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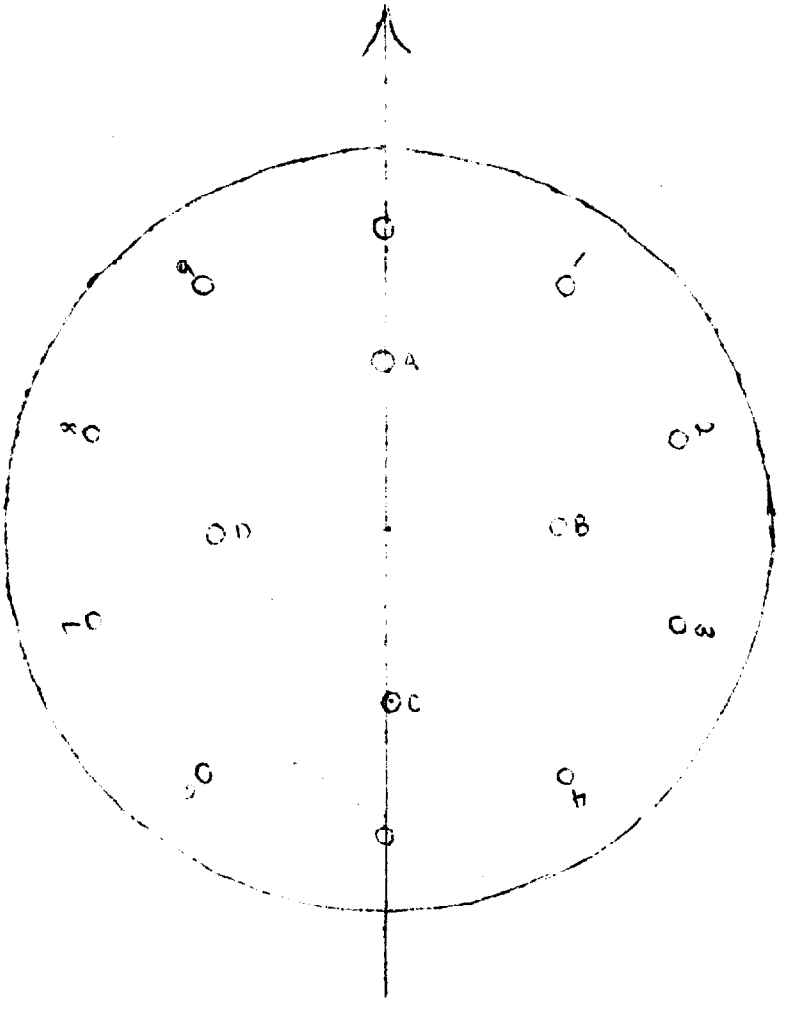
NEW HAVEN, CONNECTICUT

EXPERIMENTS IN THE GENETICS OF BACTERIA

1946- 1947.

Joshua Lederberg.



AUXANOGRAM STENCIL -

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Abbreviations:

F(x) = Fries supplemented ē x. (Neurospora minimal).
CM = complete medium (essentially aminoac + vits.)

Ca = HC = Hydrolyzed Casein.
Vit = Vitamins B + hydrolyzed YNA
YNA = yeast nucleic acid
EAA = EA "essential" amino acids =
NEAA = NA non - EAA.
⊘ = minimal
⊛ = complete
T(x) = coll ⊘ = Tatum + Gray's + min. suppl.

Amino acids.

1 leucine
2 isoleu
3 valine
4 phen.
5 trypt.
~~6 tyrosine~~
7 lysine
8 arginine
9. methionine
10 histidine
11 ~~hydroxyl~~
12 aspart
13 glutam
14 proline
15 HO- "
16 glycine
17 alanine
18 cysteine
6 tyrosine

Ind = Indole
Anth = Anthranilic.

Vits. √

1 Thiamin
2 Riboflavin
3 PAB
4 NIC
5 Folicac
6 Pyridoxin
7 Pant
8 choline
9 inositol
10 Biotin

Purines + Pyr.

3/23/46. – 3/24 Y 160.

Test 156 – Strains. Dil. surface growth in H_2O + test as indic.

Incr 9P23. 33°
1st Reading: 12 h.
2nd 24h. 9A24.

#	O		C-M		HC		Vct		Eaa		Naa	
1	–	–	++	+++	++	+++	–	–	+	+	–	–
3	–	–	++	+++	++	+++	–	–				
4	–	–	++	+++	++	+++	–	–				
7	+	++	++	+++	++	+++	+	++				
9	±	++	++	+++	++	+++	–	+				
10.	+	++	++	+++	++	+++	+	+++				

check!

(This agrees ε the previous interpretation that 7–10 may readily backmutate. 1, 3, 4 should be tested further. of (1).
Note poor growth in e 9. 9. Evidently strong inhibition is present. Wh. is inhibiting?!)

Incr: dil. from HC
9P24. 36h.

1: T( )

trypt	–	
thr	–	
lys	–	
arg	–	
val	–	
meth	–	
v-i	+	+++
leuc	–	+
hist	–	
HC	+++	
O	–	

ser. – ++

Repeat 9: Incr dil from CM. 24h. 1st. 36h 2

T (Vct)	–	+	T (O)	++	+++
Vct	–	+	O	++	+++
Vct	+	++	O	++	++

T (HC) +++

Test 160 [156-1] for adaptation. 161.

9P26. 37°.

162

	Incr. into 0.	12h.	24h	Therefore these responses are by way of growth, rather than adaptation.
1	~~HC~~	0	—	
2	V-iso.	0	—	
3	lc	0	—	
4	cool.	0	—	
156-9.5	from HC			into CM slants for testing
6	" V.i.t			
7	" 0.			

1130A28 V 330 — 1130A28 0 330

				+	
				-	
				-	

dilute + test 2P27.

5		±	+	++
6	-	±	-	+
7	-	-	-	+

Test 160 for growth vs. adaptation - 9P26. into T(0)

		12h	24h.	48h	930A29	10A30	test 0	A29	10P29	10A30
1.	from HC	—	—	—	+++				±	+++
2	" V.i	—	—	—	2P27					
3	lc	—	—	—	—	—				
4	cool	—	—	—	—	±				

Test 156-9 substrains.

a. to CM slants — 5 from HC — 6 from V — 7 from 0

			1130A28	330P28	8P28	A29	Test 0		10P29	10A30
to vit + 2P27 min.	HC	5 - vit	—	±	++				±	+++
		0	+	++	+++				+	+++
	V	6 - vit	—	±	++					
		0	—	+	+++					
This behavior is remarkable. What is inhibitory? What is the type of genetic modification?	0	7 - vit	—	⊕	±					
		0	—	+	+++					

V

See 164.

Mutant identification.
"Auxanography"

Dec 7 1948

	histidine	+ c. Hydrolyzed Casein →
E-8		
161-6		

ca. $10^{4.5}$ colonies per plate. They are visible for a radius of 1 cm in both cases around the HC, then thin out somewhat.

161-6 – not scattered large colonies, but quite numerous small. ∴ not "adaptation". ∴ vitamin effect is directly on growth, not adaptation. Sp. of g.a. not clear. Confluent faster on e-a-a. than on s.f.a.a. but this may not be a specific response. Need L-15 for control. Inhib. by boric?

P 3 → ca 10·12 "colonies" full size are seen in the e-plate, presumably adaptants

Try heavier inoculum.

Dec Auxanography – p. 168.

3/28 Mutant identification; plating. 163a

Plate histidineless (E-8) and 161-6 heavily into T7 agar. When solidified, add a loopful of

(a) HC
(b) histidine .1% to each.

7P28

Tart "washed agar #1" for biotin. Agar was had by 10 transfers through dest. H_2O, + 2× ē 95% alcohol, dried in adsorcator ē $ZnCl_2$.

Plate "58" (biotinless E coli) in 2% agar unwashed
" " washed
P31 — no colonies! " washed + 1× biotin

Incr. on surface (streak) 8P31.

A2 — Well developed colonies only where biotin was added. Nine clear-cut!!

Auto-answer

Test various T-L. mutants.
Retest 156-1 142-17.
Test 156-2, 3.

4 1130P 28 incr.
1 930A 29 1
2 12N
3 9P 29

		O	TL	TL(HC)	+eaa	TL+neaa			
1	142-17	- - -	- - -	+ ++ +++	- + +	- - -		= Y10	
2	-36	- - =	- - = 10A30 +++	++ +++	+ ++ ++	- - = +++		(adapted??	
3	-54	- - -	- - -	- + +	- ± ±	- - -		= Y9)	
4	-57	- - -	- +++	++ +++	+ ++	- + +++	n.g.		
5									
6		O	HC	neaa	leuc ①	isoleuc ②		val+ile (23)	val+leuc (13)
7	156-1	- - =	++ +++	- - =	± ++++	- ± ±		- + +++	± ++ +++
8						↖ check!			
9		O	HC	neaa	eaa				
10	156-3	- - =	++ +++	- - =	++ +++				
11									
12	156-4.	- - =	++ +++	- - =	++ +++				
13									
14	E-1	O 10A30	HC	neaa	eaa 10A30				
15		- - -	++ +++	+ ++ +++	- - -				
16									
17	Test 156-9	eaa	O	HC	V1+s.	V1	V2	V3	V4
18	on various vitamins.	+ ++ +++	- - - +++	++ +++	- - - +++	- + +++	- + +++	- + +++	- + +++
19					- - ± +++ 10A30				
20		neaa	////////	V6	V7	V8	V9	V10.	V11-yeah.
21		- + +++	////////	- ± +++	- + ++	- + ++	++ +++	- - +	- + +++
22	E-6	O → - - -	-		✓ P3				
23	E-1 + E-6 (Sept).	O → - - -	- 10A30						
24									

Incr. 530P 9. Suppl. A 10A30 ? Retest ~~156~~ 142-17 - Auxanogram.

9A 10 - D +++ A +. Suppl. ± vitamins.
Many adapted colonies. 12M 10. Norm. response. - Control plate somewhat turbid
to new plate 4P 10. Incubate 4h. before suppl ± vits. etc.
12M 10 - Thiamin.

3/29 -- Identify mutants on hand

1. 12N30
2. 4P30

12M30 Bmoc.

12M31 Bmoc. 1) 4P31 2) 10A1. 3/29

		TL	val TL+3	ø TL-4	try TL-5	lys TL-7	arg TL-8	meth TL-9	hist TL-10
1	142-17						-±	± ± +++	
2	142-36 N.G.	- +++	+++	- +++	± +++	- +++	- +++	- +++	- +++.
3	142-54	- - -	- - -	- - -	- - -	- - -	- - -	- + ++	- - -
*	- Postpone								
8	not.		Check 4/2/46 ✓ 4/3/		TL-9 +++		no others.		
*							∴ probably both methionine.		

0 conts. ↓		11	10	1	2	3	4	5	7	8	9
4	156-1				± ± * ✓ +++	± -					
- 5	156-3	-	- - ✓	+ +++	± ± +++	- ✓	- ✓	- - ✓	✓	✓	✓
- 6.	156-4. ± ±		- -	± +++	± ± +	-	-	- -	-	-	-
				No further growth by 10A1.							
(1)	10A30.										
			Check: 4/2 — OK.								
* mycoin prepn.											
other is Tatum's. may not be enough vol.											

6P4. -- Plate 142-17 + 142-54 into T(TL) agar. Incl more: incubate to 9P4, then do auxanogram on Essential e.g. on 54, only "9" = 17 ~~is~~ using double depth agar.

See infra for recheck on 142-17 + 54.

	HL	B	1	2	3	4	5	6	7	8	9	10	A	C	D	
	++	+++														Check!
17			-	-	-	-	-	-	-	-	-	-		-	++	8P5
54			-	-	-	-	-	-	-	-	+	-		-	-	8P5-

Check 54 on Lyrid 10P5. - 12N7(1)

∴ 54 is TL Meth.

>	M	MT	ML	MTL	TL
	-	-	-	++	-

Hydrolysate C. 166a

200 (2×100) T(O) 72 hour culture. Centrifuge cells down, put in 10 ml 6 N HCl, seal tube + keep in boiling H_2O for 20 hr.

Lost during hydrolysis.

Try again.

2/31 - 4/

Mutants by ultra-violet irradiation.

167a.

10A 31 Incr 50ml/125 ml flask coli C-M ē 58 (Tatum's biotinless coli) and grow on shaker, slowest speed, at room temperature.

① 9A1 – 1ml sample to 50ml coli CM.

②. Irradiate in quartz tube, 11 cm from tube, 15 ~~sec~~ min. Incr. 1ml into 50ml coli CM. Grow 1, 2 on shaker.

No appreciable growth in 24 hours. Dosage too high. Try 5 mins. (1 colony at 1:50. dil.)
P2 finally came up.

A2. Use 167A1 + irradiate as above, 5 min. Do in tupl.
11A2.

Estimate (a) before irradiation.
Dil $\frac{1}{500 \cdot 500 \cdot 50}$ + plate into YBG. 1a.

b. after irradiation.

1:1	50^0	710.
1:50	50^1	9
1:2500	50^2	
1:125 000	50^3	
1:6250 000	50^4	

c. In (a) prepare last dilutions in saline also. Irradiate + compare sal. + H_2O after 48h. Do in tupl. 1c.

~~d. Test colonies from b (1:1) and use for 58/2 studies~~:

2a+2c 10P2. O O
3a+c 9[30]P3.

~~d~~. 15 minute irradiation A1.

Before irradiation, plate counts not made
after
from c-M broth 1 ml. 2P#3 dilute 1:25x10⁶ + use method II,
plates 1-5 for contacts (T(o) + drug to ti)
Colonies first appeared 9P4. 10A5 Layer YBG. (Agar too soft).

1
2 Plates so soft as
2P4. 3 to be almost useless.
4
~~#5~~
T(o) 5. Pale colonies before zygal. t₁ > 8/radiation -
YBG resistant.
test on T(o) - all gone —

5 minute irradiation. A2 See 172
i ii iii

Control plate counts: *1a — (last dil in 0.9)
1: 12,500,000. *1c — (last dil in saline).

* sand triality study.

After irradiation, 1b : 1 ml 7 10 } nothing different. Incubate +
1:50 9 } plate alternately.

c) Viability of control in sal, H₂O. — Apparently very low in this dilution.
i ii

Incubate last dilution	1CP2	2a	0	0
flasks 1a, 1c + sample.	10P2	2c	0	0
plate in YBG	P5	3a	0	0
	P5	3c	0	0

Auto anamnesis

I 10A1. halo 2 cm diam. 2 1cm fringe around H.C.

Nothing over 10^{-3} dilutions. By 8P, there was a very faint response to H
At 8P, >3 cm diameter.

II Supplement 10A1. By 2P., a distinct turbidity was visible over HC & a faint one over histidine. By 8P this was very distinct & H. less impressive over histidine. Both ca 2.5 cm diameter..

B). Supplement other portions of both plates, as above, 8^{39}P. (after prolonged incubation.

10^{30}P — 1B-dist turbidity under HC.
— 1 colony.

12 hour incubation probably optimal
maximum size is about or less optimal + maybe reduced for frequently occurring types.

3) Add HC qp 4. — No response desired.

Incubation, lawn at ca 35° unless stated.

~~Auxo~~-auxanography

8P31 - Plate heavily (ca 10^6) into 1(0)2% agar ε·8 (histidine)

I Add, as cooling drops of HC + histidine (10%, .1% resp.) + dil. 1:1000 resp.

II Incubate 1½ h. fast.

III Cover + incubate to P4. add HC. then ~~[illegible]~~ to determine survival.

P2 - ~~#~~ 45 adapted colonies. ←
Try more conc. agar.

P4 ←

IV 4/2-3. Try as above ε 3, 4, 6% agar.

No diffusion to speak of.
∴ 2% is ogt.

V 4/3 - Use of indicator - plate ε·8 as above ε 10r/cc
Methyl Red (Also 20r/ 50r/). Medium is alkaline to the indicator.

156 - 9.

See 161.

4/2/46.

I

Plate 161 - Theavily in 20/0 7(0) agar plate. Add suppl. HC & Biotin to surface 10P

10A - Turbidity increased over HC.
~~Practically~~ clear ~~as~~ under biotin. (Loopful 1v/ml)

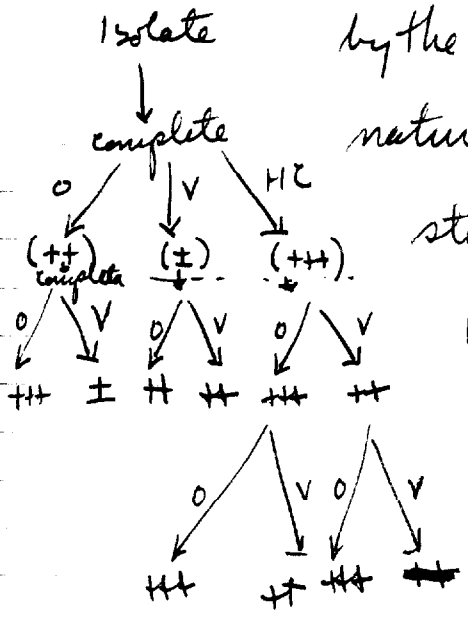
5P. do. The biotin area is definitely less turbid than the rest of the plate. The plate is ~~fairly~~ dense but somewhat darker under HC.

9P4. Differentials essentially disappeared. ∴ 156-9 is not an adapter, but perhaps a slow grower & perhaps inhibited by biotin. Check ē 115! (At best adaptation is not genetic or "mutational")

See 173.

Study of 156-9

1. Isolated by Method C. from V-resyd L15.
2. Responds rapidly to HC, but grows on min.
3. Is slower on vitamins, or on biotin, but eventually grows up.
4. No "adapted" colonies seen on auxanography on 1st attempt (163.)
5. Exp. 161. Grown on HC, Vits and O. (Exp. 160). Then transfund to complete slants, growth tested on O + V. To all appearances, that grown on minimal was most sensitive to biotin. Apparently an exposure to HC or to V limits response to V, but not as up. to complete. Also, growth on minimal reinforces susceptibility. This is not supported by the trial results on the HC line!!! The fundamental nature of the phenomenon is not clear. It should be more striking on plates than otherwise. Also, all these results have to be checked.



I

Inoculum ca 10^6

Data

	Medium	Date	Require-					
1. 58.	T(0)	4/3	Biotin	4/4. 0	4/5 0			
2. E-6	T(0).	4/3	Methionine	4/4. 0				
3 58-5198	T(0)	4/5	indole	0				
4 58-5417			uracil	9				
5 58-5636			indole	>10 (plate minimally turbid) compare 5198				
6 679-680	T		[illegible]	7				
7 "	L		[illegible]	1				
8 "	0		[illegible]	0				
9 679	0		[illegible]	2				
10 58-5631	0		[illegible]	1				
11 58-681	0		[illegible]	0				
12 58-5621	0		[illegible]	0				
13 58-5173	0		[illegible]	0				
14 58-118	0		[illegible]	0				
15 58-336	0		[illegible]	0				
16 679 662	0		[illegible]	0				
17 679-183	0		[illegible]	1				
18 58-4991	0		[illegible]	0				
19 58-3214	0		[illegible]	[illegible]				
20 58-3316	0		[illegible]	0.				

Mutant Reversions

Incr ca. 1 ml of inoculum (ca. 10^6) into T/C, irradiated and incubate 4+ hours; look for adapted colonies. Data on 1104.

Mustard.
E-3-Histidine exp 168

58 (Bertani).
E-6 Methionine. } Probably excess carryover: plates turbid.

Mustard induced — consequently each mutate also.

$(-N\text{-}et)_n + H_2O \rightarrow -NH + EtOH)$

Check for auxotrophic requirement: 58-36 sa

Dex.

4/3/46.

5P3 – Cross-streak on minimal plate:

E-6 (methionine), 58 (biotin) + TL.

P5 no growth.

9:30 P5 – In minimal liquid, the following:

	Exp.				
1	679-680	T-L			
2	"	"			
3	"	"			
4	58-278	B-6	No growth in any by N9.		
5	"				
6	Both	—			
7					
8			10P10	(1 drop × 10^8/cc)	

N9. Repeat above & heavier, fresher inocula. in T(0).

1		TL	±	±			
2		TL	±	±			
11		TL + Bϕ_1	+	++			
12		TL + Bϕ_2	+	++			
13		TL + Bϕ_1	+	++			
14		Bϕ_2	±	++			
21		Bϕ_1	±	++			
22		Bϕ_1	±	++			
31.	In complete	TL + Bϕ_1	–		+	See 176. + +++	
32		TL + Bϕ_2	–		+	++ +++	
33	~~In T(0)~~ test in T(0)	Bϕ_1 + Bϕ_2	+ B	++ B	+ +	+ +++	
			T(0) 1030 P9 – P10	930 P10	P11.	T(0) 930 P10 – P11.	

Note: Try plating out from C-M.

2P9 Cross streak on T(0) agar

41.	Bϕ_1 × TL	nothing by P14.	. . something happened in the complete cultures between the 1st + 2nd tests in min.
42.	Bϕ_2 × TL.		

1927 ...

	Plate #	Total.	Mutants 1230 A.7	Mutants 1130 A.7	#s.Y.		Plate.
	1	218	3 [1-3]	1 [4]			1 [5]
	2	213	4 [6-9]	5 [10-14]			2 [15-16]
	3	220	2 [17-18]	3 [19-21]			1 [22]
	4	194	5 [23-7]	3 [28-30]			2 [31-2]
lost	~~5~~	~~209~~	~~2~~ [33-4]	~~2~~ [35-6]			0
	6	160	1 [37]	0			0
37/	~~8~~	1214	17	14.			6
		Retest					
Y(58--)		T(6)	HC	Vits.	Autoradiogram.		
1		+ ✓					
2		+ ✓					
3		+ ✓					
→ 4	58-Y11	— ✓			HC; D?; ?;		
5		+					
6		+					
7		+					
8		+					
9		+					
Proline 10	Y12	+ +			A-B C?		
11	~~S[redacted]~~	− X+			tentative plate Sl. inh. HC		
→ 12	Y13	+ −					
13	~~S[redacted]~~	− x+			tentative plate		
→ 14	~~S[redacted] Y13~~ Y14	+ −			B?		
15		+					
16		+ ✓					
17		+ ✓					
18		+ ✓					
20		+ ✓					
21							
22							
23							
24							
25							
26							

Ultra-Violet Irradiation: See 167A.

Irradiate 58 coli. 5 mins at 11cm vigorously tube on shaker.

Inoculum before irradiation / ml. ~~(4 x 10^8)~~. (8×10^{-8}).

i	1a. (455) = 5.7×10^9	1c	(249) = 3.12×10^9
ii	1a 97. = 1.23×10^9	1c	(129) = 1.64×10^9
iii	1a. 300 = 3.75×10^9	1c.	(252) = 3.1×10^9
	107		7.8
	3		3
	$= 3.6 \times 10^9$		2.6

3×10^9 = mean.

Survivors = ~~710~~ = ~~7 x 10^2~~ $\frac{7 \times 10^2}{3 \times 10^9}$

$= 2 \times 10^{-7}$

.00002 % survival. in 5 min.

[Ann. these were ca 50/ml after 15 mins, there must be heterogeneity in u-v susceptibility.]

Shake 48 hours + dil: 12.5×10^6. (9p4). Plate 4 in T(13).

2:30 P6. Layer.
12:30 A 7. Pick mutants (ca. 1%.) see data.

[See 183]

All mutants this time were picked 21 hours after layering.

Score in this group is 5/12.

3 probable.

5 mutants in 1200 cells.

comp. ε spont.

5 / 6000.

	found	Exp.
sp	5	1.7
ur.	5.	8

$\frac{10}{7200}$ — Exp. are too small.

$\frac{(8-5)^2}{5} = 1.8 = \chi^2$ too small for sign.

		T(Bod).	HC	Vits.	Durango.	✓
Proline ~~27~~	Y15					
28	~~58-Y14~~	– ✓			HC lz; ?;	
also test: Proline - 29	Y16	+ ✓				
30	~~58-Y15~~	– ✓			HC large zone; D;	
31		+ ✓				
32		+ ✓				
33		+				
34		+				
35		+				
36		+				
37		+ ✓				
Tatum's 58	cantrol.	+ ✓				
58-278	"	– – ✓				
Total: 5 stable						
1214 colonies.						
5580.					HC; AAA???	

4/5/46

156-9 vs. L15.

Biotin

Incr. 3P 9 asmd.

(1) 9A10.
(2) 9P10

	O	O	V.+	Biot.	valuic			
L15	+++ ✓	+++ ✓	− ++	+ +++	+++ ✓			
156-9.	+++ ✓	+++ ✓	− ±	++ +++				
K12	+++ ✓	+ ++	− +++	− +++				

Biotin is then not the only factor + there is some inhibition by a vitamin of 156-9.

Add 1 drop 1N HCl / 10cc
for preservation?

Also consider:

Eff. pH with drop (for pump. preservation).
benzene

Concentration & amt. of substrate.
(Res might be sharper after pre-incubation).

4/4/46. Auxanography—

Optimal inoculum size. Plate into T(B) varying amounts of an inoculum conty. ca 10^8/cc. of 58-278 (B-Φ)

Incubate 12 hours & supplement ē HC + ē Φ. [B4: Phenylalan]

Inoc: 530P.9 Suppl. 1630 9A10:

		Diam. Φ	Diam HC.
1 ml	1	1.8 ++	2.3 +++
.1 ml	2	2.5 ++	3cm ++
.02 ml	3	2cm ++	3cm +
.002 ml	4	? ±	2cm +
1:25,0000 .000004 ml	5	#2 +	4cm +

distinctness

The method can be used at any inoculum size, but is most useful [illegible] varying amounts. For very valuable strains, it may be important.

OPTIMAL AGAR DEPTH. 4/10. Inoc. 1 ml undil culture into varying agar depth. Inoc ~~1130~~ ~~Incubate~~ to 1130P9.

Supplement

A - Φ - at t = 0. B: Φ at 1230P11.

		A (11A.) 730P	B. 230P
1	5 ml	= !	
2	10 ml	nothing	practically nothing
3	15 ml		(pin response)
4	30 ml	nothing	

It makes very little difference what depth agar is used 10–15 ml is quite OK.

TIME OF INCUBATION. Inoc. 58-278 into T(B) agar 9P11

Suppl.		t	9A12	7P12
	①	10P	0	±
	②	12M	+	+
	③	~~9A~~		
quality not.	④	10A12	~~##~~	−
	⑤			
	⑥			
	⑦			

← optimal < 12h.

sample is 6000.

eg. 5 mutants

compare 175.

~~hydrolysate~~ A 1:10.

* Contam. ? Neurospora + thermoph!

4/9 - 10... 1/6.

Spontaneous mutants in 58.

5.30P9 broz t.f. ē coli CM ē 58. Shake at R.T.								
9P10 – dil 1:12,500,000 + plate out by method II in T (biotin).								
Incubate at 35° to 11P11								Plates crowded!
	Count 1130A12	6P13.						
*1								
*2								
*3								
4	1000	1						
5	"		2-5					
6	"	6						
7	"	7	8-9					
8	"	10						
9	"		11,12					
*10								
		126	304.					
Add .1ml of "A/10" in lieu of biotin:								
11.								
Prick to CM 8.30P14 n.v. satisfactorily								
None of the 1st series would have qualified except by statistical criterion.								
	1(B).	v.sm. inoculum from cell pellet.						
1	+							
2	+							
3	–							
4	+							
5	+							
6	+							
7	–							
8	–							
9	–							
10	vg growth in CM							
11	–							
12	vg. gr. in CM.							

4/12/46

Broc. C-M E: 4 shelves A12 — P13

1. TL
2. B ϕ_1
3. TL+B ϕ_1.

12 M13.		T(0)	P16.	P17	P17: add	A18	P19.
Test 1 } 1 loopful	4	−	−	−	1 loopful of	−	−
2 }	5	−	−	−	complete	−	−
3 }	6	±	+++		coli medium		
1+2 }	7	±	+++		to tubes		
					4, 5 and 9		
3 } v.sm. inoc	8	−	−	−	13, 15 + 17		−
1+2 }	9	−	−	≠ −		−	− killed?

See 171. Plate 31. into T(0) + cover. M13. (TL×B ϕ_1 in C-M).

1: 25000 ~~150~~

10 }	No colonies P16	Correct ϕ A1 + B.	
1: 12,500,000 11 }		Not turning over	
	P17	A18	P18
12	3 into CM, 5 shaking at 35° 1030 A13		
small inoc. 13	Test: 0). −	− ≠	+
large inoc. 14	++	+++	

P16.

Test 6 sm	15	−	+++ ≠	∴ the carryover is as much as in culture count of cells!
6 large	16	−	+++	These have no recombination.
7 sm	17	−	− ≠	−
7 large	18	−	−	−

Oceanography.

Preservatives.

I Plate K-12 in T(0). Add drops of HCl – diluted.

Center	Conc HCl (10N)	4 cm inhibition	
2	1:10	1 cm inh.	
4	1:100	No inhibition	try 1 drop HCl/ 10 cc bacteria, etc.
6	1:1000	No inh	
8	$1:10^4$	No inh.	

I. Benzene.

1.	Center –	pure Benzene	clear zone
2.	10 –	Benzene-water.	OK
3.	5 –	Alcohol 95%.	OK
4.	~~Detection~~	Chloroform	OK.

Staphylococcus

Pour plates ~ ca 10^6 organisms aerial + hyph. when hard, streak ~ on surface.

1.	B Φ		
2.	B Φ		
3.			
4/17	V	hyph	II
1.	B Φ	0	K-12

See N-363 for expt. on symbiosis with N: B3757.
E 181!

A19. About each streak is a clear zone, ringed ~ a range of greater density, fading off to ~ small turbidity.

Ans complete P19 — No diffusible zone formed. K-12 somewhat inhibited.

Salt resistance mutants. 150

after Stevens + Tatum.

4/17/46. 58/

1. Plate [redacted], ca 10^8, into complete plates +

1. $CuSO_4$. [redacted] 10 mg
2. $HgCl_2$ .1 mg

A19. Nothing came up. use less.

A Penicillium sp. contaminant did grow on D!

Syntrophism

58-161 + 58-273
14 ϕ,

10 ml T(b) in desm. tubes. Biotin .01γ. Suppl. + inoculate as indicated (in γ/10 ml): 1030 P19. Incubate at ~~37~~ 28°

Desm. readings (galv., uncor. tubes).

#	Inoc	Sup: ϕ	M	930P20.	1P21	330P22	9P22	9P23	Tube #
1	M	10	10	—	+	80	80,	+1.5	
2	ϕ	10	10	—	—	~~93~~	95	—	!
3	M	0	0	—	—	93²	93²	—	
4	ϕ	0	0	—	—	94²	93²	—	
5	K-12	0	0	++	+++	58²	57²	+++	
11	ϕ+M.	1	0		—	94	93²	—	
12	"		1		±	90	83	+++	
(13)			3		±	68	61²	+++	
(14)			10		—	83	75²	+++	
21	"	3	0		—	94'	94²	—	
22			1		—	93	93	±	
23			3		—	75'	63	+++	
(24)			10		+	66	67	+++	
31	"	10	0		±	88	84	+++	
32			1		—	93	92'	±	
33			3		+	84	13³	+++	
34			10	-	±	81³	80²	+1.5	
41	"	0	0		—	92'	92²	—	
42			1		—	92	91'	±	
43			3		—	93	93	—	
44			10.	—	—	95'	do.	—	
		75% = ½ max.				73²	73'		

45. Plate ϕ+M into plates ± Sr methionine; 100γ phenylalanine/plate to see whether this is a good separation. ϕ el. inadequate

~~M~~ 22 to 38°. This does sum to spud things up.

#13

Dil 1:50,50,500 = ~~1:1,250,000~~ = 1:2,500,000. + inoc. into

A. T(B)	0	
B. T(B+M)	40	21.5%
C. T(B+ϕ)	146	

186

FPA.

4/14/46
26 old!

Dr before:

		Supplements.		
	broc	∅	Tyr.	FPA.
51	K-12	0	0	10r
52		0	0	100r
53		10r	0	100r
54		100r	0	100r
55		0	10r	100r
56		0	100r	100r
57		10r	10r	100r
58	58-278	1r	0	0
59		10r	0	0
60		100r	0	0
61		10r	100r	0
62		10r	0	10r
63		10r	0	50r
64		10r	0	100r
65		0	0	100r
66		100r	0	10r
67		100r	0	50r
68		100r	0	100r
69		10r	10r	100r
70		0	0	50r
				100r
71	58-4899	0	0	100r
72	58-5030	0	0	100r
73		0	10	0
74.		100	10	0

Many aspects of this experiment are coextensive with the investigation of the aromatic a.a. mutants + are to be postponed until this is carried through by ELT, et al.

4/19. Utilization of FPA:

			430P20	3 30 P22	9P22	P23
81	58-278	10	–	96		–
82		100	–	97		–
83	58-4899	10	–	96[2]		–
84		100	–	97		–
85	58-5030	10	±?	95	75[3] ∓	+++ (green tint)
86		100	–	67	65' ∓	+++

∓ test on minimal: they grow.

Mutant detection:

Viability

(24h shaken)

Pour 58-278, dil. to 1:12,500,000 into T(6) plates + cover as in mutant detection. 830 P19. Count complete at time t_1. Colony * counts recorded at t_n. Incubate at 38°.

	38°.	hours	h^1 430P20	h^2 830P	10A21	4P21	8P21	12M21	Count.
1	9P19	1	19 5		+++ (creditable variation.				
2	"	1	4			← do			362
3	1130	3	17		+++				352
4	"	3	6			← do			
5	930A20	12	7	< #1.	+++				379
6	"	12	3			← do.			407 (41?)
7	830P20	23		—	++		+++		363
8	"	23		—					5
9	10A21	36			—	±+	+	++	ca. 380. ← too many bottom colonies
10	8P21	48.						++	349
	~~11 2145 48~~							9A22	353
	12+ 1mg ∅	∅	6	∴ 7 hours is barely too long for period II. Past rems 4.9.					

Bottom colonies troublesome!

Repeat 9P20: clearer again as above. Pour bottom layer. Inoculum from 48 hour shaken culture is complete. Incubate 30°.

21	9P20	9	+	++	++	+++		0	131	−9
22	9P20.	9	+	++	++	+++		0	139	−1
23	1P21			±	+	++		16	138	−2
24	1P21.			±	+	++		16	151	+11
25	8P20	2/3			—	+	+++	23	154.	+14
26	8P20	2/6			—	+	+++	23	133	−7
27	930A22			Uneven pour: lumpy.			++	36	139	−1
28	"			" " "			++	36.	123	−17
29	3P23	7:15P.		(5) colonies quite distinct micr. indicates (3-5h)				64	156.	+16
** 30	1130A24.	26°	130P: colonies micr. dist. ing. !!!	—	(7) sm. colonies				145	+5
31	"	30°			(2) sm colonies			3½ da. 86.	135	−5
32	" (2x)	38°			(8) " "				340. (2x)	
33	CM.-P.C.	+		+++				0	146	+16
34	Plate cast	+		+++				0	143	+13
35	"	+		+++				0	138	+8
		11A21.		8P21	12M21	10A22	P23			Δ.

Count. $\Sigma\Delta^2$ =

m = 140. 1097.

σ = ~~9.16~~ 8.85

$\sigma^{140}_{calc.}$ = 14.

Viability of 58-278 at 38° is excellent for ± 48 hours.

* 7 small colonies noted 10P22. punctiform.

Rick colonies 11A30. see 194.

* 15 units = 1 mm.

Auxanography: pre-incub term series,
use Φal.

Put only 2 zones/plate.

Inoc T(B) plates heavily ~ 58-278. ~~Inc~~ 230 P21. Incubate to t~~x~~ at 28°. Then suppl. ~ drop of dl Φelan. 6:50 to 38° incub.

A2L

1A 3:30
B. 6P >A.

2C 8P >>A. Best: 6 hr. preincubation
D 8P: K-12. Inhibited around streak; then zone of growth as supra.

3.

4.

Auxanograms var. unidentified mutants. As above.

8P: ABCD.

58-5880 A,C,(B) C9.,(6+7?) Methionine? Recheck.
175-3 - Inh. HC
175-7 A.
175-8 D.
175-9 C?
175-11 Inh. A,C.
175-10 Inh. hydr. casein. Seems proto.
— 175-12 A✓ D1 inh D7 Proline inh tyrosine
175-14 —
— 172 28 A,D. D1 inh. D7,8 Proline " + cyst
— 172 30 A,D. D1 " " Proline. " + cyst

not a v.g. series.
Need better working.

Check densitometer calibration

4/22/46

Use culture of 278 which has given plate counts of ca. 3×10^9.

1:3 = ca. 10^9.

Green filter 540.

(73) Absolute = 0. Stand = 0.

A. 42'

St. 91^2

Red filter 660

74.

A 53

St. 20mg/ml 25

10mg/ml 48^2

Green filter Abs = 0.

71'

	Galv.	Dens.	Dens/dil
A.	43	367	367
A/2	65.	187.	374
A/3	75'	124	372
A/6.	87	060.5	363
A/9	91	041.0	369.

mean = 369.

m.d = 5

= <1%.

$367 = 3^2/_3 = 11/_3 \doteq 10^9$ cells.

1d = ca. 3×10^6

ca. 10^6

12.3×10^6

1 1 1

50 50 50

Ultra-violet mutations : tryptic

4/22/46.

24 hour shaken tube culture 58-278. Irradiate at 11cm. for 2 mins. Incr 1 ml into another 10ml and shake 24 hours. ( ).
9P22.
Incr 1 ml into coli car + ~~diffuse~~ da Growth quite clumpy, very slow. (sterilization?)

60
125
7500 dil 1:500 - ca. 7500

1:75,000 - 126 These figures checks.

50
0

∴ survival is ca. $500 \times 7500 = 3750000$ out of 3×10^9
$= 1\%$. = 2 mins.

N25. Dil $1:2.5 \times 10^6$ and plate into T(Φ,B). 38°. 5 plates.
1230P26 Larger coli complete.

O
4P26. 9P26. 3P27. 10P28.

1
2
3
4
5
73

nothing *

growing rather slowly.

* There are 12 prototroph colonies on this plate. In addition ca 50-60 new colonies have appeared, first noted at this time. The other plates are similar. Made prototroph list & continue incubation. In addition there is a single colony of intermediate type. 7P27 -

(see also 183)

Dec 194.

4/23/46. no good! Streptococcus FPA. 187.

I 58-278 (ϕ) & 58-161 (methionine). As. 181. Incub. 38°.
Inoc. 1230 A24. T(B) medium. .01 r. 10 r = 1 cc. Add 10 cc.

		ϕ	M.	24 hr. 38 hr.	G d.		
				M24 2P25	11P25	11P25.	11P26
1	M	0	0	−	−	100	
2	"	0	1	−	±	98	009
3	"	0	3	−	+	98	009
4	"	~~5~~	5	−	++	95	022
5	"	0	10	−	+	98	009
6	"	10	10	−	++	95	022
7	ϕ	0	0	−	−		
8	"	1	0	−	−		
9	"	3	0	+	+	97	013 +++
10	"	5	~~5~~	−	−		−
11	"	10.	0	−	−		−
12	ϕ	10	10	++	++	92^2	034 +++
13	ϕ	250	0	−	−		−
21	ϕ + M.	0	0	−	−		−
22	"	~~5~~	~~5~~	++	+++	73	137 +++
23	"	~~5~~	~~5~~	±	++	92	036 +
24.	"	~~5~~	~~5~~	−	±	99	004 +

every thing still clear

II ~~679~~

		ϕ	FPA				
31	ϕ.	100 r	0	±	−		
32		100 r	100 r	−	−		
33		200 r	100 r	−	−		
34.		200 r	200 r.	++	+++	64.	114
		200 r	0. See 13.	−			
		0	0 See 7.				
		0	+ See 181.				

77.

N. G. Old culture medium.
See 193.

58/r - Mutations + Killing.

4/26/46. Incr incomplete. U.V. radiation 11cm etc.

		KRAD			
26.		0. 7×10^8	1:12,500,000.		56.
A.	1	2 MIN.	250,000.	1	ca. 10^5. Killing. 10^{-3} surv.
	2		2,500,000.	0	$\rho S = 3/2\mu$.

					7P27
B.	1	5 MIN.	1:1	.1	1
	2		1:100	1:100	2
	3		1:5000	1:5000	0
	4		1:250,000	1:250,000	
	5		1:4,500000	1:2,500,000	0

Incr flasks of 50 ml Complete Coli ē 1 ml of each dosage above.

D	1	6.	.1	
	2	7.	.1	
	3	8.	.1	
dil c	4	9	1	
	5	10 ✓	1	
	6	12 ✓	1	0 : 2
	7	15 ✓	1	
	8	20	1	
		30		

practically sterile.

This strain is certainly less resistant than 58-278, and should hardly be designated 58/r.

19[illegible] A. 1:12,500,000 1030P27. into T(B, ∅) bottom + cover.

11					0	51
12		1 (31)	Y23-Arginine		1	48
13		1 32				55
14		2 33,34			2	
15.					0	
16 complete.						

250.

3P290 4A30X 10P30 Θ 1030P1 △

Sm colon. Total
4 250

Picks [illegible]. 197

Triple mutants.

4/26/46

24 hour culture shaken at 30° 58-278. ~~Plate~~ .02 ml into CM

Plate unmix. culture 1:12,500,000. into T(B, Ø). 3P26. 192 A.

4P26

	1S0A28	123OP28	10P28	10A29	10P30	
1	—	—	—	—	(35)1	P1
2	—	—	—	—	N. crassa contam.	
3	—	—	—	—	0	
4	—	—	—	—	2	36, 37.
5	—	—	—	—	2	38, 39

Duplicate 2 mins. → 5 total

26	
37	
29	
38	
31	
161	

Layn ē complete 10P27 exc. 3+13.

10. 1:25,000. in coli complete. centrifuge + resuspend. 750 ca. ½% sucrose.

1:25,000 in T(BØ,) etc.

11°	—	—	—	—	4
12	—	—	—	(2)	6
13	—	—	—	—	0
14	—	(1)	—	—	1
15	—	—	—	—	0

0 (not layed).

172	
253	
227	
247	
198	
1099	

10P27. 1 ml into 50 ml coli ( ). 192 B. 1:12,500,000. Bottom ~~T~~ T(ØB)

3P29 O 4A30 X 10P30 Θ 1030P1. △. — 5P3

18h. 36h.

21	°	1	
22	°	3	
23			
24	1	1	
25	0	0	

32

~~x25000~~ ~~750~~

38 x 12,~~500,000~~ / ~~50~~ 2

240 tested

N - contamination e.g. for pich.

26°	°	1	
27°	0	3	
28°	°	2	
29°	0	0	
30.	°	2	
31 complete.			

20.

35
51

1/238 =

Cover ē salivie - glucose - agar.

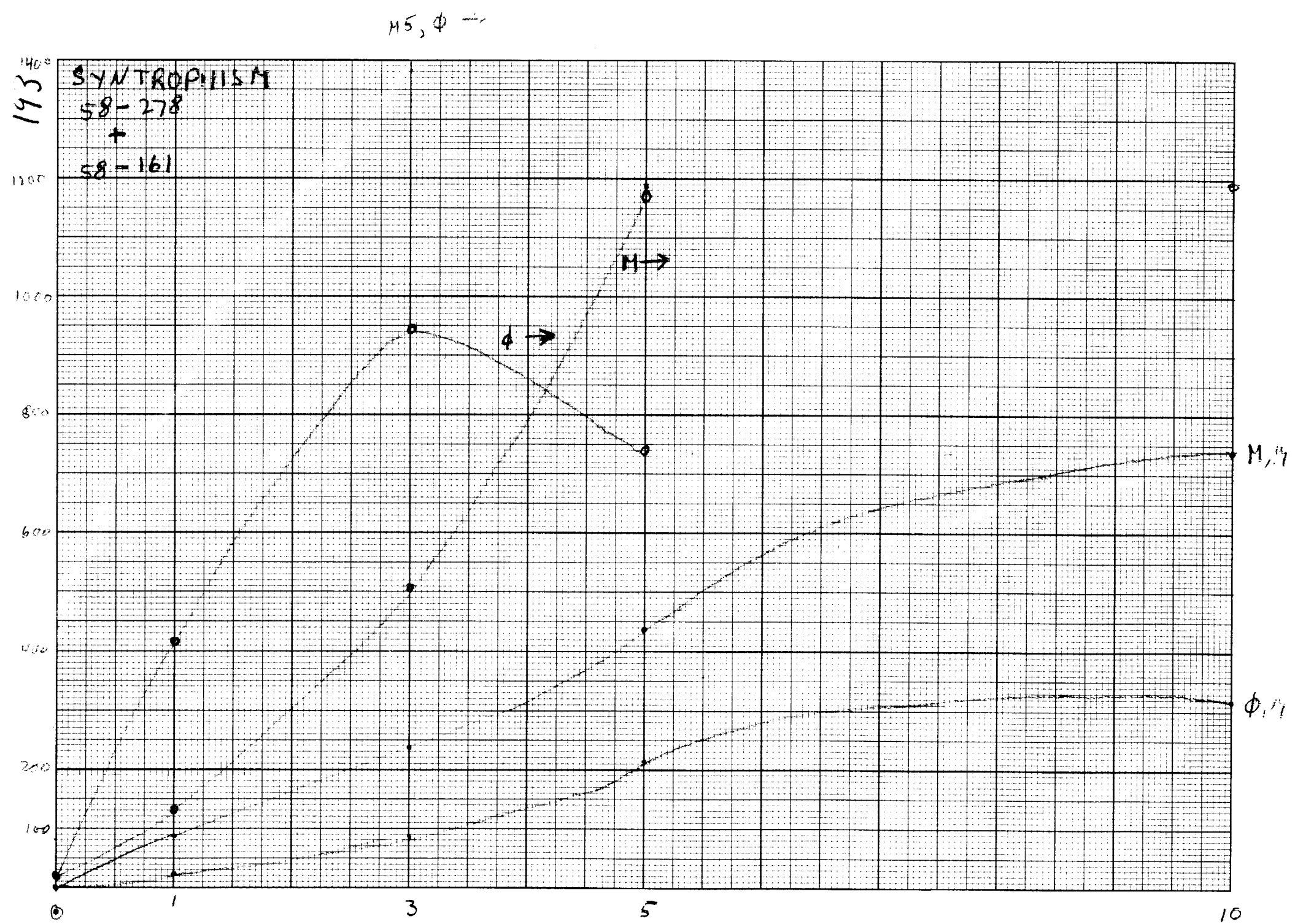
Layn ē complete 10A29.

Prototrophs unusually large here!

Incubate 1-15 at 31° after layering. do. 21-31.

Pick 1947

M5, Φ —



EUGENE DIETZGEN CO.
NO. 340-20 DIETZGEN GRAPH PAPER
20 X 20 PER INCH

Syntrophism

Meth. – ØAl.
Proline.
Use fresher cultures for inoculum.
4/27. Inoc 1230A28. 31°.

58-278 (Ø) and 58-161 (M.) As before 10 ml T(B)+.

	Inoc.	Suppl Ø	M.	10P28 11A29 (77) G	D'	34h. 48h. 12M29. (77)	60h. 72h. 12N30. 11P30 72² 77	% M.
1	Ø M	5	0	— 100		100	100 100	
2	"	5	1	± 98	88	98³	98³ 98	
3	"	5	3	+ 94³	235	94²	94 94³	
4	"	5	5	+ 90²	434	90	91² 90	
5	10	5	10	+± 84²	731	84'	85 86²	
6	"	5	100	++ 63²	1973	66	67² 66	
11	Ø	0	5	– 99²	22	100	100 100	
12		1	5	– 99²	22	98³	98³ 98³	
13		3	5	± 98	88	97²	96 92³	
14		5	5	± 95'	212	93³	88' 77² *	No protoh. on plating!
15		10	5	+ 93	315	90.	79 * 73 *	
16		200	5	+++ 61'	2129	60²	61' 61	
21	M+Ø	0	5	+ 90'	446 -M5 12	89'	81 72³	54
22		1	5	+ 82'	849 414	71	74³ 77³	44
23		3	5	+ 72³	1082 948	71	72' 72³	
24		5	5	+ 76'	1111 743	70	73² 73	
25	~~10~~		5					
31		5	0	+ 96	177 -D5	95'	62' 63	
32		5	1	+ 42'	321 139	74	67 69³	41
33		5	3	+ 84³	718 506	67	71 73	
34		5	5	++ 72³	1352 1170	67²	72' 72	50
35		5	10.	++ 72'	1412 1200	71	73² 74	

plug wit

679-183 (P) + 679-662 (G). in T(the. ½ mg). N2

		Proline.					
41	G	~~Ø~~ 0	— 100	100	100 100	—
42		3	— 100	100	100 100	+++
43		10	— 100	100	99² 97'	+++
44		100	— 92²	84	83² 83	+++
~~45~~						
51	P.	0	— 100	100	100 100	—
52		3	± 97'	97³	98² 96² 97²	+
53		10	± 96	95	96² 93² 93³ *	+
54		100	+± 69'	68'	68³ 68²	+++
61	P+G	0	— 100	100.	100 100	—
62		3	± 98'	96	81 79'	+++
63		10	± 96³	81³	74' 79 *	76³ +++
64		100	+± 66³	68	68 66'	+++

Note – change to EDST. 2A28.

* Adaptation.

1 A.M. 4/20/46.

Remove .1 ml of culture ~~[illegible]~~ from tubes 22 + 3 ~ and plate into 6 T(x) at a dil. by ca 1,250,000. Bottom + conn. After counting 1 mutant, agar + other supplement + count like other. Dil. only ~~[illegible]~~ 500 for 1, 3) plates.

		3P2 L	S.	Total ✓	M %.	
etc						
22a.	52. (→ 117) (r. small colonies) ~~[illegible]~~	117	125	(251).	46.5	later: 42.5
b.	114. 10P30. (→ 125) ~~[illegible]~~	w.T. 0 (!)	125	94?	43.0	
0L a.	67. (later 114 →) ~~[illegible]~~		114.	149 d = 1308.	43.2	39.3
b.	141 ~~[illegible]~~	w.T. 00.1%				
		145 ~~[illegible]~~			do.	

3 22c 3 colonies/sem 10P30. (1:500.) ✓ Dilute 1 to 2500 for later c mutants (later, at 4P1, d = 1739.).

22c. 0. ✓

Notes Bror. 1A30.

Layer c later: 4P1 (i.e., 22a c phenylalanine).

At 3P2, examine + recount. Small colonies of the heterologous mutant have appeared.

Early counts are erroneous. Methionineless are much slow to develop initially on overlaying. At 3P2 they are however quite distinct but too small to be counted readily.

930P2: Φ smaller colonies intergrading with large but still distinguishable.

M/Φ small colonies still minute, but enumerable. Count A3

N3 - M colonies still v. small. Not properly enumerable

Analyze syntaplue cultures.

4P1.

Culture	M v	∅ v	G	d Dilution	% M.	Colony counts on: T(B)	T(M)	T(∅)	T(M)+∅	T(∅)+M
Two single progeny lines.	193-21	5	0		54	10^3 >10^4-5$10^6$ ✓	194	≠10^6 170	369	380 ~~369~~
	-22	5	1		44	" 0	106	≠ 144	230	247
	31	0	5		—	>10^5 (N)	— (N)	—		
	32	1	5		41	ca 3000 (N)	133	" 202		357.
	34	5	5		50	" 7	(N) 158 ✓ ≠ 161			330.
last 58-278 adapted.	14	5	5,			(106) - 0 ! /////	≠10^6 - 246			

What is legit. of adaptation here.

62 10v

T(G)(T.) T(T). T(0 + ✓). (ca ¼ missing) (partplete.)
(N) 392 166 0 ✓ 380.

∴ assume that another ¼ = prolineless.

1st counts 930P2

tagged complete 12 Nov. these marked ✓

Eye differential is maintained indefinitely.

Thus, culture 31 was adapted (no detection in kind of M or ∅ cells)
21 is also adapted, and contains, undoubtedly, 3 cell types.

		% M.			% M.
21	M	52.5	34	M	
21	∅	55.3.		∅	~~54.6~~ 49.7
	R	53.2		R,	49.5
22	M	46.0			46.0
22	∅	~~43.5~~ ~~44~~			43.7
	R	42.5			42.5
31	M				
31	∅				
	R	**			
32	M	—			
32	∅	43.5			
	R	39.3			

W.T. = .9

Spontaneous mutants.
small colony variants.

58-278

Azo Pick colonies on 183 plates to 1 ml coli complete Eq.

		Complete	Test on T(B,Ø).	to CH plants P7.	Test on Ind	Test BØ 5/11	BØ 5/18	– Azo
183-32	1	+	–		–	+	+	+
	2	+	–		–	–	–	–
	3	–						
183-29	4	+	–		–	–	–	✓
	5		–		–			
	6	"	–		–	–	–	✓
	7		–					
183-31	8		–	v. sparse	incr. in cavity	–	–	✓
	9	"	–	n.g.				
	10		–	do.		±	±	✓
183-30	11		–	ng P22				
58-278	12		–		–	±	±	✓
spont ↑	13	"	–	ng P22				
	14		–					
			P1 – 7.					
186-15.	21	+	–		–	–	–	✓
	22	"	–	ng		±	±	
>70%	23		–	ng	–	–	–	–
	24		–					
on 58-278	25		–		–	–	–	–
+ uv	26		–					
	27	"	–		–	–	–	✓
	28		–					
	29		–		–	–	–	✓
	30		– *			This half a gd count		

21a. to Sml complete; 10 P1; grow on shaker at 31°.
Came up slowly + to a low level.

* suspicious consistency.
medium later found n.g.

58-278 ++ ~~++~~

Every O was a photograph.
These are the 5 hour colonies.

Are must examine plates daily

for 3-4 days.

Syntagheum

58-278 + 58-4899.

4/30/46. 530PM

T(B) 10 ml +

		10 P I	N 2
1. 5 r del	58-278	++	++
2. "	58-4899	++	+++
3.	58-278 + 58-4899.	++	+++

4.9. - too much del

4/29/46. Synthoplasm - plate pour. 1945a

Pour plates of T(B) or T(T) ± various organisms as indicated 230P29. Incubate at r.t. to 230A30. Inoc. surface ± homologous & heterologous E. coli + ± Neurospora 5801. & 21863.

Inplate:	Inoc:	1	2	4	3	
58-161		HC	-278	homologous	N. 21863	
		+	Sl. growth of streak	"		5P1.
			considerable "P3.			
58-278		HC	-161	"	N. 21863	
		+	—	—	—	
679-183		HC	662	"	N. 21863	
		++	sl. growth str.	—	—.	Clear in plate ± stim. zone
679-662. (turbid-camphor)		HC	183	"	N. 21863	
		+	—	—	—	
-662. no penicil.		+	N. 5801 growth; no coli stim.			
0					N. 21863	

679 – 662

Response of [redacted]
to glut + prol.

4/30/46. Is response to prol, which is delayed, an adaptation?
5 30 PM.
hroc T(T) + suppl. ε 679-662

1. proline 200 r
2. glut. ac. 200 r

abandon temporarily.

Used 1 – 2 transfers in each soln.

* different colonial appearance. Stain à l'acide-fuchsine: épiplocoques : ++

58-278

Cystinelees

1) Spontaneous:	5 / 161.	.15		.031
2) U.V. – (t=0)	10 / 1099	.001	.014 197-61	.0099
3) U.V. (t=24h)	(24 / 240.)			.10

χ^2 tests:

	f_o	f_e	
1) – 2)	2	5	161
	13.	10	1099
		15	1260

$$\chi^2_{unc} = \frac{(2-5)^2}{2} + \frac{(10-13)^2}{13}$$

$$= 5,2. \quad p = .023$$

$$\chi^2_{cor} = \frac{(3-5)^2}{3} + \frac{9}{13}$$

$$2.0.$$

$$p = .15.$$

	f_o	f	
1) – 3).	12	5	161
	17	24	240
		29	401

$$\chi^2 = \frac{(5-12)^2}{12} + \frac{(24-17)^2}{24}$$

$$= 4.1 + 2.0$$

$$= 6.1.$$

$$p = .014$$

	f_o	f	
2) – 3	28	10	1099
	6	24	240
	34		1339

$$\frac{(10-28)^2}{28} + \frac{(24-6)^2}{6}$$

$$= 11.6 + 54$$

$$= 66.$$

$$p = << .001$$

Pick "mutant" colonies to coli complete

197

See 194

5P3

All colonies except O were of desired type at picking.

Plate.	Design.	#	Complete test.		BΦ (again) c.d	BΦ test	BΦ		BΦC
91-12	X	31	+	A1	From plate 5/11 ↓	+ A12	−	−	✓ Arginine
	X	32	+	2		+	±	±	✓
91-13	X	33	+	3		+	−	−	✓ Leucine
91-14	X	34	+++ !	4	+	+++	Seems to grow unusually rapidly. *		
92-1	Δ	35	+	5		−	−	−	✓ +
92-4	Θ	36	"	6		−	±	−	✓ +
	Θ	37		7		−	±	−	✓ +
92-5	Θ	38	"	8	—				+
	Θ	39		9		−	±	−	✓ +
		40		10					
92-11	Θ	41	"	11	×				+
	Θ	42		12	×				+
	Θ	43		13	?	−	−	−	✓
	Θ	44	"	14		−	−	−	✓
92-12	O	45		15		−	++	✓	✓
	Θ	46	"	16	×				+
	"	47		17	×				+
	"	48		18		−	±	+	✓ +
	"	49	"	19		−	±	±	✓
	"	51		20		−	+	+	✓
	"	52		21		−	−	−	✓
	[illegible]	[illegible]	"	22					
	Δ	54		23		−	−	−	✓
	"	55		24		−	−	−	✓
	"	56		25		−	−	−	✓
	—	57		B1	✓	↓	+	+	✓
92-[illegible]	[illegible]	58	"	2			−	−	✓
92-14	Θ	59		3			+	+	✓
	Δ	60		4			−	−	✓
	O	61		5			−	−	✓ −
92-15	Δ	62	"	6			−	−	✓
	Δ	63		7			−	−	✓
92-21	O	64	++	8		±	++	✓	++
	Θ	65	+	9			±	−	✓
	Δ	66	+	10			+	+	✓
	—	67	−	11			+	+	✓
92-22	Θ	68	[illegible]	12			+	+	✓
	Θ	69	[illegible]	13			[illegible]	−	✓
	Θ	70	+	14			+	+	✓
	Δ	4c. [illegible]	[illegible]	15					
92-23	—	71	[illegible]	16					
92-24	—	72		17			±	−	✓ +
	X	73		18		++	±	++ ✓	✓
	Θ	74		19				−	✓
	Δ	75		20			+	+	✓
92-25	Θ	76		21			±	++	✓
	Θ	77		22		++	±	++	✓
92-27	Θ	78		23				−	✓
	Θ	79		24			[illegible]	[illegible]	✓
	Θ	80		25			±	±	✓

Transferred from plate 5/11 to 5/18. — A20.

remeasured, broken disp 58-278 by P12.

1976.

							B ϕ C
192-29	—	81	+	C1	broken.	± ±	✓ +
-30	⊖	82	+	2			+
17-22	—	83	+	3		− ±	✓ +
				4		++	
58-278				5			
58							

An exceptionally high proportion of mutants is indicated.
These have to be auxanographed now.

~~With~~ By series 197- 35 to 82, 58-278 treated ō u.v.

