

E. D. 2/6/60
 11/1/61
 11/1/61

A1
 (Agrobacterium)

Cultures

- | | | | | |
|----------------------|-----------------------|---|--|----------|
| CG-1 | <u>A. tumefaciens</u> | AG - virulent | } Ribier | |
| CG-2 | <u>A. tumefaciens</u> | AG-6 - attenuated | | |
| " | " | AG PEN penicillin resist. | } Klemmer
(see PA1a) | |
| " | " | AG P5a polyamycin resist. | | |
| " | " | AG C5 chloramphenicol resist. | | |
| CG-3 | " | AG-S ² | } probably identical } #A5 ff | |
| CG-4 | " | AG-S ² | | |
| CG-5 | " | AG-6-S ² | | |
| CG-6 | " | AG-6-S ² | | |
| | <u>A. radiobacter</u> | 1001 | | } Ribier |
| | " | 1005 | | |
| | " | 1007 | | |
| CG-7 | <u>A. tumefaciens</u> | } spores as a staple from CG-2 | | |
| CG-8 | <u>A. tumefaciens</u> | } would require (?) sub-identical | | |
| CG-9 | " | } suitable from CG-1-B, UV; ^{genotype} YVA | | |
| CG-10- ¹⁵ | <u>A. tumefaciens</u> | from CG-1-S, UV. | Ribier ceptos, not run down | |
| CG-16 | <u>A. tumefaciens</u> | " " " | Biotin - | |

Antibiotic resistant cultures of *Agrobacterium tumefaciens*

Resistant cultures were developed to five antibiotics from a sub-culture (A6K/6) 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 Joyln & Stahlbratt, J. Bact., 59 (1950)], using a 50% inhibition in broth, approx. 8 hour endpoint. The 50% inhibition levels (I.D.50's) of the A6 strain, and, where known, the 100% levels are given below in the table

Culture resistant to	Level of resistance $\mu\text{g/ml}$	% loss of virulence	Parent A6 culture - inhibited at ($\mu\text{g/ml}$)	
			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 Maksman

<u>Medium 79</u>	AGI	<u>Modification I (DCG)</u>	
Mannitol	5.0 g	Sucrose	5.0 g
MgSO ₄ ·7H ₂ O	0.2 g (anhyd. 0.1 g)	MgSO ₄ ·7H ₂ O	0.2
K ₂ HPO ₄	0.2 g	K ₂ HPO 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, CaCO₃ & agar could be omitted. Then medium 5 agar or CaCO₃ could be used as complete broth; 2 agar but 5 CaCO₃ as complete medium for plating. Also omit sugar from mix (Sucrose ok for crown gall org. according to Dr. Pinner, mannitol used for Abryobium.)

II. Minimal medium used in glycemic attenuation experiments (van der Meer, Halderon, & Pinner, J. Bact. 63, 715-721 (1952)).

SM |
ST |
Amidure blue |

AG II



- Mannitol 5.0 g
- KNO₃ 5.0 g
- CaCl₂ 0.19 *reduce to 0.1*
- NaCl 0.2 *see p 33*
- K₂HPO₄ 0.2
- KH₂PO₄ 0.1
- MgSO₄·7H₂O 0.2
- Distilled water to 1 l.
- Adjust pH to 6.8.

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

Sterilize sugar separately?

McIntire, Baker, and Peterson (1941) found
traces of Mn, Fe⁺⁺, and 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 starts. Tested pH of mix + 0.5% sucrose (before adding CaCO_3 or agar).
 pH = 6.78 \approx adjustment.

Possible approaches: -

1. Glycine

A. Repeat glycine attenuation using A6. This would at least serve to give a second overland strain.

B. Plate on glycine to get glycine-resistant col. (Could this be used as a marker?), von Janung, Baldwin, & Sizer (1952, p 717) indicate that glycine-resistant clones are of normal overland - This argues against "glycine attenuation" being a selection of cells which are glycine-resistant and overland. [But see tryptophan paper - or what] [more sensitive than overland]

2. Markers other than overland

A. Antibiotic resistance. Cultures on hand resistant to penicillin, chloramphenicol, and polymyxin. Try to get an S^{12} ; 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 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 1ml grow culture into complete medium.
(Penassay for col.)
Add antifoam, aerate 3-3½ hrs.
3. Cfg 10 minutes, wash in saline.
Resuspend in 10ml saline, cfg 20 min.
Wash pellet, resuspend in 10ml saline
4. Add 0.1ml suspension to each of 2 DO tubes
Add 0.3ml penicillin soln to one tube (Other inoculated)
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 minimum.

1. $\frac{1}{2}$ L. strain, used previously, quality survival of 10^{-6}
or more (Meth. Med. Res.)

Variables checked:

Gen. conc.

Age of inoculum

Inc. time in pre-inoculum 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;

pon on D-O.

Try one of the regular Agar media
Growth more uniform in NSB than in Penassay.
More clumpy in latter.

Penicillin run

1. Inoc. fresh Penassays from those since 8-24.
(1ml inoc/10ml Penassay tube). (Using
Penassay rather than NSB because it is a more
complete medium).

Streptomycin

Spread 2 drops Penassay culture ^{AG 1 & 2} on B. Lee say plates
to look for S²'s

Sealed stabs of AG & AG-6

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

3-30-54

Made up AG II minimal liquid

Regulated scale incubator at 213 to 26° and mixed streptomycin plates tomorrow.

Pen run

2. Penicillin tubes inoc. 3-29 fairly well grown; aerated at incubator (penicillin 9:00 AM) to see if it would help growth.

Try repeat both for next pen run (AG I liquid)

Make up AG I and AG II plates.

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

Pen run

3. 1:30 pm. 1/2 of cultures, though they are not as well grown as I would expect at corresponding step. 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 leanest & most rapid growth in complete medium?

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

Inoculate each tube \bar{c} 0.1 ml old Penassay culture. Inoc. 10:00 AM 3-31.

Streptomyces

Plates inoc'd with broth culture 3-29:

papillae, probably S^r, against background of very scanty growth. Number per plate ranges from ca. 6 to ca. 20. ⁵⁰⁻²⁰⁰ papillae per plate

Picked 12 papillae each of A6 & A6-6; streaked from small waters onto 5 ml SM.

If these appear to be S^r, pick single colonies, streak out complete 5 SM; separate to SM medium.

4-1-54

Paracoccus run

Both parallel and control tubes turbid. In next run plate at 24 hours even if growth in control tube is very scanty.

Comparison of complete media - 24 hr growth

Parassay at 26° - poor

AGI at 26° - a little better than Parassay

AGI aerated at room temp - very good growth, especially of A6. Considerably more turbid than AGI tubes incubated at 26° & aerated for 46 hrs

Use aerated tubes to start second parallel run

4-2-54

Second par run

Good growth in control and par tubes in 24 hrs.

- 1) For next run, use at least 1000 U per ml
- 2) Spread dilutions from old par assay tubes directly on AGI plates & replicate to AGI. These old cultures have accumulated many S^{rv} 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} $\xrightarrow{1 \text{ ml}}$ 10^{-5} $\xrightarrow{0.1 \text{ plate}}$ 10^{-6}

↓ 0.1

10 ml → 10^{-6} $\xrightarrow{0.1 \text{ plate}}$ 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 & SM.

Spread plates - wait for sub. to increase until Sun or
Mon. - no visible growth at 24 hrs.

Streptococcus -

AG - Streaks from all 12 wells showed now
well grown on K and SM.

AG-6 - Several of 12 well grown; 4 more (3 deep)
showing rather scanty growth in shallow part
of streak (S^R mutations?)

Pick colonies from 2 streaks from AG-6; streak
on AG I plate & SM.

4-5-54 (Monday)

Streptomyces

Replicated S^R cultures streaked on AG I to AGISM.

Spread plates from old necessary cultures (2-4 \times each 10^{-7})

A6 - Only 15-20 col per each 10^6 plate. Plates muddy. Pick all individual colonies, spot on AG I & AG II. (Have to take too long to spot on complete, then replicate to minimal - 2-3 days required at each step)

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

Pericillium

Inoculate AG I bottles from old necessary cultures. Also inoculate bottles of new slants to use as "stock".

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

How many mutant streaks? Using prefix A would lead to duplication. Will use CG for crown gall. A6 = CG 1; A6-6 = CG 2. Will not

give CG numbers to Klammer'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 grown, one more. Looks
as though it was not picked up by velvet; however,
pick & test to be sure. Spot on men. & complete
plates = A6 cultures being tested.

Check on
markers of

Streptomycin -

Saved 2 S² each of A6 & A6-6

CG 3, CG 4 CG 5, CG 6

AGI slants & nutrient slabs.

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. (Replied to plate spread on penicillin?). Then try pen run again.

4-8-54

(A6-6)
Penicillin run - 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 SR cultures used for SRP type experiments.

4-9-54

A6-6 penicillin run NOTE: A second type of colony has appeared on the A6-6 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 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 in diluted culture in penicillin treatment. (See pp A7 ff). However, in streptomycin experiment, many colonies appeared late on SAM plates, after colonies had already been picked which were saved as S₂ stocks. Well penicillin in 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

Unfortunately, the old broth culture used to start this run has been discarded in broth inoc. 4-5.

or complete than on mineral?)

Take the following steps:

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

Arbitrarily,

Early growth on complete, slow on mineral = a

Late on complete, good on mineral = b

3) Save both types

4) Use all inocula and mineral as a control

5" pen run in Inoc 2nd 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 over crowded for picking.

4-10-54.

MAKE UP AG-I plates ✓

Recultures run on AG-6

All "type a" col. seem an ochraceous - marginal
are minimal at 48 hrs.

Make up round new plates; test the colonies picked
yesterday (ca 20 col) Save any plates.

For round ones, am using AA groups, YE and
undisturbed as usual.

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

Streaks on mini & complete; (about 20 hours)

complete AG - very scanty - single colonies barely visible.

AG-6 - slightly better than AG

(these were streaked from AG (colts))

"Type a" - Growth good plenty - individual
colonies large enough so they could easily be
picked.

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

Memorial

AG - very scanty

AG-6 slightly better than AG, but very scanty

"Type a" - no growth

"Type b" - barely visible

Plated penum \approx CG 3 & CG 5. Used 1000 μ ml

AG streaked for test on penicillin - not many isolated colonies - also no plate available to which to add penicillin. Pick several colonies to small Removays & test with penicillin.

Save a "Type b" AG-6 colony as stock

4-11-54

CG 3 OK for plating - CG 5 given me
per tube. Plated 0.1 ml of 1:10 & 1:100 CG 5

4-12-54

CG 3 plating - no growth at 48 hrs. Try plating direct from penicillin tubes. (Use AGI, no AGISBY available)
No turbidity in penicillin at 48 hrs.

