start second penicillin run

4-2-54

Second pen run

Good growth in control and pen tubes in 24 hrs.
1) For next run, use at least 1000 U per ml
2) Spread dilutions from old pen assay tubes directly on AGI plates & replicate to AG.I. These old cultures have accumulated many S^r cells and probably many pen. resistant; perhaps there are auxotrophs which could be picked up directly.

0.1 to 10 ml

↓ 0.1

10 ml = 10^{-4} —1 ml→ 10^{-5} —0.1 to plate→ = 10^{-6}

↓ 0.1

10 ml = 10^{-6} —0.1 to plate→ = 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 S^{R} colonies, streak on AG I $\bar{c}$ SM.

Spread plates — wait for rel. & mucoid until Sun or Mon. — no visible growth at 24 hrs.

Streptomycin —
A6 - Streaks from all 12 [illegible] ed now well grown in K and SM.
A6-6 Several of 12 well grown; 4 now (6 days) becoming [illegible] allow scanty growth in [illegible] part of streak (S^{R} mutations?)

Pick colonies from 2 streaks from A6 + 6-6; streak on AG I plate $\bar{c}$ SM.

4-5-54 (Monday)

Streptomycin

Replicated S^r cultures streaked on AG I to AG I SM.

Spread plates from old [illegible] assay cultures (2-4 [illegible] in 10^-7)

A6 - Only 15-20 col per each 10^{-6} plate. Plates moldy. Picked all individual colonies, spot on AG I & AG II. (Would take too long to spot on complete, then replicate 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 run

Inoculate AG I broths from old Pen assay cultures. Also inoculate broths from slants to [illegible] [illegible] clubs?

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

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

give CG numbers to Klemmer's cultures until their markers are checked.

4-6-54

Spread plates –

A6 : spots are minimal not well enough grown to read

A6-6 : replicas minimal : Only 1 small colony on complete not growing on min. Looks as though it was not picked up by velvet; however, pick & test to be sure. Spot on min. & complete plates c̄ A6 cultures being tested. [illegible]

Streptomycin –

Saved 2 S^r each of A6 & A6-6

CG3, CG4 (A6) — CG5, CG6 (A6-6)

AGI slants & nutrient stabs.

Pen. run

Used 1000 U/ml

4-7-54

Penicillin run

A6 - good growth in penicillin tube (1000 U/ml!)
A6-6 - pen. tube clear. Plate out orig. & 1/10 dil.

A6 - Streak out & try to get penicillin sensitive colony. (Replica to plate spread c̄ penicillin?). Then try pen. run again.

4-8-54

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

Started broths for pen runs on CG 3 and CG 5
Auxotrophs in S² cultures used for pen. SRP type experiments.

4-9-54

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

ago. 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 grown almost as well on minimal as on complete, while the colonies which were quite evident 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 ē diluted culture s̄ penicillin treatment. (See pp A7 ff). However, in streptomycin experiment, many colonies appeared late on SM plates, after colonies had already been picked which were saved as S^R strains.

Will pen run ē CG-5 yield only the cells which grow up faster on complete? Why should a penicillin run 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

Incidentally, the old broth culture used to start this run has been discarded (in broth exc. 4-5.

on complete than on minimal?)

Take the following steps:

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

2) Test penicillin resistance of both types.

Tentatively,

Early growth on complete, slow on mini = a

Late on complete, good on mini = b

3) Save both types

4) Make all sure neither one is a contaminant!

5th pen run: Inoc. 2d broth tube from broths of CG 3 & CG 5 which have now 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 another 24 hours the type b colonies will be so well grown up that the plates will be too crowded for picking.

4-10-54

MAKE UP AG-I plates ✓

Penicillin run on A6-6

All "type a" col. seem [illegible] - magenta on minimal at 48 hrs.

Make up random plates; test the colonies picked yesterday (ca 50 col) Saving plates.

For randoms, am using AA supp, YE and hydrolyzed casein.

