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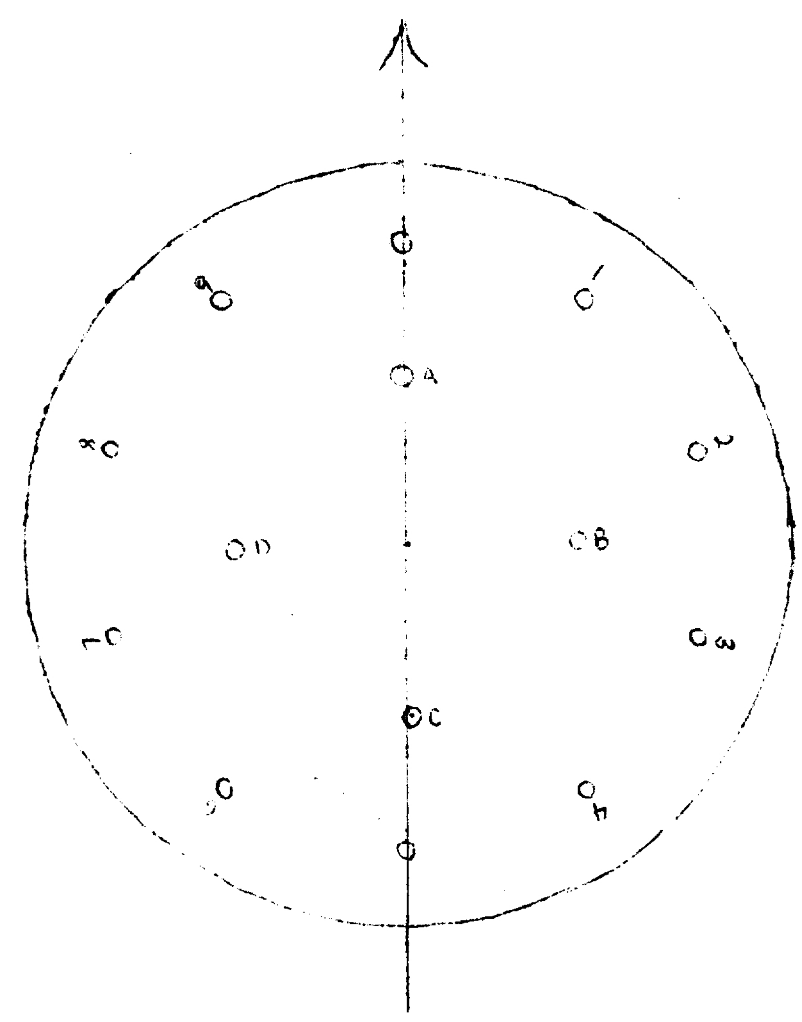
NEW HAVEN, CONNECTICUT

EXPERIMENTS IN THE GENETICS OF BACTERIA

1946- 1947.

Joshua Lederberg.



AUXANOGRAM STENCIL -

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Abbreviations:

F(x) = Fries supplemented c̄ 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.
0 = minimal
∞ = complete
T(x) = coli 0 = Tatum + Gray's + ind. suppl.

Amino acids.

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

Ind = Indole
Anth = Anthranilic.

Vits. V M 1 Thiamin
2 Riboflavin
3 PAB
4 NIC
5 folic ac
6 pyridoxin
7 pant
8 choline
9 inositol
10 Biotin

Purines + Pyr.

3/23/46. — 3/24

Test 156 — Strains. Dil. surface growth in H_2O & test as indic.

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

9A24.

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

Check!

(This agrees c̄ the previous interpretation that 7-10 may readily back-mutate. 1, 3, 4 should be tested further. cf(1).)

Note poor growth in c a.a. Evidently, strong inhibition is present. Wh. is inhibitory??

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

1: T( )
trypt —
thr —
lys —
arg —
val —
meth —
v-i ++ +++
leuc — +
hist —
HC +++
O —

isol. — ++

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

T (VcT) — +
VcT — +
VcT + ++

T (O) ++ +++
O ++ +++
O ++ ++

T (HC) +++

Test 160 [156-1] for adaptation.
9P26. 37°.

	Inoc. into 0.	12h.	24h							
~~1~~	~~HC~~	0	—							
2	v-iso.	0	—							
3	lc	0	—							
4	isol.	0	—							

Therefore these responses are by way of growth, rather than adaptation.

			1130A28 V	330	1130A28 0	330
156-9.5	from HC	onto CM slants for testing	~~+~~	++	+	
6	" Vit		-	+	-	
7	" 0.		-	1	-	
	dilute + test 2P27.	5	~~—~~	± ~~++~~	+	++
		6	-	±	-	+
		7	-	—	-	+

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

		12h	24h.	48h	930A29	10A30	tests on 0 A29 —	10P29	10A30
1.	from HC	—	—	—	+++	✓		±	+++
2	" v.i	—	—	—	2P27				
3	lc	—	—	—	—	-			
4	isol	—	—	—	—	±			

Test 156-9 substrains.

a. to CM slants 5 from HC 6 from vit 7 from 0

			1130A28	330P28	8P28	A29 Test: 0 9A29	4P29	10A30	V
to vit + min.	2P27 HC	5 - vit	—	±	++	±	+++	— —
		0	+	++	+++	+	+++	+ ++
	V	6 - vit	—	±	++			
		0	—	+	+++			
	0	7 - vit	—	(±)	±			
		0	—	+	+++			

This behavior is remarkable. What is inhibitory? What is the type of genetic modification?

See 164.

Mutant identification.
"Auxanography"

Mar 7 P78

	histidine		+ C Hydrolyzed Casein →				
E-8							
161-6							

ca. $10^{4.5}$ colonies per plate. They are visible for a radius of 1cm 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 a.a. not clear. Continues faster on e.a.a. than on s.e.a.a. but this may not be a specific response. Need L-15 for control. Inhibit by biotin??

P37 ca 10-12 "colonies" full size are seen in the E-8 plate, presumably adaptants.

Try heavier inoculum.

See Auxanography – p. 168.

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

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

7P28

Test "washed agar #1" for biotin. Agar was washed by 10 transfers through dist. H_2O, + 2x c̄ 95% alcohol, dried in a desiccator c̄ $ZnCl_2$.

Plate "58" (biotinless E coli) in 2% agar unwashed
" " washed
" washed + 1γ biotin

P31 — no colonies!

Inoc. on surface (streak) 8P31.

A2 — Well developed colonies only where biotin was added. None elsewhere!!!

Auto-ansvar

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

4 1130P28 inoc.
1 930A29 ·1'
2 12N
3 9P29

		O	TL	TL(HC)	+eaa	TL+neaa			
✓1	142-17	− − −	− − −	+ ++ ++	− + +	− − −		= Y10	
✓2	-36	− − =	− − − 10A +++	++ +++	+ ++ ++	− − +++		(adapted??)	
✓3	-54	− − −	− − −	− + +	− ± ±	− − −		= (Y9)	
✓4	-57	− − −	− +++	++ +++	+ ++	− + +++ n.g.			
5									
6		O	HC	neaa	leuc (1)	isoleuc (2)		val+isol. (23)	val+leuc. (13)
✓7	156-1	− − =	++ +++	− − =	± ++ +++	− + ± check!		− + +++	± ++ +++
8									
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	Vits.	V1	V2	V3	V4
18	on various vitamins.	+ ++ +++	− − +++ / − − +++	++ +++	− − − ++ / − − ± ++ / − − ± ++ 10A30	− + +++	− + +++	− + +++	− + +++
19									
* 20		neaa	V5	V6	V7	V8	V9	V10.	V11-ynah.
* ✓21		− + +++		− ± +++	− + ++	− + ++	++ +++	− − +	− + +++
22	E-6	O →	− − − −						
✓23	E-1 + E-6 (3ex).	O →	− − − − 10A30		✓ P3				
24									

Inoc. 530P4. suppl A B C D 1130P Retest 142-17- auxanogram.

9A10 − D +++ A+. suppl. c̄ vitamins.
Many adapted colonies! 12M10. No sp. response. − Entire plate somewhat turbid
to new plate 4P10. Incubate 4h. before suppl c̄ vits. etc.
12M10 − Thiamin.

3/29 — Identify mutants on hand

1. 12N30
2. 4P30

12M30 inoc.

12M31 inoc. 1) 4P31 2) 10A1. 3/2P1

		val	ϕ	try	lys	arg	meth	biot
	TL	TL+3	TL-4	TL-5	TL-7	TL-8	TL-9	TL-10
1 142-17						- ±	± ± +++	
2 142-36 N.G.	- +++	+++	- +++	± +++	- +++	- +++	- +++	- +++
3 142-54	- - -	- - -	- - -	- - -	- - -	- - -	- +++	- - -

~~7~~ ~~8~~ ~~9~~ Postpone inoc.

Check 4/2/46 TL-9 +++ no others.
✓ 4/3/ ∴ probably both methionine.

0 conts. ↓

		11	10	1	2	3	4	5 ~~6~~	7	8	9
	4 156-1				± ±* +++ ✓	• — —					
—	5 156-3	—	— — ✓	+ +++	± ± +++	— ✓	— ✓	— — ✓	— ✓	— ✓	— ✓
—	6. 156-4.	± ±	— —	± +++	± ± +	—	—	— —	—	—	—

No further growth by 10A1.

(1) 10A30.

Check: 4/2 — OK.

* my own prep. other is Tatum's. may not be enough val.

~~1. Try 8.~~

See infra for recheck on 142-17 + 54.

8P4. — Plate 142-17 + 142-54 into T(TL) agar. 1ml inoc. Incubate to 9P4, then do auxanogram on essential a.a. on 54, only "9" = 17 using double depth agar.

Check!

	HZ	B	1	2	3	4	5	6	7	8	9	10		C	D	!
17	++	[illegible]	-	-	-	-	-	-	-	-	-	-		-	++	8P5
54			-	-	-	-	-	-	-	-	+	-		-	-	8P5

Check 54 on lysine 10P5. — 12N7(1)

∴ 54 is TL Meth.

→ M	MT	ML	MTL	TL
—	—	—	++	—

Hydrolysate C.

200 (2×100) T(0) 72 hour culture. Centrifuge cells down, put in 10 ml 6 N HCl, seal tube + keep in boiling H_2O for 2 1/4.

Lost during hydrolysis.

Try again.

Mutants by ultra-violet irradiation

10A 31 Inoc 50ml/125 ml flask coli C-M $\bar{c}$ 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 ~~secs~~ min. Inoc. 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 tripl. 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 dilution in saline also. Incubate + compare sal. + H_2O after 48h. Do in tripl. 1c.

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

2a + 2c 10P2. O O
3a + c 930p3.

U.V. irradiation;

~~#.~~ 15 minute irradiation A1.

Before irradiation, plate counts not made
after 1:50 — 1.

Incubate 1 ml. 2P3 dilute 1:25 x 10^6 + use method II, plates 1-5 for mutants (T(o) + dr bacteria)

Colonies first apparent 9P4. 10A5 layer YBG. (Agar too soft).

	1	
2P4.	2	Plates so soft as to be ~~almost~~ useless.
	3	
~~#5~~	4	
T(o)/YBG	5.	Pick colonies before ... to >8/radiation-resistant.

Test on T(o) — all grew —

5 minute irradiation A2 See 172

i ii iii

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

After irradiation, 1b : 1 ml 710
1:50 9 } not very different. Incubate + shake alternately.

+ send to viability study.

c) Viability of control in sal, H_2O. — Apparently very low in this dilution

			i	ii
Incubate last dilution	10P2	2a	0	0
flasks of 1a, 1c + sample.	10P2	2c	0	0
Plate in YBG	P3	3a	0	0
	P3	3c	0	0

I 10A1. halo c̄ cm diam. c̄ 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 & sl. less impressive over histidine. Both ca 2.5 cm diameter.

B). Supplement other portions of both plates, as above, 8³⁰P. (after prolonged incubation.
10^{30}P — 1B - dist turbidity under HC.
— 1 better.

12 hour incubation probably optimal
Inoculum size is close or less optional + maybe reduced for frequently reverting types.

3) Add HC 9P4. — No response dead?

Incubations, hence at ca 35° unless stated.

~~Auto~~ - auxanography

8P31 - Plate heavily (ca 10^6) into T(o) 2% agar $\bar{c}$ -8 (histidine)

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

II Incubate 14h. first.

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

P2 - # 48 adapted colonies. ✓
Try more conc. agar.

P4 ✓

IV 4/2-3. Try as above $\bar{c}$ 3, 4, 6% agar.

No difference to speak of.
∴ 2% is gd.

V 4/3 - Use of indicator - plate $\bar{c}$ ·8 as above $\bar{c}$ 10γ/cc Methyl Red (also 20γ/ 50γ/). Medium is alkaline to the indicator.

156 – 9.

See 161.

4/2/46.

I Plate 161 – 7 heavily in 2% T(0) agar plate. Add suppl. HC + Biotin to surface 10P

10A – Turbidity increased over HC.
~~Questionably~~ clearer under Biotin. (loopful 1γ/ml)

5P. do. The biotin area is definitely less turbid than the rest of the plate. The plate is ~~[illegible]~~ 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 c̄ L15! (At least adaptation is not "genetic or mutational")

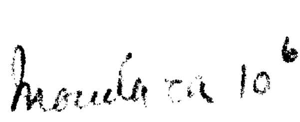
See 173.

Study of 156-9

1. Isolated by Method C. from X-rayed 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 transferred 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 an exp. to complete. Also, growth on minimal reinforces susceptibility. This is not supported by the serial 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.

Isolate
complete
O V HC
(++ complete) (±) (+++)
O V O V O V
+++ ± ++ ++ +++ ++
O V O V
+++ ++ +++ ++

I



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 unusually turbid) compare 5198				
6 679-680	T		leucine	7				
7 "	L		threonine	1				
8 "	0		? L	0				
9 679	0		threonine	2				
10 58-5631	0		adenine	1				
11 58-161	0		methionine	0				
12 58-7651	0		proline	0				
13 58-5773	0		adenine	0				
14 58-218	0		[illegible]	0				
15 58-336	0		isoleucine	0				
16 679-662	0		proline	0				
17 679-183	0		proline	1				
18 58-4899	0		[illegible]	0				
19 58-3214	0		proline	0				
20 58-3336	0		meth	0.				

Mutant Reversions

Inoc ca. 1 ml of inoculum (ca. 10^6) into T/C, or as indicated and incubate 48 hours; look for adapted colonies.
Data on 170a..

Mutant.
E-8-Histidine see p. 168

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

Mustard induces — Coincidentally reds mutate also.

$(-N-Et)_n + H_2O \rightarrow -NH + EtOH)$

Check for biotin requirement: 58-56a

Sex.

4/3/46.

SP3 – Cross-streak on minimal plate:

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

P5 no growth.

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

		Req.
1	679-680	T-L
2	"	"
3	"	"
4	58-278	B-Φ
5	"	
6	Both	
7		
8		

No growth in any by N9. P11

(1 drop x 10^8/cc)

N9. Repeat above c̄ heavier, fresher inocula. in T(0). 10P10

1		TL	±	±					
2		TL	±	±					
11		TL + BΦ1	+	++					
12		TL + BΦ2	+	++					
13		TL + BΦ1	+	++					
14		BΦ2	±	+±					
21		BΦ1	±	+±					
22		BΦ1	±	+±					
31.	In complete	TL + BΦ1	–		+	See 176.	+	+++	
32		TL + BΦ2	–		+		++	+++	
33	test in T(0)	BΦ1 + BΦ2	+	++	+	+	+	+++	
			T(0) 1030 P9 –	P10 930 P10	P11.	T(0) 930 P10 –	P11.		

Check minimal plates

Note: Try plating out from C-M.

2P9 Cross streak on T(0) agar

41. BΦ1 × TL
42. BΦ2 × TL.

nothing by P14.

∴ something happened in the complete cultures between the 1st + 2nd tests in min.

19+27 ...

	Plate #	Total.	Mutants	Mutants	# S.Y.			Rate.
			1230 A7	1130 A.7				
	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 [31]	0			0	
37/		1214	17	14.			6	
		Retest						
Y(58--)		T (b)	HC	Vits.	Auxanogram.			
1		+ ✓						
2		+ ✓						
3		+ ✓						
→ 4	58-Y11	— ✓			HC; D?; ?;			
5		+						
6		+						
7		+						
8		+						
9		+						
Proline 10	Y12	+ ±			A · B C ?			
11	~~58-Y13~~	− X+			hybrid plate sl. inh. HC			
→ 12	Y13	+ −						
13	~~58-Y13~~	− x+			hybrid plate			
→ 14	~~58-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 11 cm in quartz tube on shaker.

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

i 1a. (455) = 5.7×10^9
ii, 1a 97. = 1.23×10^9
iii 1a. 300 = 3.75×10^9

10.7 / 3

= 3.6×10^9

1c (249) = 3.12×10^9
1c (129) = 1.64×10^9
1c. (252) = 3.1×10^9

7.8 / 3

2.6

3×10^9 = mean.

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

= 2×10^{-7}

.00002 % survival. in 5 min.

[Since there 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 II in T(B).

2:30P6. Layer.
12:30A7. 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 proline.

5 mutants in 1200 cells.

5 / 6000. comp. c̄ spont.

	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.	Aucxanog.		✓	
Proline ~~27~~	Y15							
28	~~58-Y14~~	– ✓			HC Iz; ?;			
also test: Proline - 29	Y16	+ ✓						
30	~~58-Y15~~	– ✓			HC large zone; D; A			
31		+ ✓						
32		+ ✓						
33		+						
34		+						
35		+						
36		+						
37		+ ✓						
Tatum's 58	control.	+ ✓						
58-278	"	– – ✓						
Total: 5 stable								
1214 colonies.								
5580.					HC; AAA????			

4/9/46 156-9 vs. L15.

Biotin

Inoc. 3P9 asind.					(1) 9A10. (2) 9P10
	0	0	V.+	Biot.	valine
L15	+++ ✓	+++ ✓	− +++	+ +++	+++ ✓
156-9.	+++ ✓	+++ ✓	− ±	++ +++	
K12	+++ ✓	+ +++	− +++	− +++	

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

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

Also consider:

Eff. pH in drop (for prep. preservation).
enzyme

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

4/9/46.

Auxanography –

Optimal inoculum size. Plate into T(B) varying samples of an inoculum culty. ca 10^8/cc. of 58-278 (B-Ø)

Incubate 12 hours & supplement c̄ HC + c̄ Ø. [B4 = Phenylalan]

Inoc: 530P9 Suppl. 1630 9A10.

		Diam. Ø	Diam HC.
1 ml	1	1.8 ++	2.3 +++
.1 ml	2	2.5 ++	3cm ++
.02 ml	3	2cm ++	3cm +
.002 ml	4	? ±	2cm +
1:250000 .000004 ml	5	2 +	4cm +

↑ distinctness

The method can be used at any inoculum size, but is most [illegible] c̄ heavy inocula. For very adaptable strains, it may be important.

OPTIMAL AGAR DEPTH. 4/10. Inoc. 1 ml undil culture into varying agar depth. Inoc ~~[illegible]~~ ~~Incubate~~ to 11^{30}P9.
Supplement
A – Ø – at t = 0. B: Ø at 1230P11.

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

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
quality of zone	①	10P	0	±
	②	12M	+	+
	③	~~[illegible]~~		
	④	10A12	~~[illegible]~~	–
	⑤			
	⑥			
	⑦			

← optimal < 12h.

sample is 6000.
ca. 5 mutants.

compare 175.

~~hydrolysate A~~ 1:10.

* Contam. c̄ Neurospora + Thermomonas!

4/9 – 10 . . . 1 46.

Spontaneous mutants in 58.

530P9 broz t.t. c̄ coli CM c̄ 58. Shake at RT.

9P10 – dil 1:12,500,000 + plate out by method II in T(biotin).
Incubate at 35° to 11P11 [illegible] 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			
		12h. O	30h.

Add .1ml of "A/10" in lieu of biotin:

11.

Pick to CM 830P14 n.v. satisfactorily
None of the 1st series would have qualified except by sterility criterion. T(B). v.sm. inoculum from CM liquid.

	T(B)
1	+
2	+
3	–
4	+
5	+
6	+
7	–
8	–
9	–
10	no growth in CM
11	–
12	no gr. in CM.

Save (–)'s (4–8)

Iex.

4/12/46

Broc. C-M c: + shakes A12 — P13

1. TL
2. Bφ1
3. TL+Bφ1.

12 M13.		T(0)	P16.	P17	P17: add	A18	P19.
Test 1 } loopful inoc	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×Bφ1 in C-M).

1:25000 ~~1:50~~	10	No colonies P16	Cover c̄ φA1 + B. Nothing came up.
1:12,500,000	11		

			P17	A18	P18
	12	3 into CM, c̄ shaking at 37° 1030 A13			
small inoc.	13	Test: (b).	—	— ‡	+
large inoc.	14		++	+++	
P16. Test 6 sm	15		—	+++ ‡	
6 large	16		—	+++	
7 sm	17		—	— ‡	—
7 large	18		—	—	—

∴ the recovery is as rapid as in medium count of cells! There has been no recombination.

Auxanography.

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 ~~3~~ 1:100 No inhibition try 1 drop HCl/ 10cc biotin, etc.
6 ~~4~~ 1:1000 No inh.
8 ~~5~~ 1:10^4 No inh.

II, Benzene.

1. Center - pure Benzene Clear zone
2. 10 - Benzene-water. OK
3. 5 - Alcohol 95%. OK
4. ~~[illegible]~~ Chloroform OK.

Synthophenins

Pourplates c̄ ca 10^6 organisms and + sugg. where hard, streaks on surface.

~~1. BΦ~~
~~2. BΦ BTL~~
3.

4/17 I suppl II
1. BΦ₁ 0 K-12

~~[crossed out]~~

See N-363 for expt. on symbiosis with N: 33757.
E 181.

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

An complete P19 — No differential zone found. K-12 somewhat inhibited.

Salt resistance mutants.

after Severens & Tanner.

7/17/46. 58/
1. Plate ~~[illegible]~~, ca 10^8, into complete plates +

1. $CuSO_4$. ~~5mg~~ 10mg
2. $MgCl_2$ .1mg

A19. Nothing came up. use less.

A Penicillium sp. contaminant did grow on (1)!

Syntrophism

58-161 + 58-278
14 φ.

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

Dens. 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~~ 9?	95	−	!
3	M	0	0	−	−	93	93	−	
4	φ	0	0	−	−	94	93	−	
5	K-12	0	0	++	+++	58	57	+++	
11	φ+M.	1	0		−	94	93	−	
12	"		1		±	90	83	+++	
(13)			3		±	68	61	+++	
(14)			10		−	83	75	+++	
21	"	3	0		−	94	94	−	
22			1		−	93	93	±	
23			3		−	75	63	+++	
(24)			10		+	66	67	+++	
31	"	10	0		±	88	84	+++	
32			1		−	93	92	±	
33			3		+	84	13	+++	
34			10	−	±	81	80	+1.5	
41	"	0	0		−	92	92	−	
42			1		−	92	91	±	
43			3		−	93	93	−	
44			10.	−	−	95	do.	−	

75 = ½ max. 73 73

Held 45. ~~Plate φ+M into plates c̄ 5γ methionine; 100γ phenylalanine (plate to see whether this is a good separation~~ φel. inadequate

(22) to 38°. This does seem to speed 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

~~4/19/46~~
~~Hold!~~

Dr before:

		Supplements		
S	Broc	Φ	Tyr.	FPA.
51	K-12	0	0	10γ
52		0	0	100γ
53		10γ	0	100γ
54		100γ	0	100γ
55		0	10γ	100γ
56		0	100γ	100γ
57		10γ	10γ	100γ
58	58-278	1γ	0	0
59		10γ	0	0
60		100γ	0	0
61		10γ	100γ	0
62		10γ	0	10γ
63		10γ	0	50γ
64		10γ	0	100γ
65		0	10	100γ
66		100γ	0	10γ
67		100γ	0	50γ
68		100γ	0	100γ
69		10γ	10γ	100γ
70		0	0	50γ 100γ
71	58-4899	0	0	100γ
72	58-5030	0	0	100γ
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] ǂ	+++ (green tint)
86		100	–	67	65[1] ǂ	+++

ǂ test on minimal: they grow.

Mutant detection:

Viability

Pour 58-278 (24h shaken), dil. to 1:12,500,000 into T(b) plates + cover as in mutant detection. 830 P19. Cover c̄ complete at time t₁. Colony diameter recorded at t₂.* Incubate at 38°.

	t₁	hours	h¹ 430P20	h² 830P	10A21	4P21	8P21	12M21	Count
1	9P19	1	19	5		+++ considerable 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			—				
9	10A21	36				—	±++	+++	++	ca. 380. ← too many bottom colonies
10	8P21	48.						++ 9A22	349
~~11 ... ~~					Bottom colonies troublesome!				353
12 + 1mg ∅		0		6	∴ 7 hours is barely too long for period II. Past runs n.g.				