Streaks on minimal & 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 penicillin 1. Summary of colony
morphology; looks more like the translucent
than the dense type in the 6. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100. 101. 102. 103. 104. 105. 106. 107. 108. 109. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120. 121. 122. 123. 124. 125. 126. 127. 128. 129. 130. 131. 132. 133. 134. 135. 136. 137. 138. 139. 140. 141. 142. 143. 144. 145. 146. 147. 148. 149. 150. 151. 152. 153. 154. 155. 156. 157. 158. 159. 160. 161. 162. 163. 164. 165. 166. 167. 168. 169. 170. 171. 172. 173. 174. 175. 176. 177. 178. 179. 180. 181. 182. 183. 184. 185. 186. 187. 188. 189. 190. 191. 192. 193. 194. 195. 196. 197. 198. 199. 200. 201. 202. 203. 204. 205. 206. 207. 208. 209. 210. 211. 212. 213. 214. 215. 216. 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1014. 1015. 1016. 1017. 1018. 1019. 1020. 1021. 1022. 1023. 1024. 1025. 1026. 1027. 1028. 1029. 1030. 1031. 1032. 1033. 1034. 1035. 1036. 1037. 1038. 1039. 1040. 1041. 1042. 1043. 1044. 1045. 1046. 1047. 1048. 1049. 1050. 1051. 1052. 1053. 1054. 1055. 1056. 1057. 1058. 1059. 1060. 1061. 1062. 1063. 1064. 1065. 1066. 1067. 1068. 1069. 1070. 1071. 1072. 1073. 1074. 1075. 1076. 1077. 1078. 1079. 1080. 1081. 1082. 1083. 1084. 1085. 1086. 1087. 1088. 1089. 1090. 1091. 1092. 1093. 1094. 1095. 1096. 1097. 1098. 1099. 1100. 1101. 1102. 1103. 1104. 1105. 1106. 1107. 1108. 1109. 1110. 1111. 1112. 1113. 1114. 1115. 1116. 1117. 1118. 1119. 1120. 1121. 1122. 1123. 1124. 1125. 1126. 1127. 1128. 1129. 1130. 1131. 1132. 1133. 1134. 1135. 1136. 1137. 1138. 1139. 1140. 1141. 1142. 1143. 1144. 1145. 1146. 1147. 1148. 1149. 1150. 1151. 1152. 1153. 1154. 1155. 1156. 1157. 1158. 1159. 1160. 1161. 1162. 1163. 1164. 1165. 1166. 1167. 1168. 1169. 1170. 1171. 1172. 1173. 1174. 1175. 1176. 1177. 1178. 1179. 1180. 1181. 1182. 1183. 1184. 1185. 1186. 1187. 1188. 1189. 1190. 1191. 1192. 1193. 1194. 1195. 1196. 1197. 1198. 1199. 1200. 1201. 1202. 1203. 1204. 1205. 1206. 1207. 1208. 1209. 1210. 1211. 1212. 1213. 1214. 1215. 1216. 1217. 1218. 1219. 1220. 1221. 1222. 1223. 1224. 1225. 1226. 1227. 1228. 1229. 1230. 1231. 1232. 1233. 1234. 1235. 1236. 1237. 1238. 1239. 1240. 1241. 1242. 1243. 1244. 1245. 1246. 1247. 1248. 1249. 1250. 1251. 1252. 1253. 1254. 1255. 1256. 1257. 1258. 1259. 1260. 1261. 1262. 1263. 1264. 1265. 1266. 1267. 1268. 1269. 1270. 1271. 1272. 1273. 1274. 1275. 1276. 1277. 1278. 1279. 1280. 1281. 1282. 1283. 1284. 1285. 1286. 1287. 1288. 1289. 1290. 1291. 1292. 1293. 1294. 1295. 1296. 1297. 1298. 1299. 1300. 1301. 1302. 1303. 1304. 1305. 1306. 1307. 1308. 1309. 1310. 1311. 1312. 1313. 1314. 1315. 1316. 1317. 1318. 1319. 1320. 1321. 1322. 1323. 1324. 1325. 1326. 1327. 1328. 1329. 1330. 1331. 1332. 1333. 1334. 1335. 1336. 1337. 1338. 1339. 1340. 1341. 1342. 1343. 1344. 1345. 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2010. 2011. 2012. 2013. 2014. 2015. 2016. 2017. 2018. 2019. 2020. 2021. 2022. 2023. 2024. 2025. 2026. 2027. 2028. 2029. 2030. 2031. 2032. 2033. 2034. 2035. 2036. 2037. 2038. 2039. 2040. 2041. 2042. 2043. 2044. 2045. 2046. 2047. 2048. 2049. 2050. 2051. 2052. 2053. 2054. 2055. 2056. 2057. 2058. 2059. 2060. 2061. 2062. 2063. 2064. 2065. 2066. 2067. 2068. 2069. 2070. 2071. 2072. 2073. 2074. 2075. 2076. 2077. 2078. 2079. 2080. 2081. 2082. 2083. 2084. 2085. 2086. 2087. 2088. 2089. 2090. 2091. 2092. 2093. 2094. 2095. 2096. 2097. 2098. 2099. 2100. 2101. 2102. 2103. 2104. 2105. 2106. 2107. 2108. 2109. 2110. 2111. 2112. 2113. 2114. 2115. 2116. 2117. 2118. 2119. 2120. 2121. 2122. 2123. 2124. 2125. 2126. 2127. 2128. 2129. 2130. 2131. 2132. 2133. 2134. 2135. 2136. 2137. 2138. 2139. 2140. 2141. 2142. 2143. 2144. 2145. 2146. 2147. 2148. 2149. 2150. 2151. 2152. 2153. 2154. 2155. 2156. 2157. 2158. 2159. 2160. 2161. 2162. 2163. 2164. 2165. 2166. 2167. 2168. 2169. 2170. 2171. 2172. 2173. 2174. 2175. 2176. 2177. 2178. 2179. 2180. 2181. 2182. 2183. 2184. 2185. 2186. 2187. 2188. 2189. 2190. 2191. 2192. 2193. 2194. 2195. 2196. 2197. 2198. 2199. 2200. 2201. 2202. 2203. 2204. 2205. 2206. 2207. 2208. 2209. 2210. 2211. 2212. 2213. 2214.

Auxotrophs replicated to roundabouts: No growth on AA's except for 4 poor variants or slow growers. Scanty growth on YEX and long-term casein. Try penicillin and vit. From how Gordon's experiment - also try reduced sulfur.

4-13-54

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

Pen. run as CG3 - colonies present at 48 hrs. in appearance - the col. appearing at 48 hrs in previous pen exp. Ca 50/plate from 1:100 dilution. This corresponds to nos. in AG-6 exp. Replicate to mineral. This is Sr deriv. of 46.

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

4-14-54

AB-6 auxotroph - no growth at 20 hours in minimal
+ YNA or minimal + vits. Incubate at least one
more day.

To summarize:

Poor growth in hydrolyzed casein

Trace of growth in yeast extract

No growth in any AA group

" " (24 hrs) in YNA or vits.

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

CG 3 penicillin -

Colonies mucoid, not large enough to score. No

further colonies are complete beyond no. at 48 hrs.

These colonies all are the small "type 4" (27 rough?)

It seems distinct that each culture is mucoid (AB & AB-6) or
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 "rugged" survivors of a penicillin run were either autotrophic or very slow in their growth on minimal.
- 4) CG3 (A65²) is "rough"

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

Also, streak A6 + A6-6 both types, on AGIM. Compare to CG3 + CG5 probably streaked.

4-15-54

CG3 pen run: One possible autotroph. Inoc. tubes of minimal & complete.

A6-6 autotroph: inoc. with 100 into 2 YNA at 48 hrs. Try single cross-stains to AA groups.

4-16-54

CG3 possible autotroph: at ca 20 hours, growth in complete, none in minimal. Inoc. sl. 1; containing inoculation of bottles.

Ab-6 auxo (sl. 1#1) - AA single omissions of groups
Growth in -AA4; also in hydrolyzed casein.

Test 2: HC + AA+

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+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 streaks on complete medium

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

A few colonies smaller, white, translucent

Ab-6 original (CG-2): Moderate size & dense, resistant
centers. Seems homogeneous

AG "R" : Small, white; mass growth looks rough

AG "M" : Large, \bar{c} yellowish centers. Gummy.

AG-6 "R" : Like AG "R"

AG-6 "M" : Like AG "M"

Small (prototrophic) from AG-6 penicillin; Small but not rough. Centers yellowish.

Large (auxotrophic) from AG-6 penicillin; Colonies not very large. But more yellow & gummy than AG-6 "M".

AG PEN (Klammer) } Much like AG "M"
 AG P-5 " " }
 AG C5 " " }

CG 3 (AG S^r) - Seems intermediate between AG "R" & AG "M"; colonies somewhat yellowish, but mass growth looks slightly rough

CG 4 (AG S^r) - Both large & small colonies; large have yellowish centers, Mass growth smooth, quite gummy.

CG 5 (AG-6 S^r) - Like CG-3.

CG 6 (AG-6 S^r) - Like AG-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 - As on AG-I SM except colonies are
smaller. This could be because medium is
rather old.

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

Cross-brushing to penicillin (loop of 10,000 CFU
solid); Pen. conc. apparently insufficient for
any definite effect.

Brush of A6-6 autotroph from penicillin looks so
yellow compared to other cultures that it seems
wise to try to make sure it really is

A. tumefaciens.

4-17-54

CG-3 possible autotroph; at 2 days, growth in
minimal. So far (24 hrs) no growth in minimal
or in complete - i.e. at same time (inoculum probably
very small). Streak from 48 hr complete into
complete plate & try several individual colonies

before discarding as no yield.

Ab-6 as autoph:

Original single-omission tubes (now 2 days):
 Good growth in -AA+; poor growth in
 hydrolyd casein; near slight growth in
 -AA 5. Still no growth in other
 single omissions or in minimal.

Subculture 4-16 (24 hrs)

HC	+	complete	++
HC+A4	+	AG II-0	-
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 autotroph: 3 days: Slight
 growth in all tubes, but good growth only in AAS.
 Will pick & test several col. from plate streaked 4-17
 in men, complete, & AAS.

AB-6 auxo; original single omission tubes, now
 4 days; there is some growth in all tubes except
 minimal and -AA1 (includes small amount),
 Sulfide??

Second run done test (now 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; also K_2S

4-20-54

CG-3 picked from separate colonies

At 24 hrs, no growth in any of the auxo + K_2S
 still turbidity and complete, faint red coloration

AB-6 auxo auxo growth in A1 (Why didn't this happen
 on plate?) but not in individual AAs of the group or K_2S .
 Try single omissions auxo auxo & growth at high conc.