5 - grow on minimal

39 . grow on biotin + dal + cyst.

1 (#61) - ?

Auxanography: 194 + nutrients. 197 C

Plates w obstacle. Pour plates 10 PB. Inc 30°
1230 AP – A) ø 10x B) etc. Inc 35°

5/18/46.

	5A19.9A	Sp			A24 T(BCφ) Final identity		Y–
Y11	A D	D SA	1	Proline –			
Y13							
Y14							
718	A C	C SA		Turbid			
Y19	A B/ C	B SA		– 1. (Thiamin) ✓			
Y20	A; D, B C	D SA		Turbid			
2							
4	A						
6	A B?						
Y21	A D A turbid.						
25	A						
29	A —						
Y12 D6 AD 31	A C	C SA		– 8 Arginine.			(23)
5580 A 02	A —						
37	A AD	D IIA		1??			
38	A D: A						
35	A —					+	
36	A —					+	
37	A —					+	
39.	A —					+	
43	A C	C IIA				+	
44	A C	C IIA		———		+	
48	—					+	
12 N – put φ alat 49	A —					+	
C, B, D. 51	A —					+	
52	A —					+	
Proline del. 54	A —					+	
55	A —				Growth on	+	
Error! 56	A —				T(cyst)	+	
57	—				liquid.	+	
58	AD	D IIA	6		BφC		24
59	AD AD	D IIB	6		"		25
60	A				"	+	
61	turbid A					—	
62	AD	D IIA	6		BφC		26
63	AD	D IIA	6		"		27
65	A D	D IIA	6		"		28
66	AD	D SA	–6?	(Cyst) ✓	"		29
where? 67							
68	A D AD	D IIA	6		BφC		30
69	— A				"	+	
70	AD	D IIA	6		"		31
72	A —				"	+	
74					"	+	
75	A D	D IIA	6		"		32
78	AD	D IIA	6		"		33
79	A AD	D IIA	6	Cyst	"		34
80	AD	D SA	6?	Cyst	"		35
81	A, D: D:	D SA	6		"		36
83	AD	D SA	6		"		37
21	A —						

Method A: Bact. hydrolysate C 198.

Inoc. 200 ml in 500 ml Erlenmeyer ~ K-12, on shaker 31°. OP 5/3

1. Coli complete

2. T(o).

① Harvest OP 6 (& wash superficially) into 20 ml 6N HCl. reflux 1A7 – 1P7 (calc 6 x 10" cells)
distill off HCl & water to volume of ca 5 ml.
Centrifuge humin down, dilute to 20 ml & store as hydrolysate 198.
(light golden brown color.) for future titration & assay.

The T(o) has not been growing well.

B/r mutants.

5/8/48

Inoc 10ml exp ī B/r from const slant. 1130A8.
1130P9 d = 1.4900

Irradiate as above for ~~20 min~~. t. (ps. calc. as 4.9/min.).
Construct killing curve points.

	Rad. t.	Dil.	Count.	pS.	pS/t
1.	0	$1:12½\cdot10^6$	86.	1.2×10^9	0
1 "	"	"	83.		
2.	20 sec.	10^2	>>		
a 3		10^4	ca 10^4		
4.	.	10^6	~~508~~ ca 900	$.9\times10^9$	.12
5	60 sec.	10^2	>>		
6		10^4	ca 10^3		
6. 7.		10^6	558	$.56\times10^9$	.33
8		.1	>>		

Inoc 1 ml of a and b into 10 ml soln.

Use. b. only. Apply mutant method. 1130P10.
dopt = 1.6120
dil. $2:12.5\times10^6$
104.0 28h. Δ 60h.
12M12 6P13 3A15

1	1,2,3,4		marked colonies all still dist. small
2			
3			
4			
5			
6	5	2	not very sem.
7		7	
8		8	
9			
10. not layered.	6		
	6.	2	

650.
~~165~~ 650

1130P11 (24 hours) - colonies in T/O visible, but very small. Hold ∞ layer.
145 P12 38 hours. - good colony spread; sight sl. small but uniform.
layer ī ∞ 2U P12.

1130P12 colonies sl. larger than (10).

Pick to complete liq. 1ml:

Pick to complete. A21.

	G(∞)	G(0).
1.	O	+
2.	O	+
3.	O	+
4.	O	
5.	O	
6.	O	h.g.
7.	Δ	
8.	Δ.	
3/2		++

Kymograph 5/23.

3 - C8 - Arginine
4 - C1-2 - Leucine or valine!

Bact. Hydrolysate D. 201.

5/9/46.

Broz K-12 into 200ml T(0) es198. Use fresh culture.

A. - unshaken 9P9.
B - unshaken

medium n.g. Repeat All.

see 200, 202 ff.

Precursors + bacterial mass activity.

Remove alcohol from bacterial suspensions 2 and 7 by centrifuging + decanting, and hydrolysing by refluxing ~ 6N HCl 4 hours.

Calculate product in "density units"/ml by dividing original optical density by the ~~dd~~ ultimate concentration. e.g. #16 hydrolysate if final volume is 20 ml and 100% recovery is assumed would be:

$848 \times \frac{25}{20} = 1060$ d/ml. This may be useful in calculating recoveries of various substrates.

Precursors and filtrate activities

5/11/46.

1. Inoc ≡ K-12 the following. 10P11 31° (on shaker = ↵)

	Coli Minimal +	Inoc.		2P12 Growth	1130P Growth	Final growth G 1:10.	d
1.	+0	500 ml	250 unsh	+	2+	96	177
			250 shaker		3+	82'	848
2.	glutamic ac. 5 mg.	50 ml		+++	++++	67[2]	1707
3.	anthranilic ac. 5 mg. Harvest 10A16	"		—	±	86	655
4.	citrulline 5 mg.	"		++	4+	71[3]	1442
5.	~~anthran~~ pantoyl-lactone 1 mg.	"		++	4+	76[2] lost.	F163
6.	phenylalanine 5 mg.	"		+++	4+	not done.	
7.	0	"		++	4+	71' (74)	1472

The most efficient growth is evidently in small shaker flasks.
Harvest N14. Save filtrates & bacterial mass.

(Pool bacteria of # 1, ≡, ≡ for hydrolysis. Preserve filtrates in alcohol. Collect in 25 ml 6N HCl + reflux 4P14. — 3A15 ca 11 hours lost P14.

E. coli K-12 50 ml.
8. pantolactone 1 mg
β-alanine 1 mg

84 ... lost on centrifuging (73+)

N. crassa ≡.SY7 in Fries + 50 ml.
9. pantolactone 1 mg
β-alanine 1 mg.

Harvest. 11A17.
Sample 5 ml (st.) from 9 add to 5 ml F(0)
pantolactone & inoculate ≡ 5531.

BA15. 30°

(79)

Droc.	1	2	3	4	5	6
	0	202-7		1 ml	1 ml 202-7	1 ml 202-7
1 58-3214.	"	1 ml	.1 mg glut.	202-2	.1 mg pro	.1 mg gl
	−	− ± ±	−	0 −	±	++++ − ±
	100		100	100		99
2 RS72-228	−	" "	.1 mg cit	202-4	.1 mg arg.	.1 mg cit
	−	± ±	−	0 ±	++++	++++ ± ±
	100		100			
3 58-5030	"	"	.1 mg pel	202-6	.1 mg tyr.	.1 mg pel
	−	− ± ±	−	− ±	± ++++	± 0 ±
	100		100			
4 K-12	"	"				
	+	+++ ++ +++				
	+++	+++				
5	"	"				
6	"	"				
7	"	"				

all ± are ca 79, indicating traces of substrates. Larger quantities of pitrates should be tested, since these are def. by 1:10.

1st reading "P15.
2d reading A16.
3d reading 9P16

Interaction of nutritional requirements

5/10/46.

679 – 183.

Inoc 1130P10. (10hour culture!)

	T. (r)	P. (r)	830P11. 21.4.	
1	500	0	100	
2	"	10	93	
3	"	30	(81')	proline limiting value.
4	"	100	62	
5	"	300	63	
6	1	500	100	
7	10	"	97	
8	30	"	93'	
9	100	"	–	
10	300	"	(83.5)	threonine limiting value.
11	1000	"	71'	
			59	
12	100	10	94	
13		30	84	← not depressed below T limit.
14		100	±	
15		300	±	
			±	
16	1	30	100	
17	10	30	97.3	} 11 to T response at higher proline.
18	30	30	95	
19	100	30	83 ←	
20	300	30	81'	
21.	1000	30.	82.	

(78')

mis nion?

The requirements seem to be independent, with a sharp cut-off when the limited growth is reached. Set up another, cleaner expt. to establish this, using levels of

Proline = 40 r

Threonine = 100 r.

α = 6 79 18 5

5 = 5 8 · 16 1.

5/13/46. Inoc BA 45. 30°

		Inoc	Biotin mg	Meth.	T. r	P d r	11P15	10P16	8 M17 (to M17)		
1		—	—	50	(10)	±	+	89	505		
2	α	—	—	(500)	10	±	+	98	281		
3	3	3	(100)	—	—	+	++	83'	796		
4	3	(10)	3	—	—	±	+	94'	259		
									D4.	ΣD calc.	
11	α + β	(10)	3	(500)	10	last.	head grown to tip				
12	"	(10)	3	50	(100)	+	++	77'	1121	538	
13	"	.3	(100)	500	100	+	++	76'	1177	1077	
14	"	.3	(100)	50	100	+	++	73^2	1337	1301	
15	"	(10)	(100)	—		+	++	70^2			
16	"	—	—	50	100	+	++	74'			
21	β	.3	3	—	—	+	+	$+92^3$,	339		
22	α	—	—	50	10	+	+	+ 91	398		
23	α +	.3	3	50	10	++	++	$++79^3$	982 > .		
									A18	A18	
31	α + β	10	—	500	—	—	—	—		—	
2		"	.01	"	—	—	—	—		+++	Aco
3		"	.03	"	—	—	—	100		—	+++
4		"	.1	"	—	—	—	100		—	
5		"	.3	"	—	±	±	+++59,	+++	+++	
6		"	1	"	—	±	±	± 96,	+++	+++	
7		"	3	"	—	+	++	$+++65^3$	+++	+++	
41	β	"	—	"	—	—	—	100		—	
2		"	.01	"	—	—	—	— 100		—	
3		"	.03	"	—	—	—	— 100		—	
4		"	.1	"	—	—	—	— 100		—	
5		"	.3	"	—	±	±	± 99		±	
6		"	1	"	—	±	±	± 97^2		±	
7		"	3	"	—	+	+	+ 93^2		+	
51	β	"	3	"		+		+		+++	
52	"	"	3	"		+		+		+	
53	"	"	3	"		+		+		+	
54	"	"	3	"		+		+		+	
61	58-278	"	balance. 3			±		±	+	+++	
62	"	"	3			±		±	+	+++	
63	"	"	3			±		±	+	+++	
64	"	"	3			±		±	+	+++	
65	α +	soli <									
66	β	—	100			—		++ 77^2		! adapt?	

10P16 - mice 51 and 52 = 58-3214. (α) 77^3

See 207

Synthophism - Sex. 20%.

679-183 x 58-161.

½ values - 81 ca. ongalv.

This experiment is designed for:
a) critical conditions of synthophasm:
b) substrates in culture medium
c) recombinations.

679	200 r	T
183	30 r	P
58	.7 mr	B
161.	10 r.	M

1st. Need data on interaction of requirements. — Independent.

a. 4 1:1 interactions: use excess and .1 optimal

a. BT	+ PM
b. BP	TM
c. MT	BP
d. MP.	BT.

Analyse for recombination types.

2:2 .1 optimal for each.
e
f
g
h.

3:1. , 1 opt for
BM
TP

Critical conditions:
a. delayed inoculum
b. excess BT. provide M in range 0-5 r.
c. 278 adapt. series at 5 r.

Dysentophism.

10γ proline

All 58- cultures ± .01γ Biotin

679- ± .5 mg threonine

#1130P10. 30° 5/10/46.

	hor A	hor B.	Supp.	1130P11.	1130P12	1130A13	ka (78)	
1.	58-3214.	—	BT.	+	+	+	85	
2.	—	679-183	BT.	+	+	+	89 3	
3.	—	679-662	BT.	−	±	±	98	
4.	58-3214	679-183	BT.	++	+	+	88	
5.	58-3214	679-662	BT.	+	+	++	74 2	Syn. !!
6.	58-3214	—	B	+	+	+	90'	
7.		Y1	"	+	+	+	93 3	
8.		Y1	"	+	+	+	93 3	
9		Y13	"	−	−	−		proline req?
10		Y13	"	+	+	+	89	
11		209-301R	"	++	+++	(autol?)	77	
12		209-301R	"	++	+++		74'	
13		58-2651		+	+	+	90'	
14.				+	+	+	90	
15		58-3232	"	+	+	+	90'	
16				+	+	+	89	
17		58-52.55	"	+	+	+	87 2	
18				+	+	+	84 1	
19		5450	"	−	+	+	91	
20				+	+	+	91'	
21		6049	"	−	+	+	91'	
22				+	+	+	92	
23		6177	"	+	+	+	89 2	
24				+	+	+	91	

All available prolineless are identical exc 679-662 which uses glutamine.

Note 1 cell contains ± 10^{-12} g H_2O.

at pH 7. the $[H^+] = 10^{-7} \times 6 \times 10^{23} \times 10^{-3}$ g.

$= 6 \times 10^{13}$ molecules/g.

This is ± 60 H^+/cell. at pH 7

.1% of the ions, there will be only 30

A daily requirement of 30 r / 6000 r is quite reasonable for protein 10-40% of the dry wt.

dry wt./cell $d = 3 \times 10^6$ cells. = ca 1 r

$\therefore$ 1 cell $\doteq$ .3 rr dry $= 3 \times 10^{-3}$ dry.

ca. 1 rr wet.

This is less than previous estimates: $(8 \times 10^{-13}$

Bacteria production.

T(0) in 4 liter lots in 4 l. Pyrex bottles.

1.
2.
3. Without asparagine.

Aerate by aspirator suction; cotton - in glass air filter.

Incr 14-12 2A15. 5/15/46. Room temperature.

11A16 — 1, 2 ++ 3 ± | A17. 3+++.
12M16 — 1, 2 are nearly opaque; 3 ++. growing slowly
930P17 — all indistinguishable. Harvest - pool in large carboys + put in cold box.

1. 1.
2.
3.

Both are 90^2 at 10:1

Remove 250 ml samples of 2 + 3 for centrifugation.

Allow to settle in cold room at 0°C. for two weeks.

P2 - separate by siphon into (1) 4l., (2) 4l., (3) 3l., (4) 1l. fractions, ca.

and reject fraction 2. Allow others to settle further; centrifuge 500 ml samples from 3 + 4 and collect + accumulate the harvest.

PS. Decant accumulated harvest after adjusting in saline - note strikingly (orange) Cells highly pitted - and wash ± H_2O. Dry 1) over $ZnCl_2$ 2) at 100° 2-3 hours. Extract ± 100cc Et_2O overnight.
Dry weight was 3.406 g. Assume ca 75% yield of the bacteria, approx.
After extraction 3.318 ca 3%
There were $12000 \times 10 \times 43 = 5,160,000$ 5.2×10^6 density units ≡ 3.406 gm. or 1 density unit ≡ 1γ dry wt. bacteria. and average culture as minimal 10 units ± G = 60 has about 2 mg dry bacteria in it.

Assays of Yhom hydrolysate Neurospora.

Mor 1A6. 30°.

A. Biotin. SY7A. 9A8.

1. ~~206~~ c "Biotin"-free Fries. ±
   SY7.
2. do. + [redacted] +++
   1ml 206A.
3. do. + 1ml 206A + .05 r biotin. +++

B. Inositol. 34701 a.

1. 10cc Fries. —
2. + 1ml 206A. —
3. + 5 ml 206A. —
4. + 5ml 206A + 5r inositol. +
5. 5r inositol. +

C PAB. 1633A

1. 25cc Fries. —
2. " + 1ml 206A. +++
3. " + .1ml 206A +
4. " + 1r pab. +++
5. " + 1ml 206A + 1r pab. +++

While "appreciable" biotin and pab are available, there is no detectable inositol in this fraction of E. coli.

6/6/46.

Take ca 1 gm sample (1.014 g.) + reflux c. 18% (6N) HCl at 100-110°.

at 4 hours digestion, 20.5 cc was present. Remove 5 ml sample + continue digestion. — make up to 20 cc. lost.

Remove another 5 ml. $= \frac{1}{4} \times \frac{3}{4} \times 1.014$ g.

$= .190$ mg

neutralize c NaOH. Store as 1% bacterial hydrolysate Concentrate + assay 4 hr. hydrolysate 206a.

Continue hydrolysis of remainder to 24 hours. Concentrate, neutralize and dilute to a conc. of 20 mg/cc. Store in cold

See 234 for proline, arginine assay of hydrolysate

Sex; syntrophic ratios.

Use 204 - syntrophic cultures:

Mixtures of BM. + TP.

P. 5/17/46.

#	Growth excess:				P19	Layn:	%BM.
1.	11. Lost						
2.	12 BT.	Plate	1:1000	in T(O).	0		
3		"	1:1000	in T(BT)	3	1-3	
4		"	10^{-6}	BT	0		
5		"	10^{-6}	BM -			∞
6		"	10^{-6}	BM. ✓			PT
11	13 MT		10^{-3}	O.	0		
12			10^{-3}	BP	0		
13			10^{-6}	BP	0		
14			10^{-6}	BM	-		∞
15			10^{-6}	BM.	✓		PT
21	14. MP		10^{-3}	O	0		
22			10^{-3}	MT	0		
23			10^{-6}	MP	0		
24			10^{-6}	BM	✓		∞
25			10^{-6}	BM.	✓		PT

~~15.~~ Wash:

31	65.	~~10^{-7}~~	~~∞~~			
32		10^{-3}	O		0	
33		10^{-3}	BT		4	4-7
34		10^{-3}	BP		0	
35		10^{-3}	MT		0	
36		10^{-3}	MP		1 cont? ✓ 8	
37		10^{-7}	BM.	- ✓		∞
38		10^{-7}	BM.			PT.

Where possible recombinations colonies are present, pick to a complete ~~Bq~~ plate (BT). A21.

Note: only B-T. Why?

See 212.

P21. to slants (A, B, C) + test on:

Retest on variants = #5.

	T(O)	T(B)	T(T)	T(BT
	1922 P922			
1	— ✓	± ✓	± ✓	+++ ✓
2	— ✓	± ✓	+ ✓	— ✓
3	— ✓	± ✓	± ✓	— ✓
4	— ✓	± ✓	± ✓	— ✓
5	± ✓	± ++	± ✓	+++.
6				
7				

A BT strain? Call it 58-619 x.

Why no growth? trace neg.

Streak out 1 on a complete plate.

Sex

v. 204. 679-183 α × 58-161 3

5/21/46. 30° 2A 23.

	hrs.		Biotin mγ	Methionine γ	Threon. γ	Proline γ	12h.	
	1	α	10 ✓	3	500 ✓	10	+	
	2	3	10 ✓	3	500 ✓	10	++	
	3	α + 3	10 ✓	3	500 ✓	10	++	
	4	α + 3	10 ✓	3	500 ✓	10	++	
	5	α + 3	10 ✓	3	500 ✓	10	++	
BT.	6	α	10 ✓	3	500 ✓	100 ✓	+++	
	7	α	10 ✓	3	500 ✓	100 ✓	+++	
	8	3	10 ✓	100 ✓	500 ✓	10	++	
	9	3	10 ✓	100 ✓	500 ✓	10	++	
	10	α	10 ✓	100 ✓	500 ✓	100 ✓	+++	
	11.	3	10 ✓	100 ✓	500 ✓	100 ✓	++	
	12	α + 3	10 ✓	100 ✓	500 ✓	100 ✓	++	
	21.	α	.3	100 ✓	500 ✓	10	+	— do not use
		3	.3	100 ✓	500 ✓	10	++	? autolysis or phage??
MT.		α + 3	.3	100 ✓	500 ✓	10	+	
	31.	α	10 ✓	3	30	100 ✓	+	
BP.		3	10 ✓	3	30	100 ✓	+	
		α + 3	10 ✓	3	30	100 ✓	+	
	41.	α	.3	100 ✓	30	100 ✓	+	
MP		3	.3	100 ✓	30	100 ✓	+	
		α + 3	.3	100 ✓	30	100 ✓	+	
	51.	coli α, 50 ml flask, shaken			α		+Y	Taken off shaker
	52	"			3		+Y	
	53	"			α + 3		+Y	11A28

Culture titrates.

50ml cultures, shaken at 30° K-12 mor 1030A21.
6 - d.

2 ≠. glut 5mg, putkactone 1mg, β-alanine 1mg
3 ≠. prol 5mg put .5mg

1. T(0).
4. See 202-9.

To desk top N22
sample a 10P22.

1. Ta 5ml + F(0) 5ml. N. casea 5531.
2. 2a 5ml + F(0) 5ml N. casea 5531.
3. put series 1r, 5r, 10r " "
4. 3a .1ml + 10ml F(0). " "
5. " .5ml
6. 4a 5ml + 5ml F(0). " "

11. 1a 5ml + T(B) 58-3214.
12 2a " " 25
13 Proline 25r + 1a .5cc + glut 25r.
14 ~~3a .25cc + T(B)~~
3a 0.25cc + T(B) 10ml.

Mor N23.
1
1
1
1

hydrolyze 1-3 (wash 3 cells) in 6N HCl. 14 hours.

	total o.d.u.
1.	10.400
2.	12.000
3.	4.240

Dup: analyse 207-1.

Streak out 207-1 on a coli + plate P22. Isolate 10 colonies to H_2O, and test; transfer to + slants. 3P23.

∴ ~~thr~~ threonine not required.

		T-B		T-T		T-BT.	
1		+	++	−	−	+	++
2		+	"	−	−	−	"
3		+	"	−	−	−	"
4		+	"	−	"	+	"
5		+	"	−	"	+	"
6		+	"	−	"	−	"
7		+	"	−	"	+	"
8		+	"	−	"	−	"
9		+	"	−	"	−	"
10		+	"	−	"	+	"

Test 207-2-4. 11P23.

	O	B.P.M.T.	BM	BT	MP	MT.	BP	TP
*								24h 32h.
2		− ++	− −	− −	− −	− −	− −	− − ++ ✓ ++ May be a multiple mutant.
3		± ++	− −	− −	− −	− −	− −	− ++
4		+ ++	− −	− −	− −	− −	− −	− + ++
5		− ++	− −	− −	− −	− −	− −	− − ++
8		± ++	± ++	± ++	± ++	± ++	± ++	± ++

Finally: +.

2 -	BPMT, PT.	679-183. ✓	212 B.
3	BT, PT, MT, BPMT.	679-183+	
4	BPMT, BT, PT. MT(-)!	679-183+ 679-58x?	679-183+
5	BPMT, PT	679-183	
8			

Note: all these strains were isolated from BT plates!

Retest:

	BPMT	BPM	PMT	MTB	BPT	MT.
207-1						
207-1A.						
207-2						
207-4						
BM						
BP						
PT						

See 212.

Sex in bacteria. Prospectus. 210N.

5/25/46. P25.

From mixed cultures, strains have been obtained which behave peculiarly in their nutritional requirements, behaving for a time like recombination types. For a demonstration of sex, a stable recombination type is essential. An analysis must be made of cultures 207-1 and 207-2.

207-1 behaves like a culture of BT with a small % of B cells. Therefore plate heavily + lightly into T(B) + layer ẽ "T" after B is detected.

207-2 behaves like a culture of BMPT ẽ some PT cells still present. Plate into 1) PT 2) BM. Afterwards layer.

207-4 may have BT cells Plate from 210-4-BT into B. After-layer ẽ T.

4-A Mutants

5/26/46

I Cystine Requirement of Y24.

1426. A. 10mcl T(B, ϕ) + B: .01r ϕ 300r

	Cyst.	8P27
1.	10r	89
2.	30r	84
3.	100r	83^2 ← ½ opt.
4.	300r	76^3 like 58·309.
5.	1mg.	68

11. Methionine: 1mg. ± not parathiotrophs.

12. Meth. 1mg+Cyst 1mg. 65.

II Otherstrains:

			T(0)	Ht	Vits.
1	B	Y13.			
2	B	Y14			
3	B	Y18			
4	B	Y20			
5	B	Y21			
6	B	Y22			
7	ϕB	197-61.			
8	B	197-32			
9	B.	197-33			
10	Bϕ	-5			
11	Bϕ	-7			
12	Bϕ	-21			
13	Bϕ	-23			

Analysis of 207- cultures
Six.

5/26/46.

Scrape growth from slant, suspend in H_2O & dilute as indicated. ✓ (rodes well)
To obtain cleavage, autoclave 3% agar + T(0) 200% in quantity. Thurs mng. Mutant selection procedure.

12M-1A 26. ~~Use T(0) + ½ usual phosphate [T½]~~

A. 207 - 1. P27. Layer Addnl col.
11 P207. 11A28.

1:1000 in:

	1.	B	+++				
B^-M^+	2.	B	+++				
	3.	BT	+++				
	4	T	0				
	1:10^6						
	11	B	51	: T	0		
	12	B	39.	: T	1	±4	
(N)	13	BT	43				
	14	T.	0				

Pick to complete 1128.

For analysis see 219.

requires B.

207 - 2.

1:1000 in:

B.

21	PT	+++				
22	PT	+++				
23	BMBT	+++				
24	BM	—				
1:1000000						
31.	PT	9				
32	PT	8				
33	PT	15	: ∞	1	±1	
34	PT	14	: ∞	1	±2	
35	BPT	12				
36	MPT	12				
37	BMP	0				
38	BMT	0				
39	BMPT.	14				

C. 207-4

1:1000

41.	T	++				
42.	B	0				
1:10^6						
51	B	0				
52	T.	3	B	1	±3	

FPA.

dl-fluorophenylalanine

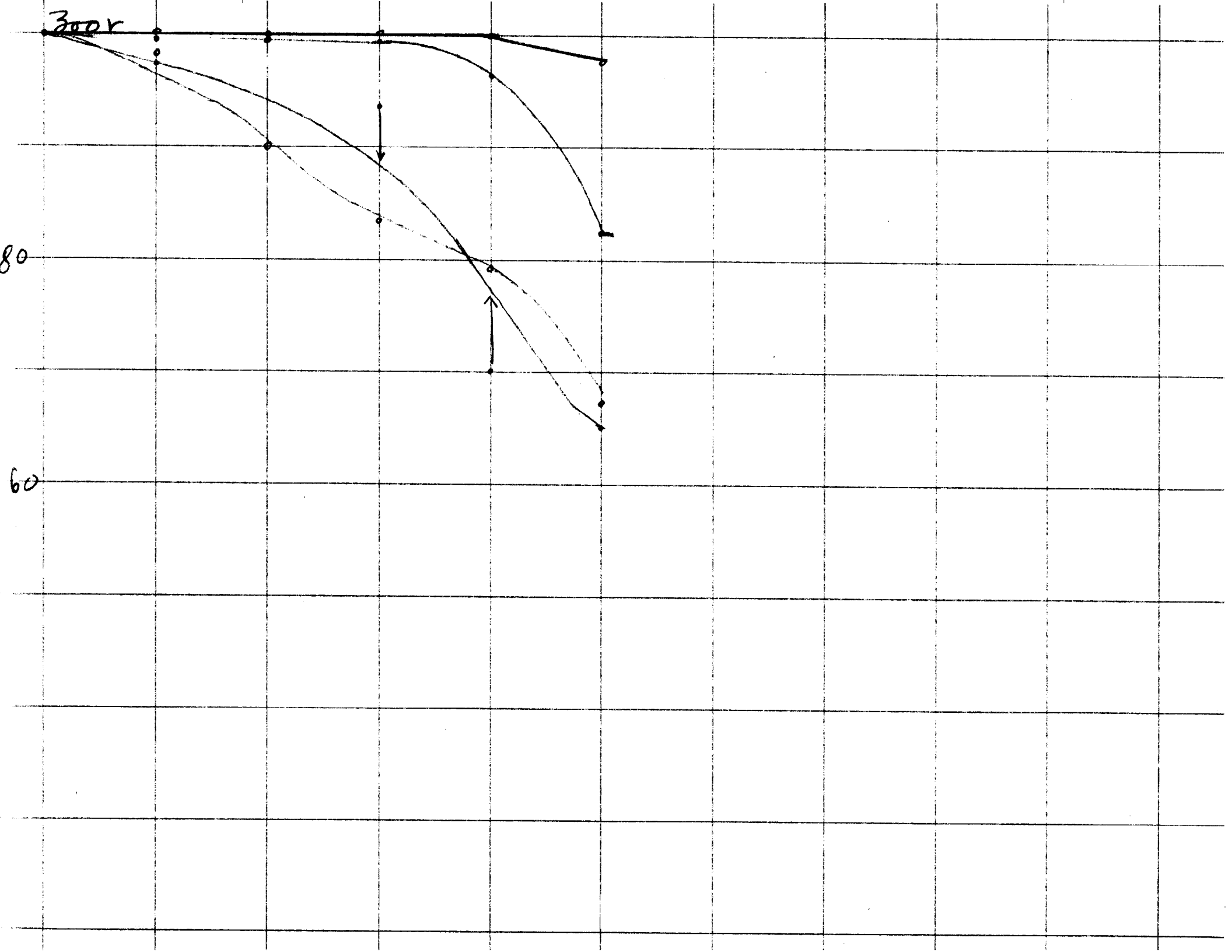
5/24/46.

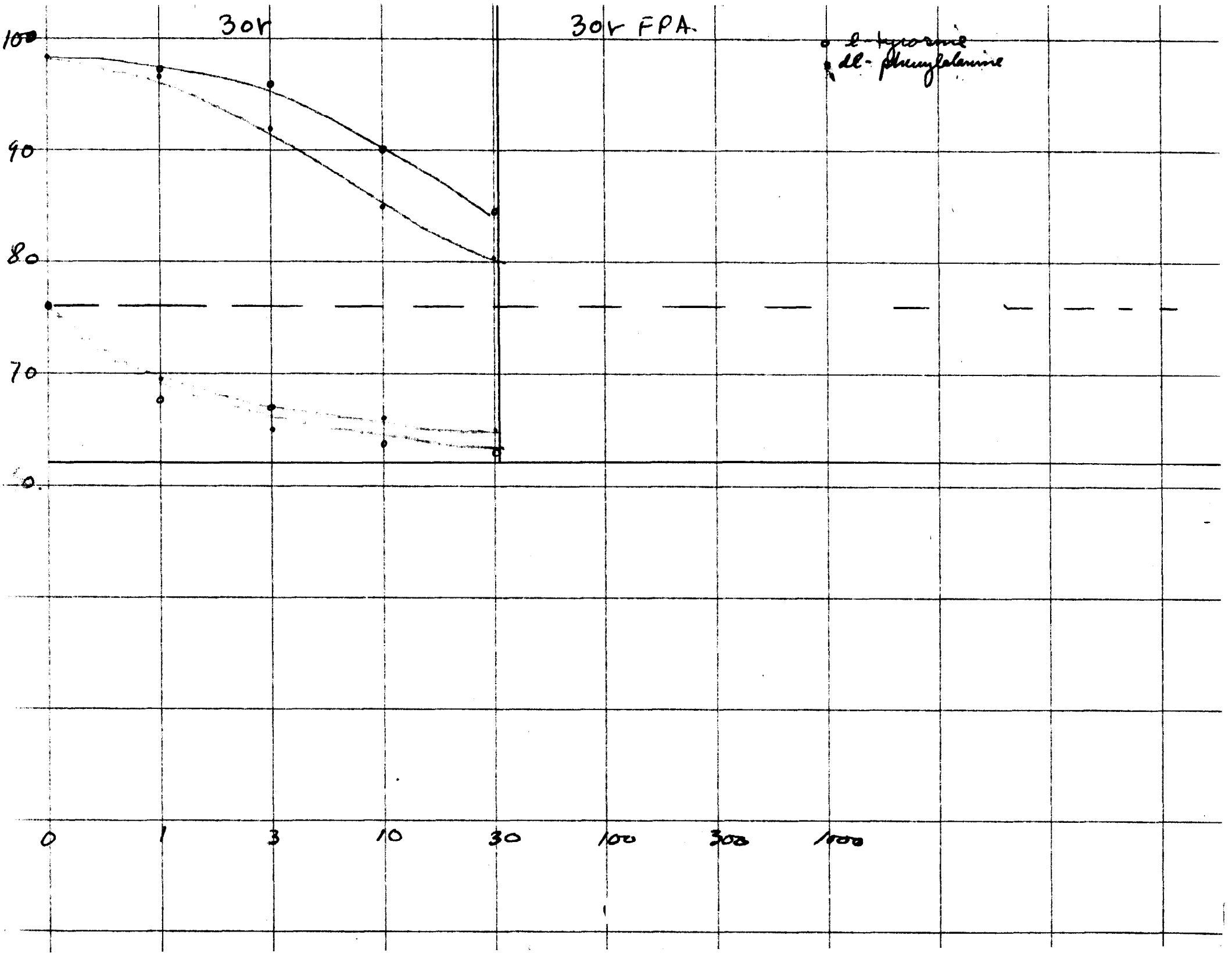
10ml T(B). 30°. in colorimeter tubes. broc. 1A26.

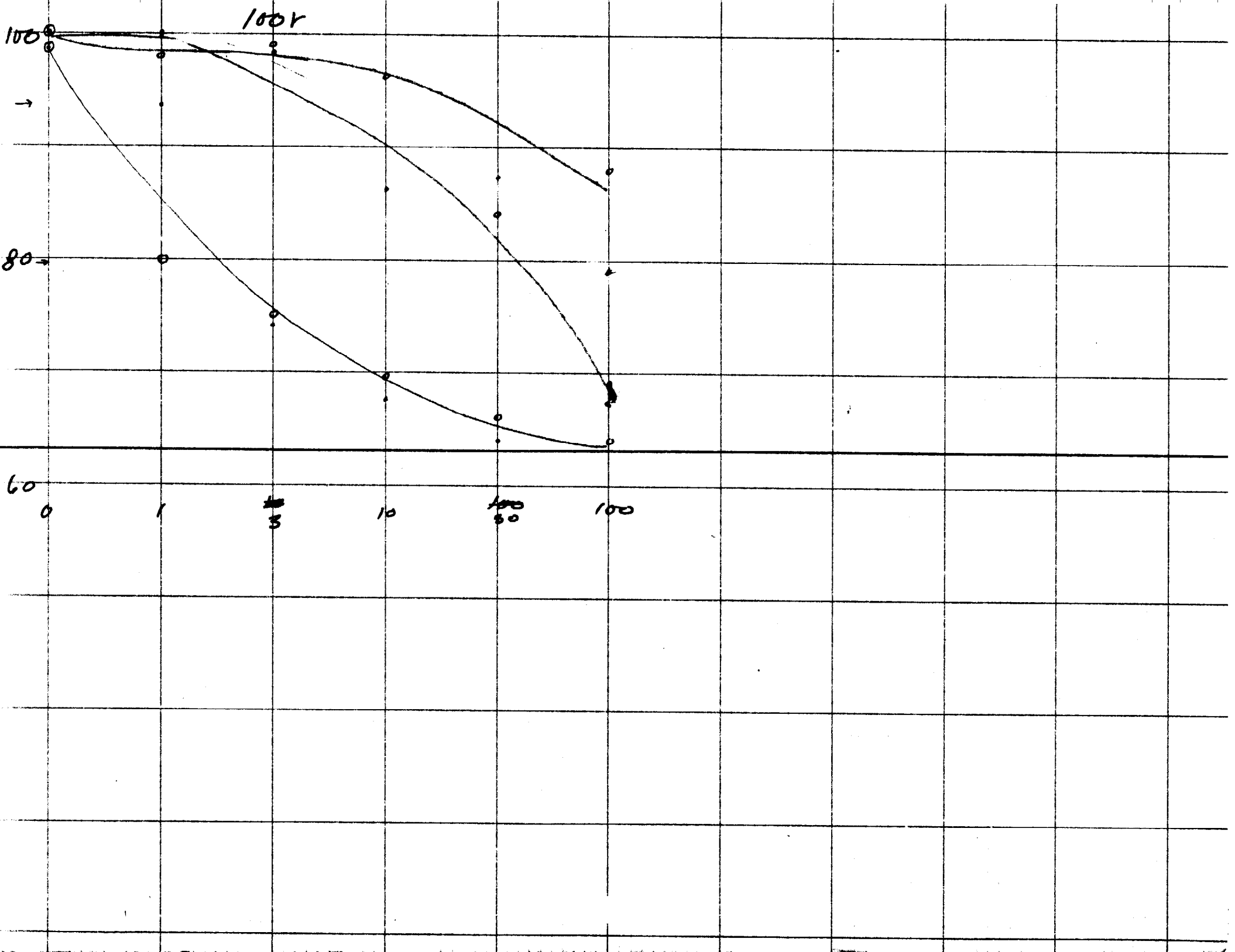
broc. dl ΦAl. dl FPA. l-tyr. 604.

					8P27.	1P28
1.	R-12	0	0		67	68'
2.	"	"	10 r		80	68'
3	"	"	30 r		97	75^2
4	"	"	100 r		100	89'
5	"	"	300 r		100	100
6	"	"	1 mg.		100	100
11.	58-278	100 r	0		70	70
12	"	"	10 r		66^3	67'
13	"	"	30 r		67	70
14	"	"	100 r		69	67
15	"	"	300 r		70	69^3
16	"	"	1 mg.		99	96^3
21	58-278	200 r	0		72	68^2
22	"	"	10 r		66'	67^2
23	"	"	30 r		67'	68^2
24	"	"	100 r		69	68^2
25	"	"	300 r		69'	70
26	"	"	1 mg.		71^2	71^2
31	58-5030	0	0	0	— 100	100
32	"	"	0	60 r	+++ 77'	77^2
33	"	"	10 r	60 r	77	78'
34		"	100 r	60 r	78^3	79
35		"	1 mg.	60 r	75'	75^3
36.		100 r	100 r	0	— 100	100
37.		100 r	0	0	— 100	100
38.		0	100 r	0	150.	100
					76^5	75^3

compare E 133.


300r
80
60


30r
30r FPA.
l-tyrosine
dl-phenylalanine
100
90
80
70
0
0
1
3
10
30
100
300
1000


100
100 r
80
60
0
1
3
10
50
100

FPA.

Inhibition of K-12 and Reversal by phenyl-alanine + tyrosine.

6/2/46.	Inoc K-12				11P2.	25h.	36h.		48h	60h.
	FPA + dl Phe + l-tyr.				4P3	12M3.	1FA4		11P4.	11A5
1	30	0	0	±	98[1]	76			63[2]	
2		1		±	96[3]	69[2]			65	
3		3		+	92	65			68	
4	→	10		+	85	66				
5	—	30		+	80[1]	65				
6			1	+	97[1]	70 / 67[3]			67[1]	
7			3	+	96	69 / 67[3]				
8			10	+	90	70 / 63[3]				
9			30	+	84[3]	~~71~~ / 63[3]			68	
						63[3]				
11	100	0		±	100	98[3]			97[1]	71
12		1		±	100	93[3]			67[2]	
13		3		±	99[1]	74			67[3]	
14		10		+	86[1]	67[3]				
15	→	30		+	87[1]	64				
16		100		++ slc	69	67[1]				
17			1		98	80			70	67
18			3		99	75			66	
19		→	10		96[1-2]	69[3]			73	
20		→	30	+	84	66			68	
21			100	+	88	64			69[1]	
31	300	0			100	100			99[3]	90[3]
32		1			99[3-]	97[1]			78[1]	69[2] ←
33		3			100	100			100	97[1]
34		10			99[2-]	93[3]			72[2]	71
35		30			96[2]	70			67	
36		→ 100		+	82[1]	65			70	
37			1		100	98[2-]			98[2]	74[2]
38			3		99[3-]	90			70[1]	70[2]
39			10		100	83[2-]			68[1]	
40	—		30		100	79[1]			68[1]	
41			100.	±	97[3-]	67[1]			67[2]	
51	0	1		±	85	61[2-]			65	66
52		10		+	76	62[3]			66[2]	
53		100		+	76	65			69[2]	
			*							
61			10	+	77	62[3]			67[2-]	
62			100	+	85	60[1]			69[2]	
63			1000	+	74.	66[1]			67[2]	
				(80)	(80.)	(283)			80	80.

2M 9-43 BIOMSTAT FORM NO. A-2788
SUBJECT

FPA. – Growth Curves. 30°.

SHEET NO.
BY 215a

Inoc. 11305

	Inoc.	Medium	1145 A6	210 P6.	450 P6	750 P6	11 P6.	220 A7	945 A7	1150 A7	350 P7	810 P7	Lag	mgt.
	FROM 11A6.													
Hours + mins	→		45	190	350	530	820	920	1365	1490	1730	1990		
1	K-12	0	96	92	84^3	69	58^3	57^1	59				lag 10.34. mgt = 2½4.	
2	K-12	0	97^1	92^3	84^2	69	59^2	58^1	61					
3	K-12 v.small	0	98	98^3	98^2	98^2	98	$96^{2\leftrightarrow 8.5}$	73^3	66	58			144.
4	"	0	99^2	100	100	99^2	99	95^3	72	64^3	57			
5	K-12	FPA 100r	99	99	99^2	98^2	95^1	90	67^3	68^1	65^3	67^2		220?
6	K-12	FPA 300r	99	99^2	99^2	98^3	97	96	81^3	77^2	68^1	67^1		390 ok.
7	K-12	Img. FPA 10	100	100	99^3	100	100	99^3	98^3	100	97^2	96^3		
8	K-12	ΦA 10	100	98^3	93^3	88^2	76	67	69^1	69^2	69^3			175
9	K-12	"	99^2	98	93^3	83^2	74	67^3	70^3	76^2	70			"
10	K-12 v.sm.	"	100^2	100	100	100	100	100^T	99^T	96	87^2	72^2		140; 205.
11	K-12	FPA 100 Tyr 10	99^3	99^1	95^3	87^1	77^2	68	64^3					220
12	"	"	100	99^1	(96^2)	87^3	75^3	68	64^1					"
13	" v.sm.	" FPA 300	100	100	100	100	99	99^T	96	94^3	83	68^3		
14	K-12	ΦA 10	97^1	93	82^1	66^3	59^2	62^1	66^3	65^2	66			
15	K-12	FP 300 Tyr 10	44^1 but(T)	99^1	99	98^2	97^2	97^1	91^3	89^2	77	67^1		
16	K-12	50%	100	96^2	88^1	74^2	69	71	73	73	73	73		
			77	77	76	77								
					77									
							16^2	76^2	76^3	77	76^2	78^2		

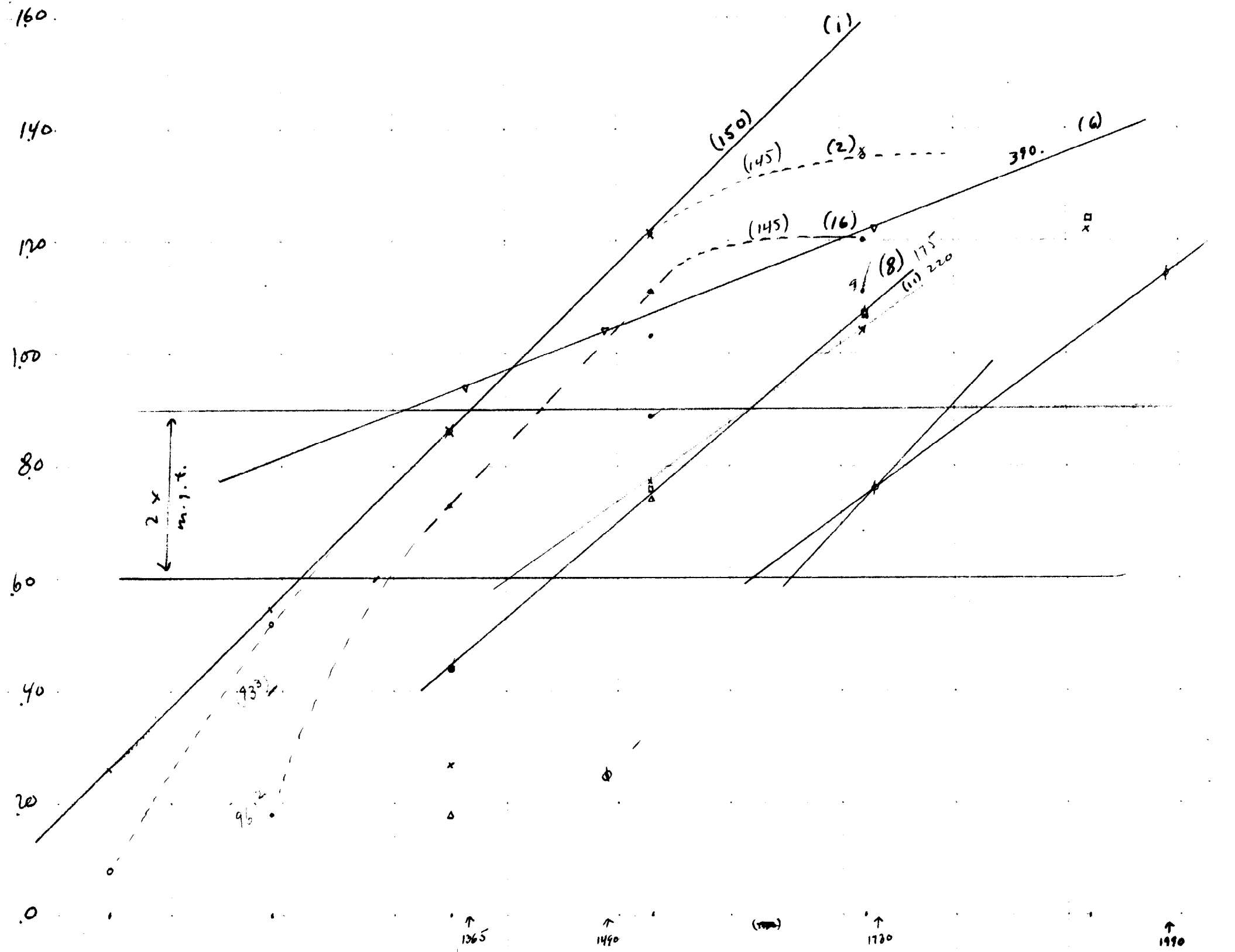
should have a separate blank.

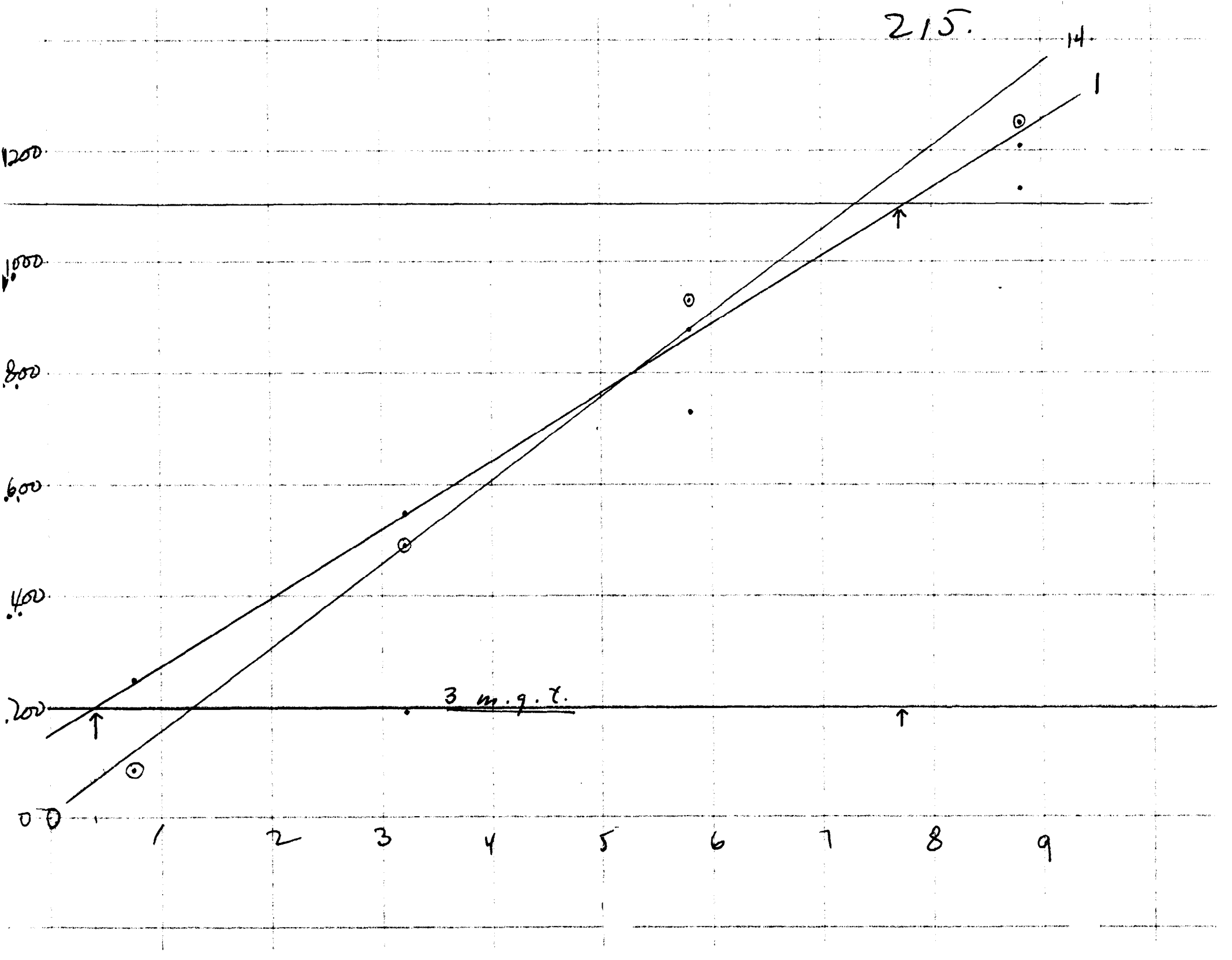
$$d = 2 - \log G$$

$$t = k \log d.$$

$$t = k \cdot \log(\log G/100)$$

$$= k - \log \log G/100$$


160
140
120
100
80
60
40
20
0
(1)
(150)
(145)
(2)
390.
(6)
(145)
(16)
(8) 175
(11) 220
2 x m.i.r.
93
96
1365
1490
1730
1990


215.
14
1
1200
1000
800
600
400
200
0
3 m.g.t.
1
2
3
4
5
6
7
8
9

13/2 mutants.

Inoc. 12 45A 28.

		Arginine	Leucine	Citrulline	21h.	48h.	
Y-38	1	0			99[3]		good response curve.
	2	1			94[1]		1/2 max: ca .03 mg
	3	3			92[1]		
	4	10			83		
	5	30			78[2]		
	6	100			61		
	7	300			57		
	8			1000	100	all up.	
Y-39	11		0		100	100	
	12		1		"		
	13		3		"	"	
	14		10		"		
	15		30		"		
	16		100		"		
	17		300		"	"	

(78)

Y-39. 21. L-leucine 100γ

dl-leucine 100γ

dl-isoleucine 100γ

Inoc Y38 in coli of 12M28. Grow on shaker @ 30°.

Mix after growth together →

Controls are questionable —

synthesis in plate 13 or carryover?

Dez. Analyses of 212 cultures. 219-

			BPTM	−B	−P	−T	−M
		M28 Pick ∞.					
x P.T.?		1 ✓	+	+	+	−	+
"	?	2 ✓	−	−	−	−	−
BT ?		3					
BT ?		4 ✓	+	−	+	+	+

Analyse 219-2

5/31/46.

1. Incr 50 ml coli ♂ i α (679-183) and β (58-161) and shake at 30° 11P30 - 11:30P31.

T/= Wash and resuspend in 25 ml H_2O.

Turbidity – P4.

Turbid Cell count:

1. α 10^{-7} in α: 24
2. β " " " : 53

Back-mutation rates: Incr 1 ml. of suspensions.

3	α	in	0.	0	—
4	α	in	Threonine exc.	0	±
5	α	in	proline " .	2	surface maybe cant test looks like coli
6	β	in	0	0	—
7	β	in	Biotin	0	—
8	β	in	Methionine	0 (T)	+

Syntrophism. Incr in BT.

9.	β	10^{-7}	Control	1	—	
10	α	10^{-7}	"	0	—	
11	α	10^{-7}	+ 1 ml β ~~control~~ Broth	0	—	11 very small colonies.
12	β	10^{-7}	+ 1 ml α " .	0	—	—

	α	β			
13	1 ml.	1	0 (T)	++	
14	"	10^{-3}	1	surrounded by local stim. zone ±	
15	"	10^{-5}	1	" " " " " ±	
16	"	10^{-7}	0	±	no colonies.
17	10^{-3}	1	0	—	
18		10^{-3}	0	—	
19		10^{-5}	0	—	—
20		10^{-7}	0	—	
21	10^{-5}	1	0		num. v. sm. col.
22		10^{-3}	0		
23		10^{-5}	0	—	
24		10^{-7}	0	—	
25	10^{-7}	1	0	—	num v. sm. col.
26		10^{-3}	0	—	
27		10^{-5}	0	—	
28		10^{-7}	0 (N)	—	

→ The conclusion that may be drawn from this are limited:
in general diffusion must be limited by the agar to the point
where synthesis is less effective. It is not due to cells sticking
together confirm by plating wild type into concentrated mutant.
delete
Does not adaptation serve the same purpose ??

—

Y38 Ultraviolet killing.

As above but expose by removing transite shutter after 5 min. warm-up period. 5" from tube. Plate initially at $1:10^7$, then take 1 ml samples and determine terminal slopes of eq. 7.

5:15 P31. 1 min. intervals for 10 min.

t=0	10^{-7}		65	650,000,000

1	1 ml	turbid		
2	1 ml	turbid		
3	1 ml		10^5	
4	1 ml		ca 10000	
5	1 ml		ca [redacted] 1200	
6	.1 ml		ca [redacted] 40.	→ 6
7	.1 ml		7	
8	.1 ml		0	
9	.1 ml		0	
10	.1 ml		0	
15	1 ml		0	

Because of clumping after [redacted] ing, the counts at lower mortality (?) may be disregarded + the initial count should be considered to be

Y38 – Multiple Mutants.

6/1/46.

~~1130 P31~~. Broc 50 ml coli ∞ and shake at 30° - Y38.
4. ~~14~~ P1

1. Dil 1:10^7 into coli ∞. ca 2000.

Irradiate 5 mins as exp. 221. Broc 5 ml into 50 ml coli ∞ and incubate on shaker. (A). Centrifuge + wash 25 ml & resuspend in saline. Inoculate 0.1 ml into T (arginine) by detection technique, 5 cores.

Immediately after irradiation:
.1 ml = ca 2000 colonies.

1
2
3 $pS_{5\,min.} = 6.$
4
5
6
7
8
9
10.

Dil. A 1:10^{-7} and/nior readme.

11
12
13
14
15
16
17
18
19
10.

opt. arginine = 300 r dl – .

Phage T-1.

1/2/46. labelled titre 2×10^{10}

Dilute T-1 as received from Demerec 10^{-9} and plate ± ca 10^9 cells of:

740P2

1. B/r — Uniform turbidity
2. Y38 (B/r) Agr inconclusive. — Uniform turbidity

3, 4} Plate ca 10^8 Y38 ± 10^{10} T-1 in coli ∞. for Y38/T1: Large characters ± ca 400 secondary colonies; periphery turbid. Turbid!

5. 10^8 Y38 in coli ∞ aq. When solid, streaks
6. 10^9 ≠B/r in coli ∞ aq. T-1 over surface.

} no response!

Liquid culture —

4P4 — broc coli ∞ ± Y38 shaken at 37°. ca. 10^8/ml.

1016P — Hold 1cc of Phage T-1 "2×10^{10}":

12M - no change

9A5 - no change.

Dex;

Broc 50ml coli of flasks + shaker @ 30°. 11P2 6/2/46.

Shaken broke down A3.

1. 58-161 —
2. 679-183. —
3 58-161 + 679-183. feebid; ca 16 colonies.

11P4 - Centrifuge + wash. Broc into T(0) ≐ 2cc. culture. p. plate + incubate. Also 208-51-2-3. → 0, 0, 0.

As above. Voshaking 1130P5. – 1230A7. plate out on T(0).

	P7	
11 58-161	0	
12 679-183	0	
13 } grown	4	! Fission or sexuality ??
14. } together	6	
15 } grown	0	
16. } separately	0	

P7. Pick 6 colonies from (5) ~~to~~ coli ~~L~~ ; p8 to slants. 1-6.

See: 227

P10 – numerous additional colonies appear in 13+14
Definite halation around colonies for a diam 2-4mm.

6/5/46.

Incr. 5 flasks coli ∝ ē Y38. Grow shaken 37° 4P4

1030P 5:

Inoculate individually 5 min. in same quality tube + determine killing.

	t = 0. 10^{-7}	t = 5. Incr 1 ml.
1.	ca 100.	ca 19,000
2		
3		
4		
5		

Killing pS at 6 mins. can be taken as: ∝ log : $10^4/10^9$ = (5.)

Y38 - mutants.

6/6/46.

1130 PS Inoc 50 ml coli ∝ 2 Y38 Shake at 37°.

Irradiate 6 min. Wash by centr., dilute 1:100 + plate in T (aq.) for mutant detection: ~~#200 A7.~~ 2A7

1
2
3
3
4
5
6
7
8
9
10

Too heavy

!! ** ☺

ca 10^{4-5}

Inoc 1 ml into F/o) and cultivate at 37°.

6/9/46. 9 JUN 1946

1A9 - Plate cultures 1-4 of 224 (1-6) at 10^{-6} and 10^{-7} into T(0).

1a
b

2a
b

3a
b.

1A10 - numerous colonies on plates per repetition. Pick sample colonies from surface each plate ~~and~~ to H_2O + to slant colis + test on minimal

Plate:		T(0).	T(0).
1.	1	++	
	2	"	
	3	"	
2	11	"	
	12	"	
	13	"	
3	21	"	
	22	"	
	23	"	
4	31	"	
	32	"	
	33	— →	

See 231.

A10. from os slant to tube of ∞. Wash. Use in PM for plating.

Phage T-1.

1 9 JUN 1943

1. Isolated colonies from plate 223-3. to coli ∞. Transfer to slants.
2.
3.
4.

11. Picks agar between colonies from plate 223-3 to the surface of a coli ∞ plate seeded with B/1. No lytics. Probably agar too old.

≡ phage ≡ !!?

12. Incc coli ∞ tube ≡ B/1 heavily transplanted. 8 P. 10 P. Incc T-1 (D) by 1130 considerable clearing?

13. " agar plate as above ≡ T-1 (D). → lytic zone.

2A 13A. Y38 + T-1. → lytic zone. Use YB5 agar for coli ∞ in

9. 14. Streak agar plate of B/1 ≡ 12. this expt.
disturbed lytic zone.

15. Streak agar plate of Y38 ≡ 12 lytic zone (?); later somewhat turbid

all streaks gave clear areas:

∴ Phage C is effective on coli B/1 + Y38; also Y38 is susc. to T-1.

21. 11A9. Incc 5 ml coli ≡ T-1 "10^10" + 5 ml 10 hour E. coli B/1 from coli ∞ Incubate at 37 in shaker. 1230 P9. - largely cleared.