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

Streaks are even & complete; (about 20 hours)

complete

A6 - very scanty - single colonies barely visible

A6-6 - slightly better growth than A6

(These were streaked from old colls)

"Type a" - Quite good growth - Individual colonies large enough so they could easily be picked.

"Type b" - growth very scanty - comparable with A6

Minimal

A6 - very scanty
A6-6 slightly better than A6, b1 very scanty
"Type a" - no growth
"Type b" - barely visible

Plated pen rev c̄ CG 3 & CG 5. Used 1000 u/ml

A6 streaked for test on penicillin - not many isolated colonies - Also no plate available to which [illegible] pen. [illegible] Pick several colonies to small [illegible] & test when convenient.

Save a "Type b" A6-6 only, on slant

4-11-54
CG3 OK for plating - CG5 grown in pen tube. Plated 0.1 ml of 1:10 & 1:100 CG5

4-12-54
CG 3 plating - no growth at 24 hrs. Try plating direct from [illegible] tube. (Use AGI; no AGISM available)
No turbidity in pen tube at 48 hrs.

Streaks on minimal & complete – 3 days.

Complete – A6 and A66 – both have 2 colony types;
One small & dense; other larger and more translucent.
Pick and save both types.

"Type a" – from [illegible] : [illegible] [illegible]
margins, looks more like the translucent
than the dense type in A6 [illegible].
Centers dense & [illegible], [illegible] translucent.

"Type b" – White; in form [illegible] (quite dense),
margins [illegible] & regular.

<u>Minimal</u> – A6 & A6-6 ; 2 colony types, one
moderate size & translucent, second dense but
very small – hardly [illegible] [illegible] [illegible] pinpoint
size.

"Type a" – like first type of [illegible]
"Type b" – virtually no growth [illegible] [illegible]
[illegible], but [illegible] [illegible] shadows or
ghosts; [illegible] [illegible] dense [illegible] [illegible],
but they are transparent, not visible unless
light is just right.

Auxotrophs replicated to rundowns: No growth on AA's except for 4 poor mutants or slow growers. Scanty growth on YEX and beginning of casein. Try purine etc and vits. From Dave Gordon's experience - also try reduced sulfur.

4-13-54

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

Pen. run on CG3 - Colonies present at 48 hrs. ~~in appearance~~ the col. appearing at 48 hrs in previous pen expt. Ca 50/plate from 1:100 dilution. This corresponds to nos. in A6-6 expt. Replicate to minimal. This is 5th deriv. of 46.

A6-6 run i choose 1 auxotroph & use YNA, V175, & control AG II tube.

4-14-54

A6-6 auxotroph . no growth at 50 hours in minimal + YNA or minimal + vits. Incubate at least one more day.

To summarize:

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

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

CG 3 penicillin -
Colonies on minimal not large enough to score. No further colonies on complete beyond no. at 48 hrs. These colonies all are the small "type b" ([illegible])

It seems clear 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 in a penicillin run.
3) All "mucoid" survivors of a penicillin run were either auxotrophic or very slow in their growth on minimal.
4) C63 (A65 [illegible]) is "rough"

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

Also: streak A6 + 46-6 both types, on AG I [illegible]. Compare c C63 + C65 [illegible] [illegible] streaked.

4-15-54

C63 pen run; One possible auxotroph. Inoc. tubes of minimal + complete.

46-6 auxotroph [illegible] [illegible] with [illegible] vits or YNA at 48 hrs. Try single omissions c AA groups.

4-16-54

C63 possible auxotroph: about 30 [illegible], growth in complete, none in minimal. Inoc. [illegible]; continuing incubation of both.

A6-6 auxo (sl. 1#1) - AA single omissions of groups.
Growth in -AA4 ; also in hydrolyzed casein.
Test c̄ ; HC + AA4
AA 1, 2, 3, 5 + the individual components
of AA4.
also - to find actual requirements, test all combinations
of AA groups, excluding 4.