4-21-54

AB-6 auxotroph At ca 24 hrs, no growth in A1 single
 omissions or in A1. (~~Can't make random set and not
 a suitable group checked total of 1/2 the used, not
 acid~~)

Original A1 medium (2 days)
 A1 group - quite good growth.
 Slight cloud of turbidity and mottle, most of No. 2.
 Inoculated at 1st time day.

CG-3 "auxotroph" from single colonies, growth equally
 good in mix & mix + AS. T.O.

4-23-54

AB-6 auxotroph; At 3 days, moderately good
 growth in AA1 - arginine & - lysine. Poor growth
 in AA1 complete group. No growth in AA1 - meth
 a - cpl. Bill. Try sulfide again.

4-24-54

J.L. reports that Klett's results were due to water content
 & cannot be repeated. Hence, there can be less
 explanation on transfer of water - turbidity
 can be considered just another marker.

Try first time in medium use AG only, see if
it is not necessary to have auxotrophs in both
AG 4 & 6. Use AG R & AG M to see if any
difference in behavior in penicillin.

Inoculate both of the following

AG "8" } independent mixed
AG "9" }

A. radiobacter 1001 } to be compared with
" " 1005 }
" " 1007 }

4-27-54

Broths inc. 426: Moderately good growth in all. The
"rough" AG 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

Sticks of A. radiobacter (48 hrs):

1001 : 2 types of colonies: ① small
② large & runny.

1005 : like another uniform colony of ②
& runny.

1007 : 2 types, same as 1001.

Stick 3 stick of both types active present.
Also pick 8 stick 1 stick of 1005 to have
stick arising from single colony.

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

Inoculate bottles for procedure on A6 'R' & 'M'.
Acid.

4-30-54

Started and run on A6 'R' & 'M'. 100 U/ml peroxidase

5-1-54

Both grew in pen tubes.

Hendrickson, A.A., Baldwin, I.L., and Piker, A.S. 1954 Studies on certain physiological characters of *Streptococcus hemolyticus*, *S. rheginus* and *Bacillus anthracis*. Part II. J. Bact. 58, 547

5-3-54

Absorption of radiation line as a marker.

Start broth (no. 161) 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 old 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.

(to killing curve when more plates available)

5-6-54

Irradiation exp. 40 sec. UV gave noticeable killing. 20 plates after irradiation too cloudy 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 at 24 hrs and is large, yellow, & gummy like previous autotrophs. Strain

out to test.

5-7-54

Struck from col picked from UV plate grow well on plate in 24 hrs. Pick one col. to move to complete bottle.

5-8-54

at about 20 hrs, good growth of "auxotroph" in complete, more in minimal

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

"R" and "M" cultures of A6 & A6-6
S² from A6

Also - spot A6-6 S² with A6 "M", recover S² to test. Do control plating of A6 "M" to check rate of mutation to S².

CG-1-M

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

5-10-54

CG-1-M

Inoc A6-J both to A6 "M" & CG-5 separately

UV autograph. No growth on material in
3 days. Same, see above. AG UV-1

Cultures to use for plant inoculation:

- 1) S² from mixture expl.
- 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) ~~AG UV-1~~ CG-5

Strained mixed culture on AGEI & AGI SM

[At 48 hrs. these were streaked on both complete & complete + SM at ca. 48 hrs. in complete medium at same temperature without aeration.]

5-11-54

Save recultures of AG UV-1 (tubes).

Started growth of AG for UV testing above.

In mixture experiment, should have spread plates & 5 SM c appropriate dilution, rather than streaking, to get estimate of proportion of S² to S¹. Start same 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 there
a virulent, 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 $\bar{c} + \bar{s}$ dilution of
2 day non aerated broth cultures of CG-1-M and
CG-5.

5-13-54

(48 hrs)

AG UV-1 sundowns: fair growth in mini. + YNA; none
in other additions, but slight turbidity in minimal;
Beckie. Also check parvines & pyramidenis (as 2 groups)

Mixture streaked 5-10 on complete & 5 SMA:

Complete; no SMA: Colonies predominantly "M" type;
some "R" type. Pick a number of both
types, test for S character. ~~There are a~~
~~few of the large, common colonies which~~
~~have always turned out to be auxotrophs~~
~~Pick + test.~~ Don't know whether 1-M or 5

Complete + SMA: Great majority of colonies are
the "R" type, like the S² 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. Use both
in overabundant 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).

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

Spread AG-1⁴ & AG-1 SM plates & 10^{-5} dil. of 1-M+5 mixture into both 5-11.

5-14-54

AG-1 remains: Still no growth (3 days) except in Y11⁴, and for some reason, minimal.

Set up: Minimal, YNA, peptones, pyrimidines

Plates of CG-1-M and CG-5 used 5-12;
 (Spinal 0.1 ml 10^{-4} inoculum of 2 day,
 unacrated broth)

CG-1-M; low complete inhibition of growth
 size stable, clusters of 10^6 cells
 low ST, no colonies.

CG-5; low complete inhibition of growth
 and less common than CG-1-M. 997 col.
 low ST, also some mixed colonies, but
 much smaller. Lack of growth in
 may be too high for the medium.

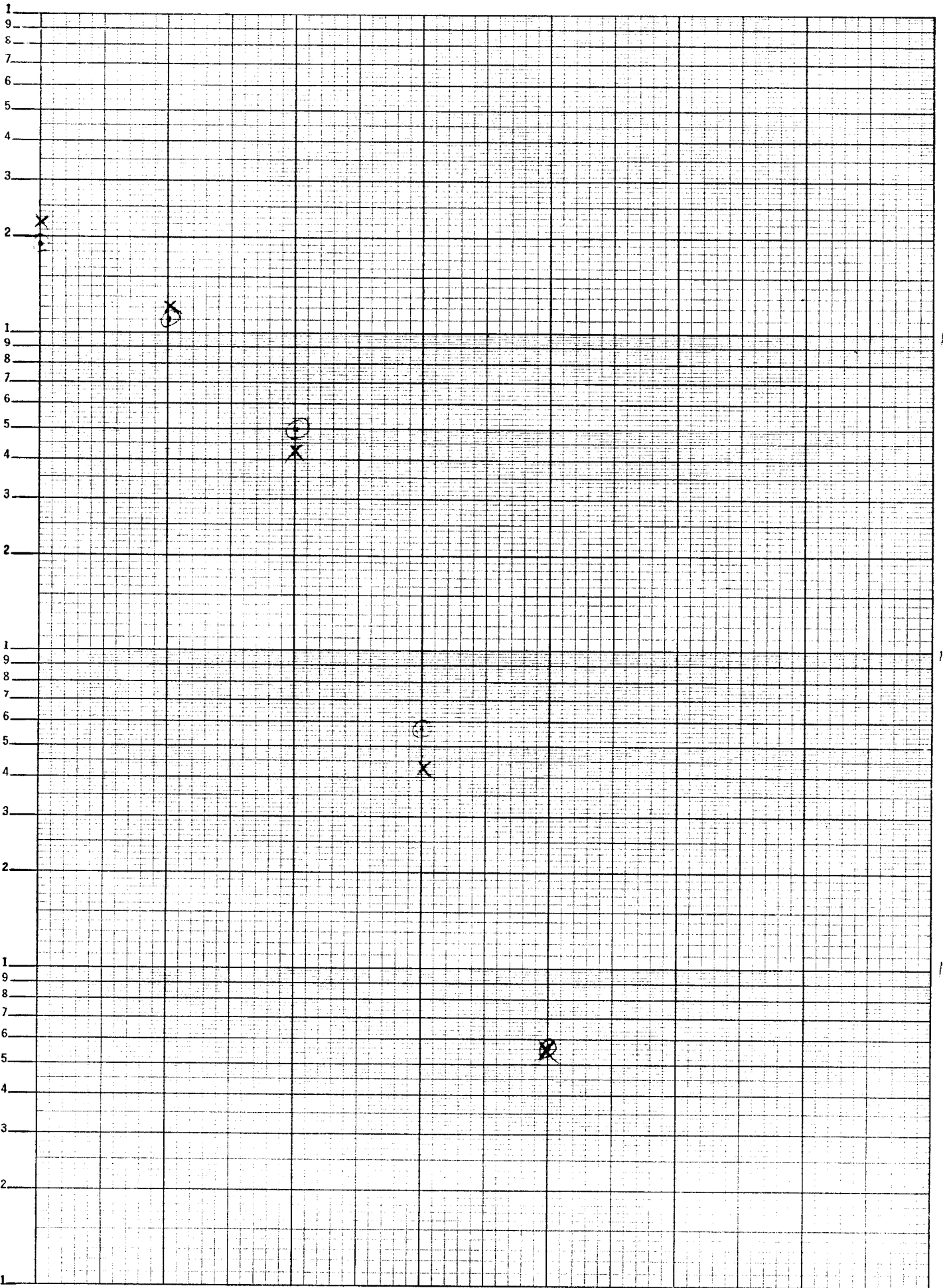
Colony types should be quite stable; on
 each AG-1 plate there is one colony which
 looks although it might be of the opposite
 colony type (1 "R" on 1-M plate; 1 "M" on
 5 plate)

UV experiment

Exposure	10^{-6}	10^{-7}	10^{-8}	Exposure	10^{-6}	10^{-7}	10^{-8}
0	—	1465	219	120	54	2	—
30	—	1025	116	180	0	0	—
60	—	417	58				
90	432 165	70 20	—	3 days unacrated			

KEUFFEL & ESSER CO., N. Y., NO. 356-81
Semi-Logarithmic, 4 Cycles X 10 to the inch, 5th lines accented

MADE IN U. S. A.



10

10¹⁰

10¹

10⁴

10⁷

0.0010 0.01 0.1 1 10 100 1000 10000

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 swimming colonies, one of which is regular and dense across \bigcirc , one looks as though it arose from 4 cells stuck together ⊗ , and one which has opaque and transparent sectors ⊗ . Pick a streak from these.

After one more day, replicate plates in appropriate areas to see if it checks for auxotrophy.

Pick 100% swimming possible areas from H1 + 5 plate streaked 5/10. Test for auxotrophy also whether S^2 to determine domain.

Plant inoculations: Growth of the following very poor;
Desmodium illinoense small: 1-R 3 5 XM2 XM4
Staph. pl. G. d. black 2-R 4 XM1 XM3 XR1
 2-R 2-R XR2

5-15-54

UV killing curve exp.

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

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

Streaks from large col on 90 sec plate

One type of colony from the large, ^① rounded col - spreading, diffuse

Centred col. 2 types, one as above, the other small & translucent. ^②

Irregular col: all individual col spreading & opaque; but mass growth does not look uniform.

Save all these, test col. types for anisotropy

Possible anisotropy from mixture plate: very scanty growth in 24 hrs, could prove as cups on exp. plate.