22. Incc 50 ml ≡ 21-1 ml + B/1 5 ml. 1230 P9. Incubate at R.T. 11P9: - Cleared (faint residual turbidity). Control completely turbid! Use for stock T-1. See 230

23. Plaque count T-1 (D) on B/1. 10^{-3} to 10^{-9}

- results obscured by vigorous fermentation + bubble formation.

No plaques found at 10^{-11}, 10^{-9}, 10^{-7}

Numerous at 10^{-5} (ca 10^3).

Innumerable at 10^{-3}. Should use medium with less glucose for phage enumeration?

Gas bubbles in the agar are cones or lenticulate.

Try thrombin. ± recovery added Thrombin in view of

very high requirement.

9 JUN 1948

As 202. K-12 in 50ml T( ) + suppl. Shaker at 37°. Inoc 145A 9.

		G (1:10)	d.	mg/25 cc	Hydrolysate at =		Recovery %
	1. 0	84[1]	.744	[redacted]	85[1]	.693	93%
qual	2. glut. 5mg	81[3]	.875	[redacted]	forcing (743) = 83[2]	.783.	89%
soluble	3. arg. 5mg	83[2]	.783	[redacted]			
put	4. pantolact 1mg, β-alanine 1mg.	84	.757	[redacted]			
req.	5. citrulline 5mg.	83[2] (73)	.783		(78[2])		

See 234.

Harvest 1130P9. Separate 25 ml samples. (a). 16 = 15cc.
Start hydrolysis in 6N HCl 20cc but discontinue due to severe bumping: — for 1, 2, 5 only. Estimate recovery from volume ←

	Volume after hydr.		to	
1.	~~4.5cc~~ 11	: %	to	13.5 cc
2.	4.5 cc	22.5%		5.6 cc
5.	6 cc.	30%.		7.5 cc

Neutralize. Dilute to % of 25 cc ind. by recovery
1 cc of each solution should give mutants responses of magnitude indicated above, in 10 ml medium. See 234. for assays

Glutamic does not increase (proportion) production of proline in wild type
Citrulline " " " " " " arginine

Note Y38 is a B strain and therefore >100% recovery does not signify anything.

∴ Requirement is ∝ content. (Proline ∝ = c. 3% dry wt.).

Phage T-1

6/10/46.

Steele-filter 228-22 for T-1 stock. Pool ē 228-21

Respread elgiots variously; some to K.W. for lyophil preservation.

Plaque out on B/r at indicated dilutions. 1A1B for titer.

1. 10^6 ca 300
2. 10^8 1
3. 10^{10} 0
4. 10^{12} 0

Aer. 231.

9/11/46.

Plate out 227-1, etc. detection plates, for mutant segregants.
Dil 10^{-6}, 10^{-7}. Unshaken culture virulent from x slant.

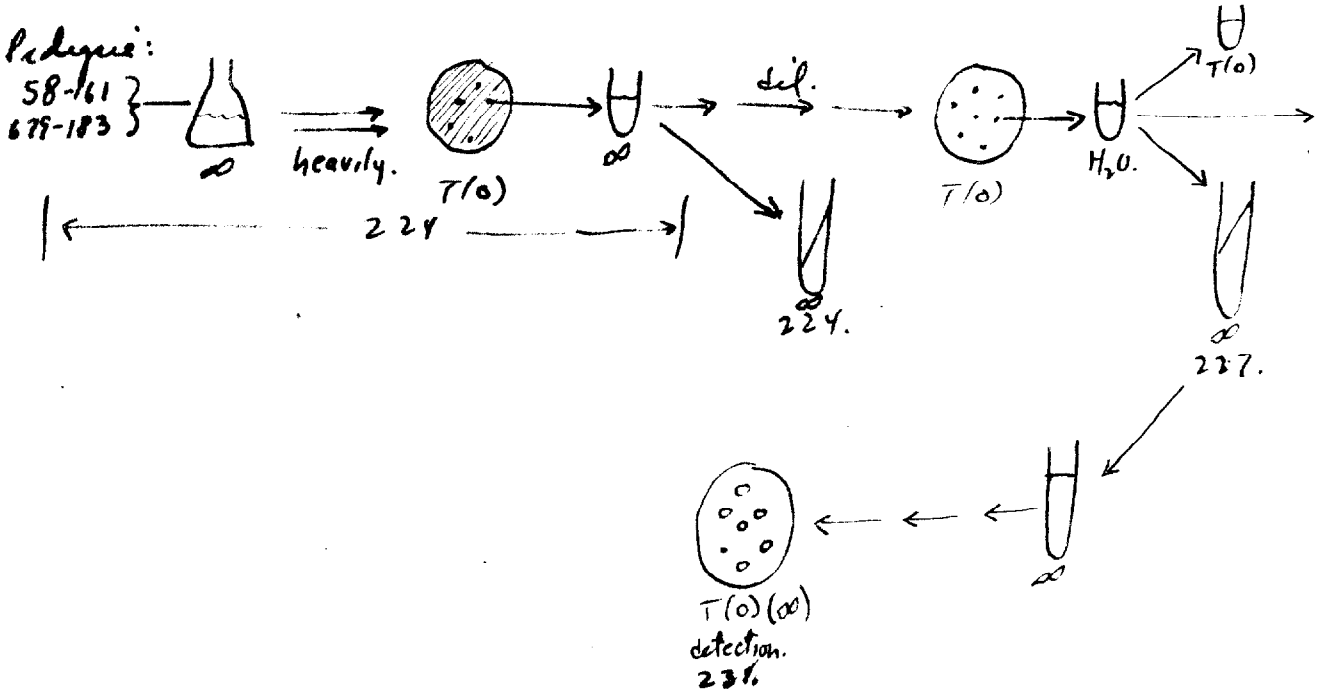
227- 1A11. Taya E coli # 12N12.

1
2 1

3
4 11

5
6 21

7
8 31.



1230A13 - Nothing segr. seen.
11A13 - do. (1 col 0?)

Y38 14 cultures.

6/12/46.

Inoc coli 00 4 ml ± Y38 + skelsea t 30° 1/P 11

Lebistrides

Sex

6/11/46.

Wash and plate heavily into T/O) etc. The cultures:

Inoc PS colinoc

1. SR-161 — heavily = 1ml grown culture/plate.
2. 679-183.
3. SR-161 + 679-183. — SP 11↓ (6 day cultures).

			1230A13	11A13
1.	1	in O	O	
2.	1	m O	O	
3	2	in O	O	"
4	2	in O	O	
5	3	m O	O	
6	3	m O	O	
7	1+2	m O	O	
8	1+2	m O.	O	"
9.	1	m biotin	O	
10	1	m meth	O	
11	2	m thiocrine	O	
12	2	m proline.	O	
13	3	in BP	O	"
14	3	" BT	O	
15	3	MP	O	
16.	3	MT.	O	

OK / throw out.

6/11/46.

Assay medium + hydrolysate of cultures grown in excess precursor. Use 50% medium filtrate; hydrolysate as 1 ml equivalent of the completely grown culture / 10 ml. 8P18.

Broc annidisted, 30° very little in ~~hydro~~ filtrates; considerable in hydrolysates

1	229-1	Medium 50%	Blank.		
2	"	" "	+ Biotin - Y38	58-3214. for proline.	100
3	"	" "		for arginine.	100
11.	229-2	Medium 50%	Blank		
12		" "	+Biotin	58-3214 for proline.	100
21	229-5	" "	Blank		
22		" "	Y38 -	arginine.	100
31.	229-1	hydrolysate 1 ml.	Blank		
32	"	" "	" +biotin	58-3214 prol.	85[1]
33			Y38	arg.	94[3]
41.	229-2	hydrolysate 1 ml		58-3214 ++	83[2]
51	229-5	"	"	~~58~~ Y38 arg. ++	78[2]
61.	206.	hydrolysate 1 mg.	Blank		
62		"	+biotin	58-3214 prol	77[1]
63.			Y38	arg.	72[3]
71	206	filtrate 50%	blank		
72			+biotin	58-3214	100
73			Y38.		100
81	- T(0)	Y38			—
82	T(B)	58-3214.			—

Broc IIP 6/17/46.

K-12 Phage

12 JUN 1946

① 3P. Bror 50ml coli ∞ ē K-12. Shake at 30°.

1130 A13 - Bror 1ml of ① + ind. phage sources into 50ml coli ∞.:

1. T-1
2. C
3. Sewage
4. Cole.

Incubate at 35°.

2P. - #1, 2 clear; 3, 4 turbid.

Bror coli ∞ ē 1ml of grown K-12 + Inc. 35°.

11. 1. cleared
12. 2. cleared.
13 —

Streak Phages on a K-12 plate (coli ∞).

14) T-1 C "Cole."

∴ T-1 and C → K-12

Prepare 58-161 /1:

15. [redacted]

Cross streak on coli plate:

	K-12	58-161	679-183	B/r	~~Bkt.~~
T-1	—	—	—	—	
235-11	—	—	—	—	ē secondary growth along streak.
~~235-~~					
235-12	do.				
C	do.				

12M 11 Inoc coli → flasks; shake at 30°.

♀ 58-161
♂ 679-183
3. Both.

Plate into minimal, heavily* after washing 1130 P12.
P15

* → inf	1.	1	– No colonies.	0	
grown	2	1 + B	– No colonies.	0	
culture	3	1 + M	– Turbid plate. No colonies.		
	4	2	– No colonies!	0	
	5	2 + T	v. distinct halation around adaptants .... 23. N14.		#
	6	2 + P	3	6.	
				(Some colonies may adapt in agar.)	
	7	1+2	– 2 seen N14.		
	8	3	11P13. N14	again, some colonies come up secondarily (after the first.) pick one colony – (236-9) to water + ∞ slant	
	9	3	13		
	10.	3	13 va 100.		
			12		

same cultures. 1130 P13 (.48 L.). T(0).

11	1	P15 0	
12	1		
13	2	0	
14	2	0	
15	1+2	0	
16.	1+2		
17	3	4.	
18	3	3	

To recapitulate, in the following expts. wilds were found by interaction only:

Date	1	2	1+2	3	Expt
5/31.	0	0			220
6/2	0	0	0	4+; 5+	224
6/11	0	0	0	0	233
6/12	0	0	3	10^2	236a
6/13	0	0	0	4.	236b.

In 5 attempts, no double revertants have appeared, while prototrophs have repeatedly appeared in mixed cultures.

halation = turbidity around colonies. Consists of v. small colonies with diminishing density.

K-12 - doubles - T-1 resistants.

13 JUN

Use ~~cult~~ 236 ① and 236 ② as inocula. Inol $\cong 10^9$

1130P13

1. 58-161 10^9 + T-1 10^7 in coli ∞ plate
2. 679-183 do.
3. 58-161 10^9 + T-1 10^7 in coli ∞ ~~flask~~ flask. Incubate . Then plate 1 ml into coli ∞.
4. do. 679-183. all plates

~~5. 58-161 10^9 + T-1 10^7 in coli ∞ flask~~

10AM N14 7P14

5 Flasks of 3 turbid. Streak out on coli ∞.

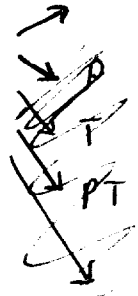
6. " 4. " "

Isolate colonies from streak plates to BM + PT salts respectively to ~~avoid~~ "type" ~~transfers~~ resistants. Also, inoculate flasks 5+6 directly.

	BM. 5		
11	colony	−	
12	"	±	Streak out on coli ∞ N16. See 245.
13	"	−	
14	"	−	12N17 as infra.
15.	liq.	−	

↘ BM
↘ BM Type.

	PT. 6		
21	colony	+	Streak out on coli ∞ 12N16. slant
22	"	−	
23	"	+	12N-17. colony to water
24	"	+	
25	liq.	+	



Cross streak 12 + 21 ~~+~~ ~~K-12~~ 58-161

test for lysogenicity ~ 161.
on coli ∞ plate.

Evidence re heterocaryosis —
Mutants in Recombination studies.

2

① 1145 P13. Incr 50 ml coli ∞ ē 227-1

1030 P14. Irradiate 4 mins. Incr. 1 ml into 50 ml koli ∞. (A). (calc ρS = 5.)

Wash Ice: Dilute 10^{-2} and plate into coli ∞. and detection plates. immediately.

1 P16.

Conclude that survivors are only ca 10/cc.

1	—
2	—
3	1
4	—
5	—
6	—
7	—
8	—
∞	0

1 tested.

Dilute ① 10^{-1} + Apparently killing was > anticipated
Layer ē to 1 P16. Circle numerous minute colonies. Detect: (sp. mutants 2 / cover ē F(0)!).

S	1	302.
	2	Examine 4P, 11P16. 11A17. 10A18
	3	
	4	
	5	1 colony found A17. 238-2.
	6	
	7	no growth on picking.
	8.	
	∞	316.

2400 total tested.

Dil 1: 10^7 11P15. cover ē T(0) previous.
Layer 1130 A17.

A	1	
	2	Examine 9P17, 11A18. 12M19.
	3	
	4	
	5	1 colony - surface? cent? 238-1
	6	
	7	mici. not ē coli. Saccin?
	8	
	∞	91

700 tested.

pick variants to ∞ 12M19.

~~239~~ Sex-plating. 23?

15 JUN 1946

Inoc 183+ 161. (separately) 11 P 14. ~~Attempting~~

Inoc ~~1 ml~~ 5 ml each into coli ∞. 50 ml. 30°. Plate at varying intervals.
G (1:10) (73³) Wash (separately in 1st) + plate as indicated. Regularly.
9:45 P 15. Mix: A 17. cells/cale.). 1/del of culture.

0 time.			0	96¹	5×10⁸/ml. 1st plating is 5 ml each original culture = ca 5×10⁹ added.
	1	161 only	0		
	2	183 only	0		
.5 ml each	3				
1 ml each	4.		1		
1 hour.	11		0	96²	
2 ml	12.		0		
2½ h.	~~15~~ 21		4.	95²	
3 ml.	~~16~~ 22		3		
	31		3	95 (94³)	
19 h.	41				
	42.				

~~Use 241 cultures for inoculum + repeat. Mix ~~15~~ P 16.~~ 6

Transfer cultures to coli ∞ slants when possible. Designate as, e.g. 239-4a.

Designate Surface col.

~~K 12~~ ; phage C. ; Segregants (successive serotypes) 240

16 JUN 1946

1230 A16. Pick a colony from 236-9 to 1 ml H_2O.

0. Streak a T(0) plate N17. to H_2O → slant 240-1
→ T(0).

From comparison C diluted 239, dil 1/2 : 100 : 1000 +
plate 1 cc dil. into detection plates for recombinants.
Unfortunately, ca. 1200/plate ~~1%~~ 2% small colonies in minimal (B)!
Layer 1230P17.

1. BP 2 new.
2 BT
3 TM
4 MP
5 —

Sterile filter 235-12 + dispense in 10 ml tubes.
Plaque on 183. (241 moi.) 6P16. H.9.

Test C again on K-12, 183, 161 by cross streak 1A:8.

activity on K-12
non-activity on 58-161
activity on 679-183 ??

Pick colonies 12M19. to 0. See 245 for tests
all prototroph

Ultraviolet induced reversions.

16 JUN 1946

1A from 161, 183 small coli ~ 30° sh.

$1/S = 3 \times 10^5$

5P16. Irradiate 2 mins. $pS = 5.5$

Wash both aliquots + dilute + plate as indicated.

	1.	10^{-7} in ∞		79.	7.9×10^8
A – unirrad.	2.	10^0 in P		3	
	3	10^0 in P	—	3	
	4	10^0 in T	—	0	
	5	10^0 in T	—	0	
	7	10^0 in 0		0	
	8	10^0 in 0.	—	0	
	11.	10^{-2}, 10^{-4}, 10^{-6} } ∞		27	2.7×10^3
	12.	10^0 P		0	
	13.			0	
	14	10^0 T	+++	7 many small.	
	15		2 large, ~~nn~~ sm. 3 + many small.		What are the small colonies?
	17	10^0 0.	—	0	
	18		—	0	

4P17. A18

What are the small colonies ?? Can conclude anyhow that u-v increases reversion rate markedly.

6/16/46. 17 JUN 1946

Inoc 50ml T(0) K-12 30° sh. 10P16.
3P17 harvest, centrifuge + sterile filter 25ml sample. = X_1

1. X_1 5ml + T(B) 5ml. Add X_1 sterilely 5 autocl. ++
2. X_1 5ml + T(B) 5ml. Autoclave together. ++

9P17 harvest second sample = X_2

3 X_2 5ml 5 autocl X_2. ±
4. X_2 5ml autocl. ±

Inoc ε 58-3214. 1220A18. 30°.

on 183 + T plates — filter paper tech.

a. .1cc X_1 —
b. .1cc X_2 —
c. (a) 10γ proline +++
d. .1cc X_1 boiled. —

There is evidently a considerable derivation: no growth proceeds.

Add 10γ proline to 4 1130P19. +++

Sex. plating

1 JUN 1955

Broz 50ml ∞ K1,183 1A17 30° sh.

3 P17. (14h.) ca 25ml. each + 50ml ∞ 30° S shaking.

930 #P17. Plate ord: 1ml equiv. after washing. Plate in thin layer.

		TP19
1	0	10
2	0	11
3	0	9
4	.5ml	13
5	.2ml	4
6	MP	turbid;
7	MT	"
8	BP	+++ colonies
9	BT	+++ colonies.
		10^4?

Isolate 20 colonies from surface of each. Satellite colonies quite distinguishing in both cases.

See 145 for tests

U-V Induced reversion.

~~243~~ 244.

17 JUN 1946

Use 679-183 cells of exp. 243

430P. Irradiate in medium 1 min. Shutter exposure

Unirradiated: 1/S = 53
ρ S = 1.7

1. 10^{-7} ∞ 30- not properly countable.
Wash both:
2. 10^{-7} ∞. 80 (8×10^8)
3. 10^0 T 21
4. 10^0 T 12
5. 10^0 T 11
6 10^0 P. Turbid!?

11. 10^{-2}, 10^{-4}, 10^{-6} } ∞ 10^4 (1.5×10^7)
15.

12 10^0 T 0
13 10^0 T 2
14 10^0 T 0
15 10^0 P Turbid Turbid!?

16 10^{-2} T 0

Broz. coli ∞ 50 milkj. ē 10^0. (A). 5P17. Sh. 30°

Effect here is very slight. Use longer killing.

Wash put in ē T.

19 JUN 1946

Test:	B	M	BM	P	T	PT	BMT syp
237-12			++			++.	OK.
243-8-	=B	=P	=T.	BTPM. -M	=-0	0	
1	++	+	++.				
2	++	+	++				
3	++	++	++				
4	++	++	++				
5	++	++	++				
6	"	"	"				
7	"	"	"				
8	"	"	"				
9	"	"	"				
10	"	"	"				
11	"	"	"				
12	"	[redacted]	+				Streak out
13	"	+	++				
14	—	—	++				(Short rods) (Hooray!). See c.
15	++	—	++				Streak out.
16	++	++	++				
17	"	"	"				
[redacted]							
[redacted]							
[redacted]							
238-1						· — · —	Not coli.
238-2 n.g. ∞						· ·	

243-9. From BT Plate.

21	++	++	++	
22	do.			
23	do.			
24	do			
25	do			
26	++	—	++	Streak out.
27	++	+	+	
28	++	+	++	
29	++	—	++	Streak out
30	++	+	+	
31	++	+	++	
32	++	++	++	
33	++	+	++	
34	++	++	++	
35	++	++	++	
36				
37				
38				
39				
40.				

Recombination tests, etc.

19 JUN 1945

BMPT. O

240-1/41 42
240-2/43 44
240-3/45

240-1	41	++	++	Small colonies on T/O
	42	"	"	but not biochemical mutants.
240-2	43	"	"	Morphological ??
	44	"	"	
-3	45	"	"	
	46	"	"	
	47	"	"	
	48	"	"	
	49	"	"	
4	50	"	"	
	51	"	"	
	52	"	"	
	53	"	"	
5	54	"	"	long rods; hazy internal structure.
	55	"	"	
	56	"	"	
	57	"	"	

Recombination tests

Analysis of a possible recessive recombination

#14. BP?

N21. Streak out on ∞ plates; inoc slants to keep it.
Clones to H_2O: ∞ slants N22.
Test on large tubes B: − P: − BP: − (medium?) add M to each.
BM!!

	B	P	BP	T	BT	O
141			−			
142			−			
143			−			
144			−			
145			−			
146.			−			

Check on def. & deg: − B − − M = − P = − T. = − O

679-188	++	++	−	−	+	
58-3214	−	++	−	++	++	
58-161.	−	−	−	−	++	Is M generally lacking?

151		−		
152		−		
153		−		
261			−	
262			−	
263			−	

These recotypes are n.g. See ext 249.

Sex: plating Exp. N.9. c46

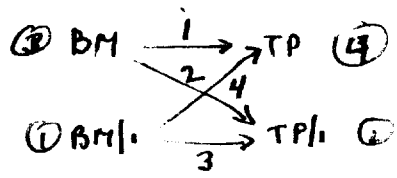
Segregation of resistance to T-1

19 JUN 1948

1A20 Incr 5 ml coli ∞ 30° sh.

(1) 2372/2 BM/1 (trypt.??)
(2) 23721 TP/1

(3) 58-161
(4) 679-183.



1130A20. 1 ml each in sterile test tube. 30°.

1. (3) + (4)
2. (1) + (2)
3. (2) + (3)
4. (1) + (4)

5P20. Plate 1 ml eq. (after washing) in thin layer. T(a) a = 0, ... x.

#		a	
1.	(1)	0	++
2.	(2)	0	
3.	(3)	0	
4.	(4)	0	++
5.	(1)	BM.	T.
11.	2	0	
12	3	0	+
13	4	0	
21	1.	0	++
22	1.	0	
23	1.	B	++
24	1. 10^{-2}	B	
25	1	M	T
26	1 10^{-2}	M	
27	1	P	
28	1 10^{-2}	P	
29	1	T	
30	1 10^{-2}	T	
31	1	BP	
32	1 10^{-2}	BP	
33	1	BT	++
34	1 10^{-2}	BT	++
35	1	MP	T
36	1 10^{-2}	MP	
37	1	MT	T
38	1 10^{-2}	MT	
39.	(3)	B	−
40	(3)	M	T
41	(4)	P	10^3 "
42	(4)	T.	"

3 pl.

1/2

1/2

1/2

see 247 - use these for entries.

Colonies through depth of agar
Must assume contamination of agar or solutions, etc.
Also lack of diffusion between 33+34, etc.

∓ = surface colonies. Not appl. to 1-5.
39-42 which were not found thin or surface.

q-V reversion.

24 JUN 1946

Inoc 50 ml coli ∞ sh 30° 8A 7 4. 5P 25. Wash + irradiate in H_2O. in Q. tube

1. 679-183. add 1 ml = to T (Threm) agar.

	Quad.	
1	0	4
2	0	3
3	½ min	
4	1 "	
5	2 "	
6	~~4~~ "	
7	4 "	+++ but only very minute colonies. Possibly only survivors
8	~~4~~ "	using product from killed cells.

Sex: other recombinations

Prototroph recombinations

Incr. [redacted]. 12M 2[redacted].0 50ml ea sh 30°

A. 58-161
B. 58-336

C. 679-183
D. 679-680.

5ml each. 3P2[redacted] 30° wash.

1. A+C
2 A+D
3 B+C
4 B+D.

Plate out 1ml eq. 11P2[redacted]. Use washed agar.

	T(0)	+	8P22.	5P23	
1.	A		—		
2	A	M	T	—	
3	B		—	O	
4	C		—		
5	D.		—		
11	1		—		
12	2		—	T	
13	3		—	T	
14	4		—		
21	1	BT	Turbid.		
22	1	PM	—	—	
23	1	BP	—	6	Incubate for later picking. very marked halation.
24	1	TM	T.		
31	1 10^{-2}	BT	—	T; ①	
32	"	PM	—	—	
33	"	BP	—		
34	"	TM	—		
41	.5	B	T.		
42	"	M	—	—	
43	"	P	—		
44	"	T		T	
45	"	O	—	—	
51	A	B	—	—	
52	A	M	T		
53	B	B	—	10 - compare 3 Marked halation P25.	
54	C	T	—	6	
55	C	P	6	7	
56	D	T	—	3	
57	D	L			

This may not be a good method to cross these bugs!

Other recombinations
Phage Resistance Segregations.

22 June 1946.

11P21. Broz. 50 ml coli ∞ sh 30°:

A 58-161
B 679-183
C 58-161/1
D 679-183/1
E 58-278-Y24 (in yx-pept + cystine 1 mg).

4P22. 5 ml each. as in 248.

1 A+B
2 C+B
3 A+D
4 C+D
5 B+E

9P abandon in view of 248

8Y5P22 Broz 10 ml each into 50 ml coli ∞ as above T
- rattin - inoc tubes 1-5 into 50 ml coli ∞.

2A23 - Harvest + plate as before.

1.	1	0	–	
2.	2	0	–	
3	3	0	–	
4	4	0		
11.	5	0	–	
12.	5	BØ	1	
13	5	BC	7	
14	5	T	1	32
15	5	P	6	
17	5	BØT	2	13
18	5	BØP	4	
19	5	BCT	2	++ cont?
20	5	BCP	38.	
16	5	0	1	
21	E	0	–	
22	"	BØ	A24	A25
23	"	BC		

many plates look contaminated. Do not keep.

June 24, 1946.

8A24. Incr together into coli ∂ (or ♂ + cyst − glucose = C).
30° 5 hr.

1. 58-161 + 679-183 C
2. 58-161/1 + 679-183
3. 58-161 + 679-183/1
~~4. 58-161/1 + 679-183/1~~ C
5 ~~×~~ 79-183 + Y24. C

3P25. Harvest + plate. Inel = .

	Incr.	T(0) + 6P26.	
1.	1	0	ca 10^2
2.	2	0	(5)
3.	3	0	0
4.	5	0	(4)
5	5	0	(4)
6	5	0	7
11	5	P	13
12	5	T	19
13	5	Bϕ	6
14.	5	BC	6
15	5	BϕP	(12)
16	5	BϕT	(29)
17	5	BCP	(18)
18	5	BCT.	(1[illegible])

Streak out Sec 254.

No quantitative evidence of [illegible] recombination.

S. pombe. 751

24 JUN 1946

8A24. Inoc into 10 C. 50 ml; T(0) + pombe. 50 ml
& 10 ml.

5P25. tube +++ oxygenation ??
flasks ±. mds glucose ? doubt.

930P25. Estō hemocytometer: 2.6×10^6/ml.
Use 2×10^{-4} dilution + plate in F(pv)

1. In thin layer –
2. In thin layer, covered ✓
3. In coli ∞. n.g!!!

Colonies first noted in F(pombe vits) A 28. (2½ days). Thus are rather variable. Large colonies near surface. Maybe intrinsic heterogeneity. Isolate colonies from baseplate.

P 28; A 29. Good size colonies. More uniform. Pick from single colony + streak out on coli ∞.

Ad; recombination

2521

26 JUN 1946

Use 1 ml grown cultures as inocula.

6P26.

1. Y40 + Y41.
2. Y40 + 183 Compare 250-2
3. Y41 + 161. Compare 250-3
4. Y24 + 183

12M26. Wash cts + plate in T(0). 1 ml = .

			10P27	12M26 P28.
1	1		3	22
2	1		1	14
3	1		1	63
4	2		10'	30
ampl. 5	2		10'	11
6	2		10'	11
7	3		17	13
8	3		1	7
9	3		0	9
11	4	B	ca 30	
12	4	B	30	
13	4	B		
14	4	B		
15	4	Bϕ		
16	4	BC		
17	4	P	10²	
18	4	T	10²	
21	4	BϕT	10²	
22	4	BϕP	10²	
23	4	BCT	10²	
24	4	BCP.	10²	

Segregate 1A29.

Abandon tests on these in favor of the more efficient Y24 (BϕC) × Y41 (PTG-IR)) with the additional character R.

12M30. Streak out 1, 3 & colonies. see 257

Bacterial "nucleoproteins".

26 JUN 1946

A.M. Exps. ± 12 hours ∞ culture K-12. Marked increase in stickiness of bacteria noted after 5 freezings + thawings in .9% NaCl. Considerable material extractable ± .9% which ppted ± alcohol in fibrous form (RNP?) Residue still sticky + fibrous. Treatment with 6% NaCl removed stickiness partially, but supernatant failed to ppt on dilution + apparently still had many intact cells. Probably freezings should have been repeated more.

11 P.M. Incr. col ∞ ± 58-161 for exps. next day of similar nature.

Conclusion: considerable amount ± .9% Nothing then removed ± 6% NaCl

100 ml culture 10 hours old. Centrifuge. Pipet supernatant. Suspend residue in .9% & centrifuge again. Suspend residue in .9% (ca 20 cc) and freeze + thaw 7 times in a CO_2 bath. Centrifuge. Supernatant - 1.

Residue + .9% extr + centr. Supernatant 2

S1,S2 + alcohol. no ppt. Residue not as sticky as yesterday

Residue + 6% Residue much stickier.

nothing extractable

27 JUN 1946

Suspend colonies of 250 in H_2O + streak out on coli ∞; mor. slants.

250- Test 1 A29!

21	2	BM/1 x PT. T(α). +	T-1 Resist. series: a + b + c + d + e + f + ~~g +~~	
22	2	+	+	
23	2	+	+	K-12 ~~+~~ −
24	2	+	+	Y40 +
				Y41 +
11	1	BM x PT. Streak out again.		227-1 −
12				controls
13				
25	2	+	T-1 resist +	
26	2	+	+	
27	2		+	
28	2	T-res. series: + a + b + c + d + e + f + g + h +		
~~21~~	~~2~~	all grew (−) on T(α)! Check on neg.		See below: ~~250.~~ 255.

		T(α)	T(β)	
41	4	+	+	
42	4	−	+	* Proto
43	4	+	+	
51	5			
52	5	−	+	* Proto
53	5	+	+	
54	5	+	+	
61	6	+	+	
62	6	+	+	
63	6	+	+	
64	6	+	+	
65	6	+	+	
66	6	−	+	

* transfer to slants + check later. (260)

~~Total tested for B.~~

grant. not valid: plated on minimal medium.

Total tested for B, - 28

B - (test.) 5

131	13	+ ~~(B+)~~	(B) +	(O) +	
PT x Bδc on Bδ vs on Breq.	2		+	−	* Biotin-less. Later test - did not grow on 7/19/46 B alone.
	3		+	+	
	4		+	+	
	5		−	−	
	6		+	+	
	7				
141	14		+	+	
20 BC.	2		+	+	
	3		+	+	
*	4		+	−	Proto.
	5		+	+	
*	6		+	+	Proto.
	~~7~~		~~+~~	~~=~~	
	8		+	+	
	~~9~~				
	10		+	+	

+ growth in (O) not in B - may be incomplete.

Read 830P29.

(3) Repeat from Column 3.

	Bφ	P	BφP.	
151	—	+	+	P
2	—	+	+	P
* 3	—	+	+	P
(4)	—	—	+	BφP2 BP
5	+	—	+	Bφ
6	—	+	+	P
* (7)	—	—	+	BφP?
8	—	+	+	P
9	—	+	+	P
0	—	+	+	P

(BφP.) – Repeat in small tubes:
Bφ –
P –
BPφ +

(P)
Repeat again in 10cc tubes
in uniform nicolla formol-starch:
B –
φ –
P –
Bφ –
Pφ –
BP +++
BPφ +++

7/1/46.

There can be no doubt from that this is $B\bar{P}$ which would be a recombination type for the cross:

$\underline{B^-\phi^-C^-P^+T^+} \times B^+\phi^+C^+\underline{P^-T^-}$

	BC	P	BCP
171	+	+	+
2	—	+	+
3	+	+	+
4	+	+	+
5			
6			
7	—	+	+
8	+	+	+
9	—	+	+

	BC		T		BCT.		
181	+		+		+		—
2		+		+	+		—
3		+		+	+		—
4		—		+	+		T
5		—		+	+		T
6		+		+	+		—
7		—		+	+		T.
8							
? 9		—.		+			Check: (P.)

from test plate: Requirements of 254-28. known 1.

	BM.	PT.	BPMT.
28.	—	—	

Later check: B-M-

12M 26. See
Inoc coli ∞ c 227-1. (Ultra-Violet.)
5P 29 (40h.) Irradiate ½, 1, 2, 5 min + inoc 1 ml in coli ∞ 50 ml. Inoc 1 ml each of these dilutions in coli ∞ plates for approximating killing 8h. liquid cultures; incubate plates 30°

2 - ca 10000 surv. (×50) pS = log $10^5/10^9$ = 4.
5 - ca 2 ×50.

Plate out ② at 10^{-7}. in T(0) detection plates. 11P 30.

330P2. Layer + ~~irrigate~~. (CSH)
11P12. Made numerous small colonies. Incubate.
ca. 1%
10A14. Picks to complete (not all, only those most convenient by way of isolation). Stock. A15...

Test Recombinants Exp. 252

29 JUN 1946

Streak out on coli ≈ plates. Number in range to test from >55

			T-1 cracked only	T(0) isol. col.
Y40+Y41	1	11	R ±	T-1 cracked
		12		
		13	all resistant: 6.	
	2	21	R ±	
		22		
		23	all resistant: 7	

Y41+161.	7	71	S ✓	S	
		[redacted]			
		73	S	S	
		74	S	S	
	8	81	S ✓	S	
		(82)	R	R	—
		83	S	S	
	&				
	9	91	S ✓	S	
		92	R	R	
		93	S	S	
		94	S	S	
Y40+183.	4:	41	[redacted] S ✓	R	
		42	R	S	
		43	R	R	
		44	R		
		45	R		
		46	[redacted] S.	S	
		47	R	R	
		48	R		
		49	S	S	
		50.	R.		

Linkage of R to BorM?

Isolate several colonies from 82+test:

	92
821	R
822	R
823	R
824	R
825	R
921	S
922	S
923	S
924	S
925	S

all +

all x

Phage analysis of Prototrophs.

30 JUN 1948

N30 Inoc coli ∞ Ē mid. cultures for incr. below. 4h. 30° 12 4.

1130P. Inoc sonic coli [redacted] 1 ml: + T-1 10^4 >4. 30°

" 1030 A 1. "

1. 257-71a. (183R × 161S) S. Complete lysis.

2. 255-24 (183S × 161R) R. Full growth.

N1. Plate and streak out –

1.	1.	10^0	
2.	1.	10^{-2}	
3.	1.	10^{-4}	ca 10^2 → See 262. Isolate colonies + test for T-1 res. + T(0) growth.
11.	2.	10^{-7}	T(0) d. ca 10^2. } no mutants present.
12		"	ca 10^2
13		" ∞	ca 10^2
14.		Streak ∞.	do not use.

330P2 Layer ∞ + refrigerate at 12 N 3.

259-C2 team B+/1 x B-... → B-/1

259-C6 P-/1... x P+... → P*-

[B+ φ+ C+ T- P- T1R] × [B- φ- C- T+ P+ T1S]

JUN 1946

Use Inl mac: Y41 (679-1831) + Y24 (BφC) into 50 ml colin C
Sh. 30°. 12M30.

12N. 7/1/46. Wash + plate into T(0). Save culture.

	P2	P12	
①	4		{ 7 S to T-1.
②	5		{ 2 R.
③ BCT.	27		
④ BφP	14		

A. P12. Pick colonies from 3 and streak out. P13. Test coil colonies.
T-1. = BCT+ BC − BT. ≡ T(0) LR probably (T- P+ R.)].

	T-1.		
1	T-1.		+
2	S		
3	R		
4	R	P̄T̄R ✓ parental	BCφPT+. See 272
5	R		
6	S		BCφPT+ See 272.
7	R		
8	R		
9	R		
10	R		
11	R		
12	R		
13	R		
14.	R		
15	R		
16.	R		

must pure on test / perhaps should streak out on OC again.

B. P13. Streak out colonies of 1, 2.

	T-1
1	S
2	S
3	R
4	S
5	S
6	S
7	S
8	S
9.	R

C: streak out P13. Test P14. A14 Test ④

	T-1	T(0)	BP —	Bφ —	BφP — BP	P
1	R	−	+	−	+	
→ 2	S R ✓	−	+	+	+	Protinless
3	R	−	+	−	+	
4	R	−	+	−	+	
5	R	−	+	−	+	
→ 6	S ✓	−	+	−	+	Prolinless
7	R	−	+	−	+	
8	R	−	+	−	+	
9	R	+	+	+	+	
10	S	+	+	+	+	
11	R	−	+	−	+	
12	R	−	+	−	+	

Segregation of Biotinless: Etc.
$B^-d^-c^-$ × $B^+P^-T^-$

Check. neg.

	T(0) room	T(B).
1. 254-144.	+	+
2. 254-146.	+	+
3. 254-52	+	+
4. 254-132	−	+
5. 254-42	+	+

Test other colonies from 254.

254-28.

1. BM − PT− BMPT+ BPT −? from BM/1 × PT

By auxan: BM 1. check. parental.

Recotypes

12 JUL 1946

10P12. Establish Dracula. in 50ml cols @ 30°C.

1. Y9 (LM)
2. Y40
3 Y41
4 58-161
5 679-183
6 Y24 n.g.
7. ~~Y33~~ Y38 } for radiation.
8 Y39.
9 58-183X n.g.

~~Dra 50 ml colid~~ Store in refrigerator 7P13.

Dra 50 ml cols:

① 161 + Y41
② 183 + Y40.

1. ① 6
2. ① B. 8
3 ② 9
4 ② B 19. See infra. Isolate and check for Bug, 11.
5 ~~See 262~~ Y40 + Y41 X-strain. 2 surface colonies. Will have to repeat procedure.
6. $5\ 10^{-2}$

Sex: cross-streaking

13 JUL 1946

7P13. Using loopful from 261, crossstreak on colic∞.

A. 1. Streak 1 2.
3.
TLM 1. Y9 TLM 161 BM
2 183 TP
3 Y41 TPR
4. Y40 BMR
5.

B. 11 Y40 BMR 183 PT
12 Y41 PTR

C 21 Y41 PTR 161 BM. Smeared.
22

D 31 Y38 Y39.

Scrape growth from B 11 B12, suspend in H_2O and plate into T(0).

no colonies.

B/s: double mutants.

13 JUL 1946

Irradiate Y38 and Y39 cultures (see 260) [redacted]. and incor: colis.

11p13. Sh.35°.

Y38 1 min
2 "
5 "

Test Y39 — dl leuc
dl isoleu.
l-leuc.

Y39 1 min
2 "
5 "

Detection plate Y38 5min. into T(arg.) at 10^{-7}. 4p14.
Y39 " into T(lc + isole.

Y38 - 2min
Y38 - 1 min 2 plates.

Y38 5min
1 600 cells. 4 colonies.
2
3
4
5
6
7
8
9
10

1 min 11 350 cells 1. 1 colony.
" 12

2 min. 13 500 cells. 9 colonies.
14

Y39
21 did not grow
22 evidently [redacted] is not leuc or isoleuc strain.
23
24
25 Test by auxan: e.g.g: histidine
26
27
28
29
30
31
32

Specific for tests on Y38 —

Sex: triple cross

TLM × BØC

9P. 7/14/46. 1 ml mix into colicin + C Sh. 30°

Y9 × Y24

N15 Plate nito:

1	0	0	
2	0	0	
3	0	0	
4	0	0	
11	B	0	
12	Ø	0	
13	C	4	turbid
14	T	0	
15	L	4	
16	M	0	
21	BT	0	
22	BM	0	
23	ØT	0	
24	ØL	6. v. sm.	
25	ØM	0	
26	CT		turbid
27	CL		turbid
28	CM		turbid
31	TLØ	ca. 16	
32	TMØ		
	BLT		turbid
	CTL		turbid

very desappointing. (resistance? or strain?)

Throw out plates.

679-680-Y9. 265

Y10.

July 16, 1946.

Check on requirements:

P16: Y9.

TLM	++
TL	−
TM	−
LM	−
TLM+Cyst	++
TL+Cyst	−

Growth is however not optimal at all: methionine; something else may be required. (Consider pab, homocysteine, choline, etc.

by TL + EAA, NEAA, YE, Vits.

TLM

TLYE +++. others + or −.

Y10:

TB_1	−
LB_1	+.
TL	−
TLB_1	+++

probably some $T^+L^-B_1^-$ in the population. Re-isolate — streak out from TLB_1.

2/5 isolates tested came up on LB_1 as well as TLB_1. same as Y45. Other three - same 1. as Y10a. (or after 7/27 as Y10.

Killer E. coli.
Resistance

7/15/46.

P 15 Imoto & Hershey's "T" and "R".

A 16. Filter "T" and test for activity as R in plates.

1	T + R 1 ml ea.	+++
2	T 10^{-3} + R	+++
3	T + R·10^{-3}	++
4.	T 10^{-3} + R 10^{-3}	++.

no demonstrable killing.

17 JUL 1946

"Reacting" strains "3" and "14" received from Dienes AY7.

Transfer to subculture slants D3 D14.

streak plates 10A17. D3 swarmed only. Rodes in D14?

Nutritional Requirements: 11P17.

grows very rapidly compact colo

		9A18	9A19	
D3.	Put Mic Cyst	++	+++	
	PN	−	++	
	PC	+	+	to C. + slowly ++.
	NC	++	+++	
	Cyst – Vits.	+++	+++±	
D14	PNC	++	+++	
	PN	−	++	
	PC	±	+	to C + slowly ++.
	NC	++	+++	
	Cyst-Vits.	+++	+++±	

Repeat for asp. vit. req.

	Cyst + 10 B vits =	D3	D14	10P18. 35°
1		++		
2		++		
3		++		
4	+	+	f. (mic)	
5		++		
6		++		
7		++		
8		++		
9		++	+++	
10.		++	+++	

cystine is stimulatory; probably not adaptation.

Coincidental reversion

1 [illegible] 1956

Recd. from Ryan a "prototroph" obtained directly from 679-680. Subculture

1. A17 streak out on T(0). No colonies
2. Incr loopful in T(0) No growth.

P19 - Incr ca 10^7 cells into T(0). Use loopful to incr T, L, TL:

0	+
T	—
L	—
TL	+++

Not prototroph!

M20. Incr coli ~ 2 % Use v. large inoculum. 30° C.

10P21. Plate out 1ml ea into:

1. T(lc)	10^3
2 T(thr)	0
3. T(0)	0
4 T(0)	0

240-5
Size variant (-)

17 JUL 19[illegible]

P16. Broc cocktail 30°C 4. ± 240-5a ①
K-12. ①

P17. Dil 10^{-7} and plate on detection plates. T(0)

A18.

1. K-12 +
2. 240-5 ~~+~~ ++
3. both. +: ++.

Phage: T-1 susceptible.

Pedigree:

236: ~~58~~ 58-161 × 679-183 as minimal. Pick to H_2O + streak a minimal plate. Pick a colony to water + plate d. (240-1). 2% small colonies. But all prototrophic. Check now for unstability of colony size.

If anything 240-5 is the faster growing colony type
240-5 large colonies at 24h
K-12 small v. distinguishable.

Repeat. Broc 12M18.
Plate 6P19.

1720- same result as above - K-12 colonies appear more slowly than 240-5 on T(0). They are indistinguishable on d! [Why was 240-5 first picked up as a small variant?].

TLM x BØC.

BØC x TLM

N17 Inl mor ea. into coli — > 30°

S30P18. Wash + plate Inl ÷.

		SP2a		
1	O	0		
2	B	0		BL
3	Ø	1	?	
4	C	57	T	[C]
5	T	0		
6	L			[Ø]
7	M	0		
8	BT	0		
9	BL	20	T	
10	BM	0		Compare — 264.
11	ØT	0		
12	ØL	0		
13	ØM	?2		
14	CT		T	
15	CL	52	T.	
16.	CM		T	

BCL?

Try Y18. BØC - TLB;

Sex: cross streak.

271

17 JUL 1946

N17. cross streak on colin 58-161 x 679-183.

1,2

3. Streak = mixed inoculum.

10P19. Plate inter T(0). ① @ ca. 10^8
② ● ca 10^9

no dup colonies. ∴ this is not a good lead.

259 – Recotypes? 272

July 18, 1946.
P17. P18' , 10 P 18

	BΦCTP	BΦCT^{-P}	BΦCP^{-T}	BΦPT^{-C}	BCPT$^{-Φ}$	ΦCPT^{-B}	BΦCPT^{-O}	
A4	++	–	–	+	+	+	+	$\bar{P}\bar{T}$/, parental.
A6	++	–	–	+	+	+	+	$\bar{B}\bar{T}$/, "

	BΦP	B	P	BP.	T–1	
C2	++	++	–	++	R	Recombination Types!
C6	++	–	++	++	S	

Y38 - mutants.

See 263.

	Arg.	
1	++	
2	++	
3	Spreader (not coli	
4	—	
5	—	T (A)
6	—	T
7	++	
8	—	C - 9 - Methionine. check in liq. Y43 ✓
9	—	A only. → glutamic. Y49
10	—	T
11	—	T
12	—	T (e)

256 - 1	T(o) +	aux: turbid; A.C.		(not coli)
2	+		ACD	
3	+++	—		lost.
4	+.	turbid		

	T(Arg)	T(Meth)	T(A.M.)
Y43.	—	—	++

Y39 - mutants.

July 19, 1946.

10 P 19. Irradiate 24 hour culture Y39 in ∞ 2 mins. uv. in medium.

① plate 1 ml in coli ∞ pS = 2 to 3.

② broz 1 ml in 10 ml coli ∞ 5 h 30°

Tape N 22.
1130 P 20. Detection plates - T (hist-leu) 10^{-7} and 5×10^{-8}
ca 1200 colonies total. 10 small colonies. pick. 8 P 23.
to ∞ slants. T(H)

1		++	probably not coli
2	not coli	—	
3		—	T
4		—	A
5		—	B? —
6		—	B — B-3 Y44
7		—	
8	~~sp.~~ r pseudomonas coli ∞.		+ on minimal (E2)
9		+	
10	n.g.		

uv xanthogaaph P. 25. - No vitamin sp. resp. in 6. checks in liquid + for yna. B-3 pab.

	12 h.	24 h.	
H	—	—	
H+ pab.	—	+++	10 r.
" yna	+	+++	
" M	±	±	
" M+yna	+++	+++	
-H+pab.	—	—	

Try 1. more pab
2. pab sterile filtered. yna replaces pab.
slow on pab

Sex conditions

July 21, 1946.

Inoc 1 drop each of Y24, Y41 in medeq of 275a.

30° ~~sh~~ sh. 11P21 Plate into T(0). 3P. 22.

		Growth	Sing. (1:10)^6 plated	Cells/cc	Colony count	x/cell.	
30° 1.	Coli ∞	+3	86^2	2×10^9	20	10^{-8}	+
2.	-glucose	+3	89	1.5×10^9	200	10^{-7}	
3.	-yx	+2	1:5 88	7×10^8	2	10^{-8}	+
4.	pH variation	pH=8. → a	78^2	3×10^9	100	3×10^{-8}	+ − ++
		b	72^1	4×10^9	40	10^{-8}	
		c	75^2	3×10^9	30	10^{-8}	
		d	85^3	2×10^9	10	10^{-8}	
5	Beef x.	+3	79	3×10^9	5×10^3	10^{-6}	++++
6	T(HCl)	+3	68	5×10^9	200	10^{-7}	++
7	MaltEx	? + sing.-matter	1:2 81	6×10^8	0	0	−
8	Coli hydrolys. T(0)	+3	79	3×10^9	10^4	10^{-6}	++++
~~9~~							
10	.2% peptone	±	1:2 93	2×10^8	0		−
11.	Coli ∞ vary salt. a 1%	++	74	3×10^9	10	10^{-8}	+
	b 2%	++	82	3×10^9	10	10^{-8}	+
critical point? →	c 5%	+++	80	3×10^9	0	0	−
	d 10%	−					
12	coli ∞ unsh.		93^2	1×10^8	10	10^{-7}	++
13	coli ∞ + cyst.		86^2	1.5×10^8	1 - 10 ?	10^{-8}	+
31.	unsh. 25°		95	10^9	10	10^{-8}	+
32.	38°		95^2	10^9	0	0	−
33	10°		93	10^9	1	10^{-9}	±
41	u.v.		86 (73^2)	1.5×10^9	10	10^{-8}	+

[Salt inhibits recombination?!]

Δ.

25-30° opt.
unsh
-glucose.

inoculate 50 ml of the following media ± Y24 + Y41. wash.

30°

1. Coli ∞ (yx .3%; peptone .5% glucose .5%). See 276
2. yx .3% peptone .5% ~~#~~
3. Peptone .5%; glucose .5%
4. Peptone - yx - (glucose) in T(0) adjusted to various pH's. — 8 a, 7 b, 6 c, 5 d, 4 e. no growth
5. Beef extract - yx. broth. .5%.
6. T(0) + N-Case + ~~[redacted]~~ Vit S.
7. Malt extract 1%. (~~[redacted]~~).
8. T(0) + E coli hydrolysate .1%.

~~9. Corn Meal agar stock.~~

10. .2% peptone + biotin

~~11~~ 31. Coli ∞ 25°

32. coli ∞ 38°

33. coli ∞ 10°.

41. Irradiate 1 min. ± U.V. then into coli ∞ 30° sh.

41. coli ∞ + 1% NaCl a
2% NaCl b
5% NaCl c
10% NaCl d. no growth

Conclusions:
optimal:
pH 7-8 } buffered
- glucose
- shaking
low salt
high nutrient N.
25-30°

Y10 = TLB.
Y41 = TPR

July 21, 1946.
Incolino, 1030 P21 1 drop each of:

1. Y10 x Y41
2. Y10 x Y24
3. Y41 x Y24
4. Y43 x Y41
5. D3 x Y41
6. D14 x Y41 agglutination!
7. D3 x Y43
8. D14 x Y43. agglut!
9. " Y43
10 Y41.

~~Tabloid~~ T(0) T(B) T-1 (1:d). etc. see cuffs.

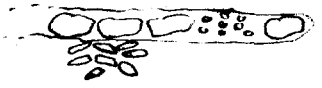
PTR, x BΦC	1.	B	③	36
	2.	B		37
	3.	B		64
	4.	B		55
	5.	0.		33
TLB, x BΦC	11	0	②	- 1
	12	B_1		+ 7 -
	13	T		+ 8
	14	L		20
	15	B		+ 11
	16	Φ		+ 4
	17	C		T 5
	18	B_1 B		++ 17
	19	B_1 Φ		++ 17 √
	20	B_1 C		T 7
	21	T B		1 9
readily hits	22	T Φ		9, many small
	23	T C		14 many v. small
	24	L B		14 "
	25	L Φ		++ 15
	26	L C		

See 276

TPR	31.	O	(4)		O
	32	A			4_3
AM	33	M		T	10^3
	34	T			15
	35	P			27
	36	AT			10^1
	37	AP			10^2
	38	MT		T	10^3
	39	MP			10^3
Y43	41.	O	(9)		O
	42	O			O
	43	A			50
	44	M	T. ca 10^2		100
Y41	51	O	(10)		O
	52	O		-	O
	53	T			100
	54	P			35
B3 Y41	61	O	(5)	T	
	62	O	(6)		
	63	O	(7)		
	64	O	(8)		

- star-like threads. —

microscopically long bundles of filaments & large cells of varying length + sometimes thicker up:



B. mycodes
see infra

Segregation of virus-resistance (T-1) 278

23 JUL 1946

Use plates 4C, 5, and 8 of the cross B♀C × PT♂. Test surface colonies directly for resistance to T-1 See p. 275

4C. 5 R
13 S.

5 7 R
15 S

8 4 R
16 S

Total: 16 R /60 total. Ca 25% recombination of R ē either B+, T+ or P+

See 284. 5R/20.

Summary.

me	16	60
284	5	20
p.279	2	9
	23	89 = 26%
284	5	21
	28	110

Stored overseas?

Aux conditions

23 JUL 1946

According to 275, a buffered meat-extract or coli-hydrolysate enriched medium is best for producing new prototrophs. Check on this with other mutants.

Inocula: ① Y41 + Y24 as 275. Media: ~~T~~ = "Yeast Buf" broth = M×Y
② Y41 + 58-161 Ba = Bact. hydrol. 1 mg/ml
PMx = Nutrient Broth.
③ ~~Y9 + Y24. (which has yielded no prototrophs hitherto)~~. Y10 + Y43. (4-12 × B/4.)

Inoc. 1 drop each (5 stock cultures). Incubate 30° ⑤ shaking.
1245 A 24. Plate 4P24.

	Medium.	Inoc.
1.	M×Y	①
2	M×Y	2
3	PMx	1
4	PMx	2
5	T(PMx)	1
6	T(PMx)	2
7	M×Y Ba	1
8	M×Y Ba	2
9.	M×Y	3.

Results are not encouraging.

How different from 275? — tense? shaking?

Try Y3-cross

See 279

For soil conditions: Plate 4P24. (15h.)

11	1.	100	(100)
12	1 10^{-7}	1	
21		10	10
22	10^{-7}	0	
31 ~~23~~		100	(100)
32 ~~24~~	10^{-7}	1	
41 ~~25~~		100	100
42 ~~26~~	10^{-7}	0	
51 ~~27~~		200	(200)
52 ~~28~~	10^{-7}	2	
61 ~~29~~		50	50
62	10^{-7}	0	
71		10	
72	10^{-7}	0	
81		10	
82	10^{-7}	0	
91		0	
92	10^{-7}	0.	

For recombination types: 1030 P24

(2). 10^{-3}

1	O
2	B
3	M
4	P
5	T
6	MP
7	MT
8	BP
9	BT
10	O
11	O
12	O

too dilute

n.g.

(1) 10^{-3}

21	O
22	O
23	O
24	B
25	B
26	B

K-12 x B/2.

11P24. 1 ml Y41 + Y43 in YB. Sh. 30°

N28. Draw into T(O). plates.

O

to 7P25, on desktop. Backs on shaker.

1P27. Plate out. O

Y43 x Y44 O

Proteus + coli.

11P24. Inoc YB D14, Y41 Sh 30°

10A25. Inoc YB 1 ml each of above Sh 30°

to A27. only typical bacilli.

[Repeat in yeast ext - peptone medium]

P27 Repeat in dilns.

a) D1614 Y41 — only bacilli

b) agar plate only atypical forms → practically exclusively the unusual organism (vastly in excess!) (actinomycete?) But true filaments of long cells like subtilis, staining well & saprophytic

Isolate + determine nutr. req., phage resistance, to exclude origin from Proteus [How about Proteus x coli?] Streak out from supernatant after large masses have settled.

Grows on plate like filamentous fungus. Refer to 283

B. mycoides according Tatum

Tests on 276 crosses

1. Biotin + phage-resistance segregation.

Y411 x Y24. m T(0)

Plate 5. T-1

	T-1	T(0)	T(B)	
1	R	−	−	
2	S	−	+	
3	R	−	+	
4	S	−	+	
5	S	−	+	
6	S	−	+	
7	S	+	+	Prototroph
8	?	−	−	not coli

5 R
15 S.

8 presumptive B⁻ mutants

∴ 4 B⁻ out of 32 attempts.
This is not a random segregation.
284-1 + 284-3 maybe original mutants. see infra for determination

Streak out + test for biotin.

Plate 4 ~~T+~~ ~~T(0)~~ ~~T(B)~~ T-1 T(0) T(B)

m Biotin

21	+		
22	+		
23	+		
24.	+		
25	+		
26	+		
27	+		
28	−	+	
29	−	+	
30	+		
31	+		
32	+		
33	+		
34	+		
35	+		
36	+		
37	±		
38	−	+	Yeast? contamination
39	+		
40.	+		
41	+		
42	+		
43	+		
44	+		
45	+		

17 total

5 R
15 S.

2

Plate 3

3

15 total.

of 21 prototrophs from ... 5 ... R

Biochemical ecotypes.

		Plates		Tubes		
		T(B,)	T(ϕ)	T(B,ϕ).		
TLB, x BϕC on B, ϕ. 19	1	+ +	+ +	+ +	−	This is very suspicious. There should not be so many prototrophs per original plating data
	2	+ +	+ +	+ +		
	3	+ +	− +	+ +		
	4	+ +	+ +	+ +	−	
	5	+ +	− +	+ +		
	6	+ +	− +	+ +		
	7	+ +	+ +	+ +	−	
	8	+ +	+ +	+ +	−	
	9	+ +	+ +	+ +		
	10	+ +	+ +	+ +		
	11	+ +	− +	+ +		
	12	+ +	− +	+ +		
	13	+ +	− +	+ +		
	14	+ +	− +	+ +		
	15.	+ +	− −	+ +		B_1^-

		B	BT	T	
on BT. 21	21	−	+	+	T^-
	22	−	+	+	T^-
	23	−	+	−	B^-T^-?
	24	−	+	−	B^-T^-?
	25	+	+	−	B^-
	26	−	+	−	B^-T^-?
	27	−	+	−	B^-T^-?
	28	+	+	−	B^-
	29.	+	+	−	B^-
	30.	−	+		B^-T^-?
	21b	−	+	+	T^-

		Bϕ	BϕT	T	
ϕT. 22	31	− +	+ +	−	ϕ^-
	32	−	+	−	ϕ^-T^-?
	33	+	+	−	ϕ^-
	34	+	±	−	ϕ^-
	35	+	+	−	ϕ^-
	36	+	+	−	ϕ^-
	37	+	+	−	ϕ^-
	38	+	+	−	ϕ^-
	39				
	40				

Check serum.

	ϕ	T	ϕT.	
32. large tubes	−	++	++	but came up late on T. streak out + test isolates. ↓ 4 isolates behaved similarly ϕ is just slow. or $T^- \rightarrow T^+$ very unlikely in this stream Drop it.

	B	T	BT	
23	−	+	+	
24	−	−	+	
26	−	+	+	
27	−	−	+	
29	−	−	+	

~~286.~~ Filtrate - transformation. 286

N 25. Inoc YB ≡ 58-161.

Filter Y41 culture in YB (from 283). Dil ca 1:3 ≡ YB. (1).

1. Inoc ≡ 1 ml 58-161. Sh. 30° 1P25.

2. Inoc YB ≡ 1 ml 58-161; 1 ml Y41 (culture above).

Plate 1P27. ca 200

Inoc YB ≡ Y41 P25. Filter 1P27 & as above 1.

Inoc 58 1(1 1-1)

Plate in T(o) 7P28.

(1).

7/26/46.

YB medium (ml each. Sh. 30° P26 - N28 Plate in T(0).

3. Y10 + Y24

10.

4. Y41 + Y24

300

5. Y41 + 161

100

6. Y41 + Y43 0. (K-12 + B/r)

48 hours. But not quite optimal numbers.

Sex - conditions

July 28. 1946

7P28. Inoc: a drop of mixture. 8h. 30°
– 1130 A 29.

			Growth	Clones ('/10 ml)
1.	Y24 + Y41	T(Ba)	++	36
2.	do.	YB	+Y	6
3.	Y24 + Y10	T(Ba)	++	0
4.		YB	+Y	150 ~
BM+TL 5.	Y40 + 679-680	YB	+Y	33
	6. Y40 + Y45	YB	+Y	0
B⁻P⁻ TLB.	7 58-183x + Y10	YB	+Y	30

8 679-680 (tgt a 680).	—	in O	O
		in T	O
		in L	10

Plate 0.1 ml ÷ 1-7 into T(O). 8 into O, L, T.
Same water suspensions.