A1 + A2
A1 + A3
A1 + A5
A2 + A3
A2 + 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 stocks on complete medium

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

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

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

A6 PEN (Klemmer) ; } Much like A6 "M"
A6 P 5 "
A6 C5 "

CG3 (A6 5 R) - Seems intermediate between A6 "R" & A6 "M"; colonies somewhat yellowish, but mass growth looks slightly rough
CG4 (A6 5 R) - Both large & small colonies; large have yellowish centers, Mass growth smooth, quite gummy.
CG5 (A6-6 5 R) - Like CG-3.
CG6 (A6-6 5 R) - Like A6-6 "R".

Streptomycin resistance; A6 R+M and A6-6
"R" + "M" failed to grow at all on AG-I SM in 48 hrs.

CG 3 - Grows on AGI $\bar{s}$ SM except colonies are smaller. This could be because medium is rather old.

CG 5 - Looks rough on SM medium, colonies very small.

Cross-breeding $\bar{c}$ penicillin (1 cc of 10,000 u/ml soln); Pen. conc. presently insufficient for any definite effect.

Brush of A6-6 auxotroph from pen. run looks so yellow compared $\bar{c}$ other cultures that it seems wise to try to make sure it really is A. tumefaciens.

4-17-54

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

before discarding as no good.

A6-6 auxotroph:
Original single-omission tubes (now 2 days):
Good growth in −AA4; poor growth in hydrolyzed casein; now slight growth in −AA 5. Still no growth in other single omissions or in minimal.

Inoculated 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 rundowns, 3 days: Slight growth in all tubes, but good growth only in AA5. Will pick & test several col. from plate streaked 4-17 in min, complete, & AA5.

A6·6 aupo; original single omission tubes, [illegible] 4 days; There is [illegible] growth in all tubes except minimal and − AA1 (amides [illegible]).
Sulfide??

Second [illegible] test ([illegible] 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; [illegible] Na_2S

4-20-54

CG·3 picked [illegible] separate colonies.
At 24 hrs, no growth in [illegible] min + Na_2S;
[illegible] turbidity [illegible] plate. Continue [illegible]

A6·6 [illegible] Growth in A1 (Why didn't this happen on plate?) but not in individual AA of the group + Na_2S.
Try single omissions [illegible] [illegible] at higher [illegible]

4-21-54

A6-6 auxotroph At ca 24 hrs, no growth in A1-single omissions or in A1. (~~[illegible] rundown [illegible] [illegible] faint [illegible] [illegible] of [illegible] the [illegible], and acid~~)

[illegible] A1 [illegible] (2 days) A1 group - quite good growth. Slight [illegible] turbidity in [illegible]; [illegible]. [illegible] day.

CG-3 "auxotroph" from single colonies: growth equally good in min. & min + A5. T. O.

4-23-54

A6-6 auxotroph; At 3 days, moderately good growth in AA1 - arginine & - lysine. Poor growth in AA1 complete group. No growth in AA1 - methionine - cystine. Bill [illegible] subculture again.

[illegible]-54

J.L. reports that Klein's results were due to [illegible] & cannot be repeated. Hence [illegible] can be less emphasis on [illegible] -- [illegible] can be considered good [illegible] other mutations.

Try penicillin [illegible] [illegible]. Use A6 only, since it is not necessary to have auxotrophs in both A6 & [illegible]. Use A6 "R" & A6 "M" to see if any difference in tolerance in penicillin.

Inoculate broths of the following:

A6 "R" } in penicillin medium
A6 "M" }

A. radiobacter 1001 }
" 1005 } to [illegible]
" [illegible] }

4-27-54

Broths inoc. 4-26: Moderately good growth in all. The "rough" A6 has a pellicle. Growth of *A. radiobacter* in this medium (AG-I) very similar to "smooth" or "mucoid" *A. tumefaciens*.

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

4-29-54

Streaks of A. radiobacter (48 hrs):

1001: 2 types of colonies: (1) small
(2) large & gummy.

1005: [illegible] all uniform [illegible] &
gummy.

1007: 2 types, same as 1001.

Pick & streak of both types where present.
Also pick & streak 1 col of 1005 to have
stock arising from a single colony.

The large type is opaque, the small is trans-
lucent. In mass growth the mounting
appearance is much like plaques.