Spread plate of 1-M + 5 mixture M-type seems predominant, difficult to tell the two types apart. Continue incubation. (85)

One S41 plate All col. very small, considerably lower count than one non-S41 plate. (281)

5-17-54

90 sec. irradiation; streaks from large colonies; (non-3 days)

All except sectorid (#2) show only one type of colony - usual large gummy type.

Streak from sectorid 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 aerotrophs from mixture plate: Pick, spot on complete for replication to minimal.

5-18-54

Possible auxotrophs from mixture expt; Duplicate from AG-1 to AG-24 AG-1-519 to check for auxotrophy and to tell whether CG-1-19 or CG-5.

AG UV-1; Attempt to run down specific growth requirements; At 24 hrs, growth only on YNA, not in peptone mix nor peptone dense 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; one looks same as "normal"; the other shows very scanty growth at 24 hrs. No color. [EML had yellow colonies 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.

Streaked broths (AG-1) 3:30 pm of all A. radiobacter cultures for microscope examination. Inc. at same temp 5 aeration.

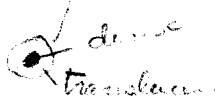
5-19-54

AG UV-1 medium. At 48 hrs, growth only in YNA; not in peptic or casein medium.

Before going into identity of requirement, include on AA's + on Na₂S.

Also include AG-6 mutants on AA's, YNA, and Na₂S. In adding Na₂S to sterile medium, was directly from sterile stock solution and also diluted 1:10 and 1:100.

AG UV, cultures from streaked colony

(1) "Normal". Quite a number of colonies (about a quarter of the well separated colonies on plate) now show small patches of transverse growth. There is no "pure" transverse colony and several colonies, instead of being streaked, are of this "normal" form. 

Peak: (1) translucent

(2) streaked (several)

(3) "pure" type; streaked out.

- 2) Translucent: Colonies are scarcely distinguishable from the opaque "normal". No scales.
 However, on one of the three streaks, very small translucent colonies of what I judge to be the "R" type have now app. and (48 hrs).
 From this streak, pick & streak clonally and "R" type.
 The opaque colonies & their "normal" "M" type are characterized by their rapid growth, forming colonies 1.2 mm in diameter in 24 hrs.

- 3) Yellow: The yellow colonies are also growing in a confluent mat - They have a ^(very faint) yellowish a peculiar swirled appearance. No scales are appearing on this plate (48 hrs). The colonies present at 24 hrs (see 5/10) are also many are sectored as described under D.

Streak CG-1 (R) for comparison to CG-2.

Possible origin of mutation plate after 48 hrs. 4 picked from orig. plate & streaked on complete, 4 more were picked from each of the 4 streaks. Of these, 2 from #1 and 1 from #4 are S^r, presumably

indicating that they originated from CG-5. I don't
know whether any are anastrophs so far it appears
not. In a small culture repl. to several parts.

Present medium does not seem very satisfactory,
especially if it is liquid

Try: 1) liquid or aerobically

2) Addition of ferric ions (see source
(Medullary tissue - Peterson, 1941)

Fe⁺⁺⁺ 5.8 mg/ml

Mn⁺⁺ 0.18 mg/ml

Check whether these conc. refer to
the vol. of the salt (some toward MnSO₄)

Med of MnCl₂ · 4H₂O soln 1.24 mg/ml

Fe₂(SO₄)₃ soln 72.0 mg/ml

Use full 1:200
(Both should be available;
avoid pH. mostly for use)

5-20-54

Ab 14-1 ... 3 days Still growth only on YNA.

... of Ab 14-1 ... growth
also 20 hrs except in complete.

... from mixture plate ...

Ab ... from a liquid ...
Streak ... 5/19 ...
... (24 hrs).

From "band 1" streak on 5/17 plate, what I called "R" type colonies yesterday are actually the yellow enteric 1.

CG-1-R & M streaked for comparison

No visible growth on A5-1 on 24 hrs.
All the descendants from the second colony (except the contaminant) grew up with colonies

Repetition of UV plates (Sept 2) to see results

Found possible auxotrophs on only 2 plates

Six suspected auxos; 3 are the large colonies which appeared at 24 hrs.

1. → Check all derivs. from second colony see if all are auxos. Pick up other 2 col.

Other 3 possible auxos were noted as being of dense colonial appearance - small, less dense than "normal".

Numbering:

2 UV-1 (≠ 1 from 2nd identification)

Up one, non-sectored from 24 hrs. col.

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

2 UV-3 }

2 UV-4 } small col from 90 sec plate

-5

-6

Stain slide of *A. radiobacter* with phase scope:
 2 day, full medium, 4000x, diluted 1:20,
 examined under phase.

↑ 1001 S IR 5 75 7R

Very good and clear for strain. Except for 1007R,
 the same as other isolates of any kind; 1007R had
 a few clumps. Mobility, poor.

100 U/ml

Streptococcal resistant strains were obtained in 1947;
 could be used as marker. Also prepared, 1000 U/ml
 Inj. cells, SA1, ST resistant and Ab-6 ampres;
 ABIV-1, new ampres (2 UV series)
 Try cross breeding SA1 to see whether it can be
 secondarily.

5/21/54

hundreds of Ab-6 ampres and ABIV-1: No growth
 in any supplement (48 hrs). Doubt if any
 double mutants. This is the first time.

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

Derivatives from selected colony ($\approx 2 UV-1$):

Selected colonies streaked done 5/19, both from selected and non-selected colonies. This culture is being saved as $2 UV-1$, so can be checked again later for continued stability of colony type.

Inoculated soft agar tubes \pm to Strickland 1007S, 1007R, and 1007K to see if they will grow; if so, can get more highly variable cultures.

Have stock numbers to find as yet

AG-6 (1) = CG-7 } sample?
AG-6 (2) = CG-8 }
AGUV-1 = CG-9 (YNA)

5/22/54

Incubation: (4 days; 1 day since Mn & Fe added)

CG-7 & CG-8: faint growth in YNA, possibly in highest Na_2S conc.

CG-9: good growth in YNA; possibly stable in highest Na_2S conc.

5/22/54

Overruns (6 days since Mn & Fe added)

CG-1 - Fair growth in AA-1; no growth in YNA; slight in Na₂S org. conc. and 1:10.

CG-9 - Possibly slight growth in YNA in a drifting slot.

(The Mn & Fe was added before autoclaving, & after autoclaving had a flocculent ppt.)

5/28

Plant inoculations (now 2 weeks):

Balls on all plants since 2 CG-1-M, CG-1-R, CG-3, CG-4. 5th day. All show little or no loss of verucence.

Spotted CG 7, 8, & 9 and 2 UV-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 & 2 UV-1 to supplemented plates

Spread aerated over night cultures of CG 7 & 9 on SM and ST plates (SM 1/4 the conc. used in ^{YNA} _{0.05% (2/ml)} ~~media~~ ^{media}); ST 100 U/ml.)

5/31/54

Reinoculations of auxes (plates)

	<u>O</u>	<u>A1</u>	<u>A2</u>	<u>A3</u>	<u>A4</u>	<u>A5</u>	<u>HC</u>	<u>VITS</u>	<u>YNA</u>	<u>Sulfide</u>
CG 7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	+	+	+	+	±	+	-	-	+	+
2UV 1	+	+	+	±	±	+	+	-	+	+
2	-	-	-	+	+	±	+	-	-	-
3	+	+	+	+	+	±	+	-	+	+
4	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-

All done in replication to complete.

Can only get a sense of these results by assuming sufficient incubation for 11 plates from one original.

Continue incubation. For 2UV 4, 5, & 6 try AA prep & re-examine. AA + will yield extract.

Pickup CG 9 and 2UV 1 & 3 in liquid medium may be due to something in agar. Also check in minimal medium.

ST and SO4 expts:

CG 7: S.M. too low, background growth quite heavy, no resistant papillae visible.

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

CG 9: SM: 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

Remover 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 AG2 SM.

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 would be very useful. Check slant - streak out & test individual colonies; if St^s , grow in broth & spread plates.

6/2/54

Run down plates; CG 7 & 8 now well grown on

H.C.; faint growth of these & of 2 UV 3, 4, & 5 on YIVA.

CG 9 S^r : Pick 1 col.; streak on AG-1 & 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. Bushing on AG-1 & SM or St.

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

5/3/54

Final readings on recond plates (5 days)

	<u>0</u>	<u>A1</u>	<u>A2</u>	<u>A3</u>	<u>A4</u>	<u>A5</u>	<u>HC</u>	<u>VITS</u>	<u>YNA</u>	<u>Na₂S</u>
CG 7	-	-	-	-	-	-	+	-	-	-
8	-	-	-	-	-	-	+	-	-	-
9	+	+	+	+	+	±	⊖	⊖	+	+
2UV1	+	+	+	+	+	+	+	⊖	+	+
2	-	-	-	±	±	+	+	-	-	-
3	+	+	+	+	+	±	+	⊖	+	+
4	-	-	-	-	-	-	±	-	-	-
5	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-

6/4/54

Tests of autos for S & SE sensitivity by cross-breaking

	<u>S</u>	<u>SE</u>		<u>S</u>	<u>SE</u>
CG 7	R	R	2UV1	S	S
8	R	R	2	S	R
9	R	S	3	R	S & R
			4	R	R
			5	R	R
			6	R	R

However, spread plates indicate that CG 7 is not uniformly S & R nor CG 9, uniformly SR. CG 7 did appear to be uniformly SR. CG 7 has been checked out on No. 1; pick & test at least

10 col. for *St. nactini*.

6/5/54

Pinked col. of CG7, cross brushed \bar{c} *St.*

CG9; 2 UV1 and 3; Check for anaerotrophy.

Try spotting on minimal

2) Growing in minimal tubes,
 \bar{c} & \bar{s} aeration.

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

6/8/54

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

cupos, since none of the requirements is completely determined

Spotted cupos on AG 1 for repl. to sundrows

6/9/54

Cupos on AG-1 (24 hrs); Good growth of CG 7, 8 + 9; 2UV1, 2, + 3; fair growth of 4, 5, + 6. Replicated to AA group single omission sundrows

6/10/54

Strucked all CG and radiobacter cultures on AG-1 \bar{c} 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 5'
 Moderate growth (CG 9, 2UVI & 3) Should
 have used prototroph control. Still no
 growth in minimal tubes inoculated at the
 same time.

Experiments replicated 6/9

	0	-A1	-A2	-A3	-A4	-A5	AA+	HC	Y.EX	complete
CG 7	-	±	+	+	+	+	+	+	+	+++
8	-	±	+	+	+	+	+	+	+	+++
9	±	±	+	+	±	+	+	-	++	+++
2UVI	±	-	+	+	+	+	±	+	+	+++
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 circled plants.