YB is OK but not entirely consistent from one culture to another

EG Y40+Y45 Should be repeated.

Streak out 679+680 and use thereafter as Y47.

Y9; Y44.

7/28/46

Test Y9m:

		24h.
MV	+++	+++
MV−yna	++	+++
M yna	−	−
V	±	+++
M	−	−

Methionine may be mildly stimulatory as Roepke suggests it is in wild type.

Evidently Methionine + some vitamin may be needed. (choline??)

Try series ± vit. left out.

TLM+Vits. 12h.

1.	− B_1	+
2	B_2	+
3	pab	−
4	niacin	+
5	folic	+
6	B_6	+
7	niacin	+
8	pant	+
9	inos	+
10	biotin	+
11.	+V+yna	+
12	TLM yna	−

Yne is a pab-less, thus, which is not completely replaced by [redacted] methionine + yna. Compare Y44.

Y44: 8°25

	24h.	36h
1-H		
H + 100γ pab (streaks filtered).	+++	+++
+10γ	+	+++

Linkage of Virus - Resistance.

8 PM. 7/28/46.

Plate. Incl 287-4 into (Shred in 1/4, 0.2 V4 in 2nd)

				Δ over 0.	Δ/0	Look for
1.	0	30, 25, 26	Average: 27			
2	B	28, 33, 30	30	3	.1	R
3	ϕ	38 35	37	10	.3	R
4	C	29	29	2	.1	R
5	P	[redacted], 150, 136.	143	116	4.0	S
6	T	50 52	51.	24.	1.0	S

Summarily one might think: T^- unlinked
P^- linked either to B, ϕ, or C.

B, ϕ, C also linked to each other. Need other data. Analyze phage linkages. Test for resistance to T-1 + test biochemical req. of those ind.

P30.

		# tested	# resist	fraction.	Calc. R in mutant.	
1.	0	27.	2	.075		
2	B	23	5	.22	>0	Check R. $2B^-$ $3B^+$
3	ϕ	30	0	.00	0	
4	C	15	0	.00	0	
5	P	43	32	.75	.98	Check S. ✓ $8P^- 2P^+$
6	T	26	8	.31.	.5	Check S, R. ✓

5 T^- S
4 T^- R,
10 T^+ [redacted] S
1 T^+ R,

7/29/46.

M28 Incr 50 ml coli → : D3, D14. Sh. 30°.

Irradiate each 1, 2, 5 mins @ u-v in quartz tube.

Incr 1 ml into coli → sh 30°; .1 ml into → plate & Cover.

		Surv.	
A. D3	1 min	—	++
	2 min	10^3	
	5 min	10	
B D14	1 min		++
	2 min	10^5	
	5 min.		

Use D3 1 min. D14 2 min.

Cyst Nuc Pour detection plates in T(CN) at 10^{-8} dilution 6 P 30.

D3 - 12 × 40 = ca 500 colonies. 1 small colony.

D14 - 12 × 9 = ca 100 colonies 1 small colony.

Plates contam. do not pick.

Small Colony Variants in Detection Method. 292

7/30/46.

Going over old stocks, select all available which tested + on minimal (for parent) but which were picked from small colonies. Determine inheritance of this characteristic

A. 58-series. P29. Broth 50 ml coli ∝ ē stock. 54. 30°

1. 172-1	ng	
2 172-25	ng	
3 172-13		
4 172-32	ng	
11. 6303	*	>> col.
12 6321		large + small colonies on T(0). 1/500 sm. colony on layering.
13 6325		" " " " " no more on layering
14 6323		[>> " colonies on T(0). Do not repeat.]
15 6319	ng	
16 58	*	
17 6320	0	Numerous colonies appeared 10 P1.
18 6329	ng	

* Plated too heavily

Δ Large. 3P1.

→ auxanogaph.

Y10/1 × Y24.
TLB, R, BΦC

30.JIII.1916

1 drop ea. YB & 30° 7P 30.

Wash and plate: Use 1 cc in 1-7 · Rec chamber.

Calc. typeface 22a ~~BΦC~~

1.	O	46, 47, 64.	52	*	23	BΦT	12	17
2.	B	70, 66	16	*	24	BΦL	13	33
3	Φ	49, 61.	3		25	BΦB,	14 v. v. very small.	—
4	C	22+., 31+.,			26	BCT	11	
5	T	28+32=60	8		27	BCL	10	
6	L	44+44=88.	36		28	BCB,	13	
7	B,	94+70: (164)	112	*	29	BTL.	73[41]	302.
* 8	BΦ	9	4	*	30	BTB,	39	52
9	BC	7	0		31	BB,	41	—
10	BT	6	0		32	ΦCT	5	
11	BL	12	0		33	ΦCL	5	
12	BB,	19	0		34	ΦCB,	14	
13	ΦC	5	0	*	35	ΦTL	56+ 144 sm.	158.
14	ΦT	3	0	camp? 20.	36	ΦTB,	20	—
15	ΦL	10	0		37	ΦLB,	43	—
*	16 ΦB,	23	104		38	CTL	39	
	17 CT	11 (+)	0		39	CTB,	30	
	18 CL	10	0		40	BΦTL	56.	—
	19 CB,	12	0		41	BΦTB,	28	—
					42	BΦLB,	53	—
					43	BCTL	60 [24].	
* phaqe.	20 TL	35 +	196		44	BCTB,	28	
* ph.	21 TB,	22	82		45	BCLB,	41 [incl 6]	—
* ph.	22 LB,	46	350		46	ΦCTL	23 (small)	
					47	ΦCTB,	26	
					48	ΦCLB,	41	

680 – mutants.

August 1, 1946

Irradiate 36 hr. culture of 679 + - 680 u.v. quartz tube, 2 mins. 1P1
Incor 1 ml into colicin. (YB)
N 2. Detection plates.

Pick 8 colonies:

	T(0)	HC	V
1	+		
2	+		
3	+		
4	+		
5	+		
6	–	+	+
7	+		
8	+		

Phages T2 – T7.

August 1, 1946

Received, possibly contam., A 31 c titres ca 10^9.

Incr 1 ml ea. + 1 ml K-12 culture into YB flasks. sh. 30° 1130 P31–

Centrifuge off cells + sterile filter.

Plague out T7 + T3 on K-12 and on B/r deriv. at dil. 10^{-4}, 10^{-7}.

A1. T-3, T-7 clear; others turbid (secondary growth?)
Filter T-3, T-4.

Repeat with others. v.q.

Plate T3, T7 of above c mutants for resistance. (titer?)

T3 Y40 = do many resist. T7. not mixed well: no lysis?
Y41 good lysis on most of plate " " ; lysis; resistants as in control
161 do.
Y10. N.G.

Culture phages c B/r. A2. + K12 as liquid.

3½ h:

Filter	T2	K-12 ± ✓	B. ±
Filter.	3	±	—
	4	∓	T?
	5		T+
	6	F	T
	7	T	—

High titres developed, but activity seems to be lost after filtration exc. T2 which leaves no resistants. T6 could not be developed. Titers of other phages not consistent

Redevelop T7 on K-12.

2 7'

Incr K, B c culture 1P2.

Tests on 290 - Various R-linkage

See 290. August 1, 1946.

A. From 290-2. (on B) test 5 R.

	T(0)
1	+
2	+
3	+
4	−
5	−

B. From 290-5 (on P). test 20 s.

	T(0)	
	1	−
	2	− ✓ not B-.
	3	+
	4	−
	5	−
2+	6	−
2−	7	−
✓OK	8	−
	9	+
	10	−
	11	+
	12	+
	13	+
	14	+
	15	+
	16	+
	17	−
	18	−
	19	−
	20.	−

C From 290-6 (on T) Test 10.

	T(0)	T-1
	1	+ S
	2	+ R
	3	+ S
	4	+ S
	5	+ S
	6	+ S
9−	7	+ S
	8	− S
11+	9	− S
OK.	10.	− R

Check:

7 +
8
9 ✓ T−S
13 ✓ T−S
15?

	T(0)	T-1
11	−	R
12	+	S
13	−	S
14	−	R
15	−	S
16	−	R
17	+	S
18	+	S
19	+	S
20.	+	S

T−	S	5
T−	R	4
P+	S	10
P+	R	[illegible]
		1

Analysis of 293: Y10/1 x Y24.

1. Phage resistance from o plates. T-1. 25/35 susc.
2. do. B plates. 6/20
3. do. L plates 6/10
4. do. B_1 plates ? smeared.
5. B ϕ. [Exp. 1:20].
6. B_1 ϕ [Exp. 2:3] 2 ++ 1 B_1^-
7. T-1 on TL, TB_1, LB_1
8. ~~23~~. B ϕ T [Exp. 1:6] 4 ++ 3 T-
9. B ϕ L [Exp. 1:3] 5 ++ (1 BL) = 297-9. R_1.
10. B T L [Exp. 1:2]
11. B TB_1 [Exp. 1:6] 3 ++ 1 T-
12. ϕ T L [Exp. 1:3]. 4 T-L- 2 T- 1 L-

Phage analyses: 297.

	Bioch.	T-1	
1.	T-	R	
2	T-	R	
3	T-	S*	✓ Recheck biochemical reg.
4	T-L-	R	
5	T-L-	R	
6	T-L-	R	
7	T-L-	R	
8	T-	R	
9	T-	S*	✓
10	L-	R.	

297 8, 12.

6 T-
25
4R.

11	B_1- ✓	S *	✓	
12	B_1-	S *	?	
13	B-L- ✓	R -	297-9.	} 297-6, 11.
14.	T-	S *		

T- S 3
R 4

Rilling: K-12 on plates: u.v.

8 AUG 1946

Spread ca. 10^4 bacteria on surface of 5 plates.
Irradiate 0-120 secs. under lamp @ 17cm.
Check on amt. lost to spreader.

temp. 30°

0
0'
0/100
0/100'
0

0, respread after 3h. incubation.

time:
5
10
20
30
40
50
60
80
100
120

Plates smeared + contaminated.

Repeat: 2P9. Count most plates for dup colonies. Use 2% agarbase. 58-161.

Use complete cultures 10^{-4} .1 ml

1. 10^{-6}, 1ml 75,
   residue on smearing rod: 0
2. 10^{-4} .1ml 0 +++ (compatible ~ 7500)
3. 1 sec. ++
4. 2 sec ++
5. 5 sec. ca 200.
6. 10 sec. ca 10. (some may have been shielded by edge of plate).

$\frac{2}{75} = 2.7\% / 5$ sec.

$\frac{10}{7500} = .0013. / 10$ sec.

5 secs is diff. to control. Use 10 secs. and a higher conc. back.

$(.027)^2 = .0007$

Proteus mutants

August 8, 1946.

Irradiate 1.5 mins. in quartz tube.
P 3, DK1. Inoc 5 ml into 50 ml coli broth. 1118.

Detection plates 2A11. in T/cyst; nic)

Picks 12 03, 10014.

all grow on P(0) = [T + nic + cyst]

Y24 × Y10/1 ; ~~Y24~~ Y24/1 × Y10.

~~200~~
300.

8 AUG 19[illegible]

P8 mor. YB.

5PG plate into 0, etc. .5 ml

A

Y10/1 × Y24

	0	28	28.
	0	26	
	0	30	
	BT	30	
	BL	8T.	
	BB,	~~ca 100.~~ 82	
ϕT	~~[illegible]~~	34	
ϕL		16T.	
ϕB,		~~ca 100~~ 85.	

10/29 R_1 = .34

9+ T 1

9+ B_1 1

8+ ϕ 3

3+

9+ B_1 1

B

Y10 × Y24/1

	0	35
	0	15
	0	27
	BT	56
	BL	5?
	BB,	ca. 100.
	ϕT	36
	ϕL	29
	ϕB,	ca 120.

19/26. R_1 = .73

7+

3+ (BL) $1^{T\ 2}$ (300 - 4)

2+

Sample colonies + test for ecotypes.

Associated Mutations
Reversion.

August 10, 1946.

P10. Incc 50 ml coli 0 in 67% [illegible] Sh. 30°.

P11 Wash & incc 5 ml into T(0) + NEAA + Vits + EAA −
Plate 1 ml into T(0), T(Lc) T(Thr)
a) Leucine
b) Threonine.

Plates: L. 22
T. 4
O. 0

Recotypes.

August 15, 1946.

Incr YB P12 plate M14. .3cc each.

1. Y24 x Y10/1 29/49 = ~~sus.~~ 59%
2. Y24/1 x Y10
3. Y24/1 x 679-183
4. Y24/1 x 679-680.

301-1 "B,ϕ" — gave on B_1.
2 BB, " "
3 BB_1. " "
4 B_1C gave on C.
5 " " " "
6 BB_1 " "

301-1-3.

15	1. BϕTB_1 :	+ 6	ϕ B_1 6	(Bϕ 1) (BB 2)
15	2 CL :	+ 14	1 C (301-6)	
15	3. CB_1 :	+ 5	B_1 8	~~2~~ (B_1C) 2. 301-4,5
3	4. ϕL	+ 3		
5	5. ϕTL	+ 4	T_{R_1} 1	
7	6. BTL	+ 6	T 1	
15	7. BB, ϕ	+ 5	B_1 9	(BB_1) 1 (301-5)
13	8 B_1 ϕT	+ 4	B_1 9.	
13	9. BB_1L.	+ 8	L 2 B_1 2 B_1L 1	
3	10 BB_1T	B_1 1	B 1 + 1	
15	11 Bϕ T.	+ 12	B 3	Bϕ 2
15	12 Bϕ L.	+ 12	L 2	ϕ 1
12	13. BϕB$_1$L	+ 8	B_1L 2	B 1 B_1 1

2. Minimal " " " "

3. 0 BLϕ BLB BLD

4. 0 BLϕ BLB BLD

Collect B_1 and test for R_1. ~~+12/11~~ ~~7/20~~ resistant!! from Y24 x 110/1 !
χ^2 = ca 2.

Collect ++ and examine for heterogeneity. Select a ++ which appears to be recio but has, app., a recessive component. : 301-7.

See 305

Compare ± wilds on plates where each mutant could emerge

These ratios mean little.

Summary	+	B_1	T	L	B	ϕ	C	B_1L	(BB_1)	Bϕ
	86	36	2	4	5	1	1	3	3	2
ratio to wild	1.	1	.06	.07	.09	.02	.05	.2	.1	.05
counted		37.	31	55	56	52	19	16	28	41
+ on av. plates	146	~~86~~	58	70	~~89~~					

Strains:

K-12		L15	6522	B/r	Proteus
58	679	148-334	Y1	B/1	(Dienes)
58-161 ✓	679-680*	532-171	Y2	B	D3
58-278*	679-680A	209-301	Y3	T	D14.
58-309	679-183 ✓	152-171	Y4	R	
58-336	679-440	558-228	Y5		
58-580*	679-662*	572-228	~~Y6~~	C	
58-593*	~~679-680~~	1250-228		C-phage	
58-610*	679-680-Y9*	823-304.			
58-741	679-680-Y10*				
58-2651					
3214					
3232		*66-489 lys.			
3356		*152-171 lys.			
4899*		*18-152-171-meth.			
5030					
5255					
5273					
5298					
5417*					
5450					
5580					
5631					
5636*					

58 — Shigella paradysenteriae
6049 — Schizosaccharomyces versatilis
6177 — Endomyces albicans
Y17 — Endomyces fibuliger.
Y12 — Schizosaccharomyces pombe (Wickerham)
Y15 — S. pombe (Spiegelman).
Y16

Alkaligenes fecalis - Yale M-cl. 35 — proteh.
Aerobacter aerogenes - Yale M cl 12. — proteh.

* strains which adapt readily
" do not "

Penicillin.

15 Aug.

Drop 2 drops Y40 into 5 ml YB contg: 2P15 30°

a 50 u/ml — 11P15, N16 - filaments; "zygosp" common.

b 100 Markedly inhibited.

c 150 Strongly inhibited.

d. 200 " "

penicillin.

Repeat:-

10P21.

1. Penicillin 2500 u. /50ml. + 1ml mor. Y10/1. Sh. 30°

4P22. Filaments + beaded [redacted] rods. V. rare "zygosp."

4P31. Drop 1ml Y10/1 into YB 10 ml Sh 30°

Salmonella strains.

August 20, 1946.

Received from P+S diagnostic labs. to fresh slants

						12 h:	EN	NV	EV	ENV
EN	S1	para A	(I) II XII	a			+	−	−	+
E	S2	para B	(I) IV (V)	b		1, 2	+	±	+	+
E	S3	Cholera suis	VI, VIII	(c)		1, 5	+	−	+	++
E	S4	"	"				+	−	+	+
E	S5	enteritidis	I, IX, XII	g, m			+	±	+	+
E	S6	"					++	±	++	±
E	S7	oranienburg	VI, VIII	m, t			++	+	++	+
E, N	S8	montevideo	VI, VIII	g, m, s			++	+	+	+
E, N	S9	newport	VI, VIII	e, h.		1, 2, 3	++	±	+	+
E, N.	S10	"					++	±?	++	++
EN	S11.	typhi murium	(I) III (V)	(i)		1, 2, 3	+	+	+	+++
							+++	+	+	+++

24 hour readings:

		A −V	B −E	C −N	D −O	
E	1	+	−	+	+	para A
	2	+	+	+	+	
E	3	+	±	+	+	cholera suis
E	4	+	±	+	+	cholera suis.
	5	+	+	+	+	
	6	+	+	+	+	
	7	+	+	+	+	
	8	+	+	+	+	
aggl	9	+	+	+	+	
	10	+	+	+	+	
	11	+	+	+	+	

methionine, tryptophane

++ on EAA; ~~[illegible]~~.

methionine

3 pullorum strains - see infra. as above in analysis

Cross strain Salmonella pullorum, and S1, S4 ≡ T1, T3, ...

Recombination E coli 304.

10PM. 8/26/46.

Sh. 30° YB. 1 ml incc.

(1) Y24/1 × Y10

(2) Y24/1 × Y101 No colonies!!

(3) Y24/1 × 679-68C

(4) Y24 × Y1C 20/20 susc.

---

(1) Plate 5P28. (½ml.) Examine P30.

1	0	52	
2	0	51	
3	B,	211	
4	B,	~~129~~ (142 but smeared)	
5	BΦ	TB,	
6	BΦ	LB,	very crowded.
7	BΦ	TL	
8	BC	LB,	very crowded
9	BC	TL	turbid.
10	BC	TB, ~~EE~~	
11	ΦC	TB,	Too turbid.
12	ΦC	TL	too turbid
13	ΦC	LB,	too [illegible]

(3) 359

371 Test 50 isolates 78 ++
423 on T(0). Keep incc. 2 ?—
tubes in cold for v. Check. ++

This run may have many T- revertants.

304-3A
-3B

cystine contains too much B, B, etc. evidently.

data:

Plates.	Colony types:				
BØTB,	15 +	6 Ø	5 B,	2BØR	1B.! 1B?

BØLB, none taken

BØTL	20 +	4 Ø	1 BØR	1? ~~1? BØL~~ ~~1? BØT~~ BØ?	ØL?
BCTB,	5 +	3 B,	~~1 B,B?~~	1 B, T? R.	∴ mucotype.

+ 27/37 R. 8/9 R.B,. 10/10 R Ø

Imissed (304-1) See 305

40+	1 B	1 ~~BB,~~
10 Ø	3 BØR	
9 B,	1 B,T	
(8B,R; 1B,S)		

304-1 · streaked out + test:

10/10 S!!

ØL: app. OK but checked in detail. same growth as Ø alone!

Analysis of 301-7

30 AUG 1946

P30 - streak out and test colonies for T1 resistance.

1/15 resistant ① ⟶ 20/20 R.

1 clear lysis + colonies in zone of streak. ② ⟶ 1/10 R. (streak out.) = 26.

a. Streak out ① + ②

Test with seq. of several types.

1.	①	++
2.	②	++
3.	S	++
4.	S	++
5.	S	++
6.	S.	++

2' = resistant component of 2. (Later 1 - slow on -C ?] ++

a: test (a'1) + (a'2) colonies for resistance:

Compare 304-1.

2b. all resistant.

∴ 301-7 is evidently a mixture of R + S, 1/10 colonies from which was also contaminated.

Salmonella pullorum
leucinless mutant.

305

10 SEP 1946

48 hour 512 in YB. Broth subcs. in:

1. T(0) no colonies
2. T(6) not turbid!

later found nuds cystine

Strains for linkage study.

September 4, 1946, ff.

The 6 C_2 combinations of B, B_1, T, L are available. Streak out on NSA plates and incubate single colonies into 50 ml YB. Incubate to CC slants for inocula to confirm growth factor requirements. Incubate with excess T1, T3 in NSA plates for virus-resistant mutants.

	Sources:		Nutr. Req.	Virus.		✓
Mut.	"TL"	679-680		S_1	S_3	Y30
Recomb?	"TB_1"	304.	H.q. T−	R_1	S_3	Y31
Recomb	"BL"	300-1.	✓	R_1	S_3	Y32
Recomb	"BT"	285-24	✓	S_1	S_3	Y33
	"BB_1"	301-2	✓	S_1	S_3	Y34
Rev.	"LB_1"	[redacted] Y45.		S_1	S_3	[redacted]

Use vacant Y numbers.

BB_1
- 1
- 11 R
- 13 M

BT
- 11 S
- 13 M

BL
- 11
- 13 R, S.

TL
- 11 M
- 13 M

LB_1
- 11 R
- 13 R

Strains used

Shift. mutations.

8 SEP 1946

Recd. from Roepke + Langham:

α 15L-171 lys
β 18-15L-171 meth.
(γ) 66-489 lys.

According to covering letter, α: 5 single colony isolations
β: 1 isolation away from α.

"a single colony culture of α contains a few cells which require methionine."

Test α and β on:

		lys	meth	lys+meth.	O
1A8.					
6P.	α	+++	−	+++	
1A10		+++	++	+++	−
6R.	β	−	−	−	
		+	+	+++	−

Reversion Y10.

308

8 SEP 1946

Normal 36h. Y10 into

		colonies (48h.)
1.	O	—
2.	B,	—
3.	T.	1-(cont? — tehcont+ test)
4.	L	—
5	B, T	—
6	B, L	—
7	TL.	—

no reversions! (viability?)

11 SEP 1946

S12	L,I,H,C. ±	L,I,C ±	L,H,C ±	H,I,C. −	L,I,H,M. −	Leucine, cystine ...
S13	L,C ±	C ++	L −	L,M ±±		leucine, ~~cystine~~
S14	L,A,M −	L,M,C −+	L,A,C ++ −	L.AMC −++	~~AM.C~~ ++ −	Leucine, methionine, cystine
S15	L −	O −	LC +	C −	LM. +	leucine, ≠S
S16	L −	C +−	LC −±	++		leucine ≠S
S17	L −	C −	LC −			
S1 (R) ~~S~~	M −	T −	MT −+	++		methionine, trypt
Y50.	O	N,C				

Salmonella - Serv.

510

1 SEP 1946

Ind mor. S1, S17 (S15?) in AB = single controls. Sh. 30°

1230A 11

Plate N13. into F(O) dupl.

no colonies many plates.

Salmonella: N.R.

3 11

12 SEP 1946

	ENV	EN	NV	EV	O	thioglyc. +2
S18	+	+	+	+	— + R.	+
19	+	+	+	+	— ±	+ +
? 20	+	+	+	+	— +	+
21	+	+	— +	+	+	+ ±
22	+	+	± +	+	+	+ +
23	+	+	+	+	—	+ +
24	+	+	+	+	— +	++
25	+	+	± +	+	— +	++ ±
26	+	+	+	+	+ +	++ ±
27	+	+	± +	+	—	+ +
28	+	+	+	+	— +	+
29	+	+	± +	+	+	trough
30	+	+	+	+	— +	+
31	+	+	+	+	— +	+
32	+	+	+	+	+ —	± + -
33	+	+	+	+	— +	+ + ±
34	+	+	— ±	— +	—	± +
35	+	+	— +	±	—	— +
36	+	+	±	+	—	—
37	+	+	± +	+	—	±
38	+	+	± +	+	—	— +

These must be a substitutes to acct. for these results; probably cystine + methionine. Check with thioglycollate also

(also: arginine - prol; glut ald. - t; ϕ-alanine - tyrosine; tryptophane - serine)

S18 paathiotroph.

S19 paathiotroph

S36 paathiotroph

Ev. no good mutants here!

S19, S32, S34 } paathiotrophs; others are prototrophs

S35-8 may be mutants.

These tests were not too careful.

On retest, only S36, S37 held up as mutant types

Strains for linkage Study.

18 SEP 1946

1P18 Incr NSB to prepare inocula.

A19 – plate ± T1, T3 to obtain resistant mutants.

A22: Titer of T3 is very low + continuous lysis is not obtained!

Y41, TL, BL, BT, no sens. Y10/1 completely lysed!
Y24, BB, LB, * (~~no~~ rechecks).

Y10/3 OR for resistance.

TL/1 and TL/3 plates have 4 mucous dense white colonies.
do. LB.

Y24/1 on T3 showed no lysis. (cross-resistance ⇒ — contam!)
3 strains before used.

BM/3 OR. R. Pick to YB tubes. Test for
BB/1 — mucoid. resistance to 1; 3.
Y10/3 OR R.
BT/1 Mucoid.
LB/1 v.s. colonies v. dry - probably cont.
LB/3 } look like rdi, but green!
TT/3 }

Linkage: BMR x PT; BM x PTR.

18 SEP 1946

1P18. Inoc YB to prepare inocula. 679-183 from lyophil.

3P24. ~~Inoculate~~ YB. Plate P26

1. PT x Y24/1 5 tubes. -

2. PT x Y40 5 tubes

3. Y41 x 58-161 5 tubes.

R to T1.

1: 27/30, 20/20, 27/30. } 74/80 = .92 PT x ~~BMR~~ BØC/1

2: 18/20, 26/30, 5/7 } 49/57. .86 PT x BM/1

3. 0/3, 0/1, 4/10, 1/10 } ~~5/28~~ 5/24 .21. PT/1 x BM.

Resume linkage data – phage resistance 314

Ref.

257	BM×PT/1	2/10 R.	0.2
	BM/1×PT	7/10 R	.7

compare 313

259	BΦC × PT/1	2/9 R	.2
278	"	16/60 R	.27
284	"	5/20 R	.25
284	"	5/21 R	.24

313: 30/137 = .22 .78

BΦC/1×PT 27/30, 20/20, 27/30; 24/80 = .92 .92

287-	BΦC × PT/1	2/27 R	.1
	on B.	5/23	.22
293-	BΦC × TLB,/1	10/35 R.	.29
300A	"	10/29 R	.34
300B	BΦC/1 × TLB,	19/26 R	.73
301-1.	BΦC × TLB,/1	20/49 R	.41
	BΦC/1 × TLB,	27/37	.73

SUM: A.	BΦC × TLB,/1	40/113	.35
B.	BΦC/1 × TLB,	46/63	.73

χ^2_1 = 0.8 for .35 vs. .27 (1-.73)

χ^2_1 = 9 for .35 vs .50

χ^2_1 = 5.5 .73 vs. .50

1.08

315-	BΦC × TLB,/1	13/73 R	.18
318	BΦC/1 × TLB,	34/40	.85

R: Y40 × Y64 Y64 × 58-161

SUM:	A. Y24 × Y10/1	53/186	.28
	B. Y24/1 × Y10.	80/103	.77

353	47/57
358	16/18
364	49/70
359	32/50

313	BM × PT/1	0/3	
		0/1	
		4/10	
		1/10	
		5/24	.21
313	BM/1 × PT	18/20	.86
		26/30	
		5/7	
		49/57	

19 SEP 1946.

A. BΦC x TLB | 1 B', A': is mixture in std medium
B. BΦC/1 x TLB.

Use 1ml inocula for flasks; .1 for 10 ml medium in tubes

a). 5 of A; 5 of B in 50 ml YB 30° shk. (A_L, B_L)

b) 10 each in 10 ml YB in tubes, 30° shk.

Week + plate N20.

A series: some lactose col. many small heavily ruled mucoid

		Prototrophs.	T-1 R..
A	a:1		2/8
	2		0/3
	3		0/14
	4		0/13
	5		~~2/7~~

B series: all heavily turbid; maybe contam, or accom. rate/may be very high.

B	a.1	
	2	
	3	
	4	
	5	

A' numerous col. - not coli exc. 1/10 s.
B' like std B. plates

A	D. 1	0/3
	2	0/6
	3	2/7
	4	2/5
	5	1/3
	6	0/5
	7	5/10
	8	1/6
	9	
	10	

Sum total: 13/73 = .18

B.	1	
	2	
	3	
	4	
	5	
	6	
	7	
	8	
	9	
	10.	

Y10/1 x Y24/1.

20 SEP 46.

10 ml. YB at 4.30° Also controls

N.g. — Y24/1 cont
Stained Bodies

Staph. flavoviridis

20 SEPT 1946

ENV	NB.	NV	EV	EN
++	+	+	+	−

tryouts – e.g. ~~Beat~~ Repeat!

BØC
TLB, - 1

24 Sept 1946

In 10 ml YB, 1P24. do. - spread .1 ml mixture on 12 plates.
(A) + (B).

1. Y24 x Y10/1 (A)
2. Y10 x Y24/1 (B).

Wash + plate p. 26. with T(0).

controls: Y24 - no colonies
Y10 - no colonies
Y24/1
Y10/1
in broth

(A) (B). P28 - colonies as in both tests.
No evidence of marked increase.

B: T-1 R.
28 | 33
2 | 3
1 | 1
1 | 1
1 | 1
1 | 1

34 | 40 = .85

A- 1/9
4/10

September 29, 1946.

P29. BM/3 x PT/1 in YB Broth 10 ml.

P1. Plate into T10). only a few colonies.

Effects of dilution and of u-v killing on recombination

30 SEP 1946

N30.

A. To 10 ml YB, add 1 drop 24hr. Y24/1 and

10^{-2} 1. 1 drop 679-183 (PT) * compare ± final PT population. 1 drop ≈ .1 ml.

10^{-3} 2. 10^{-2} drops. "

Dilution. 10^{-2} 3 10^{-4} drops "

15. 4. 10^{-8} drops. " (plate .1 ml.). original = ca 10^9/ml OK.

~~5. 10^{-8} drops~~ " ~~plate~~

B. To 10 ml YB, add 1 ~~drop~~ 24hr. Y24/1 and 1 ml of the following.

6 see A1.

0 7. 1 ml u-v irradiated 5 mins. plate .1 ml. 10^4

0 8. 1 ml u-v irr. 10 mins. 20

0. 9. 1 ml u-v. irr. 15 mins. 20

Plate plate to T(0).

∴ sublethal doses of ultra-violet prevent recombination!

Heterozygote Isolation ?

30 SEP 1946

11 P30. Broc 10ml YD Y10/1 ; Y24.

P1. 1. Mix cultures, centrifuge and wash. Plate into T/0);

A. Centrifuge together 3-4 hours to pack cells together. 0, 1, colony.

B. Wash ~~together~~ separately + plate together. 4, 2, 0 colonies. too dry to pick.
This, therefore, is the better procedure.

Salmonella typhi-murium

Mutants - (u.v.).

30 SEP 1946

P1. Inoc S20 and S21 into YB.

11P2. Irradiate each culture 1, 2, 5 mins. + inoc 1 ml samples into 10 ml YB. Detection plates SP3. 25°.

	1	Y20 - 1m	2000 cells sampled.	7 small colonies.
	2	2m		
A	3	5m	Use.	
			Picks A 8.	
	Y 5	Y21 1		
		2		
B	6	5	Use. 1200 sampled	2 small colonies.

		YB.	O.	E	N	V	ENV	
A	1	+	+	→				green 2 cont.
	2	+	+	→				green pink. cont.
	3	+	+	→				OK. mi color yellow
	4	+++		→				
	5	+		→				
	6	+++	−	+++	+++	−	+++	← OK. S 70.
	7	+++		→				OK. mi color.
B	8	+		→				green cont.
B	9.	+		→				yellow

some app. do not grow well even on

S70:

paratriosophe

Salmonella - N.R.

30 SEP 1946 Al:

	T(o)	ENV	EN	EV	NV	
SR4 abeq.	−	− ++ +	++ +	++ +	++ ±	++
36 gall.		− −	++	± +	++	± ±+
37 dub.	−	+	++ ±	++ +	++ ±	++
42 t.n.	−	+	++ ±	++ ±	++ −	− ✓
45 ent.	−	−	±R ++	±R ++	±R ++ −	− ✓
50 para A	−	− −	++ −	− ✓ −	(±) ✓ −	− ✓
51 B	−	− ✓ +	++ ✓ +	++ ± ✓ +	++ ✓ +	++ ✓
52 B	−	− −	− −	− −	− −	− −
55 chols.	−	− ✓ ±	++ ✓ −	++ ✓ −	+ ✓ −	− ✓
56 chols.	−	++ ✓ ±	++ ✓ ±	++ ✓ ±	++ ✓ −	++ ✓

? (Estim) grows well on T(o). [Test later]	
V, E.	Thiamin
( )	Thiamin.
(E)	Typ
E.	Arginine ✓
E, N, V.	~~[illegible]~~ BIOTIN, TRY.
(V)	
?	- slip?
E (N)	TRYPTOPHANE; ~~(methionine)~~, ~~arginine~~ SERINE.
( )	

The cleanup assay:

342 - typhimurium - (E)
345 - enteritidis (E)
350 - para A (ENV)
555 cholerae suis (E) (N)

others are:

S24 - pullorum

gallinarum V, E
dublin,
S56 chol suis } strin? no gr eq.
S50 para B

552 - did not grow

42 no gr. Trypt.
45 Arginine. (citr ✓) (urea)
50 Biotin; TRYPT; grows N.E.A. (~~methionine~~; meth) BTCM n.g.
55 TRYPTOPHANE; SERINE T+S n.g.
no!! 36 ENV +++ V ++ E ++ N − O − ? V or E + − B,
37 ENV +++ V ++ E ++ N − O − Try V alone V or E + − B,.
56 ENV +++ V − ++ E + ++ NE ++ O − ~~T~~. E or NE
~~[illegible]~~
52 ENV +4 EV+ EN− NV− ~~[illegible]~~. O − Hey all.
51. ENV +++ V+ E+ NE− O −

Penicillin

1 OCT 1946

add ind. amts. of Penicillin (Oxford units) to 10 ml YB broth.
add 2 drops of Y24 + Y10/1. 10 P 1. Sh. 30°.

		Penicillin (per 10 ml).	10 A 2.	
3	1.	0		
1	2.	100.	some very motile motility diminished/a few filaments.	
0	3.	200	v. "stringy" turbidity	v. filamentous
0	4.	500.	partial inhibition.	v. filamentous; many "zygospores"

Wash + plate.

10 A 2 Also anon. 1 ml each into 10 ml YB.

1	1'.
7	2'.
2	3'.
8	4'.

penicillin is suppressed.

Edwards 10/9/46.

	S		O	ENV	EN	EV	NV	
57	typhi Smith		—	++	—	—	—	ENV
58	abortus ovis		—	+++	++	+++	—	E
59	sendai		—	+++	+++	+++	+ ++	Try E. (usually 9 EAH)
60	sendai 571		—					
61	Taube I		—					E or N? methionine. Campaic 56 etc. do.
62	Taube II		—					
63	P-1		—					
64	P-2			+++	++	+++	+++	
65	P-11		—					
66	1181-1		—					
67	1181-2		—					
68	H66		—					
69	Pigeon 1366.		—					
70	322/6 S 20.			E; N. + methionine. (SH.).				

Bath Israel 10/21/46

71	typhi 2943 Smith	
72	sendai 3007	
73	para A 3280	
74	para A 3089	
75.	typhi 3166	

Kaufmann

76	S. para A	
77	" Durazzo	
78	typhi 2	
79	" Wolfram	
80	S. sendai	
81	" negram	−
82	"	+

Linkage.

20 OCT 1946

1. P2 YLY x Y10/1.

[2. YLY x 183]

[3. 183]

4. YLY x Y41

5. Y41.

Plate .5 ml eg. (1) .2 ml eg. 4,5 into T(0) and suppl. for detail linkage of R.

Plate .5 ml eg. Y41 into O, T, P. 20 colonies on [illegible]! crowded on [illegible].

Exp 1. 1:

Salmonella crosses. 325

12 OCT 1946

Inoc 1 drop of 12 hr. cultures into 10 ml H/B. Plate 10/14/46.

S1 x 13
1 x 45
1 x 50
1 x 55
coli) 1 x TL.

1 colony formed. [para A + para A !!!!]. Needs rechecking.
Later found that S1 sweets.

13 x 45
13 x 50
13 x 55
coli) 13 x BT

45 x 50
45 x 55
coli) 45 x TL

50 x 55
50 x TL
coli) 55 x TL

1
13
45
55
TL
BT

repeat. S1 x S50. 10/16/46. Plate into T(0), T(tupt).

very many minute colonies in presence of tryptophane. Should be repeated ē
(ca ½) a more diluted inoculum. v. much smaller (a few cells / colony) ∃ tupt.

Evolutions

20 Oct 1946

Mix 2 grown cultures. N2. (YB) 30° 2h.

Y24 x Y10 /1 Plate 3P3

~~Detection plates - 7(0). 10^{-7}. P3~~

2 colonies. / 1 mfg.

Trilateral combinations test for transformation.

12 OCT 1946

Inoc 1 drop 12 hour cultures into 4B Plate 10/14/46.

	O	T	L	B.
TL	—	—	ca 10^4.	
		B	L	
BL	—	—	—	
		B	T.	
BT	—	+	+.	

BT + TL + BL } 1/4 plates (turbid). turbid. ca 10^3 not so numerous →

This expt. is inconclusive, since there were a large number of L⁻ cells present. Quantitatively, it seems to support the recombination hypothesis.

Reisolate TL.

Salmonella - N.R.

S45, S50, S55.

	1	2	3	4	5	6	7	8	9
	O	PROL	GLUT	ORN	CITR	ARG.			
S45. ✓	—	—	—	+++	+++	+++		arinthine	

	NV-TR. ✓	NV-TRM. ✓	V-TR ✓	V-TRM. ✓	N-E ✓	BIOTIN+E. ✓	E ✓	NE+E ✓	N.E.V. ✓
S50. ✓	—	±	—	±	±	—	—	++	+++

	SERINE-E.	NE-TR.	Ser-TR.	NE-E ✓	E	NE ✓		
S55 ✓	+++	+++	—	+	+++	+	++	+++ ?	try Trypt+meth.

S56. ✓ NE- Tyrosine (??) 0.

S36 ✓ V■ (B_1) also. 0.

		B_1	B_2	3	4	5	6	7	8	9	10
S37 ✓	V+.	B_1 +++	B_1 only OK.					Threonine ✓			

S42 E- Tryptophane.

S58 E-

S59 E- Grows on any combination of 9 EAA.

S61 E- Grows on any combin. of 9 EAA.

(Heterozygotes) 329.

4 OCT 1946

Dror 24 hr. cultures of Y24 and Y41 into T10 varying grams. (washed separately. Dr water together ca. 3 mins. Mix with agar before pouring. A4.

most plates sterile. A few have many non-coli bugs.

15 OCT 1946.

Add Y40+671-183. & varying chloral hydrate

		Growth.	Plate:	
1	1%	±		sl. (?) inhibition, but not feasible for exp. use.
2	.1%	+++	ca 100.	
3	.01%	+++	ca 500	
4	.001%	+++		
5	.0001%	+++	ca 500	
6	.00001%.	+++		
7.	—	+++	ca 10^3	

plate. H18. Compare, however, effect of 5% salt.

But swarming in motility-gelatin-agar is only partially inhibited by .1% chloral hydrate (1-2cm/24h.) Try .2%, .5%.

Saccharomyces sp. (ss.)

V.t. Rey. – mai. Fruits.
no growth – pab
delayed growth – mi.

Salmonella NR

529

21 [illegible] 1945

S36. O B, V, Ts.
− +++ +++ OR. Thiamin.

S56 O tyr tyr+glyc V,Ts Tyr+V,Ts ØA+V,Ts
− ++ ++ ++ ++ ++. which V Ts?

S50: O ENV EN NV EV V tryp tryp luce tryp luce tryp meth NV tryp luce
+++ − +++ − − −

S55. O Ser+E E Ser V Tr. NE Tryp. NE-Ser. + tryp. NE
− − + ++ ± ++ − ± + +++ ± +++ ∴ Tryp. + {Ess.

S42. O Tryp. n.g. 24h ~~very slow!~~
− − ± +++ Tryptophane.

S58 O Tryp.

S59 O Tryp. Single amino ac: E: amyg. a. +++ O ++. adapted??

S61 O ~~Tryp.~~ Single amino ac. E: meth +++ others −

S077
S67
S64.
O
tryp

S70. O Tryp. Single amino ac E: meth +++ others −

Sarah. Lwid. gamma B vits etc
− pab, folic OR on − folic. ∴ − pab, − nic!
− nic.

Salmonella, N.R.

23 Oct 1946

	O	EN	ENV	ENB	ENB.	NV	BN	
S50.	−	−	++++	+++	−	+	+	∴ Biotin. can desp. 2.a.a.

	O	SH	Cyst	Meth.	Trypt	Bridde Panther
S61	±	−	−	+++		

S70. — ± +++ +++ +++ ++ +++. two paratricotroph!

S42 — ±++ ✓ ± ++ ✓ — — another → mf → trypt. ∠ ∈ F. ideo.

S62
63
64
65
66
67
68
69

59. ± ++ heavy accr ++. (mod. adepts!)

S56. Try as all amino acids + vitamins separately. V +++ E +.
Biotin ++ ✓ not E. alanine ± lys. ± trypt ± tyros. ± || 48 hr: Biotin +++ alanine ± tyrosine +++

S55. Trypt + NE (all +; letatrine) and ~~Trypt~~ NE (all): ✓ OK.
T + N ++
N ++
individually: — — exc. cystine ++ Try cyst. alone.
O — heavy accr. in O +++

later O: — Test growth in proline, for transfer growth
any NE. ++
all NE do. in minimal, proline; compare original culture.

E coli mutants.

24 OCT 1946

Selective media (lactose)

1. EMB Difco. (OK.)

2. Endo: lactose 1% pul.
   Na_2SO_3 2.5
   Fuchsin .4 g
   K_2HPO_4 3.5 g
   Nutr. agar
   n.g. as made up.

3. Purple lactose:
   Nutr. agar
   lactose
   BCP .025 g/l. n.g.

Streak K-12, S1 and K-12+S1 on each of these plates, for decision as to most appropr. medium.

S50 Biotin +: (N)— (EAA) BV + nonegrow.

S56. O Biotin Tyrosine dl-tyrosine Biotin? ?
— ± ± +++
± +++

S55. O Trypt. Cyst. Tr+Cyst. Tr+Prol.
S55. — — — — —
S5a. — — — +++ — muds Trypt, cyst adapts readily.

S57. EN V−: nonegrow! -Leucine found later (tubes allowed to stand)
EV N−:
NV E−:

S58 E− : nonegrow!
O ENV NV EV.

S60

S51 NE+ proline +++. others — NE +++ Serine ±

S52. NV E−

S61. O Meth. NV N-cyst Cyst.
+++ +++ +++ +++ +++ all adapt!

O Meth Tryp.
62 ++ +++ ++
63 + +++ +
64 + +++ +
65 ± +++ —
66 ± +++ —
67 ± +++ —
68 ± +++ —
69. — +++ —

Sugar mutants – E coli.

Yeast

Endo-lactose	K-12	S-43
a. Med School	+++	– I
b. made up	+++	–
c Endo-glucose	+++	+++
d. Endo-sucrose.	–	I
e maltose	+++	slow +

EMB.

N.A. + 1% sugar + 2g K_2HPO_4, .4g Eosin Y; .065g MB/l.

a. lactose	–	+++ –
b. glucose	+++ slow	+++ rosheen!
c. sucrose	I	
d maltose	+++	–

Try: E coli suppl.

Coli (T10))
½% glue — n.g.

Fries (N10))
2% sucr. — requires pab.

* Burkholder's
5% gluc. — only on vits.

Coli T10)
5% glue — n.g.

*/liter
glucose 20
aspar. 2
NH_4PO_4 1.5
$MgSO_4$ .5
$CaCl_2$ .3
$NH_4{}_2SO_4$ 2.0

work?

	O	glut	prol	orn	citr	arg.	E-arg.	Ho prol	E	N
S51. ✓	+++	+++	—	++	±	~~+~~	—	—	+++	+++

later, all up- adapted (!?)

	O	trypt	T+ cyst	T+ meth	T+ S-MeOH.
S55 ✓	—	++	+++	+++	+++

S52. ✓ NV E- all +++ exc. trypt. ++.

S50. ✓ Biotin + N- all — BN, BE ++ !!

	ENV	EN	NV	EV	E	N	V	O
S57.	—	~~+++~~						

	ENV	EN	NV	EV	E	N	V	O	
S58	+++	++	+	+	—	±	—	—	(may have adapted on slant (large + small colonies on Rb slant).
~~S60~~	"	"	"	"	"	"	"	~~+++~~	

S56. ✓ O. ~~+++~~ Biotin l-tyrosine dl-tyrosine. alcohol

— +++ (lodoslike a contam.) +++ —

	l-tyr.	l-Øal	gly-tyr	tyr-gly	glut-tyr	tyr tyr	gly	dehydro Øal	pOH-o pOH-glyc	glyc	de-hydro Ø glyc.
	+++	—	+++	—	+++	+++	—	—	—	—	

rhodamalcohol.

	mg per 5cc
Blank	
l-tyrosine	0.10
l-phenylalanine	0.10
glycyl-l-tyrosine	0.16
l-tyrosyl glycine	0.15
l glutamyl-l-tyrosine	0.25
l-tyrosyl-l-tyrosine	0.20
glycyl dehydro phenyl alanine	1.5

filtered solutions

	p-OH phenyl pyruvic	1.0
*	phenyl pyruvoyl glycine	1.5
x	glycyl dehydro phenyl-alanyl glycine	1.5

* old solutions.

Factor mutant: Y10 335

29 OCT 1946

washed suspension 10⁹

Inoculate Y10 1, 2, 5 units. SP 10/28.

Incr. 2 ml into 10 ml YB shake 11 H 29. Spread 5 min. ca 100 each on Endo-lactose + EMB-lactose plates. Incubate at 30°.

~~2~~ no mutants / 3000 on EMB.

Incr 1 ml 2 min. culture. Re-inoculate 1, 2, 5. spread on ~~100 plates~~ (EMB-lactose).

Spread 2 units:

161 × ~~III~~ =

64 "

480 × ~~III~~ "

200

75 "

ca 200/plate average.

80

16,000

6 pink colonies which are not obviously contaminants.

Pick to complete for further identification.

1 Yeast — cont
2 morph. typical coliform.
3 came up v. slowly on YBagar
4
5 Yeast cont.
6 came up v. slowly on YBagar.

∴ 2 is only likely possibility of a lactose – coli mutant:

335-2:

T_1^S

requires T_1 L, B_1 ✓

Y 53

Activity on various sugars: K-12, Y53.

11/13/46

The strains were tested on EMB plates & sugars:

	glucose	sucrose	maltose	lactose	galactose	
K-12	+~	−±	+	+	+	
Y53.	+~	−±	+	−	+	& this is interesting!?

Salmonella crosses.

28 OCT 1946

Plate (washed) 24 hour cultures of:

S1 x S50. ○ typ.

S1 x S50 deleted. + T. Latent virus??? (phage ??) — no secondary.

↓ → typical prototroph? colonies

42 x 70
42 x 37
= 42 x 61 (T.) —

13 x 36
1 x 45
42 x 45.
61 x 70 -

Tryagain - many (++++)
do. v. small colonies

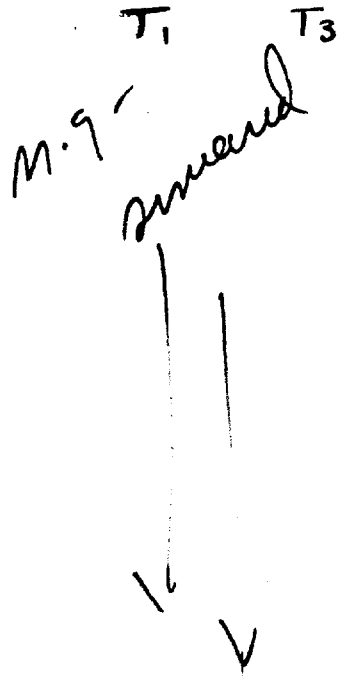
28 Oct 1946

Plate 24 hr. cultures:

(A) Y10/1 + 58-161/3 only ca 5 colonies/plate

(C) Y10/3 + 58-161/1. ca 25 col./plate.

Y10/1
Y10/3
BM/1
BM/3



(A) (1)

(B) (2)

⋮

28 OCT 1946

Plate 24 hour cultures of: mix-

	B*	T*	L*	
B⁻T⁻L⁺	0*			* same minute colonies
B⁻T⁺L⁻	0	0	10	probably B⁻T⁺ on agar bacteria
B⁺T⁻L⁻	0	0	400	- pick bacteria colonies for nurse tests.
3 strains:	0	100	1	0
	0			

B⁻L⁻ × B⁻T⁻ apparently only B⁻ appeared in the mixture. (from B⁻T⁺L⁻ × B⁺T⁻L⁺)
must repeat + demonstrate recombinability of all three strains!

Try B⁻L⁻ × T⁻L⁻ etc. But supports thesis of recombination.

Y10: revision.

28 OCT 1946

Plate washed Y10 in.

O

TL ca 100 colonies.

TB$_1$ 1 dup colony, n.g. for pick.

LB$_1$. (plated too heavily.) ca 20 colonies.

Time of recombination.

29 OCT 1946

add .1ml old Y10 and Y41 into ca. 4ml YB. at various incl. times. At terminal time, wash all cultures + plate; compare prototroph frequency. Plate .5ml agar. 30° ch. also compare YB + 5% glucose, YB + 5% NaCl, YB + 10% Blood

24h.	1030P29.	1.	+++
12h.	10A30.	2	+++
10h.	12N30	3	+++
9h.	130P30	4	+++
7h.	330P30	5	+++
	Centrifuge 1030 P.		
2h.	11 P.	6	+++
1h.	1245A	7	++
½h.	1245.	8	−
	Cents. 1245.		

The area -2 hours. should be explored in more detail with comparative cell counts. Selective growth can not be a critical factor in so short a time. Also grow cells separately + 2 hours + mix — hours + plate.

conditions:

glucose	++
NaCl	++± although growth was very much diminished!
Blood	++
" 1:100	−
control	++

29 OCT 1946

Sc 0.

S. cerevisiae haploid - (S.c.) on B(0) [Burkholder's + 5% glucose].
37° ; shaking preferable

V-

B_1 - stimulatory (carryover??)

pab-
Pyridoxine
pantothenate
inositol
biotin.
} required on this medium.

cf. Fries (may contain B_6, inositol in the lactate.)

came up in 48 hours on overflowing but:
- biotin
- pab - pant.

6 vits above - 0 ++ | B_1 +++ | pab + | B_6 +++ | pant + | inos ++ | biotin ±

- pant + alan ++ | + pntate + | - pab + Bc +.

also

Biotin pab pant	Biotin pab	pab pant	Biotin pant	Biotin pntate pab	Biotin β-alanine pab	V	-pab +meth +yna
+++	+	±	±	+	+++	+++	+

carryover evidently too large for critical work; however, it is clear that Biotin and pab are essential; and β-alanine limiting.

S1 x S50.

342

1 NOV 1946

5 cultures each - S1, S50, S1 x S50. mix mainly P 1.

Plate N4 ½ ml ≅ or .01 ml ≅.

S1 — dil

S50 — dil. no colonies.

S1 + S50 — dil.

colonies appear which are the same in approx. numbers as those which occur in the S1 + S50 mixture. It is difficult to account for them on the basis of a double inversion!

v. supra.

S1 x S50. – phage 3 4/3

Plate 3 loopfuls of S1 + of S50 as follows.
on nutrient salmonella agar.
S1 10^{-7} + S50. = S1/50 etc.

1. 1/50
2. 50/1
3. /1
4. /50
5. 1/
6. 50/
7. /1, 50.

no evidence of phage action.

when each area previously inspected, it showed no ~~zone~~ transmissable lysis. Therefore this hypothesis is probably not tenable !!

Recombination: Time of Occurrence

Y10 x Y40.

4 NOV 1946

Prepare fresh cultures for inocula. YP4.

Inoc 10 ml YB with 1 ml each inoc. at various times as indicated.
(Calculate from beginning of centrifuging) 28°

Read initial and final optical densities to measure amount of growth.

Transfer cultures to smaller tubes for centrifuging and washing.
Inoc. .5 ml each washed cultured into a minimal agar plate
Inoc. 1 ml into 10 ml prepared H_2O to measure the inoculum size.
Plan experiment to last 2 hours plus 1 hour for wash and plate

	Time:	Initial d		Final d.	growth	Inoc.d. (cells) (1:6)	colonies ×10⁹		R/10⁹
* 1.	2h	10:07	66' 66	2882	1.60		1.7	ca 20.	10
				2059	1.14	882	1.24	11	9
2.	1.5	10:37	66' 66	55 2596	1.44	87 (1.5)	2.02	20	10
				55 2596	1.44.	87	1.35	14	10
3.	1.0	11:07	65 64	59 2291	1.27	86	1.46	ca 20	15
				53 2366	1.31	882	1.24	18	15
4.	.5	11:42		63 2007	1.11	813	2.04	25	12
			1800	62 2059	1.14	87	1.32	14	11
5.	15m	11:57		64 2 1905	1.05	91	.96	20	20
6*		Mixed 12:10.		64 2 "	1.05	91	.94		
				66 ———	—	93	.74	—	0
7					1.00	90 2	1.07	25!	25!
					—	~~81~~	~~2.35~~	—	

832:660 filter.

* Mix immediately before washing; in 7. mix after washing.

#4 on 54

Washing commenced at: 15: 77

Centrifuging 12:24. 73°

Readings at 12:10

* contains Neurospora cont.; individual second 1 colony coll 341-1 Neuro

by this run, there seems to be an appreciable frequency of recombination on mixing in the plates!!!

Later: separate cells from medium before testing

Y53 x ~~Y47~~ Y40

Segregation of lactose fermentation.

5 NOV 1946

Prepare inocula P5 in modified YB broth (NYB): per liter-

Nutrient Broth	8
Yeast extract	1
K_2HPO_4	3
KH_2PO_4	1
NaCl	5
Glucose	10.

Plate after 4 hrs. growth.

a) ~~P5 glucose~~ NYB-glucose

b. NYB-lactose

c. NYB-no sugar.

all colonies are similar on lactose + bar on glucose plates!

1. Y53a x Y40a in NYB-glucose.
   Plate in: glucose- 187! v. variable!
   lactose 247! v. variable size.

2. Y53c x Y40c in NYB-
   glucose no colonies.
   lactose no colonies.

3. Y53a x Y40b in NYB-
   glucose
   lactose

4. Y53b x Y40b in NYB-lactose
   glucose
   lactose

5 Y53a x Y40a in NYB-lactose
   glucose
   lactose

6. Y53b x Y40c in NYB
   glucose no colonies
   lactose no colonies.

7 Y53b x Y40b in NYB.
   glucose 115 do.
   lactose 120. uniform size

	$L^+ T_1^R$	$L^+ T_1^S$	$L^- T_1^R$	$L^- T_1^S$
7 gluc.	4 ✓	–	6 ✓	–
lac lact.	3 ✓	1 ✓	1 ✓	2 ✓
4 gluc	3 ✓	1 ✓	4 ✓	2 ✓
lac lact	2	–	7	2
1 gluc	1		7	2
fuc lac	4 ✓		6 ✓	
	16	4	31	6

Test Lac^+ after replating on glucose medium.

	L^+	L^-	
R	16	31	47
S	4	6	10
	20	37	57

18% S
$\chi^2 = 23$
$p = < .001$

35% L^+

20% S 17%

$\chi^2 = 4.6$
$p = .02$

7+4: lac / gluc.

	L^+	L^-
lac	5	12
gluc.	8	12
	13	24

	4	6
	3	7
	7	13

The presence of asparagine in the plating medium might be responsible for loss of fermentative ability and segregation of the gene. Must the media contain no other carbon source?? Grow cells on synthetic medium, 4% 53 on glucose; 4% 16 on lactose; mate in presence of lactose only 3 growth; plate in lactose minimal (5 asparagine) and in glucose and compare numbers which appear; also test Lac^+ for dissimilation.

Salmonella Crosses
S42 x S45; S61 x S70.

346.

8 NOV 1946

In YB broth 11A5 Crosses YB4PB.

— dil.

S42
S45 v. numerous colonies.

S42 + S45 turbid dil

S61 turbid. numerous
S70 — v. minute colonies.

× turbid
no colonies.

Dissimilation of lactose fermenters

1 C NOV 1946

Streaked out fermenters from 345 - 4L and - 7L on nutrient - salmic agar plates. Retest isolates on EMB - lactose.

all as before. (4)

Non-Genetic, Nutritional Adaptation

Disproportionate segregations can be interpreted on the basis of the transfer of ~~cy~~ plasmagenes, or cytoplasmic entities. Since growth rate on minimal medium is less than on complete, the efficiency of plasmagenes in biosynthetic processes is a limiting factor and one expects that there will be, on minimal, selection for those cells which by virtue of essentially non-genetic variation have the most numerous and efficient cytogenes; on complete medium, there will be no selection at this level; if anything, it will be for efficiency at later synthetic steps (e.g. protein synthases). This might be revealed by comparing cells grown on synthetic and on supplemented medium with respect to the lag which they exhibit when transferred, after washing, to minimal medium. Briefly, can cells become adapted to minimal medium? (in the adaptive enzyme sense, as opposed to gene mutation.)

For media use - coli (o) + 1% glucose; as supplement, add vitamins, hydrolyzed yeast nucleic acid, and hydrolyzed casein

Wash cells in coli (o) + glucose or asparagine or NH_4 ions.
i.e. in phosphate buffer
K_2SO_4
NaCl
trace of
$MgSO_4$; $CaCl_2$

Nutritional adaptation

3415a

11/7/46

a. Large inoculum from agar slant. Shaken at 37° for 15 hours. resuspend in H₂O. wash. 3 ml.
b. 1 ml from a in same medium. (930+18) Shake 37° 1 hr.

1. 2	Lactose adapted	a.	into lactose ferm.
3 4	Glucose adapted	a	into 1 5
5 6	x adapted	a	into x 0
7 8	0 adapted	a	into x 0.

11 etc. is as above = b.

3.1 b

L/L	L/G	G/L	G/G	∞/0	0/∞	0/0		Time — hours.	
1	2	3	4	5	6	7	8		
88	88^{3}	92^{3}	92	96	98	91		0	1045A
~~89~~ 89^{1}	89	92^{3}	91	95^{1}	97^{3}	91^{2}	93^{2}	30m.	~~1115A~~ 1115A
89^{3}	89^{2}	93^{3}	90	92^{2}	97^{3}	90^{1}	94^{1}	2h.	1245A
90^{1}	90^{1}	88	76^{2}	94^{3}	97^{2}	59^{1}	61^{1}	4 1/4	3P
90^{3}	90^{2}	75^{1}	67^{3}	94^{3}	98	38^{3}	39^{1}	5 1/2	420P
90^{1}	91	62^{2}	54^{1}	94^{3}	98	34^{1}	34	6 3/4	530P
+	+		29	+	+		30		12M

log 2 1/2 hr log 1 hour

log 1 = 1 hour

(30 = 1 hour)

			73	80^{1}	80	80^{1}	80
11	12	13	14	15	16	17	18

9 NOV 1946

Grow Y54 + Y41 in YB 1½ days. Plate in T(0) p 9.