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

			11A21	8P21	12M21	10A22	P23		Count	Δ
21	9P20	+	++	++	+++			0	131	−9
22	9P20.	+	++	++	+++			0	139	−1
23	1P21		±	+	++			16	138	−2
24	1P21.		±	+	++			16	151	+11
25	8P20			—	+	+++		23	154.	+14
26	8P20			—	+	+++		23	133	−7
27	930A22	Uneven pour: lumpy.				++		36	139	−1
28	"					++		36.	123	−17
29	3P23	7:15P — (5) colonies quite distinct mici. indicates (3-5 h)						64	156.	+16
30**	1130A24.	26°	130P: colonies mini. destroyed !!! —		(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 Count	+	+++					0	143	+13
35	"	+	+++					0	138	+18

Count. m = 140. ΣΔ² = 1097.

σ = ~~9.~~ 8.85

$\sigma_{calc.}^{140} = 14.$

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

* 7 small colonies noted 10P27. punctiform.

Pick colonies 11A30. see 194.

* 15 units = 1 mm.

Auxanography: pre-incub time series,
use ϕal.

Put only 2 zones/plate.

Inoc T(B) plates heavily c̄ 58-278. ~~me~~ 230 P21. Incubate to t_x at ~~2~~8°. Then suppl. c̄ drop of dl ϕalan. 6:50 to 38° incub.

A22

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.
172-10 Inh. hydr. casein. Seems proteol.
— 172-12 A✓ D1 inh D7 Proline inh tyrosine
172-14 —
— 172 28 A, D. D1 inh. D7,8 Proline " + cyst
— 172 30 A, D. D1 " " Proline. " + cyst

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

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[1]	
St	91[2]	

Red filter 660

	74.
A	53
St. 20 mg/ml	25
10 mg/ml	48[2]

Green filter Abs = 0.

71[1]

	Galv.	Dens.	Dens/dil
A.	43	367	367
A/2	65.	187.	374
A/3	75[1]	124	372
A/6.	87	060.5	363
A/9	91	041.0	369.

mean = 369.
m.d = 5
= <1%.

367 = $3^2/_3$ = $^{11}/_3$ ≑ 10^9 cells.

1 d = ca. 3×10^6

ca. 10^6

1/2.3 $\times 10^6$

1/50 .1/50 .2/50

4/22/46.

24 hours shaken tube culture 58-278. Irradiate at 11 cm. for 2 mins. Mix 1 ml into another 10 ml and shake 24 hours. ( ).

9P22.
Mix 1 ml into coli Car + ~~dilutions~~ de Growth quite clumpy, very slow. ('intibition')?

60
125
7500

dil 1:500 – ca. 7500

1:25,000 – 125 These figures check.

50

∴ survival is ca. 500 × 7500 = 3750,000 out of 3 × 10^9
= 1%. ≐ 2 mins.

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

	0 4P26.	4P26.	3P27.	10P28.
1		nothing*	growing rather slowly.	
2				
3				
4				
5				
73				

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

(See also 183)

See 194.

no good

4/23/46.

Syntrophism
FPA. 187.

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

		ϕ	M.	24h. M24 2P25	38h. ||P25	G 11P25.	d. ||P26
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	+

everything still clear

II	~~679~~	ϕ	FPA				
31	ϕ.	100γ	0		±	—	
32		100γ	100γ		—	—	
33		200γ	100γ		—	—	
34.		200γ	200γ.		++	+++	64. .194
		200γ	0.	See 13.	—		
		0	0	See 7.			
		0	+	See 181.			

77.

n.g. Old culture inoculum?
See 193.

58/r - Mutations + Killing.

4/26/46. Inoc in complete. U.V. radiation 11cm etc.
T(RAD)

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 ρ^S = 3/2m.

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 1:2,500000 1:2,500,000 0

Inoc flasks of 50 ml Complete Coli c̄ 1 ml of each dosage above.

D
1 6. .1
2 7. .1
3 8. .1
dil c̄ 4 9 .1
5 10 ✓ .1
6 12 ✓ .1
7 15 ✓ .1
8 20 .1
A 30 ? ...

0 - 2 probably out.

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

197 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 } 250.
14 2 33,34 0 2
15. 0
16 complete.

3P29 0 4A30 x 10P30 ⊖ 1030P1 ▥ △

Sm colon. Total
4 250

Pick ~~196~~. 197

Triple mutants.

4/26/46

24 hour culture shaken at 30° 58-278. ~~[illegible]~~

4P26 Plate unir. culture 1:12,500,000. into T(B, ϕ). 3P26. .02 ml into CM 192 A.

	150A28	1230P28	10P28	10A29	10P30 (35)	P1	
1	—	—	—	—			26
2	° —	° —	• —	• —	0	N. crassa conidia.	37
3	—	—	—	—			29
4	—	—	—	—	2	36, 37.	38
5	—	—	—	—	2	38, 39	31

Irradiate 2 mins. → 2 5 total — 161

10. 1:25,000. in coli complete. Centrifuge + resuspend. 750
ca. ½% survival.
1:25,000 in T(B ϕ,) etc.

Layer c̄ complete + 10P27 exc. 3+13.

11	° —	• —	° —	° —	4		172
12	—	—	—	! (3)	6		253
13	—	—	—	—	0	0 (not layered).	1099 { 227
14	—	! (1)	—	! —	1		242
15	—	—	—	—	0		198

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

	3P29 O	18h. 4A30 X	36h. 10P30 ⊖	1030P1. Δ.	— 5P3
21	!	°	!		
22	•	°	3		32
23					
24		1	1		
25²		0	0		

x ~~25000~~
~~750~~
38 x ~~12,500,000~~ 502

240 tested

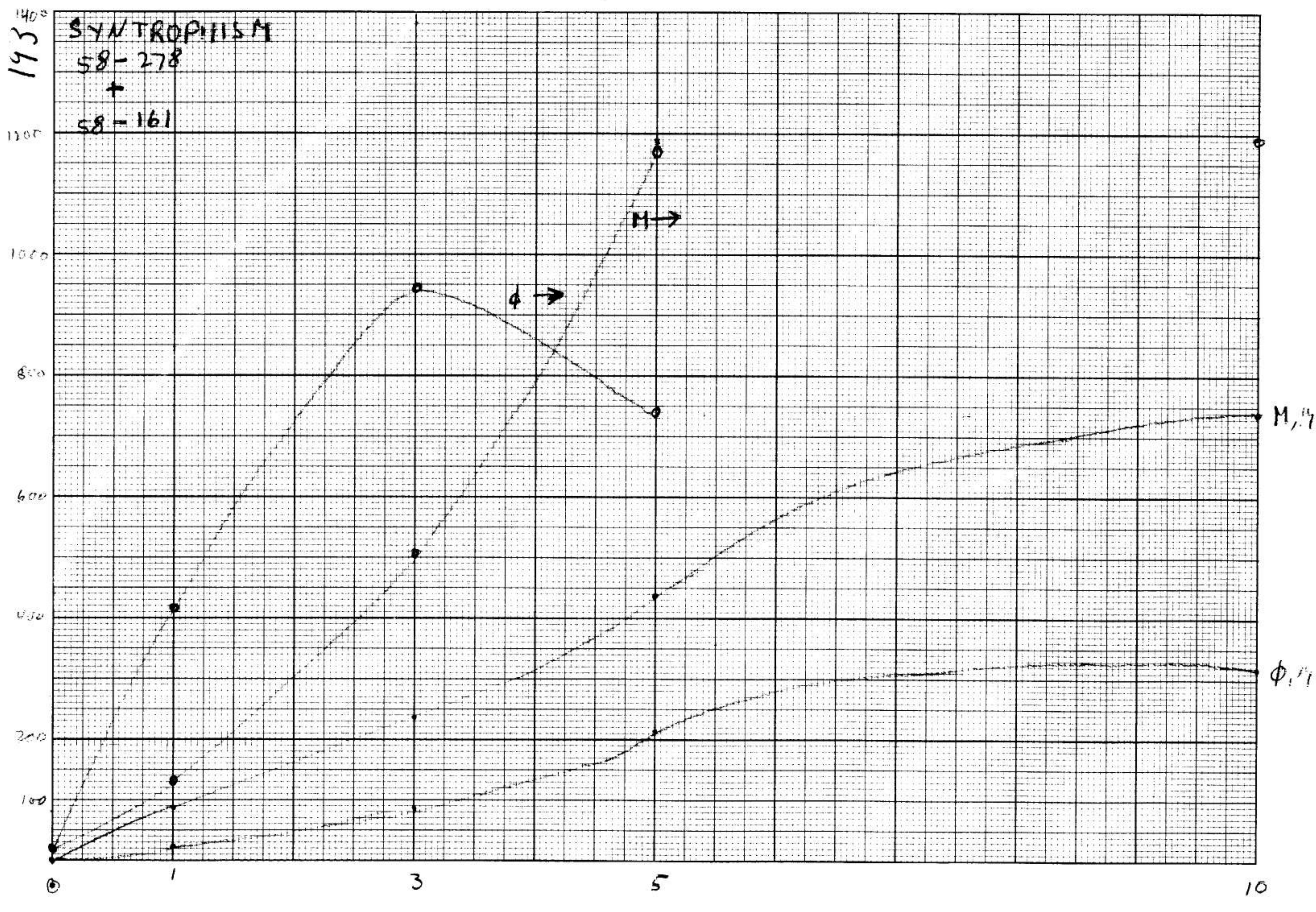
26°	°	1	N - contamination n.g. for pick.
27°	°	3	
28°	•	2	20.
29°	°	0	
30.	°	2	35
31 complete.			31

1/238 :

Cover c̄ saline - glucose - agar.
Layer c̄ complete 10A29.
Prototrophs unusually large here!

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

Pick 19/47



M5, Φ →
143
SYNTROPHISM
58-278
+
58-161
1400
1200
1000
800
600
400
200
100
M→
Φ →
M,4
Φ,4
0
1
3
5
10

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 (v.)	M.	10P28	11A29 (34h.) (77) G	D'	12M29 (48h.) (77)	12N30 (60h.) 72²	11P30 (72h.) 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	1/10 "	5	10	+±	84²	731	84¹	85	86²	
6	"	5	100	++	63¹	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	±	95¹	212	93³	88¹	77² *	No prototr. replating!
15		10	5	+	93	315	90.	79 *	73 *	
16		200	5	+++	61¹	2129	60²	61¹	61	
21	M+Ø	0	5	+	90¹	446 (-M5) 12	89¹	81	72³	54
22		1	5	+	82¹	848 414	71	74³	77³	44
23		3	5	+	72³	1382 948	71	72¹	72³	
24		5	5	+	76¹	1117 743	70	73²	73	
~~25~~		~~10~~	~~5~~							
31		5	0	+	96	177 (-65) —	95¹	62¹	63	
32		5	1	+	42¹	301 139	74	67	69³	41
33		5	3	+	84³	718 506	67	71	73	
34		5	5	+±	72³	1352 1170	67²	72¹	72	50
35 (plug wet)		5	10.	++	72¹	1412 1200	71	73²	74	

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

		Proline						N2
41	G	0	—	100	100	100	100	—
42		3	—	100	100	100	100	~~+++~~
43		10	—	100	100	99²	97¹	~~+++~~
44		100	—	92²	84	83²	83	~~+++~~
51	P.	0	—	100	100	100	100	—
52		3	±	97¹	97³	98² 96²	97²	+
53		10	±	96	95	96² 93²	93³ *	+
54		100	+±	69¹	68¹	68³	68²	~~+++~~
61	P+G	0	—	100	100.	100	100	—
62		3	±	98¹	96	81	79¹	~~+++~~
63		10	±	96³	81³	74¹	79 * 76³	~~+++~~
64		100	+±	66³	68	68	66¹	~~+++~~

Note - change to EDST. 2A28.

* Adaptation.

1 A.M. 4/00/46.

Remove .1 ml of culture ~~medium~~ from tubes 22 + 32 and plate into Γ(x) at a dil. of ca 1,250,000. Bottom + cover.
After counting 1 mutant, layer with other supplement + count the other. Dil. only ~~[illegible]~~ 500 for (.3) plates.

22a. x = biotin + methionine.
b. x = " + phenylalanine.
c. x = ".
etc -

3P2

			L	S.	Total ✓	M %.	later:
22a.	52. (v. small colonies) (→ 117)	~~[illegible]~~	117	125 (251).		46.5	42.5
b.	114. 10P30. (→ 125)	~~[illegible]~~	w.T. 0 (1)	125 ~~[illegible]~~ 94?		43.0	
32 a.	67. (later 114 →)	~~[illegible]~~	114.	149	d = 1308.	43.2	39.3
b.	141	~~[illegible]~~	w.T. 00.1%	145 ~~[illegible]~~		do.	

32c ✓(1:500.) 3 colonies seen 10P30. Divide by 2500 for ratio c mutants (Later, at 4P1, d = 1739.).

22c. 0.✓

Notes from. 1A30.

Layer c lateis: 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 slower to develop initially or on layering. 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 syntrophic cultures.

4P1.

	Culture	M r	ϕ r	G	d Deletion	Colony counts on: T(B)	T(M)	T(ϕ)	F(M)+ϕ	T(ϕ)+M
F two-sized groups here.	193-21	5	0	% M. 54		10^3 $>10^4$-$5\cdot10^6$	194	F 10^6 170	369	380 ~~369~~
	-22	5	1	44		" 0	106	F 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 F 161		330.
but 58-278 adapted. What is sign of adaptation here.	14	5	5,			(10^6) - 0 !	/////	F 10^6 - 246		

62 10r — T(G)(T.) 10^6 (N) 392; T(T) 10^6 0 ✓; T(0 + ✓).(part of plate, ca 1/4 missing) 380.

∴ assume that another 1/4 = prolineless.

1st counts 930P2

Layer c complete 12 AH. those marked ✓

Size differential is maintained indefinitely.

Thus, culture 31 was adapted (no detection tried of M or ϕ cells)

21 is also adapted, and contains, undoubtedly, 3 cell types.

	% M.				% M.
21 M	52.5		34	M	
21 ϕ	55.3.			ϕ	~~51.8~~ 49.7
R	53.2			R,	49.5
22 M	46.0	46.0			
22 ϕ	~~43.3~~	43.7			
R	42.5	42.5			
31 M					
31 ϕ					
R	**				
32 M	—				
32 ϕ	43.5				
R	39.3				

W.T. = .9

Spontaneous mutants.
Small colony variants.

58-278 A 3a Pick colonies on 183 plates to 1 ml coli complete liq.

		Complete	Test on T(B,φ).	to CH slants P7.	Test on 1 ml Bφ 5/11	Test Bφ 5/18		– A20
	1	+	–		–	+	+	+
183-32	2	±	–		–	–	–	–
	3	–						
	4	+	–		–	–	–	✓
	5		–		–			
183-29	6	"	–		–	–	–	✓
	7		–					
183-31	8		–	v. sparse	incr. heavily	–	–	✓
	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 had a gd control		

21a. to 5 ml 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.

One must examine plates daily
for 3-4 days.

Syntrophesin
58-278 + 58-4899.

4/30/46. 530PM
T(B) 10ml +

		10P1	N2
1. 5γ pal	58-278	++	++
2. "	58-4899	++	+++
3. "	58-278+58-4899.	++	+++

4.9. - too much pal

4/29/46. syntrophism - plate proc. 195a

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

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

(circle diagram: 1, 2, 3, 4)

Response of ~~[illegible]~~ 679 – 662
to glut & prol.

4/30/46. Is response to prol, which is delayed, an adaptation?
530PM.
Inoc T(T) + suppl. c̄ 679-662

1. proline 200γ
2. glut. ac. 200γ

abandon temporarily.

Used 1-2 transfer in each soln.

* different colonial appearance. Stain c̄ acid-fast...: diplococci: ∷

58-278

Cystineless

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

χ^2 tests:

1) – 2)

f_0	f	
2	5	161
13.	10	1099
	15	1260

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

$= 5.2.$ $p = .023$

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

2.0.

$p = .15.$

1) – 3).

f_0	f	
12	5	161
17	24	240
	29	401

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

$= 4.1 + 2.0$

$= 6.1.$

$p = .014$

2) – 3

f_0	f	
28	10	1099
6	24	240
34		1339

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

$= 11.6 + 54$

$= 66.$

$p = \ll .001$

Pick "mutant" colonies to coli complete

See 194

5P3

All colonies exc. O were of dest.-size at picking.

Plate.	Design.	#	Complete test		BΦ (agar) CS (From plate)	BΦ test 5/11 (From plate)	BΦ 5/18		— A20.	BΦC
91-12	X	31	+	A1	↓	+ A12	−	−	✓	Arginine
58	X	32	+	2		+	±	±	✓	
91-13	X	33	+	3		+	±	−	✓	Leucine
91-14	X	34	++!	4 + |||		P11+++				
						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	x					+
	Θ	42		12	x					+
	Θ	43		13	?	−	−	−	✓	
	Θ	44	"	14		−	−	−	✓	
92-12	O	45		15		−	++	✓	✓	
	Θ	46	"	16	x					+
	"	47		17	x					+
	"	48		18		−	±	+	✓	+
	"	49	"	19		−	±	±	✓	
	"	51		20		−	+	+	✓	
	"	52		21		−	−	−	✓	
	"	~~53~~	"	22						
	Δ	54		23		−	−	−	✓	
	"	55		24		−	−	—	✓	
	"	56		25		−	−	—	✓	
	—	57		B1	✓	↓	+	+	✓	
~~92-13~~	~~Θ~~	58		2			−	−	✓	
92-14	Θ	59	"	3			+	+	✓	
	Δ	60		4			−	−	✓	
	Θ	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	−	13			−	−	✓	
	Θ	70	+	14			+	+	✓	
	Δ	uc. ~~71~~	~~+~~	15						
92-23	—	71	+	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			+	±	✓	
	Θ	80		25			±	±	✓	

none grew, but no diff. 58-278 by P12.

						BφC			
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~~ In series 197- 35 to 82, 58-278 treated c̄ u.v.

5 - grow on minimal

39 grow on biotin + φal + cyst.

1 (#61) - ?

Auxanography: 19 ? nutrients. 197C

Plates not sterile. Pour plates 10 PH. Inc 30°
1230 A — A) ⌀ 10X B) etc. Inc 35°

5/18/46.

	5A19.9A		Sp			A24 T(BC⌀) Final identity	Y—
Y11	AD		D SA	1 Proline —			
Y13							
Y14							
Y18	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?					
YY21	A D	turbid.					
25		A					
29	A	—					
31	A C		C SA	— 8 turbid Arginine.			(23)
32	A	—					
33	A	AD	D 11A.	1??			
34	A D!	A					
35	A	—				+	
36	A	—				+	
37	A	—				+	
39.	A	—				+	
43	A	C	C 11A			+	
44	A	C	C 11A	————		+	
48		—				+	
49	A	—				+	
51	A	—				+	
52	A	—				+	
54	A	—				+	
55	A	—				Growth on +	
56	A	—				T(cyst) +	
57		—				liquid. +	
58		AD	D 11A	6	B⌀C		24
59	AD	AD	D 11A	6	"		25
60		A			"	+	
61	turbid	A				—	
62		AD	D 11A	6	B⌀C		26
63		AD	D 11A	6	"		27
65	A	D	D 11A	6	"		28
66	AD		D SA	-6? (Cyst) ✓	"		29
67							
68	A D	AD	D 11A	6	B⌀C		30
69	—	A			"	+	
70		AD	D 11A	6	"		31
72	A	—			"	+	
74					"	+	
75	A D		D 11A	6	"		32
78		AD	D 11A	6	"		33
79	A	AD	D 11A	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	—					

Margin notes: Y22 turbid (beside 4–6); Y12 D6 AD 5580 A (beside 31–32); 12 N — put palat C, B, D. Proline del. Error! (beside 48–57); where? (beside 67)

Method A: Bact. hydrolysate C 198.

Inoc. 200 ml in 500 ml Erlenmeyer c K-12, on shaker 31°. 8P 5/3

1. Coli complete

2. T(0).

① Harvest 8P 6 (& wash superficially) into 20 ml 6N HCl. reflux 1A7 – 1P7 (calc 6 x 10¹¹ 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(0) has not been growing well.

B/r mutants.

5/8/48

Inoc 10 ml c∞ c̄ B/r from c∞ slant. 1130A8.
1130P9 d = 1.4900

Irradiate as above for ~~[illegible]~~ t. (ρS. calc. as ~~[illegible]~~.9/min.).
Constant killing curve points.

		Rad. t.	Dil.	Count.	ρS.	ρS/t
	1.	0	1:12½·10⁶	86.	1.2×10⁹	0
	1 "	"	"	83.		
	2.	20 sec.	10²	>>		
a	3		10⁴	ca 10⁴		
	4.	•	10⁶	~~508~~ ca 900	.9×10⁹	.12
	5	60 sec.	10²	>>		
	6		10⁴	ca 10³		
b.	7.		10⁶	558	.56×10⁹	.33
	8		.1	>>		

Inoc 1 ml of a and b into 10 ml coli ∞.
Use. b. only Apply mutant method. 1130P90.
d opt = 1.612
dil. 2: 12,5 × 10⁶

	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	7	
7		7	
8		8	
9			nothing seen.
10. not layered.	6		
	6.	2	

650.
~~145~~ 6500

1130P11 (24 hours) - colonies in T/0) visible but very small. Hold ∞ layer.
145 P12 38 hours. - good colony spread; sig. sl. small but uniform.
Layer c̄ ∞ 2 P12.

1130P12 colonies sl. larger than ⑩.

Picks to complete liq. 1 ml:

Picks to complete. A21.

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

Auxanograph 5/23.

3 - C8 - Arginine
4 - C1-2 - leucine or isoleucine!

Bact. Hydrolysate D.

5/9/46.

Inoc K-12 into 200ml T(0) as 198. Use fresh culture.

A. - unshaken 9P9.
B - unshaken

medium n.g. Repeat A11.

see 200, 202 ff.

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

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

$\frac{848 \times 250}{20}$ = 1060 d/ml. This may be useful in calculating recoveries of various substrates.

Precursors and filtrate activities

5/11/46.

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

Coli Minimal +	Amt.	2P12	Growth. 1130P	Final growth G 1:10.	d
1. + 0	500 ml (250 unsh / 250 shake)	+	2+	96	177
			3+	82'	848
✓ 2. glutamic ac. 5 mg.	50 ml	+++	++++	67²	1707
✓ 3. anthranilic ac. 5 mg. Harvest 10A16	"	—	±	86	655
✓ 4. citrulline 5 mg.	"	++	4+	71³	1442
5. pantoyl-lactone ~~ornithine~~ 1 mg.	"	++	4+	76² lost.	F163
✓ 6. phenylalanine 5 mg.	"	+++	4+	not done.	
7. 0	"	++	4+	71' (74)	1472

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

(Pool bacteria of # 1, ~~2~~, ~~3~~ for hydrolysis. Preserve others in alcohol. collect in 25 ml 6 N 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 ~~[illegible]~~.5Y7 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)
autoclave + inoculate ē 5531.

3A15. 30°

(79#)

Inoc.	1	2	3	4	5	6
	0	202-7		1ml	1ml 202-7 +	1ml 202-7 +
1 58-3214.	"	1ml	.1mg glut.	202-2	.1mg prol	.1mg gl
	− −	± ±	−	− ±	++++	− 0 ±
	100		100	100		99
2 R572-228	"	"	.1mg citr	202-4	.1mg arg.	.1mg cit
	−	± ±	− 0	± ++++	++++ ±	±
	100		100			
3 58-5030	"	"	.1mg phel	202-6	.1mg tyr.	.1mg phel
	− −	± ±	− −	± ±	++++ ±	0 ±
	100		100			
4 K-12	"	"				
	+ +++	++ +++				
	+++	+++				
5	"	"				
6	"	"				
7	"	"				

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

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

Interaction of nutritional requirements

5/10/46.

679 – 183.

Kroz 1130P10. (10 hours culture!)

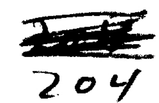
	T. (γ)	P. (γ)	830P11.
			21.4.
1	500	0	100
2	"	10	93
3	"	30	(81') proline limiting value.
4	"	100	62
5	"	300	63
6	1	500	100
7	10	"	97
8	30	"	93'
9 (mis nor?)	100	"	←
10	300	"	(83³) threonine limiting value.
11	1000	"	71'
			59
12	100	10	94
13		30	84 ← not depressed below T lim.
14		100	±
15		300	±
			±.
16	1	30	100
17	10	30	97³ } ‖ to T response at higher proline.
18	30	30	95 }
19	100	30	83, ←
20	300	30	81'
21.	1000	30.	82.

(18)

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 = 40γ

Threonine = 100γ.



α = 679 · 185

β = 58 · 161.

