Cultures

CG-1  A. tumefaciens  A6 = virulent
CG-2  A. tumefaciens  A6-6 = attenuated

CG-3
CG-4
CG-5
CG-6

A. radiobacter

CG-7  A. tumefaciens
CG-8  A. tumefaciens
CG-9

CG-10  A. tumefaciens  from CG-1-8, UV.
CG-16  A. tumefaciens

A6 PEN penicillin resistant
A6 P5a polymixin resistant
A6 C5 chloramphenicol resistant

1001  1005  1007

Klemmer (A1a)
Antibiotic resistant cultures of Agrobacterium tumorfaciens

Resistant cultures were developed to five antibiotics from a sub-culture (A6K76) of a single-celled culture of the parent A6 strain of A. tumorfaciens. Three of these cultures are still in stock (on antibiotic-free yeast extract-mannitol medium 72) after 2 months. Three remaining were not tested for resistance for at least 2 months. All five cultures are listed below, along with the level of resistance obtained and the 50% inhibition level (in %) compared with the parent A6 strain.

The parent A6 strain has been assayed against several antibiotics by Dr. Rupp [Phytopath., 39 (1949)] using a 100% inhibition for 20%, 50% four end points and by Kliommer [method of Jagger and Kliommer, J. Bact., 63 (1952)] using 2 1/2% inhibition in both, about 8 hour end point. The 50% inhibition levels (20-50) of the A6 strain and culture shown the 100% levels are given below in the table.

<table>
<thead>
<tr>
<th>Culture resistant to</th>
<th>Level of resistance</th>
<th>50% Toxin</th>
<th>Parent A6 culture inhibited at (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin AUPEN</td>
<td>1000</td>
<td>80</td>
<td>8.0</td>
</tr>
<tr>
<td>Streptomycin look</td>
<td>800</td>
<td>20</td>
<td>3.5</td>
</tr>
<tr>
<td>Polymyxin A6P5a</td>
<td>150</td>
<td>80-90</td>
<td>5.6</td>
</tr>
<tr>
<td>Chloromycetin A6C5</td>
<td>40</td>
<td>50</td>
<td>0.24</td>
</tr>
<tr>
<td>Terramycin look</td>
<td>15</td>
<td>20-30</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The culture still in stock; resistant to penicillin [A6(Pen)], to polymyxin [A6(P5a)] and chloromycetin [A6(C5)]

If you want more information, phone 2318 for Howard Klommer.
**Medea**

I. To maintain stock cultures, adaptation of "Medium 29" of

Fred and Nakamura

*MEDIUM 29*

- Mannitol 5.0 g
- MgSO₄·7H₂O 0.2 g (and 0.1 g)
- K₂HPO₄ 0.2 g
- CaCO₃ 1.0 g
- 2% trypticase
- yeast extract 100 ml
- Agar 15 g
- Water to 1000 mL
- pH 6.8 (adjusted)

**Modifications**

1. (DEC)

<table>
<thead>
<tr>
<th>AGI</th>
<th>Modification 1 (DEC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucrose 5.0 g</td>
</tr>
<tr>
<td></td>
<td>MgSO₄·7H₂O 0.2 g</td>
</tr>
<tr>
<td></td>
<td>K₂HPO₄ 0.2 g</td>
</tr>
<tr>
<td></td>
<td>CaCO₃ 1.0</td>
</tr>
<tr>
<td></td>
<td>Difco YE 5.0</td>
</tr>
<tr>
<td></td>
<td>Agar 16.0</td>
</tr>
<tr>
<td></td>
<td>Water 1000 mL</td>
</tr>
<tr>
<td></td>
<td>pH 6.8 (adjusted)</td>
</tr>
</tbody>
</table>

In making mix, CaCO₃ or agar could be omitted. Then, medium 5 agar or CaCO₃ could be added as complete broth. 10 agar but 5 CaCO₃ as complete medium for plating. Also omit sugar from mix (Sucrose or for cornstarch, galactose according to both Dec, mannitol used for glucose.)

II. Nutritional medium used in glycerol attenuation experiments (Smith et al., 1953; Smith, et al., J. Bact. 62, 715-721 (1952)).
According to the above establishment of materials and processing conditions, anodizing is achieved at a suitable current density. The specific anodizing solution and current density are to be determined experimentally. Further details on the processing conditions and materials are presented in the following section.
3-28-54
Made up mix for complete medium (Modification 1 of Medium 79 = AGI). Made up 200 ml for plates. Tested pH of mix + 0.5% sucrose (before adding 3% agar). pH = 6.78 ± adjusted.