Macerate galls in sterile H₂O, streak heavily on
 B glu and AG-1 + on pl.

Plant readings
6/11/54

control

000

CG 5 (+)

" +

" ±

CG 4 +++

+++

+++

CG 3 (+++)

+++

++

CG 1-R + (poor growth of plant)

++

(++)

CG 2 M ±

" ±

" ±

CG 1 M (+++)

+++

+++

AG UV-1 0

(+)
0

-1M+5 R1 ± (one good fall)

1M+5 R1 +

" R4 +

1M+5 (R2) +

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

(M3 +)
M3 +
(M3 ++)

R2 ±

R2 +

M4 ±

M2 +

M4 ±

M1 +

M4 +

M1 ++

M2 ±

M2 +

M1 ±

6/12/54

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

6/14/54

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

Readings - final reading (repeated 6/9)

	<u>0</u>	<u>A1</u>	<u>A2</u>	<u>A3</u>	<u>A4</u>	<u>A5</u>	<u>AA</u>	<u>HC</u>	<u>YEX</u>	<u>Complete</u>
CG 7	-	±	++	+++	+++	++	++	++	+	+++
8	-	±	++	+++	+++	++	++	++	+	+++
9	±	+	+	+	+	+	+	-	+	+++
2UV1	±	±	++	++	+	+	+	+	+	+++
2	-	++	++	+++	+	+	++	+	+	+++
3	±	++	++	++	+	+	+	+	+	+++
4	-	-	+	+	+	±	+	+	+	++
5	-	-	+	+	+	+	±	+	+	++
6	-	-	+	+	+	+	+	-	+	++

Being fed at one edge by mixed colony.
Note that most cultures grew best in the absence of A3, and CG 7 & 8, in the absence of A4.

2UV 4, 5, & 6 Try on A1 alone & various other samples, singly. Also try this on CG 7 & 8. In this expt,

CG 9 & 20V143 grew little if any and unsufficiency
 minimal, 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 inc. to
 SR (CG 4 + CG 5) absorb aniline blue
 strongly.

Pick at least 10 col. each. Test for prototrophy/
 auxotrophy and SA. Save for tests of
 virulence. CG-1-R as reserved is still
 rough.

Save actinomycete on 1-M+5 (M3) 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
 SA 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 in streaks; most are highly pigmented, yellow, orange, and pink.

R2 from mixture experiment came out smooth, unlike CG-1-R, which was rough when recovered. 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 growth was pecked, 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 mirrorid, 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 indole blue absorption.

Made up 20x salts solution for AG-2, incorporating Fe & Mn in soln. (from conc. stock Fe & Mn salts).

6/18/54

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

6/19/54

Spotted all auxos 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.

X M3: 1 yellow, very gummy; 2 moderately gummy remainder less gummy and rather translucent.

X R2: All colonies moderately smooth; not so smooth

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

6/21/54

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

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

Replicated auxotrophs to complete, mini, and
AI + other AA groups singly.

6/22/54

Put in slabs:

CG-1-S

CG-1-R

CG-2-S

CG-2-R

} Have slabs only of AB and AB-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 onto
plants. Struck out R2 & M3, pick 10 or more
colonies, test for purity & respect to SM.

6/23/54

All isolates

	<u>Type of growth</u>	<u>SM</u>		<u>Type of growth</u>	<u>SM</u>
CG-1-S	10 S	10 S ^S (all note)	CG 9	10 S	10 S ^R
CG-1-R	2 S, 8 R	10 S ^S	X M3	10 S	2 S ^S , 8 S ^R
CG-4	3 S yellow, 7 S white	10 S ^R	X R2	10 S	10 S ^R
CG-5	8 S, 1 R	9 S ^R			

S^S more common 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 damp) . CG-1-R : no growth at all on S^H.

All isolates grew very well on minimal.

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

Randoms inc. 6/21

	<u>0</u>	<u>A1</u>	<u>A1+2</u>	<u>A1+3</u>	<u>A1+4</u>	<u>A1+5</u>	<u>A1+HC</u>
CG 7	-	-	-	-	-	-	+
8	-	-	-	-	-	-	+
9	-	-	-	-	-	±	±
2 UV-1	±	±	±	±	±	+	+
2	±	±	±	±	+	±	+
3	±	±	±	±	±	±	+
4	-	-	-	-	-	-	±
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-

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

	<u>S</u>	<u>SE</u>	<u>Source</u>	<u>Arrangement of test plates</u>	
CG 7	R	R	CG-2		
CG 8	R	R	CG-2	1 S	2UV1
				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
				8	
2UV-6	R	R	"		

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

In testing SM & St status, include 2UV1, previously recorded as S^SSt^S, as control.

Steps - CG 7 stab appears to be contaminated (yellow).
Inoc. new stab from slant.

6/24/54

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

Randomness mic. 6/21:

	<u>0</u>	<u>A1</u>	<u>A1+2</u>	<u>A1+3</u>	<u>A1+4</u>	<u>A1+5</u>	<u>A1+HC</u>
CG 7	±	±	±	±	±	±	++
8	±	±	±	±	±	±	++
9	-	-	-	-	-	-	⊕
2UV1	±	±	±	±	±	±	+
2	±	±	±	±	+	±	++
3	±	±	±	±	±	±	+
4	-	-	-	-	-	-	±
5	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-

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

Need a better minimal medium.

Try medium based on Hodgson, Peterman, & Piller, 1951

$(\text{NH}_4)_2\text{SO}_4$ 1.0

NaCl 0.2

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 8.8 mg

ferric alum 43 mg

$\text{Mn}_2\text{SO}_4 \cdot 4\text{H}_2\text{O}$ 0.8 mg

Use $\text{K}_2\text{H}_2\text{PO}_4$ 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 stock soln.

This differs from present medium chiefly in N source ($(\text{NH}_4)_2\text{SO}_4$ instead of KNO_3); also has less CaCl_2 and includes zinc.

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

6/25/54

6/21 rundowns:

	<u>0</u>	<u>A1</u>	<u>1+2</u>	<u>1+3</u>	<u>1+4</u>	<u>1+5</u>	<u>1+HC</u>
CG 7	±	±	±	+	+	±	++
8	±	±	±	+	+	±	++
9	±	-	-	-	±	-	±
ZUV 1	±	±	±	±	±	±	+
2	±	±	mold	±	+	±	+
3	±	±	±	±	±	±	+
4	-	-	-	-	±	±	+
5	-	-	-	-	±	±	±
6	-	-	-	-	±	±	-

Checks of streptomycin and streptothricin resistance:

	<u>S</u>	<u>St</u>		<u>S</u>	<u>St</u>
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. & aupos. [2UV-4 had been scored S^R on previous test]

2UV6 looks like a good aupos to try in SRP test.

Problem: How make sure it is really A. tumefaciens?

- 1) Microscopic
 - 2) Growth in complete broth, aerated and anaerated.
 - 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, CG-1-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 unacrated; slight difference between 1-R acrated and acrated; and little or no difference between 2UV6 acrated and unacrated. (ca 20 hrs)

Growth at 37°: CG1 S & R - slight growth; 2UV6 very little growth. Growth of all unacrated cultures at room temp much better than at 37°.

Yesterday made up 5 plates AG1 c̄ 1:10,000 aniline blue (Coleman-Bell). Have been using National Aniline, same conc. Natl Aniline → pale greenish blue medium; Coleman-Bell → grayish purple.

Streaked CG1 S & R on plate made c̄ Coleman Bell dye.

Motility checks 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. broth cultures of 2UV6 and CG-2-S, together and separately, to be inc. at room temp c̄ +5 aerations.

X M3 & X R2 from slants (1-5) + 5 replicates each.
Picked 1 col from each strake (12 from each culture),
spotted on AG-1 for replication to SM.

6/28/54

Test of Gram - Cell outline blue; Neither CG-1 or
16 colored on AG-1 with dye 1:10,000.

2UV-6 identity. Have reservations about this culture
being Corynebacterium 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 \bar{c} aeration
2UV-6 grows almost as well \bar{c} as \bar{c} aeration
(Final opacity of culture about the same, but
requires 48, rather than 24 hrs. CG-1
never does grow very well in un-aerated
broth).

X M3 & X R2 ^{from slants} Replicate to AG-1-SM.

Plated CG-2-S / 2UV-6 crosses on AG-2-SM.

Grow together - aerated	Grow separately - aerated
Grow together - not aerated	Grow 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

X R2 & X M3 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.

S^R P plates (now only ca 20 hrs)

Controls: 2 UV6 aerated & un-aerated, no growth.

CG-2-S un-aerated; 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 large papillae.

When papillae better grown, quite
test for autotrophy. Probably autotrophs being fed by background
of CG-1-S. If prototrophic - !!! A36

Too early to try reaching any conclusions. Results
observed so far could be explained by feeding of
20V6 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 S¹⁴ conc, rather than confluent growth
of S^R mutants, since papillae are readily
seen on plates spread in un-aerated cultures.
S¹⁴ 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 in
CG-9).

Use CG 7 (from CG-2) and CG-1-S.

Grow as before, mixed & separately, aerated &
un-aerated. Also mix extra mixture tube,
inc. in aeration for several days, rather than
only 48 hrs.

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

6/29 1:30 pm 6/29

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

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-2-S 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

phage plaques in the area of heaviest growth. The plaques are present only on 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-size 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 AG-2 SM
 no growth in 24 hrs. Continue incubation. Also:
 check SM level of new batch of AG-2 SM by streak
 on CG-2 S and CG-5 (or CG-2-S & CG-3 or 4)

Pick more papillae from "cross" plates, streak on AG-1
 (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 un-aerated
culture. However, should be OK to use at 48
hrs.

7/1/54

2S / 2UV6 SRP expt. i: papillae which were
tiny yesterday now full-size colonies, evidently
SR mutants of CG 2S, since nos. are about the
same on "cross" plates & CG 2S control plates.

About 200-300 per plate from un-aerated inocula;
several thousand from aerated inocula, which gave
heavier suspensions for plating.

Discarded plates. (Most were moldy)

Phage

✓ 1) Aerated cultures: a) mix direct from plate; b) 2UV6
+ mix from plate; c) CG 2S + mix from plate.

None show evidence of lysis. Cross-brush
each on complete med. c̄ 2UV6 & CG 2S.

2) EMB plates streaked c̄ 2UV6 (6/28); 2 kinds

of colonies, one very translucent, one 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 ordinary
 complete medium. \bar{E} mold present.

4) Spread plates of complete medium \bar{E} mixture of
 CG2S and 2UV6.

CG-1-S/CG7 SRP expt: Cfg, wash, plate. SRP's picks
 from previous SRP experiment are growing rather poorly
 in ^{present batch of} AG2SM; probably had the usual E. coli con
 of SM added.