P12 – numerous yellow + white colonies (cont??) —
check for T_1^R, Lac$^+$.

Segregation of Lac⁻; Evidence on transformation.

18 NOV 1946

6 PM prepare media. Mix (1-2ml mix / 2ml YB) and sh. 10 A 11. Wash + plate 1 P.

1. Y53 × 58-161
   TLB, Lac⁻ T_1^S BM Lac⁺ T_1^S

2. (Y1011) × 58-161
   TLB, Lac⁺ T_1^R BM Lac⁺ T_1^R

   yellow colonies present in all plates. Contaminant somewhere!! (Wash-water ????)

3. ✓ Y53 + Y1011 + 58-161.
   TLB, Lac⁻ T_1^S TLB, Lac⁺ T_1^R BM Lac⁺ T_1^R

4. ✓ Y53 × Y4D.
   TLB, Lac⁻ T_1^S BM Lac⁺ T_1^R as usual. ignore

   do not use, of course.

5. ✓ Y53 × Y40 (lactose) in YB-lactose plate in lactose-minimal: L
   and in glucose- " : G
   (5 asparagine).

N.B.: If mixing of factors can take place (i.e. transformation) +++ should be found which are Lac⁺ T_1^R (such as are found in cross 4.)

Conditions for Sex: terminal salt

9 NOV 1946

Y10; Y40. prepare inocula separately 1) P8.
2) 1 P8.

6 P8: inoculate 1.5 ml of mixture into YB + ascending NaCl conc. (4 each)
830P. wash 9P plate .5 ml ± 0 into T(0) agar. 4 YB mix
d 1140c.

1	35.
2	60
3	35
4	20
5	27
6	13
7	5
8	5
9	10
10	4.

Best is to grow in ca 2-3% NaCl
or to grow separately + mix after washing ± no growth!
use relatively young inocula.

Also, inoculate 0, 5% ± separate cultures, 1ml. washes above
11 - mix 0's in 5% and rewash. 20.

~~12. mix 0's after washing~~

12 - mix 5's after washing. 30.

Effects of saline: Test for agglutination by salt of mixed culture —

5 hours:

	agg.	gr. nits.
0	—	—
1	—	—
2	++	—
3	++	—
4	++	—
5	++	—
6	+++	+
7	+++	+
8	+++	+
9	++++	++
10	++++	++

Sex conditions

Wash 10 ml 16 hr. cultures (YB) of Y40, Y53 ≈ water. 10 AM.
Mix 1 ml samples at various times; and dil part mixture → 2.5% Na Cl.
plate at: 3PM. Incubate). shake gently at 30°.

		H_2O.	Na Cl.
1.	10 A.M.	20.	6
2.	1130 A.M.	8	5
3.	130PM.	14	1
4.	3PM.	2 plate.	0.

Note: they were more dense in the H_2O than in Na Cl
$(\frac{c_1}{c_2})^2$ = ca 4. which might account for the results.

5. Mix 1 ml sep. culture ≈ 5 ml agar + pour successively. 0.

Next use younger cultures. No confirmation of effectiveness of Na Cl in increasing rate.

Segregation of Lac⁻

Repeat 350. Prepare inocula in YBS 3:30P 14
Incubate in YB 11A-2P4 31.

1. Y53 - 58-161 Too few to pick
$T_1^S Lac^-$ or $T_1^S Lac^+$

2. Y10/1 - 58-161 Too few to pick.
$T_1^R Lac^+$ $T_1^S Lac^+$

3. Y53 - Y10/1 - 58/161 11 $T_1^S Lac^-$ 4 $T_1^S Lac^+$
no $T_1^R Lac^-$
total {Lac⁻ 41
Lac⁺ 13
54

4. Y53 - Y40. Most plates too smeared to be readable.
9 $T_1^R Lac^-$ 2 $T_1^R Lac^+$ 3 $T_1^S Lac^-$ 0 $T_1^S Lac^+$

5. Y54 - Y10/1 no colonies. total {Lac⁻ 41
Lac⁺ 13
54 = 24%

get Y53/1

See previous exps.

Phages on var. coli strains

		T1 ✓	T2 ✓	T3	T4 ✓	T5 ✓	T6 ✓	T7	φ-C
Y54 ~~B₁ φ²~~ / Y40 T¹ B₁ Lac⁻									
1	Y54	S	S±	S ✓		S	S	S	S
2	Y40	R	S+	R		R	S	S	
3	Y53	S	S±	S		S	S ?	S	S all lac⁻
4	58-161	S	S	S		S	S	S	
5 —	Y10/1	R	S	R ?		R	S	S	
6	" B14/3"	R	S	R	S	R	S	S	
7	" Y10/3" —	S. ✓	S	S	S	S	S	S	

titer too low? 1 plaque each

plaques v. small !!

OK.

5) to Y10/1,3,5 singly

Throw out these "B14/3", "Y10/3"

T1 = (R/1,5)

Plate phages + bacteria on surface of EMB plates to secure other resistant types.

Y10/1/7 ca 10^3 K-12/1 ca $10^{3.5}$

58-161/7 ca 10^2 lac⁺?

Y53/3 ca 10^2 smeared

Y53/7 ca 10^2

Y40/7 smeared - ca 10^3 some on each on 1st streak. Picks.

58-161/3 ca 10^3 smeared. do. " " " picks

Y10/1/3 smeared — called T3 small — is Y10/1 T_3^S ??

Y40/3 smeared. — do. Y10/3

Phage Resistance groups.

555

Parent.		Phage.	T1	2	3	*	5	6	7	
Y53	1	T1	R ?	R	R		R		R	resistance may be due to flora of mucoid growth??
	2		R ?	R	R		R		R	
	3		R	R	R		R		R	
M	4		R ?	R	R ?		R		R	
	5		R ?	R	?		R		R	
	6		R	S	R		R	S ?	S	
	7		R	S	R		R		S	
S	8		R	S	R		R		S	
	9		R	S	R		R		S	
	10		R	S.	R		R		S	
	11		R	S	R		R		S	
	12		R	S			R		S	
	13		R	S			R		S	
	14		R	S			R		S	
	15		R	S			R		S	
	16		R	S			R		S	
	17		R	S			R		S	
	18		R	S			R		S	
	19		R	S			R		S	
	20.		R	S			R		S	

Y10/1		T7.								
	1		R		R		R	not available;	not free of phage	resistant as to whole host culture although lysogenic
	2						R			
	3						R			
	4									
	5									
	6		do.	not available do.	do.		do.			
	7									
	8									
	9									
	10.									

Y53		T3								
	1	M	R		R		R		R	
	2	M	R		R		R		R	
	3	S	R		R		R	not available.	R	
	4	M	R		R		R		R	
	5	S	R	S?	R		R		R	
	6	S	R		R		R		R	
	7	S	R	S?	R		R		R	
M	8	S	R	S?	R		R		R	
	9	S	R		R		R		R	

Phage resistance groups

		T1	T2	3	5	6	7	
Y53 mucoid	1			R	R		R	
	2			↓ with eaten through	R		R	
E	3				R		R	
	4				R		R	
	5				R		R	
	6				R		R	
	7	S	↑ do. S	later mucoid	S	no plaques (To mucoid) S?	R	less mucoid.
	8				R		R	
	9	?			R		R	
	10				R.		R.	

all with eaten through (not fuzzy edge)

Test isolate for strokes.

G	1	Y10/1	R	S	R	R	S	S	
	2	Y53	S	S	S	S	S	S	
	3	Y55	S	S	S	S	S	S	
Y53/3	4	Y57	S	S	(S)÷R)	S÷R	?	S	mucoid.
Y53/7	6	Y58	S	S	S	S	S	S	! – compare E. (mucoid).
Y10/1/7	6	Y59	R	S	R	R	S	R	
~~no~~	~~7~~	~~Y60~~	~~R~~	~~S~~	~~R~~	~~R~~	~~S~~	~~R~~	
Y497	8	Y61	R	?	R	R	S	R	
58-161/3	9	Y62	S	S?	S !	S	S	R.	plaques v. small.

The 13 resistants seem to become sensitive very rapidly when removed from phage!

	1	R	S?	R	R	? S	S↓
	2		?	R	R		
F=Y53/1	3	↓	?	R	R	↓	
	4		?	R	R		
	5		?	R	R		
	6		?	R	R		
	7		S	R	R		
	8		S	R	R		
	9		S	R	R		
	10		S	R	R		

all are 1, 3, 5

Phage resistance patterns.

Conclusions.

A. } all (smooth) T_1^R are also T_3^R T_5^R T_2^S T_6?
B. } S mucoid T_1^R are resistant to all phages, incl. T_2, T_7..
F. }

C. an fresh isolation, Y101 ; Y531 give T_7^R which contain phage, and
E. which are plaque infected, suggesting either reverse mutations, or loss of resistance by bacteria.

on streaking out, Y53/7 has given rise to $Y53T_7^S$. (reversion?)

Y40/1/7 ; Y10/1/7 → $Y53T_7^R$ OK.

D. Y53/3 shows equivocal resistance to T3; T5 after purification. KJ.
1,3,5,7. appr. only does obt. on immediate streaking.

G.

Sex: conditions.

a) 36 hour known cultures: Y53, Y40.

b) prepare fresh cultures from these 1130 A18.- compare when mixed in H_2O (after washing) for 1 hour. (see 352). Prepare the b) mixtures in 1:5 dilutions also. c.

a). 1ml eq. each in 1ml total

b.) do.

c.) as above + 4 ml. H_2O.

Plate comparable nos. of cells known.

d 1:5 — a. 75
b. 91
1.5×10^9 R = 1.4

∴ Test cultures will be known

1) old

2) post [illegible] in H_2O is not favorable.

3) dilution effect insignificant.

	time 0		time 1 hr.	
	.5	1.0	.5	1.0
old A	3	6		20
new B	90		22	
mixed c		6		

(ca 10^{-7}).

Test for segregations:

T_1^R lac − 9

T_1^R lac + 2

T_1^S lac − 3

T_1^B lac + 0

Y61 x Y53

Segregation of Virus Resistance

Prepare inocula P21. Plate P22 — also surface

A24: - pick from colonies to minimal agar to avoid contamination; also test 15 directly.

		T1	T3	T5	T7	Lac	B- M-				× T L B, Lac -
A	✓1	R	S	S	S		T1 T3 T5	T7			
	✓2	R ?	R	R	S						
	✓3	R	R	R	S						
	✓4	S	R	R	S		R S S	S		2.	
	5	?	R	R	S?		R R R	S		4	
	6	?	R	R	S		S R R	S		3	
	✓7	S	S	S	S		S S S	S		2.	
B	✓8	S	R	R	S	—					
	✓9	R	R	R	S	—					
	✓10	R	R	S??	S	—	R RS?	S		1	
	✓11	R	S	S	S	—					
	12	S?	R?	?	S	—			?	2	
	13	R?	R?	?	S	—					
	✓14	S	S	S	S	—					

A1 and B1 were confused ? as demonstrated by Lac test!! should be:

	S	S	S	S	-
	R	R	R	S	-
	R	R	R	S	-
A2.	R	R	R	S	+
	R?	R	R	S	+
	R?	R	R	S	-
	S	S	S	S.	-

and these is only one possible discrepancy! A5. should be — R.

otherwise: 10R/14.

	R	R	R	S	-
	R?	R	R	S	-
B	R	R	S??	S	-
	S	S	S	S	-
	?	R?	?	S	-
	?	R?	?	S	-
	S	S	S	S	-

Virus - Resistance segregation

357.

Y61 x Y53.

From same plating as 357:

	T1	T3	T5	T7	Lac
1	R	R	R	S	−
2	R	R	R	S	−
3	S	S	S	S	−
4	R	R	R	S	−
5	S	S	S	S	−
6	R	R	R	S	−
7	S	S	S	S	−
8	R	R	R	S	+
9	S	S	S	S	−
10	R	R	R	S	−
11	R	R	R.	S	−

	T1	T3	T5	T7	Lac
1	R	R	R	S	−
2	R	R	R	S	−
3	R	R	R	S	+
4	R	R	R	S	−
5	S	S	S	S	−
6	R	R	R	S	−
7	R	R	R	S	+
8	R	R	R	S	+
9	S	S	S	S	−
10	R	R	R	S	+

D

1	S	S	S	S	−
2	R	R	R	S	+
3	R	R	R	S	−
4	S	S	S	S	−
5	R	R	R	S	−
6	R	R	R	S	+
7	R	R	R	S	−
8	S	S	S	S	−
9	R	R	R	S	+

	S	S	S	R S	−
	R	R	R	S	−
	S	S	S	S	−
	S	S	S	S	−
	S	S	S	S	−
	S	S	S	S	−
	S	S	S	S	−
	S	S	S	S	−
	R	R	R	S	−

X

A

1	R	R	R	R↓ ?	−
2	R	R	R	R	−
3	R	R	R	R	+
4	S	R?	R	R	−
5	R	R	R	R	−
6	R	R	R	R	−
7	R	R	R	R	−
8	R	R	R	R	+
9	R	R	R	R	+
10	R	R.	R	R	−

C

22 R | 40. all T_1^R are $T_3^R T_5^R$

T_1^R Lac −	T_1^R Lac +	T_1^S Lac −	T_1^S Lac +
21	11	18	0

Virus resistants.

Streak out to purify:

Y63 Y53/Muc from A.

Y64 Y53/1 from A.

Y65 Y10/1/7M from C.

Y66 Y53/3,1,5,7M from D.

Y53/3,1,5,7S from D.

Y67 ~~Y53~~/7 M from E

Y68 Y53/7 S from E

After 4-platings, restagain 12/10.

		T_1	T_3	T_5	T_7	Morph.	#
A	Y57	R	R	R	S	Y53/3	M
	Y58	S	S	S	S	Y53/7	SR
	Y59	R	R	R	S	Y10/1/7	SR
	Y61	R	R	R	S	Y40/7	SR
	Y62	S	S	S	S	58-161/3	SR
	58	S	S	S	S	✓	SR
D	Y63	S	S	S	S	"Y53/1"	SR
	Y64	R	R	R	S	Y53/1	SR
	Y65	R	R	R	R	Y10/1/7	SR
	Y66	R	R	R	S	Y53/3	SR
	Y67	S	S	S	S	Y53/7	M
	Y68	S	S	S	S	Y53/7 S	M

also ~~R~~ T4, T6 R. probably contaminant.

Prepare plates for:

58-161/3

58-161/7

Compare $\frac{B\bar{}}{B^{+}} = \frac{50}{212}$ with $5/20$ on p. 364.

but need better information.

5 hour cultures, washed, mixed, and plated into various media.

Turbidity	Medium.	Colonyets.	Mean	m.d.	Excess.	R/prot.
±	O	217	212 ±34			1.00
	O	193				
	O	279				
	O	234				
	O	137				
++	B_1	760 ✓			548	2.58
	B_1					
−	B	100		d.n.u. note: thin agar layer	50?	<< 1.
		282				
±	L	421	389		177	.85
		367				
+	L	304	350		148	.65
		395				
++	M.	0 ?!		Does not seem to be so turbid that serum should be inhibitive!!		
				Repeat & added proteoleptus.		
+	BB_1	764.			0.	
	BTL				—	
	ML	0				
	MT	0				
++	BL_B.	v. small cols.			?	
	BT_B,	+++			—	
	MB_1	0				
	MTL	0				
	MTB_1					
	BL				—	
	BT.				—	

Y40 x Y53

Reversion controls

			stab.
Y53 ini:	TL.	0	++
	TB$_1$	12	+
	LB$_1$	2	+
Y40	M	0	++
	B	0	+

Conclusions:

Plate count determinations may be in error due to variable increases in cell density. B$_1$ seems to be a limiting factor in synteplasm. (Try it in aB$^+$ x bB$^+$.).

B$_1$ independent, or linked to: B$^+$; M$^+$

B linked to M.

L independent?

T. independent or linked to L.

∴ B$^-$ should be linked to Lac

and in this cross, we may find that the B$^-$ acpcd. Lac + compared to B$^+$.

Similarly ē B$_1^-$

5/20 B⁻ Chap. 10.

5	15
10	10

$$\chi^2 = \frac{25}{10} + \frac{25}{10} = 5$$

$p = .025$. Need more data!

Recotypes Y40 x Y53. 364

Test colonies on ●, B, B_1; T; L medium appropriately & segregate together various single mutants for lysis & ferm. tests.

~~1/31 L− ??~~

		T_1^R Lac−	T_1^R Lac+	T_1^S Lac−	T_1^S Lac+
		17	9	13	1
46/48	B_1−	~~26~~	~~2~~	~~20~~	~~0~~
2/30	L−	2	0	0	0
~~5/20~~	~~●~~ B−	2	2	0	1
~~4/76~~	~~●~~ T−	2	2	0	0

17	9	13	1
100	55	50	4.

all recotypes of B_1, Lac, T_1^R.

~~Protolysates: See 362.~~

Protolysates.

Ser 1.	4	4	6	0
Ser 2.	~~25~~ 24	17	15	0
	28	21	21	0.
Summary. Y. 357:	51	23	11	4.
B_1− may be deficient in T_1^R Lac+ class (parental type). Y. 359	~~79~~ 79	44	31	4
	21	11	18	0
	~~95~~ 100	55	~~45~~ 50	4

Prepare medium P 2 4.

Plate Y 55 (lactose – from Y 53 × Y 40) into lactose – minimal.

10^4 colonies tested

Galactose mutant

366

Inoculate 58-161 P24. 2 mm's. Incr 2 ml 110 YB. Quad 1 min Benzer.

20-24,000 colonies examined.

No typical gal- colonies. Several sectorial colonies + some rather mucoid gal- were seen.

Streak out on ~~lactose~~ galactose EMB.

No mutants.

1 mucoid form.

Penicillin-resistance.

Y53 x Y54. grow separately. Pick protoplasts

1. ferment.
2-16 mass-ferment.

give to Truffaut.

Y10/1 x453 x 58-161.
"3"

November 24, 1946.

(Naguoutb.) test prototypes.

38 T_1^S Lac$^-$
16 T_1^R Lac+

no { T_1^R Lac+
T_1^S Lac+.

Dry conditions. YAC x YS3. 369.

26 NOV 1940

a) Synthetic medium preparation (T+B, BM); —

much more turbid; no protoplasts. Occ. on surface. ca 10^8:...

b) YB. 10^{-7} on surface. as many in dup agar.

suggests YB better than synth. However, must be repeated!

plate

11/24/46.

Y40 x Y53. Plate is growth unlike B_1,

Pulse to H_2O; test on B_1+, B_1-.

33/39 = B_1-

6/39 = B_1+ = ~~6%~~ 15%.

Test B_1^- for Lac, T_1.

12/3/46: Tests:

T_1^R Lac -	T_1^R Lac +	T_1^S Lac -	T_1^S Lac +
1	3	3	—
6	3	1	—
2	1	2	
4	1	2	—
13	8	8	0

T_1^R	T_1^S	Lac +	Lac −
		6	4

3-way crosses, etc.!

26 NOV 1946

a) BTL.

BT }	0.	0
BL }	B	10^2 turbid.
BT }	0	1 !!
TL }	T	≠ 0
BL }	0.	0
TL }	L	10 ? T
BT+TL+BL }	0	0 1 plaque ??
	0	0. These plaques!??
	B	10^2 turbid
	T	0
	L	0 ?

Plaques are probably absorbed antibubbles

b) B TB.

BB_1 }	0	
BL }	B	? turbid.
BB_1 }	0	0
B_1L }	B_1	0
B_1L }	0	0 — clear not turbid
BL }	L	1?
3	0	0.
~~BT+TL+BL~~	0	
	B	10^2 1 plaque?
	B_1	0
	L	plaques??

$BB_1 \times TL$. 0.

December 4, 1946.

YS3 × Y0 in YB. (5 growth) Plate in various + test. Compare $\bar{c}$ do. grown in Nutrint Saline Glucose.

NB } 0 ✓ 10^{-2} just as good as YB.
NB } 0.

YB:

0
B
B
B
B_1
B_1
B_1
$\bar{T}$
T
T
T
L
L
L
L
BLT
BLT
BLT
BLT.

} most colonies do not resemble E. coli. Broth is fresh seems contam.

cent! ✓✓ colonies not so large as BLT.

$\bar{B}_1$	$\overset{+}{B}$	$\overset{+}{M}$	Lac −	T_1^S	$\bar{T}$	$\bar{L}$
B_1	B	M	Lac +	T_1^R	T	L
+	−	−			+	+

↑

Look for types which are $B_1^+ M^+$, i.e. recombinant colonies and study progeny.

December 5, 1946.

$10^3/10^9 = \underline{10^{-6}}$!

YB. a) Plate Y40; Y53 on B_1 plate. Select colonies and plate entire smear of ~~colony~~ into BMTL. If any colonies appear they may be either BM or the complementary recombinant.

P5. Test for linearity ... L- should be tested thoroughly. [Use detection procedure?]

↓

$\frac{\bar{B}_1 \; \overset{+}{B} \; \overset{+}{M} \; Lac- \; T_1^S \; \bar{T} \; \bar{L}}{\underset{+}{B_1} \; \underset{-}{B} \; \underset{-}{M} \; Lac+ \; T_1^R \; \underset{+}{T} \; \underset{+}{L}}$ complementary type is $\bar{B}\,\bar{M}\,\bar{T}\,\bar{L}$ and may have any Lac, T_1 configuration, particularly Lac+ T_1^S.

b). Assuming that M is relatively far from L or T, so that (as in 4-strand) 2 double exchanges can be expected to occur in this region, plate out for such an exchange (e.g. $-M, -T$ or $-M-L$ [$\underline{BB_1L}$; $\underline{BB_1T}$] and examine for heterogeneity in Lac. or T_1 (particularly the former).

BB_1L: as ~~above~~ + v. turbid. do not use!
below (371)

Linkage –

Compare B(P) x Y53 vs (P) B_1 (cancel P- with proline)

and BB$_1$ x TL.

12/5/46. BB$_1$ x TL.

Repeat. 12/9/46.

A. BB$_1$ x TL.

O ~~No~~ 3 colonies. ?? coli.

B_1 No prototrophs.

B. B x TL B_1.

O

B_1

} No colonies.

rather turbid!

~~Doubage~~. 4 strand hat

December 9, 1946.

Y40xY53: into BB, L. (A) and BB, T (B)

e.g. like 375

12/9/46.

Cultures ca. 8 hours.
(too old???)

Y40x453. into BTL.

ca. 10 colonies. Latter inhibited by tumbrid growth.

6315 x Y53.

December 9, 1946.

Y53 x 58-6315. (Biotin – "ϕ-alanine?" + cystine, ~~stop~~.)

Gave a very high frequency ($5 \times 10^3 / 10^9$) of prototrophs; ca. same number of colonies on a ϕ-alanine plate. To Carl

~~Few prototrophs on T₁-Cai plates.~~ Carl – Scored ϕ-...+ type as indicating separability of P, Cyst ag.

Y4③ x Y53

$T^- + L^-$

10/5 – 10/46.

Plate on T, L resp. test clo. Need more data.....

T: 5/20. $T_1^R Lac-$ $T_1^R Lac+$ $T_1^S Lac-$ $T_1^S Lac+$

(.25) 3 0 1 1

2 2

5 2 1 1

Cf 364 4/26.

9/46 T− ✓ 1 (✓) ? 2 ✓ ++ ng 3 ✓ ++ n.g. 4 ✓ 5 ✓ (✓)

T^-

T^+ also all ++.

$\therefore T^- T_1^R Lac-$ & $T^- T_1^S Lac+$

L: 3/26. 1 2

(.11)

Cf. 2/30. Both $T_1^R Lac-$ 2 0

364.

3 2 0 0

5/56.

n.g. (?) ✓ 1 ✓ ✓ 2 (✓) 3 (✓) 4/5

$T_1^R Lac+$ (2).

~~mixture LT titre Hay T sera.~~

ca 10% L^- OK.

ca 10% T^-

Complementary type

10 December 1946.

8 Dec. plate colonies of (Y53 x Y40) from B, agar, into BTLM agar.

Most plates have 1-200 colonies, & many non-proliferating B_1^- in between.

12/10/46. Pick colonies a) to BLTM; BTM small tubes 10 tubes x 10 plates.

b) to BLTH large tubes (for detection plates)

Tests. (only BLTM+ BTM- or ? recorded).

Plate no:		T_1^R Lac-	T_1^R Lac+	T_1^S Lac-	T_1^S Lac+
	1	0			
	2	0			
	3	0			
	4	0			
	5	0			
	6	1			
	7	1			
	8	1			
	9	2			
	10.	2			

378-1
-2
-3
-4, 5
-6, 7.

c) Pick colonies to EMB lactose (1 plate):

15 + (8)
4 - (9)

1 BM
2 BM
3 BM
4 BM
5 BM
6 $B^{M+?}$
7

December 13, 1946. Plate following asinidi.

A 1. Y53 x Y40. (Shows)

B 2. Y64 x 58-161

C 3. Y65 x 58-161. (Y10 4/7 x 58-161).

D ~~GT.~~

4. Y67 x Y40

E 8. Y53 x ~~58-161~~. Y68.

F 9. Y67 x Y68.

most moulds too large

Best method: surface spreading!

A: Yield rather low! B. too turbid. BTL OK but >> than O.

B: also too heavy. V. low yield.

C: (O. none B_1: ca 20 ± very wide zones of stimulation) all dysp. (contam?)

D O. Rhizopus sm. amount.

E ca 10^2-10^3 colonies. Not very much like coli, but test on EMB lac all mucoid, lac +

F O.

December 16, 1946.

12/16. Use B_1^- / BMTL plates of Exp. 378. Pick colonies from lettered plates to EMB lac to eliminate Lac+ which from 378 are probably B-M-.

Streak out Lac- colonies on EMB-lac to obtain pure cultures & avoid pitfall of dysentheria. Test on:

	BMTL	B,MTL	B,BTL	B,ATL	B,BMT	B_1
	BMTL B_1	BMTL	BMB,	TLB,		B_2
C1	+	+	+	+	—	
C2	+	+	+	+	—	
D1	+	—	±	+	+	
E1	+	+	+	+	—	
E2	+	+	±−+	+—	+	
F1	+	+	+	+	—	
F2	+	—	+	+	+	
G1	—	—	+	+	+	
H1	+	+	+	+	—	
J1	—	—	+	+	+—	
K1	+	+	+	+	—	
M1	+	+	+	+	—	
378-8	—	—	+	+	+	
378-9.	±	+	+	+	+	
	−B	−M	−T	−L	$-TB_1$	
			$+B_x$			

Lac- (A, B, C, D, E, F, H, J, K, L, M)

Lac+ (G, C, H, J)

? B-- Lac+

A. 14/15 +

B. no growth

C. 8/8 − 1 or 2

D. 1/1 − 1

E 17/17 − 1 or 2

F 8/8 − 1 or 2

G ++ − 1 (Lac+)

H 4/4 1

J 1/1 − 1

K 6/6 − 1

L ——— no viability in streaked plates. colonies.

M 1/5 − 1

15.

Picks 1: 380 - D1
380 - E2 (or 0)
380 - F2

378-8
378-9.

See 380.

		Lac	small colonies.	
		–		✓
	C1	B,	–	E1
	C2	B,	– ?	
*	D1	M (T)	– ✓ –	H1
	E1	B,	– ✓	K1
*	E2	(T)	– – ?	
	F1	B,	– – ?	M1
*	F2	(B) M	– – ?	
	G1	BM	+ –	-!
	H1	B,	– ✓✓	C2
	J1	BM	+ —	
	K1	B,	– ✓	E2
	M1	B,	– ✓	F1
378-8		BM	+	F2
378-9.		(B?)	–	

Pick small colonies to colicin, ... 4 lot

		O	B	M	T	BM	TM
check on	D1	+		+ –	+		+
	E2 pr	+			+ –		
	F2.	+	+	+		+	

			C	B1	M	T	L	B
1	C2		—	+	+	+	+	
2	E1		± +	+	+	+	+	
3	E1		—	+	+	+	+	
4	E1		—	+	+	+	+	
5	E1		—	+	+	+	+	
6	F1		+	+	+	+	+	
7	E2		+	+	+	+	+	
8	F2		+	±	+	+	+	
9	F2		—	—	±	+	—	?? hold! → + + + + +
10	F1		—	+	+	+	+	
11	H1	B,	—	+	+	+	+	
12	H1	B,	—	+	+	+	+	
13	K1	B,	—	+	+	+	+	
14	K1	B,	—	+	+	+	+	
15	K1	B,	—	+	+	+	+	
16	K1	B,	—	+	+	+	+	
17	M1	B,	—	+	+	+	+	
18	M1	B,	—	+	+	+	+	
19	M1	B,	—	+	+	+	+	

8 F2	–	–	–	–	+	+	+	+	– (+)
9. F2.	–	+	–	±	–	+	+	+ –	±

Bye - inhibition of E. coli. 38 c

January 5, 1947

1 ml, 36 hour broth cultures in YB agar Y40 1 ml A
Y40 .1 ml B
Y53 1 C
Y53 .1 D

+++ = uniform turbidity.

		A	B	C	D
Proflavine	$1:10^3$	+++	→		
	4		"		
	5				
	6			"	
	7				"

n.g. at all from broth

Cryst. Viol.	$1:10^3$	—	do.	do.	ca 10^3 cols.
	4	+++	→		
	5		"		
	6			"	"
	7				

Proflavine is n.g. under these conditions
Survival ± crystal violet is OK in range 10^{-4} to 10^{-3}.
This should be extended. Also washed cultures?

January 9, 1947.

V x y p

Irradiate in flask, varying times. Dilute YB-1 and Hao plate on EMB .01 ml undiluted portions

	S 1/100	S.	pS.
0	+++		
15 sec	+++		
30	++±		
60	ca n×10³	10⁵	3?
120	ca p×10³	10⁵	4
300	0?5	10³	6

1 min

pS/10 - ca 1/15 [illegible]

i.e. calc 15 ms. kills

10³'s of the organisms

survivors.

non-[illegible] kill.)

P10 Dilute 120 sec: 1:10⁷ on EMB plates + spread.

P14. Pick colonies which seem to be non-papillate. Sample is not clear-cut because plates are crowded and entire population could not be screened. Estimate ca 5-10×10⁴ tested to finish EMB for further test. 50,000

Pick 6 colonies to YB slants which seem to be non-papillate. 1 is mucoid.

See 716

Transformation control

January 9-10, 1947.

P9. Inoc YB - Y53, ~~Y57~~. Y40

10A10. Inoc ($\frac{100}{50}$ ml NSB) Y53, Y40 (A)

1P10 Inoc YB - Y40 (B)

4-5P10. Wash (A) cells.

1. Mix Y53-Y40 cells. ✓

8P10. Resuspend Y53 (A) cells in T-minimal. Incubate 3 shaking 3½.
Sediment (C,D) and mix with washed Y40 (B). 2. plate 0.

3. ~~Mix~~ supernatant of C,D + mix + washed Y40 (B) 3. plate 0.

1. 5×10^2 prototrophs.
2. C $> 10^2$ "
   D No? turbid for count.
3. filtrate:

   C - 1 prototroph ??
   1 ??
   } supernatant was not entirely freed of cells by the centrifugation. Repeat + controls on influence of dilution of 1 cell type on prototroph yield.

Recombination types

January 10, 1947.

Y40 + Y53 on T(0), T(B,) agar

Pick colonies to EMB lactose. 1/12/47 1-15. 8, 13 + others are −

Streak out directly on (A) BMTL-lactose (B) BMTL lactose + glucose.

Compare the B_1^- types & $\bar{E}$ types isolable from these plates.

Colony-Plate:	Colony: 0 = B_1^- orig.	A	B	C	D	E	F	G
				on EMB-lac.				
Loc 1	$-S^{(+R)}$	$-S$	$-S$	$-S$	$-S$.			
Loc 2	−							
* Loc 3	$-R$	$+S^{(+R)}$	$-S^{(+R)}$					
* Loc 4	$-S$	$+S^{(+R)}$	$-S^{(+R)}$	$\pm S^{(+R)}$	$\pm S^{(+R)}$			
Loc 5	$-S^{(\pm R)}$	$-S$	$-S$	$+R^{(-R)}$				
Loc 6	$-R^{(\pm R)}$	$-R$	$-R$	$-R$	$-R$	$-R$		
6 8	* $-S$	$-S$	$-S$	$-S$	$-S$	$-S$	$-S$	$+R$
6 11	$-R$	$-R$	$-R$	$-R$	$-R$	$-R$	$-R$	$-R$
6 12	$-S$	$-S$	$-S$	$-S$	$-S$	$-S$		all mixed + R
6 13	$+R$	$+R$	$+R$	$+R$	$+R$	$+R$	$+R$	
* 6 15	$-R$	$-S$	$-S$	$-S$	$-S$.			
	↓ B_1^-	↙ B_1^-	↘ BM+R		↙ B_1^-	↘ BM+R		

BM + R
TLB$_1^-$ S

January 11, 1947.

Y64 × 58-161.

TLB₁lac- T_1^R × BM lac+ T_1^S

good material.

a. prototrophs

	T_1^R lac- R	+ S	- S	+
	42	1	53	23

R = 36%   64%

Lac- = 80%

b. B₁ plates. Much more numerous colonies (10 ×)

(not well readable). (colonies impure!).	8	0	5	10

Segregation of Mucoid.

January 11, 1947

a. Y67 (Y53M) × 58-161 (Try × Y40.)

shunon lac+! Muc lac- Sm lac- Muc lac+ Sm lac+

P) 17 P) 9 Lac- = .66

~~Y67 (Y53 3,11, M) × 58-161 (Try × Y40)~~

c Y68 (58-161M) × Y53. (Try × Y64)

segregation: ~~M L- M L+ m L- m L+~~

6 P 22 P 12 7

M_{68} linked to lac⁻

Interaction of expression of Lac- + Muc+ on EMB lac medium?

note variation in shunon. Genetic character?

attempted Transformations.

January 11, 1946.

P10 from 100 ml / 125 fl. YB – Y53.

N11. Centrifuge 250 ml (step 25 – 1. Suspend cells in 15 ml .9% NaCl. Add lysozyme & incubate for autolysis on shaker at 25°. (12:45 PM – 3 P.M.) Centrifuge "free cells" and

Mix 5 ml & 1 ml Y40 suspension & plate 3 x 2 ml samples into T(0) agar.

P14. 2 dishes:

ca 10 large $10^{2.5}$ small & halos. v. clear plates. sign?

See 394

January 11, 1948.

Plate Y55⁻ (+1... Lac-) into Lactose-minimal at various dilutions: (Assay. = 10^9)

est. cells	
10^8	Discretely crowded.
10^6	about 10^4 visible colonies
10^4	about 200 large colonies, with haloes of small ones. Small cols. much smaller than below.
10^2 about 5 x.	10^3 small colonies; 6 typical colonies (probably Lac+). Diffluent

1) The reversion frequency, as estimated from EMB plates is very high (ca 10^{-4} to 10^{-3}/generation??)

2) At best on this medium, Lac- is capable of developing to some extent. Since they develop haloes, it is likely that there is a limiting factor in the agar which faintly permits growth.

Test large colonies on EMB:

Segregation of T_7^R 390

Jan 11, 47

Y65⁻ x 58-161 No colonies!! (Repeat!)
not turbid - prob mix error.

Repeat Jan 15.

Loaded! Hydrate ????

Recombination with P/2 (cont.)

January 11-12, 1976.

P11 Bronx YB- Y43, Y44.

230P12- Bronx YB ± Y43, Y44, Y43+Y44, Y43+Y53.

4P12. Wash + plate.

(Y43)+(Y44)	0,0	not forbid (exc.)
(Y43+Y44)	0	
(Y43+Y53)	0	
(Y43)+(Y53)	0	

January 12, 1947

Plate Y40 + Y53 (cultures as in 391) in T(0) as initial controls.
9-10 P12. .5 ml. ~~25~~

T.0: – ca 2-300.

a. Keep Y40, Y53 in water (.9% NaCl) 25°. Mix 4P13.

b. Keep (Y40 + Y53) in water. Plate 4P13

c. Keep Y40, Y53 in T(0). [Add 1 ml to 10 ml T(0)]. Mix + plate 4P13

[d.] P12. Plate Y40, Y53 in superficial layers of agar. — 4 colonies.

e fresh Y40 × Y53.

a	$10^{-2.5}$
b	10^{-2}
c	10^{-2} clear plate
d	4.
e.	10^{-2}

Cells will react if kept in water for 24 hours + then mixed.
but not many more are formed if they are kept together. ∴ Recombination takes place in the agar.

Differential centrifugation of Bacteria
Preliminary Expts.

0. Density — in sucrose buffer, centrifuge 1 hr. eq. washed YSS bact., at 10 10 mins, etc. Sediment mid.

	time.	1.0	1.04	1.08	1.12	1.16	~~E20~~	
~~Mark~~								10
Mark		+	+	+	+	+		10
	time							10 ~~25~~
Mark		++	++	++	++	++		10
								20
n.q.		++	++	++	++	++.		50

1.16 = 20g sucrose / 100cc water

1:4 bacterial susp. in H_2O. 4 g for density.

Repeat, using 20g sucr / 20g H_2O as d = 1.25. (actually 1.23)

($\frac{1\text{ ml}}{4\text{ ml } 1.25}$)	1.0	1.05	1.10	1.15	1.20	
diffuse in bottom layer, i.e. a ring at junction	++	++	+	±?	—	20m 50.
						+ 30m
	+++	+++	+±	+	±	50
						+ 1hr.
	1.15	1.20			do.	
Use heavier resp. cells.				+	±	1:30

This might achieve some separation.

Tuy K1
Tuy Hg504

Y53 x Y40 - deletion effect.

1/13/47.

½ ml of vaccinia dilutions.

	1ml + 1ml Y53	Y40.		
1.	10^{0}	10^{0}	ca. 100	
2.	10^{-2}	10^{0}	6	
3.	10^{-4}	10^{0}	0	
4.	10^{0}	10^{-2}	8	
5.	10^{0}	10^{-4}	1	
6.	10^{-2}	10^{-2}	0	— 0.
7.	10^{-4}	10^{-2}	0	
8.	10^{-2}	10^{-4}	0	

Y40 - Lac - mutant.

January 15, 1947.

Inoculate Y40 2 ml. broth 1 ml/10 YB incubate 18 hours + dilute + plate on EMB lact. 20,000 colonies examined.

3 colorless, but rather small colonies were found. Pick + test further.

1 Lac+ Mucoid colony was found. Pick + streak out to isolate.

all Lac+ Lac+ Muc = Y69

January 17, 1947

See 383 (1-6)

P21. Colonies have taken a blue tenge. Make streaks + compare ē Y53. All show coloration in lytic zone ē T1 vernis.

5, particularly, shows few or no papillae. Y70.

1	very few papillae
2	papillae.
3	papillae.
4	few, but some papillae
5	~~few~~ no papillae.
6	few but some.

Y71.

Y70. — Further study suggests that fewer colonies have papillae, & fewer of them than 11-12, but some papillae are formed. Comparison should be made of some prototroph segregants. This allele may work to Y53-Lac-.

coli Novoffi — papillates very readily

Attempt at transformation

January 18, 1947.

P17 - P18. 24 hour cultures Y53 autolyze ± 300 ml
benzene
washed cells in Va. Cl under ~~toluene~~. 3 hours. Shaken at 28°.

Sediment cells. Remove supernatant fluid by vacuating chamber. Suspend Y40 cells in autolysate - Plate ~~1:~~ 1:3 to 5 Y40.

Control - use washed cells of above ± autolysis x Y40.

See also 399.

Turbidity of autolysate was ~~more~~ < 1/2 than that of the 1:100 dilution in 399.

Incub. ~~Plate~~ sample overnight ± ~~heavy~~ heavy layer of benzene and repeat later.

Hold autolysates overnight in cold.

6P19 - remove benzene from sample by evacuation.

A. Y40 + D2-autolysate 0, also in EMB. 0

B. Y40 + autolysate 0, 0

C. autolysate ± ~~+~~ Y40. 0, 0.

autolysate is sterile; no protoplasts.

3-way cross.

January 20, 1947.

A : Y40, Y10, Y64. −S not wilic.

BM+R × TLB, F1 +S −R

+R	−R	+S	−S
9	7	1	11

B. 58-161, Y46, Y53 −R wilanticr.

BM+S × TLB, F1 +R −S.

+R	−S	+S	−R
20	34	5	40

Some mistake??

See 4/11 for repeat

3-way cross.

Y10

BM Lac+V$_1^R$ × TLB, Lac+V$_1^S$ → +++

Y40 Y64

Lac+V$_1^R$	
Lac+V$_1^S$	
Lac−V$_1^R$	
not Lac−V$_1^S$	

Y46 Y53.

BM Lac+V$_1^S$ × TLB, Lac−V$_1^R$ → +++

58−161

Lac+V$_1^R$	
Lac+V$_1^S$	
Lac−V$_1^S$.	
not. Lac−V$_1^R$	

Controls: BM Lac+V$_1^R$ × TLB, Lac−V$_1^S$ → all types
Lac+V$_1^S$ rare.

BM Lac+V$_1^S$ × TLB, Lac−V$_1^R$ → all types,
Lac+V$_1^R$ rare.

Already done!

Dilution Effect 1:10

January 18, 1947.

½ mlreach:

	Y53	Y40.	
1.	10^{0}	10^{0}	120
2.	10^{-1}	10^{0}	120
3.	10^{-2}	10^{0}	13
4.	10^{0}	10^{-1}	60
5.	10^{0}	10^{-2}	8
6.	10^{-1}	10^{-1}	23
7.	10^{-1}	10^{-2}	16
8.	10^{-2}	10^{-1}	8
9.	10^{-2}	10^{-2}	1

f(Y40) f(Y53)

Y53: 10^{0} 0 120, -1 60, -2 8 — Y40: 0 120, 1 120, 2 13 (10^{0})

10^{-1} 0 120, -1 23, -2 16 — 10^{-1} 0 60, 1 23, 2 8

10^{-2} 0 13, -1 8, -2 1 — 10^{-2} 0 8, 1 16, 2 1

Y53+Y40 10^{0} 120 10^{-1} 23 10^{-2} 1

Mucoid segregation 400

January 17, 1947.

Y57 x Y68 (TLB, -Lac - $V^{R}_{1,3,5}$ x BM-Muc)

No prototrophs!

See 404 & 387 for mucoid segl

Y53M

Y67 x 58-161 ~~is~~ ok.

Y68 x Y53 ~~is~~ ok.

~~58~~-161M

Toxicity of benzene and removal.

January 19, 1947.

7P19. Layer 1/2 ml benzene on 1 ml Y40 in water. Keep on dark.
do in H_2O.

N20. Remove water layer; evacuate to remove benzene.

1. Plate to determine killing of Y40. — 0.
2. Add 1 ml fresh Y40 to free aqueous layer + let sit for 24 h. Plate.

January 20, 1947.

A. Plq. broz Y40, Y53 into YB + Tween ; A 20 transfer Lilleuine ; plate

A. A. 1%
B. .1%
C. .05%

no growth effect!

on T(0) agar + 1% Tween

B. Plq broz Y40, Y53. into YB. etc.
Plate into T(0) agar +

A .1%
B 1%
} Tween.

all ca 10^2

no particular effect of Tween could be established.

January 20, 1947.

5 1 ml samples 58-161 grown 18h. in YS. Wash + irradiate 2 mins. bro 1:100 in nutr. sal.

1.
2. 1/5
3.
4.
5. 1/5 survivors

58-161 is evidently more sensitive than YS3. (which has had 1 further X-Ray + u.v. exposure).

~~Y64x68.~~
segregations

January 22, 1947.

1. Y65 x 58-161 (Y10/1/7) (in 1:100 del.)
2. Y57 x Y68 (~~Y10~~ Y53/1 x BM Muc)

1. Shows no recombination prototrophs. (Is Y65 unable to recombine??)

See 379, 390

2. 1 plate ~ ca 1/10 (no selection). (Try at See 400

Try Y64 x Y65

BM +R × TLB, -S

See 385.

P21. Streaks out 385-3, 4, 15. Test 1 colony isolates on T1.

		385			
1-2	3- 0	~~-R~~	— R 1 + R 2	-R	(B_1^-)
3-6	A	+S	+ S 5,6 — S 3,4	+R	
7-10	B	-S	— S 7,8 + S 9,10.	-S	
				+S	
1	4- 0	-S	— S 1	-S	(B_1^-)
2-5	A	+S	+ S 2,3 — S 4,5	+S	?
6-9	B	-S	— S 6,7 + S 8,9		
11-14	C	± S	— S 11,12 + S 13,14		
15-18	D	± S	+ S 15,6 — S 17,18		
1-2	15- 0	-R	— R 1,2	types -R (B_1^-)	
3-4	A	-S	— S 3 + R 4	-S (B_1^-)	
5-6	B	-S	— S 6 + R 5	and +R are present.	
7-8	C	-S	— S 7 + R 8		
9-10	D	-S	— S 10 + R 9		

(Replates 3-0 also.)

Test samples of above:

P: parental

above #			Nutr	Comment	
3-1	1	-R	B_1 ✓		
3-2	2	~~+R~~	~~BM TL?~~	P (BM)	
3-3	3	-S	B_1		
3-5	4	+S	B_1 ?		
3-7	5	-S	X B_1		
3-9	6	+S.	++		
4-1	11	-S	B_1 ? ✓		
4-2	12	+S	+? ✓		
4-6	13	-S	B_1 ?		
15-1	21	-R	B_1 ?		
15-3	22	-S	B_1 ✓		
15-4	23	+R	BM ✓	P.	
15-9	24	+R	BM ✓	P.	
15-10	25.	-S	B_1 ✓		
3-4	31	-S	B_1		
3-6	32	+S	B_1		
3-8	33	-S	B_1		
3-10	34				

B_1 -R
B_1 -S — 3 tests BML definitely, 1 test BM

[B_1 +S]

[++ +S]. See 408.

of 21.

of 21. (B_1-R)(B_1-S).

Transformation - autolysate.

January 21, 1947.

250 ml eq. 24 hour cells of Y53 harvested from YB + washed. ~~with~~ autolyze 24 h. under Thymine at room temp.

P22 add Y40 cells + plate.

P24 - no colonies.

January 22, 1947.

Plate "B_1^-" colonies into T(0) agar + BMTL. Use plates which contain relatively few, isolated prototrophic colonies.

0 Y65 × 58-161

1 Y40 × Y53.

Test original colonies for V, R, lac⁻ :

	"0"	"1"
1	+S	−R
2	−S	−S (+R)
3	−S (+R)	−R
4	−S	−R
5	−S	+R (−R)
6	−S	−R
7	+S	−R
8	+S	−S
9	+R	−S
10	−S	−R

Plate colonies into BMTL. Pick + test samples of colonies which arise.

		# colonies		
1	+S	10⁵		
2	−R	1000	8 + S	+S = BM type
3	−S; +R	1000	8 + S	−R = TLB$_1$ type
4	−S	200	3~~4~~ +S	+R = lac type.
5	−S	1000		
6	−S	500	~~7 +S~~ 7 + S	all +S. 1 case (BM) Test for a
7	+S	300	7 +S	lac⁻ segregant +.
8	+S	300		
9	+R	10⁶		
10	−S	200.	8 + S	
11	−R	200	10 + R	+R = BM type
12	−S (+R)	500	10 + R	−S = TLB$_1$ type
13	−R	50	2 −R 7 + R.	lac type
14	−R	500	8 + R	
15	+R (−R)	300	9 + R	[−R = B$_1$ type]
16	−R	20	1 − R 5 + R.	
17	−R	10⁶		
18	−S	200	10 + R	
19	−S	500.		
20	−R	200	9 + R.	

(labelled "into T(0)")

+S × +R BM TLB$_1$

+S × +R TLB$_1$ BM

How explain "10" − Reversion of B_1^- ?? Label mislabeling?

Recombination types - see 383

January 25, 1947.

Retest 405-2. (475) in plates. Dil to ca 100/ml + pour plates ~

1. BM 346
2. BMT do
3. MBL do
4. BMTL do.
5. BMTLB, 365

January 25, 1947.

Test types of 407 in BMTB (-L) on very light streaks of the purified cultures.

Streak out colonies of 407 on EMB-lactose. Test on:

			BMB.	BMTL.	
1.	407-	2 +S	+	+	P
2.		3 +S	+	+	
4.		4 +S +R (use a colony in line of prior streak (maybe mutant from S to R))	+	+	
5.		6 +S do.	+	+	
6		7. +S	+	+	
7		10. +S	+	+	
8.		11 +R	+	±	P
9		12 +R.	+	+	P
10	13	+R	−	−	
11	13	−R	+	+	
12	13	−R	+	+	
13	13	+R	+	+	P
14	14	+R	+	+	P
15	15	+R	+	+	P
16	16	−R	−	+	+
17	16	+R	+	±	
18	18	+R	+	+	P
19	20	+R	+	+	P.

Transfer 1, 9, 11, 12 to slants + test further.
16, 17

January 27, 1947

1. BM + R / Y40 < TLB, −R Y64 → −R, +R
TLB, +S Y10 → +R, +S.

−S	−R	+S	+R	
0	16	7	28.	] 51

Therefore one can assume that an error was made in the previous experiment.

See 368, ~~410~~ 398.

58-161 < Y10/1 Y53 → 38 −S
16 +R
0 +S. (rare!)
0 −R

January 27, 1947

BM + S 58-161.

TLB, −S	Y53	→	−S, +S
TLB, +R	Y46	→	+S, +R.

−S	−R	+S	+R
3	0	0	0.

Tests for >2 strand cross-over.

Tests for phenotypic lag.

January 27, 1947

1. Plate Y40 + Y53 in B, agar.
(Use colonies of 407) Stand in coldroom.

a. Streak out on EMB agar. & b. plate remainder of colonies in T(0) agar.
Use well separated colonies.

		Mut.	Mut. variability in $\times 10^3$	
	1	−	0/10^3	0/3 G-turbid
	2	−		0/3
Y40 +	3			colonies
Y53	4	+		0/3 0
and A	5	+		0/3 0
	6	−		0/3 turbid
	11	−		0/3 turbid
Y64 +	12	−		0/3 0
SR-161	13	−		0/3 colonies
	14	−		0/3 0
and B	15	−		0/3 ca 20 colonies.
	16	−		0/3 0

b. Streak out 18 other colonies on EMB, looking for variation.

	1	+	0/10^3
	2	−	0/3
	3	−	0/3
A	4	−	0/3
	5	−	0/3
	6	+	ca 1/3 ca ½ each.
	7	−	0/3
	8	−	0/3
	9	−	0/3
		−	0/3
		−	0/3
		−	0/3
B		+	0/3
		−	0/3
		−	0/3
		−	0/3
		−	0/3
		+	0/3
			0/3

Total: 6 + / 31 ca 80% −

only 1 heterogeneous.

Mucoid segregations.

January 27, 1947

1. Y57 × Y68 (Check on previous latter) No colonies

2. Y64 × Y68 in B, No colonies!
   Y53/1 × 58-161M

3. Y53 × Y68 (test for recombinability). No colonies

4. Y67 × Y40. in B, very numerous, very elongate colonies.
   Y53M × 58-161/1

5. Y69 × Y53. in B, Fair, v. elong. colonies.
   Y53 × 58-161/1/M

6. Y67 × Y68 in B, No colonies
   alleleins

7. Y67 × Y69. in O rather few (< 10^{-8}) colonies. all deep.
   alleleins.

1	Y53 × 68	n.g.	
2	Y57 × Y68	n.g.	∴ Y68 is n.g.
3	Y64 × Y68	n.g.	
6	Y67 × Y68	n.g.	
4	Y69 × Y53 (Y40M, Y53S)	OK.	
5	Y40 × Y67 (Y40S × Y53M)	OK.	
7	Y67 × Y69	OK, best prop.	

Y67 = Y53 M
Y68 = 58-161 M
Y69 = Y40 M

Single cell isolates of Y55.

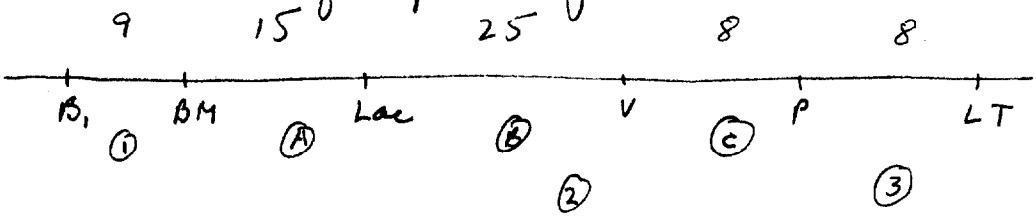
January 30, 1947.

Pedigree:

Received from Dr. Max Zelle, N.I.H., Bethesda, Md.

	Var	V_1
B	–	S
C	–	S
	–	S
	–	S
	–	S
	–	S
D	–	S
	–	S
	–	S
	–	S
	–	S
	–	S
	–	S
	–	S

An basis of map theory:



1. Prototrophs are ①③ types or ①③②^2. By the cross: BP × Y64:

−	+	−	R	+	−
+	−	+	S	−	+
①		Ⓐ	Ⓑ	ⓒ	③

the most frequent prototroph class, by far, should be −R.

The other types all require double crossovers. The relative frequencies of types should be of the order of:

a)

−R	.87
+R	.08
+S	.03
−S	.04

The position of P is inferred from rather complex data. It can be confirmed by showing that LT does not affect the segregation, nor appr. increase the yield. [Unf. B_1^- may want + it would likewise be difficult be use only B_1 as the marker.

b) $B_1^- > B_1^+$, ∴ influence on segregation.

$B^- > B^+$ ∴ following distribution (ca.)

−R	.30
+R	.50
+S	.15
−S	.05

BLT, B_1P, LT, should be readily recoverable.

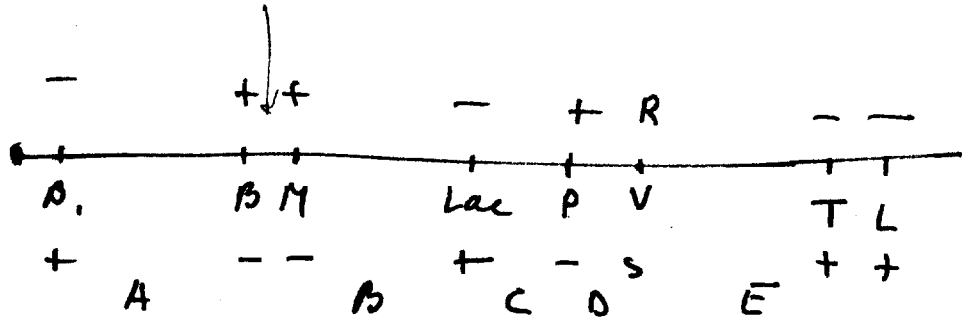
BP x TG

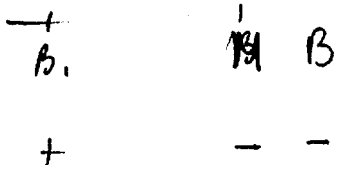
4/6

To demonstrate genetic as well as biochemical distinctness of prototrophs (glut -) and prototrophs (glut +)

58-5255 x 679-662 Rather small moi. ($< 10^8$)

8 colonies found.


R
Lac
P
V
T
L
A
B
C
D
E



58-5255 x Y64.

1. Plate 5255 in O, Biotin alone for R test. O: no colonies. B- " "

2. Plate into O, B_1; B.

P- too turbid } Use more dilute inocula
B_1 P.

BLT - very turbid, but normal colonies recoverable

B_1 >> O. (5-10 x)

B ca 2-3 x O.

Prototrophs:	−R	−S	+R	+S	Total	Total −
	25	27	0	4	56	
from B_1 plates:	62	41	2	1	106	.93
						.97
from B. plates	74	69	10	12	167	.85

χ^2_3 B/B_1 = 11.1

of which 8.3 is +R, +S classes. ∴ B is actually misdefining the distn

On basis of map B. B Lac V P TL, the prototroph distribution should have been predominantly −R, while B⁻ should be ½ +R, which is not suggested by these data. Also need data on the frequency of B^-/B^+. This distribution suggests the map order:

B_1 B Lac P V TL.

Lac$^{R}_{-}$ x Lac+ ~~Y6~~
Y76 x Y40.

418

B$_0^-$	35/35	Lac+	
B$_1^+$	10/10	Lac+.	Y5 tested all Lac+

Streptomycin Resistance

Recd. 200,000 Units of Streptomycin·HCl, Merck, Lot 277
Potency 250 U/mg unofficially from H. Robinson.

A. Dissolve 100,000 units in 10 ml H_2O for stock solution: 10^4/ml
Dilute serially for stocks of 10^3 & 10^2/ml.

Use 10^4, 10^3, 10^2 u./plate & controls on washed Y53
(standing 24 hours in H_2O prev.).

10^4	—
10^3	—
10^2	ca. 100, very small "resistant" colonies at 18 hours. Incubate further.
0	+++T
0	+++T.

Recotypes - see 407

January 25, 1947.

Test types of 407 in BMTB, (-L) ~~in~~ with very light inocula of the pure-purified cultures.

Streak out colonies of 407 on EMB-lactose. Test on:

		BMB,	BMTL.	
	407 –			
1.	2 +S	+	+	P
2.	3 +S	+	+	
4.	4 +S +R use a colony in line of prior streak (maybe mutant from S to R).	+	+	
5.	6 +S do.	+	+	
6.	7. +S	+	+	
7.	10. +S	+	+	
8.	11 +R	+	±	P

Deoxyribonuclease and recombination. ~~450~~ 420

Dissolve 10 mg DRNase (gift of Avery & McCarty) in 10 ml 2x coli minimal. Sterile filter — filtered well. Store in cold. Presumably no denaturation.

Plan: Add .1 ml of DRNase (1 mg/ml) to 1 ml of cell suspensions, separately. ~~Mix~~ Mix cell suspensions + plate ½ ml. Also, hold cells in DRNase, in minimal medium

A.	.1 ml	Total 200 r/4 plates	17, 8, 19, 15	Av. 15 ✓
B	.5 ml ea.	2 mg/4 plates	6, 11, 12, 33	15.5 ✓
C	control		9, 7, 23, 13.	13. ✓

By this expt., DRNase has had no appreciable influence on recombination.

.5 mg 200 r [illegible]

Indicate T L ✓

2/10/47

BT/1 × B, L.

i.c. 42 16

B_1	B_2	V	T	L
+	−	S	+	−
−	+	R	−	+

Prot: S > R. ↑ ↑ ↑

T^- R >> S ↑ $T^- >> T^+$

L^- S > R ↑ ↑ $L^- < T^- > L^+$

or.

B_1	B	V	L	T
+	−	S	−	+
−	+	R	+	−

Prot: R > S. ↑ ↑

T^- R > S. ↑

L^- ↑ ↑

Plate mixture into 0, T, L.

0: 1/4

T 1

L 1.

n.v.g. at recombination.