Possible approachs:

1. Glycine
   A. Repeat glycine treatment using AB. This would at least serve to give a second control strain.
   B. Plate one glycine to get glycine-resistant col. (Could this be used alone or base?) on Glycerol Glutamate, Gela (1951, p. 71) indicate that glycine-resistant clones are of normal serogroup. This argues against "glycine treatment" being a solution of cells which are glycine-resistent and resistant [one quarter these resistant?]

2. Markers other than serolence
   A. Antibiotic resistance. Can these be back selected to penicillin, chloramphenicol, and polymyxin. Try to get one of these also choose others.
   B. Nutritional - Penicillin minus?
3. Actual repetition of Klein's experiments, especially the U-tube experiments.

4. If markers are obtained, test for recombination of markers at mixture and U-tube experiments.

3-24-54

Transfer all Agrobacterium cultures to fresh AG2 medium.

Try 46 & 46-6 on some routine E.coli media to see whether they can be used at all.

Bouillon method as used for E.coli

1. Grow up culture

2. Incubate 1 ml gram negative culture into complete medium
   (Renassay for E.coli)
   Add antifoam, aerate 3-3 1/2 hrs

3. Cfq 10 minutes, wash & saline
   Resuspend in 10 ml saline, Cfq 20 more
   Wash pellet, resuspend in 10 ml saline

4. Add 0.1 ml suspension to 0.1 ml of 2 80 tubes
   Add 0.2 ml per centulin solution to one tube (other omitted)

5. Incubate at least 4 hours.

6. Spread on complete plates (0.1 ml culture and 0.1 ml of 1:10 dilution). Replicate 3 minimum.
Variables studied:
- Sex
- Cycles of amendment
- Duration and preparation methods
- Complete medium
- Nutritional medium
- Temp., aeration?

3-26-54

Test of growth on culture media:
- A6: excellent, steady, poor, no growth, and 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.; more on EMS.
- A6-6: very well and stable; A6, little or no growth.
  (Isolate A6-6 from fresh plants)

3-29-54
- A6-6 well-grown and NSB & penassay growth
  scanty in D.O. Good growth on EMB media
pre-190 D.O.

Try one of the regular bacterial preparations, not the more suspense in NSE than in Peromyscus. More chemically reactive later.

_Peromyscus remark_

1. Store fresh Peromyscus from these mice. 5 24.
   (1 ml more/1 hour Peromyscus tube). Clearly
   Peromyscus rather than NSE because there is no
   complement medimir.

_Striped to edge_

Sprinkle 2 drops Peromyscus culture in 8.5% soya milk
to look for S+ S

Sealed stats of A6 & A6-C

Made up 20x males for AB III (nonmonoid)
3-30-54
Made up AGII nutrient liquid
Regulated at 30°C, incubated at 213 to 26°C and
mixed routinely plates three

2. Clumping tube series
3.29 faEc (pig) was aerated
in 40ml flasks (bacterial count 9.10E4) to check for clumping
in growth.

Try Agar blood for neutral presumptive (AGI liquid)

Make up AGI and AGII plates.

Inoculated hedgehog cultures 1 Ab + Ab 6 into 10ml tubes AGI. Incubate at 26°C.

Pen summary

3. 1:30pm: Hedgheogs, though they are not as
well grown as last week corresponding step, Ab
furry no firmly tented; Ab 6 already beginning.

3-31-54
Pen summary: 4 tubes barely tented, 1 medium tubes
clear. (Used AGII as minimal). Plate out at ca 48
hours.
How get fastest & most rapid growth in complete medium?

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

Incubate each tube to 0.1 ml old Penassay culture, Inc. 10:00 AM 3-31.

Streptomycin:

Plates and a broth culture 3-29:

papillae, probably S
2, against background of very scanty growth. The larger plate ranges from ca. 10 to ca. 20. Size # 2: 50 - 200 µm.

Picked 12 papillae each of AB & A6-8, stained from small cultures with E. and SM.

If these appear to be S
2, pick single colonies,
struck on complete & SM; replicate to SM medium.
4-1-54

Pericillin run

Both pericillin and control tubes tilted. 3 hours on round plate at 24 hours view of growth. 2 control tubes very scanty.

Comparison of complete media - 24 hr growth

Pericillin at 26° - poor
AGI at 26° - a little better than Pericillin
AGI at 30° - no temp - very good growth, especially of AGI. Considerably more limited than AGI tubes incubated at 36°. Observation for 24hrs.

Use acid tubes to start second pericillin run.

4-2-54

Second pericillin run

Seed growth on control and pericillin tubes in 24 hrs.
1) For next run, use at least 1500 U per ml.
2) Spread dilutions from old pericillin tubes directly on AGI plates & replicate to AGI. These old cultures have accumulated many S cells and probably many pen-resistant, perhaps there are auxotrophs which could be filleted up directly.
4-3-54

Replicate spread plates to AG II.