5/13/46. kroz 3A vs. 30°

	kroz	Biotin	mr Meth.	Thr. r	P d r	11P15	10P16	8A17 (to M17)		
1	1	—	—	50	(a)	±	+	89	505	
2	α	—	—	(500)	10	±	+	98^3	281	
3	3	1/3	(100)	—	—	+	++	183^1	796	
4	3	1(10)	3	—	—	±	+	94^1	257	
									D+.	ΣD calc.
11	α + β	(10)	3	(500)	10	lost. had grown to				
→ 12	"	(10)	3	50	(100)	+	++	77^1	1121	538
13	"	.3	(100)	500	100	+	++	76^1	1177	1077
14	"	.3	(100)	50	100	+	++	73^2	1337	1301
15	"	(10)	(100)	—		+	++	70^2		
16	"	—	—	500	100	+	++	74^1		
21	β	.3	3	—	—	+	+	+92^3	339	
22	α	—	—	50	10	+	+	+ 91^1	348	
23	α +	.3	3	50	10	++	++	++ 79^3	982 > .	
									A18	A18
31	α + β	10	—	500	—	—	—	—		—
2		"	.01	"	—	—	—	—		+++ A20
3		"	.03	"	—	—	—	100		— +++
4		"	.1	"	—	—	—	100		—
5		"	.3	"	—	±	±	+++ 59^1	+++	+++
6		"	1	"	—	±	±	± 96^3	+++	+++
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	"	β alanine 3			±		±	+	+++
62	"	"	3			±		±	+	+++
63	"	"	3			±		±	+	+++
64	"	"	3			±		±	+	+++
65	α +	coli								
66	β	—	100			—		++ 77^2	? adapt?	

Margin notes: 4; 6; 5 dual exchange; 7

10P16 - nice 51 and 52 c 58-3214. (α) 77^3

See 207

Synthophism - Sex.

679-183 × 58-161.

20/9.

1/2 values - 81 ca. orgals.

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

679	200γ	T
183	30γ	P
58	.7 mγ	B
161.	10γ.	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 0-5γ.
c. 228 adapt. series at 5γ.

Syntrophism.

10γ proline
All 58-cultures c̄ .01γ Biotin
679- c̄ .5mg threonine

1130P10. 30° 5/10/46.

	Inoc A	Inoc B.	Supp.	1130P11.	1130P12	1130A13	Ro (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[1]
7.		Y1	"	+	+	+	93[3]
8.	✓	Y1	"	+	+	+	93[3]
proline req? 9		Y13	"	—	—	—	
✓10	✓	Y13	"	+	+	+	89
✓11		209-301R	"	++	+++	(autol?)	77
12	✓	209-301R	"	++	+++		79[1]
✓13		58-2651		+	+	+	90[1]
14.	✓			+	+	+	90
✓15		58-3232	"	+	+	+	90[1]
16	✓			+	+	+	89
✓17		58-52.55	"	+	+	+	87[2]
18	✓			+	+	+	84[1]
19		5450	"	—	+	+	91[1]
✓20	✓			+	+	+	91[1]
21		6049	"	—	+	+	91[1]
✓22	✓			+	+	+	92
23		6177	"	+	+	+	89[2]
24	✓			+	+	+	91

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

Note 1 cell contains ≮ 10^{-12} g. H_2O. [illegible]

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 time, there will be only 30]

A [illegible] requirement of 30 γ / 1000 γ is quite reasonable

for protein 10–40% of the dry wgt.

Dry wt. / cell d = 3×10^6 cells. = ca 1 γ

∴ 1 cell ≑ .3 γγ dry = 3×10^{-3} dry.

ca. 1 γγ wet.

This is less than previous estimates: (8×10^{-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.

Inoc 11-12 ZA15. 5/15/46. Room temperature.

11A16 — 1, 2 ++ 3 ±
48h. 12M16 — 1, 2 are nearly opaque; 3 ++. growing slowly | A17. 3+++.
930P17 - all indistinguishable. Harvest - pool in large container + put in ice box.
Both are 90[2] at 10:1

l. 1.
2.
3.

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 4l. (1), 4l. (2), 3l. (3), 1l. (4) fractions, ca.
and reject fraction 2. Allow others to settle further; centrifuge 500 ml samples from 3 + 4 and collect + accumulate the harvest.

P5. Dry accumulated harvest after collecting in saline - note swirling, native cells largely (?) intact - and wash $\bar{c}$ H_2O. Dry 1) over $ZnCl_2$ 2) at 100° 2-3 hours. Extract $\bar{c}$ 100 cc 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 × 10 × 43 = 5,200000 5.2 × 10^{6} density units ≙ 3.406 gm. or 1 density unit ≙ 1 γ dry wt. bacteria, and average culture remaining 10 ml $\bar{c}$ G = 60 has about 2 mg dry bacteria in it.

Assays of 4 hour hydrolysate
Neurospora.

Mor 1A6. 30°.

A. Biotin. 5Y7A. 9A8.

1. ~~25~~cc "Biotin"-free Fries. ±
5Y7.

2. do. + ~~[illegible]~~ +++
1ml 206A.

3. do. + 1ml 206A + .05γ biotin. +++

B. Inositol. 34701 a.

1. 10cc Fries. —

2. + 1ml 206A. —

3 + 5ml 206A. —

4. + 5ml 206A + 5γ inositol. +

5 5γ inositol. +

C PAB. 1633A

1. 25cc Fries. —

2. " + 1ml 206A. +++

3. " + .1ml 206A +

4. " + 1γ pab. +++

5. " + 1ml 206A + 1γ 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 20cc.
lost.

←

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

≗ .190 mg Concentrate +
neutralize c NaOH. Store as 1% bacterial hydrolysate

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

Use 204 - syntrophism cultures:
Mixtures of BM. + TP.

P. 5/17/46.

#	Grown in excess:				P19		Layer:	%BM.
1.	11. Lost							
2.	12 BT.	Plate 1:1000	in	T(0).	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}		0.	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}		0	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}~~		~~∞~~	~~[illegible]~~			
32		10^{-3}		0	0			
33		10^{-3}		BT	4	4-7		
34		10^{-3}		BP	0			
35		10^{-3}		MT	0 cont? ✓			
36		10^{-3}		MP	1	8		
37		10^{-7}		BM. ✓			∞	
38		10^{-7}		BM. ✓			PT.	

See 212.

Where possible recombination colonies are present, pick to a complete liquid (BT). ~~A~~. A21.

Note: only B-T. Why?

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

	T(0)		T(B)		T(T)		T(BT	
	PP22	PP22						
1	—	✓	±	✓	±	✓	+++	✓
2	—	✓	±	✓	+	✓	—	✓
3	—	✓	±	✓	±	✓	—	✓
4	—	✓	±	✓	±	✓	—	✓
5	±	✓	±	+++	±	✓	+++.	
6								
7								

Retest on various = #5.

A BT strain? Call it 58-619x. (1)

} Why no growth? trace req. (2–4)

Streak out 1 on a complete plate.

Sex

v. 204. 679-183 (α) x 58-161 (β)

5/21/46. 30° 2A 23.

P2

	Inoc.	mγ Biotin	γ Methionine	γ Threon.	γ Proline	12h.
	1 α	10 ✓	3	500 ✓	10	+
	2 β	10 ✓	3	500 ✓	10	++
	3 α + β	10 ✓	3	500 ✓	10	++
	4 α + β	10 ✓	3	500 ✓	10	++
	5 α + β	10 ✓	3	500 ✓	10	++
BT.	6 α	10 ✓	3	500 ✓	100 ✓	+++
	7 α	10 ✓	3	500 ✓	100 ✓	+++
	8 ~~α~~ β	10 ✓	100 ✓	500 ✓	10	++
	9 β	10 ✓	100 ✓	500 ✓	10	++
	10 α	10 ✓	100 ✓	500 ✓	100 ✓	+++
	11. β	10 ✓	100 ✓	500 ✓	100 ✓	++
	12 α + β	10 ✓	100 ✓	500 ✓	100 ✓	++
	21. α	.3	100 ✓	500 ✓	10	+
MT.	β	.3	100 ✓	500 ✓	10	++
	α + β	.3	100 ✓	500 ✓	10	+
	31. α	10 ✓	3	30	100 ✓	+
BP.	β	10 ✓	3	30	100 ✓	+
	α + β	10 ✓	3	30	100 ✓	+
	41. α	~~10~~ .3	100 ✓	30	100 ✓	+
MP	β	~~10~~ .3	100 ✓	30	100 ✓	+
	α + β	.3	100 ✓	30	100 ✓	+

— Do not use. ? autolysis or phage??

51. coli α, 50 ml flask, shaken α +Y
52 " β +Y
53 " α + β +Y

Take off shaker 11A28

Culture filtrates.

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

2 #. glut 5 mg, putlactone 1 mg, B-alanine 1 mg
3 #. prol 5 mg put .5 mg

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

To desk top N22
sample a 10P22.

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

11. 1a 5 ml + T(B) 58-3214. mor N23.
12 2a " "
13 Proline 25 r + 1a .25 cc + glut 25 r.
14 ~~3a .25cc + T(B)~~
3a 0,25 cc + T(B) 10 ml.

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

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

Try: analyse 207-1.

Streakout 207-1 on ac coli [illegible] plate P22. Isolate 10 colonies to H_2O, and test; transfer to ∞ slants. 3P23.

∴ threonine not required.

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

Test 207-2-4. 11P23.

	0	B.P.M.T.	BM	BT	MP	MT.	BP	TP (24h)	TP (32h)	
2		− ++	− −	− −	− −	− −	− −	− −	++	Maybe a multiple mutant.
3		± ++	− −	− −	− −	− −	− −	− ±		
4		+ ++	− −	− −	− −	− −	− −	− +	++	
5		− ++	− −	− −	− −	− −	− −	− −	++	
8		± ++	± ++	± ++	± ++	± ++	± ++	±	++	

Finally: +.

2 - BPMT, PT. 679-183. ✓ 212B.
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 ~~[illegible]~~ BPT MT.
207-1
207-1A.
207-2
207-4
BM
BP
PT

See 212.

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 c̄ "T" after B is detected.

207-2 behaves like a culture of BMPT c̄ 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 c̄ T.

5/26/46

I Cystine Requirement of Y24.

1A26. A. 10ml T(B, φ) + B: .01tr φ 300γ

	Cyst.	8P27
1.	10γ	89[1]
2.	30γ	84[1]
3.	100γ	83[2]
4.	300γ	76[3]
5.	1mg.	68

← ½ opt. like 58-309.

11. Methionine: 1mg. ± not parathiotroph.

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

II Otherstrains:

			T(0)	HC	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
Sex.

5/26/46.

Scrape growth from slant, suspend in H_2O + dilute as indicated. ✓ (works well)

12M-1A26. ~~Use T(o) c ½ usual phosphate [T_1]~~

To obtain clean agar, autoclave 3% agar + T(0) 200% quantity. Thursday. Mutant selection procedure.

A. 207-1. P27. Layer c Added col. 11 P207. 11A28.

1:1000 in:

B^-M^+ 1.	B	+++			
2.	B	+++			
3.	BT	+++			
4	T	0			
1:10⁶					
11	B	51	: T	0	
12	B	39.	: T	1	#4 → requires B.
13	BT	43			
14	T.	0			

Pick to complete M28.

For analysis see 219.

207-2.

1:1000 in:

B. 21	PT	+++			
22	PT	+++			
23	BM~~B~~T	+++			
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 ~~[illegible]~~

1:1000					
41.	T	++			
42.	B	0			
1:10⁶					
51	B	0			
52	T.	3	'B	1	#3

FPA.

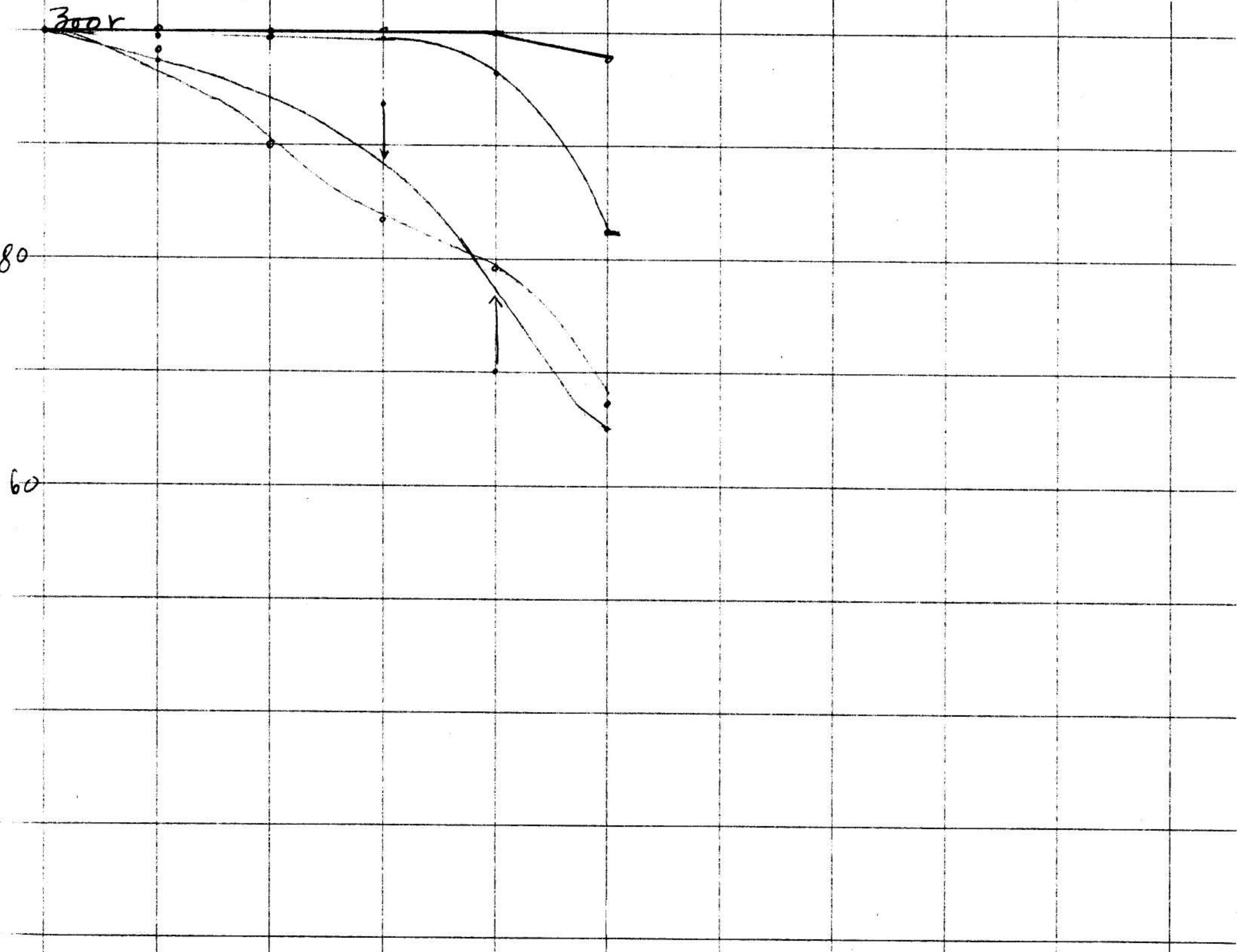
dl-fluorophenylalanine

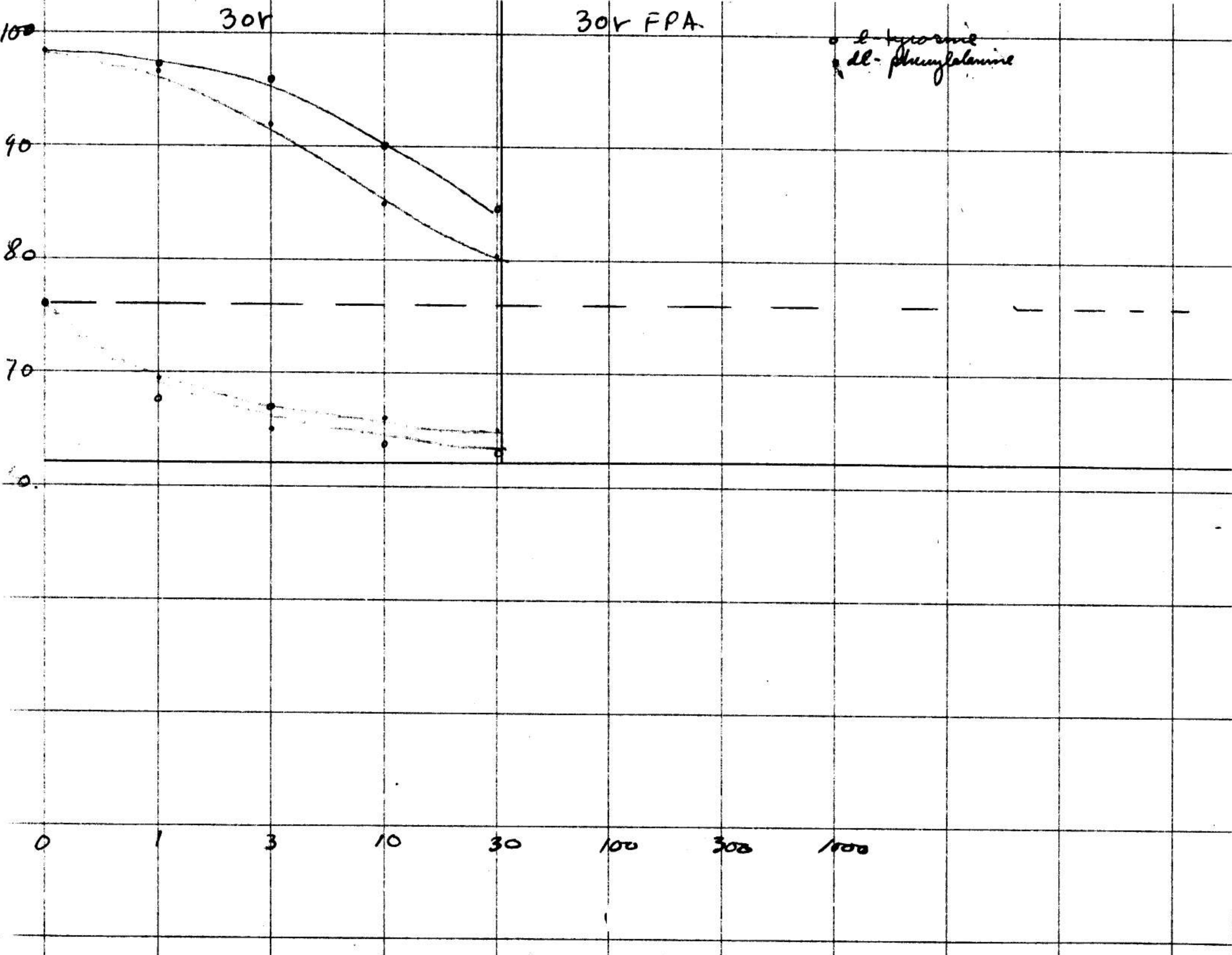
5/24/46.

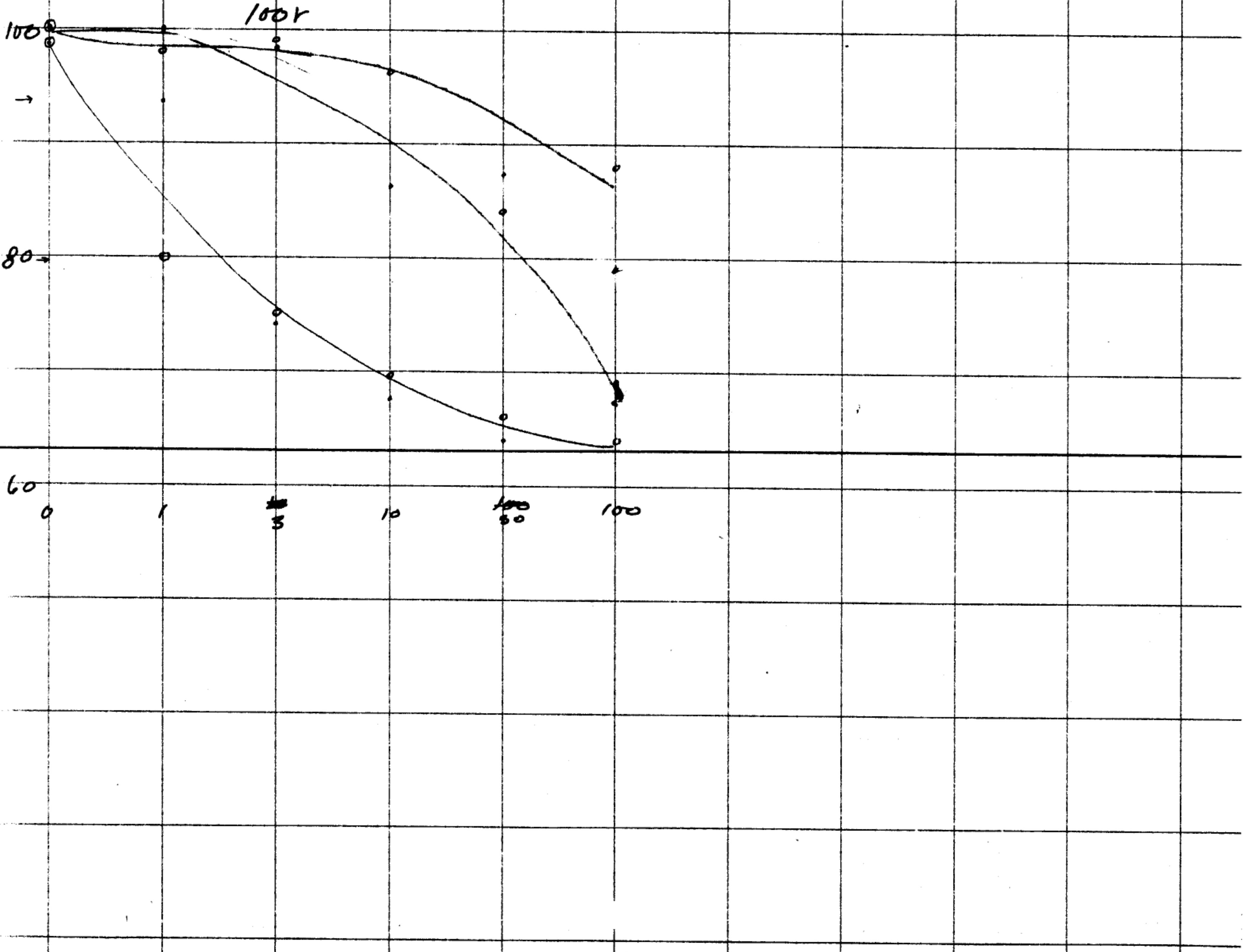
10 ml T(B). 30°. in colorimeter tubes. Proc. 1A26.

	Proc.	dl ΦAl.	dl FPA.	l-tyr.	604. 8P27.	1P28
1.	R-12	0	0		67	68'
2.	"	"	10γ		80	68'
3	"	"	30γ		97	75²
4	"	"	100γ		100	89'
5	"	"	300γ		100	100
6	"	"	1 mg.		100	100
11.	58-278	100γ	0		70	70
12	"	"	10γ		66³	67'
13	"	"	30γ		67	70
14	"	"	100γ		69	67
15	"	"	300γ		70	69³
16	"	"	1 mg.		99	96³
21	58-278	200γ	0		72	68²
22	"	"	10γ		66'	67²
23	"	"	30γ		67'	68²
24	"	"	100γ		69	68²
25	"	"	300γ		69'	70
26	"	"	1 mg.		71²	71²
31	58-5030	0	0	0	– 100	100
32	"	"	0	80γ +++	77'	77²
33	"	"	10γ	80γ	77	78'
34		"	100γ	80γ	78³	79
35		"	1 mg.	80γ	75'	75³
36.		100γ	100γ	0	– 100	100
37.		100γ	0	0	– 100	100
38.		0	100γ	0	100.	100
					76³	75³

compare c̄ E 133.


300r
80
60


30r
30r FPA
100
90
80
70
60
l-tyrosine
dl-phenylalanine
0
1
3
10
30
100
300
1000



100 r
100
80
60
0
1
3
10
50
100

FPA.

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

	FPA +	dl φal +	l-tyr. +	6/2/46. Inoc K-12 11P2. 4P3	25h. 12M3.	36h. 11A4	48h 11P4.	60h. 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		
8			10	+	90	70 / 63^3		
9			30	+	84^3	71 / 63 (63^3)	68	
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		++ sic	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	100^2	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	
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-2733

SUBJECT: FPA. - Growth Curves. 30°.