Three-way cross. Y22

February 10, 1947

Y64 x Y10 x Y40. —

Yield very poor. Do not use ~~it~~ for testing.

cf. other experiments this date !!! — minimal medium ??

February 10, 1947

Repeat part B and controls of 420. Y40 x Y53.

B: ½ml cells + ½ ml DRNase separately + mix cells. Plate into minimal + B, agar. (½ ml of mixture).

B: 0 20
B, ca 150.

C: 0 2
B, 8

controls did not do well here!
(agar base cloudy!)

Test various polymerases against Y41, etc. for gene homology.

			No. prot./10⁹
1.	Feb. 10 '47.	58-3214 × Y41.	0
2.	Feb. 13	6177	0
		3232	0
		6049	0
		6317	0
		5450	0
		5255	0, 0
		679-440 × 5255	0

Test Roepke's mutants for recombination. 425

February 10, 1947.

			24h.	48h.	
1.	Thr × bid	—		5	
2.	meth × arg	2000		$10^{3.5}$	
3.	lys × leuc	~~trace~~ —		0	
4.	pro × cit-ura	~~trace~~ —		0.	
5.	~~[illegible]~~ meth × Y64.	—, —, —, —			Later 3/4 plates!
* 6.	thr	—		0	558-228
7.	bid	—		5	1250-228
8.	meth	—		2	532-171
9.	arg	2000		$10^{3.5}$	572-228
10.	lys	—		40	R152-171
* 11.	leuc	—		1	(probably contam!). Y5
12.	pro	—		100	209-301
13.	cit-ur.	~~trace~~ —		0 (tubiff)	823-304

no evidence of recombination.
threon + leuc seem to be most stable types in this series.

Recessives in Y53.

Febr. 10, 1947.

Treat cell suspension of Y53, Ȳ40 .01% HN_2 (bis-β-chloroethyl methylamine-HCl) in phosphate-citrate buffer, pH 6.0 for ½ hour at room temperature. Terminate treatment by diluting with broth + centrifuge + wash into fresh YB broth. Incubate overnight.

A. Streak out EMB agar after 4-hour incubation.
Y53 has proliferated considerably; Y40 has not!
Take isolated colonies to YB liq. | do. below.
Y53: 21-40 → Y40 - 1-20.

B. Streak out cultures after 20 hour incubation.

Also, take slants from entire population.

P13. Cross 10 cultures from A, each, Ȳ Y40 + Y76 resp. in O + B_1 agar.

H15.

	O	B_1		O	B_1		O	B_1		O	B_1
1	10	100	11			21	20	100	31		
2	14	100	12			22	10	100	32		
3	26	800	13			23	60	300	33		
4	15	100	14			24	20	500	34		
5	20	100	15			25	8+	100	35		
6	0	60.	16			26	14	50	36		
7	1	100	17			27	20	100	37		
8	2	100	18			28	40	500	38		
9	7	30T.	19			29	50	500	39		
10.	3	50	20			30	10	500	40		

all OK.

[Repeat 6, 7, 8 + compare Ȳ appr. controls.] 467 O

Febr. 13, 1947.

Y76 x J8-161. in T(0) + T(B.)

B_1^- 44 lac+

B_1^+ 9 lac+

53 lac+ add to 418: 45 tests.

= 98 tests. This tests for only 49 recombinations, since ½ would be E^+ lac+.

Resistance. 428

Febr. 13, 1947

1/2 ml eq. 426. per plate: NSA.

Streptomycin —

10 u. + turbid plate.

50 u. As below!

100 u. ca 10^2 small resistant colonies. (did not mix adequately in agar).

~~1000 u.~~ med > 5 u/ml.

Brilliant Green (1:1000)

1 ml —

.5 ml — no resistants found!

~~.2 ml~~

.1 ml + turbid.

$HgCl_2$

10 mg —

1 mg —

.1 mg not well diffused; evidence of resistance in some regions.

Tyrocidin (in alcohol)

500 γ + turbid (ca 10^7 colonies) = 50 γ/cc no inhibition.

200 γ do.

100 γ do.

∴ Tyrocidin ~~mg~~.

B.G. OK at ca + 1: 500,000

Hg OK at ca 5 γ/ml.

Streptomycin OK at 5-10 u/ml.

Fermentation tests.

February 17, 1947.

Repeat — [Used mustard treated cultures].
streak out Y40, Y53 on sugar-EMB media.

	Y40	Y53
Lactose *	+++	—
Maltose	+++	+++
Mannitol	variable.	variable, predominantly —. later - pred +
Glycerol	— (1+).	variable late +!
Alcohol	±	
Sucrose	— to ±	— to ± (faint blue colonies not a + reaction).
Citrate	pH too low	

* some — colonies ? - pick + retest.

Vote diff. Y53 + Y40.

Note 3/18: Xylose: K12 is ++

Maltose is definitely +.

Sucrose seems distinctly — - select for + mutants ??
Mannitol + glycerol may be too variable to be useful.

Sucrose — = E. coli communis.

Inversions in Y40.

February 18, 1947.

Cross 426-6, 7, 8 $\bar{c}$ Y53; Y76 $\bar{c}+\bar{s}$ B1.

		A × Y76 O	A × Y76 B1.	B × Y53 O	B × Y53 B1.
1	426-6.	40, 50	ca 200, ✓	50, ✓	200T, ✓
2	426-7	ca 50.	400,	50	500
3	426-8	150,	++T;	150,	++T;
4	~~#10~~ 58-161! (no Y40m).	100T	++T;	200,	++T

Also inversion

Inhibitors

February 18, 1947.

per "20" ml plate NSA.

Y64 Hg 100γ "1 resistant colony. not too well diffused
Y46 do. clear. — "secondary colonies!!

Y64 Streptomycin 50 u turbid
100 u. clear in parts. Not wellcloth.

Y7*

58-161. 50 u. turbid
100 u. as above.

Y64. Brilliant Green .5 ml 1:1000 clear! (1:40,000). pick drug resistant
" Malachite Green 1:500 1 ml clear! (1:10,000). ca ½ large; 40 sm.
.5 ml " 1:20000
.1 ml (1:100,000). poorly diffused.
clear when light is visible

Y46. Sodium Azide 10γ turbid
100γ turbid
1 mg. irregular clearing.

Y1, AG from fluorene

Y64 "Spergon" (1/20) .1%. no inh.

Y64 tri-iodobenzoic ac. (1/20) 1% " .

58-161 / Y64 } $HgCl_2$, 1 mg. autoclaved with 100 ml N.A. — not inhib.

" $Bi.Br_3$. 2 mg/100 ml. N.A. — ~~colorless~~ only faintly inhib.
= 1:50,000. (need more!)

" Streptomycin 5 u/ml. OK.

Y64 NaN_3 2 ml/20 .1% inh. — later grew uniformly.

Y64, 58-161. Mal. Gr. 2 mg/100 NA — not enough!

Sex in L15 mutants?

February 17, 1947.

Grow separately; plate together.

Y5	2
"thern.	0
Y5 x thern	0.
Y5 x Y44	0
Y5 x 58-161.	0

no evidence for sexuality under these conditions. Try growing together!

Y40 x Y53. 432

~~Recombination~~

2/21/47

Mix Y40+Y53 in water + add to agar, mixing. Add 5 ml aliquots ± ca. ½ mls. to various suppl. plates. (see 433 for notes on media).

5 ml. 0 12
10
10
3
11
10.

B 11
10
4
15
10

B_1 >25 T.
" T.
" T.
" T.

This expt. illustrates influence of conditions on detection of recombinants.

10 ml - 5 sublayer.
0 —
0 —
0 —

c sublayer. 0 5
0 4

B_1 T. B. 12.

Tests for inversion in B_1-B_{region}.

2/21/47.

Cross mutant treated (426) isolates of Y40, Y53 ♀ Y53 × Y40 resp. on O, B_1 medium resp.

Plate in medium lacking NH_4NO_3 (inadvertent omission).

	O	B_1
Y40 T. 11	++	T ++
12	++	T ++
13	++	T ++
14	++	T ++
15	T +	T ++
16	++	T ++
17		
18	++	T ++
19	++	T ++
20	++	T ++
Y53T. 31	++	T ++
32	++	T
33		
34	++	T ++
35		
36	++	T
37	++	T
38	++	T ++
39	++	T ++
40	++	T

no evidence of inversion in any of these isolates —
17+20 = 37 tests.

numbers of prototrophs on this minimal seem quite unusually high.

In Fabco test tubes, Y40 × Y53 ♂ spp. inhibited by anaerobic conditions

Fermentation tests. 434
Mutants.

Spread out mustard-treated Y40 (see 426) on EMB-lactose + a few maltose, sucrose, glycerol plates and examine for mutants ca 400 per plate.

Lactose: 36. # of plates. 1 mucoid found

∴ ca 15,000 colonies examined.

no fermentation mutants.

~~Maltose~~ Mannitol 2 plates + 2 streak plates.
ca 1000 examined.

2 very small M- colonies noted. Streaked out on fresh medium.
all M+.

Glycerol - slow utilization. — compare passages on glycerol & peptones.

Sucrose - very slow but definite utilization

Fermentation contents — enrichment cultures.

Feb. 25, 1947.

Broth 50 ml NB + 2% sugar + Brom Cresol Purple A25. Y53.
symbols mid. acidity.

	A26	A27	A3	A4	A5	
glucose	+++					
glycerol	+	+++ Y81.				growths heavier than in glucose — no % black on EMB.
lactose	-	-	+	++	*+++	* colonies from EMB
sucrose	-	-	-	-		— all white colonies!

* growth mic. in standing at room tem.

Streak out some apparently Gly - (a) colonies from 434 + compare with atypical Gly ± — (b).

	Y84.	
a)	—	Y80.
b)	+	

Compare a, b + gly+ enrichment culture above.

	EMB	
Y80	—	N/s evidence of papillae.
(from glucose above) Y53 gly ±	+	
Y53 gly+	+ a ++	diffuent from Y53?
enrich.		

on BCP — medium is not changed in color, cells show slightly diffuent shades (+, ± pinkish, — more translucent or violet.)

on Gly - BCP broth — Gly -, Gly ± and Gly + all show slow acid + gas.

(see over)

1. Enrichment for gly+

A5 - streak from gly tubes to new Gly EMB

A8 - scoring OK, as before!

Streak out susp. of on N Agar + Mol/Lum. 10^{-4}. Streptomycin 5 u/ml

	M.G.	>H.	B.G.	
Y77	++ *	—	—	!
Y78	—	++		

* dye is decolorized.

after 3 days, several hundred colonies appeared on the streak. Y79.

Streak Y77 over Y88/MG. to determine if decolorization is due to effect of dye. No evidence of stimulation of previously streaked (Mg^3) culture. Probably due to pH change.

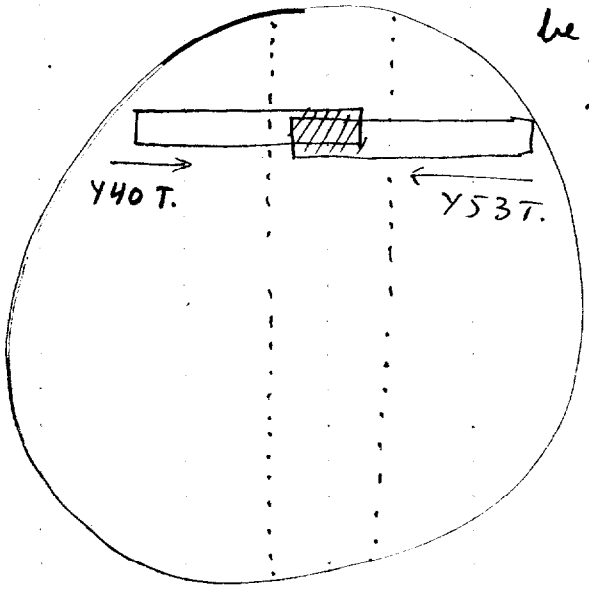
Inversion Detection

See 437a for summary.

1 MAR

Streak out Y40, Y53 (T from 426) on NA plates.

P1. Pick single colony and streak on NA, overlapping a streak of the other type in the center of the plate, and mix well in center. This can then be picked after growth and inoculated into minimal.



P3. Test combinations by suspending growth from center in 1 ml water, ~~and streaking~~ pouring ion minimal plates. (i.e. 84 tests!) ca 10^9 inoculum.

Tests. log prototrophs

1	1+	13	1+	26	2	39	1
2	2	14	1	27	1+	40	1
3	2	15	1+	28	2	41	1
4	1	16	1+	29	2	42.	1
5	1.5	17	1+	30	1		
6	1.5	18	1+	31	1+		
7	2	19	1	32	1+		
8	1	20	1.5	33	1		
9	1	21	1+	34	1		
10	1+	22	1+	35	1		
11	1	23	1+	36	1		
12	1	24	1+	37	1		
		25	2	38	1		

1-6 also tested by streaking 1 loopful on T(0) plates.

	colonies
1	0
2	3
3	1
4	2
5	7
6.	10
7	1
8.	1

84 tests - no inversions

Streak technique not as reliable as desired!

Inversion Tests : Summary.

exp.	material.	tests:				Cumul. Tests.	Yield
426	MN_2 ht.	Y40 x Y53T;	Y53x Y40T, by st. tech.,	O+B,	20 tests.		0
433.	MN_2 ht.	do.			17	37.	0
437.	"	Y40T x Y53T.	mi only, x mags;	2x42 =	84	121	0
508	X-ray	Y40T x Y53T.	mi only. mags.	2x14 =	28.	149	0

Time of prototroph initiation.

Pour Y40×Y53 plates in T(0). To exp. add also 10^{-6} K-12 cultured + washed similarly in order to compare rates of colony development.

See 445.

Y65 } test for inversion.
Y68 }

Plate Y65 x Y40

Y68 x Y68.

in T(0) + T(3,).

no prototrophs at all!

∴ not due to suppression of X in B,-B4 region.

Segregation of Gly –

March 4, 1947 –

Y80 x Y81. cf. 435.

$B_1^- > B_1^+$ as usual. Pick 4 plate on EMB lac + EMB gly agar.

					B_1	
	Lac – R	Lac – S	Lac + R	Lac + S	Gly – / Gly +	Lac – R – S + R + S.

Gly segr.

Y80 x Y80.

A. In min.

Gly	Lac	V
+	−	R
+	−	S
+	−	R
+	−	R_S
+	−	R
+	−	S
+	+	S
+	−	S
+	−	S
+	−	R
+	−	R
+	−	S
+	−	R
+	−	S
+	−	R_S
+	−	S

B.

	Gly	Lac	V
T(0)	+	−	R
	+	−	R
	+	−	S
	+	−	R
	+	−	R
	+	−	R
	+	−	R
	+	−	R
$T(B_1)$	−	+	R
	−	−	R
	−	−	R
	− ?	−	S
	−	−	S
	− ?	−	R
	−	−	R
	−	−	R
	−	−	S
	− ?	−	K

[scoring maybe inadequate]

When real again 4 hours later, series B had gotten considerably darker!

C.

	Gly	Lac	V
B_1	−	−	R
	−	−	S
	−	−	R
	−	−	R
	−	−	S
	−	+	R
	−	−	R
	−	−	S
	−	−	R
	−	−	R
	−	+	R
	−	−	R
	−	−	S
	−	−	S
	+	−	R
	−	−	S
	−	−	R

D.

	Gly	Lac	V
	−	−	R
	−	−	S
←	[? +	− +	S R]
	−	−	R
	−	−	S
B_1	−	−	S
	−	−	R
	−	−	S
	−	−	S
	−	+	R
	−	−	R
	−?	−	S
B_1	−	−	R
	−	−	S
	−	−	S
	? −	+	R
	? −	−	S

Note: B_1^+: 25/25 Lac+, Gly+ close linkage? These results are difficult to reconcile with the map!

$B_1^+ + B_1^-$: 44/46 Lac+.

*

Lac, V segregation.

March 6, 1947

Lac− V^R Lac− V^S Lac+ V^R Lac+ V^S

B_1^+: ~~15~~ ~~9~~ 0 1 25 Faulty segregation of Lac+?

all Gly+.

B_1^-

Gly− 24 19 5

Gly+ 1 (1?)

This scoring of Gly, done at 2 da. is not borne out by readings at 3 days, when in both groups, all were Gly+, $\frac{VS}{c}$ some variation in intensity.

Note: 24 Lac− : 1 Lac+ in B_1^+ (95%!)

46 Lac− : 5 Lac+ in B_1^-.

1) B_1^-/B_1^+

25	24	1	25
41	40	4	45
64	6		70.

$\chi^2 = .04 + .02 + .04 + .03 = .13$

compare ī standard.

18	24	7	25.
51	~~6~~ 19	6	70

$\chi^2 = \frac{13^2}{51} + \frac{13^2}{19} = 3.3 + 8.9 = 12.2$.

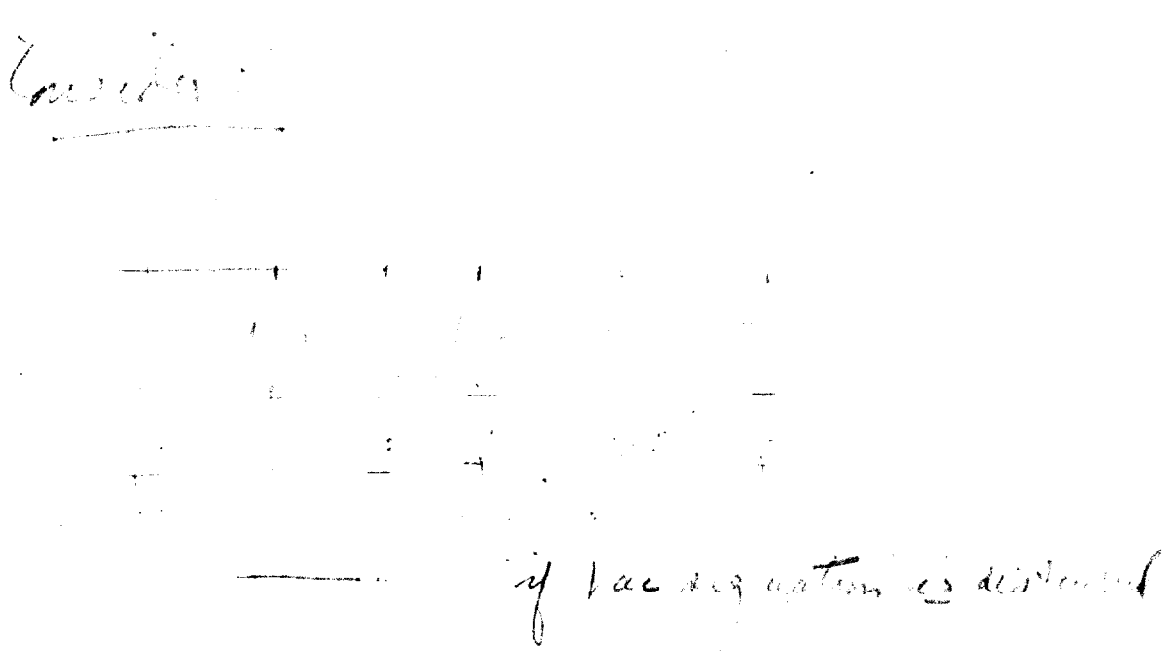
a) All data: 72% Lac−

b) new data 64% Lac−

$\chi^2 = \frac{36}{18} + \frac{36}{7} = 7$ $P = < .008$.

efficiency of Lac+

Peculiar segregation may explain peculiarities of Gly segregation.



Segregation of drug-resistances

~~Y78 x Y80~~

A Y77 x Y78. – in 7/10 OK!

Streptomycin 5 γ/ml
Novobiocin 25. 100 γ/ml

B Y53 x Y78 OK! ±6 Lac– 1 Lac+.

C. Y40 x Y77 OK! no ±

A. Y77 x Y78.

Lac	V	Str	M.G.
+	R		R
± +	R		R
+	S		R
–	S		R?
± +	R		R?
± +	R		R?
± +	R		R
–	R		R

± precedes dug blue color

? probably S.

scoring uncertain due to selection of resistant residue

Lac	V	Str	M.G.
–	R		R
–	S		R
–	S		R
–	R		R
–	S		R
± +	R		R
± +	R		R?
± +	R		R?
–	R		R?
–	S		R.

do.

M.G. resistance 10^{-4}.

	~~Lac~~	Lac+MR	Lac+MS	Lac–MR	Lac MS.
C.		2	1	~~8~~ 8	2
total.		0	2	16	3
Lac+S x Lac–R.		2	3	24	5

Scoring not certain

mid. cont.

nd=no. of resistants. If valid, indicates linkage to B4.

should score on minimal plates to avoid selection for resistant contaminants.

Selection of recombinants with drugs. 442

P3. - mix YB cultures of Y77 (Mg^{-1}) and Y78 (Str_5^{-1}). 30°
also use mixture of mult. plating technique of 441.

			A6	A8.
1.	Mix culture	in Mg + Str	0, 0	1 colony;
2	Y77	"	0	2 colonies; also as 3.; 4 cols.
3	Y78	"	∞	1 colony ca 100 minute clearings, mucoid cols.
4	Y77 + Y78	"	ca 10 v. small	A - as 3. B > 10 large colonies innumerable minute clearings

A6. - incubate at 35°.

Indeterminate whether the mult. resistant colonies represent recombinations.

Compare 4 (cols) c 2 mutations of $Sm^S Mg^R$ to $Sm^R Mg^R$

There may be some synergism in view of the long lag before colonies are detectable!

[Use Y84 × Y79. and plate in brilliant green. + streptothricin.]

Resistance steps; resistance to streptothricin.

443

March 4, 1947.

Recd. 127,000 u. streptothricin from Woodruff, Merck. non-sterile ampules.
Suspend in 2.7 ml 95% alcohol for 3 hours. Add 10.3 ml sterile H_2O → ca 10,000 u/ml in 20% alcohol. Dilute further as required.

u/100 ml = u/ml

100 u	1	
Y78		T
Y53		T

500	5		
Y78		T	streak Y78 on 5u agar for —
Y53		1-200	Y82 - streakout on 5u agar

1000	10		
~~Y78~~		50	Y83 - streak on 10u agar
Y53		1	Y84 — do.

10,000	100	
Y78		0
Y53		0

Y78 — Streptothricin on 5u agar gives colonies but not so large as Y82.

Y83, Y84 OK on 10u agar.

Segregation of B⁻, etc.

See 452.

Y40 × Y53. Mix cells in agar pour 5 ml each plate.

O:	B:	B_1:
11	12	124
15	11	136
9	14	
10	12	m = ~~10~~
15	9	130
	13	
60	6	more turbid.
m = 12.0	12	
	m = 11.1	

A).

B) 20 B tested: 18 B^+

2 B^- (Lac−R; Lac+R)

5/20	364
2/20	445
5/50	452
12/90	13%

C. Segr. of Lac, UV^R in O, B_1:

O:	−R	−S	+R	+S
	24	~~11~~ 9	~~1~~	0
B_1	22	~~5~~ 15	14	0

	−R	−S	+R	+S
O	17	10	9	0
B_1	16	12	13	0

2 | 44 | 5 | 1.

more O plate, streak out 11-12 on surface. These colonies appeared at same time as prototrophs (24 hours) and were of comparable size.

Lac – V segregation.

March 6, 1947.

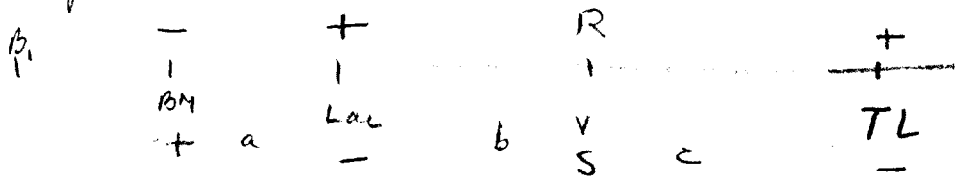
Use colonies from Expt. 437 (grown together – chance for selection) [Y40 x Y53 (T) by plate number].

	Plate #		Lac – R	Lac + R	Lac – S	Lac + S.	Σ	* V scoring not dependable – phage apparently very low titer.
	9	10	2	5	1	0	8	
	20		4	4	2	0	10	
–	* 27	40	9	6	0	0	15	
–	* 22	40-50	8	10	0	0	18	not significant by itself
	* 3		8	3	0	0	11	
	8		3	1	0	0	4	
	3		7	3	3	0	13	
	42		1	2	1	0	4	
	6		6	2	2	0	10	
	4		4	2	0	0	6	Lac – = 135/214
	14	20	6	1	0	1	8	= 63%
	40		1	1	2	0	4	compare ± 70% pur.
	21		2	1	1	0	4	V^R = 190/214
	7	40	6	3	2	1	12	= 85%
	23		3	2	0	0	5	comp. ± .74 pur.
	2		13	7	1	0	21	
=	25		5	14	2	0	21	
	11		1	3	0	0	4	
	10		11	1	3	0	15	
	19		1	1	1	0	3	
	17		5	1	0	0	6	
	29		7	4	1	0	12	
			113	77	22.	2	/214.	

The agreement of the Lac+R; Lac – S classes with exp from previous expts is very poor. Reexamine crosses of aberrant cultures.

There is a shift from lac − S to lac + R

the wrap-box is



<lac − bvc < >a

>v^R bva <. <c

(<c. [illegible])

a = +R

b = −R

c = −S.

[− I would not be augmented by the diminution of c? .]

---

or, another interpretation, is that

the previous strains were revertible for T or for L, i.e. a longer sentence in the interval V−(T or L)

∴ compare types i.e. short or long interval

i.e. low or high-S) biochemically.

contd. 446.

#	Lac-R	Lac+R	Lac-S	Lac+S.	Σ
15.	3	9	1	1	14
33	2	1	2	0	5
34	~~6~~	0	~~0~~ 0	0	5
30	6	2	2	0	10
31	7	7	4	0	18
24	3	3	1	0	7
32	7	3	1	0	11
35	7	2	1	0	10
1	6	4	4	0	14
36	4	2	2	0	8
16	12	10	3	0	25
26	14	9	3	0.	26
	83-76.	52 53	22 20	1	/ 151. ~~214~~
	106-113	77 76	22 21	2	/ 214
	189	129	44	3	/ 365

cf. 445.
compare ē remarks
& rept.

These samples agree.

Homogeneity??

$\chi^2_2 = \frac{49}{83} + \frac{49}{106} + \frac{1}{53} + \frac{1}{76} + \frac{9}{70} + \frac{9}{27} = 1.86$ $p = .4.$

.59 | 1.86
.46
.02
.01
.45
.33

analysis of
446 vs 359 summarized.

−R	+R	−S	+S.	Σ
189	129	44	3	365
100	55	50	4	209
289	184	99	7	574

$$\chi^2 = \frac{25}{105} + \frac{25}{184} + \frac{144}{67} + \frac{144}{117} + \frac{196}{36} + \frac{196}{63} + \frac{1}{4} + \frac{1}{3} \ldots$$

$= .2$
$.1$
2.2
1.2
5.4
3.1
6.33

8.5 by this component. $p = .005$.

∴ it is the difference in the frequency of −S which differentiates the distributions.

$12.7 = \chi_3^2$

$\frac{8.5}{4.2} = \chi_2^2$ $p = .04$

compare

194 - 189	129	124	318
95 - 100	55	60	155
289	184		473

should be 194:124
95:60

$\chi_1^2 = \frac{25}{189} + .25 + \frac{25}{124} + \frac{25}{60}$ = ca 3.

Heredity of segregation types.

March 7, 1947

Recover 446 – 22, 25 + 27. in order to ascertain heritability of disproportions in ratios. Compare segregation of Lac + V ♀ Y40, Y53 stand. in previous test:

A 22:– 8:10:0:0
B ~~24~~:~~25~~ 9:6:0:0
C ~~25~~:~~27~~ 5:14:2:0 Lac, V.

D. Y40 × Y53.

		−R	+R	−S	+S
A		47	19	9	1
		44	20	7	1
B		26	12	16	0
		[illegible]	13	16	0
C		7	5	[illegible]	1
		80	36	32	2
D.	–	30	21	19	0
	O_2	10	7	5	0
	N_2	6	5	6	ϕ
	M.B.	4	4	6	ϕ
	S.H.	2	1	2	0
D. total:		52.	38	39	0
Total		132	74	71	2

This is homogeneous with the previous tabulation: 100:55:50:4.

279.

$\chi^2 = \frac{100}{37} + \frac{100}{62} = 4.3$

$+ \frac{4}{21} + \frac{4}{36} = .28$

$+ \frac{64}{18} + \frac{64}{31} = 5.6$

$\chi^2_2 = 10.1$

$p = .007$

Compare A ♀ D..					
	47	19	9 +1	0	76
	52	38	39	0	129
	99	57	49		205

See over.

Compare A & S.

58	66	10 (18)	76
98	90	39 (31)	129
	156	49	205

$$\chi_1^2 = 64\left(\frac{1}{58} + \frac{1}{98} + \frac{1}{18} + \frac{1}{31}\right)$$

$$= 64(.017 \quad .010 \quad .055 \quad .032)$$

$$= 64(.114)$$

$$= 7.3 \qquad p = .007.$$

Therefore the total discrepancy is due to a difference in the proportion of V^R cultures. In practice, this means a deficiency of $-\underline{5}$ cultures in the new group.

		−R	+R	−S	+S.	
A. 22		~~44~~	20	~~7~~	1	. This is the "abnormal" destribution;
B. 25		25	13	16	0	1 plate showed 13:14:0:0.
C. 27		7	5	2	1	(perhaps scoring of V^R was not proper.' Rechecks!
		~~76~~	38	~~25~~	2	
D.	—	30	22	19	0	
	O_2	10	7	5	0	
	N_2	2	1	2	0	
	SH	6	5	6	1	
	MB	4	4	5	1	
	$\bar{2}$.	52.	39	37	2	4·7
		69	38	31	2	

Plate by plate

A	6	2	0	0
	6	14	6	0
	8	1	3	0
	17	3	4	1
B	4	1	6	0
	12	10	8	1
	9	2	2	0
C	7	5	2	1

compare +, −

89	41	130
100	40	140
189	81	270

Caution! Beraufal of scoring V^s.

Interpretation of 447.

Compare $T^- \rightarrow T^+$ in 447 Lewis + standard.

March 7, 1947

Using cells from Exp. 447, plate the Y53 components at a 10^{-2} dilution into LB$_1$ agar.

Y53	turbid	10 c.c.
(Y53-Y27)	clear	30 c.c.
25		10 "
22		1-2

There is no stable diffuse negative, but apparently due to scoring difficulties.

Plating medium for recombination

March 7, 1947

Factors: buffer: phosphate, citrate, acetate, phthalate. Near alkaline.

R_H: thioglycollate; ascorbic acid; pyruvate-lactate; O_2; Methylene Blue. Hydroquinol??

B_1 ? — Try on BM x TP.

T(0) — V40 x Y53 mix in agar + pour on pre-poured plates.

1. Controls
2. "O_2 atmosphere" least turbid plates
3. N_2 atmosphere turbid plates; colonies v. small, countable near rare.
4. Na thioglycollate 2 mg = .2 mg =
5. Ascorbic acid 1% no colonies (pH 4-5) No turbidity
6. Methylene blue 100γ Turb. ≤ [illegible]
   10γ " "

The number of apparent colonies is correlated inversely ∴ the gross turbidity, and was least in O_2, most in N_2. SH had no apparent effect, however. There is, however, no marked increase of colonies in the O_2 atmosphere plates, but those which do appear are larger.

See 441. Test Y78. on EMB-Lactose. Cf. Y53.

-disc 441A - lactose - on Lac → glycerol + blue colonies

Y78	(58-161 Sm^R)	++ gas.	Gly. ++ - gum sheen !
58-161			- (±??)
Y80	(58-161 - V_1^R - Sly -)		-

Note, Y80 on v. long incubation turns a faint violet color, à la succose from previous expts + now

Y53	++
Y81	++
Y40	++
Y78	++

? relative amts.

Y80	—
58-161	±

58-161 is slow, but wilfscore vs. Y80. see Y53.

Y80 x Y81.

Gly. segregation.

March 9, 1947.

A. Repeat 440 See 451A.

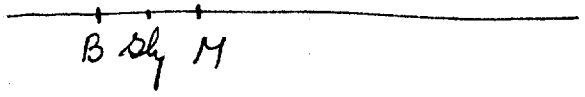
Y80 x Y81.

T(0).	16/16 Gly+	12/14 Lac−	86%
T(B,)	13/13 Gly+.	56/72 Lac−	78%

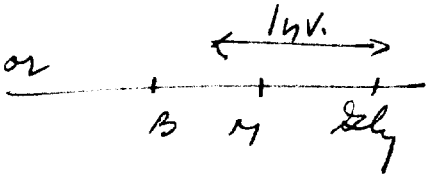
~~B~~ Note, this denotes absolute linkage of Gly to BM. This is predictable either as the mapping:

written Y80 x Y53
not Y81 x Y40
seems to be disturbed!

typo!



or



Note (also) the segregation for Lac may be disturbed. Comparing ē accum. data for Y40 x Y53: (71% Lac−) $p = .06$

ē 440	$p < .025$
(This + 440) ē 71%	$p = < .001$
440 ē 71%	$p = .008$
B_1^+ / B_1^-	$p = .3$

Glycerol segregation.

March 9, 1947.

B. Y80 x Y53. Test $B_1^+ + (B_1^- + B_1^+)$ on Gly; Lac.

These plates were allowed to stand 3 days before testing

a. B_1^+ 27 Lac− : 9 Lac+ 35/35 Gly+

b. B_1^- 50 Lac− : 16 Lac+ scoring uncertain: 1++; 30+/31

77 Lac− : 25 Lac+ · ca 75% Lac−

C. ~~A+B~~. Y81 x Y40. Test on Lac

B_1^+ 104 Lac− : 43 Lac+

B_1^- 28 : 13

132 : 56 188 70% Lac−

Gly 16/16 +
(not weak)

This experiment is not homogeneous with the earlier Y40 x Y53. mes.

D. Y53 x Y40.

	−R	−S	+R	+S	
B_1^+	33	32	28	25	14 18 0 → 75
B_1^-	18	19	12	15	13 10 1 → 41

→ stand $\chi^2_2 = 3.2$, $p = .2$ data not alternate

→ $\chi^2_2 = 3.5$, $p = >.2$

51. 40 27 1 119 of standard. $p = .06$

91 : 28 76% Lac−

Segregation of B−

March 9, 1947.

See 445

Y40 × Y53 in Biotin medium. Rel. colonies & H_2O, + moi. hopeful in T(0) + T(B). 50 isolates.

50 isolates. At 12 hours, 5 def. B⁻ & did not grow in cotton. 5 at first.

Lac, V segr.:

	Lac	V
1	+	R
2	+	R
3	−	R
4	−	R
5	−	R.

P13− 5 grew on B_2 not O. Ancest, 1−4 contained primarily −S, E + R as well. 5 was uniformly −R. see 456.

To summarize: B⁻

~~Lac R − S + R + S.~~

−R	+R	−S	+S.
					1							0	1

∴ 0 − S / 12.

	6	5	0	1	12
A (−)	6	3	3	.36	
B (M−B−)	1.4	9	.8	1.4	

$\chi^2_2 = \frac{4}{3} + \frac{9}{3}$ 4.3

$P = .14$

$\chi^2_2 = 5 + \frac{16}{9} +$

$= 7 +$ .03

∴ maps

B.9 B 13 M

Glycol reactions, various strains.

March 11, 1947.

P11. Streak on a single Glycol plate (EMB - 2%) the following:

		A12	P12	A13	4P13	A14
1	K-12	−	+	++	+++	+++
2	58-161	−	−	−	− + shreds.	
3	Y40	−	−	±	± + shreds.	
4	Y10	−	−	±	+	
5	Y53	−	−	+	++	
6	Y46	−	−	+	++	
7	Y64	−	−	±	++	
8	Y78	−	±	++	++	
9	Y77	−	−	±	++	
10	Y80	−	±	±	± translucent, creamy shade, not opaque purple.	
11	Y81	−	−	+	++	
12	Y82	−	−	±	+	
13	Y73	−	−	+	+	
14	Y74	−	−	+	+	
15	Y82	−	−	+	++	
16	Y83	+	++	+++	+++	
17	Y84	−	−	+	++	
18	Y79	−	−	+	++.	

— resistance of bacteria

4/11/47

Ars various plates, streak: Serratia marcescens 1
Salmonella 20 2 incubate
" 21 3
Phytomonas tumefaciens 4
Staph aureus 5

			1			2			3			4			5		
Vor u./ml.																	
Malachite Green.	M.G.	1	++	✓	++	✓	–	++	✓	–	±	++	–	–	✓		
		10	+	++	–	✓	++	✓	++	✓	–	–	✓	–	–	✓	
		50	–	–	–	–	–	–	±	–	–	–	–	–	–	–	
		100	–	–	✓	–	(– colo)	±	✓	±	++	✓	–	–	✓	–	–
								+									
Brilliant Green.	B.G.	100.	–	–	✓	–	–	✓	–	–	✓	–	–	✓	–	–	✓

			1			2			3			4			5		
Streptothricin	Sth	1	++R	✓	+	–	✓	+	–	✓	+	++	–	++V	✓		
		5	+	+	colo.	–	–	±	±	papillae	±	+	++	–	colonies. ✓	O/K.	
		10	–	✓ cols.	good colo.	–	–	–	–	✓	±	+	✓	–			
Streptomycin	Sm	* 1	–	–	✓	+	+	papillae	+	+	±	+	✓	–	✓ cols.	✓ grow large.	
		5	–	–	–	–	–	–	–	±	–	–	✓				
Penicillin	sii	10	++	✓	–	✓	–	✓	–	✓	–	✓					
	sii	100	++	✓R	++	✓	++	–	±	++	++	✓V	✓				
Control	N.A.		++	++R	++	–	++	–	±	++	–	++	++V	–			

Incr 10P11. Reading 1st. 1130A12.
6P12
9A13

Concl.–[Verb. of selective conc.]:

	M.G.	B.G.	Sth	Sm	Pen	
Serratia	10-50	– <100	10	10	<1.	–
Sal 20	50	50 <100	5	5	1-5	>1
S21	>100	<100	>5	>5	1-5	>1
Phyto	1-10	<100	>10		>5	
Staph	<1	<100	5	5	1	1

10-100

order of activity:

M.G.	Sm	Sth	Pen.
Staph	Serr.	Staph	
P.t.	Staph	S 20	
S.m.	S 20	S 21	
S. 20	S 21	Serratia	
S. 21	Phyto	Phyto	Serratia

↓ incr: resistance

Use studies following papillae on higher drug conc for higher steps:

S20/MG 50 on B.G. 100
M.G. 100.

S.m / Sth 10

S20 / Sth 5

S21 / Sth 5 on higher Sth.

Staph / Sth 5

S20
S21 | Sm on higher Sm.
Staph

Resistance mutants

Staph.	M.G.	1	turbid.
—		5	ca 10 Mg^R/cc; gd. inhibition of residue
—		10	perfectly clear!
—	Sm	1	ca 10^4 Sm^R.
—		5	ca 10 Sm$_5$^{R}
	Proteus	100.	Turbid.
		10	clear at 48h.
— Serratia	M.G.	20	clear at 24 hrs.
		50	a. ca 20 Mg^R (ctd?)
—	Sm	1	clear. no Sm^R seen. ; some v. small Sm^R.
—		5	~~ca. 10 appr. colonies~~. 1 Sm^R? white!
—	Sth	100	ca 5 Sth^R/ml col. v. small
—		20	ca 100 Sth^R/.1 ml Wide range of color.
	Prof.	100	turbid
— S20	Bg	100	clear, no Bg^R!
	Sm 1		turbid
—	Sm 5		turbid; but some possibly Sm^R, v. small ✓
	Proff 100		turbid.
— S21	Bg 100		clear, no Bg^R.
	Sm 1		turbid.
—	Sm 5		as S20. inhibition incomplete.
	Proff 100		turbid.
Phyto.	M.G.	10	inhibition incomplete
	Sth	50	inhibition incomplete; some selection
	"	100	clear! ca resist.
	Sm	10	incomplete inhibition ca
		50	clear! ca 100.
	Prof	100	clear! — ca 300

Available:

~~Starts~~: Sth 100
Sm 50
Prof 100

Phyto

St: Sm 5
Sth 10
Mg 5

820 B.G.
Sth 10
Sm 5

S21 Bg
Sth 10
Sm 1

Senate Sth 20
50
100
} colonization

Sm 5
Mg 50

Reversion detection

4/11/47.

As above on N.A. and T(0).

photographs show up as papillae in T(0) streak, justifying this technique. Throw out NA plates.

T(0)	colonies in center zone
1	++
2	++
3	+
4	++
5	++
6	++
7	++

Streak out further 48 hr. colonies of 440T + 453T.

e.g. too irregular. NA mix + plate is more reliable.

"Syntrophic colonies?" 456

See Y52 for sources.

Colonies from Y40 x Y53 on Biotin were picked to water + T(O), T(B) inoculated. Those reported are 5/50 which grew on T(O) only after 2-3 days. The T(O) and T(B) tubes were both streaked on Lac-V agar: (↓ no turbidity)

	T(O)	T(B)
1	−S ; +R	−S ; +R
2	−S ; +R	−S ; +R
3	−S ; +R	−S ; +R
4	−S ; +R	−S ; +R
5	+R	+R.

Since −S and +R are the parental configurations, the delayed growth [and the original colony formation] might be due to syntrophism ?

∴ streak out T(O) tubes on EMB lac to purify.

Test − (a) and + (b) colony of each on B, O mediums.

	O	B
1a	−	−
1b	−	−
2a	++	++
2b	−	−
3a	−	−
3b	−	−
4a	−	−
4b	−	−
5a	−	−
5b		−

Expl. 1. Syntrophic colony

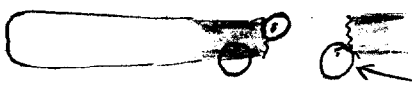
2-Colony not picked; only interstitial growth in agar. Requires repeating.

Mucoid variation.

19 MAR 1947

Streak Y53 across T1 on EMB agar.
Suspend slimy growth from "[redacted]" intersection of bacteria + phage in H_2O + streak out.

Note, at intersections of bacteria + phage, a zone of coloration of the bacteria as if there were there some enzymatic activity.



N21. Pick from red region + from mucoid colonies to water and streak out on EMB lac. (Y53/1).

Y53/1 same growth (probably resistants; ~~both~~ lac − + lac +) (standard type).

1. Y53M − all mucoid. pick one colony + test on T1 V^R
   also streak out →

2 $Y53M_2$ − all mucoid. P23. Streak out →

3 $Y53M_3$. all mucoid. Pick to slant and label for subsequent analysis. Y86

Selection: Y40 x Y53. 458

Plate Y40 x Y53 as usual. At 48 hours pick 5 largest (>) and smallest (<) colonies from each of 7 plates & compare the distributions

−R	−S	+R	+S.
4	1	0	0
4	0	1	0
3	0	2	0
2	2	1	0
2	1	2	0
2	0	3	0
1	0	4	0
18	4	13	0
27	12	30	1

In large, +R > −S.

In small +R > −R.

$\chi^2_3 = 5.14$ $p = .16$.

But. compare all 3 groups,

$\chi^2_4 = 12.61$

$p = .013$

selection may play a role.

Random selection from these plates gave:

27	13	13	1.
221	136	142	6

is cumulative data.

Note that both in in large + small selection types, there is a marked deficiency in −S as compared to random selection + cumulative data!

Ratio of types:

15 Mg

A. 5 ml Y40 : 1 ml Y53. Group by plate (1-4) and colony size (L & S).

	−R	+R	−S	+S.	
1L	5	4	2	2	
1S	7	2	3	0	
2L	7	3	2	0	
2S	6	1	2	1	
3L	8	1	4	1	
3S	3	4	4	1	
4L	6	3	1	0	
4S	6	4	1	0	
L:	26	11	9	3	49
S:	19	11	10	2.	42
Σ :	45	22+	19	5.	/91.

no ex. of selection here!

O.K.

B. 1 ml Y40 : 5 ml Y53.

	2	0	1	1
	~~1~~	1	~~3~~	0
	5	1	0	0
L	7	1	0	0
S	7	2	0	0.
	~~21~~	5	~~2~~*	0
	22	5	4	1

same plate! ~~check~~ check for virus

no S/plate!

do not use this expt. no too small anyhow

	-R	+R	-S	+S
B_1^+ ..	7	1	7	5
	4	0	6	1
	2	0	3	3
B_1^-	4	0	5	1
	17	1	21	10

	Lac-R	Lac+R	Lac-S	Lac+S
TL	2	0	3	2
BTL	2	0	0	1

Y80 x Y81.

~~Y80~~
Y61

Repeat 451 for Lac segr. test + for gly- segregation.
Plate is T(0), T(B), T(B_1).
1 plate – Y80 in B_2 to check on reversion of 14.

$\bar{B_1} >>> B_1^+$

++. 31/31 Gly+
27/27
10/10
32/32
100/100.

from B plates: 42/42
20/20
8/8
28/28
98/98

Totals of Gly+ segr.

Cross	
461.	198
451	29
441	70
	29.7

∴ 1. Linkage is very tight, perhaps requires a double X against interference
2. Cytoplasmic inheritance
3. Triploid reversions.

Y80/B → no reversions

Y80 x Y81.

Y62

March 20, 1947

Grow Y80 + Y81 in mixed culture, and plate out on EMB medium. Select Lac + colonies and test for Gly reaction to determine possibility of transformation.

plate too crowded. take up scrapings.

Fermentation mutants.

March 22, 1947

Incr Y40T (mustard see Y40) and Y53T in YB P 22

Spread dilute (10^{-7}) cultures of Y40 on EMB-lactose 1% P23
of Y53 on EMB glycerol 2%

and incubate at 35°.

Y40 - lactose. ca. 13,000 colonies. no mucoid.

1. lac - 1. translucent pigmentation. Recover for test as mutants.

Read P24. ↑

Y87. Lac - ✓
V_1^R ✓
V_1^S ✓
Nutrition:

Y53 - glycerol. ca 29,000 colonies.

mucoid - 1 (2?)

gly - 1? (mucoid?)

gly - probably contaminant. Test by streaking on lac, V.

March 21, 1947

A. 440 x Y53 B. 58-161 x Y64.

phage ratio. Lac- unreliable as they were scored on second transfer to lac. V plate for checking on phage

		Lac-	Lac+
T(0)	rand.	20 +30	5 +15
	small	23	4
	large	18	6
B_1	random	27	17 !
	small	31	8
	large	39	14
		168	54 total 198:69

These data are too uncertain to be used.

B.

	−R	+R	−S	+S.
L	0	0	4	5
S	2	0	5	1

difference A + B $p = .1$

T(0)	rand	33	12
	small	15	13
	large	22	8
B_1	small	16	8
	large	15	7
		101	48

269	102	371	= 72.5%

Hemoresistance as a means of selecting recombinants. 465

March 22, 1947.

Pick various resistance mutants directly to YB+ incubate 48 hours. Plate as indicated.

Phy / Pro. 100	M.G.	10.	turbid.	Phy/Sth	turbid Pen 100.
		20	turbid		
		50	turbid.		
		100	sl. turbid. - keep.	ca. 100 resistants.	
	B.G.	100	turbid!		
Phy / Sth 100	Sm 50		clear - some resistants.	ca. 10^3	

Phy / Pro seems M.G. resistant also, to a certain ext.

Staph / M.G. 5	M.G.	5.	Irregular turbidity. some "resistants"	
		10	clear plate. ? "	
		20	" "	
		50	" "	
		100	clear	
	B.G.	100	clear	
	Pen.	1	turbid.	
		5	spots clear & resists.	
		10	turbid	
		50	turbid	
		100	turbid.	
Staph / Sm. 5	Sth	10	clear & resist. —	
		~~20~~	clear S	
Ser / Sth 100	Sm 5.		clear - small resists.	
Ser / Mg 50	B.G. 100		turbid.	
	Pen 100		turbid.	
S20 / Bg 100	Mg	0.5	irreg. turb.	S21 / Bg.
		1	clear - one is comm.	clear one is center (not mixed?)
		5	clear!	
		10	clear!	
	Pen 100		turbid.	
/ Sth 10	Sm 10, 5		turbid	/ Sth - turbid Sm 10, 5 turbid.

Chloroacetic acid resistance.

March 22, 1947.

See Penfold ~~1914~~ 1911.

Streak Y53 on NA + various conc. Monochloroacetate meath. ± NaOH conc. expr. as free acid.

	Y/per cent
50	continuous growth
250	dense growth of streak ± ca 10 papillae of large size per streak.
500	Background growth less. do.
1000 = 1	" " very slight.

Pick papilla of 1 to new 1 CLA plate. P24.
Isolated colony to ∞ slant: Y88

Grow into fermentation tubes P26. (Bethesda Bl.)

		glucose 1%		glycerol 1%.	
	Y53	A++	++G	A±	±G
12h.	Y88	A++	-G	A±	±

see 468.

	Y53	A++	/+	A+	+G
36h.	Y88.	A++		A+	±G

Segregation of Mucoid Resistance.

467

March 26, 1947

by T(O).

A Y66 × 58-161 — protoplasts rare (2/7 plates!)

B Y86 × Y40.

A. 8 mucoid, all resist. — 7 Lac−, 1 Lac+. — 1 Smooth. Lac− $V^R S$! [Mucoid diffrnt from resistance ?]. — check.

B. 28 mucoid, all resistant. — 8 Lac+, 20 Lac− — 1 Smooth Lac− V^R

---

9 Lac+ : 27 Lac− — Smooth 2 Lac− : 0 Lac+.

Prepare Smith fermentation tubes ± Nutr. Broth + various supplements as ind.

Formate includes M/20 phosphate buffer.

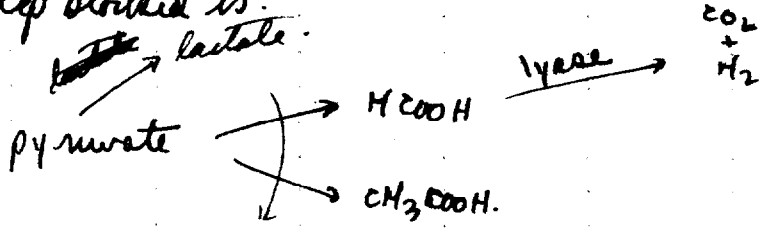
12 h.	control	formate ½%	F+gluc 1%	F+man 1%	gluc 1%	man. 1%	malt 1%	suc 1%	glycerol 1%
			H_2:CO_2 ca 2:1						
Y53	— ✓ —	+ + ++	+ +	+ ++	+ ++	+ +	± ∓	±?	— —
		++			++	++	++	±	± ±
Y88.	— ✓ —	+ +	++±	+ ++	— ++	± ±	— ∓	—	— —
		++			—	+±	±	—	± ±

	pyruvate 2%	lactate 1%	malate 1%	acetate 1%				
Y53	++ +	+	—	—	—	—	—	—
	++		✓		✓		✓	
Y88	± —	—	—	—	—	—	—	—

second reading 72 h.

Formic hydrogenlyase is apparently intact.

∴ step blocked is:



Try: utilization of pyruvate in synthetic medium.

Carbon source utilization (T(0) — asparagine + TLB$_1$).

Suppl: 1 — 2 pyr 2% 3 lact 2% 4 gluc 2% 5 mannitol 1% acetate 1% 7 glycerol 1% 8 malate 1% 9 formate 1%

Y53:	—	+	+	++±	+	++	? +	+ ++	+ +++	—
Y88:	—	+	+	++	+	++	? ±	+ ++	+ +++	—

malate, mannitol } were most eff. carbon sources → major difference.

~~wild type : cell type test~~

W.T.	Mutant.		
	++		++ formate
+	++	+	— glucose
+	++	—	— pyruvate } major differentiation
±	±	±	± glycerol
+	++	+	++ mannitol
+	++	±	± maltose
±	±	±	— sucrose
—	—	—	— acetate &c.

utilizes AcO ? ut. AcO

collect gas (if any) in Durham tubes & estimate gas ratio).

		acid	gas	ratio $CO_2 : H_2$
Y53.	glucose	+	+	< 1/5 H_2 ✓
	mannitol	+	+	< 1/5 no poss. hand.
	pyruvate	±	+	< 1/5 H_2 ✓
~~Y70~~ Y88	pyruvate	−	−	~~< 1/5 H_2 ✓~~
	glucose	+	−	
	mannitol	+	+	< 1/5 H_2 ✓

March 28, 1947

Y77 x Y78. (= Y6Y x 58-161)

not a good expt.

	−R	−S	+R	+S.
0.	8	8	0	3
	3	1	0	0
	4	8	2	0
	15	17	2	3
B1.	6	12	0	3
	21.	29	2	6

This distribution is entirely different from the standard ~~Y6 x Y3~~.

R : S = 23 : 35 = 40% (1-60%)

− : + 50 : 8

= 86%.

Probably a definite alteration of frequencies. Look for a lethal recombinant or for an additional mutn. req.

Test for M.G.^{R}; Sr^R.

	MG S	R	Sr S	R.
0: −R		16/16.		4/16.
+S	Lost; mostly R.			
+R	S; R		S; S.	
+S.	R; R; R		S; S; S.	

B1

−R
−S
+R
+S. ~~S; R; S~~ S; S; R. ~~all~~.

good + some

not certain as at best only a dozen colonies appeared at site of streak.

−R are all MG^R

−S are > MG^R

+R variable.
+S

Formic Hydrogenlyase.

~~469~~
470

March 27, 1947.

Inoc Y53 heavily into Nutr. Broth + 2% glucose + ½% formate.
Grow 12 hours. Wash cells once + suspend 100ml in 5 ml of buffered (6.8 M/10 phosphate) ½% formate, aseptically in Smith tube.
Gas production was marked within ½ hour. NaOH abs. ca ⅓ of gas.

Repeat in K-12.

Inoc. 1) glucose-formate-broth + 2) 2% pyruvate broth heavily in K-12 5 P 28.

Washed cells of 1) gave ++ gas on formate
2) gave no gas on pyruvate.

Segregation of Cla^R

March 28, 1947.

Y88 x Y40. on T(o).

Test 20 isolates.

	Lac V	Cla	Gas-gluc	pyr EMB
1	−R	S−	+	−
2	−R	S	+	−
Yv 3	−R	R✓	+	−
Yv 4	−R	S	+	±
Yv 5	−R	R✓	+	−
6	−R	S	+	−
7	−S · −R	R	+	−
8	−R	R	−	−
9	−R	R	−	−
Yv 10	−R	R	+	±
11	+R ②	R		−
12	+R −	R	+	−
13	+R	R		−
14	−S ③	R ①		− ②
15	−R	R		−
✓ 16	−R	R		−
✓ 17	−R	S	+	± (OK.)
18	+R	R		−
19	−R	R		−
20.	−R.	R.		−

Many of the lac colonies in groups 1 appear to be yellow suggesting possibility of contamination.

Repeat cross.

#14 seems OK however.

Y89 Streals out on NA and Cla for use in metabolic studies.

Isolate further, avoid "yellow" colonies.

all 3 sets show same no. of colonies on NA and on Cla ±.

~~Pick colonies from NA to streaks as Y89 – 1, 2, 3.~~

Reverse Crosses

March 28, 1947.

A. Y40 x Y53 B. Y64 x 58-161
on minimal.

A

Plate	— R	+ R	— S	+ S	small
1.	6	1	2	0	
2	6	2	1	1	
3	3	1	4	0	
4	7	1	3	0	
5	6	1	8	0	
6	3	3	3	0	
rand.	6	7	2	0	
	37	16	23	1	

53:24 = 69% R/S.

B

	— R	+ R	— S	+ S.
	2	4	5	0
	3	5	0	0
	0	0	1	0
	3	1	0	0
	6	3	0	2
	3	1	3	0
	3	1	4	0
	20	15	13	2

R:S = 35:15 = 70%

B.	8	1	12	8	
F2					

9:20 = 31%

r.

Compare A_L & A_S. totals.

	.11		.53		.04
	37	16	23	1.	77
2	20 .18	15 .75	13	2 .06	50
	57	31	36	3	127
			39		

χ^2_2 = .11
.18
.53
.75
.04
.06
1.67

OK. compare

	R/S	+/−
A	.69	.22
B	.70	.35
r	.31	.31.

$\chi^2 = 3.14$ $p = .08$ ≈ cumul. data —
< .07 for fit

perfect fit in reverse!

Cross-resistance - Y88.

April 3, 1947.

Agent.	conc./20	conc mg/ml	Y53 (16 hours.)	Y88
Fluoracetate		.05	++	++
		2.5	++	++

F intermediate conc. do.

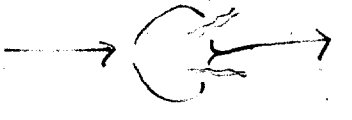
Chloracet.	1.0	±	++
dl lysine.	10.0	++	++
iodoacetate .1 mg	5γ	++	++
	50γ	−	−
	250γ	−	−
	500γ	−	−
	1 mg	−	−
	2.5 mg	−	−

showed no resistants

resistant colonies appeared profusely in 36 hours (Da^R). Y90.

a) mutational effect in manifestation

b) lethality of double mutant due to a metabolic cycle like.



Streak suspensions on surface of poured NA plates. ++ indicates heavy confluent growth.

Y90: Sa-resistance.

April 7, 1947.

1. Streak Y53, Y88, Y90 on Sa plates (~~50~~, 100, 250 r/ml)

~~2. Streak out Y90 on 50 r/ml. Sa.~~

No growth on any plates by any of the cultures.

Acetate utilization

aerogenesis.

April 8, 1947.

Broc. (lightly) T(m) + suggst. c K 12.

		24h	36h.	48h.	72h.	84h.	
1.	Acetate 1%	—	—	—	+	+	
2.	Acetate + ~~glycine~~ + malate .01%	—	—	—	+	+	
3.	Malate .01%	—	±	+	+	+	
4.	Glycine 1%	—	—	—	—	—	
5.	Glycine 1% + malate .01%	—	—	—	—	—	
6.	Malate 1%	±	++	✓	++	++	
7.	Glycine 1% + malate 1%	—	—	—	—	—	glyc inhib
~~8.~~	~~T(s) + glucose 2% (+ acetate)~~						
~~9.~~	~~T(s) + glucose 2% + acet. .5% (+ yeast tube)~~.						
8.	Glycine ½% + Acetate ½%	—	—	—	—		
9.	Pyruvate 1%	—	±	+	+++	+++	
10.	Pyruvate + Malate .1%	—	±	+	+++	+++	
11.	Acetyl-glycine		±	+	+	+	

Acetate seems to be inhibitory (cf. 1, 2 + 3). ∴ try 1-12 on various acetate, glycine concentrations. Broc P11.

		T(m) +.	A13	P13	
	Acetate.				
1		1%	—	—	
2		.5%	±	+	
3		.1%	+	++	More opt. conc.
4		.01%	±	±	
	Glycine				
5		1%	—		
6		.5%	—		
7		.1%	—		
8		.01%	—		
0		0	—		

Nutrition of 679-~~5~~ 662

April 10, 1947.

r/10	αkG	glut	etc.		12h	24h.	36h.
1.	0	—				++	++ ++
2.	0	5r				+ ±	++ +
3	0	200r				++	++ ++
4	5mg.	—				++	++ ++
5	5mg.	200r				++	++ ✓
7	2.5mg					++	++ ✓
8	2.5mg		1mg alanine		±	++	++
9	2.5mg		1mg alanine + 100r B_6		±	++	++ ✓
10.	5mg	5r				+++	++

adaptation?