(They have to wait until Sunday.)

Pick 5th colony, streak on AG I 5 SM.

Spread plates - wait for about a minute until the lens is down.

Wet - no visible growth at all.

Stephanoecia:

A6 - Stinks from all 12, a few red now well grown in Kral (SM).

A6:6 - Several of 12 well grown, 4 large today.

Looking for other colony growth at second part of streak (5th replication?)

Pick colonies from 2 streaks from A6 + 6 by streak on AG I plate 5 SM.
4.5 subculture day

**Staphylococcus.** Replicate 5th cultures streaked on AG I to AG EISM.

Spread plates from old necessary cultures (2.4.5 sub. 10-7)

**Ag - Only 15-20 col. per each 10^-6 plate. Plates should have at least two to three individual colonies, separate AG I and AG II.** (Check colonies long to separate completely, then subplate to another - 2-3 days required at each step)

**Ag - C.** Replicate to morcellate (9 to 15 col./plate in 10^-7 dilution; 10^-6 to 10^-5)

**Pseudomonas.**

Incubate AG I broth from old necessary cultures.

Also incubate broth from cultures to ensure that colonies are lifted off the plate completely.

Note: Replicate plating may not be very satisfactory. This bug - Coliforms either don't stick to the medium at all, or these are lifted off the plate completely.

How many zero mutant colonies? Using prefix A would lead to duplication. i.e. Will use CG for crown gall. A6 = CG1; A6-6 = CG2. Will not
give CG members to Heineken's cultures until their markers are checked.

4-6-54

Spread plates -

A6 4 plates are minimal and most cannot grow to read

A6 4: duplicates are minimal. Only 1 small colony on complete media growing on one. Looks as though A was picked up by oscillating shaker, not 4 and tested to be sure. Spot on min. 4 complete plates = A6 cultures being tested.

Streptomyces -

Saw red 2 Std. red of A6 x A6-6

/  \ CG3, CG4  CG5, CG6

AGI checks are natural status

Pen run

Used 10% CMC
A6 - good growth in penicillin tube (unstippled) 
A6-6 - pen. tube clear. Plate outgrowing 7/10 dil.

A6 - streaked out & try to get penicillin sensitive colony. (Replicate to plate spread in penicillin?). Then try penicillin again.

4-8-54
(A6-6) 
Penicillin run - undiluted from pen. tube too crowded; 1:10 dilution OK for replication (ca 200 col/plate)

Startled broth for penicillin run C6-3 and C6-5

Anthrax in 50 cultures would period 20 P type experiments.

4-9-54
A6-6 penicillin run NOTE: A second type of colony has appeared on the AG-I plates as 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
They are about 5 times as numerous as the earlier colonies. There is not a gradual increase in size of colonies at 48 hours, like a sharp, distinct difference.

Although these later colonies were not visible when the plates were replicated to memorial, they have now appeared and are as well as memorial as in complete, while the colonies which were quite as well as in complete when replicated, are now barely visible on memorial. The latter behaved as in other slow growth of memorial after replication from complete, as more like that observed when plates were spread to diluted culture 5 percent in treatment. (See pp. 117 ff.) However, in streptomycin approach, many colonies appeared later on SMM plates, after colonies had already been picked which were saved as E 7 stocks.

Well preserved E 6-5 yield only the cells which grow up further as complete. Why should a preparation from such a large yield of colonies which grow as rapidly as main as complete, where the mass culture needed as complete, consist mainly of cells which grow more closely.
Basically, the red broth culture used to start this runs has four different red broth cases 4-5.

are complete then are unusual?

Take the following steps:

1) Shake at a colony of each type and AG I and AG II, along with mouse culture of AG 6-6. Why not as well include AG 6-2 for comparison.

2) Test penicillin resistance of both types.

Each day:

Each, one well on complete, the others are a table on complete, and one more = 2

3) Same both types

4) Unavailable. One mouse plate is a contaminant

8th passing. Later 2d broth tube from both of CG 3 & CG 5 which have been aerated 24 hours.

Pick a large one of the type or colonies spotted directly (no suspension) on AG I plates. They may all be autotrophs, and if another 24 hours the type 2 colonies will be so well grown so that the plates will be overcrowded for study.
PERARENT RUN ON AG 6

All "like a" etc. seem analogous - somehow not so marked at 48 hrs.

Make up secondary plates (not the ones from p.6) yesterday (on 10-30 hrs) Same avg. plate.

For standards, use using AA, YO, YE and 1% hyde.

"Type A" still small and inhibits at 72 hrs.

Streaks abundant & complete (about 20 hrs).

Complete AG - very scanty - single colonies hardly visible.

AG-6 - slightly better. Note this AG.