SHEET NO. BY 215a

Inoc. 1130 P5

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 10.3h.	mgt = 2½h.
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} ↔ 8.5	73^{3}	66	58			144.
4	" 10	99^{2}	100	99 (100)	99^{2}	99	95^{3}	72	64^{3}	57			
5	K-12 FPA 100γ	99	99	99^{2}	98^{2}	95^{1}	90	67^{3}	68^{1}	65^{3}	67^{2}		220?
6	K-12 FPA 300γ	99	99^{2}	99^{2}	98^{3}	97	96	81^{3}	77^{2}	68^{1}	67^{1}		ok. 390
7	K-12 1mg. FPA	100	100	99^{3}	100	100	99^{3}	98^{3}	100	97^{2}	96^{3}		
8	K-12 FPA 100 φA 10	100	98^{3}	93^{3}	87^{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}	70^{2}	70			"
10	v.sm. K-12 "	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	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. " "	100	100	100	100	99	99^{T}	96	94^{3}	83	68^{3}		
14	K-12 FPA 300 φA 10	97^{1}	93	82^{1}	66^{3}	59^{2}	62^{1}	66^{3}	65^{2}	66			~~[illegible]~~
15	K-12 FP 300 Tyr 10	99^{1}	99^{1}	99	98^{2}	97^{2}	97^{1}	91^{3}	89^{2}	77	67^{1}		
16	K-12 50% but(T)	100	96^{2}	88^{1}	74^{2}	69^{1}	71	73	73^{1}	73	73		
		77^{1}	77^{1}	76^{2} / 77^{1}	77								
						76^{2}	76^{2}	76^{3}	77	76^{2}	78^{2}		

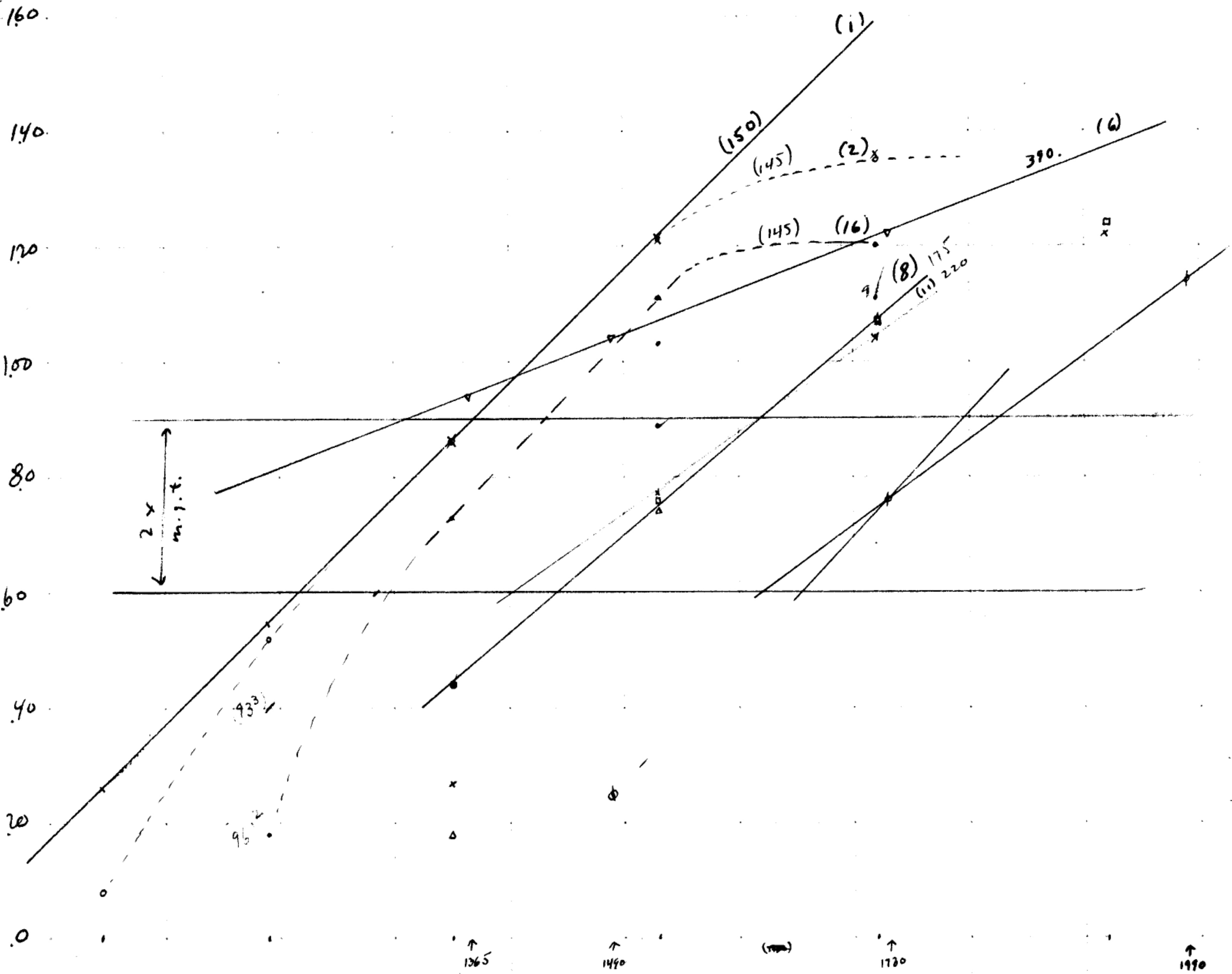
should have a separate stock.

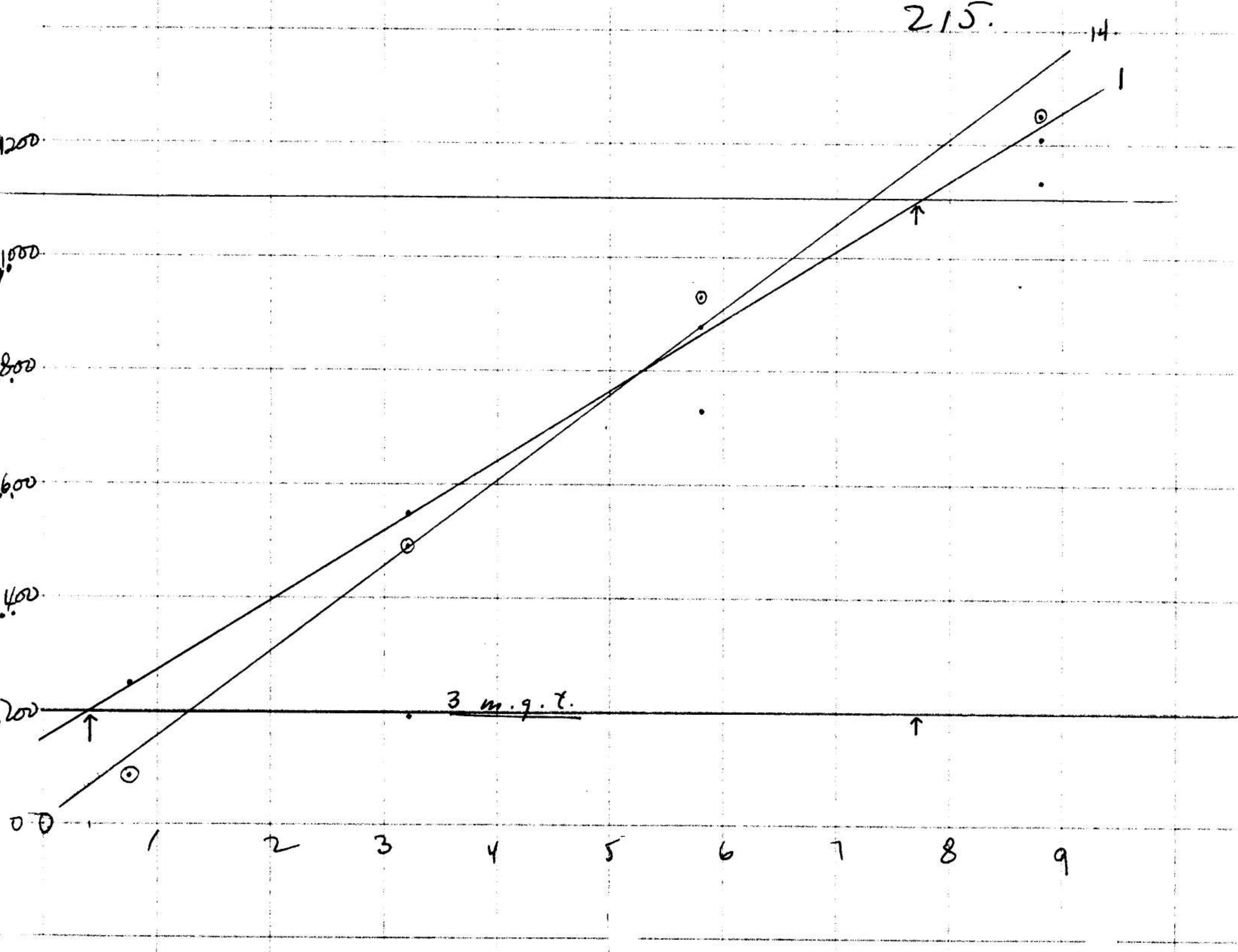
$$d = 2 - \log G$$

$$t = k \log d.$$

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

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


160
140
120
100
80
60
40
20
0
(1)
(150)
(145)
(2)
390.
(6)
(145)
(16)
(8)
175
(11) 220
2 x m.g.t.
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

B/r mutants.

Inoc. 12Y5A28.

goodresponse curve.
1/2 max: ca .03 mg

		Arginine γ	Leucine	Citrulline	21h.	48h.
Y-38	1	0			99[3]	all up.
	2	1			94[1]	
	3	3			92[1]	
	4	10			83	
	5	30			78[2]	
	6	100			61	
	7	300			57	
	8			1000	100	
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 ∞ 12M28. Grow on shaker @ 30°.

Mix after growth together.

Controls are questionable —

syntrophism in plate 13 or carryover?

Sex: Analyses of 212 cultures.

M28 Picked to ss.			BPTM	−B	−P	−T	−M
x P.T.?		1✓	+	+	+	−	+
"	?	2✓	−	−	−	−	−
BT?		3					
BT?		4✓	+	−	+	+	+

Analyse 219-2

5/31/46.

1. Inoc 50 ml coli ✓ c̄ α (679-183) and β (58-161) and shake at 30° 11P30 - 1130P31.

T/= Wash and resuspend in 25 ml H_2O.

Turbidity — P4.

turbid Cell count:

1.	α	10^{-7}	in ✓:	24
2.	β	"	" ":	53

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

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

Syntrophism. Inoc in BT.

9.	β 10^{-7}	Control	1	—	
10	α 10^{-7}	"	0	—	
11	α 10^{-7}	+ 1 ml β ~~B...~~ Boiled	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 conclusions that may be drawn from this are limited: in general diffusion must be limited by the agar to the point where syntrophism 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 time for pS of ca. 7.

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

					pS
t=0	10^{-7}		65	650,000,000	
1	1 ml	turbid			
2	1	turbid			
3	1		10^{5}		
4	1		ca 10000		
5	1		ca 1200		
6	.1 ml		ca 40.		→ 6
7	.1		7		
8	.1		0		
9	.1		0		
10	.1		0		
15	1 ml		0		

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

Y38 – Multiple Mutants.

6/1/46.

~~#30 P31~~. Inoc 50 ml coli ∞ and shake at 30° – Y38.
4. ~~#~~P1

1. Dil 1:10⁷ into coli ∞. ca 2000.

Shaker ... Irradiate 5 mins as exp. 221. Inoc 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, c̄ cover.
Immediately after irradiation: 100 ...
.1 ml = ca 2000 colonies.

1
2
3 $\rho^S_{5 min.} = 6.$
4
5
6
7
8
9
10.

Dil. A 1:10⁻⁷ and inoc as above.

11
12
13
14
15
16
17
18
19
10.

opt. arginine = 300 γ dl – .

Phage T-1.

1/2/46.

labelled titre 2 x 10^{10}

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

740P 2

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

3} 4} Plate ca 10^8 Y38 c̄ 10^{10} T-1 in coli ∞. Turbid! for Y38/T1: large clear center c̄ ca 400 secondary colonies; periphery turbid.

5. 10^8 Y38 in coli ∞ ag. When solid, streak } no response!
6. 10^9 B/r in coli ∞ ag. T-1 over surface. }

~~B3.~~ Liquid culture —

4P4 — Inoc coli ∞ c̄ Y38 shake at 37°. ca. 10^8/ml.

1016P — Add 1cc of Phage T-1 "2 x 10^{10}".

12 M - no change

9A5 - no change.

Sex;

Inoc 50ml coli flasks + shake @30°. 11P2 6/2/46.

Shaker broke down A3.

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

11P4 - Centrifuge + wash. Inoc into T(0) ÷ 2cc. culture. per plate + incubate. Also 208-51-2-3. → 0, 0, 0.

As above. No shaking 1130P5. — 1230A7. plate out as T(0).

	P7	
11 58-161	0	
12 679-183	0	
13.} grown	4	! Fission or sexuality? ?
14.} together	5	
15 } grown	0	
16.} Separately	0	

P7. Pick 6 colonies from ~~to~~ ③ ~~[illegible]~~. coli S. ; 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.

Inoc. 5 flasks coli α c̄ Y38. Grow shaken 37° 4P 4

10 30P 5:

Irradiate individually 5 min. in same quartz tube + determine killing.

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

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

Y38 - mutants:

6/6/46.

1130 P5 Inoc 50 ml coli $\mathcal{B}$ c̄ Y38 Shake at 37°.

Irradiate 6 mins. Wash by centr., dilute 1:100 + plate in T(arg.) for mutant detection: ~~1200 A7.~~ 2A7

1
2
3
4
5
6
7
8
9
10

Too heavy !! * *?

ca 10^{4-5}

Inoc 1 ml into F/0) 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 expectation. Pick sample colonies from surface each plate ~~and~~ to H_2O + to slant coli + test on minimal

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

see 231.

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

Phage T-1.

19 JUN 1946

1. Isolated colonies from plate 223-3. to coli. Transfer to slants.
2.
3.
4.
5.

11. Pick agar between colonies from plate 223-3 to the surface of a coli plate seeded with B/r. No lysis. Probably agar too old.

12. Inoc coli tube c B/r heavily from a slant. 8P. c phage C !!! 10P. Inoc c T-1 (D) .. by 1130 - considerable clearing?

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

2A 13A. Y38 + T-1. → lytic zone. Use YBG agar for coli in this expt.

9. 14. Streak agar plate of B/r c 12. distinct lytic zone.

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

All streaks gave clear areas:

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

21. 11A9. Inoc 50 ml coli c T-1 "10^{10}" + 5 ml 10 hours E. coli B/r from coli.
Incubate at 37 in shaker. 1230 P9. - largely cleared.

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

23. Plaque count T-1 (D) on B/r. 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 threonine. ̄c recovery added Threonine in view of

very high requirement.

9 JUN 1946

As 202. K-12 in 50ml T() + suppl. Shake at 37°. Inoc 145A9.

		G (1:10)	d.	mg / 25 cc eq.	Hydrolysate at =		Recovery %
	1. 0	84[1]	.744	[illegible]	85[1]	.693	93%
prol	2. glut. 5mg	81[3]	.875	[illegible]	for eq. (743) = 83[2]	.783.	89%
indole	3. anthr. 5mg	83[2]	.783	[illegible]			
put	4. pantolact 1mg β-alanine 1mg.	84	.757	[illegible]			
arg.	5. citrulline 5mg.	83[2] (73)	.783		(78[2])		

See 234.

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

	Volume after hyd.		to	
1.	~~4.5c~~ 11c	[illegible]		13.5cc
2.	4.5cc	22.5%		5.6cc
5.	6cc.	30%.		7.5cc

Neutralize. Dilute to % of 25 cc ind. by recovery
1cc 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 " " " " of arginine

Note 438 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.

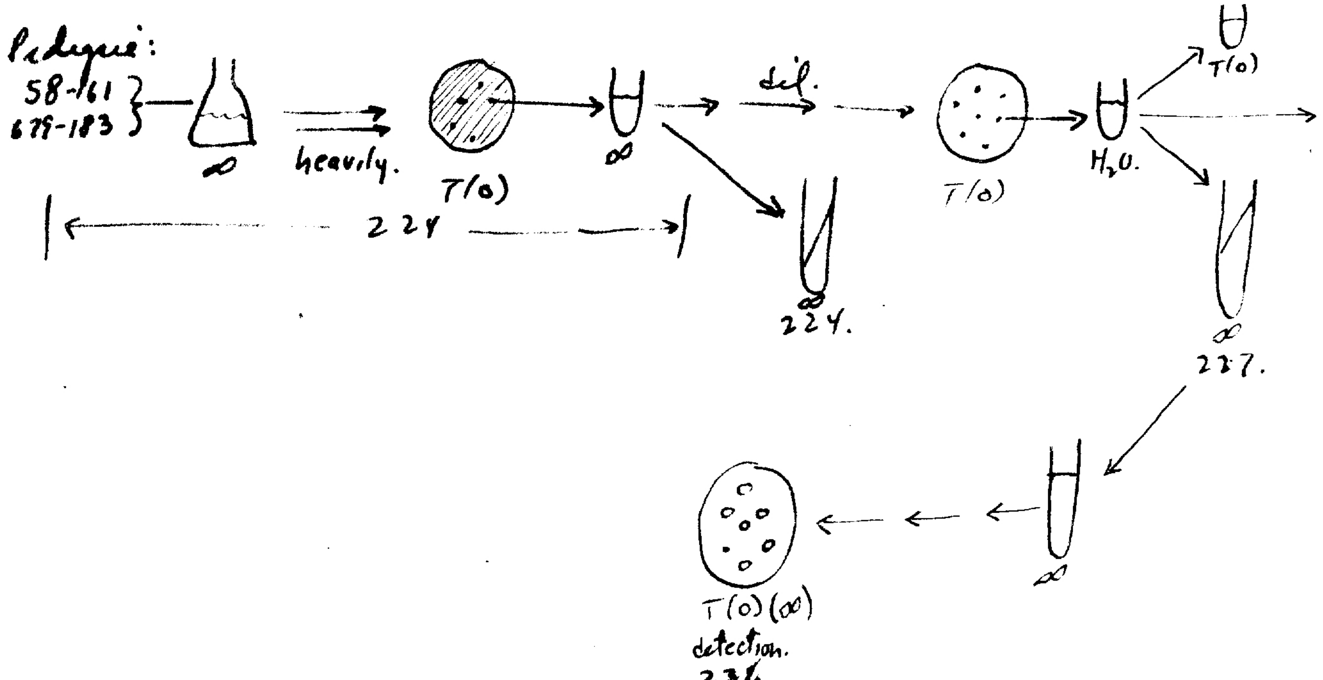
Sterile-filter 228-22 for T-1 stock. Pool c 228-21
Dispense aliquots variously; some to K.W. for lyophil preservation.
Plaque out on B/r at indicated dilutions. 1A18 for titer.

1. 10^6 ca 200
2. 10^8 1
3. 10^{10} 0
4. 10^{12} 0

1/11/46.

Plate out 227-1, etc. Detection plates, for mutant segregants.
Dil 10^{-6}, 10^{-7}. Unshaken culture inoculated from slant.
227- 1A11. Layer E coli 12N12.

	227-
1 2	1
3 4	11
5 6	21
7 8	31.



N30A13 - Nothing sign. seen.
11A13 - do. (1 col 0?)

Y38 Mutants.

6/12/46.
Mac coli 40 ml c Y38 + shake at 30° 11PM

Lebitrude

Sex

6/11/46.

Wash and plate heavily into T/0) etc. The cultures:

Mor P5 coli ∞ c

heavily = 1ml grown culture / plate.

1. 58-161

2. 679-183.

3. 58-161 + 679-183.

5P11↓ (6 day cultures).

		1230A13	11A13
1.	1 in 0	0	
2.	1 in 0	0	
3	2 in 0	0	"
4	2 in 0	0	
5	3 in 0	0	
6	3 in 0	0	
7	1+2 in 0	0	
8	1+2 in 0.	0	"
9.	1 in biotin	0	
10	1 in meth	0	
11	2 in threonine	0	
12	2 in proline.	0	
13	3 in BP	0	"
14	3 " BT	0	
15	3 MP	0	
16.	3 MT.	0	

OK / thrown 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.

Bros amino acid tested, 30° very little in ~~hydro~~ filtrates; considerable in hydrolysates

1	229-1	Medium 50%	Blank.		
2	"	" "	+ Biotin - 58-3214.	for proline.	100
3	"	" "	Y38	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.	74[3]
41.	229-2	hydrolysate 1 ml	58-3214	++	83[2]
51	229-5	" "	Y38	arg. ++	78[2]
61.	206.	hydrolysate 1 mg.	Blank		
62		"	+ biotin 58-3214	prol	77[1],
63.			Y38	arg.	72[1]
71	206	filtrate 50%	blank		
72			+ biotin 58-3214		100
73			Y38.		100
81	- T(0)		Y38		=
82	T(B)		58-3214.		

Bros 11P 6/17/46.

K-12 Phage

12 JUN 1946

① 3P. Broth 50ml coli ∞ c̄ K-12. Shake at 30°.

1130 A13 – Broth 1ml of ① + ind. phage sources into 50ml coli ∞:

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

Incubate at 38°.

2P. – #1,2 clear; 3,4 turbid.
Broth coli ∞ c̄ 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. [illegible]

Cross streak on a coli ∞ plate:

	K-12	58-161	679-183	B/r	~~B/t~~.
T-1	–	–	–	–	
235-11	–	–	–	– c̄ secondary growth along streak.	
~~235~~					
235-12		do.			
C		do.			

12M 11 Imoz coli ∞ flasks; shake at 30°.

1. 58-161
2. 679-183
3. Both.

Plate tests on minimal, heavily,* after washing 1130P12.
P15

*
1 ml grown culture

1.	1		– No colonies.	0
2	1	+ B	– No colonies.	0
3	1	+ M	– Turbid plate. No colonies.	
4	2		– No colonies!	0
5	2	+ T	v. distinct halations around adaptants....	23. N14. ‡
6	2	+ P	3	6.

(Some colonies may adapt in agar.)

7	1+2	–	2 seen N14.	
		11P13.	N14	
8	3	13		
9	3	13	ca 100.	
10.	3	12		

again, some colonies come up secondarily (after the first) pick one colony – (236-9) to water + ∞ slant

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

		P15 O
11	1	
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	236 a
6/13	0	0	0	4.	236 b.

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 ~~...~~ 236 ① and 236 ② as inocula. 1ml ≅ 10^9

1130P13

1. 58-161 10^9 + T-1 10^7 in coli ∞ plate
2. 67~~9~~-183 do.
3. 58-161 10^9 + T-1 10^7 in coli ∞ ~~tube~~ 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~~

10A14 N14 7P14

5 Flasks of 3 turbid. Streak out on coli ∞.
6. " 4.

Isolate colonies from streak plates to BM + PT medium respectively to avoid "tryptophaneless" 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 Trypt.

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

→ slant
→ ~~B~~
→ T
→ PT
→ test for lysogenicity c̄ 161. on coli ∞ plate.

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

Evidence re heterocaryosis —
Mutant in Recombination strains.

(1) 1145 P13. Inoc 50 ml coli ∞ ē 227-1

1030 P14. Irradiate 4 mins. Inoc. 1 ml into 50 ml coli ∞. (A). (calc. pS=5. killing).

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

1 P16.

Conclude that survivors only ca 10/cc.

1 tested.

1 —
2 —
3 1
4 —
5 —
6 —
7 —
8 —
∞. 0

Dilute (1) 10^{-7} + detect: (sp. mutants 2 / cover ē F(0)!). Apparently killing was > anticipated
Layer ē ∞ 1P16. Circle previous minute colonies.

S
1 302.
2
3
4
5
6
7
8.
∞. 316.

Examine 4P, 11P16. 11A17. 10A18

2400 total tested.

1 colony found A17. 238-2.

no growth on picking.

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

A
1
2
3
4
5
6
7
8
∞ 91

Examine 9P17, 11A18. 12 M19.

1 colony - surface? cont? 238-1 700 tested.
micro. not ē coli. Sarcina?

pick variants to ∞ 12M19.

~~239.~~ Sex-plating.

15 JUN 1946

Inoc 183 (679-) + 161 (58-) (separately) 11 P14. ~~Aerating~~

Inoc ~~[illegible]~~ 5 ml each into coli ∞ (50 ml.). 30°. Plate ^heavily at varying intervals.
G (1:10) (73³). Wash (separately) + plate as indicated. ^in 1st 1 ml of culture.

9:45 P15. Mix:

			A17.		cells (calc.).
0 time.				96¹	5×10⁸/ml.
	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.	~~13~~ 21		4.	95²	
1 ml.	~~14~~. 22		3		
	31		3	95 (94³)	
19h.	41				
	42.				

1st plating is 5 ml each original culture = ca 5×10⁹ added.

disregard surface col.

~~Use 241 cultures for inoculum + repeat. Mix ~~[illegible]~~ P16.~~ (6)

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

~~K-12~~ ; phage C. ; Segregations (recessive recotypes)

16 JUN 1946

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

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

From comparison c̄ diluted 239, dil ½ : 100 : 1000 + plate 1cc dil. into detection plates for recombinants.

Unfortunately, ca. 1200/plate in ~~2%~~ 2% small colonies in minimal (B?)

Layer 1230P17.

1. BP 2 new.
2. BT
3. PM
4. MP
5. —

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

Test C again on K-12, 183, 161 by cross streak 1A18.
active on K-12
non-active on 58-161
activity on 679-183 ??

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

Ultraviolet induced reversions.

16 JUN 1946

1A fr 161, 183 small coli ~ 30° sh.

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

$pS = 5.5$

5P16. Irradiate 2 mins.

Wash both aliquots + dilute + plate as indicated.

	No.	Dilution	Plated in	Unirrad.	Irrad.	Titre
	1.	10^{-7}	in ∞	79.		7.9×10^8
A –	2.	10^0	in P		3	
unirrad.	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.	~~[struck out]~~ 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, inn sm.	3 + many small.	
	17	10^0	0.	—	0	
	18			—	0	

What are the small colonies?