Inoc. 679-183 into T(0) + threonine + indicated supplements to test nature of block of this glutamicless mutant.

indicates strongly the utilization of α-keto-glutaric acid by this mutant.

Test 10 single-colony isolates of 679-662.

Values:

	T + 0	T + glut	T + αkGlut	glut	24h
1	—	+++	+++	—	✓
2	++	+++	+++	—	✓
3	—	+++	+++	—	✓
4	++	+++	+++	—	✓
5	+	+++	+++	—	✓ ++
6	++	+++	+++	—	✓
7	+++	+++	+++	—	✓
8	—	+++	+++	—	✓ +
9	—	+++	+++	—	✓
10	±	+++	+++	—	✓

no doubt of utilization of α-keto glutaric

T- OK.

~~transfer~~ 1 and 2 as T-G- & T-GK resp.

Y89; K-12.

477.

April 10, 1947.

1.) m acetate 1%

	12h	24h	48h	72h.
K-12	−	±	++	++
Y89.	−	−	±	±

2) m glucose −

K-12 A 5
Y89 A mg.

Formic H-lyase Activator

April 11, 1947.

pellets.

NH_4Cl	5
Na_2SO_4	2
K_2HPO_4	3
KH_2PO_4	1
Malic acid	5g.
NaOH	3g.
Trace Elements	
$MgSO_4$	.2g.

= Formic hydrogenlyase basal. = FM(0).

Use = Durham tubes.

— = 11
— = gas.

	12h.			36			
	K-12		Y89.				
1. FH(0).	—	—	—	+	—	+	—
2. FH - glucose 2%	±	—	±	—	++	—	++	—
3. FH - formate ½%	—	—	—	—	±	—	±	—
4 FH - glucose 2% + formate ½%	—	—	—	—	±	—	±	—
5. FH - glucose + formate + y.ex.	+	+	+	+	++	+++	++	+++
6. FH - glucose + formate + vits.	—	—	—	—	±	—	±	—
7. FH - glucose + formate + HC.	+	±	✗	—	++	+++	++	+++
8. FH - glucose y.ex.	—	+		—	++	++	++	—
9. FH - formate y.ex.		✗		✗	++	++	++	++
10. glucose 2% + formate ½% + y.ex.		✗		✗	++	+++	++	+++
11. T(0)	—	—	—	—	++	—	++	—

Formate is inhibitory, reversed somewhat by something in HC or in y.ex. perhaps by way of formation of formic dehydrogenase.

= Enzicase .5%

Hydrogenlyase coenzyme

April 13, 1947.

		K-12 12h. growth	K-12 12h. gas	Y89 12h.	Y89 12h. gas	K-12 24h.	K-12 24h. gas	Y89 24h.	Y89 24h. gas	K-12 36h.	K-12 36h. gas	Y89 36h.	Y89 36h. gas
1	FH 10) + glucose.	−	−	−	−	+	−	+	−	++	−	+	−
2	+ formate	−	−	−	−	−	−	±	−	±	−	±	−
3	+ glucose + Y.Ex.	++	+	++	−	+++	++	+++	−	✓		✓	
4	+ formate + Y.Ex.	±	+	±	++	++	++	++	✓	✓			
5	+ glucose + N2 Case	±	++	−	+++	++	+++	−	✓	✓			
6	+ formate + N2 Case	±	+	±	++	++	++	++	✓	✓			
7	+ glucose + HC	±	+	±	++	+	++	−	++	++	++	++	−
8	+ formate + HC	−	−	−	−	−	−	−	−	±	−	±	
9	+ glucose + EAA	±	±	±	−	++	−	++	−	++	±	++	−
10	+ formate + EAA	±	−	±	−	+	−	+	−	+	±	+	±
11	+ glucose + NAA	±	✓	−	±	−	++	±	+	−	++	+	+
12	+ formate + NAA.	−	✓	−	−	−	−	−	−	−	±	−	±
13	+ glucose + EA + NA + vits.	±	−	+	−	++	±	++	−	++	±	++	−
14.	glucose + tryptophane.	±	−	±	−	++	−	++	−	++	±	++	−
15	formate	−	−	−	−	±	−	−	−	−	−	−	−

Compare poor activities of NA. & high activity - N2 Case & intermediate activity of acid-hydolyzed casein.

NA, tryptophane have slight activity.
by individual NA. & more by tryptophane

Oxidation tests.

Grow in Acetate 1% broth. Wash & adjust to ca. = densities.
1 ml (≅ 5 ml original) bacteria in 1/10 phosphate + substrate.

	K-12	Y89
	ca 1:50	ca 1:50
Acetate .1%	ca 10 min	ca 35 m (not complete)
Pyruvate .2%	ca 2 min	ca 3 min.

4:05.

K-12	1	—	ClAc.	
	2	Ac	—	
	3	Ac	ClAc	
contrs?	(4)	~~Ac~~ Pyr	ClAc	4:30.
	5	Form		< 4:20
	6	Form	ClAc	< 4:20

Carbon metabolism of 679-~~[illegible]~~ 662

	K-12	679-662	
1. T(G)(100r/10ml).	—	—	
24h.			
2. T(G) + .1% glutamic ac	++	++	pellicular growth.

Quantitative response.

T(G) +	gluc	α-ketogl.	
	0	0	+++
	3r		
	↓	do.	
	1mg/10ml		

∴ this strain has fully reverted & data as retrojation are fallacious.

Reisolate from lyophil & check rigourously. OK ~

~~[illegible]~~ mg/ml & kilo glutamic acid in lieu of glutamic for growth OK.

Utilization of Acetate

480

24 APR 1947

T(m) + Acetate	Glucose		36h. K-12	48h.		36h. 489 ~~490~~		
Proc P24								
.1%	—		+	+	✓	±	—	✓
.1%	1%		++	++	✓	+++	✓	✓
.2%	—		+	+±		±	—	
.2%	1%		++	++		+++	✓	
.5%	—		++	sic. ++		—	—	
.5%	1%		±	" ++		++.	✓	

? ↑ Mutant is acetate −. Not inhibitory.

are acetate + glucose inhibitory when auto claved together ?] Cf. 475. In pres. expt. test was in phosphate buffer.

Glycerin utilization data are needed

autoclaved together.

T(m) +	Acetate	Glucose	Glycerin.
	.1%	—	—
	—	—	.1%
	—	1%	.1%
	.1%	—	.1%
	.1%	1%	.1%.
1	.2%	—	—
2	—	—	.2%
3	—	1%	.2%
4	.2%	—	.2%
5	.2%	1%	.2%
6	.5%	—	—
7	—	—	.5%
8	—	1%	.5%
9	.5%	—	.5%
10	.5%	1%	.5%
11	.5%	1%	—
12	—	1%	—

IAc Resistance etc.

2 APRIL 1947

Prepare plates of NA + IA 50γ, 100γ/ml. etc. Streak thickly.

		Cla ± mg/ml	Cla ±	IA 50 γ/ml.	NaN_3 100
Y53, Y40, Y88	24h.	± pap.	± pap	−	−
		± "	± pap	− pap	−
		+++	+++	−	−
Y53, Y40, Y88	36h.	✓	✓	−	−

do. → isolate for B-M-$V_1^R Ia^R$ Y90 (acid and gas on glucose!) test on 100γ/ml

isolate for B-M-$V_1^R Ca^R$ Y91 (acid, gas ± on glucose.)

1 small colony on Y40 streak. (Too conc. NaN_3).

Lack of Ia^R from Y88 + Y53 may be due to the more recent origin of these isolates, ∴ a smaller chance of accumulating mutants.

Y53, Y40, Y88 60h. — pap. — 1 small, 1 large colony } some residual growth in very tiny colonies.

Y92 A_2^R. note: zone of adjacent streak was v. strongly stimulated. pH effect likely.

Y93 = Y53 Ia^R

No Ia^R from Cla^R?? Are very nearly Y24 — confluent growth. see Y97.

Segregation of cla^R.

~~Apl~~ APR 4/1947

V40 x V89.

Held on ice box

Comp. 0; B, on Lac U segr.

T(0).

	Lac− V^R	Lac− V^S	Lac+ U^R	Lac+ V^S
	24	9	(24)	2
cla^R cla^S				
Gas+	14	6	3	2
Gas−	5	2	7	0

scoring is highly uncertain as tests were done on complete medium allowing the contaminants to go ahead. No. is from sample tested.

T(B$_1$).

	20	7	9	0
	10	6	4	0
	30	13	13	0
cla^R cla^S				
Gas+	4			
Gas−	1			

Total 4/78 Surv. 2 − R
1 − S
1 + R.

Segregation of Cla^R

Y40 x Y88.

T(10) Pick tan Lac-v testr to water. Streak on Cla (1–2 mg/ml)

		+R	Cla
1 –	1	Lac-v	R
	2	+R	R
	3	−S	R
	4	−S	(S)
	5	+R	R
	6	−S	R
	7	−R	R
	8	−R	R
	9	−S	R
	10	−S	R
	11	−R	R
	12	+S	R
	13	−R	R
	14	+R	R
	15	+R	R
	16	+R	R
	17	+R	R
	18	−R	R
	19	+R.	R

		Lac-v	
2 –	1	+R	R
	2	−R	
	3	−R	
	4	+R	
	5	−R	
	6	−S	
	7	+R	
	8	−S	
	9	+R	
	10	+R	
	11	+R	
	12	−R	
	13	−S	
	14	+R	
	15	+R	
	16	−R	
	17	−R	
	18	+R	
	19	+R	
	20	+R	
	21	−R	
	22	−R (S?)	
	23	−R	

Total: 56/58 = S
+ 18/20
74/78 = S.
R = 5%

T(B_1)	1	−S	R
4 –	2	−R	(S)
	3	−R	R
	4	+R	R
	5	−S	R
	6	−R	R
	7	+R	R
	8	−S	R
	9	−R	R
	10	+R	R
	11	−R	R
	12	+R	R
	13	−R	R
	14	−R	R
	15	−R	R
	16	+R	R

5	1	−R	(S)
	2	−R	R
	3	+R	
	4	−R	
	5	−R	
	6	−S (R)	
	7	+R	
	8	+R	
	9	−R	
	10	−S	
	11	−R	
	12	−R	
	13	−S	
	14	−S	
	15	−R	
	16	+R	(S)
	17	−R	
	18	−S	
	19	−R	
	20	±R	

test this group
in B_1 – T(0).

+		−	−	S	+	R	+	+	[redacted] S
B.		B	M	Ca	Lac	V	T	L	... Ca
−		+	+	R	−	S	−	−	[redacted] R

mostly R. ∴ R near [redacted] BM

~~R's are S; R~~

S's are − S; − R.

R between B, M?

Homogeneity of B_1^- : B_1^+

Y40 x Y53; Y6Y x 58-161.

483

April 14, 1947

22 APR 1947

Use light mixtures Y40 x Y53. Add B_1.

Held in cold room after 2da.

ca 1 – 5 colonies / plate.

4/19 Streak out on EMB Lact

1. all lac –
2. " 6/6
3. "
4. "
5. "
6. "

6/6 const Lac –

Should use B_1^+ on B_1^- plates.

Repeat with Y40 x 488 (Y53-Cla^R)

Reverse Crosses —

28 [illegible]

A. Y40 × Y53.

		−R	−S	+R	+S.	
T(0).	1.	7	7	6	0	
	2.	3	1	4	0	
	Σ	10	8	10	0	28
T(B,)	1.	2	3	4	0	
	2.	4	3	4	0	
	3.	11	2	6	0	
	4.	9	4	7	0	
	5.	10	5	2	1	
	7.	15	6	9	1	
	8	7	5	7	1	
	9.	5	7	7	0	
	10.	2	4	1	1	
	11.	8	7	3	0	
	Σ	73	46	50	4.	173.

Expressed as percentages.

	−R	−S	+R	+S.	
A (0).	35	30	35	0	28
A(B,)	42.3	26.6	28.8	2.3	173
B(0)	31.9	47.4	4.3	16.4	116
B(B,)	34.9	39.0	2.6	22.4	312

See summaries of data

B. Y64 × 58-161.

T(0).	1.	4	4	0	0	
	2.	2	3	1	1	
	3.	2	5	0	2	
	4.	1	4	1	0	
	5.	6	9	2	2	
	6.	5	9	0	4	
	7.	3	5	0	2	
	8(2)	2	4	0	2	
	9.	5	8	1	3	
	10.	7	4	0	3	
	Σ	37	55	5	19	116.
T(B,) —						
	Σ	109	125	8	70	312.

see next page.

Y.4x58-161 B

Y40 x Y53 A

April 20, 1947

A :

−R	−S	+R	+S.	
7	9	3	0	
4	8	3	1	
7	5	4	2	
20	22	10	3	55

In previous expts, +R >> −S occasionally.

A(B₁)

9	4	4	1	18
8	4	4	0	16
8	8	4	0	20
11	4	3	1	19
7	4	8	1	20
16	10	8	0	34
59	34	31	3	127

B :

8	9	1	2	20

B(B₁)

6	7	0	6	
4	9	1	2	
10	16	1	8	
10	5	0	1	
20	21	1	9	1
2		2	11	51 B
				A
8	1	1	2	A(B(0)

1492
22
1214

484 ctd. Y64 × 58-161. T(B1)

	−R	−S	+R	+S.	
1.	7	4	1	7	
2a	5	7	1	7	
b.	8	10	1	4	
c.	9	5	0	4	
3a	4	14	1	1	
b.	6	7	1	5	
4a.	5	12	0	3	
b.	6	8	1	4	
5a	8	10	0	2	} Retest - phage n.g.?? 13-R: 6+R.
b.					
6.	11	6	6	1?	} appearance very poor!
7b	10	6	0	0	
7a	5	8	0	6	
8a	9	5	1	5	
8b.	2	10	0	7	
9a	7	10	0	2	
b.	10	3	0	5	
10a	~~10~~ 9	12	0	3	
b.	7	4	1	7	
Σ	109	125	8	70.	312

numerals designate separate recombination plates. letters are testing plates.

N. tetrasperma

A
Pw 16 / Sub. + B. Sub

Spread spores (on petri dish) on agar surface.

2/26/47. Irradiate spores ~ ca 20,000 r (courtesy of Pollard)
Isolated 2/27 Transfer spores to small corn meal agar slants + heat-activate.
Do. ~ unirradiated controls.

Fruit scoring —

Sub.								
Control	100	80	31	+ 1/2	53	25	80%	
X-ray (J.)	100	69	9	7	16	51	= 76%	
25000 r								
Sub. 16	J.	47	34	69	+ 23	10	3 6	
	E.	21	—	25	4	0	1	
		= 70	45	=	27	5	7	
X-ray	J	50	14					
	E	77	35		13		6	
			42 / 41		19			

X: 41 / 124 ; 2/41 ⊕
C: 40 / 10 ; 22/40 ⊕

Sub. / Dummy

10	4	+ Sporulo

Sub. 16 : [illegible] stock to lab — 2 MA

C → 28
X → 51

C → 18
X → 13 >

April 15, 1947.

FH(2% glucose+). Incr 5 PM 5

	HC ½%	NZ case ½%
	++	+
	+++	+++
	growth 12h.	
1 Glutamic ac.	++	++
2 Glycine	++	++
3 Serine	++	+
4 Aspartic ac.	+	+
5 Asparagine	+	++
6 Glutamine	+±	++
7 Proline	+	+
8 Hydroxyproline	++	++
9 Cysteine	++ H_2S	++
10 Alanine	±	
11. Tyrosine	++	++
12. O.	+	++

1 mg/10 cc.

The production of gas in the minimal medium is at odds with previous results, and perhaps speaks for some error.

Repeat: OK. — gas produced on FH (0) by K-12 in 36-48 h.

abandon momentarily. One could seek an ~~at~~ antagonist of formate, however, which seems to be present in NZ Case.

Streptothrix?

Phytomonas - Recombinations.

4/85

April 1947

Strains: **B**. A6/Pro/MG. **A**. A6/Sth/Sm. Inoc separately and together after growth on ∞ slants, into canot medium. — 4 wks. Sediment + wash pellicles.
1: canot 2: slant. Inoc from canot into YB liq: 3.

A = Proflavine 100γ/ml.
B = Malachite Green 100γ/ml.
C = Streptomycin 50u/ml.
D = Streptothricin 100u/ml.

On 1AB/Nutr. Agar plate, 1 "rough" colony noted. Picked to recover

		1A ①	1B ①	1AB ②	2A ④	2B ⑤	2AB ⑥	3A	3B	3AB. Read at 48hrs
11	A	− −R ✓	+ ++ ✓	+ ++ ✓	− ✓	− ++ ✓	− ++ ✓			+
12	B	− − ±R	± ++ ✓	− ++ ✓	− −	− − 2R	− − 2R			++
13	C	+ ++ ✓	− −R ✓	± ++ ✓	± ++ ✓	− − ✓	± ++ ✓			++
14	D	+ ++ −	− −1R ✓	± ++ −	± ++ −	− − ✓	± ++ −			++
5	AB	− − ✓	− + but pof ++	− ± ++	− −	− −	− −			++
7	AC	− −R ✓	− −R ✓	− −R ✓	− −	− −	− −R ✓	✓10		many R
8	AD	− −R −	− ±R ✓	− ±R ✓	− − ✓	− − ✓	− − ✓			±R of 1B
(9)	BC	− − −1R	− − −	− − −	− −	− −	− −			−
(10)	BD	− − −	− − −	− − −	− −	− −	− −			−
6	CD	+ ++ Pof	− −R ✓	± ++ ✓	− ++ ✓	− − ✓	− ++ ✓			+
1	ABC	− − ✓	− − ✓	− − ✓	− − ✓	− − ✓	− − ✓			−
2	ABD	− −1R ✓	− −	− −	− −	− −	− −			−
(3)	ACD	− −R± ✓	− −	− −1R ✓	− −	− −	− −			−1R
4	BCD	− 1 ✓	− − ✓	− −R± ✓	− − ✓	− − ✓	− − ✓			−
15	ABCD.	− − ✓	− − ✓	− − ✓	− − ✓	− − ✓	− − ✓			−
	NA.	+	+	+						

Inoc series ~~A+B~~ 1+2 P 21; also inoc on NA plates for series 3.

Streak on plates in order:

Read at 1) 12h.
2) 48h No evidence of recombinations. Pofla + H.G. apparently intract. (see 5).
3) 60h.
5 and 6 OK. (see CD). But 3 and 4 do not suggest recombinations

R = individual resistant colonies (1–10)

Inoc 3AB N24.

Use ACD & B in combinations.

Segregation of Cla^R.

48h.

Y40 x Y88.

Compare c 1R1A.

A. T(O) B. T(B).

Streak out on CLA - minimal or CLA - biotin agar.

A. 1R/6S. = 1/7 S for R!

B. 1R/6S = 1/7.

2/16 = R. conclusion: mutated in segregation c B$^-$
Use selection of Cla^R on B plates, c reverse cross to establish
conclusion.

#14/16 Res.

23 Oct. 1947

(A) Plate Y40 × Y88 on B_1^- Acetate medium. This should select for B_1^- la^S [redacted] segregants. By plating these colonies into SMG-la medium it should be possible to eliminate the parental types (B^-M^- req. la sensitivity; $T^-L^-B_1^-$ Req B_1 requirement) and find any complementary segregants.

Growth is meth-eaten, suggesting phage! very strikingly.] probably not. See 489. 4-9.

(B.) Plate Y40 × Y88 on B_1 medium, spreading very lightly (to avoid contamination) Pick 100 colonies carefully to minimal (ca 20 colonies/plate). and test: Streak out original isolates of those only a B_1^+ component to find any possible B_1^- types

Scoring as B_1^- or B_1^+ not very clear cut. Group A. more definitely B_1^+ Group B doubtful.

A. – 15. B. – 17. Streaks out on Lac plates.

1	all –	
2	all +	+ / –
3	+	10/4
4	–	
5	–	
6	+ ;(–)	
7	–	
8	–	
9	+ ((–))	
10	–	
11	–	
12	–	
13	–	
14	–	
15	no cols.	

all phage homogeneous
numbers var reaction
different. Test
these mutationally

1	100+:1 –	11 – / 5 +
2	100+:1 –	12 –
3	all –	
4	"	
5	"	
6	"	
7	all +	
8	[redacted]	
9	all –	
10	all –	
11	all +	
12	all –	
13	"	
14	"	
15	no colonies	
16	all –	
17	all –.	

T la.

Yellow.

Streak various plates ē 4 cols. each plate.

A) mostly +R Cla^R

1 - S. Cla^R TLB$_1^-$

parental.

B) mostly −R Cla^R (B$_1^-$)

1 + R Cla^S (B$_4$)

no new combinations aside from main component in these columns!

A. Y40 x Y86. (to compare V^R loci)

B_1. (to reduce inoculum required). B-M- Lac+ V^R_{1A} Muc$^-$ x T-L-B_1- Lac- V^R_{1B} Muc$^+$

(-S Sm.)	$V^R M^+$ Lac-	16	$\therefore V^R_1 \neq V^R_{1B}$
	$V^R M^+$ Lac+	3	
	$V^R M^-$ Lac-	1	
488-1	$V^S M^-$ Lac-	1	This Recombination suggests that
488-2	$V^R M^-$ Lac+	1	$V^R_{1A} \neq V^R_{1B}$
		2R.	$V^R_{1B} = T5$ S.

B. ~~58~~ 58-161 x Y86. (to test complexity of Muc$^+$-V^R). Lac+ V^R_{1A} M$^-$ x Lac- V^R_{1B} M$^+$

$_1$ (to reduce inoculum required.)

-R Sm.	$V^R M^+$ Lac-	13	$\therefore$ Muc$^+ \neq V^R_{1B}$
	$V^R M^+$ Lac+	3	
	$V^S M^-$ Lac-	1	
488-3	$V^R M^-$ Lac-	1.	This recombination suggests that Muc$^+ \neq V_{1B}$.

[resistant to all phage. Contam? I "diplococci"]

Compare the resistance patterns of Y88-1; 2; 3; Y40; Y86

Compare recombination values of Mucoid / Lac c Standard V_1 / Lac.

From accumulated data: 45.29

	V^R Lac-	V^R Lac+	
Y40 x Y53.	846	546.	/ 1392 = 60.6% · 39%.
Y40 x Y88	56	15	/ 71 = 79% · 21%

$\therefore V^R_{Muc}$ is [redacted]

Remarks of Dr. R.

488

24 [illegible] 1937.

.1%
Plate 487 into Acetate - negative.
Indefinite background growth. no definite colonies. probably Ac too low.

~~490.~~

Allelism of V_1^R

April 23, 1947

allelism of V^R. Y40 x Y64. all resistant.

	Lac−	Lac+	
T(0).	4	1	(Mucoid)

159/159

T(B_1)	11	4	
	15	4	
	11	7	
	13	4	
	13	5	
	18	0	(!)
	15	3	
	9	0	
	13	5	
	122	32	/ 154. = 79% Lac−

This distribution fits Y64 x 58-161 better than Y53 x Y~~54~~0

Compare fit ± accumulated data.

a).

	106	48 ✓	
	122	32	154
		562	
122²	1206	578	1784
	1328	610	1938

$\chi^2 = 8.44$

$\chi^2 = 16^2$ χ 9/

= .25

2.42

5.32

.45

8.44

for Y40 x Y53

$\chi^2_1 = < 1.$

119	122	55 32	154
540	537	136 159	696
	659	191	850

Linkage Relations of Cla.

24 APR 1944

Plate 440 x 488 into Biotin$_{B_1}^{-}$ - Acetate - agar [select for $Ac^+ = Cla^s$ and compare segregation of $\underline{B}^-$ and $\underline{B_1}^-$ in the Cla^s class I.

h.g. see 489

Mitchell-Westergaard medium. 493

24 APR 57

1. Cf. 490. 1 drop of Y40 x Y64 mixture in B, suppl medium, to compare c Y90 for rate: (grown in YB; plate in MW) 0.

2. Y40 x Y53. grow in M-W: ∞ Plate do:

3. Y40 x Y53 grow in M-W Plate in T(0) } adjusted to ca. same inoculum. > 200.

~~4. Y40 x Y53 grow in YB~~

4. Y40 x Y53 grow in YB Plate T(0). } 39; 35. ca 40

5. do. do. Plate M-W 0.

The medium is n.g. for plating, but may be OK, or better than YB for growing cells peroxidy. Try $\frac{1}{5}$ buffer., $\frac{1}{5}$ KNO_3. Difference is within range of normal variation 1 expt. to another.

Medium: per liter

KNO_3	1
glucose	10
NaCl	5
K_2HPO_4	3
$NH_4H_2PO_4$	1
$MgSO_4$	.1
trace + $CaCl_2$	

I (N2Case 5, Yeast Extr 2.5) for 10.

April 26, 1947.

A. Y87 (B-M-V_1^R·Lac−) × Y10 (T-L-B_1-V_1^S·Lac+). for segregation of Lac−. (prediction: +R > +S = −R >>> −S.).

B. Y87 × ~~Y64~~ Y64 (B-M-V_1^RLac− × T-L-B_1-V_1^RLac−). for test of alleleism.

B). 134 tests of prototrophs all Lac− ∴ loci are allelic.

A) Segregation:

	−R	−S	+R	+S.	
Plated on T(0)	7	1	7	6	
	5	3	7	3	
	3	0	9	9	
	7	0	6	6	
	2	1	11	7	
	4	1	6	6	
Σ	28	6	46	37	117

on T(B_1)	9	1	16	9	
	5	0	16	10	
	7	0	8	4	
	5	1	13	3	
	4	0	10	3	
	4	0	10	4	
	4	0	9	4	
	5	0	12	1	
	8	1	10	5	
	4	0	6	6	
	4	0	8	4	
	2	1	14	6	
	6	0	12	3	
	6	1	6	5	
	4	1	7	4	
	4	0	13	4	
	9	1	5	3	
	6	0	7	6	
	6	0	9	4	
	1	0	10	3	
	102	7	201	91	401.

Y91 x Y53.

Y91 x Y53 ($B\text{-}M\text{-}Cla^R V_1^R$ x $B_1\text{-}T\text{-}L\text{-}Lac\text{-}V_1^S Cla^S$)

Minimal plates too crowded.

B_1 - Streak on B_1 · Cla or Cla NA. to classify mutants/susc.

4/56 ~~Resistant~~ Persistent

Use plating on B. Cl agar for transductions.

April 26, 1947

Streak on indicated plates.

		Backgr	Papillae
Y64	Cla 1	+	+++
58-161	Cla 1	+++	—
Y90		+	+++

4/27.

Y64	Cla 2	±	±	+	++ Y94
58-161	Cla 2	±	±	+	++ Y96
Y90	Cla 2	±	±	+	++ Y95
Y92	Ac 50	+++		—	
Y92	Ac 100.	++		—	
Y53	Ac 50	++		—	
Y53	Ac 100	-	±	—	
Y40	Ia 25	++		—	
Y93	Ia 25	+++		—	
Y40	Ia 50	—		++	Y97
Y93	Ia 50	+++		—	

need a buffered test medium probably.

	Bgr	Pap.
Salm. 20	±	++
Salm. 21	±	++
Serratia	+++	—
Phytomonas	++	—
Staph	+++	—

	Cla	Ia	Cla+Ia.
Y95.	++	++	(-)

Interaction??

Streak out all mutants on N.A. Test on inhibitor and transfer to plants.

Note - terminology: unless otherwise stated, figures are r/ml. Underlined figures are mg/ml.

Virus Resistance Pattern.

498

		T1	T3	T4	T5	T6	T7.	
	Y40	"R"	R	S	S	S	S	note!
	K-12	"R"	R	S	S	S	S	
"V_1^S 4 -lac-"	488-1	"R"	R	S	S	S	S	
"V_1^R 4-Lac+	488-2	"R"	R	S	S	S	S	
	488-3	"R"	R	R	R	R	R	contam!?
2	Y86	"R"	R	R.	R	R	S	
	Y65	"R"	R	—?	—	[redacted]	R	too light inoc.?
	Y68.	"R"	R	S	S	S	S	

This plaque ors on other plate. Repeat! plaque?

Y40 V_1^R V_3^S V_5^S. compare original description

K12 = S...

488-1 = S...

488-2 = V_1R, S..

Y86 = V_1^S! (unstable: reverted??)

Y65 = R....

Y68 = S....

Repeat T3, T1.

	T1	T3	
Y40	R	S	
K12	S	S	
488-1	S	S	
488-2	R	S	
! Y86	S	S	Unstable resistant?? Less mucoid on this plate
Y65	R	R	
Y68.	S.	S	

! streak out

[redacted] stock Y86 is predominantly mucoid; a few smooth colonies

this w/streak is predominantly smooth; a few mucoid colonies.

April 25, 1947.

Add varying amounts of 30% camphor in 95% Alcohol to plates, to give following "concentrations" of camphor. Incubate 3 days. Strain 53:

A. 0
2. 100
3 1
4 2
5 5
6. 10

very little growth inhibition was noted except in # 6 (10% camphor!) where there was considerable retardation. Comparison of cells from 6 and 1 reveals the presence of many beaded, ~~elongate~~ slightly elongate forms.

Streak out 6 as EMB to isolate clones and test for diploidization by the suppression of recessive mutations (e.g. Cla^n). Many smooth-mucoid colonies noted.

	Papillae on CA 2		
1	±	16	+
2	±	17	++
3	+++	18	+++
4	—	19	++
5	—	20	+
6	++	21	+
7	+	22	+
8	—	23	++.
9	—		
10	++		
11	+++		
12	±		
13	+++		
14	±		
15	±		

Recover 4, 5, 8, 9 to test for polyploidy.

Utilization of Acetyl-Glycine

~~447~~ 500

April 27, 1947

Sol 480. Glucose Glycine
Acetate ~~Glycine~~ Glucose

48 h. - 62 h.

					~~Y~~ Y89	K-12	Y89.	K-12
A. .1%	1	✓			−	− −	± ±	± ++ ✓
	2			✓	−	− −	−	−
	3	✓	✓	✓	+	− −	+++	+++
	4				−	− −		
	5	✓	✓	✓	++	− −		
B ~~B~~	1	✓			± ~~±~~	− ±	± ±	+ ++
	2			✓	~~±~~ −	± ✓	− −	− −
	3		✓	✓	++ ++	± ++	+++ −	+++ −
	4	✓			− ✓	± ✓	± ±	+ ++
	5	✓	✓	✓	++ ++	− ✓	+++	+++ −
~~B~~ .2%								
C ~~C~~	1	✓	ACETATE		−	−	±	±
	2		GLYCINE	✓	±	−	−	− ✓
	3		✓	✓	++	−	++	++ ✓
	4	✓			±	−	±	±
	5	✓	✓	✓	−	−	−	−
~~C~~ .5%								
D	1	✓			−	−	− ~~±~~	± ++
	2			✓	−	−	−	− ✓
	3		✓	✓	++	+	++	++
	4	✓			−	−	± ~~++~~	± ++
	5	✓	✓	✓	−	−	− ±	− −
~~D~~ 1%								
E	1.		✓	~~+++~~	+ ++	+ +++		

F. 1 Acetyl-Glycine .5% + +

2. " + glucose. ++ +++
both + in 11 hours
all others −.

Glucose 1% fluorescent Readings at 24 h;

Cont. Glycine is not utilized; not inhibitory
Acetylglycine is utilized by both
Acetate is not utilized by unbank comp/mild

Staining in presence of lysis. 501

April 27, 1947.

Compare Y53 lysed by T'on:

EMB - lactose :

- sucrose :

- blank. :

all show coloration in margins. lytic zone, suggesting that it is mostly staining of debris.

Segregation of A2 and Ia

April 28, 1947

A. Y90 x Y53 (Y40/Ia) B. Y92 x Y53 (Y40/A2)

A.

T(0).

readings on complete are available. Technique for tests on synthetics should be developed.

>> T(B).

B: Lac V1

		R	S
T(B1).	−S.	29	2
	−R	8	1
	+R	12	1
		49	4

Use 100 r/ml NaN_3 in T(0)+B1. Screen at two concentrations:

B.	BM	Lac	V	TL	A2
−	++	−	S	− −	S
+	− −	+	R	++	R

Mostly R. ∴ A2 is near TL.

ca 8% recombination.

either beyond or between T−L Use selection to locate

April 28, 1947.

A.) Y86 x 58-161 ~~B) Y86 x Y40.~~

not useful. Interesting types could be merely mutants. [Accelerate mutation?]

T(o) - no colonies (contaminated??)

T(B,) -

Mucoid character too poorly expressed, although many of the colonies picked looked as if they should be Muc Is there progressive "attenuation" of this character??

A 1. 1. Streak out Y86 stock on EMB-lactose; ~~[illegible]~~.
34 Muc : 31 Smooth.

P2 2: Streak out: A. Muc from 1. B. Mix pop. from 1.
A: "all" mucoid ~~[illegible]~~.
B: 19 Muc : 70 Smooth.

P4 3 A - mucoid from 2A. B. Mix pop. from 2B.
all mucoid. ca 100:1 smooth : mucoid

A6 4. A - mucoid from 3A. B Mix from 3B.
all mucoid. > 200:1 smooth : mucoid

P7 5. B. (mix) from streaks of 4. all mucoid

P10 6 streak from mass streak of 5: ca 10 Mucoid : 1 smooth.

May 15, 1947.

A15. 7. Steak from mass-steak of 6.
ca 45 : 20 M: 5m.

A17 8. Steak from mass-steak of 7.
ca 23 : 43 M: 5m.

P18 9. do. 9 : 21 M: 5m.

P20. 10. do. 19 : 48 M: 5m.

P22 11. do.

Acetyl utilization

April 29, 1947

	Ac.	Glucose	Glycine.	12-24h. K-12	12-24h. Y89.	36-48h. K-12	36-48h. Y89	60h.	
1.	1/2%			−	−	−	−	+	±
2.	1/4%	1/4%		−	+	±	+	++	++±
3.	1/4%		1/4%	−	−	−	−	±	++ ±
4.		1/2%		±	++	±	++	++	+++ +++
5.		1/2%	1/2%	±	+++	+	++	++	+++ +++
6.			1/2%	−	−	−	−	−	−
7.			1/4%	−	−	−	−	−	−
8.	Acetyl-Glycine 1/2%.			±	±	−	±	+	+ +
9.	o	o	o	−	−	−	−	−	−

Autoclave separately from medium. Adjust acetate to pH 6.8 ± AcOH before using.

The differential between K-12 and Y89 on acetate is not complete; there is a definite residual growth. Stimulation by glycine (not used by itself) accentuates the difference.

(Use static aerobic + anaerobic conditions?)

8 h: K-12 Y89.

1. Ac. ++ + !
3. ++ ±
8 + +.

} eventually the bug does better on acetate than on Ac Gly!

diacetyl-diketopiperazine 1/2%
neither K-12 nor Y89 showed any response!

Test of Camphor Treatments

May 1, 1947.

See 499.

Recover precumptive strains.

a) Streak again on CA agar. 4, 5, 8 finally three off from mixtures 9 only ≠ 2.

b) Cross × Y40.

c) streak out on EMB lactose all isolates are lac - V_1^S both semi-mucoid character intifume with determination of resistance.

b. P2. ~~Y cross~~ .1 ml mixtures into B_1, plain agar respectively.

1. 499-4 2. 499-5 3. 499-8 4. 499-9. × Y40.

Rib capsules between O and B_1, plates are only ca. 3-fold rather than 10-fold.

		Smooth.				Mucoid			
1-CO).		+R	-R	+S	-S	+R	-R	+S	-S.
(1)	T(O)	1				2	9		1
	B_1.	11	5			9	22		3
(2)	T(O) —								
	$T(B_1)$	14	32	1	11	typical segregation.			
(3)	T(O)	1					2	1	
	$T(B_1)$	13	1			1	21		
(4)	T(O)	2	8			2	10		
	$T(B_1)$	16	21			2	16		2

The Y53 2x doubled is not a good test; better would be Y40 which carries more dominant alleles.

Resistance Patterns.

	① T-1	② T_3A	③ T_3B	④ T_3 Batch 2	⑤ T_5	⑥ T_5 Batch 2
K-12	S	R	S	S	S (large + small plaques)	S
Y40 (from 140 phil)	R	R	S	S	S	R
Y40 pure.	R	R	S	S	S (large + small)	R
Y53	S	S	S	S	S	S
Y64	R	S	S	S	S	R
~~Y87~~	R	R	S	S	S	R

probably contam. with T_1. Start new stock. —

OK. OK.

N.G.

We phage stocks which have varied, not original cultures since Y40 = Y40 now in all respects. T_3A must be fallacious. T_5 Batch 2 behaves like T_1 and is similar to previous responses. Could it be contaminated??

Phages need purification! Rebuild T_3A. Present indications favor the interpretation that the results of last fall were due to gross contamination of T_3 and T_5 & T_1.

Program: Purify T_5 - ~~the~~ and isolate components.

⑤ was streaked out and exhibited both large and small plaques. Pick from a large and a small plaque and streak each with K-12 and Y40.

P 5 T-5 from original culture (Demerec) was plated with K-12 but gave confusing lysis. several resistants appeared; test these with T_1, etc. Use this to reisolate T5 stock.

Phage stocks

1. Start new T5 stock from 1) lysate using original T5 on K-12
   2) small colony picked from existing T5.

2. Other stocks OK. Renew T1 on K-12.

3. Test a large-plaque component of old T5 on K-12, Y40, K/5.

4. Test T1 on K-12, Y40, K/5. (from 506 #5).

	T1	"T5 large"	"T5 small"	"T5"
K-12	S	S	S	S
Y40	R	R	R	R
K/5.	R	R	R	R
"	R	R	R	R
"	R	R	R	R

T5 from original bottle!

three are isolates from 506 - (5) which, previously, lysed Y40.

Inversions. 888.

May 2, 1947.

~~Plate~~ Pour ca 10^9/.1ml on middle of NA plates + irradiate at ca 2500 r/min 20 min ≅ 50,000 r. Wtaget 60kv 25ma.

1. After shaking agar strip in H_2O ca 3h., streak out on EMB.

~~2. Streak out original sample on EMB.~~ (Y40 only.

3. Killing very great. Probably only ca 10^2-10^3 survivors.

Streak out proliferated cultures on EMB.

Isolate 14 colonies each from Y40X and Y53X and streak across each other. Plate mixed growth on T(0) agar. (28 tests).

	# of prototr.
1	20
2	30
3	20
4	10
5	30
6	20
7	20
8	20
9	200
10	10
11	20
12	20
13	100
14	10

No crossover suppression here!

Selective Segregation: Cla^R.

A. Y88 x Y40. B. Y53 x Y91 C. Y40 x Y53.

Plate into T(0) +:

	0	Cla 500	Biotin	Biotin + Cla.
A.	ca 80	26 30.	see 0.	> Cla.
	do.	smallish		ca 70-80

were too turbid. Rb may provide a source of Cla^R cells.

In A, the diffuse between 0 and Cla is not clear cut. In B, it is. There are relatively few resistants in x B as compared to A. Clear plates will be needed: use B_1 supp.

This experiment is not entirely valid because the plates less turbid.

ca 40

B	ca 40 - turbid.	3	see 0.	all Cla 500
	ca 50 - turbid	5		
		12		
		19		40
				12
C	P3: 12	0		0
	25	17		24

A + B B, have large numbers of isolable c

Test A to determine % sensitive col

clonies.

clonies.

demonstration of
use B, plates.

ps.

later, more colonies
appear in B
probably m.
mid direct
retic

Cla segregation.

509a

See 509

See 496.

A. Y88 x Y40.

	B₁	Bd-Cla	Lac	V	TL
	−	+ R	−	S	−
T(o) 17/17 Res.	+	− S	+	R	+.
B₁ 18/18 Res.					

35/35 Res.

Y53 x Y91.

T(o)

T(B₁) 74/76 ~~Res~~ Sens.

2(?) ~~Sens~~ Res.

∴ Reverse cross is supported.

Summary.

A. Y91 x Y53

496. 4/56 T(B₁)

509 ~~74/76~~.

2/76 T(B₁)

6/132

4.5%

Y40 x Y88

486 14/16 < 1/7 T(o) / 1/7 T(B₁)

35/35 T(o); T(B₁).

~~49~~/51

4%.

Camphor

May 3, 1947.

Streak Y40, Y87, Y64, Y10 on 10 Camphor./NA.

May 5 - Streak out as EMB

May 7 - Isolated colonies. Test by streaking heavily on Cla 2 agar. Following streaks no papillae + were recovered:

Recovered streak out on EMB.

Y40	2 / 15
Y87	1/18
Y10	+0 9/9
Y64	1/5
Y53	0/8

Phage resistance patterns.

5 11

May 5, 1947.

Spread on EMB lactose plates, Y53 + phages:

T1 many resistants; some smooth; a few mucoid.

T3 do. mostly mucoid.

T4 scattered small plaques. and bitter stocks.

T5 many smooth resistants. 30 tested on T1 all resistant.
present stock from Demerec's bottle

T7 all mucoid?

T1+T5 as T1; no mucoid.

T3+T4 scattered small plaques! (protection by T4???) Repeat!!!

T3+T7. as T3.

T6 confluent plaques; not continuous lysis except (?) in center. Streak out to obtain Y53-V_6^R

T1/Y40. no plaques (mucoid mutants?)

Y53-mutants.

1. Pick colonies from /T3 and /T7 and test reciprocally.

2. Test and compare /T1S and /T1M on T1 + "T5"

6. Purify + cross-test /T6. < of 20 tested ca 3 were sensitive!

7. Repeat: /T1, T3 /T4 /T3, T4.

/3 on T7: Some sensitive. Streak out and compare with Y53/3/7 No.: turned out Y53/3, 7

May 4, 1947.

streak out on NAagar +

	B.G. 50	M.G. 100.
Y77	A few isolated colonies	only a small part of the inoculum. Background shows ± growth.
Y79.	A few isolated colonies.	

streak out Y77/Hg on Hg + BG same as above!

Y79/Bg on BG.

Identification of "T5".

Enumerate stocks.

α: 506 - ⑤ K-12; Y40 S.,

β "T5 - Batch 2" 506 - ⑥ Y40 R.,

γ. "small plaques" from 506 ⑤

δ "large plaques" from 506 - ⑤

[wait for indicator stocks from Demerec.]

1. Streak out stocks with K-12; Y40 for plaque size determination.

2.

	T1	T5	T1+T5	α	γ	δ	γ+δ
Y40	R	R	R	S	R	R	R.

∴ α has another component not yet isolated. Isolate on Y40.

Location of *cla*^{R}.

Plate Y53 × Y91 on B$_1$ Cla agar. Pick ons second day +
TLB$_1$- BM Cla^R

test the resistant recombinants.

With considerable lag, varying from colony to colony, an equal yield ($ca > 10^{-6}$) was obtained ~~for~~ on Cla-B$_1$ and B$_1$ plates. The "~~resistants~~ susceptibles" formed tiny colonies early, & evident frequent ~~adapted~~ mutations! Need higher conc. of Cla??

May 15, 1947.

1). Streak 58-161 ⊂ T1.

2) Streak 58-161 alone.

no mucoids developed!!

~~Hot plate~~.

Mucoids sometimes develop on old plates on lactose agar. both into this!

May 16, 1947.

Test isolates from 510 by following crosses. on T(0).

A: Y10/Cam$_m$ x Y87/Cam. T-L-B,-B+M+Lac+V^S x ...

m T(0) 1
2
3
4

B. Y40$_m$/Cam x Y88 B+M+Lac+Cla^{S}V^R

1
2

401). -R 5 -> 2 +R 2 +S. 0 / 9. Cla all R. normal segr.

402)

*	-R	-S	+R	+S.		Cla*
8		2	7	0	17	all R.
	7	5	5	0	17	1) -R 2) -R are Cla^S
	4	4	5	1		
	19.	11	17	1		normal segregation

2/43 Cla^S.

Phage - Resistance Patterns.

May 17, 1947.

	T1	T3	T4	T5	T6	T7	"T5"
K-12	"R"	"R"	S	"R"	S ?	"R"	"R"
Y40	"	"	S	"	S	"	"
Y94 (Y53/6)	"	"	S	"	R ?	"	"
Y95 (Y53/6)	"	"	S	"	R ?	"	"
Y53/3	"	"	R	"	?	"	"
Y53/3,7.	"	"	R	"	?	"	"

These tests are obviously fallacious. Probably phage strains were allowed to "set" too long before adding bacteria

Repeat. P18.

		T1	T3	T4	T5	T6	T7	"T5"
K-12		S	S	S	S	S	S	S
Y40	/1	R	S	S	R	S	S	S ← small plaques only.
Y94	/6	S	S	S	S	R	S	S
Y95	/6	S	S	S	S	R	S	S
Y53/3		S	R	R	S	R	R	R
Y53/3,7	=	S	R.	R	S.	R	R	R.

Resistance to T6 seems to be included in the 3,4,7 pattern. probably T4.

$\therefore$ V^R_{5}; V^R_{6}; $V^R_{3,4,6,7}$ are available!

Y10/1 cam x Y87/cam.

+R	+S	-R	-S.
3	6	8	0
9		8.	

	Lac+	Lac-	
Y10/3.	15.	1.	
Y10/4.	22	6	
	17	1	
	39.	7	/46. ?
Y10/2.	22.	5	

Is $Y10_4$ aberrant?

Complex-Resistance Patterns

Add mixtures of phages + Y53 and spread on EMB agar.

T3 ca 200 R.

T4 lysis patchy at circumference. scattered resistants.

T5 complete lysis only in center; occasional resistants.

T6. " " ". occasional resistants.

T1+T3.	Complete lysis :	Ca 10-12 R	* lysis in confluent zone; nibbled colonies. Some do hole!
T1, T4.	"	1 surviving colony. _ ?	* Some nibbling! mostly OK.
T1 T6	"	1 surviving colony?	* small colonies.
T3, T4	"	Many R.	
T3, T5	"	O- — R; many tiny	* OR. plaques in regions of confluent growth
T3, T6.	"	Many R!	
T4, T5.	lysis patchy.	Occ. mucoid R.	* somewhat mucoid; no nibbling
T4, T6.	lysis patchy	No resistants.	
T5, T6	Complete lysis.	3 clones???	* v. small colonies. see (1, 6.)

* streaked.

Segregation of V_6^R

H B

Y94, Y95 × Y40. (T-L-B_1-Lac-$V_1^S V_6^R$ × B-M-Lac+$V_1^R V_6^S$)

O_1 B_1.

Y94-O. Lac	T_6	T_1
+	R	R ✓
+	S	R
+	R	R
−	R	S
+	R S	R ✓
−	R	R
−	R	R
−	R	S
−	R	S
−	R	R
−	R	R ✓
+	S	R
−	R	S ✓
−	S	R
−	R	R
−	R	R

Y94-B_1 Lac	T_6	T_1
−	R	S ✓
−	R	S ✓
−	R	R ✓
−	R	R ✓
−	R	S ✓
−	R	R ✓
−	R	R ✓
+	S	R ✓
+	S	R ✓
−	R	R ✓
−	R	R ✓
+	R	R ✓
−	R	R ✓
−	R	S ✓
−	R	R ✓
−	R	R ✓
−	R	R ✓
+	R	R ✓
−	S	S ✓
+	R	R ✓
−	R	S ✓
+	R	R ✓

Lac	V_1	V_6^R	V_6^S	
V_1^R 43 −	R	23	1	} 41 Lac−
	S	17	0	
V_1^S 18 +	R	5	14	} 20 Lac+
	S	1	0	
		46.	15	
		V_6^R	V_6^S.	

Cum:

Lac	6 R	6 S	T1
−	44	2	R
−	28	0	S
+	9	20	R
+	1	2	S

82 24

Jne 528

Y95 x Y40.

T(0)

Lac	T_6	T_1
−	R	S ✓
−	R	R ✓
−	R	R ✓
+	R	S ✓
+	S	R ✓
−	R	R ✓
−	R	R ✓
+	S	R ✓
−	R	S ✓
−	R	R
−	R	S ✓
−	R	S ✓
−	R	R ✓
−	R	R ✓
+	S	R ✓
+	S	R ✓
−	R	S ✓
−	R	S ✓
−	R	R ✓

Lac T_1	V_6^R	V_6^S
− R		
− S		
+ R		
+ S.		

T(·B1)

Lac	T_6	T_1
+	S	R ✓
+	S	R ✓
−	R	S ✓
+	S	R ✓
−	R	S ✓
−	R	S ✓
+	S	R ✓

Phage effect

Y40 x Y53

May 19, 1947.

(T1)

A. Add phage to Y53. Absorb 10 mins. Mix ± Y40 and wash
good yield of colonies!!

B. Mix Y53 + Y40. ~~let stand ... hours~~. Let stand in H₂O overnight.
Add phage before final wash + plate

C. Mix as above, no phage.

(Repeat)

V segregations.

May 21, 1947.

A Y94 x Y40 ($V_6^R \times V_1^R$).

T(a).

Lac	V_6	V_1
−	R	R
−	R	S
+	S	R
+	S	S
+	S	R
−	R	S
−	R	R
−	R	R
−	R	R

T(B_1)

Lac	V_1	V_6
−	S	R
−	R	R
−	R	R
−	R	R
−	R	R
−	R	R
−	R	R
−	S	R
+	R	R
−	S	R
−	R	R
−	R	S
−	R	S
+	S	R

T(B_1)

+	R	R
−	R	R
−	R	S
−	R	S
−	R	R
−	R	R
−	R	R
−	R	S
−	R	R
−	R	R
−	R	R

Lac	V_6	V_1
−	R	S
−	R	R
−	R	R
−	R	R
−	R	S
−	R	R
−	R	R
+	S	R
−	S	R
−	R	R
−	R	S
−	R	S
+	S	R
−	R	R
−	R	R
+	S	R
−	R	R

	V_6^R	V_6^S	
−	4+17	0+1	V_1^R
−	2+9	0+0	V_1^S
+	0+4	2+4	V_1^R
+	0+0.	1+1	V_1^S

Total:

	6 R	6 S	
−	21	1	1R
−	11	0	1S
+	4	6	1R
+	0	2	1S

Cum. total: See 518.

B.) Y96 x Y40. (Y53 - $V_4^R V_6^R$ x Y40 - V_1^R). Test on 1, 4, 6.

12 asexuales - all rattenschwanz · all resistant n-g.

mucoid.

Tests on complex mutants.

May 19, 1947.

Y53/	T1	T3	T4	T5	T6.	
1, 3	S	R	R	R?	R	
1, 4	S	R	R	R	R	
1, 6	?	?	?	?	?	Y99.
3, 5	R?	R	R	R	R	
4, 5 MRC.	R	S	R	R	R	
5, 6.	S	S	S	S	S.	!

Do not use these mutants further; their origin as independent mutations is not excluded.

Y53/3 on T6. 19 R. comparable to Y94.
pick at random. 1 good sensitive. Y98.
(3 nibbled throughout.).

check on 16 types.

~~Y53/~~		T1	T3	T4	T5	T6	T7	
Y94	Y53/6.	R	R	S	R	R	R	
Y98.	Y53/3, 6	R	S	S	S	S	R	
Y99	Y53/1, 6	S	R	S	S	R?	S	
Y8[illegible].	Y53/1 M.	R	S	S	R	R	R	
Y53/3		R	R	R	R	R	R	
Y53/3		R	R	R	R	R	R	probably contam.

Mix Y53 + phages in a tube + plate on EMB.

16,1 No numerous. See Y99.

16,5 2 v. mucoid colonies; a few tiny ones.

13,5 Numerous mostly mucoid.

13,6

11,4 Very numerous colonies (just) as 1,4. (V. considerable growth before lysis!) Probably invalid.

14,5 several mucoid colonies; occ. smooth.

(11,3) several "mucoid" colonies. Test as 11,3. Mostly very mucoid + nibbled. Isolate apparently $T_1^R T_3^R$. = 521-1

Y53/6. Mostly patchy lysis, but many well-defined resistant colonies. Test for T_3^R.

Y53/1 Test for T_5^R. large colonies: 65/67 = T_5^R. small col. pick Y100. = $T_1^R T_5^S$. 5/18 = T_5^R. Pick both var.

Y40/6 Test for T_3^R. smooth insusceptible; mucoids generally ~~s~~-resistant. isolate one as Y101

Y53/1,4. Mostly nibbled. Lysp are $T_1^R T_4^R$ as Y102. larger colonies are mucoid! V. watery or nibbled.

Y53/4,5. Test on 1,4. Mucoids are doubly resistant. Do not use.

Tests on Resistant mutants.

Y53/(1,4). on T1, T4.

Y40/6 on T3.

Y53/1 on T5.

		T1	T3	T4	T5	T6	T7	
~~Y53~~	Y40/6	R	R	S	R	R	R	T4S! T3R!
"Y53/(1,6)	Y99	R	S	S	S	S	R!	T7R! stock??
Y53/3 6^{5}!	Y98	S	R	(S)	R	R	R	
Mucoid!	Y86	R	S	S	R	R	R	
Mucoid!	58-161 Cam.	R	R?	R	R	R	R	
Y53/3	Y96	S	R	(R)	R	R	R	

Papillation of the L- Reversion.

May 20, 1947.

To 20 ml plates of T(0) + excess B, and threonine, add varying amts. of leucine. Streak Y53 on these plates to determine suitability for assay of mutation frequency. Inoc: A21.

Leucine, per plate.	24h.	48h.	72h.	84h
0	0	0	✓	
1γ	mic. colonies.	——→ do.	✓	no change!
2	"	Pinpoints	✓	
5	"	—— "do.	✓	
10	Bare visible pinpoints	v. tiny	(1 colony)	
20	> " "	tiny but visible	(1 colony)	
50	> " "	v. small; fairly uniform (Nuccleopora contaminated)		
100	tiny colonies.	small colonies. No papillae! fair sized.		
1 mg.	v. small colonies. papillae in gross streak?	Good sized (1-2 mm.) Some variation. no obvious papillation.		

range for further tests.

Sincerity Y26 x Y87 523

May 19, 1947.

ϕ-c- B+M+ X ϕ+c+B-M-Lac-V_1^R

a) Y26 grew poorly in YB. [OK c cystine supplement or c "Protease Peptone 1%"

b) Y87 in B gave no M+ colonies. ∴ Probably OK as a single factor.

c) in T(0). 5 in 6 plates. (ca 10^{-8}).

d. in T(B) ca 10/plate (ca 10^{-7}).

These testify to the predicted tight linkage of

B - M c ϕ - C.

523 B.	-R	-S	+R	+S.	
	9	1	1	1	

	Lac	V R
C, ϕ+	−	
c, ϕ−	+	S

↑ +S ↑ −S ↑ −R.

+R.

May 21, 1947

A. Y26 x Y53. B. Y46 x Y87.

B. - No colonies in 9 T(0) plates
2 colonies in 4 T(B) plates !

A T(0). 75. T(B,) 184.

~~B~~ Φ-C- x T-L-B,-Lac- all V,s.

Compare with previous intermediates of 1:1 T(0): T(B,).

T(0) 6/51 Lac +.

T(B,) ~~12~~ 14/43 Lac +.

The 43 tests are divisible into 2 parts:

(9)	14	(34) 29	43
(11)	6	(42) 46	51
	20	75	94

a) 75/184 x 43 = 17.5 ≐ B,+ ≐ 2 Lac - ; 15+Lac+
25.5 ≐ B,- ≐ 12 Lac - ; 13+Lac+

∴ the B,- may have ca. ½ Lac - ½ Lac +.
This may not be precisely true.....
i.e.

$\chi_1^2 = 25(.091 + .11 + .03 + .02)$

$= 25(.09)$.

.11
.03
.02
(.25)

$= 6.2$ $p = .01+$

B, — Lac — TL.

Camphor.

May 16, 1947.

Add 3mg/ml Camphor to NA plates. Streak with Y40, etc. Growth not markedly inhibited, but ca 3-4 day. papillae are noted. On 5th day, streak out. Test colonies by streaking on Cla 1 plates. Reject those that show papillae. Inoculate others in broth for further test.

Y40C"	Pap.	No pap.	
	8	8	(too heavily streaked)
	14	0.	
	12	0	
	2	0	
Y10 (pure culture)	5+5	0	
	11	11.	(too heavy!
58-161C.	0	17.	(too heavy).

all these eventually gave papillae

May 24, 1947.

A. Y26 × [redacted] [redacted] [redacted]
×
Y87

B. Y26 × Y64.

No colonies in 4 minimal plates.
ca. 50 colonies/plate ± keotin.
Test ± V₁; Lac.

Reversion of Y87 to M⁺ not checked.
∴ compare Lac | V

ØC +S × BM −R

−R	−S	+R	+S.
21	1	1	—
18	2	0	1
18	0	0	0
23	0	0	0
19	1	0	1
15	1	0	0
114.	5	1	2

reversion of BM to B not checked; perhaps suggested by relative frequency of B⁻ in this series!

[redacted] M⁺C⁻ + S
[redacted] M⁻C⁺ − R

↑ +S ↑ −S ↑ or none. −R.

B. Plates very turbid; samples poor.

	−R	+R	−S	+S.
T(0).	3	1	8	10
	12	10	1	0
T(B₁)	9	1	3	6

indicators; phage n.g.

Total, recently Lac segregation

			compare 524:	(n.g.).
T(0) :	29− : 31+	48% Lac−	46− : 6+	!
T(B₁) :	70− : 15+	82% Lac−	29− : 14+	

Segregation of Lac, V_1, V_{15}

May 24, 1947

Y40 × Y99 [B-M-V_1^R × T-L-B_1-Lac-V_{1T}^R].

T(0) too turbid; auto-inhibited

T(B_1): rather turbid, but colonies can be picked.

T(0). → T(B_1)

Lac	T1	T5	V_{15}	V_{1T}
−	R	R	R	−
−	R	R	R	−
−	R	S	S	R
−	R	R	R	−
+	R	R	R	−
−	R	R	R	−
−	R	R	R	−
+	R	R	R	−
+	R	S	S	R
−	R	R	R	−
+	R	R	R	−
+	R	S	R	R
+	R	R	R	−
+	R	R	R	−
−	R	R	R	−
+	R	R	R	−
+	R	R	R	−
+	R	R	R	−
−	R	R	R	−
+	R	R	S	S
+	R	S	R	−
−	R	R	R	−
+	R	S	S	S
−	R	R	R	−
−	R	S	S	R
−	R	R	R	−
−	R	R	R	−
+	R	S	S	S
+	R	S	R	−
−	R	R	R	−
+	R	R	R	−
+	R	R	R	−
−	S	S	S	S

		V_{15} R.	V_{15} S
Lac-V_{1T}^S	16 P		5 *
Lac-V_{1T}^R			2 P
Lac+V_{1T}^S	18 P		1 *
Lac+V_{1T}^R			3 P

* = visible recombinations between V_{1T}^R and V_1^R.

Segregation of V_1 and Lac is:

−R	−S	+R	+S
16	7	18	4

V_{1T} can be expressed only in V_{15}^S. It is clearly not closely linked to V_{15}

Segregation of Lac, V_1, V_6

Y94 x Y40.

T(0)

Lac	V_6	V_1
+	S	R
–	R	S
–	R	R
+	S	S
–	R	S
–	R	S
–	R	R
–	R	R
–	R	R
–	R	S
–	R	R

See 588.