These were isolated from AG (flowers)

"Type A" - Create good shape? - Individual colonies large enough so they can be picked.

"Type B" - grows very scanty comparable with AG.
Memorandum

A (very poor quality)
A6 is slightly better than A6. 6/1 very poorly.
"type a" - moderate
"type b" - barely visible

Plated pen new to CG 3 & CG 5. Used 1000 Yard

A6 stucked for test on perminator - not many
assisted colonies. Also one plate available to which
traced pen. - about several colonies to want
Pease suggest test colony tomorrow.

Save a "type b" + 6-6 with pen about

4-11-54
CG 3 OK for plating - CG 5 not used.
Plated 2.4 oz of 1:10 & 1:1000 CG 3

4-12-54
CG 3 plating - none well done. Less dry plating direct
from paraffin. (Due A.G.I. no AGES4 available).
No turbidity on perminator at 48 hrs.
Strokes are minimal & complete - 3 days.
Complete - AC & AGC - both. Classic 2 classic types:
One small & dense, then larger and more handshaped.
Pick and carry both types.

"Type A" firm, prominent, filling 1-2 margins, looks normal in the translated.
"Type B" firm, type on the left. Lift,</p>

"Type C" - Type's very firm, dense in globed type.
Margins clear & regular.

Marginal - AC & AGC-0:2 on types, more circular, may indicate transition or end stage. Likely small - hardly on day 5.

"Type A" - like field type in type.
"Type B" - virtually same as above, large, more dense.
"Type C" - dense, more, but not as obvious. However, not as dense in type, but they are transformed, moveable in less time.
Anastopathes replicated to quadrums. No growth on AA's except for 4 per cent tubes on day one. Scantly growth on YFA and glycerol agar. Try pH 7.4 and 7.6 tubes. From here Zilva's agar and also try reduced sugar.

4-13-54
(Tuesday) - Make up 2X AG-II and some mel tubes.

Petunia on CGA - Chloris present at 48 hrs.

10% of suspension the cell appears; 12-13 species per sq/ft. Ca 50 plates from 1,000 cultures. This corresponds to 10.3 AG-6 agar.

Replicate to memorial. This is 50 debr of 100.

AG-6 run in glucose 1 anastaph to YVA, V175, + control AG-II tube.
4-14-54
AB-6 auxotroph, one growth at 30 hours on minimal + YNA or minimal + sucrose, 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 w/vitamins.

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

CG 3 pneumunt
Colo;ies were not small; large enough to see. No further colonies are complete beyond week 7. These are all of the small "type b" (C & D)
It seems likely that each culture is mixed (AB & AB-6) a mixture of a "tough" smooth colony type and a very gummy, mucoid type.
To be verified:
1) The "tough" grows more slowly than the gummy on the present minimal medium (AG-11).
2) Despite this, the "rough" was selected for in a particular strain.
3) All "mutant" survivors of a particular strain were either 
    "mutanttype" or very small, their growth was minimal.
4) C63 (A655) is "rough".

Strike out all strains on complete. Compare every type. Where 
adequate, order those (in all) components.

Also: struck 51 + 46-6, both type, as AG1450. Compared 
C63 + C65 to both strains.

4-16-54

C63 parent is one possible autotroph. Need other 
possibilities to complete.

46-6 autotroph is of the type of Y4A at 48 hrs.

Try single strains + series of AN-gens.

4-16-54

C63 possible autotroph is also 80 hours, growth and 
compete, more autotroph. June 21, continue 
incubation of 24 hrs.
AG-6 ampo (etc. #1) - AA single ascus of group

Growth on -AA4; almost duplicated oogonia

Test 1: 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  
- A2 + A3  
- A1 + A3  
- A2 + A4  
- A1 + A4  
- A3 + A5  
- A1 + A2 + A5  
- A1 + A3 + A5  
- A1 + A2 + A3

Just need to do single ascus tests:

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

Check of colony types of strains on complete medium:

AG original (CG-1) : Predominantly large, dense, yellowish

A few smaller, smaller, white, translucent

AG-6 original (CG-2) : Moderate size, dense, yellowish
center. Some chromo spheres
A6 "R": Small, white; mass growth looks rough.
A6 "M": Large, tawnyish centers; glumous.
A6-6 "R": Like A6 "R".
A6-6 "M": Like A6 "M".

Small (protophase) from A6-6 personae: Small cut through; centers yellowish.
Large (anaphase) from A6-6 personae: Colossal and very large. But more yellow & glumous than A6-6 "M".

A6 P.E.N (Klemmer): Much like A6 "M".
A6 P.S.
A6 C.S.