4P17. A18

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

6/16/46.　17 JUN 1946

Inoc 50 ml T(0) K-12 30° sh. 10P16.
3P17 harvest, centrifuge + sterile filter 25 ml sample. = x_1

1. x_1 5 ml + T(B) 5 ml.　　add x_1 sterilely ē autocl.　++
2. x_1 5 ml + T(B) 5 ml. Autoclave together.　++

9P17 harvest second sample = x_2
3 x_2 5 ml　　ē autocl x_2.　±
4. x_2 5 ml　　autocl.　±
Inoc ē 58-3214.　1220A18.　30°.

On 183 + T plates — filter paper techn.
a. .1 cc x_1　—
b. .1 cc x_2　—
c. (ca) 10γ proline　++
d. .1 cc x_1 boiled.　—

There is evidently a considerable diminution as growth proceeds.

Add 100γ proline to 4 1130P19.　+++

Sex : plating

1? JUN 1946

Broz 50ml ∞ 161,183 1A17 30° sh.

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

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

		7P19
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 striking in both cases.

See 245 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

$1/S = 53$

$\rho S = 1.7$

Unirradiated:

1. 10^{-7} a 30- not properly countable.
   Wash both:
2. 10^{-7} a. 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} } a 104 ... 15. (1.5×10^7)

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

Inoc. coli a 50 ml liq. c̄ 10^0. (A). 5P17. Sh. 30°

Effect here is very slight. Use longer killing.

Was P put in c̄ T.

19 JUN 1946

Test:	B	M	BM	P	T̄	PT	BMTryp	
237-12			· ++			·	++.	OK.
243-8-	—B	—P	—T. BTPM. —M	= —0		0		
1	++	+	++.					
2	++	+	++					Most of this is clearly syntrophism.
3	++	++	++					
4	++	++	++					
5	++	++	++					
6	"	"	"					
7	"	"	"					
8	"	"	"					
9	"	"	"					
10	"	"	"					
11	"	"	"					
12	"	—	+.					Streak out
13	"	+	++					
14	—	—	++					(short rods) (Hooray!). See c.
15	++	—	++					Streak out.
16	++	++	++					
17	"	"	"					
~~18~~								
~~19~~								
238-1				· — ·		· — ·		Not coli.
238-2 n.g. ∞								

243-9. From BT Plate.

Test	B	M	BM	
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.

245
b.

19 JUN 1946

BMPT. 0

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

240-1	41	++	++
	42	"	"
240-2	43	"	"
	44	"	"
-3	45	"	"
	46	"	"
	47	"	"
	48	"	"
	49	"	"
4	50	"	"
	51	"	"
	52	"	"
	53	"	"
5	54	"	"
	55	"	"
	56	"	"
	57	"	"

Small colonies on T(0)
but not biochemical mutants.
Morphological ??

long rods; hazy internal structure.

Recombination tests

Analysis of a possible recessive recombination #14. BP?

N21. Streak out on ∞ plates; mor slants to keep it.
Colonies to H_2O. ∞ slants N22.
Test on large tubes B: − P: − BP: − (medium?) add M to each.
 + − + BM!!
B − P − = BP T = BT = 0.

	BP
141	−
142	−
143	−
144	−
145	−
146.	−.

Check on def. media: −B −−M = −P −M −T = −0

	−B	−M	−P	−T	−0
679-180	++	++	−	−	+
58-3214	−	++	−	++	++
58-161.	−	−	−	−	++

Is M generally lacking?

151	−
152	−
153	−

261	−
262	−
263	−

These recotypes are n.g. See set 249.

Mix: plating Exp. n. 9. 246

Segregation of resistance to T-1

19 JUN 1946

1A20 Inoc 5 ml coli 30° sh.

(1) 23712 BM/1 (trypt. ??)
(2) 23721 TP/1

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

(3) BM —1→ TP (4)
(1) BM/1 —3→ TP/1 (2)
(2 and 4 are the crossed arrows)

1130A20. 5 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 pl.)	3	0	+
13	4	0	
21	1.	0	++
22	1.	0	
23 (½)	1.	B	++
24	1. 10^{-2}	B	
25 (½)	1	M	T
26	1 10^{-2}	M	
27 (½)	1	P	
28	1 10^{-2}	P	
29 (½)	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.	"

see 247 - use these for controls.

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

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

24 JUN 1946

Inoc 50 ml coli [illegible] sh 30° 8A24. 5P25. Wash + irradiate in H_2O. in Q. tube

1. 679-183.

add 1ml = to T (Threon) agar.

Irrad.

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 using protein from killed cells.
8	~~4~~ "	

Sex: other recombinations

Prototroph recombinations

Mos. [illegible]. 12M 2?.0 50ml ∞ sh 30°

A. 58-161
B. 58-336

C. 679-183
D. 679-680.

5ml each. 3P2? 30° incub.

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

Plate out 1ml eq. 11P2?. Use washed agar.

	T(0)	+	8P22.	8P23
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. Inoc. 50 ml coli ∞ sh 30°:

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

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 Inoc 10 ml each into 50 ml coli ∞ as above T
-rather- 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	Bϕ	1	
13	5	BC	7	
14	5	T	1	32
15	5	P	6	
17	5	BϕT	2	13
18	5	BϕP	4	
19	5	BCT	2	++ cont?
20	5	BCP	38.	
16	5	0	1	
21	E	0	–	
~~22~~	"	Bϕ	A24	A25
~~23~~	"	~~BC~~		

many plates look contaminated. Do not keep.

June 24, 1946.

8A24. Inoc together into coli (or +cyst-glucose = c).
30° s sh.

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

5 679-183 + 424. c

3P25. Harvest + plate. 1ml = .

	Inoc.	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	(20)
17	5	BcP	(18)
18	5	BcT.	(1[illegible])

Streak out See 254.

No quantitative evidence [illegible] recombination.

S. pombe.

24 JUN 1946

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

5P25. tube +++ oxygenation ??
flasks ±. needs glucose c̄ doubt.

930P25. Est c̄ 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) A28. (2½ days). These are rather variable. Larger colonies near surface. Maybe intrinsic heterogeneity. Isolate colonies from trace plate.

P28; A29. Good size colonies. More uniform. Pick from single colony + streak out on coli ∞.

Sex; recombination

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 etc + plate in T(0). 1 ml =.

			10P27	12M26. P28.
1	1		3	22
2	1		1	14
3	1		1	63
4	2		10^1	30
5 (ampl.)	2		10^1	11
6	2		10^1	11
7	3		1?	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	ca 30	
16	4	BC		
17	4	P	10^2	
18	4	T	10^2	
21	4	BϕT	10^2	
22	4	BϕP	10^2	
23	4	BCT	10^2	
24	4	BCP.	10^2	

(1–9:) ~~Replace~~ 1A29.

(11–24:) Refrigerate 1A29.

Abandon tests on these in favor of the more efficient Y24 (BϕC) + Y41 (PT(T-1R)) with the additional character R.

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

Bacterial "nucleoproteins".

26 JUN 1946

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

11P26. Inoc coli ∞ $\bar{c}$ 58-161 for exps. next day of similar nature.

Conclusion: considerable comes out $\bar{c}$ .9% Nothing then removed $\bar{c}$ 6% NaCl

100 ml culture 10 hours old. Centrifuge. Reject 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 ∞; min. slants.
250- Test, A29.

21 2 BM/1 x PT. T(0). + T-1 Resist. series: a + b + c + d + e + f + ~~g +~~
22 2 + +
23 2 + +
24 2 + +

K-12 -
Y40 +
Y41 +
227-1 -
controls

11 1 BM x PT. Streak out again.
12
13

25 2 + . T-1 resist +
26 2 + +
27 2 +
28 2 : T-res. series : + a + b + c + d + e + f + g + h +
~~29 2~~
all grew (-) on T(0)! Check on neg. See below: ~~256.~~ 255.

		T(0)	T(B)	
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.~~
quant. not valid: plated on minimal medium.
Total tested for B, - 28
B-(tent.) 5

PT x BφC on Bφ w on Breg.

		(B)	(0)	
131	13 + ~~(B+)~~	+	+	
2		+	—	* Biotin-less later test - did not grow on 7/19/46 B alone.
3		+	+	
4		+	+	
5		—	—	
6		+	+	
7				

		(B)	(0)	
141	14.	+	+	
10 BC. 2		+	+	
3		+	+	
* 4		+	-	Proto.
5		+	+	
* 6		+	+	Proto.
~~7~~		+	=	
8		+	+	
~~9~~				
10		+	+	

growth in (0) without B may be mould.

Read 830P29.

(3) Reg.

Retest from column 3.

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

	Bφ −	P +	BφP −	P
161				
2	+	+	+	P
3	−	+	+	P
4	+	−	+	Bφ
5	+	+	+	P
6	−	+	+	P
7	−	+	+	P
8	−	+	+	P
9	−	+	+	P

(BφP.) − Repeat in small tubes:

Bφ −
P −
BPφ +

(P)

Repeat again in 10cc tubes c̄ uniform inocula from coli stand:

B −
φ −
P −
Bφ −
Pφ −
BP +++
BPφ +++

7/1/46.

There can be no doubt then that this is BP̄ 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	−.	+	[struck out]	Check: (P.)

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

28. BM. PT. BPMT. Later check: B−M−

12M26. See

Inoc coli ∞ c 227-1. (ultra-violet.)

5P29 (40h.) Irradiate 1/2, 1, 2, 5 min then 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. (x50) $pS = \log 10^5/10^9 = 4$.

5 - ca 2 x50.

Plate out (2) at 10^{-7}. in T(0) detection plates. 11P30.

330P2. Layer + refrigerate. (CSH)

11P12. Mark numerous small colonies. Incubate.
ca. 1%

10A14. Pick to complete (not all, only those most convenient by way of isolation). Slant. A15...

1
2
3
4
5
...

Test Recombinants Exp. 252

29 JUN 1946

Streak out on coli 50 plates. Number in orange to test from 255

			T-1	T(0) 150 col.
Y40+Y41	1	11	R ±?	
		12		
		13	all resistant; 6.	
	2	21	R ±?	
		22	all resistant: 7	
		23		
Y41+161.	7	71	S ✓	S
		83	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	S ✓	R
		42	R ✓	S
		43	R	R
		44	R	
		45	R	
		46	S.	S
		47	R	R
		48	R	
		49	S	S
		50.	R.	

all +

Linkage of R to B or M?

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 +

Phage analysis of Prototrophs.

30 JUN 1946

N30 Inoc coli ∞ c ind. cultures for inoc. below. @ 30° 12 h.

1130P. Inoc 50ml coli [illegible] 1ml : + T-1 10^7! > 4. 30°
" 1030A1. "

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

2. 255-24 (183S × 161R) R. Fullgrown.

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
12 " ca 10^2 } no mutants present.
13 " ∞ ca 10^2
14. Streak ∞. do not rese.

330P2 Layn c ∞ + refrigerate at 12N3.

259-C2   B+/1 (tcam)   x B- ... $\rightarrow$ B-/1

259-C6   P-/1 ...   x P+ ... $\rightarrow$ P -

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

JUN 1946

Use Inoculum: Y41 (679-1831) + Y24 (BφC) into 50 ml coli ∞ C
Sh. 30°. 12M30.

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

	P2	P12
①	4	
②.	5	
③. BCT.	27	
④ BφP	14	

} 7 S, 2 R. to T-1.

A. P12. Pick colonies from 3 and streak out. P13. Test cool colonies.

	T-1.	= BCT	BC −	BT. ≡	T(0) [R probably (T−P+R.)].
1	T-1. R				+
2	S				
3	R				
4	R				
5	R				
6	S				
7	R				
8	R				
9	R				
10	R				
11	R				
12	R				
13	R				
14.	R				
15	R				
16.	R				

P̄ T̄ R ✓ parental

BCφPT+. See 272

BCφPT+ See 272.

more pure on tests! / perhaps should streak out on ∞C agar.

B. P13. Streak out colonies of 1, 2. A14 Test.

	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. ④

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

Segregation of Biotinless: Etc.

$B^- \phi^- c^- \times B^+ P^- T^-$

Check. reg.

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

Test other colonies from 254.

254-78.

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

By aurgan: BM 1. check. Parental.

Recotypes

12 JUL 1946

10P12. Establish Inocula. in 50ml coli ∞ 30° 6h.

1. Y9 (TLM)
2. Y40
3. Y41
4. 58-161
5. 679-183
6. Y24 n.q.
7. ~~Y23~~ Y38 } for radiation.
8. Y39.
9. 58-183X n.q.

~~Inoc 50ml coli ∞~~ Store in refrigerator 7P13.

Inoc 50 ml coli ∞:

(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 B sq., 11.
5. See 262 Y40 + Y41 x-streak. 2 surface colonies. Will have to repeat procedure.
6. S 10^{-2}

Sex: cross-streaking

13 JUL 1946

7P13. Using loopsful from 261, crossstreak on coli as.

A. Streak 1 2.

		1	2
TLM	1.	Y9 TLM	161 BM
	2		183 TD
	3		Y41 PTR
	4.		Y40 BMR

B. 11 Y40 BMR 183 PT
12 Y41 PTR

C 21 Y41 PTR 161 BM.
22 ~~Y40~~ Smeared.

D 31 Y38 Y39.

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

no colonies.

13 JUL 1946

Irradiate Y38 and Y39 cultures (see 260) [illegible] and incor: coli's.
11p13. 8h.30.

Y38 1 min
2 "
5 "

Test Y39 — dl leuc
dl isole.
l-leuc.

Y39 1 min
2 "
5

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

Y38 - 2 mins
Y38 - 1 min 2 plates.

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

1 min 11 350 cells, 1 colony.
" 12 1.
2 mins. 13 500 cells. 9 colonies.
14

Y39 21 did not grow
22 certainly is not le or iso le strain.
23
24 Test by auxan: e.a.a: histidine
25
26
27 Specific for tests on Y38 —
28
29
30
31
32

Sex: triple cross

TLM × BφC

9P. 7/14/46. 1ml mix into coli∞+c Sh. 30°

Y9 × Y24

N15		Plate into:	
1	0	0	
2	0	0	
3	0	0	
4	0	0	
11	B	0	
12	φ	0	
13	C	4	turbid
14	T	0	
15	L	4	
16	M	0	
21	BT	0	
22	BM	0	
23	φT	0	
24	φL	6. v. sm.	
25	φM	0	
26	CT		turbid
27	CL		turbid
28	CM		turbid
31	TLφ	ca. 16	
32	TMφ		
	BLT		turbid
	CTL		turbid

very disappointing. (Cis medium? or strains?)

Threw out plates.

679 - 680 - Y9.

Y10.

July 16, 1946.
Check on requirements:
P16: Y9.

TLM	++
TL	–
TM	–
LM	–
TLM+Cyst	++
TL+Cyst	–

Growth is however not optimal at all ̄c methionine; something else may be required. (consider pab, homocysteine, choline, etc.
In TL + EAA, NEAA, YE, Vits.
TLM

TLYE +++. others + or –.

Y10:

TB₁	–
LB₁	+.
TL	–
TLB₁	+++

probably some $T^+L^-B_1^-$ in the population. Reisolate — streak out from TLB₁.

2/5 isolates tested came up on LB₁ as well as TLB₁. save as Y45. Other three – save 1. as Y10a. (or after 7/27 as Y10.

Killer E. coli.
Resistance

7/15/46.

P 15 Mixed ē Heershey'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.	T10^{-3} + R 10^{-3}	++.

no demonstrable killing.

Proteus.

17 JUL 1946

"Reacting" "strains" "3" and "14" received from Dienes. A17.

Transfer to subculture slants D3 D14.

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

Nutritional Requirements: 11P17.

grows very rapidly compl. cote

		9A18	9A19		
D3.	Pnt Nic Cyst	++	+++		
	PN	−	++		
	PC	+	+	to C.	+ slowly ++.
	NC	++	+++		
	Cyst − Vits.	+++	+++±		
D14	PNC	++	+++		
	PN	−	++		
	PC	±	+	to C	+ slowly ++.
	NC	++	+++		
	Cyst-Vits.	+++	+++±		

Repeat for exp. vit. req.

Cyst + 10 B vits −		D3	~~D14~~	10P18. 35°
1		++		
2		++		
3		++		
4	+	+	fr.	(nic)
5		++		
6		++		
7		++		
8		++		
9		++	+++	
10.		++	+++	

cystine is stimulatory; probably not adaptation.

Coincidental reversion

1 [illegible] 1946

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

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

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

O	+
T	−
L	−
TL	+++

Not prototroph!

M20. Inoc coli ∞ c ϕ Use v. large inoculum. 30° c/s.

10P21. Plate out 1ml ÷ into:

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

240-5
Size variant (?)

17 JUL 1973

P16. Inoc coli in 30 ml at 30° s.h. c̄ 240-5a ①
K-12. ②

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

1. K-12 + A18.
2. 240-5 ++
3. both. +:++.

Phage: T-1 susceptible.

Pedigree:

236: 58-161 × 679-183 on 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 mutability 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. Inoc 12M18.
Plate 6P19.

M20 - Same result as above - K-12 colonies appear more slowly than 240-5 on T(0). They are indistinguishable on ∞! [Why was 240-5 first picked up as a small variant?].

TLM × BφC.

BφC × TLM

N17 1ml each ea. into coli... >6 30°

530P18. Wash + plate 1ml ÷.

		5P20	
1	0	0	
2	B	0	
3	φ	1	?
4	C	57	T
5	T	0	
6	L		
7	M	0	
8	BT	0	
9	BL	20	T
10	BM	0	
11	φT	0	
12	φL	0	
13	φM	?2	
14	CT		T
15	CL	52	T.
16.	CM		T

BCL? (margin note beside row 8–9)

BL

[C]

[φ]

Compare — 264.

Try Y10. BφC - TLBi

Sex: cross streak.

17 JUL 1946

N17. cross streak on colis 58-161 × 679-183.

1,2

3. Streak c mixed inoculum.

10P19. Plate into T(o). ① @ ca. 10^8
② ca 10^9

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

July 18, 1946.

P17. P18, 10P18

		BφCTP	BφCT -P	BφCP -T	BφPT -C	BCPT -φ	φCPT -B	BφCPT -0	
A4	[illegible]	++	—	—	+	+	+	+	P̄T̄/1 parental.
A6	[illegible]	++	—	—	+	+	+	+	P̄T̄/1 "

		BφP	B	P	BP.	T-1
C2	—	++	++	—	++	R✓
C6		++	—	++	++	S✓

Recombination Types!

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. → glutamine. Y49	
10	—	T	
11	—	T	
12	—	T (e)	

256 -			
1	T(0) +	aux: ~~turbid~~; A.C.	(not coli)
2	+	~~ACD~~	
3	+++	—	Lost.
4	+.	turbid	

	T(Arg)	T(Meth)	T(A.M.)
Y43.	—	—	++

July 19, 1946.

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

① plate 1ml in coli ∞ . p S = 2 to 3.

② Inoc 1ml in 50ml coli ∞ s.6 30°

Layer N22.

113 d P 20. Detection plates - T (histidine) 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.~~ v poor on coli ∞.			+ on minimal (E2)
9		+		
10	n.g.			

auxanograph P 25. - No vitamin sp. resp. in 6. Check in liquid + for yna. **B-3** pab.

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

Try 1. more pab
2. pab sterile filtered. (slow on pab)

yna replaces pab.

Sex conditions

July 21, 1946.

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

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

	Growth	G(1:10) of susp. plated	Cells:	Colony count	×/cell	
30° 1. Coli ∞	+3	86²	2×10⁹	20	10^{-8}	+
2. -glucose	+3	89	1.5×10⁹	200	10^{-7}	
3. -yx	+2	1:5 88	7×10⁸	2	10^{-8}	+
4. pH variation pH=8. → a		78²	3×10⁹	100	3×10^{-8}	+ — ++
b		72¹	4×10⁹	40	10^{-8}	
c		75²	3×10⁹	30	10^{-8}	
d		85³	2×10⁹	10	10^{-8}	
5 Beef x.	+3	79	3×10⁹	5×10³	10^{-6}	++++
6 T(HCl)	+3	68	5×10⁹	200	10^{-7}	++
7 Malt Ex	? + susp. matter	1:2 81	6×10⁸	0	0	—
8 Coli hydrolys. T(0)	+3	79	3×10⁹	10⁴	10^{-6}	++++
~~9~~						
10 .2% peptone	±	1:2 93	2×10⁸	0		—
11. Coli ∞ vary salt. a 1%	++	74	3×10⁹	10	10^{-8}	+
b 2%	++	82	3×10⁹	10	10^{-8}	+
c 5%	++	80	3×10⁹	0	0	—
critical point? → d 10%	—					
12 coli ∞ unsh.		93²	1×10⁸	10	10^{-7}	++
13 coli ∞ + cyst.		86²	1.5×10⁸	1-10 ?	10^{-8}	+
31. unsh. 25°		95	10⁸	10	10^{-8}	+
32. 38°		95²	10⁸	0	0	—
33 10°		93	10⁸	1	10^{-9}	±
41 u.v.		86 (73²)	1.5×10⁸	10	10^{-8}	+

[Salt inhibits recombination??]

Δ.

25-30° opt.

unsh

-glucose.

Sex: conditions

Inoculate 50 ml of the following media c̄ Y24 + Y41.
1 wash.

30°

1. Coli ∞ (yx .3%; peptone .5% glucose .5%). See 276
2. yx .3% peptone .5% ~~g~~
3. Peptone .5%; glucose .5%
4. Peptone - yx - (glucose) in T(o) adjusted to various pHs. — 8 a, 7 b, 6 c, 5 d, 4 e. no growth
5. Beef extract - yx. broth. .5%.
6. T(o) + NZcase + ~~biotin~~ VITS.
7. Malt extract 1%. (~~[illegible]~~).
8. T(o) + E coli hydrolysate .1%.
9. ~~Corn Meal agar stock.~~
10. .2% peptone + biotin

Temp:

31. Coli ∞ 25°
32. coli ∞ 38°
33. coli ∞ 10°.
41. Irradiate 1 min. c̄ u.v. then into coli ∞ 30° sh.

71. 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.
In coli, 1030 P21 1 drop each of:

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

~~[illegible]~~ T(0) T(B) T-1 (1 cd), etc. see infra.

PTR, × BΦC	1.	B	③	36
	2.	B		37
	3.	B		64
	4.	B		55
	5.	0.		33
TLB, × BΦC	11	0	②	- 1
	12	B_1		+ 7
	13	T		+ 8
	14	L		20
	15	B		+ 1[illegible]
	16	Φ		+ 4
	17	C		T 5
	18	B_1 B		++ 17
	19	B_1 Φ		++ 17 ✓✓
	20	B_1 C		⧺ 7
	21	T B		1 9
...edly ...ish	22	T Φ		9 many small
	23	T C		1
	24	L B		14 many v. small
	25	L Φ		14 "
	26	L C		++ 15

See 276

TPR AM	31.	O	(4)		O
	32	A			4 3
	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 c large cells of varying length + sometimes broken up:

B. mycoides

see infra

Segregation of virus-resistance (T-1)

23 JUL 1946

Use plates 4C, 5, and 8 of the cross BΦC × PT2. 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 c̄ either B+, T+ or P+

See 284. 5R/20.

Summary.

here	16	60	
284	5	20	
p.259	2	9	
	23	89	= 26%
284	5	21	
	28	110	

stored over superposition?

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.
(2) Y41 + 58-161
(3) ~~Y9 + Y24.~~ (which has yielded ~~no prototrophs hitherto~~).
Y10 + Y43. (K-12 × B/K.)

Media: ~~[illegible]~~ = "Yeast Beef" broth = MxY
Ba = Bact. hydrol. 1mg/ml
PMx = Nutrient Broth.

Inoc. 1 drop each (stored cultures). 1245A24. Incubate 30° (5) shaking. Plate 4P24.

	Medium.	Inoc.
1.	MxY	(1)
2	MxY	2
3	PMx	1
4	PMx	2
✓5	T(PMx)	1
6	T(PMx)	2
7	MxY Ba	1
8	MxY Ba	2
9.	MxY	3.

Results are not encouraging.
How different from 275? — time? shaking?

Try Y/B-agar

See 279

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

11	1.	100	(100)
12	1 10^{-2}	1	
21		10	10
22	10^{-2}	0	
31 ~~23~~		100	(100)
32 ~~24~~	10^{-3}	1	
41 ~~25~~		100	100
42 ~~26~~	10^{-2}	0	
51 ~~27~~		200	(200)
52 ~~28~~	10^{-2}	2	
61 ~~29~~		50	50
62	10^{-2}	0	
71		10	
72	10^{-2}	0	
81		10	
82	10^{-2}	0	
91		0	
92	10^{-2}	0.	

For recombination types: 1030P24

(2). 10^{-3}

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

too dilute

4.9

(1) 10^{-3}

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

K-12 × B/1.

11P24. 1ml Y41 + Y43 in YB. sh. 30°

N25. Drops into T(0). plates.

0

to 7P25, on desktop. Backs on shelves.
1P27. Plate out. 0

Y43 × Y44 0

Proteus + coli.

11P24. Inoc YB D14, Y41 Sh 30°

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

to A27. Only typical bacilli.