Inoculate broths for penicillin on A6 'R' & 'M'.
Aerate.

4-30-54

Started penicillin on A6 'R' & 'M'. 1000 U/ml penicillin

5-1-54

Both grew in pen tubes.

Hendrickson, A.A., Baldwin, I.L., and Riker, A.J. 1934 Studies on certain physiological characters of Phytomonas tumefaciens, P. rhizogenes and Bacillus radiobacter. Part II. J. Bact. 28, 597
Absorption of [illegible] as a [illegible]

5-3-54

Start broth ([illegible]) of A6 M for irradiation.

5-4-54

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

Plate 10^{-5} + 10^{-6} dil. of [illegible] culture (cfg., washed, resusp. in 10 ml saline)

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

(to kill [illegible] [illegible] more [illegible] [illegible])

5-6-54

Irradiation expt: 40 sec. UV gave no detectable killing. All plates after irradiation too crowded to count. Duplicate control plates of 10^{-6} dilution gave average count of 328 per plate, i.e. 3.3×10^{8}. Had estimated 10^{6}.

One plate of irradiated culture has one colony which appeared in 24 hrs and is large, yellow, + gummy like previous auxotrophs. Streak

out & test.

5-7-54

Streak from col picked from UV plate grew well on plate in 24 hrs. Pick one col. to min & complete broth.

5-8-54

At about 20 hrs, good growth of "auxotroph" on complete plate, none on minimal.

Have cultures ready for plant inoculation by end of next week.

"R" and "M" cultures of A6 & A6-6

S^r from A6

Also – grow A6-6 S^r with A6 "M", recover S^r's & test. Do serial plating of A6 "M" to check rate of mutation to S^r.

Inoculated together A6 "M" and CG-5 (CG-1-M) from water suspension of roots.

5-10-54

Gave A6 J both c̄ A6 "M" & CG-5 (CG-1-M) separately

UV auxotroph. No growth on minimal in 3 days. [illegible] mutations. A6 UV-1

Cultures to use for plant inoculation:

1) S^r from mixture expt.
2) CG-1 M
3) CG-1 R
4) CG-3
5) A6 UV-1
6) CG-2M
7) CG-2 R
8) CG-4
~~9) A6 UV~~-1 9) CG-5

Streaked mixed culture on AG I & AG I SM
[At the time these were streaked [illegible] had [illegible] about 48 hours in complete medium at room temperature without aeration.]

5-11-54

[illegible] mutations of A6 UV-1 (tubes)

Started [illegible] of A6 [illegible] UV killing curve

In mixture experiment, should have spread [illegible] of $\bar{S}$ SM $\bar{c}$ appropriate dilution, rather than streaking, to get estimate of proportion of S^r [illegible]. Start new identical mixture, to be plated at ca 48 hrs. Also plate separate cultures on complete & complete + SM at 48 hrs.

5-12-54

AG UV-1 At 24 hrs slight growth only in complete and YNA.

Mixed culture - streaked on complete $\bar{c}$ + $\bar{s}$ 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 over night, 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 $\bar{c}$ 10^{-5} dilution of 2 day non aerated broth cultures of CG-1-M and CG-5.

5-13-54

(48 hrs)

A6 UV-1 rundowns: fair growth in min. + YNA; none c̄ other additions, but slight turbidity in minimal; Recheck. Also check purines & pyrimidines (as 2 groups)

Mixture streaked 5-10 on complete c̄ & s̄ SM:

Complete, no SM: Colonies predominately "M" type; some "R" type. Pick a number of both types, test for S character. ~~There are a~~ few of the large, creamy colonies which have always turned out to be auxotrophs ~~Pick + test.~~ Don't know whether 1-M or S

Complete + SM: 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 virulence test. The "R" type could be pooled, but the "M" type should be checked 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).

Scrape colonies off agar (SM plate) from areas where there are no "M" type colonies; inoc mass into broth. Inoculate slant c̄ 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		

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

5-14-54

AG-UV 1 rundowns; Still no growth (3 days) except in YNA, and for some reason, mercurial.