CG 3 (A6 S n): Seems intermediate between A6 "R" & A6 "M";
colonies somewhat cylindrical, but mass growth looks slightly round.
CG 4 (A6 S n): Both large & small colonies; large have
tawnyish centers. Mass grows smooth, quite glumous.
CG 5 (A6-6 S n): Like CG 3.
CG 6 (A6-6 S n): Like A6-6 "R".
Streptomycin resistance: A6 R+M and A6-6 R+M failed to grow at all on AGI-SM after 48 hrs.

CG 3 - As an AGI-SM except colonies are smaller. This could be because medium is rather old.

CG 5 - Looks rough on SM medium; colony is very small.

Cross-breeding is promising (check of cross-proven strain): Parent and offspring examined for any definite effect.

Brush of A6-6 autotroph from putative look so well compared to other cultures that it seems wise to try to make sure it really is A. Teratocarcin.

4-17-54

CG-3 possible autotroph. At 2 days, growth is minimal. So far (24 hrs) no growth in medium or in complete - to ad same time (enough proto but very small). Streak from 48 hrs complete to complete plate to try several individual colonies.
before decimal as one said.

AA: 6 + streaks:

original single omission tubes (new 2 days):

All growth in AA +, poor growth in
hyperly deo avs. still slight growth in
- AA S. Still also growth in other
single omission on acid normal.

Table inc. 4-16 (24 hrs)

<table>
<thead>
<tr>
<th></th>
<th>complete</th>
<th>pH</th>
<th>AG II</th>
<th>AA-4</th>
<th>AA-4+T</th>
<th>AA-4+G</th>
<th>AA-4+P</th>
<th>AA-4+A</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2O + AA</td>
<td>+</td>
<td></td>
<td>AG II-0</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2+3</td>
<td>-</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1+2+5</td>
<td>-</td>
<td></td>
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<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
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<tr>
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<td>+</td>
<td>+</td>
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</tbody>
</table>

4-19:54

CG-3 possible + streaks 2-3/2 days. slight
growth in all tubes, but good growth only in AA S.
Wells picked + test several cel. from plate streaked 4-17
in mm. complete, AA S.
A6-6 auxo, original single omission tube, over 4 days; there is some growth in all tubes except medial and - AA1 (includes medial -). Sweet?  ?

Second count test (now 2 days):

<table>
<thead>
<tr>
<th></th>
<th>++</th>
<th>complete</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>++++</td>
<td></td>
<td>++++</td>
</tr>
<tr>
<td>HC+AA4</td>
<td>++++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1+2+3</td>
<td>++</td>
<td>AA-4+4</td>
<td>+++</td>
</tr>
<tr>
<td>1+2+5</td>
<td>++</td>
<td>-4+T++</td>
<td>+++</td>
</tr>
<tr>
<td>1+3+5</td>
<td>++</td>
<td>-4+G+++</td>
<td>+++</td>
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<tr>
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<td>-</td>
<td>-4+P+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-4+A+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Try with AA1 group in order and avoid groups over 6.

4-20-54

CG 3 picked from separate cultures
Ab. AA1 line, no growth in medium + AA1.
Will try more and complete bacterial combinations.

Ab's paper: Growth in AA1 (May 21) add 10% eppendorf or plate? but not with individual Lots of the suspension. 5 lots made, one lot not high enough.
4-21-54

AB-6 count plus AB 12:24 hrs, no growth in AB plus selection of AB Al. (Call in sunflower oil unread)

1 day

Slight Al counts in AB group - quilt and vials with. Slight tube Al; fluorescence and motility read as +. Streaked at different day.

CG-3 "anastrophe" from single strain, quite elongated.

4-23-54

AB-6 anastrophe: Al 3 days, moderately good growth in AB-1 - argonaet + - buprestis. Good growth in AB-1 euthonia group. No growth in AB-1 - super. Both try apple juice.

Import: that entire results were due to contamination & cannot be reported. Hence these must be less important mechanically of great. - Therefore, can be concluded quite for these, matter.
Try preparing agar base. Add Ab only, since it is not necessary to have a complete broth. Add Ab and Ab. The colony difference is obvious at a glance.

Incubate for three of the plates. If 
\[ Ab \downarrow \] 
\[ Ab \uparrow \]
Incuba for 3 days.

A. radiobacter 1001
1947
1937

4-27-54

Broths were 426. Noticeably good growth in all. The "rough" Ab has a peculiar. Smear of A. radiobacter on this medium (AB-1) very similar to "small, round, mucoid" A. terrificans.

Streak each radiobacter culture on AB-1, see whether more than one colony type.
4-29-54

Stokes of B. turbidus (48 hrs): 1001
1. 2 types of strains: 1. Small
2. Large + swimming.
1005: Various amounts of green forming g.