[Repeat in yeast ext - peptone medium]

P27 Repeat in coli ss.

a) D14 + Y41 — only bacilli

b) agar on plate early atypical [illegible] → practically exclusively the unusual organism (antibiosis?) (actinomycete?) But fine filaments of long cells like subtilis, staining well c̄ safranin)

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.

Y41 x Y24. m T(0)

Plate 5. T-1

1–20

5 R
15 S.

8 suspected B⁻ in large tubes

	T-1	T(0)	T(B)
1	R	−	−
2	S	−	+
3	R	−	|
4	S	−	+
5	S	−	+
6	S	−	+
7	S	+	+
8	?	−	−

(7: Prototroph not coli)

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

Streak out + test for biotin.

m Biotin

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

	T(0)	T(B)
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		

Plate 3 (31–45)

2 — 17 total
5 R
~~15 S.~~

11 S of 10.

3 — 15 total.

12

of 21 prototrophs from [illegible] plates, 5 — R

Biochemical recotypes.

TLB₁ × BφC on B₁ φ. 19

	Plates T(B₁)	Tubes T(B₁)	Plates T(φ)	Tubes T(φ)	Plates T(B,φ)	Tubes T(B,φ)	
1	+	+	+	+	+	+	✓
2	+	+	+	+	+	+	
3	+	+	−	+	+	+	
4	+	+	+	+	+	+	−
5	+	+	−	+	+	+	
6	+	+	−	+	+	+	
7	+	+	+	+	+	+	−
8	+	+	+	+	+	+	−
9	+	+	+	+	+	+	
10	+	+	+	+	+	+	
11	+	+	−	+	+	+	
12	+	+	−	+	+	+	
13	+	+	−	+	+	+	
14	+	+	−	+	+	+	
15.	+	+	−	−	+	+	B_1^-

This is very suspicious. There should not be so many prototrophs per original plating data ..

on BT. 21

	B −	BT −	T −	
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^-

[illegible] for

φT. 22

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

Check [illegible]

	φ	T	φT
32. large tubes	−	++	++

but came up late on T. streak out + test isolates.
↓
4 isolates behaved similarly

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

φ is just stim. or $T^- \to T^+$ very readily in this strain. Drop it.

 Filtrate - transformation.

N 25. Mor YB c̄ 58-161.

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

1. Mor c̄ 1ml 58-161. Sh. 30° 1P25.

2. Mor YB c̄ 1ml 58-161; 1ml Y41 (culture above).

Plate 1P27. ca 200

Mor YB c̄ Y41 P25. Filter 1P27 & as above 1.

Mor 58 161 P27

Plate in T(o) 7P28.

0.

7/26/46.
YB medium 1ml 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/1)

48 hours. But not quite optimal numbers.

Sex - conditions

July 28. 1946

7P28. Mix c a drop of mixture. Sh. 30°
- 1130A29.

			Growth	Colonies (1/10 ml)
1.	Y24 + Y41	T(Ba)	++	36
2.	do.	YB	+4	6
3.	~~Y24~~ + Y10	T(Ba)	++	0
4.		YB	+4	150 ✓
BM+TL 5.	Y40 + 679-680	YB	+4	33
6.	Y40 + Y45	YB	+4	0
B⁻P⁻ TLB. 7	58-183x + Y10	YB	+4	30

Study segregation [illegible]

8 679-680 (to get a 680). — in O 0; in T 0; in L 10

Plate 0.1 ml : 1-7 into T(0). 8 into O, L, T.
Save 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 merely stimulatory as Roepke suggests it is in wild type.

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

Try series c̄ vit. left out.

	TLM+Vits.	12h.
1.	− B₁	+
2	B₂	+
3	pab	−
4	niacin	+
5	folic	+
6	B6.	+
7	niacin	+
8	pant	+
9	inos	+
10	biotin	+
11.	+V+yna	+
12	TLM yna	−

Here is a pab-less, then, which is ~~not~~ completely replaced by ~~pab~~ methionine + yna. Compare Y44.

Y44: 8/28

	24h.	36h
M		
M + 100γ pab (steile filtered).	+++	+++
+ 10γ	+	+++

Linkage of Virus-Resistance.

8 PM. 7/28/46.

Plate. Incl 287-4 into (Stored in H2O 24h in cold)

					Δ over O.	Δ/O	Look for
1.	O	30, 25, 26	average:	27			
2	B	28, 33, 30		30	3	.1	R
3	∅	38 35		37	10	.3	R
4	C	29		29	2	.1	R
5	P	~~[illegible]~~, 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, ∅, or C.

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

P30.

		# tested	# resist	fraction.	Calc. R in mutant.		
1.	O	27.	2	.075			
2	B	23	5	.22	>0	Check R.	2B⁻ 3B⁺
3	∅	30	0	.00	0		
4	C	15	0	.00	0		
Am.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⁺ ~~[illegible]~~ S
1 T⁺ R,

7/29/46.

M28 — Inoc 50 ml coli ∞ ē D3, D14. Sh. 30°.

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

Inoc 1 ml into coli ∞ sh 30°; .1 ml into ∞ plates. Count.

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

Use D3 1 min. D14 2 min.

Expt 1/Jc — 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.

7/30/46.

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

A. 58-series. # P29. Incr 50ml coli ⊗ ē stock. SG. 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 " " (none 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 Layer. 3P1.

auxanograph.

Y10/1 × Y24.
TLB1R1 BΦC

30 JUL 1946

1 drop ea. 4B sl 30° 7P 30.
Wash and plate:

Use 1 cc in 1-7
• .1cc elsewhere.

Calc. type/cc 22

1.	0	46, 47, 64.	52	* 23	BΦT	12	17
2.	B	70, 66	16	* 24	BΦL	13	33
3	Φ	49, 61.	3	25	BΦB1	14 v. many small.	—
4	C	22+.., 31+.,		26	BCT	11	
5	T }1	28+32=60	8	27	BCL	10	
6	L }1	44+44=88.	36	28	BCB1	13	
7	B1 }1	94+70: (164)	112	* 29	BTL	73 [41]	302.
*8	BΦ	9	4	* 30	BTB1	39	52
9	BC	7	0	31	BB1	41	—
10	BT	6	0	32	ΦCT	5	
11	BL	12	0	33	ΦCL	5	
12	BB1	19	0	34	ΦCB1	14	
13	ΦC	5	0	* comp? 35	ΦTL	56+144 sm.	158
14	ΦT	3	0	20. 36	ΦTB1	20	—
15	ΦL	10	0	37	ΦLB1	43	—
* 16	ΦB1	23	104	38	CTL	39	
17	CT	11 (+)	0	39	CTB1	30	
18	CL	10	0	40	BΦTL	56.	—
19	CB1	12	0	41	BΦTB1	28	—
*phage. 20	TL	35 +	196	42	BΦLB1	53	—
*ph. 21	TB1	22	82	43	BCTL	60 [24].	
*ph. 22	LB1	46	350	44	BCTB1	28	
				45	BCLB1	41	—
				46	ΦCTL	23 (incl. 6 small)	
				47	ΦCTB1	26	
				48	ΦCLB1	41	

August 1, 1946

Irradiate 36 hr. culture of 679⁺-680 u.v. quartz tube, 2 mins. 1P1

Inoc 1 ml into coli ∞. (YB)

N 2. Detection plates.

Pick 8 colonies:

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

Phages T2 – T7.

August 1, 1946
Received, possibly contam., A31 $\bar{c}$ titres ca 10^9.

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

Centrifuge off cells + sterile filter.

Plaque 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-7.

Repeat with others. n.g.

Plate T3, T7 of above $\bar{c}$ (filter?) mutants for resistance.

T3 Y40 5 do many resist.
Y41 good lysis on most of plate
161
Y10. N.G.

T7. not mixed well; no lyses?
" ; lysis; resistants in no conc
do.

Culture phages $\bar{c}$ B/r. A2. + K12 in liquid.

3½h:

		K-12	B.
Filter	T2	±	±
Filter.	3	±	–
	4	∓	T?
	5		T+
	6	∓	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.

→ 7'

Inoc K, B $\bar{c}$ culture 1P2–

Tests on 290 - Vesicles 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	−	
8−	7	−	
✓OK	8	−	
	9	+	
	10	−	
	11	+	
	12	+	
	13	+	
	14	+	
	15	+	
	16	+	
	17	−	
	18	−	
	19	+	
	20.	−	

(Entries 11–20 struck through.)

18
8+

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	1

Analysis of 293: Y10/1 × Y24.

1. Phage resistance from 0 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. Bϕ. [Exp. 1:20].
6. B_1ϕ [Exp. 2:3] 2 ++ 1 B_1^-
7. T-1 on TL, TB_1, LB_1
8. ~~23~~. BϕT [Exp. 1:6] 4 ++ 3 T-
9. BϕL [Exp. 1:3] 5 ++ (1 BL) = 297-9. R_1.
10 ~~B~~TL [Exp. 1:2]
11. BTB_1 [Exp. 1:6] 3 ++ 4 T -
12. ϕTL [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
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.
11	B_1^- ✓	S ✓ *
12	B_1^-	S *
13	B^-L^- ✓	R ⁻
14.	T⁻	S *

✓ Recheck biochemical req.

297 9, 12.

? 297-9.

297-6, 11.

6 T-
2S.
4R.

T- S 3
R 4

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

8 AUG 1946

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

pre. 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: 2 P 9. Cover most plates for deep colonies. Use 2% agar base. 58-161.

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

1. 10^{-6} .1 ml 75,
residue on smearing rod: 0
2. 0 10^{-4} .1 ml +++ (compatible c̄ 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. bact.

$(.027)^2 = .0007$

Proteus mutants

August 8, 1946.

Irradiate 1.5 mins. in quartz tube.
P3, DK1. Inoc 5 ml into 50 ml coli so. 11P8.

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

Pick 12 D3, 10D14.

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

Y24 x Y10/1 ; ~~Y10/~~ Y24/1 x Y10.

~~290~~
300.

8 AUG 194[illegible]

P8 moi. YB.

5P9 plate into 0, etc. .5 ml

A

Y10/1 x
Y24

0	28	} 28.	10/29 R_1 = .34		
0	26				
0	30				
BT	30		9+	T	1
BL	8 T.				
BB_1	~~ca 100.~~ 82		9+	B_1	1
φT ~~[illegible]~~	34		8+	φ	3
φL	16 T.		3+		
$φB_1$	~~ca 100~~ 85.		9+	B_1	1

β

Y10 x
Y24/1

0	35	19/26. R_1 = .73			
0	15				
0	27		7+		
BT	56		3+	(BL) 1 T 2	(300 - 1)
BL	5?				
BB_1	ca. 100.				
φT	36				
φL	29		2+		
$φB_1$	ca 120.				

Sample colonies + test for aecotypes.

Associated Mutations
Reversion.

August 10, 1946.

P10. Inoc 50 ml coli 0 $\bar{c}$ 679-~~8~~80 / Sh. 30°.

P11 Wash + inoc 5 ml ~~into~~ T(0) + NEAA + Vits + EAA –
a) leucine
b) threonine.

Plate 1 ml into T(0), T(lc) T(thr)

Plates: L. 22
T: 4
O. 0

Recotypes.

August 15, 1946.
Mor J YB P12 plate M14. See cards.

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

301-1 "B,φ" – grew on B_1.
2 BB_1 ✓✓
3 BB_1 ✓✓
4 B, C grew on C.
5 " " " "
6 BB_1 ✓✓

301-1-3.

15	1. $B\phi TB_1$:	+ 6	φ B_1 6 (Bφ 1) (BB_1 2)
15	2 CL :	+ 14	1 C (301-6)
15	3. C B_1 :	+ 5	B_1 8 ~~..~~ (B_1 C) 2. 301-4,5
3	4. φL	+ 3	
5	5. φTL	+ 4	T_{R_1} 1
7	6. BTL	+ 6	T 1
15	7. $BB_1\phi$	+ 5	B_1 9 (BB_1) 1 (301-6)
13	8 $B_1\phi T$	+ 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 BφT.	+ 10	B 3 Bφ 2
15	12 BφL.	+ 12	L 2 φ 1
12	13. $B\phi B_1L$	+ 8	B_1L 2 B 1 B_1 1

2. Minimal
3. 0 ~~BLP BLP BLP~~
4. 0 ~~BLP BLP BLP~~

Collect B_1 and test for R_1. ~~13/19~~ 7/20 resistant!! from Y24 x Y10/1!
χ^2 = ca 2.

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

See 305

Compare c̄ wilds on plates where each mutant could come up

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 + on av. plates	146	37. ~~80~~	31 ~~38~~	55 ~~70~~	56 ~~89~~	52	19	16	28	41

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	~~#~~	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		Candomyces albicans			
Y17		Endomyces fibuliger.			
Y12		Schizosaccharomyces pombe (Wickerham)			
Y15		S. pombe (Spiegelman).			
Y16					

Alcaligenes fecalis - Yale Med. 35 protol.
Aerobacter aerogenes - Yale Med 12. protol.

* strains which adapt readily
" " do not "

15 Aug.
Inoc 2 drops Y40 into 5 ml YB contg: 2P15 30°
a 50 u./ml — 11P15, N16 - filaments; "zygospores" common.
b 100 Markedly inhibited.
c 150 Strongly inhibited.
d. 200 " "
penicillin.

Repeat: -

10P21.
1. Penicillin 2500 u. /50ml. + 1ml inoc. Y10/1. Sh. 30°
4P22. Filaments + beaded ~~[illegible]~~ rods. V. rare "zygosp."

4P21. Inoc 1ml Y10/1 into YB 30ml Sh. 30°

Salmonella stocks.

August 20, 1946.

Received from P+S diagnostic labs. to pest 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	cholerasuis	VI, VII	(c)	1, 5	+	−	+	++
E	S4	" "				+	−	+	+
E	S5	enteritidis	I, IX, XII	g, m		+	±	+	+
E	S6	"				++	±	++	±
E	S7	oranienburg	VI, VII	m, t		++	+	++	+
E, N	S8	montevideo	VI, VII	g, m, s		++	+	+	+
E, N	S9	newport	VI, VIII	e, h.	1, 2, 3	++	±	+	+
E, N.	S10	"				++	±	++	++
EN	S11.	typhimurium	(I) IV (V)	(i)	1, 2, 3	+++	+	+	+++
						+++	+	+	+++

24 hour readings:

		R −V	B −E	RS −N	RA −O		
E	1	+	−	+	+	para A	methionine, tryptophane
	2	+	+	+	+		
E	3	+	±	+	+	cholerasuis	++ on EAA; ~~+ thre, + methionine~~ — any single no.
E	4	+	±	+	+	cholerasuis.	methionine
	5	+	+	+	+		
	6	+	+	+	+		
	7	+	+	+	+		
	8	+	+	+	+		
aggl	9	+	+	+	+		
	10	+	+	+	+		
	11	+	+	+	+		

3 pullorum stocks - see infra. as above in analysis

Crossstreak Salmonella pullorum, and S1, S4 c̄ T1, T3, ...

Recombination E coli

10PM. 8/26/46.

Sh. 30° YB. 1ml inoc.

(1.) Y24/1 x Y10

(2). Y24/1 x Y10/1 No colonies!!

(3). Y24/1 x 679-680

(4) Y24 x Y10 20/20 susc.

---

(1) Plate 5P28. (½ml.) Examine P30.

1 0 52
2 0 51
3 B1 211
4 B1 ~~12~~ (142 but smeared)
✓5 BΦ TB1
6 BΦ LB1 very crowded.
✓7 BΦ TL
8 BC LB1 very crowded
9 BC TL turbid.
10 BC TB1 ~~TL~~ ✓

11 ΦC TB1 Too turbid. ✓
12 ΦC TL too turbid ✓
13 ΦC LB1 too turbid ✓

(3) 359 This series may have many T-reversions.

371 Test 80 isolates 78 ++
423 on T(0). Keep inoc. 2 ?✓
tubes in order for v. Check. ++
304-3A
-3B

cystine contains too much B1 B1 etc. evidently.

data:

Plates. Colony types:

BφTB₁ 15 + 6 φ 5 B₁ 2BφR 1B₁! 1B?

BφLB, none taken

BφTL 20 + 4 φ 1 BφR 1? ~~(1? BφL)~~ ~~(1? BφT)~~ φL?
Bφ?

BCTB₁ 5 + 3 B₁ (1 B,B?) 1 B,T? (R) ∴ ecotype.

+ 27/37 R. 8/9 R.B₁. 10/10 R φ
1 mixed (304-1) See 305

40+ 1 B ~~1 (BB₁)~~
10 φ 3 BφR
9 B₁ 1 B,T
(8 B,R; 1 B,S)

304-1 streak out + test:
10/10 S!!

φL: app. OK but check in detail. same growth on φ 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 slow lysis + colonies in zone of streak. ② ⟶ 1/10 R. (streak out.) = 2b.

a. Streak out ① + ②

Test mutr. reg. of several types.

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

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

a: test (a1) + (a2) 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
leucineless mutation.

10 SEP 1946

48 hour S12 in YB. Mor 1ml-eq. in:
1. T(0) no colonies
2. T(L) not turbid!

later found needs cystine

Stocks for linkage study.

September 4, 1946, ff.
The $6C_2$ combinations of B, B_1, T, L are available.
Streak out on NSA plates and inoc: single colonies into 50 ml YB. Inoc to CC slants for inocula to confirm growth factor requirements. Inoc. with excess T1, T3 in NSA plates for virus-resistant mutants.

	Sources:		Nut. Rq.	Virus.		✓
Mut.	"TL"	679-680		S_1	S_3	Y30
Recomb?	"TB_1"	304. n.g.	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"	Y45.		S_1	S_3	[illegible]

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

Stocks lost before used.

Shift - mutations.

8 SEP 1946

Recd. from Roepke + Langsam:

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:

1A8.		lys	meth	lys+meth.	0
6P.	α	+++	−	+++	
1A10		+++	++	+++	−
6R	β	−	−	−	
		+	+	+++	−

Reversion Y10.

8 SEP 1946

Inoc/ml 36hr. Y10 into

		colonies (48h.)
1.	0	—
2.	B_1	—
3.	T.	1 - (cont? — take out + test)
4.	L	—
5	B_1 T	—
6	B_1 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) M — — — T — + MT ++ methionine, trypt

~~S1~~ ~~M~~ ~~O~~ ~~C~~

Y50. O N,C

1 SEP 1946

1 ml inoc. S1, S17 (S15?) in YB c̄ single controls. Sh. 30°

12:30 A 11

Plate N13. into T(o) dupl.

no colonies many plates.

Salmonella: N.R.

12 SEP 1946

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

(S18–21:) There must be a substitution to acct. for these results; probably cystine + methionine. Check with thioglycollate also

(also: arginine - prol; glut / [illegible] - [illegible] / φ-alanine - tyrosine / tryptophane - serine

(S32:) parathiotroph.

(S34:) parathiotroph

〃

(S37–38:) parathiotroph [illegible]

Ev. no good mutants here!

S19 } S32 } S34 } parathiotrophs; others are prototrophs

S35-8 may be mutants.

These tests were not too careful. In retest, only S36, S37 held up as mutant type.

Stocks for linkage Study.

18 SEP 1946

1P18 Inoc NSB to prepare inocula.

A19 - plate c̄ T1, T3 to obtain resistant mutants.

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

Y41, TL, BL, BT, are sens. Y10/1 ~~completely~~ lysed!
Y24, BB, LB, * (~~without~~ residuals).

Y10/3 OK for resistance.

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

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

BM/3 OK. R.

BB./1 — mucoid.

Y10/3 OK R.

BT/1 Mucoid.

LB./1 v.s. colonies v. dry - probably cont.

LB./3 }
TT/3 } looks like coli, but green!

Pick to YB tubes. Test for resistance to 1; 3.

Linkage: BMR x PT; BM x PTR.

18 SEP 1946

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

3P24. ~~In 10 ml~~ 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 ~~BM~~ 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 } ~~5/23~~ 5/24 .21. PT/1 x BM.

Resume linkage data - phage resistance

Ref.

257 BM×PT/1 2/10 R. 0.2 } compare 313

BM/1×PT 7/10 R .7

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

$30/137 = .22$

.78

313: Bφc/1×PT. 27/30
20/20
27/30

74/80 = .92

.92

287- BφC × PT/1 2/27 R .1
on B: 5/23 .22

293- BφC × TLB₁/1 10/35 R. .29
300A " 10/29 R .34
300B Bφc/1 ×TLB₁ 19/26 R .73

301-1. BφC × TLB₁/1 20/49 R .41
Bφc/1×TLB₁ 27/37 .73

SUM: A. BφC ×TLB₁/1. 40/183 .35
B. Bφc/1 ×TLB₁ 46/63 .73

$\chi^2 = 0.8$ for .35 vs. .27 (1 - .73)

$\chi^2_1 = 9$ for .35 vs .50

$\chi^2_1 = 5.5$ .73 vs. .50

1.08

315- BφC ×TLB₁/1 13/73 R .18

318 Bφc/1×TLB₁ 34/40 .85

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

R: Y40× ~~Y53~~ Y64×58-161

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

Linkage-/T1.

19 SEP 1946.

A. BφC × TLB/1 B', A'. is mixture in old medium

B. BφC/1 × 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° sh. (A_L, B_L)

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

Wash + plate N 20.

A series: some large col. many small heavily seeded micro col.

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

B	a.1
	2
	3
	4
	5

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

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

		T-1 R.
A	b. 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 sh. 30° Also controls

n.g. — Y24/1 cont
starting [illegible]

Staph. flavocyanea

20 SEPT 1946

ENV	NA.	NV	EV	EN
++	+	+	+	−

try on vits – n.g. ~~Reat~~ Repeat!

BΦC
TLB, - 1

24 Sept. 1946

In 10 ml YB, 1P24.
1. Y24 x Y10/1 (A)
2. Y10 x Y24/1 (B).

Wash + plate p. 26.
nitol 1(0).

(A) (B). p28 - colonies as in both tests.
No evidence of marked increase.

do. - spread .1 ml mixture on
CC plates.
(A) + (B).

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

B:

T-1	R.
28	33
2	3
1	1
1	1
1	1
1	1
34	40

34/40 = .85

A 1/9
4/10

September 29, 1946.

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

P1. Plate into T(0). only a few colonies.

Effects of dilution and of u-v killing on recombination

30 SEP 1946

N30.

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

Dilution			
10^2	1. 1 drop 679-183 (PT)	* compare c̄ final PT population.	1 drop = .1ml. true.
10^3	2. 10^{-2} drops.	"	
10^2	3 10^{-4} drops	"	
15.	4. 10^{-7} drops.	"	(plate .1ml.). original = ca 10^9/ml OK.
	~~[illegible] drops.~~	"	~~plate~~

B. To 10ml YB, add 1 drop. 24hr. Y24/1 and 1ml of the following.

6	see A1.			
0	7. 1ml u-v irradiated	5 mins.	plate .1ml.	10*
0	8. 1ml u-v ir.	10 min.		20
0.	9. 1ml u-v. ir.	15 min.		20

Plate Pl into T(0).

∴ sublethal doses of ultra-violet prevent recombination!

Heterozygote Isolation?

30 SEP 1946

11P30. Inoc 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 damp to pick.

This, therefore, is the better procedure.

Salmonella typhi-murium

Mutants – (u.v.).

30 SEP 1946

P1. Inoc S20 and S21 into YB.

II P2. Irradiate each culture 1, 2, 5 mins. + inoc 1 ml samples into 10 ml YB. Dilution plates SP3. 25°.

1 Y20 - 1m
2 2m
A 3 5m Use. 2000 cells sampled. 7 small colonies.

Picks A8.

4 Y21 1
5 2
B 6 5 Use. 1200 sampled 2 small colonies.

		YB.	O.	E	N	V	ENV	
A	1	+	+	→				green } cont.
	2	+	+	→				green
	3	+	+	→				pink. cont.
	4	+++	→					OK. in color
	5	+	→					yellow
	6	+++	–	+++	+++	–	+++	OK. S70.
	7	+++	→					OK. in color.
B	8	+	→					green cont.
B	9.	+	→					yellow

same app. do not grow well even on [illegible]

S70: → parathiotroph

Salmonella - N.R.