Set up mercurial, YNA, penicillin, pyrimidines

Plates of CG-1-M and CG-5 made 5-12:
(Spread 0.1 ml 10^{-4} [illegible] of 2 day, unaerated broth)

CG-1-M: [illegible] complete [illegible] of [illegible] size, white, glistening [illegible]. 616 col
[illegible] no [illegible].

CG-5 [illegible] plate [illegible] a bit smaller and less [illegible] mucoid than CG-1-M. 997 col.
[illegible] also some [illegible], but much smaller. [illegible] more. [illegible] too high for [illegible].

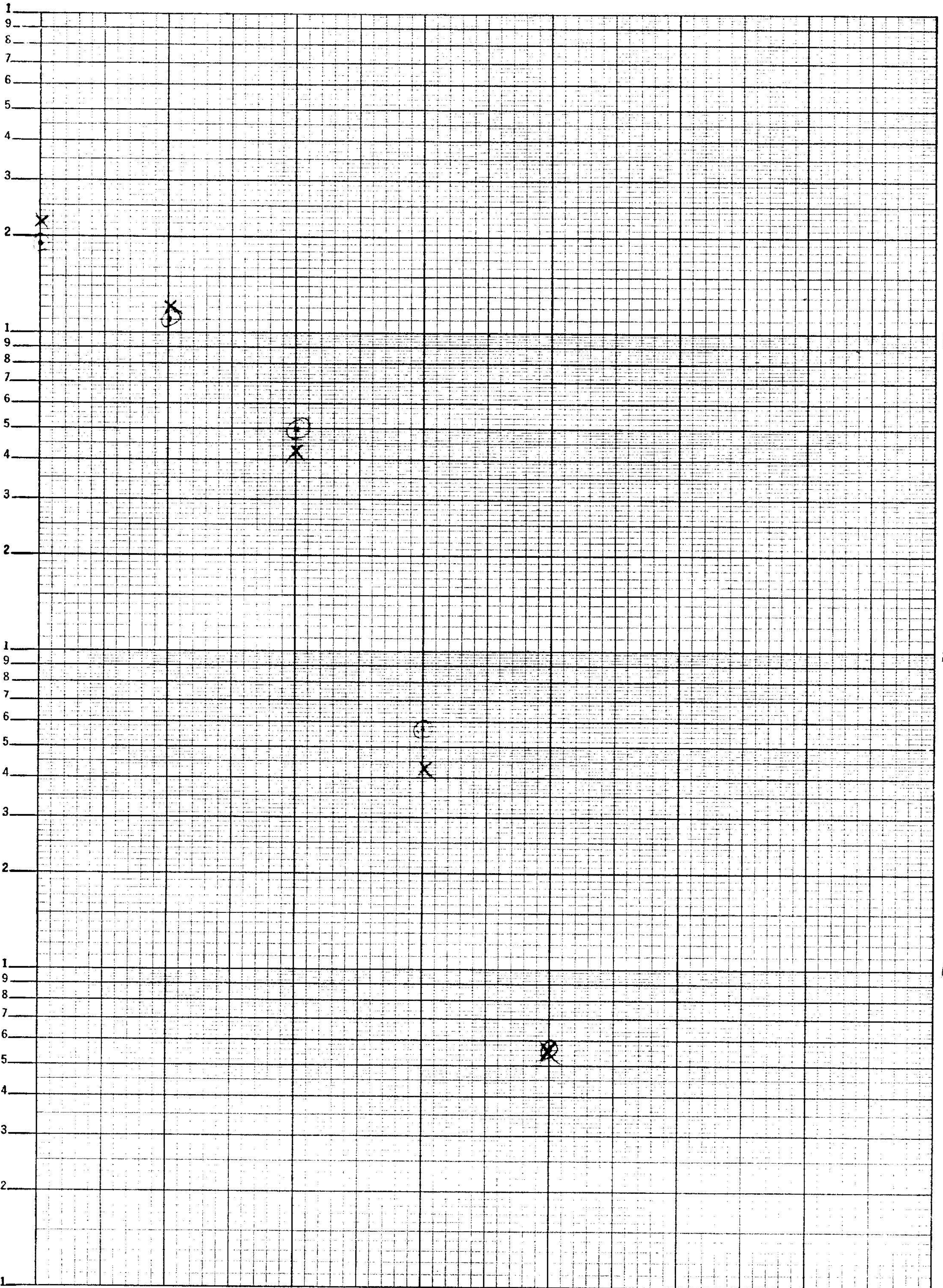
Colony types [illegible] to be quite stable; on each AG-1 plate there is one [illegible] which looks as though it might be of the opposite colony type (1 "R" on 1-M plate; 1 "M" on 5 plate)