5-3-54

5-4-54

Start broth (incl. LD) of 10% M fructose solution.

5-5-54

Pump set turned off, no culture was not added over night. Had been aerated for about 6-7
of the 24 hours 2 days previous.

Plate 10^-8 + 10^-5 dil. of 10% culture (ref. vol. vol.
resusp. ml 10 ml saline)

Graduate 40 cc. into saline & big clamp. Plate 10^-3
10^-4 + 10^-5 dil. of undiluted culture.

(60% 80% increase in viable numbers)

5-6-54

Graduates right 40 cc. UV gave no detectable
failing. 25 plates after aeration. The needed
to make replicate plates of 10^-6 dilution gave
an average count of 3.2 x 10^9 per plate, e.g. 3.3 x 10^8.
Had estimated 10^8.

Two plates of undiluted culture, the counting
made. After 24 hrs on the bench,
yellow, a fuming like previous experiments. Street
5-7-54

Starch from GC picked from UV plate grew normal plates at 24 hrs. Indicate to move complete batch.

5-8-54

At about 24 hrs, good growth of reinoculated complete, move and reincubate.

Three cultures ready for phage inoculation by end of week.

"A" and "M" cultures of A6 + A6-6
5/6 and A6
Also - A6-6S with A6 "M", receive 5/6 S strain. Do initial plating of A6 "M" to check rate of inoculation to S5/6.

Incubated together. A6 "M" and A6-5-5 from nutritive supplement plates.

5-10-54

Incubate A6-5 with A6 "M" and A6-5-5 separately.
UV-irradiated Pea-Tissue cultured on 3 days. Plating medium BA UV-1

Cultures to use 3 days post irradiation:
1) S
2) CG-1 M
3) CG-1 R
4) CG-3
5) AG UV-1
6) CG-2 M
7) CG-2 R
8) CG-4
9) CG-5

Streaked mixed culture on AG I & AG I SW
Leaves on top of the of the plates were left 37°C for 1 day in complete medium at 100 rpm on a rotator.

11-5-84
One unidentified of AG UV-1 (tubes)

Started bulk of AG for UV mutagenesis

In mutagen experiment, should have spread seeds of 5 seeds per plate in otherwise identical culture.
AG UV-1 at 24 hrs slight growth only on complete and YNA.

Mixed culture - streaked on complete + S9 (-"M" type) on complete only. 2 colony types: large, gummy, and small translucent ("R" type). The resistant sensitive culture used is an "M" type, and the avirulent, resistant, and "R" type on complete + S9 only the "R" type only. Not yet large enough to pick clearly.

UV killing curve.

Inoculated 10^-4 dilution (in saline) of over night, aerated broth (AG-2) culture of AB (M). Plate as control final dilution from original culture of 10^-7 and 10^-8. Incubate 30, 60, 90, 120, 180 seconds.

through 60 sec, same dilutions as control, 90-180 sec, 10^-6 and 10^-7

Spread AG-1 and AB I-2Y plates = 10^-5 dilution of 2 day monoxenic broth cultures of CG-1 M and CG-2.
(48 hrs)

AG UV - I sundries: fair growth and many + YMA, more
3 other additions, still slight turbidity in medium.
A check. Also include precures + precautions (as 2ampa).

Mixture streaked 5-10 on complete 3 x 3 SBA:

Complete: 5 x 5:
Colonies predominantly "M" type;
Some "R" type. Picked a number of both
types, test for S character. There are a
few of the large, creamy colonies which
seemed always turned out to be a few of the
black + tests.

(48 hrs)

Complete: 1 x 1:
Lack majority of colonies are
the "R" type, like the 5 x 5 mixture zone. There
are more a few "M" type colonies appearing.
Picked a number of the "R" colonies and as
many as possible of the "M" type. Used both
an incidence test. The "R" type could be
picked, but the "M" type almost be cleared
individually.

Plank inoculation 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 from areas where there are no "M" type colonies, and mass into broth. Inoculate blank E with this mixture.

These are the cultures to be used:
(CG-1, M) 1-M+5 (M1)
(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)
AG-3 1-M+5 (R2)
AG-4
AG-5

Spread AG-1 plate with 10^-5 dil. of 1-M+5 mixture and inoculate with 5-11.

5-11, 9/19

After 1 round, still no growth (3 days) except in Y114, and for some reason, cannibal.
Set up: Membrane, YMA, peptone, pyruvolic
Plates of CG-1-M and CG-5 were C-12.

CG-1-M: too complete; too few organisms

CG-5: too incomplete; plates were blemished

To determine the similarity to 99.7% col.