	Lac	6R	6S	T1	
	–	28	1	R	
	–	25	0	S	
	+	2	7	R	
	+	1	1	S	
Cum.	–	72	3	R	75
	–	53	0	S	53
	+	11	27	R	38
	+	2	3	S	5
					171

$T(B_1)$.

+	S	R
–	R	R
+	R	S
–	R	R
–	R	R
+	S	R
–	R	S
–	R	R
–	R	S
+	R	R
–	R	R
–	R	S
–	R	R
–	R	S
–	R	S
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–	R	S
+	R	R
–	R	R
–	R	R
–	R	S
+	S	R
–	S	R
–	R	S
+	S	R
–	R	S
–	R	R
+	S	R
–	R	R

Effect of hardness of agar on recombination.

Use plates ē underlayer of T(B) + 1½% agar. Add 5 ml of a mixture of Y53 + Y50 to 100 ml of a series of agar concentrations, mix thoroughly, and pour 10 ml quantities. Compare yields. [This should further delimit the mobility of the transforming principle.]

Agar concentrations of ½%, ¾%, 1%, 1.5%, 2% should be tried.

A 27.

½%	Underlyspread: 54+; —	
1%	16, 7	
1.5%	5, 2	
2%	32, 27	ruds-spacing.
2.5%	29?, 10.	¾% is lowest suitable concentration.
¾%	54, 36	

Acenaphthene.

May 17-1947.

Streak NA plates ± Y40; 58-161, and invert over acenaphthene crystals. Incubate 5 days 37°. No marked inhibition noted; no papillation. Streak out on EMB. A23. P24. Marked sugg dimorphism noted.

Test colonies on cla for papillation.

Streak out large + small colonies and Y40 standard:
dimorphism breaks true, but is present in standard stock!
Test biochemically.

	B	M	BM
L.C.	—	—	++
S.C.	—	—	++

This dimorphism must be pursued, as it may be responsible for the heterogeneity in segregation data previously observed.

Colony tests:

	Pap.	No ext. pap.	
58-161 Sm. col.	10	3	
	3	9	probably too heavily streaked.
161 L.C.	11	0	
Y40. L.C.	6	0	
	10	0.	
S.C.	7	5	
	6	; 6?	

Some small colony types do not papillate on first test.

Segregation of V_3^R.

May 27, 1947

Y98 x Y40. Test on T6, T3, T1.

T(0); T(B,).

	Lac	T6	T3	T1		Lac	T6	T3	T1
T(0).	−	R	R	S	T(B,).				
	−			R					
	−			R					
	+			R					
	−			R					
	+			S					
	−			S					
	+			R					
	−			R					
	−			R					
	−			R					
	−			R					
	−			R					
	−			R					
	+			S					
	−			R					
	−			R					
	+			S					
	−			R					
	+			S					
	−			R					
	+			S					
	−			R					
	−			R					
	−			R					
	−			R					

all R →

all R →

all R

R.

probably selective picking of T_3^R T_1^R types.

May 27, 1947

mar: ~~Y87 x Y64~~ Y87 x Y64. (the remaining 4 [illegible] deaths.)

May 27, 1947.

Y100 x Y40

Batal – tested, cumulously, on T6, T3, T1.

See below for Y100 x ~~Y40L~~ x 58-161L.

Phages n.g.? – all R.

874/377.
377
1251

(This Y64 stock is substantially L)

ns B_1 | $-B_1$, ca 690:302: 2.25

Compare 524
184:75 ~ 2.42

Lac, V_1.

$\chi_1^2 = < 1.$

T(6).

-R	-S	+R	+S.	
7	8	1	4	
5	13	0	7	
5	8	1	10	
5	13	2	4	
22	42	4	25	/93

no difference in distributions

T(B_1).

8	6	3	7	
10	12	1	4	
5	17	1	4	
2	3	0	3	
7	13	2	5	
32	41	7	21	/101.
54	83	11	46	/194
in %: 29	43	6	23.	

(30 u.)

B_1	ϕc	Lac	V
−	+	−	R
+	−	+	S.

↑ +S 23 ↑ −S 43 −R 29.

−S/−R should be same as before. ✓

Colony description

May 28, 1947.

Streaked out various cultures on EMB:

58-161	A24 (16h.) L+S	ca 1:5	→ all S. → all L.
Y40	(do.)	purify to Co^L and Co^S lines.	
Y87	all S. ??	→ S.	
Y53	all L? Pick 1 large	→ all L? some S??? →	
Y10	L+S	ca 10:1. Purify.	→ L OK. → S not sharply different from L.
Y64	(all?) large.	Exc.??	L → OK. Pick for stock. S? → somewhat smaller than Y64L
Y46.	(all?) large.		
Y94.	Predom. L.	Some small?	

L = large "rough"

S = small, "smooth?"

Y40 S from BM synpth. all S.

on EMB, Lac+ S do not show a gum sheen
Lac+ L do., particularly in (Y10)

Take L colonies for new stocks. label as Co^L and Co^S respectively.

Y40/6. all small.

Y87/6. large (somewhat mucoid) and small. →

(and Y87L). Ar compare Y87S × Y10L with Y40S × Y94L
58-161S × Y64L.

Some produce less sediment, more pellicle in broth

Effect of colony dimorphism on segregations

May 29, 1947.

A) Y40 · C_0^S × Y53. [C_0^L] B) Y40 · C_0^L × Y53. [C_0^L].

all T_1^S. This argues for an error in the setting-up of the experiment! Test Y40 suspensions which were kept!

A. large colony selection:

Lac−	Lac+
17	6
20	4
9	2
20	5
17	5
11	6
84	28 / 112

small colony.

Lac−	Lac+
7	5
14	8
17	5
41	38 / 56.

81	84	31. 28 / 112
122	46	168

together: 122 : 46 / 168

$\chi^2 = 9\left(\frac{1}{38} + \frac{1}{15} + \frac{1}{31} + \frac{1}{81}\right)$

= .026
.067
.032
.012
9 × .137
= 1.2

P =

b).

9	8
17	6
13	8
14	8
12	11
16	6
19	2

16	3
14	7
30	10

b) 104 100 49 45 / 149. $\chi^2 = < 1.$

A) 118 122 46 50 / 168 P = ...

222 / 95 317

May 3[illegible], 1947

A. 58 161L × Y64L.

B. 58 161S × Y64L

C. 58-161 S × Y64S.

[D.] 58-161L × Y64S.

Stock parents:

58-161 S } indistinguishable!
58-161 L }

Y64S } occasional L.
Y64L } ca 1:1 S:L.

Test parents on T1. [. also "Y40" from exp. 536.

a : a' ca 1:10 in frequency.
(T(0) : T(B1)).

B : B' do. lower frequency of prototrophs. May due to suppressors.

C very few recombinants. (S × S)

D. same. [Y64S v.g. for recombinations ???].

June 3, 1941.

Y100 x 58-161. [Y53 - V_{1T}^{R} x 58-161 V_{1T}^{S}]

plates of V-test today forward test. H. G.

T(0).

-R	-S	-R	-R	-R
-R	+R	+R	-R	-R
-R	-R	-R	+S	-R
+R	+S	-R	-S	+S
+R	-S	-R	-S	-R
-R	-S	-R	-R	-R
-R	+S	+R	-R	+R
+R	-S	-R	-R	
-R	-R	-R	-R	
+S		-R	-R	
+R	But OK.	-R		
-S		+R	-R	
+S		+R	-R	
-S		-R	-R	
+R	65:3R.	-R	-R	
-R			-R	
-R			-R	
			-R	
			-R	
			-R	
			-R	
			-R	

V_{1T} is therefore either 30 rel. units to the left of TL, just right of V_{15}, or to the right of TL. This could be settled by studying interaction ± Lac. This favors the V_{15} - TL position. Or, in the cross BP x Y100, a

B	Lac	P	change in V_1

segregation would situate an intercalary location.

T(B_1).

June 13, 1947.

T(0).

-R	-S	+R	+S.
9	7	1	4
2	3	2	1

T(B_1).

1/2	8/9	0	2/5

Total:

-R	-S	+R	+S
14	22	3	12

S = 36/51 = 70 %

R = 30 %.

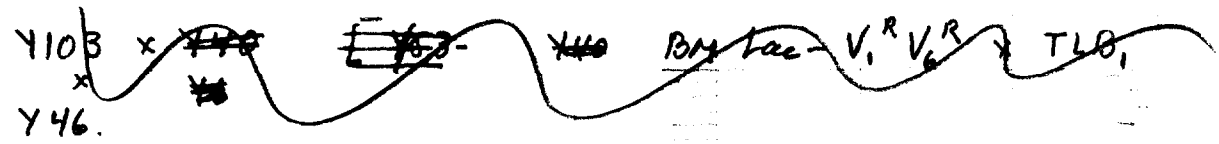
B_1	BM	Lac	$V_{15}^{R.}$	V_{1T}	TL	V_{1T}
	−	+		T	+	.....
	+	−			−	.... V_{1T}^{R}

June 3, 1947.

Reversion of B/1. Tryptophane requirement.
Plate ca 10^9 cells/plate of B/1 into T(0)

on one plate, ca. a dozen colonies on surface. [~~probably~~ contaminations].
~~Recover test on the plates~~ ∴ no reversions

Segregation of V_6, V_1 539



Y103 x Y10

no T_6 sensitives seen; phage probably n.g. (see test on T_1, T_2)

for T_1 and Lac:

	−R	−S	+R	+S.	
T(0)	2	0	4	6	
	2	0	2	7	
	2	0	4	4	
	6	0	10	17	
T(B$_1$)	2	0	3	8	
	2	1	8	3	OK.
	0	0	4	6	
	0	0	0	4	
	0	0	3	7	
	2	0	5	2	
	2	0	3	2	
	13	1	26	32	
	19	1	36	49.	

Sex activity of smates.

Prepare Y40, Y53 suspensions as usual in ~~Y53~~. saline. Treat in conic apparatus for 1 hour. X = Hd; C = control. Add ½ + ½ + plate ½ ml.

a.) Y40X in N.A.
do. 1:100 > 10^4

b). Y53X in NA.
do 1:100 > 10^4 "

c). Y40X × Y53C ++

d). Y40C × Y53X ++

e) Y40X + Y53X ++

f.) Y40C × Y53C. +++

g.) (Y40C × Y53C) + (Y53X × Y40X) fabid ++

V_2^R.

5-41

June 4, 1947.

T2R – stock received from Lucia. titre = 10^9 when made.

plate coured & colonies

Cla - titrate

Plate 58-161 on NA + Cla:

		12h.	24h.
1.	0		
2.	200 r/ml	no inhibition	
3	400	colonies somewhat smaller; no papillation	v. sl. inhibition:
→ 4	600	marked inhibition; no papillae	large and small colonies.
5	800.	very marked inhibition. colonies pinpoint colonies minute	all single colonies are small; papillae in streak.

36h.

400

600 colony dimorphism ◌○. visible papillation

800. colonies very large or very small. ○. distinct papillation.

Range between 5 - 700 r/ml probably optimal. Use mutagens??

June 6, 1941

Rec'd from A. Boivin, lysogenic C1's and C2's, recorded as Y105, Y106 respectively.

a) Test on Cla - agar: papillae found; streaked to purify and test for aerogenesis.

b) Test on T(0) - grows well both on liquid + solid T(0).

c) Lac - both strongly lact+

d) Phage reactions:

	T1	T2	T3	T4	T5	T6	"T7"
Y105	R	R	R	R	R	R	R
Y106	R	R	R	R	R	R	R
K-12	S	S	S	S	S	R	R

! cf. Hershey!

∴ available phages are n.g. find numbers?

Test Cla^R types on glucose fermentation tubes. (pick 5 colonies / plate streaked ⊼ a Cla^R papilla

gas →

105	A	+	+	+	+	+	
(C1)	B	−	−	−	−	−	Y104 = C1 · Cla^R. S
106	A	−	−	−	−	−	Y103 = C2 Cla^R. S
	B	−	−	−	−	−	Y104 = C2 Cla^R. S.
(C2)	C	+	+	+	+	+	

d) Sucrose: no definite fermentation by either. Both show slight papillae in region of second streak. When there are streaked,

Transformation Expts.

June 10, 1947.

a) Prepare extract + sterile filtrate of a 24 hour culture of Y105⁻ in YB.
Filtrate: sediment cells in centrifuge; sterile-filter.
Extract: Suspend cells ≐ ca 200 mg in 10 ml H_2O. Treat sonically for 2 hours at 0°C. Sediment debris + emulsify supernatant with benzine overnight. Remove sediment + excess benzine; remove benzine in vacuo. Should leave a sterile preparation. —
Add 1:10 to YB tubes for assay.

b). Incc Y107, 108, 109 into glucose-gas tubes + 1:1 filtrate.

24h.	107	—
	108	—
	109	—
	—	no acid

c) Incc 107, 108, 109 into YB + filtrate. grow 24h. use this to inoculate lactose gas tubes:

	6 h.
107	—
108	—
109	±

(Transformation?!)

d. Streak out 109 (da); 109/Fb (db); 109/Fc (dc); 109/Fc/Test (dd) on Cla plates to detect sensitive colonies.

ca 20% resist. Sens. with resistance not lost!

e. Sucrose — all sucrose — on second transfer on EMB plates.

Transformation

June 13, 1947.

Y109. in glucose broth	~~gas~~	gas	
	~~—~~	—	
	~~—~~	—	
	~~—~~	—	
Y109 in YB.		—	
then glucose		—	
Y109 in YB + 1:1 filtrate of Y105		~~—~~	See previous test for Y.
		—	
Y109 in YB + 1:10 extract of Y105		+	
		+	
		±	
1:10 extract Y105		—	no acid.

∴ It appears very likely that a transformation has been effected from Ac^- to Ac^+.

Lactose mutants of Y106. 546.

June 1/, 1947.

Irradiate a washed suspension of Y106 in dilute UV 4 min.
Incubate 2 hours. Plate out in GMS lactose.

ca 500 x 58 = ca 30,000 colonies examined.

No clear cut, smooth, Lac- seen. (possibly due to transformation - reversion.)

About 8 possible, small-colony Lac-? were marked for retest.

Also pick a number of small colony types in hopes of finding an R_2 transformable to S_1.

Pick 40 small 10 large colonies to small tubes of YB, and sort on basis of "autoagglutination".

a) large colo: all dispuse 10/10.

b) small colo. 23 clumped 17 dispuse. discard dispuse and incl. clumped types into large YB tubes for further tests.

In general, growth of these types is poorer.

of 23 clumped in 1st test, 15 do not show dispuse growth on second in large tubes.

4 good roughs 546- 1, 2, 3, 4

Tation in S_1, S_2.

Treat washed suspension of Y106 with 4.5% Na deoxycholate (DX) for 3 hours at room temp. Slight lysis observed. Wash & inoculate into 10 ml YB broth + 1 mg. deoxyribonuclease. Incubate overnight and plate for mutant detection P21.

A23 – look over for preliminary small colonies. Overlay with: agar 1.5%, N Z case 2%; Y. Extr. 1% 11 A23. The colonies of this form are extremely uniform, and less than .1% of the original sample are abnormally small, e.g. ● ● normal <.1% are ≤

○ red.
11 picked

Examine for small colonies.
ca 250 colonies/plate × 26 = 6,500 tests.

P23. X red 16 picked
A24. 1 red 8 picked. 6 small YB.

Grew:
○
X
Δ.
Test on T/O)

Spread treated suspension on EMB lactose. 82 × 1/5 cols. = 8000
examined

		T(0)		
Δ	1	−	Y110	
	2	−	Y111	
	3	−	Y112	
	4	−	Y113	
	5	−	Y114	valine + isoleucine
	6	−	~~Y115~~	
	7	++		
	8	−	Y116	
X	11	−	Y117	arginine
	12	±		
	13	++		
	14	++		
	15	−	Y118	arginine.
	16	−	Y119	
	17	−	Y120	valine isoleucine
	18	++		
	19	±		
	20	−	Y121	cystine
	21	−	Y122	
	22	++		
	23	±		
	24	−	Y123	
O	31	++		
	32	−	Y124	

Y106 ++

∴ at least 18/24 mutants.

18/8000 = .5%.

Requirements identified by L. Rodriguez

Later: 133 Lysine
138 Leucine
139 histidine
} from 118 ∴ arginine −

141, 142, 143: arginine from 121 ∴ cystine.

Y110 ... other gave minimal or second best on E. coli by L. R.

S_1 and S_2 Bovis

[June 20]

Chemical test for bromide:

Take 10 ml. suspension of S_1 + S_2; sediment and suspend in 60% HCl + 1:10 1% alcoholic napthoresorcinol. Boil 1 min. Let cool; add 1 ml ether shake + examine.

C_1 - red color at interface

C_2 - no color.

Both show a green fluorescence in aqueous phase.

Test for Sucrose fermentation

a) on plates (EMB). - C_1 + C_2 both negative

b) in liquid - C_1, C_2, Y109 all negative after several days.

Acetate utilization:

after 4 days - Y109 ∓

Y106 +±

C, 1P.

DX- alcohol procedure.

June 23, 947.

36 hour culture of Y105 : 600 ml YB in 1 liter flask.
shake at 30°.

Sediment + resuspend in 20 ml 4.5% DX. Add benzene and shake at 25° from 1230 P 23 to .

P23. Sediment and remove debris + ~~blue~~ benzene phase by filtration.

~~add 10 vols. 100% Alcohol.~~ Collect sediment in a ~~sterile~~ tube.

Sedimentation required ca 5 hours, due to thick emulsion. Possibly pH too low. Supernatant collected.

same DX in solution.

Upon addition of alcohol, a thick fibrous ppt. formed. Probably consists largely of desoxycholic. Sediment and resuspend in alcohol to dissolve desoxycholate. Sediment (easily done in centrifuge). Supernatant ppts in aqueous 6.8 buffer. ∴ probably desoxycholate

Try dissolving sediment in H_2O. OK - very viscous solution.

R_2 In second ppt'n attempt, add a few drops of NaOH to prevent ppt'n of NaDX & alcohol.

Test R_1; R_2 on Y109. no gas+ produced.
Sterility - OK.

medium.

(Repeat, omitting DX)

Transformation.

June 26, 1947.

Y109 in YB + 545 extract.

tests on glucose tubes.

sterility	—
Y109	—; —
Y109 + TP	+ +

transformation OK. sterile acid on Cla.

Preparation of TP: alcohol procedure.

Autolyse 500 cc = Y105 in 15 ml N/5 Cl .9% + 1 ml benzene at 30°.
Sediment + separate extract.

550 - X1 aliquot unlifyg 4 tubes of cells. Shake with benzene & store overnight at cold room.

550 - X2. Add 6 vols. 100% alc. to extract. Ppt ca 5-10 mg of material. sediment transfer to sterile tube; ~~sediment~~ resuspend in 100% alcohol for 2 mins. Sediment and redissolve in H_2O. 1 ml = ca. 25 ml culture.
Add 1:10 to YB tubes to test for sterility and activity.
Inoc with Y109. After 16 hours, add 1 2 ml culture to glucose tests.

1. Y109	-
2. Y109 + X_2	+
3. Y109 + X_2	++
4. Y109 + X_2 + 1 mg DRNase	-
5. Y109 + X_2 + 1 mg DRNase	+
6. ~~—~~ (sterility control.)	

all were inoc ± X_2 and 109!

moi. ± 109 in exp. ±

Transformations.

June 27, 1947.

1. Y109
2. Y109 + X_2
3. Y109 + X_2
4. Y109 + X_2 + 1mg DRNase
5. Y109 + X_2 + 1mg DRNase
6. Y109 + X_1
7. Y109 + X_1
8. X_1.
9. X_2.

Preparations from 550.

June 21-28 1947.

Prepare extract from 500ml of Y105 by alcohol ppte. method, after toluene autolysis 3½ hours. Add extr #10 in YB.

		2-3 hours test
1.	109	+++
2.	109+ X	+++
3.	109+ X	+++
4.	109+ X + DNase	+++
5.	109+X + DNase	++
6.	X.	

not sterile? – non very slow growth on transfer to glucose test.

P28. Add 10 vols alcohol to remainder of X to sterilize. 1 pt + store sediment in 70% alcohol.

"109" inoculum?? probably in error. · Letters:

inhibitor.

N-379

5/8/46.

Blend 368-7 (43-3 mycelium, mix exc. leuc.) [redacted], sterilely, 11P9
Transfer remainder of blendate to sterile flask + store cold.

1. 5 ml unblended medium — 43-3
2. — 10A.

Add 5 ml extract + 5 ml F(o) + asmid. 1 mg dl-leucine

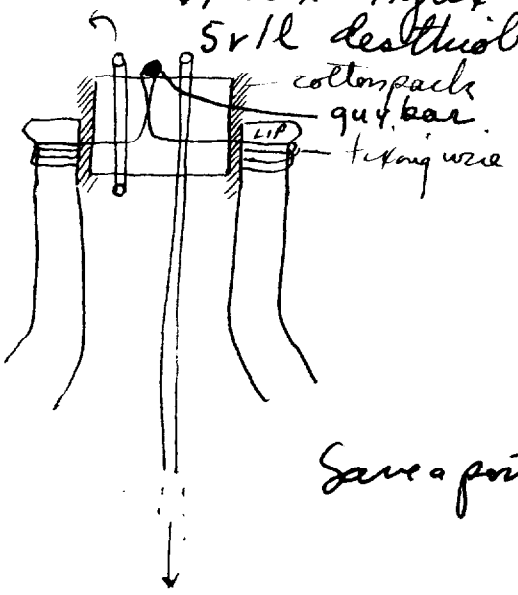
					P11.	P13	P22
3	extr.	+ F(o)		—	−	±	±
4	"	"		10A	+	+++	++++
5	"	"		43-3	−	±	±
6.	"	"	+ leuc	—	+	++	++
7	"	"	"	10A	++	+++	++++
8.	"	"	"	43-3.	++	++	++

No evidence of inhibition.

N-380

Production of Neurospora. 5/14/46.

In 20 l. Pyrex carboy, 10 l. of a Fries ≡ 2% glucose,
5 vl desthiobiotin. Thom ≡ 847



Inoculate 6/8/46. 12 M.
Harvest A12

Yield: ca 150-200 g. dry

Send to Graf in 95% alcohol (before drying)

Save a portion and dry for hydrolysis.

Mutants by selection. N-381

5/14/46. 349 - A5 x 25a.

Isolate ascospores, 1/petri dish. 7 germ. / 20.

5/14 - 20 isol.	Color	Morph.	F(o).
1	n.g.		
2	"		
3	"		
4	"		
5	"		
6	+	+	++
7	n.g.		
8	ng		
9	ng		
10	ng		
11	ng		
12	−	+	++
13	+	+	++
14	[illegible] −	−	
15	n.g.		
16	−	+	++
17	+	+	++
18	ng		
19	+	+	++
20	ng		

Isogenic N- strains
Isolations.

N382

5/28/46 SY7 x 360-6. (S series.) 4/30 prototroph

A. 1 ng
2 "
3
4
a 5 ✓
6 ng
7
8 "
9
10. "
11.

G(0)
SY7 x 1633a (70-26) 4/30 pab p

21 —
22 +
23 +
✓ 24 —
25 —
26 +
27 n.g
28 ~~n.g~~ +
29 n.g +
30 n.g.

SY7 x 378-3 5/10 mixed

a 31 —
a 32 — Store
A ✓ 33 +
34 —
35 +
36 +
37 +
38 ng
39 ng
40. ng

3740 1a x SY 1

↓

378-3 x SY7

↓

382-32

N-582
a

nic a × put A
4540 5531.

dated 4/28. Isolate:

5/14: ✓ o nic put nic-put
infected.

1
2
3
4
5
6
7
8
9
10
11

pure germinated.
Retreat.

4/27 - 5/14. 366 - 3 × 25a. (Lc-4637+[redacted]-15300).
21 isolates 2/pair. not oriented here. Only 6 germ!

#41 is giantspore		Color	F(o)
n.g.	21	+	++
	22	— (pf)	"
	23	+	"
	24	+	"
	25	+	"
	26	± (dil)	"

not so fast!

multiple mutants

4540a × 370-12 (4540 - 5531 A7. dil)

5/28/46 — G (nic) G (pnt) G (pnt-nic) Color Sex.

	1	n.g.					most spores are not colored.
~~flaky~~	2 ✓	−	−	+	±	A	
	3 ✓	−	−	+	+	a	Isp. A. select for deep pigment.
?	4 ✓	−	−	−	−		
A ✓	5 ✓	−	−	+	−	A	Isp. B. " " light " .
	6 ✓	−	−	+	+	a	
	7 n.g.						
	8 "						
	9 "						
	10 "						
	11 "						
	12 "						
	13 "						
	14 "						
	21						
	22						
B	23						
	24						
	25	n.g.					
	26	W					
	27						
	28						
	29						

pnt ~~lc~~ − pnt-lc. Sex.

5/28/46. 5531 A × 33757 a.

	31		
✓	32		
Random	33		
	34 ✓		
	35		
	36		
	37		
	41 ✓	+	−
broader	42 ✓	+	−
	43 ✓	+	−
	44 ✓	+	+
??	45 ✓	+	+
	46 ✓	+	+
	47 ✓	+	+
	48 ✓		
	51 n.g.		
	52 n.g.		
	53	+	−
broader	54 n.g.		
	55	+	+
	56	+	+
	57	−	+

1633a x SY7A.

58-6 x SY7.

N-383a

4/30-5/14.

5/14. – all spores isolates.

~~366-3 x 33757a~~.

G(0). 58-6 x SY7. third more isolates.

5/14: several pcs. 3 sporulp. soup.

P1.	1		
	2	N.	G.
	3		
P2	4	N.	G.
	5		
	6		
P3	7	ng	
	8		+
	9		+
P4	11	·	+
	12		
	13		
P5	14		
	15	·	+
	16		
P6.	17		
	18		
	19		
	29	·	+

PAB - adaptation.

384

358·6 × SY7A

5/28/46 G(6).

Random - from spore print.	1	+
	2	+
	3	+
	4	+
	5	+
	6	+
29 coll.	7	+
	8	+
	9	+
184.9. 1st heating	10	+
	11	+
all morph.	13	+

~~gametic~~.

?H. compare serological ratios.

Expt. 371. Nuclear origin of protoperithecia

Isolate peritheca as far as possible of ascospores. However some had discharged. 5/18/46 Test on F(o) A31.

		Color.	F(o).
371-11.	1	+	+
P:	2	+ −	−
5531-15300+	3	+	−
37401	4	+	−
×	5	+	+
5531-37401	6	+	−
	7	+	−
	8	+	−
	9	+	−
	10	−	−
	11	+	−
	12	+ −	−
	13	[redacted] +	−
	14	+	−
	15	[redacted] −	−
	16	+ −	−
	17	+ [redacted]	+
	18	+	−
	19	+	−
	20	+	−
	21	+	−
	22	[redacted] ng	
	23	[redacted] ng	3+ 18 −
371-12	31	+	
	32	+	+
do.	33	+ −	
	34	+	+
	35	+	−
	36	±	+
	37	±	−
	38	+	−
	39	+	−
	40	+	−
	41	+	−
	42	+	−

		Color	F(o)	F(pit)	
371-1551		+	+	−	+
	52	+	+	−	+
P: 5531 −	53	±	+	−	+
37401	54	+	+	−	+
×	55	±	+	−	+
5531-15300	56	+	+	−	+
+	57	+	−+	−	+
37401	58	±	+	−	+
heterozc.	59	+	+	−	+
	60	+	+	−	+
	61	+	+	−	+
	62	+	+	±	→ slow ++. ??
371-16	71	+		−	+
	72	+	−	−	+
5531-37401	73	+		−	+
×	74	+		−	+
5531-15300	75	+	−	−	+
and	76	+		−	+
37401	77	+		−	+
mixed	78	+		−	+
conidia	79	+		−	+
at first 12	80	+		−	+
	81	+		−	+
	82	−		−	+
	83	+		−	+

Why should pit conidia be more effective?

Nuclear origin, etc.

N 386.

6/1/46

Incor. F(0) plates ē 4545a + 37401a. to hetero caryon formation
P1.

P3. No growth. (Dead stock?)

P4. Repeat ē fresh cultures.

P6 – no heterocaryotic growth !!! see 389. Compare

N 387

L_1 and L_2 ; $L_1^- + L_2^+$. Kenrami
distribution

6/1/46. 25° Proc 4P.

Use 43-3 as L_2^- 368-22 as L_2^+. 10A as L_1^+ $L_1^- = L_2 - 4637A$.
5P1.

Proc N∞ plates ē the following ispairs, ē verified plates ij dy.
all OK.

1. $L_2^{-v} + L_1^{v+}$

prel. calib.

2. $L_2^{-v} + L_1^{v-}$

3. $L_2^{+} + L_1^{v+}$

4. $L_2^{+v} + L_1^{v-}$

5 $L_2^{v+} + L_2^{v-}$

3PM 6/2/46

Isolate 387 hyphal tips to mid. medium. ∝ is solid; o is liq.

Incubate at 30°. p4

		1 to ∝ Color	G(o) - color.	5 to ∝ Color.	G(o) - color.
	1.				
L_1^+	1		+	71	± +
	2		+	72	− +
L_2^-	3		+	73	± +
	4		+	74	± +
	5		+	75	± +
	6		+	76	± +
		1 to d			
	11	++	−	5 to o	
	12	"	−	L_1^- 81	++ −
	13	"	−	82	−
	14	"	−	83	++ −
	15	+	−	84	" +
	16	++	−	85	" ++
		½ to ∝		86.	" ++
	71		−		
L_2^-	72		−	77	+
L_1^-	73		−		
	74		−		
	75		−		
	76		−		
		3 to ∝			
	31		−		
	2		−		
L_1^+	3		−		
L_2^+	4		−		
	5		−		
	6		−		
		5 to o			
	41	+	=		
No	42	+	−		
popin.	43	+	−		
	44	+	=		
		4 to ∝			
	51		− +		
L_1^-	2		− +		
L_2^+	3		− +		
	4		− +		
	5		− +		
	6		−		
		4 to o			
	61	++	−		
	62	"	−		
	63	"	−		
	64	"	±		
	65	"	±		
	66	"	±		

The color line is quite dep. Unless a further mutation of 15300 is considered, this strain is highly suspicious. Again should be repeated.

No inhibition is demonstrated.

6/4/41. Inoc F(o) plate ≡ 94-4 + 37401a. Use edges of plate for controls.

P6 - no growth!

Repeat P10.

- initial hyphae, but no extended growth!

N 391

In vitro activity: synthesis of pantothenic acid by Neurospora.

6/5/46.

50 ml Fries + Incr SY 7 1030 A15.

1. —

2. 1 mg β-alanine + 1 mg pantolactone.

Harvest: 11P11. (6½ days).

A Medium

B. Mycelium in 10 cc H_2O. Then boil, and remove mycelium for digestion.

Use medium 50% in assay; extract B. 10 ml / 50 Fries.

Incr 5531. 12N12.

1. F(o) 50 ml.
2. 1A.
3. 1B
4. 2A
5. 2B.

no demonstrable response in 2 days.

33751 - Selection vs. dominance. N-392

9 JUN 19[illegible]

In previous experiments, color markers were used; here another biochemical mutation is employed: 33757-4540 + other single mutants.

All F(0) plates + 1mg dl leuc./10 ml: P10:

361-6 is 33757-4540 A.

~~#9~~ 11 P10. Isolate 4P 11.

α 361-6 + 5531 A.

β 361-6 + 16117 A.

γ. 361-6 + 1633-15300 A.

		To F(lc) small lg. hyphae 1230 A13	1130 A13	To F(0). 1230 A13	
γ	1	–	–		
	2	–	–		
	3	–	–		
	4		–		
	5	–	–		
	6	–	–		
	11	–	–		
	12	–	–		
β	13	–	–		
	14	–	–		
	15	+	++		
	16	–	–		
	21	–	–		
α	22	–	–		
	23	–	–		
	24	–	–		
	25	–	–		
	26.	–	–		
	51			–	
	52			–	
	53			–	✓
	54			–	
	55			–	
	56			–	
	61			–	–
	62			–	–
	63			–	±
	64			–	–
	65			–	–
	66			–	±
	71			–	–
	72			–	–
	73			–	–
	74			–	–
	75			–	–
	76			–	–

To N∞ slants.

α	81	+++
	82	+++
	83	+++
	91	+
β	92	+
	93	+
	41	+++
γ	42	+++
	43	+++

15, 62, 66 to N∞ large slants.

A24. tests on: F(0) F(lc) F(lc mic) F(flc)

15
62
66

Selection vs. Dominance N-393.

Test 392 on le, mic, etc.

Thus may have been deficient leucine in the medium.

	le	mic	le-mic
81	+ (±?)	+	+
82	+ (±?)	+	+
clusts. 83.	−	+	+
91	+	+	+
92	+	+	+
93	+	+	+
41.		+	+
42		+	+

N-394.

hiles specific heterocaryons.

6/13/46. 13 JUN 1946

12N.
See N-83. N. sitophila 299A + N. crassa 1633-15300A (70-27). on F(o) plate

Isolate hyphal tips to minimal liquid. 4P14.

1 —
2 —
3 —
4 —
5 —

Take a block of agar-mycelium + inoculate F(o) plate:

11. a few hyphae grew out and covered plate. Evidig spotted white + colored in various areas.

Syntrophism? or unstable heterocaryon?

Heterocaryon transformation
nic + pant.

N-395

14 JUN 1940

1. Incr F(0) = 5531A + 45Y0A. 1130P14.

3P15. Isolate hyphae to F(0) ~~2ml~~ 1ml tubes.

1
2
3
4
5.

1A16. Incr pnt-cornmeal = 383-3

Chlamydomonas nivalis.

Arancipondon Prospect. St. was found to bloom after every rain. Collected 5/18/46, and found Stichococcus, Bacillariae and a variety of Chlamydomonads. Purify by centrifuging + phototaxis, and incr. into Moore's/5.

P22 - some green growth noted - a few filaments + some sediment

A24 - mic exam. showed numerous flagellates. Plan to streak out when culture is heavier.

P1 - streak vertron Moore's/5 agar. + mic. liq. ē culture

Endomyces - Killing & Mutants

-- 36. hour culture in F(0). Shaken at 30°.

Irradiate in quartz tube 2 mins., etc. Incr. coli CM plates

6P26

7P27: small colonies = hyphal radiations.

1. Control. Dil. 1:25,000; 1:1,250,000 — (29) — 36,000,000.
   1:10 dens. = σ = 89 d = 0'51 d_{corrig} = 505 G_{01}. 31
   limit of d = 71,000
2. Irrad. 2 mins. /ml
   1:50
   1:2500 (11) 276,000 S = .0076
   1:125000
   1:625000 pSurvival = 2.1

✓3. 1 ml to coli CM. 6P26. Dilute to 10^{-6} (approx.) /ml and spread over coli CM plates. d = 1340 ≐ 95×10^6. 2P28.
etc.

Complete
1. Spread 1cc over
2. surface.
3. Embed in agar 50cc
4. Surface again.
5. Let cover
6. as coli procedure.

Fairly uniform. Liquid on surface w.p. spreading growth. and upon plate.

Tow

Many

6 Spread 1cc over surface
7 surface
8 Embed in surface agar
9.
10
11 Embed + cover Liquidity heavily.

11 Ycolonies. P1. Layer complete 1P2.

2A30.

11 not up yet.

Nothing came up

to 38°. 7 colonies!

F(0).

pS = -log survival This should be a dose.
= log (1/1 - killing)

A 5

Schizosaccharomyces octosporus (SO). 6/1

1. Vitamin Requirements.

1. T(O) + Biotin, Thiamin, Riboflavin, nicin, pnt, inositol.

1.

2. – biotin

3. – thiamin

4. – riboflavin

5. – nic

6. – pnt

7 – inos.

8 – pab

9 – folic

10. – B_6

6/5.	1 colis	6/6 +++	unknown factors?
F)	2 HC+V	±	Anyhow, octosporus is genetically not
	3 HC	±	satisfactory for mutant production as it di-
	4. V	±.	ploidizes very readily. Use S. pombe

which requires biotin, nic, pnt + inositol.
Compare S. cerevisiae which does not req. pnt

S. pombe. - misc. prelim. data. 44

1/7/46.

1. plating density. susp. in H_2O. G = 93. = 31.5 d.u.

Hemocytometer count. 136, 138 = 137 / 10^{-4} ml.
= 1.4×10^6/ml.

∴ 1 density unit = 4.3×10^4/ml.

Vitamin Requirements - stated as biotin, pnt, nic, mio.

			P9.
1.	Fries + vits.	+++	++++
2.	" + 4 vits.	+	+++
3	− pnt	−	++
4	− nic	−	−
5	− mio.	−	−
6.	T − + vits.	+	+++

1230A7. Inoc coli 2. / 37° sh. − growth only fair worse than use 10 vits. Omit 1 & 1.

1A9 Inoc.

1 − B_1 ~~(Biotin [illegible])~~
2 − B_2
3
4
5
6 ftc.
7
8
9
10

50 ml 11. F (vits.) 37° shaken.
12. " 37° shaking.

see A5.

Vitamin Requirements

A-5

S. paratyphi.

19 JUN 1946

19 JUN A M 1

Compare A-1. 37°.

Fries + vitamin supplement: 10B vits. — ind. F cont's. desthiobiot.

	11P 9	10A10		
1 - B_1	+	++	(put)	put
2 - B_2	+	++	nic	nic.
3 - pato	+	++	inos	vits
4 - nic	—	—	[redacted]	[redacted] OK.
folic 5 - [redacted]	+	++		
6 - B_6	—	++		
put 7 - [redacted]	-	+		
8 - chol	+	++		
9 - inos.	—	—		
10. - Biotin	+	++		

Compare shaking 5 unshaken cultures. 37°.

11 F-vits	sh.	+
12. "	unsh.	—
13 coli ∞	sh.	+
14. "	unsh.	—

Growth is poorer than in tubes.

Relatively anaerobic conditions

~~Vit Requir.~~

A10.

Fries:

21 put inos, nic, folic (biotin)

22 ~~put~~

23 ~~inos~~

24 ~~folic~~

For colony type, plate A4-2 into F(P) = Fries + paratyphi vits

G 1:10 = 78^2 d = 1051 (45,000,000) dil. 10^6.

P13 — colonies finally noted. Resemble bacterial colonies.

Moratium?? to dd.

PAB - responsiveness. E2

8/20/46.

I PE3

		0	.001	.003	.01	.03	.1	.3	1
	T L	100							
Y44	H	100							
	O								
	O								
	O								

II Y44 (#

	0					
Y44						

III #22

IV #11 Y.O

	0	.001	.003	.01	.03	.1
15	96	91.3	92		13	90
	100	87.5	88	93	91	93
		#1				

(82)

INDEX to BACTERIAL GENETICS
V. 1-3 Yale 1946-1947.

CROSSES: I

Y40

Y41: 252, 257 Y54: 349ng
679-183: 252,257,313
Y53: 345(lactose), 353,358,361,364

Y41
58-161: 252,257,260,287,313 " /3" 319
Y24 : 259,278,276,287,290,296
B/r muts. 277,282,287,391
Proteus: 277,283

Y24
679-183 252
Y9 264,270
Y10 276,284,285,287
Y46: 293,297,300,318,321,301

Y24/1
Y10: 300,304,318,301
679-183: 301.313
679-680: 301
Y53 X Y61 : 353

Three Way: B-L-T-: 326, 338

Y53-Y46-58.161 353

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X Y10

Y87 494
Y103 539

X Y40

Y53: 358, 361, 364, 370, 375(in BTL), 377, 415, 432, 445, 446, 447, 452, 458(selection eff.), 459(par. ratio eff.), 472, 484a.

Y64: 490
Y65: 439
Y76: 418
Y77: 441
Y89: 471,482,486,509a
Y94: 518,519,528
Y95: 518a,519
Y96: 531
Y99: 527

X Y53

Y26: 524
Y54: -notes to Treffers
58-6315 376
Y68: 379,387, 439
Y78: 441
Y90: 502
Y91: 496,509a
Y92: 502

X Y64

58-161: 386, 460, 472, 484a
58-prol: 417
Y26 : 526,534

Y87 X

Y26 : 523,526

58-161

Y67: 387
Y100: 537
Y65: 404

Misc:
MUCOIDS 414,467,488,503
y80 x Y81: 440,451/a,461
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L-15 mutants 425, 431.

3-way: Y46-Y53-58.161: 368, 398, 412
y40-Y10-Y64 : 398,411,422
B-L-T- 370
Misc. 554

LYOPHILIZED STOCKS Sept. 9, 1947

E. coli K-12 & 58 derivatives:

K-12	wild ✓
58	desthiobiotin ✓
58-161	" - methionine ✓
-178	Y.E.
-278	Ph alanine
-309	cystine
-336	isoleucine
-580	thiamin
- 593	thiazole
-610	thiazole
-741	histidine
-2651	proline
-3214	proline
-3232	"
-3356	meth
4447	?
4899	ph.alanine
5030	tyrosine
5255	proline
5273	adenine ✓
5298	indole to Davis
5417	uracil ✓
5450	proline
5580	A.A.
5631	adenine, guanine ✓
5636	indole
5898	methionine
6049	proline
6177	"
6313	threonine
6314	Ph. alanine
6317	proline (glut.)

E.coli 679 and derivatives:

679	threonine
" 183	?
440	?
447	?
455	?
662	glutamic acid
680	?
684	?

E.coli Lampen strain 15 derivatives:

L-1	
L-2	
L-3	arginine
L-4	meth.
L-5	threonine
L-6	proline
L-7	lysine
15 L-171	"
18-15 L 171	methionine
66-489	lysine

E.coli B

A-1	
A-2 (7-415)	
B/R	
-R	
T	

E-coli Yale mutants :

all Y-

1	(ATCC 6522)	proline
2	" "	meth. SA -resistant
3	(L-15)	leucine or isoleucine
4	"	" " "
5	"	" " "
9		threonine, leucine, pab
10		" " B1

... For continuation of this list, see Y list)

BOIVAN C1
C2

B. subtilis

hist

Staph. flavocyanae

Shigella • Hu (Hutner) uracil

79-30-2 (Weil) Flexner V
66-1-410 " " II

SALMONELLA

S1	paratyphi A	meth, tryp
S4	cholera suis	meth
S20	typhi murium monophasic (IV? V? i..)	phase 1 +
S36	gallinarum	B1
Su	S 37 dublin	B1
S42	paratyphi A	tryp
S45	enteritidis	ornithine
S-50		
S-51		
S-52	paratyphi B	req?
S55	cholerae suis	tryp+cysti
S-56	" "	tyrosine, biotin
S-61	typhimurium	IV variant meth
S70	"	--SH

ACETOBACTER M.A. 11 series:

M.A.	6.1 (11.1)		+
11.2	90		serine or gly
.4	236		glycine
.5	318		leuc

PHAGE

T-1
T-3
T-4
T-5
T-6
T- 7

Phytomonas tumefaciens

Proteus D
14
3 : - 38 -57 -63
39 58 65
40 61 66
44 62 74

available at Yale

STANFORD NEUROSPORA BIOCHEMICALS:

1A	+
25a	+
193A	pab
299	sitophila: B_6
605a	serine
830A	pab
1090	sitoph: thiazole
1298 A	uracil
1633 A	pab
"-15300	A,a
-4894	a pab-meth
3416a	nic
4540A	a nic
-10575	nic-tryp a
4545 A,a	lysine
"-5531	"-pan
-15300	A,a -al
-37401	A,a -inositol
4637 A,a	alb-1 (transl)
4711 a	isoleuc,& valine
4894 a	meth.
-15300	A,a
-34608	A,a aurescent
-37401	a inos
5359 A,a	pab
5531 A,a	pan
-4540 A	n ic
-15300 a	
-37401 A	
5801 A	scumbo
8839-4637A	leucine
9185 A	B1
10575 A	tryp
-37401	"-mic
15300 A,a	
16117 A,a	isoleuc,val.
17084 a	pyrimidine & thiaz.
-37803 a	- B6
-44602 a	- "
18558 A	thiazole
-34486	-choline
21848 A	pab
21850 A	"
21863 A	proline
27947 a	arginine
29997 a	"
30300 a	"
33026 a	valine
33050	valine A
33757	leuc A,a
"-4637	A
-15300	A
34486	chol. a
"-15300	a
-34508	A,a
-37401	A,a
34547	tryp A
35307	pab A
35420	tryp a
35810	isoleuc,val. A
36115	pab A
36607	tryp a
36703	arg. a
37401	inos A,a
-5531	A,a
-"-15300	A
37803	B6 A
37906	tryp A
37907	" A
38113	pab A
38704	valine ? A
38722	tryp a
39113	nic A
39115	tryp A
39303	nic a
39501-1452	val,tryp? a
39701	tryp A
39705	isoleuc,val a
39709-4637	" " A
39801	tryp a
40008	" A
43302	nic A
44008	tryp a
44008-65001	"
44020	" a
44210	" a
44602	B6 A
"-37401	A,a
44706	tryp A
44707	" A
44801	nic a
44802	" a
45210	tryp A
45217	" A
45219	" a
45302	" a
45303	" a
45304	" a
45503	" a

Misc. Neurospora:

S6201	val ?	A
[illegible]	[illegible]	[illegible]
Abbot 4	+	A
A" 12	+	A
Chilton	+	a
SY.	+	A,a
E 5297		a
977 R L		a

N. sit: Sands PC A
[illegible].SS F.28 PC a
56.7 PC a

N. tetra. S9

4545-5531-15300
51602-4545-37401-15300
" -5531- " "
10575-37401-15300
G 27 A al-pan-inos-B2-tryp
G 37 a

New Yale mutants:

Y-14927	lys	a
-16050	col.	A
16059	lys	A
16424	aden	a
16329	pa alan	A
16349	cyst,meth	a,A
16351	aden, hypox.	A
163 67	cyst,meth	A
16479	pab	A
16446	inos	A
16603	meth, cyst [illegible]	A
16631	adenine	A
16744	meth	A
16470	leuc.	A
16641	meth	
16695	pan	A
16644		A
16747	meth	A
16730	aden, hypox	A
16796	pab	

Penicillium

3169	proline	
4769	histidine	
6155	arginine	
6549	hist	
7286	unknown	
7288	"	
7307	hist	
9756	arg	
9929	prol	
10099	pa alan.	
10259	unknown	
10283	hist	
11117	"	
32044	unknown	
32179	arg	
40102	hist	
41272	arg	
47017	hist	
50265	isoleuc	
51775	c - m ?	(i-v?)
52204	pab	
52997	lys	
60297	choline	
69441	hist	
81414	"	
84248	unknown	
[illegible]		
84886	hist	
85880	"	
[illegible]		
86842	"	
89172	"	
96730	"	
97054	"	

Absidia glauca (Giles)

1	
10	hist
50	"
1200	
167	
1571	pan-ye.
1643	"-aden
1891	"-
2775	lys
2790	hist
2828	tryp

196 pan

STANFORD /NEUROSPORA (con't)

46109	tryp	A
46404	"	a
46405	"	a
46406	"	A
46415	"	A
46423	isoleuc,val	A
46807	" "	"
46808	"	a
47101	"	a
47203	trypto	A
47317	"	A
47711	i-v	a
47808	tryp?	a
48009	"	A
48306	"	A
48501	valine	A
48613	tryp	A
48614	"	A
48615	"	"
50005-5231	pyrim&thiaz.	a
51602	B2-temp	A, a
"-37401		A
56501	B1	a
65001	nic,tryp	a
65001-2198	?	A
-3416		A
65205	tryp	A
65612	"	"
66110	"	a
67601	val	A
70307	tryp	A
71103	isoleuc	A
71301	pab	a
75001	tryp	A,a
-39401		A
75102	"	a
80801	"	A
81001	"	a
85902	thiazole ?	A

YALE BIOCHEMICALS:

602	albino	a
1093	lys	A
1866	"	a
1870	"	a
1879	"	a
1937	YE	a
1943	YE	a
2170	al	A
2171	al	a
2198	tryp	a
2298	B6	?
2299	ye	a
2329	B6	a
2364	leucine	A
2492	ye	a

YALE BIOCHEMICALS:

2840	meth	A
2887	meth	a
3261	chol	a
3317	cyst/meth	A
3494	" & "	a
3522	meth	a
3786	hc	A
3791	meth or +	A?
4195	meth	a
4246	hc	A
4518	hc	
4617	hc	
4750	B1	A
4815	meth	a
4840	ye	A
4905	vit	a
4927	vit	a
5015	hc	
6073	meth	A
6279	"	a
6516	ye	A
6516	pab	a
6603	cyst,meth	A
7110	hc	
7142	xye	
7548	vit,color	A
7582	lys	a
8228	al?	
8552	ye	a
10626	yx	a
10654	yx	a
12239	meth	
12504		
12964	yna	
14927	lys	
16329	Pa	A
16331	trypt	a
16424	aden	a
30005	?	
30010	?	
30013	?	
30017	?	
30251		

Misc. Neurospora :

S6201	val?	A
Abbot 4	+	M A
12	+	A
Chilton	+	a
Sy F8	+ isogen	A,a
E 5297		a
977 R L		a
N.sit. Sands	PC	A
HSSF . 28	=PC	a
56.7	PC	a
N. tetra	S9	?

4545-5531-15300
51602-4545-37401-15300
" -5531- " "
10575-37401 -15300
G27 A al-pan-inos-B2-tryp
G37a " " " " "

New Yale Mutants;

14927	lys	a
16050	colonial	A
16059	lys	A
16424	aden	a
16329	ph.alan	A
16349	cyst, meth	a,A
16351	aden, hypox	A
16367	cyst, meth	A
16479	pab	A
16446	inos	A
16603	meth, cyst	A
16631	aden	A
16744	meth	A
16470	leuc	A
16641	meth	
16695	pan	A
16644		A
16747	meth	A
16730	aden, hypox	A
16796	pab	

Penicillium

3169	proline
4769	hist
6155	arg
6549	hist
7286	unknown
7288	"
7307	hist
9756	arg
9929	prol
10099	ph. alan.
10259	unknown
10283	hist
11117	"
32044	unknown
32179	arg
40102	hist

41272	arg
47017	hist
50265	isoleuc
51775	c-m? (-v)?
52204	pab
52997	lys
69297	choline
69441	hist
81414	"
84248	unknown
84886	hist
85880	"
86842	"
89172	"
96730	"
97054	#

Absidia glauca (Giles)

1	+
196	pan
10	hist
50	"
1200	
167	
1571	pan-YE
1643	" aden
1891	"-?
2775	lys
2790	

2775	lys
2790	hist
2828	tryp

YEAST

Eremothecium Ashbyii # 2
59

Sacch. cerevisiae (Lindegren)

93- 1 C haploid
Y-Yo (93-1C) orig. biotin, pan, pab
" x[illegible]r 15
19
22
24
30
35
37
39
43
48
49
[illegible]
115
122
2
5
8
26
28
29
31
125
133

33
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50
51
52
53
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56
57
58
64
66
75
78
79
83
90
103
108
99 W
99 R

Schizosaccharomyces Pombe (Wickerham)
" octosporus

Phycomyces +
4B

Summary of Y- stocks

51	274-4	Y39	UV	II	Histidine, serine
52	256-1	224-1	UV	II	Niacin (EMZ)
53	335-2	Y1o	UV	EMB-Lac	Lactose-negative; Threonin, leudine, B_1
54	TREFFERS 58-161		selection		penicillin-resistant (?)
* 55	Y40 X Y53		recombination		lactose- prototroph
* 57	Y53		seelection with T3 (sic)		resistant to T1,T3,T5 !
* 58-62	360		discarded		
* 63	discarded				
* 64	360	Y53	selection with T1		resistant to T1, T5
*65	360	discarded; preabably a contaminant			
* 66	360	Y53	selection with T3		resistant to T1,T3,T5 probably fallacious
* 67	360	Y53	selection with T7		mucoid; sensitive
* 68	366	58-161	UV	inspection	mucoid (no recomb: 414)
* 69	395	Y40	UV	inspection	mucoid
* 70	396	Y53	UV	inspection on EMB	less revertible at Lac- locus
* 71	396	Y53	UV	inspection	less revertible at Lac- locus
*72	405-1	Y53 X Y40		recomsination	B_1-Lac-V_1^r
* 73	405-3	do.		do	B_1-Lac-V_1^s
* 74	405-4	do.		do.	B_1-Lac+V_1^s
* 75	405-2	discard			
* 76	-	Y53	spontaneous; slection		Lac-reverted
* 77	430	Y64	sp.	selection	resistant to malachite green 100u/ml
* 78	430	58-161	sp	selection	resistant to streptomycin, 5 u/ml
79	436	Y77	sp	sel	resist. brilliant green 50u/ml
* 80	434	Y40	HN2	sel on EMB	glycerol-negative
* 81	discard 436:glycerol enrichment				
82	443	Y53	sp	sel	resist streptothricin 5u
* 83	443	Y78	sp	sel	resist streptothricin 10 u
84	443	like 83			
85	Y40 X Y53		recombination		B-Lac-V^r
* 86	456	Y53	selection with T1		resist T1;T5 unstable mucoid
* 87	463	Y40	HN2	sel EMB	lactose-negative
* 88	466	Y53	sp	selection	resist to Chloroacetate 2 mg/ml
* 89	Y88 x Y40		recombination		prototroph; Cla^r
90	481	Y40	sel		resist iodoacetate Ia^r
91	do.	Y40	sel		Cla^r
92	481	Y40	sel		resistant to azide(As^r)
93	481	Y53	sel		Ia^r
94	511	Y53	sel	T6	V_6^r
95					
96	511	Y53	sel	T3	V_3^s (also res. T4,T7,T6)
97	-	Y53	sp.	inspect	less revertibel at Lac-
98		Y53	sel		V_3^r (V_6^s)
99		Y53	sel	(T1 + T6)	resist to T1; T5-sens.
100	521	Y53	sel	(T1:sm col)	do.
101	521	~~Y53~~ Y40	sel		V_6^r (already res. T1,T5) [S.c.]
102	521	Y53	sel	(T1+T4)	resist T1,T4...

Aneutrophic Salmonellas

	S		type	requirements
L	1	✓	para A	methionine tryptophane.
	4	✓	cholera suis	~~tryptophane~~ methionine — lost!
	12	✓	pullorum	leucine, cystine
	13	✓		leucine cystine
	14	✓		leucine, methionine, cystine (arginine)
	15	✓		leucine, SH
	16	✓		leucine, cystine
	17	✓		leucine, cystine
L	36	✓	gallinarum	thiamine
L	37	✓	dublin	thiamine
L	42	✓ para A.	~~typhi-murium~~	tryptophane ✓ (uses indole; not anthran.)
	45	✓	enteritidis	ornithine (uses arg; citr also)
	50	✓	para A	~~tryptophene;~~ . BIOTIN;
L	52	-	para B	
L	55	✓	cholerae suis	~~Proline~~; (~~stim cystine~~) TRYPTOPHANE; cystine (adapts)
L	56	✓	cholerae suis	tyrosine, OR BIOTIN!
	57	-	typhi suis	
	58	-	abortus ovis	
	59		sendai	slow prototroph.
	60		sendai	20.
	31-69.		typhi-murium	SH METHIONINE.
	51	✓	para B	PROLINE
L	70		typhi murium	SH. u.v. mutant of S 20.

also S20 = typhi-murium

Salmonella strains

	S	type	serotype		nutrition		
P+J	1	para A	I, II, XII	a	−		* methionine, tryptophane
	2	para B	I, IV, XII	b, 1, 2	+		
	3	abortusovis	VI VII	c .. 1,5	+		
	4	"			−		methionine.
	5	enteritidis	I, IX, XII	g, m	++		
	6	"			++		
	7	typhimurium	VI VII	m, t	++		
	8	montevideo	VI VII	g, m, s	++		
	9	newport	VI, VIII	e, h ..	++		
	10	"			++		
	11	typhimur.	I, IV, V	1,2,3 i, .. 1,2,3	++		
B16	12	pullorum			−	E	leucine, ~~isoleucine~~, ~~histidine~~, (meth.) cystine
143	13	"			−	EN	leucine, (arg., meth.) cystine
138	14	"	IX, XII		−	EN	leucine, arginine, methionine, (~~histidine~~) cyst.
W17	15	"			−	E	leucine, ~~isoleucine~~, meth., hist. ← OR on leuc.
WhR	16	"			−	EN	leucine; cystine
Rettger.	17	"			−	EN	leucine (~~arg., meth.~~) cystine (slow on L C)
P.R.Ed.	18	abortusbovis	IV, XVII	b .. enx	++		
	19	" 2.	"	"	++		
	20	typhimurium	IV, V	i ... 1,2,3	++		monophasic - phase 1
	21	" "			++		monophasic - phase 2.
	22						
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	50						

* + = prototroph.

SALMONELLA - "MUTANT" TYPES.

S	Ref.	"species"			
1	P+S	paratyphi A			methionine, tryptophane
4	P+S	cholerae suis			methionine
12	Rettger	pullorum			LEUCINE, ~~ISOLEUCINE~~, ~~HISTIDINE~~ CYSTINE
13	"	"			LEUCINE, CYSTINE
14	"	"			LEUCINE, [ARGININE,] METHIONINE, CYSTINE
15	"	"			LEUCINE, ± S,
16	"	"			LEUCINE, CYSTINE
17	"	"			LEUCINE, CYSTINE
	Wheeler:				
18	227	oranienburg		++	
19	3575	oranienburg	~~±S~~	++	
219	415	kentucky	++		S8 ✓ open. →
~~221~~	421	typhi-murium		++	
~~222~~	3490	typhi murium	++		
~~223~~	3542	typhi murium	++		
~~224~~	422	abortus equi	++		
~~225~~	426	newport	++		
~~226~~	3491	newport		++	
~~227~~	429	london	++		
~~228~~	547	urbana	++		
229	1681	budapest	++ R.		
~~230~~	1916	indiana	++		
~~231~~	1918	adelaide	++		
~~232~~	3486	montevideo	±S	++	
~~233~~	3539	panama	++		
~~234~~	3573	paratyphi	±S	++	
235	3576	paratyphi		++	
236	3045	gallinarum	→	− +late	Thiamine
237	1684	dublin	→	− +late	Thiamine. motile ✓
238	3481	newport	± S	++	
	Yale:				
239		typhimur	++		
240	28		++		
241	27		++		
242	32	// para A.	−		TRYPTOPHANE type corrected by Edwards
243	25	enteritidis	++		
244	23	"	++	+late	
245	24	"	−		~~ARGININE~~. ORNITHINE
246		"	++		
247	34	para A	++		
248	33	"	++ R		
249	18	"	++ R.		
250	17		−		
251	19	para B	~~±~~ ±		✓
252	20	"	−		
253	21	"	++		
254	22	"	++		
255	31	cholerae suis		−	
256	30			−	Tyrosine;

[stim. but not ess.] + cyst or meth (sup. SH)

Virus Strains

T-1. From Demerec.

a. See 230 6/10/46. Found contaminated 7/14.
b. Recover again from Demerec suspension. Incubate @ 5 7½ -18 3 4½., centrifuge and filter. Titer on 49 = 16×10^6 Small + large plaques.
c. Recovery from lyophilization - excellent viability.

Subclones

679-680-A TL from Ryan, for better mutants (triples) 4/15/40.
Y10A Reisolate Y10 and test. (Separate from threonine cuvusion).

Y-Y0 = S. cerevisiae - haploid clone from Lindegren -
Requires pab, put, biotin. Else Bld.