<u>UV experiment</u>

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

Exposure	10^{-6}	10^{-7}	10^{-8}
120	54 0	2 0	—
180	0 0	1 0	—

3 day incubated



KEUFFEL & ESSER CO., N. Y. NO. 358-81
Semi-Logarithmic, 4 Cycles X 10 to the inch, 5th lines accented
MADE IN U.S.A.

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

On the 90 sec 10^{-7} plate there are three very large [illegible] colonies, one of which is regular and [illegible] ○, one looks as though it arose from 4 cells stuck together, and one which has opaque and translucent sectors. Pick and streak these.

After one more day, replicate plates c̄ appropriate [illegible] to minimal to check for auxotrophs.

Pick [illegible] possible auxos from 1-M + 5 plate streaked 5/10. Test for auxotrophy also whether S^{2} to determine [illegible].

Plant inoculations: Growth of the following very poor, hence inoculum small: 1-R 3 5 XM2 XM4
[illegible] to be added: 2-R (circled) 4 XM1 XM3 XR1 XR2
[illegible] 2-R

5-15-54

UV killing curve expt.
More colonies have come up (3 days) on 90 sec plates, and some now present on 120 sec plates. Counts:

	10^{-6}	10^{-7}
90	432	70
120	56	2
180	0	1

Counts on 0-60 sec plates virtually unchanged.

Streaks from large col on 90 sec plate
One type of colony from the large, [illegible] col – gummy, [illegible] ①
Mottled col. 2 types, one as above, the other small & translucent. ②
Irregular col. all individual col gummy & opaque; but mass growth does not look uniform.
Save all these, test all types for auxotrophy

Possible auxotrophs from mixplus plate: very scanty growth in 24 hrs, will do previous auxos on [illegible] plate.

Spread plate of 1 M + 5 mixture M-type seems predominant; difficult to tell the two types apart. Continue incubation. (A5)
Also S.M. plate All col. very small, considerably fewer than the on non-S.M. plate. (281)

5-17-54

90 sec. irradiation; streaks from large colonies; (now 3 days)
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-2 & 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, growth only on YNA, not on purine mix nor pyrimidine mix.

Cultures from sectored 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 (& streaked); one looks same as "normal"; the other shows very scanty growth at 24 hrs. No color. [EML had yellow contaminant on an EMS plate which looked similar to this yellow culture at 3-4 days]
3) translucent: Good growth in 24 hours. Colonies appear uniform. Very similar to "normal" but slightly less opaque.

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

Started broths (AG-1) 3:30 pm of all A. radiobacter cultures for microscopic examination. Inc. at room temp $\bar{s}$ aeration.