7/2/54

Plaque picked from 2S/2UV6 SRP plates: Streaked on
 complete \bar{E} 2 kinds of colonies: Small, translucent; a
 larger, opaque. Check both for asymmetry & S resistance
Pick to complete repl. to min x SM.

"Phage" This may not be a phage at all. The plates
 spread on \bar{E} CG2S + 2UV6 have what look like
 temp. \bar{E} colonies in large numbers, 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) Tiny, 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 one would 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, urea, HC, etc.

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

and streak 5% yeast extract to 10 ml tubes of AG 2, 0.1, 0.2, 0.4 & 1.0 ml per tube. Inoculate CG9 and 20V1. "20V1" because it has shown a reaction

specific reference to YE and YNA, and latter because it is S³, the only one among the aerotrophs, and thus has an additional marker for crosses.

Also incubated AG1 broths of CG9 and 2UV1 together & separately for crossing attempt. To be plated on minimal. Did not start aerated cultures this time.

7/3/54

CG-1-5 / CG-7 SRP: (2 days)

Cultures:

7, not aerated: Thin film, no papillae

7, aerated:

1-5, not aerated: fairly heavy background,
16 ^{large} papillae

1-5, aerated: Heavy background, no papillae

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

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

Incubed separately, not aerated: Moderate background, 50-100 large papillae per plate

Incubed separately, aerated: Moderately heavy background,

only 2-4 papillae per plate.

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

7/5/54

Plate spotted \bar{c} two col. types from 25/20V6
(1/2) Moldy; spot on fresh A61 plate for replication
to men & S4.

~~Original "phage" plate; "plaques" still visible;
Try picking from individual clear areas, see whether
→ small, clear colonies; also inc in broth
20V6 + CG25, see if any phage.~~

Look very similar to CG-15 plate - see below

CG9 + 20V1 in men. + yeast extract:

CG9: Good growth in 10 ml men + 0.1 ml 5% YEX.

(In remainder, this depo was recorded as responding to YVA).

20V1: Some growth \bar{c} 0.1, 0.2, & 0.4 ml YEX;
quite good growth \bar{c} 1.0 ml, but still not as

good as in complete. (\bar{c} CG 9, growth ≈ 0.1 in YEX is about as good as in complete). Growth in men + 0.4 ml YEX probably OK for testing for inhibition.

Add regular conc of into & AA groups to men + necessary amt of YEX.

CG-1-5 / CG 7 "SRP" ^{apf}; (plated 7/11)

Controls: 7 aerated & un-aerated; several hundred colonies; evidently this single conc not too satisfactory for "crossing" effects.

1-5, not aerated; moderate background which has "plagued" appearance - apparently due, again, to small, transparent colonies. Also ca 100 large papillae.

1-5, aerated; moderate background, several hundred papillae.

"Cross" plates: very similar to CG 1-5 controls.

20VI / CG 9: C₆ wash plate, ^{on men} (grown together for 30 min. ^{on men} in men). Also plate, ^{on men} mixture of CG 1-5 and CG 7 incubated 5 days. Not too promising.

an overview of behavior of controls.

7-5/7 Result about the same as those incubated only 2 days,
Heavy growth + numerous leakage particles

7/6/54

Cultures from 2S/2UV6 expt. Replicated to
AG2 + AG1517.

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

	CG9 48 hrs		2UV1			CG9 48 hrs		2UV1	
0	-	-	±	-	YE+AA4	-	±	±	-
YE only	+	++	+	++	AA5	+	++	+	++
YE+AA1	+	++	+	++	HC	-	-	±	-
AA2	+	++	±	++	VITS	-	+	-	-
AA3	+	++	+	+	Na ₂ S	+	++	++	+++

better growth in
YE+Na₂S 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
Agrobacterium and other growth factors.

Start this type of experiment with other auxotrophs

7/7/54

Test of "papillae" from 2S/2UV6 SRP
experiment.

Papillae on minimal SMY picked
↓ streaked on complete

small,
translucent

larger,
opaque

6 col. of each
tested

all S^R ,
auxotrophic

all S^S , prototrophic
(but inhibition on SMY
not complete -
hence gave background
which permitted auxotrophic
 S^R (2UV6) to grow).

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 in complete
AA3, 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 list p A33

7/8/54

Make up 20x salts for minimal, using only
0.2 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ per 100 ml 20x soln
(see p A33)

Then make up 2 media

1) 0.5% sucrose, 0.5% KNO_3 ; Fe & Mn
(usual A₂, except for less CaCl_2)

2) 0.5% sucrose, 0.1% $(\text{NH}_4)_2\text{SO}_4$; Fe, Mn, Zn
(~ Hodgson, Peterson, & Riker 1951)

ADD Na_2S to portions of each

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

	<u>CG9</u>	<u>2UV1</u>		<u>CG9</u>	<u>2UV1</u>
0	-	-	YE+A4	++	-
YE only	+++	+++	A5	+++	+++
YE+A1	+++	+++	HC	-	-
A2	+++	+++	VITS	+++	-
A3	+++	+	Na_2S	+++	+++

CG15 and CG25 in min. + recondens (2 days)

	<u>CG15</u>		<u>CG25</u>			<u>CG15</u>		<u>CG25</u>	
0	+	+++	+	+++	A5	-	++	-	++
A1	+	+++	+	+++	HC	-	-	-	-
A2	+	+++	+	+++	VITS	-	-	-	-
A3	++	+++	++	+++	Na_2S	+++	+++	+++	+++
A4	-	-	-	-	complete	++	+++	++	+++

Inoculated CGIS into tubes as follows:
 1 Complete 3 A3 and components
 2 Minimal 4 A4 and components
 5 A5 and components

7/9/54

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

CGIS in min + AA groups + components:

	1 day	2 days	3 days		1 day	2 days	3 days
0	-	++	++++	glutamic	-	-	-
complete	++	+++	++++	aspart	-	-	contaminated mold, growth
A3	+	+++	++++	prol	+	++	++++*
trypt	±	++	++++	A5	-	-	++
tyros.	+	+++	++++*	alan	+	+++	++++
φal	+	+++	++++*	glyc	+	+++	++++
A4	-	-	-	serine	-	±	+++
hist	-	++	++++	OH prol	+	+++	++++
thres	±	+++	++++				

In 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 AG 2 (low $CaCl_2$) \bar{c} & 5 sulfide; also the $(NH_4)_2SO_4 - Zn^{++}$ medium (AG 3) \bar{c} & 5 sulfide
 Inoc. \bar{c} CGIS

Attempted crosses of auxotrophs

	CG 7	8	9	2UVI	2	3	4	5	6
CG 7		7/5/54 1 on 8 control 1 on 9 control	7/5/54 0	7/26/54 ✓ 61; control 0					
8			7/5/54 0						
9				7/9/54 0					
2UVI									
2									
3									
4									
5									
6									

Start cultures of CG 7, 8, 9, 7+8, 7+9, & 8+9 for crossing exp

Inoc. 2 CG 15 tubes of minimal + each vitamin available in solution.

7/10/54

Test CG 15^R = individual acids of ^{AA} groups 1 and 2.
 (Used CG 1R by mistake - shouldn't make any difference in eventual result.)

7/10/54

CG 1S and CG 2S in mini + growth factors.

	<u>CG 1S</u>	<u>CG 2S</u>		<u>CG 1S</u>	<u>CG 2S</u>
0	+++	+++	A5	+++	+++
A1	+++	+++	HC	-	-
A2	+++	+++	VITS	-	-
A3	+++	+++	Na ₂ S	+++	+++
A4	-	-	complete	+++	+++

Discard

See on page 43 results of adding individual AA's.
 McIntire, 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 17/20

CG 1R in groups AA1 & AA2 and components of these groups. No growth at 20 hrs in mini. or any supplement.

CG 1S in Ag 2 & Ag 3 \bar{c} + S sulfide - no growth at 24 hrs.

CG 1S in mini + individual ams - no growth 24 hrs.

7/11/54

CGIR in A1 + 2 + components:

	<u>2 days</u>	<u>3 days</u>		<u>2 days</u>	<u>3 days</u>
0	+	+++	A2	++*	+++
A1	++*	+++	lec	++*	+++
lys	++*	+++	isoleuc	++*	+++
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 AG 2 + AG 3 \bar{c} + \bar{s} sulfide

	<u>2 days</u>	<u>3 days</u>
AG 2	-?	- (?)
2+ Na ₂ S	++ (uniform turbidity)	+++
AG 3	-	-
3+ Na ₂ S	+	+

~~REPEAT~~

CG 1 S in individual vials.

	<u>2 days</u>	<u>3 days</u>		<u>2 days</u>	<u>3 days</u>
0	++	+++	nei	+++*	+++
B6	++	+++	B1	+++	+++
Choline	++	+++	B2	++	+++
Mandelate	++	+++	pala	+++*	+++
Pentothamate	++	+++	essetil	+++	+++
	+++	+++	A12	+++	+++

7/12/54

CG 7, 8, & 9, pairs grown together & separately

Repeat of CG 15 in AG 2 + AG 3 \pm + S sulfide conc.
 (May have been an error in conc. of AG 2 + 3 salts soln. If no growth in AG 2 this time make up new 20x stock)

7/13/54

Repeat on AG 2 + AG 3 \pm sulfide:

	1 day	2 days	3 days
2	-	-	-
2 + sulfide	-	-	-
3	-	-	-
3 + sulfide	+	+++	+++

Inc. AG 2 & AG 2 + folic acid (pteroylglutamic) \pm CG 15.

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

Try CG 15 in mini + glutamic or aspartic + sulfide

7/14/54

CG 15 in 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 in AG 2 ± folic acid (PGA): equally good growth ± 0.5 PGA.

Crosses involving 7, 8, & 9: (3 days) - nothing definite. 1 possible prototroph on (7+8) plate. Plate contaminated ± yellow bug, 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

could try
stuck water mix
dated 12/15/52
also try
+8 (w/)

Start repeat run-downs of all auxotrophs, avoiding any inhibitory supplements. (Do vials individually).

Made up new 20x AG 2 salts; previous batch gave no growth \bar{S} sulfide

CGIS in AG 2 \pm glutamic \pm Na₂S (2 days)

AG 2	-	AG 2 + Na ₂ S	++
AG 2 + glutamic	-	AG 2 + g.a. + Na ₂ S	++

Try CG 2 S in run. This was earlier batch than that which gave no growth in previous exp.

Try run-downs of auxos \bar{S} Na₂S added as detoxifier

For some reason - new batch of AG 2 salts \rightarrow 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 saline be d-glutamic? The aspartic acid on hand is dl, so possibly d some could explain inhibition by both glutamic and aspartic salts.