30 SEP 1946 Al:

	T(o)	ENV	EN	EV	NV	
SR4 abq.	– –	++ +	++ +	++ +	++ ±	++	? (Estim) grows well as T(o). [Test for adapt]
36 gall.	– –	++	± +	++	± +±	V, E. Thiamin
37 dub.	– ++	+ ++	± ++	+ ++	± ++	( ) Thiamin.
42 t.m.	– ++	+ ++	± ++	± ++	– –	(E) Tryp.
45 ent.	– –	±R ++	±R ++	±R ++	– –	E. Arginine
50 para A	– –	– ++	– –	– (±)	– –	E, N, V. BIOTIN, TRY.
51 B	– –	+ ++	+ +±	+ ++	+ ++	(v?)
52 B	– –	– –	– –	– –	– –	? - clear?
55 chols.	– –	± ++	– ++	– +	– –	E (N) TRYPTOPHANE; ~~(methionine)~~, ~~arginine~~ ~~SERINE~~.
56 ch.suis.	– ++	± ++	± ++	± ++	– ++	( )

The clear-cut ones are:

- S42 - typhimurium - (E)
- S45 - enteritidis (E)
- S50 - para A (ENV)
- S55 cholerae suis (E)(N)

others are:

S24 - prototroph

gallinarum V, E
dublin, } stim? no sp. req.
S56 chol suis }
S50 para B

S52 - did not grow

42 no.gr. Trypt.
45 Arginine. (citr ±) (orn)
50 Biotin; TRYPT; grows s̄ N.E.A. (~~[illegible]~~; meth) BTCM n.g.
no!! 55 TRYPTOPHANE; SERINE T+S n.g.
36 ENV+++ V++ E++ N- O- ? V or E +- B₁
37 ENV+++ V++ E++ N- O- Try Valine V or E +- B₁.
56 ENV+++ V-++ E++ NE++ O- ~~T~~ E or NE
52 ENV+4 EV+ EN- NV- ~~[illegible]~~ O- stay all.
51. ENV+++ V+ E+ NE- O-

1 OCT 1946

add incr. amts. of Penicillin (oxford units) to 10 ml YB broth.
add 2 drops of Y24 + Y10/1. 10 P1. Sh. 30°.

Penicillin (per 10 ml). 10H2.

3 1. 0 some very motile
1 2. 100. motility diminished; a few filaments.
0 3. 200 v. "stringy" turbidity v. filamentous
0 4. 500. partial inhibition. v. filamentous; many "zygospores"

Wash + plate.

10H2 also inoc. 1 ml each into 10 ml YB.

1 1'
7 2'
2 3'
8 4'.

penicillin is ineffectual.

	S	0	ENV	EN	EV	NV	
Edwards 10/9/46.	57 typhi suis	—	++	—	—	—	ENV
	58 abortus ovis	—	+++	++	+++	—	E
	59 sendai	—	+++	+++	+++	+ ++	Try E. (use sary 9 EAA)
	60 sendai 571	—					E or N? methionine / compare 56 etc. do.
	61 Taube I	—	+++				
	62 Taube II	—	+++				
	63 P-1	—	+++	++	+++	+++	
	64 P-2		+++	++	+++	+++	
	65 P-11	—					
	66 1181-1	—	+++				
	67 1181-2	—	+++	+++	++		
	68 H66	—					
	69 Pigeon 1366.	—					
	70 324/6 S 20.		E;N. $+^3$ methionine. (SH.).				

Beth Israel 10/21/46
71 typhi suis 2943
72 sendai 3007
73 para A 3280
74 para A 3089
75. typhi 3166

Kauffmann
76 S. para A
77 " durazzo
78 typhi 2
79 " Watson
80 S. sendai
81 "regdam -
82 " +

Linkage. 325

2 OCT 1946

1. P2 Y24 × Y10/1.

[2. Y24 × 183]

[3. 183]

4. Y24 × Y41

5. Y41.

Plate .5 ml eq. (1) .2 ml eq. 4,5 into T(0) and suppl. to detect linkage of R.

Plate .5 ml eq. Y41 into O, T, P. 20 colonies on T(0)! crowded on proline.

Expt n. t.

12 OCT 1946

Inoc 1 drop of 12 hr. cultures into 10 ml ½B. Plate 10/14/46.

S1 × 13
1 × 45
1 × 50 — 1 colony formed. [para A × para A !!!!]. Needs rechecking. ~~Later~~ found ~~that~~ S1 secreta.
1 × 55
coli) 1 × TL.

13 × 45
13 × 50
13 × 55
coli) 13 × BT

45 × 50
45 × 55
coli) 45 × TL

50 × 55
50 × TL
coli) 55 × TL

1
13
~~4~~5
55
TL
BT

repeat. S1 × S50. 10/16/46. Plate into T(0), T(trypt).

very many minute colonies in presence of tryptophane. Should be repeated c̄ (ca ½) a more diluted inoculum. v. much smaller (a few cells/colony) s̄ trypt.

Incubations

2 Oct 1946

Mix 2 . growncultures . N2 . (4B) 30° sl.

424 x 410/1 Plate 3P3

~~Selection plates . 7(o). 10⁻⁷. P3~~

2 colonies. / 1 unfeq.

Trilateral combinations
test for transformation.

12 OCT 1946

Inoc 1 drop 12 hour cultures into YB   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 much as?

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
S45. ✓	O	PROL	GLUT	ORN	CITR	ARG.	arinthine		
	—	—	—	+++	+++	+++			

S50. ✓	NV-TR. ✓	NV-TRM. ✓	V-TR ✓	V-TRM. ✓	N-E ✓	BIOTIN+E. ✓	E ✓	NE+E ✓	N.E.V. ✓
	—	±	—	±	±	—	—	++	+++

S55 ✓	SERINE-E.	NE-TR.	Ser-Tr.	NE-E	E	NE ?	
	+++	+++	— +	+++	+ ++	+++	try Trypt + meth.

S56. ✓ NE - Tyrosine (??) o.

S36 ✓ V ■ (B₁) ~~also~~ o.

	B1	B2	3	4	5	6	7	8	9	10

S37 ✓ V+. B+ +++ B1 only OK. Thiamine ✓

S42 E - Tryptophane.

S58 E -

S59 E - Grows on any combination of 9 EAA.

S61 E - Grows on any combin. of 9 EAA.

(Heterozygotes)

4 OCT 1946

Mix 24 hr. cultures of Y24 and Y41 into T(0) varying quants. (washed separately. In 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. ē varying chloral hydrate

		Growth.	Prototr.:
1	1%	±	
2	.1%	+++	ca 100.
3	.01%	+++	ca 500
4	.001%	+++	
5	.0001%	+++	ca 500
6	.00001%.	+++	
7.	—	+++	ca 10^3

sl. (?) inhibition, but not feasible for exp. use.

plate. A18.

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%.

V.f. Ry. – mai. Fruis.
no growth – pab
delayed growth – mi.

Salmonella NR

21 ... 1946

S36. 0 — B₁ +++ Vits. +++ OK. Thiamin.

S56 0 — tyr ++ tyr+glyc ++ Vits ++ Tyr+Vits ++ ØA+Vits ++. which Vits?

S50: 0 ENV +++ EN — NV +++ EV — V try — try leuc — try leuc — try meth NV try leucin

S55. 0 — Su+E + ++ E ± ++ SerineTr. — ± NE Tryp. + ++ NE-Ser. + tryp. ± +++ ∴ Tryp. + {NE, Ess.

S42. 0 — Tryp. ± +++ n.g. 24h ~~very slow!~~ Tryptophane.

S58 0 Tryp.

S59 0 Tryp. single amino ac: E: any a.a. +++ 0 ++. adapted!!

S60 0 ~~Tryp.~~ Single amino ac. E: meth +++ others —

S67, S63, S64. (?)

S70. 0 Tryp. Single amino ac E: meth +++ others —

Sands. Lind. generous B vits. etc
— pab, folic OK on -folic. ∴ -pab, -nic!
— nic.

Salmonella, N.R.

23 Oct 1946

	O	EN	ENV	ENB	ENB.	NV	BN	
S50.	–.	–	+++	+++	–	(+)	(+)	∴ Biotin. can desp. c.a.a.

	O	SH	Cyst	Meth.	Trypt Middle Broth
S61	±	–	–	+++	

S70. – ± +++++ +++ ++ +++. true paratheotroph!

S42 – ±++ ± ++ – – – author → inf → trypt.

S62
63
64
65
66
67
68
69 ē Fildes.

S9. ± ++ heavy inoc ++ (and adapts!)

S56. Try on all amino acids + vitamins separately. V +++ E +.
Biotin ++ no E. alanine ±. lys. ± trypt ± tyros. ± || 48 hr: Biotin +++ alanine ± tyrosine +++

S55. Trypt + NE (all +; let a time) and ~~Trypt~~ NE (all):
T + N +++
N ++
individually: – – exc. cystine ++ ok. Try cyst. alone.
0 – heavy inoc. in 0 +++

later 0: –
any NE. ++
all NE do.

Test growth in proline, for transfer growth in minimal, proline; compare original culture.

E coli mutants.

24 OCT 1946

Selective media (lactose)

1. EMB Difco. — OK.

2. Endo: lactose 1% per l.
   - Na_2SO_3 2.5
   - Fuchsin .4g
   - K_2HPO_4 3.5g
   - Nutr. agar

   n.g. as made up.

3. Purple lactose:
   - Nutr. agar
   - lactose
   - BCP .025g/l.

   n.g.

Streak K-12, S1 and K-12+S1 on each of these plates, for decision as to most appropr. medium.

Salmonella N.R.

(S50) Biotin + : (N~~V~~)– (EAA) — BN ~~++~~ + none grew.

(S56.) ✓

	0	BIOTIN	TYROSINE	dl-tyrosine
	–	±	±	+++
	–	±	+++	

Biotin? ?

	0	TRYPT.	CYST.	TR+CYST.	TR+PROL.
S55. ✓	–	–	–	–	–
S5a.	–	–	–	+++	–

needs Trypt, cyst adapts readily.

S57. EN V– : EV N– : NV E– : none grew! –<u>Leucine</u> found later (tubes allowed to stand)

S58 E– : none grew!
<u>0</u> ENV NV EV.

(S60)

S51 ✓ NE+ <u>proline</u> +++. others – NE +++ Serine ±

S52. NV E–

	0	Meth.	N~~V~~	N~~V~~-cyst	Cyst.
S61. ✓	+++	+++	+++	+++	+++

all adapt!

✓	0	Meth	Tryp.	
62	++	+++	++	✓
63	+	+++	+.	✓
64	+	+++	+	✓
65	±	+++	–	✓
66	±	+++	–	✓
67	±	+++	–	✓
68	±	+++	–	✓
69.	–	+++	–	✓

Sugar mutants – E coli.

Yeast

Endo-lactose

	K-12	S-~~49~~
a. Med School	+++	− ±
b. made up	+++	−
c Endo-glucose	+++	+++
d. Endo-sucrose.	−	±
e maltose	+++	slow +

EMB. N.A. + 1% sugar + 2g K_2HPO_4, .4g Eosin Y; .065g MB/l.

a. lactose	− +++	−
b. glucose	+++ sheen	+++ no sheen!
c. sucrose	±	−
d maltose	+++	−

Try: c vit suppl.

Coli (T(0))
½% gluc — n.g.

Fries (N(0))
2% sucr. — requires pab.

*Burkholder's
5% gluc. — only on vits.

Coli T(0)
5% gluc — n.g.

* /liter
glucose 20
aspar. 2
KH_2PO_4 1.5
$MgSO_4$ .5
$CaCl_2$ .3
$(NH_4)_2SO_4$ 2.0

with KI.7

S51. ✓ 0 +++ | glut +++ | prol — | orn ++ | citr ± | arg. ~~+±~~ — | E-arg. | Ho prol — | E ~~arg~~ +++ | N +++

later, all up- adapted(!?)

S55 ✓ 0 — | trypt T+ ++ | cyst T+ +++ | meth T+ +++ | S-AcOH. +++

S52. ✓ NV E- all +++ exc. trypt. ++.

S50. ✓ Biotin + N- all — BN, BE ++ !!

	ENV	EN	NV	EV	E	N	V	0
S57.	—	~~+±~~						
S58	+++	++	+	+	—	±	—	—
S60	"	"	"	"	"	"	"	+++

(may have adapted on slant (large + small colonies on V-B slant).

S56. 0. — | ~~Vits.~~ Biotin +++ (Lactobacilli a contam.) | l-tyrosine +++ | dl-tyrosine. | alcohol ±

l-tyr.	l-φal	gly-tyr	(tyr-gly)	glut-tyr	tyr-tyr	gly	dehydro φal	pOH=0	pOH-glyc	glyc dehydro + glyc.
+++	—	+++	—	+++	+++	—	—	—	—	—

chuckam alcohol.

	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

<u>filtered solutions</u>

p-OH phenyl pyruvic	1.0
* phenyl pyruvoyl glycine.	1.5
* glycyl dehydro phenyl-alanyl glycine	1.5

* old solutions.

Lactose mutant: Y10

2 9 OCT 1946

washed suspension 10^9
Irradiate Y10 1, ~~2~~, 5 mins. SP 10/28.
Inoc. 2 ml into 10 ml YB shake 11 A 29. Spread
5 min. ca 100 cells on Endo-lactose + EMB-lactose plates. Incubate at 30°.

~~4~~ no mutants / 3000 on EMB.

Inoc 1 ml 2 min. culture. Re-irradiate 1, 2, 5.
spread on ~~100 plates~~ (EMB-lactose).

Irrad 2 mins:

161 × ~~|||~~ =

64 ||

480 × ~~||||~~ ||

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 YB agar
4
5 Yeast cont.
6 came up v. slowly on YB agar.

∴ 2 is only likely possibility of a lactose - coli mutant:

335-2:
T_1^S
requires T, L, B, ✓
Y53

Activity on various sugars: K-12, Y53.

11/13/46

The strains were tested on EMB plates c̄ sugar:

	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. o trypt.

S1 x S50 detected. + T. Latent virus???? (phage??) — no scum fu.
→ 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 —

Try again - many (++++)
do. v. small colonies

E coli — $T_1^R T_2^S \times T_1^S T_2^R$ ...

28 OCT 1946

Plate 24 hr. cultures:

(A) Y10/1 + 58-161/3 only ca 5 colonies/plate

(B) Y10/3 + 58-161/1. ca 25 col./plate.

Y10/1
Y10/3
BM/1
BM/3

T1 T3

M.9' smeared

(A) (1)

(B) (2)
⋮

28 OCT 1946

Plate 24 hour cultures of: on -

	0	B	T	L
$B^- T^- L^+$	*	*	*	more turbid.
$B^- T^+ L^-$	0	0		10
$B^+ T^- L^-$	0		0	400
3 strains:	0 / 0	100	1	0

* some minute colonies probably $B^- T^+$ on agar biotin.

- picks between colonies for new stocks.

$B^- L^+$ / $B^- T^-$ apparently only B^- appeared in the mix tube. (from $B^- T^+ L^-$ × $B^+ L^- L^-$)

must repeat + demonstrate recombinability of all three strains!

Try $B^- L^- \times T^- L^-$ etc.

But supports thesis of recombination.

Y10: reversions.

28 OCT 1946
Plate washed Y10 in.

O

TL ca 100 colonies.

TB_1 1 dup colony, n.g. for picking.

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-grin. 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.	~~1215~~ A	7	++
½h.	1245.	8	−
			Centr. 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 1-2 hours + mix — hours + plate.

Conditions:

glucose	++
NaCl	++± although growth was very much diminished!
Blood	++
" 1:100	−
control	++

29 OCT 1946

Sc O.

S. cerevisiae haploid - (S.c.) on B(0) [Burkholder's c 5% glucose].
V - 37° c shaking preferable

B_1 - stimulatory (carryover??)
pab -
pyridoxine
pantothenate
inositol
biotin.
} required on this medium. cf. Fries (may contain B_6, inositol in the tartrate.)

came up in 48 hours on everything 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 × S50.

1 NOV 1946

5 cultures each - S1, S50, S1 × S50. moi heavily P1.

Plate N4 ½ ml $\frac{o}{o}$ or .01 ml $\frac{o}{o}$.

S1 - 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 reversion!

S50 - dil. no colonies.

S1 + S50 - dil. v. supra.

S1 x S50 - phage

Plate 3da cultures of S1 + of S50 as follows.
on nutrient saline 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 a clear area previously was picked, it showed no ~~see~~ 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 culture 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. (1:6)	(cells) ×10⁹	colonies	Ⓟ/10⁹
*1. 2h	1007	66¹	[illegible] 2882	1.60	[illegible]	1.7	ca 20.	10
		66²	2059	1.14	88²	1.24	11	9
2. 1.5	10:37	66¹	55 2596	1.44	87 (1.5)	2.02	20	10
		66³	55 2596	1.44.	87	1.35	14	10
3. 1.0	11:07	65²	59 2291	1.27	86	1.46	ca 20	15
		64²	58 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		64² 1905	1.05	91	.96	20	20
			64² "	1.05	91'	.94		
6* ~~9P1~~	Mixed 12:10.		65 —	—	93	.74	—	0
7				1.00	90²	1.07	25!	25!
				—	~~81²~~	~~2.35~~	—	

83²: 660 filter.

* Mix immediately before washing; in 7. mix after washing.

#4 on 540 [illegible]
15: 77 / 73²

Washing commenced at:

Centrifuging 12:24.

Readings at 12:10

* contains Neurospora cont; inhibited around 1 colony call 344-1 Neurosp.

In this run, there seems to be an appreciable frequency of recombination on mixing in the plates!!!

Later: separate cells from mediums before testing

Y53 x ~~Y24?~~ 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
Sugar	10.

Plate after 4 hrs. growth.

a) ~~glucose~~ NYB-glucose

b. NYB - lactose

c. NYB - no sugar.

all colonies are smaller on lactose than on glucose plates!

1. Y53 a x Y40 a 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. Y53 a 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 Y40 c in NYB
   glucose no colonies
   lactose no colonies.

7. Y53b x Y40b in NYB.
   glucose 118 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 repleting on glucose medium.

	L^+	L^-
7+4: lac	5	12
gluc.	8	12
	13	24

4	6
3	7
7	13

	L^+	L^-	
R	16	31	47
S	4	6	10
	20	37.	57
	20% S	17%	35% L^+

18% S
$\chi^2 = 23$
$p = < .001$

$\chi^2 = 4.6$
$p = .02$

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, Y53 on glucose; Y10 on lactose; mate in presence of lactose only $\bar{s}$ growth; plate in lactose minimal ($\bar{s}$ asparagine) and in glucose and compare numbers which appear; also test Lac$^+$ for dissimilation.

Salmonella CROSSES
S42 × S45; S61 × S70.

8 NOV 1946

In YB broz 11A5   Crossin YB4PV.

— dil.

S42
S45 v. numerous colonies.

S42 + S45 turbid dil

S61 turbid numerous
S70 — v. minute colonies.

× turbid
no colonies.

1 C NOV 1946

Streak out fermenters from 345 - 4L and - 7L on nutrient - saline agar plates. Retest isolates on EMB - lactose.

all as before. (4)

Non-Genetic; Nutritional Adaptation

Disproportions in segregation ratios can be interpreted on the bases of the transfer of plasmagenes, or cytoplasmic centers. 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 variations 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 (0) $\bar{c}$ 1% glucose; as a supplement, add vitamins, hydrolysed yeast nucleic acid, and hydrolysed casein
0.3%

Wash cells in coli (0) $\bar{s}$ glucose or asparagine or NH_4 source.
i.e. in phosphate buffer
Na_2SO_4
NaCl
trace el.
$MgSO_4$; $CaCl_2$

Nutritional adaptation

11/7/46

a. Large inoculum from slant. Shake at 37° for 15 hours.
resuspend in H_2O ... ca. .3 ml.
b. 1 ml. from a in same medium. (930A18) Shake 37° hrs.

1, 2 lactose adapted a. into lactose glucose

3, 4 glucose adapted a into L/G

5, 6 α adapted a into α/0

7, 8 0 adapted a into α/0.

11 etc. is as above c b.

3'1'0 b

L/L	L/G	G/L	G/G	∞/∞	∞/0	0/∞	0/0	Time ~~hours~~	
1	2	3	4	5	6	7	8		
88	88³	92³	92	96	98	91		0	1045A
~~89~~ 89'	89	92³	91	95'	97³	91²	93²	30m.	~~1115A~~ 1115A
89³	89²	93³	90	92²	97³	90'	94'	2h.	1245A
90'	90'	88	76²	94³	97²	59'	61'	4 1/4	3P
90³	90²	75'	67³	94³	98	38³	39'	5 1/2	420P
90'	91	62²	54'	94³	98	34'	34	6 3/4	530P
+	+		29	+	+		30		12M

lag 2 1/2 hr. lag 1 hour

lag 1 1/2 hours

(30 = 7 hours ?)

11	12	13	14	15	16	17	18
			73	80'	80	80'	80

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^+.

[illegible]

Segregation of Lac$^-$; Evidence on transformation.

18 NOV 1946

6 PM prepare inocula. Mix (1-2ml mix / 2ml YB) and inc. 10 A 11. Wash + plate 1P.

1. ~~Y~~ Y53 × 58-161
TLB, Lac$^-$ T_1^S BM Lac$^+$ T_1^R

2. ~~Y53~~ × (Y1011) 58-161
TLB, Lac$^+$ T_1^R BM Lac$^+$ T_1^R

3. ✓ Y53 + Y1011 + 58-161.
TLB, Lac$^-$ T_1^S TLB, Lac$^+$ T_1^R BM Lac$^+$ T_1^R

4. ✓ Y53 × Y40
TLB, Lac$^-$ T_1^S BM Lac$^+$ T_1^R as usual. in glucose

5. ✓ Y53 × Y40 (lactose) in YB-lactose
plate in lactose-minimal ; L
and in glucose- " ; G
(c asparagine)

yellow colonies present in all plates. Contaminant somewhere!! (Wash water????)

do not use, of course.

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: time and salt

9 NOV 1946

Y10; Y40. prepare inocula separately 1) P8.
2) 1P8.

6P8: inoculate 1.5 ml of mixture into YB + ascending NaCl conc. (4 ml YB ...)
830P. wash 9P plate. .5 ml = d inoc. into T(0) agar.

#	
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 ē no growth!
use relatively young inocula.

Also, inoculate 0, 5% ē separate cultures, 1 ml. wash as 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:

	aggl.	gr. ril.
0	—	—
1	—	—
2	++	—
3	++	—
4	++	—
5	++	—
6	+++	+
7	+++	+
8	+++	+
9	++++	++
10	+++++	++

Sex conditions

Wash 10 ml 16 hr. cultures (YB) of Y40, Y53 c̄ water. 10 AM.
Mix 1 ml samples at various times; and dil part/mixture → 2.5% NaCl.
plate at: 3 PM. Mac (entries). Shake gently at 30°.

		H_2O.	NaCl.
1.	10 A.M.	20.	6
2.	1130 A.M.	8	5
3.	130 PM.	14	1
4.	3 PM.	2 plate.	0.

Note: they were more dense in the H_2O than in NaCl

$\left(\frac{c_1}{c_2}\right)^2$ = ca 4. which might account for the results.

5. Mix 1 ml sep. culture c̄ 5 ml agar + pour successively. 0.

Must use younger cultures. No confirmation of effectiveness of NaCl in increasing rate.

Repeat 350. Prepare inocula in YBG 3:30 P 14
incubate in YB 11A - 2 P4 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

Y54 ~~B-K T, p~~
Y40 T-B, Lac:

		T1 ✓	T2 ✓	T3	T4 ✓	T5 ✓	T6 ✓	T7	φ-C
1	Y54	S	S±	S ✓	titer too low? 1 plaque each	S	S	S	S
2	Y40	R	S∓	R		R	S	S	S all lac-
3	Y53	S	S±	~~S~~		S	S ?	S	
4	58-161	S	S	S		S	S	S	
5	— Y10/1	R	S	R ?		R	S	S	
6	"B M/3"	R ✓	S	R	S	R	S	S	
7	"Y10/3" —	S. ✓	S	S —	S	S	S	S	

plaques v. small!! OK.

5) is Y10/1,3,5 [illegible]

Threw out these "B M/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 [illegible] on 1st streak. picks.

✓ 58-161/3 ca 10^3 smeared. do. " " " picks

✓ Y10/1/3 smeared — [illegible] - is Y10/1 T_3^S ??

Y40/3 smeared. — do. Y40/3

Phage Resistance groups.

Parent		Phage	T1		2	3	~~4~~	5	6	7
Y53	1	T1	R	?	R	R		R	?	R
	2		R	?	R	R		R		R
M	3		R		R	R		R		R
	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
F	14		R		S	|		R	)	S
	15		R		S	|		R		S
	16		R		S	|		R		S
+F	17		R		S			R		S
	18		R		S			R	∫	S
	19		R		S	↓		R		S
	20.		R		S	↓		R	∫	S

resistance may be due to flow of mucoid growth ??

Parent		Phage	T1	2	3	5	6	7
Y10/1		T7.						
mucoid	1		R ↓	not readable	R ↓	R	not readable; not free of phage	resistant as a whole but entire streak has plaques
	2					R		
	3					R		
	4							
	5							
	6		do.	do.	do.	do.		
	7							
	8							
	9							
	10.							

Parent		Phage	T1	2	3	5	6	7
Y53		T3						
M	1	M	R		R	R	not readable.	R
	2	M	R		R	R		R
	3	S	R		R	R		R
	4	M	R		R	R		R
	5	S	R	S?	R	R		R
D	6	S	R		R	R		R
M	7	S	R	S?	R	R		R
	8	S	R	S?	R	R		R
	9	S	R		R	R		R.

should be checked!

Phage resistance groups

Y53 mucoid E

	T7	T1	T2	3	5	6	7
1				R	R		R
2				↓ moth eaten throughout	R		R
3					R		R
4					R		R
5					R		R
6					R		R
7	S				S	S?	R
8					R		R
9	?				R		R.
10					R.		