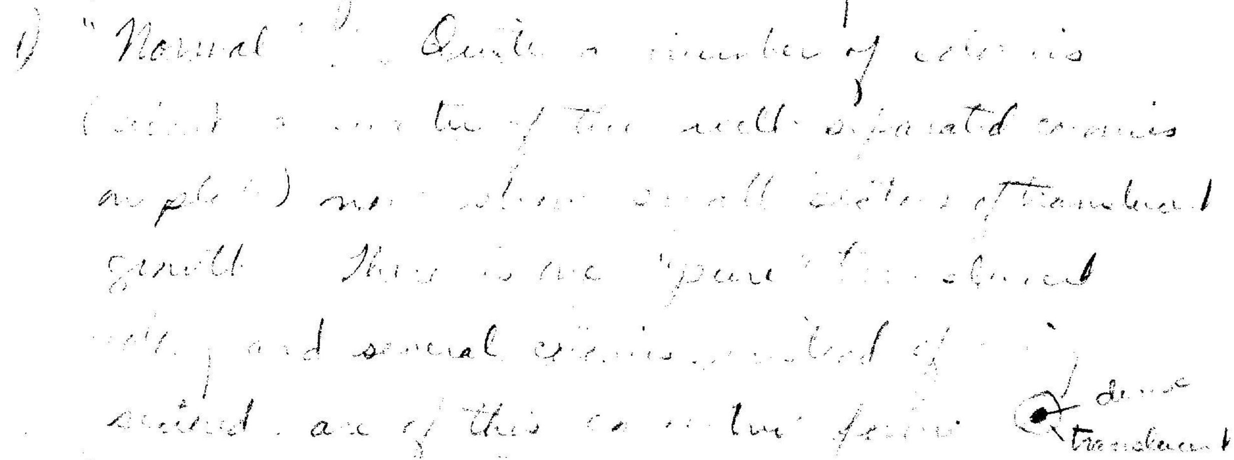
5-19-54

AGUV-1 medium: At 48 hrs, growth only in YNA; not in purine or [illegible] medium only. Before going into identity of requirement, retest on AA's + on Na_2S.

Also: restreak AG-6 mutants on AA's, YNA, and Na_2S. In adding Na_2S to sterile medium, use directly from sterile stock soln and also diluted 1:10 and 1:100.

AGUV, cultures from sectored colony

1) "Normal": Quite a number of colonies (about a number of the well separated colonies on plate) now show small sectors of translucent growth. There is one "pure" translucent colony, and several colonies [illegible] of [illegible] sectored. are of this [illegible] form



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

2) Translucent: Colonies are scarcely distinguishable from the opaque "normal". No sectors.
However, in one of the [illegible] streaks, very small translucent colonies [illegible] the "R" type have come up and (48 hrs).
From this streak, pick & streak clones and "R" type.
The clone colonies [illegible] normal "M" type [illegible] "colonies [illegible]" rapid growth, forming colonies 1 [illegible] in diameter in 24 hrs.

3) Yellow: The yellow [illegible] is a [illegible] continual [illegible] 1 - The [illegible] late [illegible] have a peculiar [illegible] (any plate) [illegible]. [illegible] appearing on [illegible] plate [illegible]. The colonies [illegible] at 24 hrs [illegible] [illegible] many are [illegible] as described [illegible] 1).

Streak CG-1 [illegible] R for [illegible] [illegible].

Possible [illegible] after [illegible] picked from orig. plate & streaked [illegible] plate, [illegible] [illegible] picked from each of the 4 [illegible]. Of these, 2 from #1 and 1 from #4 are S[illegible]. [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] [illegible] [illegible] seems very satisfactory,
especially [illegible] [illegible] [illegible] liquid

Try: 1) [illegible] acetone

2) Addition of ferric [illegible] ([illegible] [illegible] [illegible]
(MacLeod, [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 (Both [illegible] [illegible] [illegible] [illegible] [illegible] for me)

<u>5-20-54</u>

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

[illegible] [illegible] A [illegible] [illegible] [illegible] [illegible] [illegible] no growth
[illegible] 20 hrs except in complete.

[illegible] [illegible] from [illegible] plate. [illegible] [illegible]!

A6 [illegible] [illegible] (?) [illegible] [illegible] from [illegible] [illegible].
[illegible] [illegible] 5/19 [illegible] [illegible] [illegible] [illegible] [illegible]
[illegible]. (24 hrs).

From "Transduct 1" streak on 5/17 plate, what I called "R" type colonies [illegible] are actually the yellow contaminant.

CG-1-R & M streaked for [illegible]
No visible growth on AG-1 in 24 hrs.
All the derivatives from the sealed colony (except the contaminant) grew up [illegible]

Replication of UV plates (Expt 2) [illegible]
Found possible auxotrophs on replica [illegible]
Six suspected auxos; 3 are the large colonies which appeared [illegible]
∴ → Check all derivs. from sealed col, see if all are auxos. Pick up other 2 col.
Other 5 possible auxos were [illegible] being of different colonial appearance – small, less dense than "normal".