7/17/54

CG15 and 2UV6 in mediums + sulfide

no	CG 25			2UV6		
	1 day	2 days	3 days	1 day	2 days	3 days
Na ₂ S	-	+	++	-	-	-
Na ₂ S only	-	+	+++	-	-	-
A1	±	+	+++	-	-	-
A2	±	+	+++	-	-	-
A3	+	+	+++*	-	-	-
A4	+	+	+++*	-	-	-
A5	-	+	++	-	-	-
HC	++	++	+++	+	+	++
VITS	-	±	++	-	-	-
YEX	++	++	+++	+	++	+++

7/19/54

Prototypes from 7+8 cross & 8 control plate. Both grew very poorly on minimal (plate rather than tube). Colonies look identical; the one from 8 control plate does not resemble the yellow contaminant. Inoc each into min. tube & min + AA4 or glutamic acid.

Prepared the following tubes to test CG15

- min
 - min + l-glutamic
 - min + d-glutamic
- { min + d-glut. + Na₂S
 { min + " + thiosulfate
 (1:10,000)
 (as checks on whether thiosulfate is as effective as Na₂S)

Started run-ins + sulfide = CG 7 & CG 8

7/20/54

CG 15 in l-glutamic + in d-glutamic alone +
 = Na₂S + thiosulfate; no growth at 24
 hrs.

"Prototrophs" from 7x8 & 8 control; No growth at
 24 hrs in run-in or run-in + glut.

Randoms on CG 7 & 8 (black sulfide ppt in
 some tubes)

	CG 7				CG 8		
	1 day	2 days	3 days	4	1 day	2 days	3 days
Na ₂ S only	-	-	±	±	-	-	-
A1	-	++	++	+++	-	++	+++ +
A2	-	-	±	±	-	++	+++ +
A3	-	-	±	+	-	-	- -
A4	-	-	+	+	-	-	++ +
A5	-	-	±	±	-	-	+ +
HC	+*	+++	+++	+++	+*	+++	+++ ++
VITS	++?	?(-)	?(-)	-	++?	?(-)	?(-) -
YEX	+*	+++	+++	+++	+*	+++	+++ +

* Growth only
 in run-in

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

CG 1 and 2 on 7/21

7/21/54

CG 15 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 -

CG 7 & ~~CG 8~~^{CG 8} run-downs + sulfide. Heavy ppt causing turbidity in tubes. This medium was made in same salt mixture as that which gave little or no ppt in last batch of AG 2!

Started broths of CG 7, 8, & 9 and 2UVI for crossing attempts.

Also inoc 2UVI in both 7, 8, & 9.

7/23/54

CG 7 in run-downs; no growth (inoc 7/20)

Plated crosses of CG 7, 8, 9 & 2UVI

7/24/54

Inoc CG 7 into A1 run-downs; CG 8 into A1 & A2

7/26/54

CG 7 & 8 rundowns

	<u>CG 7</u>			<u>CG 8</u>		
	<u>2 days</u>	<u>3 days</u>	<u>4 days</u>	<u>2 days</u>	<u>3 days</u>	<u>4 days</u>
O	-	-	-	O	-	±
A1	+	++	++	A1	±	++
lys	-	-	+	lys	-	±
arg	-	±	±	arg	-	±
meth	-	-	-	meth	-	±
cyst	-	±	+	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 (AA rundowns)

Crossing attempts of CG 7, 8, & 9 \bar{c} 2UV1

- Controls: negative except CG 8, which is quite heavily contaminated w mold and a pink bacterial contaminant. A few col. present which look like *Agrobacterium*.
- 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 minimal. Repeat cross, this time grown together & separately, aerated & un-aerated.

7/27/54

CG 7 & 8 AA run-downs: (See table on previous page)

CG 8 seems rather "leaky"; this has happened before. CG 7: Might try A / 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 run-down attempt - 2UV1

Repeat l- & d- glutamic ppt \bar{c} CG 15, since previous ppt was lost because of mold.

Start cultures for repeat crossing attempt \bar{c} CG 7 & 2UV1.
(together & sep; aerated & un-aerated.)

7/28/54

Col. picked from 7x2 UV1 plate & repl. from complete 2
minimal: 29 picked, all prototrophs.

CG15 on d & l glutamic: no growth in 24 hrs

7/29/54

Plated 7x2 UV1 crosses (grown together & separately,
aerated & not aerated)

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

CG15 / glutamic:

	<u>2 days</u>	<u>3</u>	4
0	++	+++	
Na ₂ S only	++	+++	
d-glut	±	++	!
d-glut + Na ₂ S	++	+++	
l-glut	++	+++	
l-glut + Na ₂ S	++	+++	

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

7/30/54

CG 7 run downs

	<u>2 days</u>	<u>3 days</u>		<u>2 days</u>	<u>3 days</u>
O (Na ₂ SO ₄)	+	+	A1	++	+++
B ₂	+	+	"-lys	++	+++
Biotin	±	traced	"-arg	++	+++
B ₆	-	±	"-cys	-	++
pantoth	-	-	"-meth	+	+++
K	-	±			
B ₁	-	±	complete	+++	+++
nic	++	++ ^{traced}			
B ₁₂	-	±			
inos	-	±			
chol	++	contaminant? +++ (pullula)			
paba	-	±			
pge	-	-			

Streak out CG 7; check several colonies on anything giving 2+ or better.

7/31/54

CG 7 x 2UV1 "cross": At 48 hrs, colonies on all three plates from cultures grow together, not aerated. None on other plates.

Inoc. run downs (primary) of 2UV1

8/2/54

2 UV1 primary run-downs;

at 2 days, growth only in YNA.

Try individual vito; also p & p?

CG7: from individual colonies. Check each on
 min ($\frac{1}{2}$ sulfide) +: O; biotin; methionine; choline;
 A1 & individuals of A1 group. Try first 2 plates; 2 or three with

CG7 + 2 UV "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

2 UV1 primary run-downs; 3 days; still growth
 only in YNA; possibly trace (\pm) on A1.

8/4/54

2 UV1 primary run-downs; 4 days

O	-	A3	-	HC	-
A1	\pm	A4	+	VITS	+
A2	-	A5	+	VFX	...

Try individual
 vito.

7x20V1 "protos" (8/2): Very poor growth when streaked on minimal - very tiny colonies. Grew well on complete. Replicate from complete to mini.

Repeat 7x20V1 cross: Use several cultures of each parent, plate control from each.

8/5/54

7x20V1 "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: Protein, nic, choline, A1, & individuals of A1 group)

8/6/54

20V1 in individual vials: no growth at 2 days

Plated repeat of 7x20V1 cross.

8/9/54

Repeat of 7x2 UVI cross

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

	<u>CG 7 control</u>	<u>2 UVI control</u>	<u>cross plate</u>
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 contaminants}	0	3 + several very small
5	0	—	—
6	10 very small	—	—
7+2 UVI ①	—	—	confluent
7+2 UVI ②	—	—	0

8/10/54

Check whether filtered Ag 2 supports good growth of CG 15 (in order to get rid of confusing turbidity).
 Inc. tube (E Na₂S) & water suspension.

8/12/54

3 days growth in filtered medium only

9/3/54

3 days growth in both filtered & unfiltered,

Inoculum very small, no growth in control Ag 1 control + 8 hrs

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 aseptos

1) in min

2) in min + sulfide

3) ~~in min + sulfide~~ + VE. Complete

before starting any apts.

9/13/54

Check of aseptotrophs inoc into min ± sulfide, &
complete 9/11:

No growth in any min or sulfide tubes.

Growth in all complete tubes except 2 UV2.

Continue inc.

9/14/54

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

Transfer stabs to have one set as reserve.

9/15/54

Growth in CG7 in min + sulfide. Could be
contaminated, since another tube has mold.

1/11/55

Irradiation effd \bar{c} WCG-1-S. Used overnight aerated culture of grown in Ag 1 broth, room temp.

Irradiated 10^{-4} dilution; effd, washed, resuspended in saline, then diluted.

90 sec irradiation \bar{c} 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: $5 \times 10^{-7} \Rightarrow$ about 20 / plate. Colonies still very small; inc. another day before replicating.

Started culture for second irradiation: 2.1 ml Ag 1 broth culture (not aerated) \rightarrow Ag 1 broth aerated, room temp.

1/14/55

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

1/15/55

Exposed plates repl. to min from 1/11 irradiation: 9 possible auxotrophs from 245 colonies. Picked, spotted on complete. Some colonies very rough; use caution on identity. If possible, concentrate on typical colonies.

Second UV run, WCG-1-S:

Culture: aerated at room temp overnight; stored
3 aeration at room temp another 24 hrs.
Cfod; pellet washed in 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
 10^{-4} dilution large enough to pick from plates by replication. 2 by
5-10 fold per plate.

"Aeros" 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 cols. 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 autotroph. Test
along with other 9.

Made up 2 minimal tubes. To check medium, inoc (drop
water suspension from WCG-1-S stab into ^{1) complete, not aerated}
used 1 drop 1% sterile Na_2S / tube (10 ml) ^{2) min + Na_2S aerated}
^{3) " " not aerated}

1/18/55

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

~~Several~~ Two of the "rough" spots → mixed
 growth when replicated to men & 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 Agrobacterium.

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

More colonies now visible (3 days) on 5×10^{-6} & on
 10^{-7} plates from 2nd UV run. Pick; spot on
 complete for repl. to men.

Check on Ag 2 minimal : No growth overnight in
 men tubes 5 or 5 aeration. Un-aerated Ag 1 tube
 (compd) slightly turbid. Room temp probably quite
 low during night.

1/19/55

Check of minimal liquid (see 1/17): At 2 days, very good growth of WC8-1-S in min (\bar{c} Na₂S) aerated. About the same turbidity in unaerated mineral as in ~~unaerated~~ complete.

Why Na₂S & aeration needed for best growth? Seems contradictory. Effect of aeration may be largely agitation rather than O₂ availability; but at pH slightly below 7 should think sulfide would be rapidly lost.

Set up: min + Na₂S } aerated
min \bar{c} Na₂S }
min \bar{c} Na₂S } unaerated
min \bar{c} Na₂S }

1/20/55

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

10 picked from first run appears to be autotroph. Stab; prepare remainder 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 axepo from 2nd run. Stab,
streak on complete.

1/21/55

Effect of aeration: \bar{c} & \bar{s} Na_2S : (48 hrs)

Not aerated: Little difference \bar{c} & \bar{s} Na_2S . Poor growth.

Aerated, no Na_2S : better growth than \bar{s} aeration.

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

1/22/55

Inoc Ag 2 run-downs \bar{c} UV1-10, UV2-1, and WCG15
from water suspensions from Stals.

UV2-1 grows very poorly when streaked on complete.