Many types seem to be quite stable; one

Not all plate there is one among which darkens though it might be the opposite
colony type (1-7 or 1-17) pyl 1-17 or

UV experiment

<table>
<thead>
<tr>
<th>Spore</th>
<th>$10^{-6}$</th>
<th>$10^{-7}$</th>
<th>$10^{-8}$</th>
<th>Spore</th>
<th>$10^{-6}$</th>
<th>$10^{-7}$</th>
<th>$10^{-8}$</th>
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<tr>
<td>0</td>
<td>-</td>
<td>1405</td>
<td>219</td>
<td>120</td>
<td>54</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>1025</td>
<td>116</td>
<td>180</td>
<td>8</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>60</td>
<td>-</td>
<td>427</td>
<td>58</td>
<td>180</td>
<td>8</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>90</td>
<td>432</td>
<td>20</td>
<td>-</td>
<td>3.6%</td>
<td>0.1%</td>
<td>UV</td>
<td>-</td>
</tr>
</tbody>
</table>
This is after 2 days' incubation. The colonies on the 90 sec plates are very tiny, or hardly be counted. Further incubation of this and the 120 and 180 sec plates.

On the 90 sec 10^-2 plate there are three very large, creamy-white, one of which is angular and 1 mm in across. 5, one looks as though it arose from 4 cells stuck together. 5, and one which has 4 flagae and two central sections. 5. Both are stuck together.

After one more day, replicate plates to appropriate counts to confirm lengths for antibiotic.

Both were growing possible sparse from 171 + 5 plate streaked 5/10. And for resistance also whole 5 to determine length.

Please communicate the growth of the following very pure:

<table>
<thead>
<tr>
<th>ローンクエンス</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>3-5</td>
</tr>
<tr>
<td>2-4</td>
<td>4-6</td>
</tr>
<tr>
<td>5-7</td>
<td>7-9</td>
</tr>
</tbody>
</table>
UV killing curve shift.

Nine colonies have come up (3 days) on 70 sec plates, and some more slow on 120 sec plates.

\[ \begin{array}{ccc}
90 & 432 & 70 \\
120 & 56 & 2 \\
180 & 0 & 1 \\
\end{array} \]

Counts on 0-60 sec plates essentially unchanged.

Springs from banks of on 90 sec plate.

One type of colony from the large, white, colloidal -
granular, opaque, small 
induced only 2 layers, one smaller, darker, and another 
smaller, translucent.

Another colony, all individual col, granular, opaque, but main growth done underneath 
and form.

Some all the time, but not before for sure.

Possible an inhibitory factor inhibiting plates, may 
slowly growth up to 24 hour, visible growth 
across the plates.
Speed plate of 1 M + 5 mixture. M-type seems predominant; different to all except type apart. Centre reveals 3 mm. dish, and 1 mm. dish, with the one non-M plate.

90 sec. incubation - streaks from large colonies. 30-40
All except second (no. 2) show only one type of colony - usual large, gummy type. Streak from second col. - 8 type:
1) large, gummy
2) smaller, hand-sized
3) bright yellow, very rough, unattached (contaminated?)

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

Possible contaminant from mixture plate: Pick, spot on complete for replication to commercial.
5-16-54

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

Ab UV-1: Attempt to run strains specific growth requirements. At 24 hrs, growth only on YMA, not as pure a mix. More presumptive strain.

Cultures from section 1C, Ab UV plate:
1) "normal": Quite good growth for 24 hrs, and coloring reproducible as usual; colonies appear uniform.

2) yellow/2 col. were picked; one looked more "normal"; the other seems very weakly grown at 24 hrs. No color. LEML had yellow colony on and E M S plate which looked similar to this yellow culture at 3-4 days.

3) transparent: Good growth at 24 hrs. Colonies appear uniform. Very similar to "normal" but slightly less opaque.

Plates sent from UV irradiated 5/12: Replicated to AG-2.
Shake broth (AG - 1) 3:30 pm of all A. radiobacter cultures for microscopic examination. Inc. at room temp 5 minutes.

5-19-54

A6 UV-1 mutants: At 48 hrs, growth only in YNA salt + NaCl plates. Examine plates carefully. 

Before going into identity of requirement, melt out AAs + or NaCl.

Also I include A6-6 mutants to our AAs, YNA, and NaCl, S. Add a liquid NaCl 3 to sterile medium, verify directly from sterile stock come and also diluted 1:10 and 1:100.

A6 UV, cultures from control colony

1) "Normal": Locate a number of colonies (white or gray or the well-separated colonies on plates) more than all others of standard growth. This is the "pure" cardiac mutant and several colonies instead of clumps. It is the standard, are of this same" mutant factor. 