Numbering:

2UV-1 (#1 from 2nd irradiation): Opaque, non-sectored from sealed col.

2UV-2 } other 2 large col on 90 sec plate
2UV-3 }

2UV-4 } small col from 90 sec plate
-5 }
-6 }

[illegible] staining of *A. nidulans* with plane [illegible]; [illegible] broth [illegible], [illegible]d, diluted 1:20, [illegible]

↑ 1001 S 1R 5 7S 7R

[illegible] [illegible] are [illegible] formation. Except for 1007R, [illegible] [illegible] of any kind; 1007R had a few clumps. Motility poor.

Streptomycin resistant strains were obtained in 1947; could be used as marker. Also proflavin, 100 U/ml (circled: 100 U/ml)
Try [illegible], SM, ST resistants in A6-6 auxos; A6UV-1; new auxos (2 UV series)
Try cross breeding c̄ SM to see whether it can be scored readily.

5/21/54

Auxotrophs of A6 [illegible] and A6UV-1: No growth in any supplement (48 hrs). [illegible] [illegible] [illegible]

2UV1 - 2UV6: No growth in any, a trace on minimal. 1, 2, & 3 well grown at 24 hrs on complete, 4, 5, & 6 scanty.

Observations from sectored colony (≅ 2UV-1):
Sectored col. in streaks done 5/19, both from sectored and non sectored colonies. This culture is being carried as 2UV-1, so can be checked again later for continued instability if [illegible] any type

Inoculated soft agar tubes c̄ the cultures 1005, 1007S, and 1007R to see if they will swarm; if so, can get more highly motile cultures.

Gave stock numbers to first auxotrophs
A6-6 (1) = CG-7 } sulfide?
A6-6 (2) = CG-8 }
A6UV-1 = CG-9 (YNA)

5/22/54

Rundowns: (4 days; 1 day since Mw & Fe added)
CG-7 & CG-8: faint growth in YNA, possibly in highest Na_2S conc.
CG-9: good growth in YNA; possibly slight in highest Na_2S conc.

[illegible]/54

Rundowns (6 days with Mn & Fe added)
CG-7 [illegible] Fair growth in AA-1; [illegible] in YNA; slight in Na_2S neg. conc. and 1:10.
CG-9 Possibly slight growth in YNA [illegible] anything else.
([illegible] was added before autoclaving, & after autoclaving [illegible] 1 ppt.)

5/28
Plant inoculations (now 2 weeks):
Galls on all plants with CG-1-M, CG-1-R, CG-3, CG-4. 5 [illegible] A6 show little or no loss of virulence.

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

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

5-29-54

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

Spread aerated overnight cultures of CG 7 & 9 on SM and ST plates (SM 1/4 the conc. used [illegible] E. coli [illegible] 0.05 [illegible]/ml; ST 100 U/ml.)

5/31/54

Requirements of auxos (plates)

	O	A1	A2	A3	A4	A5	HC	VITS	YNA	Sulfide
CG 7	−	−	−	−	−	−	−	−	−	−
8	−	−	−	−	−	−	−	−	−	−
9	+	+	+	+	±	+	−	−	+	+
2UV 1	+	+	+	±	±	+	+	−	+	+
2	−	−	−	+	+	±	+	−	−	−
3	+	+	+	+	+	+	+	−	+	+
4	−	−	−	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−

All [illegible] replication to complete.
Can only [illegible] sense of these results by assuming insufficient inoculum for 11 plates from one original.
Continue incubation. For 2UV 4, 5, & 6 try AA [illegible] concentrations, AA + [illegible] yeast extract.

Recheck CG 9 and 2UV 1&3 in liquid minimal; may be [illegible] due to something in agar. Also check in minimal + [illegible].

ST and SCM expts:
CG7: S'mycin conc. too low; background growth quite heavy; no resistant [illegible] visible.