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

1/24/55

Run-downs (2 days)

	<u>WCG15</u>	<u>UV1-10</u>	<u>UV2-1</u>		<u>WCG15</u>	<u>UV1-10</u>	<u>UV2-1</u>
O	++	-	-	HC	++	+	-
A1	++	-	-	YNA	+	±	-
A2	++	-	-	VITS	++	-	-
A3	++	-	-				
A4	++	-	-	compl	++	++	++
A5	++	-	-				

1/25/55

Rundroms (3 days)

	WCG15	1-10	2-1		WCG15	1-10	2-1
0	+++	-	-	AA5	+++	-	-
AA1	+++	-	-	HC	+++	+++	++
2	+++	-	-	YVA	+++	-	+
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].

UVrun (#3)

24 hr aerated culture (Ag 1, room 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 $\bar{c} > 10$ col. Picked from plates $\bar{c} < 10$ col & spotted on compl.

One yellow (or creamy) colony. Picked to stab, labelled UV3-1. Colony was same size, shape, height, & texture as others on plate. Check motility; growth \bar{c} & \bar{s} sulfide, \bar{c} & \bar{s} aeration.

1/28/55

Rundowns, single omissions of AA groups (2 days)

	<u>UV1-10</u>	<u>2-1</u>		<u>1-10</u>	<u>2-1</u>
0	0	0	-5	+	+
-1	+	+	all	+	+
-2	+	+	all + vits	+	+
-3	+	+	HC	-	+
-4	+	+	compl	++	++

Fresh HC used (taken from freezer)

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

1/29/55

Rundowns, single omissions, 3 days

	<u>UV1-10</u>	<u>2-1</u>		<u>1-10</u>	<u>2-1</u>
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]

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

2/2/55

Reundowns of UV3 aupos: 2 days

	<u>HC</u>	<u>AA</u>	<u>AAV</u>	<u>YNA</u>	<u>Ag 1</u>	<u>0</u>
3-1	+++	+++	+++	+	++	-
2	+	++	+++	+++	+++	-
3	±	+	-	-	+++	±
4	-	-	-	-	+++	-

2/3/55

UV3 reundowns, 3 days.

	<u>0</u>	<u>HC</u>	<u>AA</u>	<u>AAV</u>	<u>YNA</u>	<u>Ag 1</u>
3-1	±	++++	++++	++++	++	++++
2	±	+++	+++	+++	+++	++++
3	±	++	++++	-	-	++++
4	-	-	-	-	-	++++

These were kept for several more days; no further growth in minimal tubes; no change in other tubes.

2/8/55

Inc. AA reundowns of UV3-2 & 3-3. (Groups, single additions)

2/9/55

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

2/10/55

Reundrns, UV 3-2 + 3-3 (2 days)

	<u>3-2</u>	<u>3-3</u>		<u>3-2</u>	<u>3-3</u>
●	-	-	A4	-	-
A1	-	-	A5	-	-
A2	-	-	WTS	+	not included
A3	-	-	compl + vito	not included	++ to check for quantity
			compl.	++	++

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

2/12/55

Set up reundrns = individual vito = UV 3-2. (B-12 may be contaminated).

2/13/55

Inv UV 3-2 vit reundrns.

2/14/55

UV 3-2

Vit reundrns; At about 28 hrs, fair growth in vito mix, trace of growth in broten.

Would pay to do individual vit = all aupos; behavior has been consistent = oil requirement, but mix may be toxic to some. Also: 3-3 = ~~long~~ AA groups.

2/15/55

Vito, UV 3-2; fair growth in broten; other vito 0

UV3-3 Inoc. randoms, AA groups.

2/17/55

UV3-3 no growth in AA groups or in all AA's (2 days)

2/20/55

UV1-10 & 2-1 in vito: no growth in individual vito or mix. (Inoc. 2/18)

2/22/55

UV1-10 No growth in individual vito, 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 reversal - see UV2-1

UV2-1 Growth in the following tubes (very slight)

Riboflavin	paba	
B-12	vit mix	Reversins?
Pentothenate		Dirty tubes?

2/23/55

(5 days)

UV1-10 Growth in: Biotin, Dirty tubes, choline, nic, paba, vit mix

UV2-1 " " : trace in those tested previously. & Very scant even in vit mix.

2/24/55

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

WCG Inc. 2/25

3/1/55

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

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

4 days 0 -

glyc ±

vito +

vito+gl ++

2-1 at 4 days: 0 -

glyc +

vito +

vito+glyc - (!?)

3/4/55

UV 3-3 (2 days) 3 days

0 - - A4 - -

A1 ++ ++ A5 - ++

A2 - - all AA's - ±

A3 - + compl +++ +++

3/6/55

(UV 3-3)

A1 runs down in 3/4. Possibly trace of growth in lysine

3/7/55

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

0 -

meth -

Lys +

A1 -

Arg -

compl ++

Cyst ±

WCG

Try e & s lysine e aeration. If really Lys - should give more clear-cut result because of more rapid growth.

3/8/55

Start culture of WCG 1^(S) for another UV exp^(#4) to try to get a readily identifiable auxotroph.

Ag 1, room temp, aerated

NO. 1111
24 hrs

TRANSFER STABS

3/10/55

Excellent growth of WCG-1-5 for irradiation. Irradiated 90 sec. (10^{-4} dose)
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 ~~the~~ 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 0.1 ml/plate of 10^{-5} dilution. Inc. 25° .

3/19/55

UV4-1 & UV3-1 (yellow): Struck on complete. 3-1 rough, 4-1 smooth. Appearance \cong rough & smooth WCG-1 except for color. Smell like Aerobacterium.

Try running down requirements

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{s} aeration \bar{c} WCG-1.

3/21/55

UV 3-1 & 4-1 Both show scanty growth in ^{complete} broth \bar{s} aeration, good growth aerated (but not so heavy as WCG-1) 4-1 spread on minimal - No papillae at 2 days.

Reindowns:

UV 3-1 ++ in A4, + in A5, \pm in vials } 2 days
UV 4-1 0 in all reindown tubes.

3/22/55

UV 3-1 & 4-1 reindowns

4-1 no growth except in complete

3-1	A1	0	A5	+++	1) 2) 3) 4) 5)
	A2	0	VTS	++	
	A3	0	0	0	
	A4	+++	compl.	+++	

3/24/55

UV 5: Of 7 possible cups picked, all show some growth in
mini at 2 days. Two have only very faint turbidity; keep these,
check especially for vits. low second check smaller inoculum, no
growth in 4 days UV 5-1 & 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 in mini for color & phototrophy.

3/26/55

UV 3-1 re-inoculation - 3 days (run temp - cold at night)

BI	0	pyri	0	A4	++	A5	±
ribo	0	pent	0	hist	0	alan	0
mic	0	<u>chol</u>	++	threo	0	<u>glyc</u>	±
paba	0	rets mix	0	glut	0	ser	0
bio	0			prol	0	<u>OHP</u>	++
PGA	0			<u>aspart</u>	++		
<small>but for maybe ppt in mini</small> Inos	++					compl	+++
K	0						

Streak out; recheck individual colonies.

3/28/55

Papilla from UV 4-1 - picked from mini, streaked on
complete - Colonies yellow. Pick several, check for
anoxytrophly.

3/31/55

UV3-1 : Roundups in. from 2 individual col. (2 days)

	(1)		(2)			(1)		(2) (3 days)	
o	o	o	o	±	A4	o	o	++	+++
vits	o	+	++	+++	hist	o	o	o	+
chol.	o	o	o	+	threo	o	o	o	o
inos	o	o	o	o	gluta.	o	o	o	++
A5	+	+++	o	±	prol.	o	o	+	+++
alan	±	++	o	+	aspart	o	o	++	+++
glyc	o	o	o	+++	campel	+++		+++	
Ser	o	o	o	o					
OHP	o	o	o	+					

Indicates erratic results prob due to reversions?

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

	<u>5-1</u>	<u>5-2</u>
o	+++	++
A1	+	±
A2	o	+
A3	±	o
A4	+++	++++
A5	o	o
Vits	+++	++++

4/2/55

Colo. from papilla from UV4-1; autotrophic: However, 1 streak -> faint growth after 5 days, 1 large colony, many small. On in. large col appears white. Pink, streak on complete.

4/5/55

Final comparison of run-downs on two col. from (mini. 3/29) UV 3-1

	(1)	(2)		(1)	(2)
0	±	+	A4	+	+++
urea	±	+++	hist	++	+
chol	±	++	threo	0	±
inos	±	+++	gluta.	++	++
A5	++++	+++	prol	+++	+++
alan	++++	++++	aspart	++	++
glyc	+++	++++			
ser	0	+++			
OH P	±	+++			

UV4-1 Picked from large col. on mini. Struck mixed, yellow col as before, also a white, rough col. type. Latter extremely rough - probably a contaminant.

Might try plating UV4-1 on 3-1 together on WCG 16. Main purpose would be to look for colonies on yellow & white sectors. First check nutrition of WCG 16; also make sure UV4-1 (on 3-1) does not respond to broken.

4/12/55

Spread water suspensions (from slants) of WCG 16 & UV4-1 on plates of mini 2 & 5 broken. Also cross-brushed ea. on SK1.

4/14/55

WCG 16 - confluent growth on mini 2 bis; none 5 bis.
 UV4-1 - no growth on either plate
 WCG 16 S^S
 UV4-1 S^R

WCG

4/22/55

Results of attempted cross of WCG16 & UV-4-1 (4 days 25°)

Controls: WCG16 can grow \bar{c} SM at 200 μ g/ml
" " no growth \bar{c} biotin.

UV4-1 Some background \bar{c} SM, none \bar{c} SM,
though culture appeared to be S^R

With mixtures, confluent growth on all media. Growth
is white, i.e. WCG16

1 mini
mini + SM
mini + SM + biotin.

3-3 AA groups

~~Plate~~ ~~crosses of K33-48~~

In loop, diluting tube plated -
 mol. color: 0.1, 1.0, 10, 100, 1000, 10000, 100000, 1000000
 10, 100, 1000, 10000, 100000, 1000000, 10000000, 100000000, 1000000000

1-10 HC - ; in AA single group omissions, grew well in all tubes except mini control. AA group - sing additives all - ; late growth (4 days) in some tubes

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

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 mini + YX
 (Have tried HC, HCV, HCYNA, YNA, YNAV, V, HCYNAV)

Possible schemes involving WCG16 & UV4-1

See whether both more or less S^S.

If could obtain:

yellow S^R x⁻ B⁺
 white S^S x⁺ B⁻

Plate on

- 1) mini
- 2) mini + M
- 3) mini + yellow + L M

What could grow?

- all x⁺ B⁺
- only x⁺ B⁺ S^R
- all x⁺ S^R

unselected:

- color, S
- color
- color, B