2) Duplicated

3) "Purified" stock
2) Translucent: Colonies are nearly distinguishable from the agar one month. No sub - 
Hence, no one of the three methods, very small
Transectural colonies of which I have seen 0.71
"A" type do remain old and (48 hrs)
true time at the peak to undergo asepsie and "A" type.
He cultures colonies of which I mention
"A" type in unchlorinated contents is rapid
growth, forming colonies 1 cm in diameter at 24 hrs.

3) Wilkins & Sutton (1996) in a curve are a comparison of x 24 hrs - 1936: (unpublished)
no peak in~~~~~~~~~~~~~~~

Possible answer: Observations of colonies after time
picked from one plate & transferred to another plate, +
and were picked from each of the 4 plates. Of
these, 2 from 71 and 1 from 134 were 84, prominent.
I think the expression "from CG-5, I only suspect that the bank was never opened" is not very satisfying, especially if it was liquid.

2) Analysis of ferrous iron from kerosene

  Fe+++ 5.8/mg
  Mn+++ 0.18/mg

High: 1000 ppm

I am not sure these figures refer to the solution in the tank (iron kerosene and MnSO4)

<table>
<thead>
<tr>
<th>Material</th>
<th>Fe+++</th>
<th>5.8 mg</th>
<th>Mn+++</th>
<th>0.18 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe+++</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn+++</td>
<td></td>
<td></td>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

5-20-85

4614.1 - T 26.1 days, Stilwell only. YNA.

- We attempted to keep the experiment going with the second control complete.

- We also ran another plate: experiment 6.

All live 26.1 days form a total of 7.
From the data, it is clear that G-1 R+R- is no longer grown.
No visible growth was observed, All the cultures from the selected strains (except the second) grew as we expected

Number of UV plates (G-1) is normal. 20 UV-1 (2 plates and 2 cultures) 20 UV-2 (2 plates and 2 cultures) 20 UV-3 (2 plates and 2 cultures) 20 UV-4 (2 plates and 2 cultures)
1) 247

The strain of A. stearothermophilus was obtained in 1947; it is resistant to 100,000 units of penicillin. A. stearothermophilus is able to tolerate 1000 units per ml. 5/21/54

Streptomycin-resistant strains were obtained in 1947; they are very resistant. Also present, 1000 units/ml. Inoculated E. coli, S. typhimurium, and A. stearothermophilus; 20% of the colonies (200/100) were resistant (200/100).

Inoculated bacteriologic media to see whether it can be cured normally.

20/21 - 20/31. No growth in any culture. A. stearothermophilus also.

1, 2, 3, and 4 failed at 24 hours on complete. 4, 5, and 6 survived.
Experiments from retarded colony (E 2 UV-1)
Saline col and sterile saline done 5/19, both from
retarded and non-retarded colonies. Third colony
is being called as 2 UV-1, so can be
checked against saline for retardation sensitivity
of any type.

Indicated agar culture 5/21 to deliver 1/07, 1007S,
and 1007R base of the experiment to work.
If so, can get more highly nutritive nutrients.

List stock numbers (final approximates)
A6-6 (1) = CG-7
A6-6 (2) = CG-8
AG UV-1 = CG-9 (YNA)

5/21/54

Results: (4 days), 1 day more Na+ has added.
CG-7 & CG-8: Plain growth in YNA, possibly a
higher Na+ same.
CG-9: Good growth in YNA, possibly Clienti
highest. Na+ same.
AY 94

Growth:  (Glycine and Mn + Fe added)

- CG-7 - very fair growth and AA in the YNA; slight at Na, Ca and Fe and 11/10.
- CG-9 - very slightly growth in YNA as well, no additional AA added.

(1087 was added before analyzing, so after maintaining media pH 1 ppm)

5/18

Plant Variations (now 2 weeks):

- Several AA show little or no loss of variation.

Spotted CG 7, 8, 9 and UV-1 the following 11/1 for one replication to supply plates.

(Note: Mineral plates now contain Mn + Fe)

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

5-29-84

- Spotted CG 1, 3, 7, 9 4 days ago to supplemented plates

Spent aerated one week cultures of CG-7 & 9 on SM and ST plates (SM 1/4 the normal amount of trace, ST 100 U/ml.)
5/31/54

<table>
<thead>
<tr>
<th>Rows: Numbers of copies (plates)</th>
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<tbody>
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<tr>
<td>CG 1</td>
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<td>Y</td>
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<td>5</td>
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<td>6</td>
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</tbody>
</table>

All new construction is complete.

Controls are some of these results by reassembling the plates from the original.

Control inoculation: For 2UV 4, 5, 6 try A4, put 0.1 mln mass, A8, 10 ml, 30 min.

Check CG 1 and 2UV 143 and bacterial controls may be necessary to see. They are again the same, but may not be identical.

ST and SS4 spots:

CG 2: Seminiferous floor, background growth and holes, no nodules visible.