T1: R ↗ do. S; T2: all moth eaten (not free of phage) NS later nonmucoid; 6: no plaques (T5 mucoid); 7: less mucoid.

Test isolates for streaks.

G

			T1	T2	3	5	6	7	
	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	5	Y58	S	S	S	S	S	S	!–compare E. (mucoid).
Y10/1/7	6	Y59	R	S	R	R	S	R	
~~Y10~~	~~7~~	~~Y60~~	~~R~~	~~S~~	~~R~~	~~R~~	~~S~~	~~R~~	
Y49/7	8	Y61	R	?	R	R	S	R	
58-161/3	9	Y62	S	S?	S !	S	S plaques v. small.	R.	

The 13 resistants seem to become sensitive very rapidly when removed from phage!

F = Y53/1

	T1	T2	3	5	6	7
1	R	S?	R	R	S?	S↓ ✓
2	↓	" ?	R	R		
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. B. F. } all (smooth) T_1^R are also T_3^R T_5^R T_2^S $T_6^?$
5 mucoid T_1^R are resistant to all phages, incl. T_2, T_7..

C. E. On fresh isolation, Y10/1 ; Y531 give T_7^R which contain phage, and which are plaque infected, suggesting either virus 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. (G).
1,3,5,7. app. only dess obt. on immediate streaking.

G.

Sex: conditions.

a) 36 hour hour cultures: Y53, Y40.

b) prepare fresh cultures from these 11/30 A18.– compare when mixed in H_2O (after washing) for 1 hour. (see 352). Prepare the b) mixtures in 1:5 dilution also. c.

a). 1ml eq. each in 1ml total

b.) do.

c.) as above + 4 ml. H_2O.

Plate comparable nos. of cells however.

d 1:5 – a. 75
b. 91
1.5 × 10⁹

R = 1.4

	time 0		time 1h	
	.5	1.0	.5	1.0
old A	3	6		20
new B	90			22
diluted c	6			

∴ fresh cultures are better than
1) old
2) post [illegible] in H_2O is not favorable.
3) dilution effect is questionable.

(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-
A	✓1	R	S	S	S	
	✓2	R ?	R	R	S	
	✓3	R	R	R	S	
	✓4	S	R	R	S	
	5	?	R	R	S?	
	6	?	R	R	S	
	✓7	S	S	S	S	
B	✓8	S	R	R	S	—
	✓9	R	R	R	S	—
	✓10	R	R	S ??	S	—
	✓11	R	S	S	S	—
	12	S ?	R?	?	S	—
	13	R ?	R?	?	S	—
	✓14	S	S	S	S	—

x TLB, Lac -

T_1	T_3	T_5	T_7	
R	S	S	S	2.
R	R	R	S	4
S	R	R	S	3
S	S	S	S	2.
R	R	S?	S	1
			?	2

A1 and B1 were confused ? as demonstrated by Lac test!! should be:

A				
S	S	S	S	—
R	R	R	S	—
R	R	R	S	—
R	R	R	S	+
R?	R	R	S	+
R?	R	R	S	—
S	S	S	S.	—

A₂.

and there is only one possible discrepancy! A5. decided — R.
otherwise: 10R/14.

B				
R	R	R	S	—
R	R	R	S	—
R	R	S??	S	—
S	S	S	S	—
?	R?	?	S	—
?	R?	?	S	—
S	S	S	S	—

Virus - Resistance segregation

Y61 x Y53.

From same plating as 359:

D

	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	–

B

	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	+
11				~~S~~	

X

	T1	T3	T5	T7	Lac
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	+
~~10~~					

A

	T1	T3	T5	T7	Lac
1	S	S	S	~~R~~ S	–
2	R	R	R	S	–
3	S	S	S	S	–
4	S	S	S	S	–
5	S	S	S	S	–
6	S	S	S	S	–
7	S	S	S	S	–
8	S	S	S	S	–
9	S.	S	S	S	–
10	R	R	R	S	–

C

	T1	T3	T5	T7	Lac
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	–

22R | 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 Y53/3,1,5,7_M from D.

Y53/3,1,5,7_S from D.

Y67 Y53/7 M from E

Y68 Y53/7 S from E

After 4-platings, test again 12/10.

		T_1	T_3	T_5	T_7	Morph.	#
	Y57	R	R	R	S	Y53/3	M
	Y58	S	S	S	S	Y53/7	SR
A	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
	Y63	S	S	S	S	"Y53/1"	SR
	Y64	R	R	R	S	Y53/1	SR
B	Y65[1]	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

[1] also ~~T~~ T4, T6 R. probably contaminant.

Prepare plates for:

58-161/3

58-161/7

Compare $\frac{B^-}{B^+} = \frac{50}{212}$ with $5/20$ on p. 364.

but need better information.

Y40 x Y53.

5 hour cultures, washed, mixed, and plated into various media.

Turbidity	Medium.	Colony cts.	Mean	m. d.	Excess.	R/prot.
±	0	217	212 ± 34			1.00
	0	193				
	0	279				
	0	234				
	0	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				
+	T	304	350		148	.65
		395				
++	M.	0!!	Does not seem to be so turbid that germ. should be inhibited !!			

Repeat ē added prototrophs.

Turbidity	Medium	Colony cts.	Excess
+	BB_1	764.	0.
	BTL		—
	ML	0	
	MT	0	
+++	BLB_1	v. small cols.	?
	BTB_1	+++	—
	MB_1	0	
	MTL	0	
	MTB_1		
	BL		—
	BT.		—

Reversion controls

			sub.
Y53 m:	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 syntrophism. (Try it in $aB^+ \times bB^+$.).

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 case, we may find that the B^- are pred. lac + compared to B^+.

Similarly $\bar{c}$ B_1^-

5/20 B⁻ Exp. 10.

5.	15.
10	10

$$\chi^2 = \frac{25}{10} + \frac{25}{10} = 5$$

$p = .025.$ Need more data!

Recotypes Y40 x Y53.

Test colonies on [illegible], B, B₁; T; L medium appropriately & segregate together various single mutants for lysis & ferm. tests.

~~10/31 L- ??~~

		T_1^R Lac-	T_1^R Lac+	T_1^S Lac-	T_1^S Lac+
46/48	B_1 -	~~26~~ 17	~~2~~ 9	~~20~~ 13	~~0~~ 1
✓ 2/30	L-	2	0	0	0
✓ [illegible]/20	[illegible] B-	2	2	0	1
✓ [illegible]	[illegible] T-	2	2	0	0

17	9	13	1
100	55	50	4.

all recotypes of B_1, lac, T_1^R.

~~Prototrophs: See 362.~~

Prototrophs.

	T_1^R Lac-	T_1^R Lac+	T_1^S Lac-	T_1^S Lac+
Ser 1.	4	4	6	0
Ser 2.	~~25~~ 24	17	1~~5~~	0
	28	21	20	0.
cf. 357: Summary.	51	23	11	4.
	79	44	31	4
	21	11	18	0
	~~99~~ 100	55	~~49~~ 50	4

~~B_1 - maybe deficient in T_1^R Lac+ class (parental type). cf. 359~~

Prepare inoculum P24.
Plate Y55 (lactose - from Y53 + Y40) into lactose - minimal.

10^4 colonies. too high

Galactose mutant

Irradiate 58-161 P24. 2 mins. Incub 2ml/10 YB. Incub 1 min Benzoi.

20-24,000 colonies examined.

No typical gal- colonies. Several sectored colonies + some rather mucoid gal- were seen.

Streak out on ~~lactose~~ galactose EMB.

No mutants.

1 mucoid form.

Penicillin-resistance.

Y53 × Y54. grow separately. Pick prototrophs

1. fermenter.
2-16 non-fermenters.

give to Tuffus.

Y10/1 × Y53 × 58-161.

"3"

November 24, 1946.

(Weymouth) test prototrophs.

38 T_1^S Lac$^-$

16 T_1^R Lac+

no { T_1^R Lac+
   { T_1^S Lac+.

26 NOV 1946

a) Synthetic medium preparation (TLB, BM); —

much more turbid; no prototrophs. Occ. on surface. ca 10^8:...

b) YB. 10^{-7} on surface. as many in deep agar.

suggests YB better than synth. However, must be repeated!

plao

Lineage.

11/24/46.

Y40 x Y53. Plate $\bar{s}$ growth into B_1

Pick to H_2O; test on B_1+, B_1-.

33/39 = B_1-

6/39 = B_1+ = 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 ~~1~~ 0

BL } 0 0
TL } L 10 ? T

BT+TL+BL } 0 0 — 1 plaque ??
0 0. — Three plaques!??
B 10^2 turbid
T 0
L . 0 ?

Plaques are probably absorbed air-bubbles

b) BTB₁

BB₁ } 0
BL } B ? turbid.

BB₁ } 0 0
B₁L } B₁ 0

B₁L } 0 0 — clear not turbid
BL } L 1?

3
~~BT+TL+BL~~ } 0 0.
0
B 10^2 1 plaque?
B₁ 0
L plaques??

BB₁ × TL. 0.

December 4, 1946.

Y53 × Y0 in YB. (5 growth) plate in various + test. Compare c̄ do. grown in Nutrient Saline Glucose.

NB } 0 ✓ 10^2 just as good as YB.
NB } 0.

YB:
0
B
B
B — cont! ✓✓ colonies not so large as BLT.
B_1
B_1
B_1
T
T
T
T
L
L
L
L
BLT
BLT } most colonies do not resemble E. coli. Biotin stock seems contam.
BLT
BLT.

$$\frac{\bar{B}_1 \quad \overset{+}{B}\overset{+}{M} \quad Lac- \quad T_1^S \quad \bar{T} \quad \bar{L}}{\underset{+}{B_1} \quad \underset{-}{B}\underset{-}{M} \quad Lac+ \quad T_1^R \quad \underset{+}{T} \quad \underset{+}{L}}$$

↑

Look for types which are $B_1^+ M^+$, i.e. crossover at arrow 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 emulsified colony into BMTL. If any colonies appear they may be either BM or the complementary recombinant.

P5. Test for leucine; any ... L- should be tested thoroughly. [Use detection procedure?]

$\overset{-}{B_1}\ \overset{+}{B}\overset{+}{M}\ Lac-\ T_1^S\ \overset{-}{T}\ \overset{-}{L}$
$\underset{+}{B_1}\ \underset{-}{B}\ \underset{-}{M}\ Lac+\ T_1^R\ \underset{+}{T}\ \underset{+}{L}$

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 (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 [BB₁L; BB₁T] and examine for heterogeneity in Lac. or T_1 (particularly the former).

BB₁L: as ~~above~~ below (371) + v. turbid. do not use!

Linkage –

~~Compare B(P) × Y53 vs (P) B1 (cancel P- with proline)~~
~~and BB1 × TL.~~

~~12/5/46. BB1 × TL.~~

~~Repeat~~. 12/9/46.

A. BB1 × TL.

O ~~No~~ 3 colonies. ?? coli.

B1 No prototrophs. ~~#~~

B. B × TLB1.

O } No colonies.
B1 } rather turbid!

December 9, 1946.

Y40 x Y53: into BB, L. (A) and BB, T (B)

n.g. like 375

12/9/46.

Cultures ca. 8 hours.
(too old???)

Y40xY53. into BTL.

ca. 10 colonies. Rather inhibited by turbid growth.

6315 x Y53.

December 9, 1946.

Y53 x 58-6315. (Biotin - "ϕ-alanine?" + cystine, istyp.)

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

~~Test prototrophs on T_1-lac plates.~~ Carl - found ϕ-...+ types indicating separability of ϕ, cyst. req.

Y4③ x Y53

T⁻ + L⁻

10/5 – 10/46.

Plate on T, L resp. test do. — Need more data......

		T_1^RLac−	T_1^RLac+	T_1^SLac−	T_1^SLac+
T:	5/20. (.25)	3	0	1	1
Cf 364	4/26.	2	2		
		5	2	1	1

T⁻

9/46 T⁻
- ✓ 1 ✓ (circled)
- ? 2 ✓
- ++ n.g. 3 ✓ (T_1^SLac−)
- ++ n.g. 4 ✓
- ✓ 5 ✓ (circled, T_1^SLac+)

T⁺ also all ott.

∴ T⁻ T_1^RLac−
& T⁻ T_1^SLac+

L: 3/26. (.11) — 1, 2

Cf. 364. 2/30. Both T_1^RLac− — 2, 0

	T_1^RLac−	T_1^RLac+	T_1^SLac−	T_1^SLac+
5/56.	3	2	0	0

n.g. (?) ✓ 1 ✓
✓ 2 ✓ (circled)
✓ 3 ✓ (circled)
4/5?

T_1^RLac+ (2).

~~[illegible] LT [illegible]~~

ca 10% L⁻ OK.
ca 10% T⁻

Complementary type

10 December 1946.

8 Dec. plate colonies of (Y53 x Y40) from B_1 agar, into BTLM agar.
Most plates have 1-200 colonies, c̄ 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 BLTM large tubes (for detection plates)

Tests. (only BLTM+ BTM- or ? recorded).

T_1^R lac- T_1^R lac+ T_1^S lac- T_1^S lac+

Plate no:		
1	0	
2	0	
3	0	
4	0	
5	0	
6	1	378-1
7	1	-2
8	1	-3
9	2	-4, 5
10.	2	-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 ascinduc.

A 1. Y53 x Y40. (Shown)

B 2. Y64 x 58-161

C 3. Y65 x 58-161. (Y10k/7 x 58-161).

D ~~G7.~~
4. Y67 x Y40

E 8. Y53 x ~~58-161~~. Y68.

F. 9. Y67 x Y68.

most mucoids too large Best method: surface spreading!

A: Yield rather low! B. too turbid. BTL OK but >> than 0.

B: also too heavy. V. low yield.

C: (0. none B_1: ca 20 ε very wide zones of stimulation) all dup. (contam?)

D OK when suc. mostly venoc.

E ca 10^2-10^3 colonies. Not very much like coli, but test on EMB lac
all mucoid, lac +

F 0.

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 dysgenesis[?]. Test on:

Headings struck through: ~~BMTL B_1MTL B_1BTL B_1MTL B_1BMT | BB M 2~~ / ~~BMTLB₁ BMTL BMB₁ TLB₁~~

		-B	-M	-T	-L	-B_1
	C1	+	+	+	+	−
	C2	+	+	+	+	−
Lac−	D1	+	−	±	+	+
	E1	+	+	+	+	−
	E2	+	+	± − +	+−	+−
	F1	+	+	+	+	−
	F2	+	−	+	+	+
Lac+	G1	−	−	+	+	+
	H1	+	+	+	+	−
Lac+	J1	−	−	+	+	+−
	K1	+	+	+	+	−
	M1	+	+	+	+	−
	378-8	−	−	+	+	+
	378-9.	±	+	+	+	+

(-B, -M, -T, -L: + B_k)

A. 14/15 +

B. nonsequer

C. 8/8 − < 1, 2

D. 1/1 − −1 ? B... lac ?

E 17/17 − < 1, 2

F 8/8 − < 1, 2

G ~~111~~ − 1 (Lac+)

H 4/14 1

J 1/1 − 1

K 6/6 − 1

L ———

M 1/5 − 1

15.

Retest: 380 - D1
380 - E2 (on 0)
380 - F2

No variability in ... in streaked plates. colony type.

378-8
378-9.

See 380.

Small colonies.

	–	Lac		✓
C1	B1	–	–	E1
C2	B1	–	– ?	H1
* D1	M (T)	– ✓	–	K1
E1	B1	–	✓	M1
* E2	(T)	–	– ?	
F1	B1	–	– ?	–!
* F2	(B) M	–	– ?	C2
G1	BM	+	–	E2
H1	B1	–	✓✓	F1
J1	BM	+	–	F2
K1	B1	–	✓	
M1	B1	–	✓	
378-8	BM	+		
378-9.	(B?)	–		

Pick small colonies to coli∞, [illegible]

[illegible]

check on	O	B	M	T	BM	TM
D1	+		+	+		+
E2 pr	+			+		
F2.	+	+	+		+	

	Type		– B1	– M	– T	– L	– B
1	C2		–	+	+	+	+
2	K1		± +	+	+	+	+
3	E1		–	+	+	+	+
4	E1		–	+	+	+	+
5	E1		–	+	+	+	+
6	F1		+	+	+	+	+
7	E2		+	+	+	+	+
8	F2		+	±	+	+	+
9	F2		–	–	±	+	–
10	F1		–	+	+	+	+
11	H1	B1	–	+	+	+	+
12	H1	B1	–	+	+	+	+
13	K1	B1	–	+	+	+	+
14	K1	B1	–	+	+	+	+
15	K1	B1	–	+	+	+	+
16	K1	B1	–	+	+	+	+
17	M1	B1	–	+	+	+	+
18	M1	B1	–	+	+	+	+
19	M1	B1	–	+	+	+	+

?? hold! → ++++++ / ++++++

8	F2	–	–	–	–	+	+	+	+	– (+)
9.	F2.	–	+	–	±	–	+	+	+	– ±

January 5, 1947

1 ml, 36 hour broth cultures in YB agar

+++ = unif. turbidity.

		A	B	C	D
Proflavine	1:10^3	+++	→	→	→
	1:10^4		"		
	5			"	
	6				"
	7				

Y40 1 ml	A
Y40 .1 ml	B
Y53 1	C
Y53 .1	D

n.g. at all from broth

		A	B	C	D
Crystal Viol.	1:10^3	—	do.	do.	ca 10^3 cols.
	4	+++	→	→	→
	5		"		
	6			"	"
	7				

Proflavine is n.g. under these conditions

Survival c̄ crystal violet is OK in range 10^{-4} to 10^{-3}.

This should be extended. Use washed cultures?

January 9, 1947.

V x y p

Irradiate in flask, varying times. Grow YB-1ml Also plate on EMB .01 ml irradiated cultures

	S /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 sec. i.e. each 15 sec. kills 90% of the remaining survivors.~~

non-linear 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 50,000 to fresh EMB for further test.

Pick 6 colonies to YB slants which seem to be non-papillate. 1 is mucoid.

See 396

Transformation control

January 9-10, 1947.

P9. Broz YB - Y53, ~~Y37~~. Y40

10A10. Broz ($\frac{100}{50}$ ml NSB) Y53, Y40 (A)

1P10 Broz YB - Y40 (B)

4-5P10. Wash (A) cells.

1. Mix Y53-Y40 cells. ✓

8P10. Suspend Y53 (A) cells in T-minimal. Incubate c̄ shaking 3h. Sediment (C, D) and mix with washed Y40 (B). 2. plate 0.

3. ~~Mix~~ supernatant of C, D twice c̄ 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?? } supernatant was not entirely freed of cells
1 ?? } by the centrifugation. Repeat c̄ controls
on influence of dilution of 1 cell type on prototroph yield.

Recombination types

January 10, 1947.

Y40 + Y53 in T(0), T(B1) agar

Pick colonies to EMB lactose. 1/12/47 1-15. 8, 13 + others are −

Streak out densely on (A) BMTL-lactose (B) BMTL lactose + glucose.

Compare the B_1^- types c̄ types isolable from these plates.

Colony-Plate:	Colony:	0 = B_1^- orig.	A	B	C on EMB-lac.	D	E	F	G
lac	1	$-S^{(+R)}$	−S	−S	−S	−S.			
lac	2	−							
* lac	3	− R	$+S^{(+R)}$	$-S^{(+R)}$					
* lac	4	− S	$+S^{(+R)}$	$-S^{(+R)}$	$\pm S^{(+R)}$	$\pm S^{(+R)}$			
lac	5	$-S^{(\pm R)}$	−S	−S	$+R^{(-R)}$				
lac	6	− R $(\pm R)$	−R	−R	−R	−R	−R		
G	8	* −S	−S	−S	−S	−S	−S	−S	+R
G	11	− R	−R	−R	−R	−R	−R	−R	−R
G	12	− S	−S	−S	−S	−S	−S		all mixed c̄ R
G	13	+ R	+R	+R	+R	+R	+R	+R	
* G	15	− R	−S	−S	−S	−S.			

↓ B_1^- ↓ B_1^- BM+R ... B_1^- BM+R

BM +R
TLB_1 −S

Segregation of T_1^{R}

January 11, 1947.

Y64 × 58-161.

TLB₁ lac- 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.)

sheen on lac+!

Muc lac- Sm lac- Muc lac+ Sm lac+

P) 17 P) 9 Lac- = .66

~~Y57 (Y53/3,15, M) × 58-161 (Try × Y40)~~

E Y68 (58-161M) × Y53. (Try × Y64)

segregation: ~~ML- Ml+ ml- ml+~~

6 P 22 P 12 7

M_{68} linked to lac-

Interaction of expression of Lac- + Muc+ on EMB lac medium?

note variation in sheen. Genetic character?

January 11, 1946.

P10 hor 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 fells" and

Mix 5 ml c 1 ml Y40 suspension + plate 3×2 ml samples into T(0) agar.

P14. colonies!

ca 10 large $10^{2.5}$ small c halos. v. clear plates. sign?

See 394

January 11, 1948.

Plate Y55 (++... Lac-) into Lactose-minimal at various dilutions: (Assoc. = 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	10^3 small colonies; 6 typical colonies (probably Lac+). Diffcntd about 5×.

1) The reversion frequency, as estimated from EMB plates is very high (ca 10^{-4} to 10^{-3}/generation??)

2) At least 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

Jan 11, 47

Y65 × 58-161 No colonies!! (Repeat!)
not turbid - prob mix error.

Repeat Jan 15.

Loaded! High rate ????

Recombination in K/12 (agent)

January 11-12, 1946.

P 11 Broz YB - Y43, Y44.

230P12 - Broz YB = Y43, Y44, Y43 + Y44., Y43 + Y53.

4 P12. Wash + plate.

(Y43) + (Y44)	0, 0	not turbid (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.o: – ca 2-300.

a. Keep Y40, Y53 in water (.9% NaCl) 25°. Mix HP13.

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 superjacent layers of agar.— 4 colonies.

e fresh Y40 x Y53.

a	10^2 – 2.5	
b	10^2	clear plate
c	10^2	
d	4.	
e.	10^2	

Cells will react if kept in water for 24 hours + then mixed. but not many more are found if they are kept together. ∴ Recombination takes place in the agar.

Differential Centrifugation of Bacteria
Preliminary expts.

0. density — in sucrose buffer, centrifuge [illegible] washed YS3 [illegible],
at 10 — 10 mins, etc. — Sediment [illegible].

		1.0	1.04	1.08	1.12	1.16	
	time / Mark	+	+	+	+	+	10 / 10
	time / Mark	++	++	++	++	++	10 / 10
n.g.		++	++	++	++	++	20 / 50

1.16 = 20g sucrose / 100cc water
1:4 bacterial susp. in H_2O.

n.g. for density.

Repeat, using 20g sucr / 20g H_2O as d = 1.25. (actually 1.23)

diffuse in bottom layer; c ring at junction.

(1ml / 4ml 1.25)	1.0	1.05	1.10	1.15	1.20	
	++	++	+	±?	—	20m / 50.
	+++	+++	+±	+	±	+ 30m / 50

+1hr.

1.15	1.20		do.
Use heavier susp. cells.		+	±	1:30

This might achieve some separation.

Tiy K1 $MgSO_4$

Y53 × Y40 – dilution effect.

1/13/47.

½ ml of various dilutions.

	1ml Y53	+ 1ml 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.

Irradiate Y40 nm. Iroz 1 ml/10 YB Incubate 18 hours + dilute + plate on EMBlact. 20,000 colonies examined.

3 colorless, but rather small colonies were found. Pick + test further.

1 Lact Mucoid colony was found. Pick + ~~streak out to isolate~~.

→ all Lac+ Lact Muc = Y69

Y53 (Lac°)

January 17, 1947

See 383 (1-6)

P21. Colonies have taken a blue tinge. Make streaks + compare c Y53.
All show coloration in lytic zone c T1 virus.

5, particularly, shows few or no papillae. Y70.

1 very few papillae Y71.
2 papillae.
3 papillae.
4 few, but some papillae
5 ~~[illegible]~~ no papillae. ✓
6 few, but some.

Y70. — Further study suggests that fewer colonies have papillae, & fewer of them

coli mutabile — papillates very readily than K-12, but some papillae are formed. Comparison should be made of some photograph segregants

This allele may revert to Y53-Lac-.

Attempt at transformation

January 18, 1947.

P17 - P18. 24 hour-cultures Y53 autolyze ÷ 300 ml washed cells in NaCl under benzene. 3 hours. shaken at 25°.

Sediment cells. Remove superficial toluene by evacuating chamber. Suspend Y40 cells in autolysate - Plate 1:3 1:10 with Y40.

Control - use washed cells of above with autolysis x Y40.

See also 399.

Turbidity of autolysate was <½ than that of the 1:100 dilution in 399.

Keep sample overnight with heavy layer of benzene and repeat later.

Hold autolysates overnight in cold.

6P19. - remove benzene from sample by evacuation.

A. Y40 + bz-autolysate   0,   also in EMB.   0

B. Y40 + autolysate   0,0

C. autolysate with Y40.   0,0.

autolysate is sterile; no prototrophs.

3-way cross.

January 20, 1947.

A : Y40, Y10, Y64.

BM+R × TLB1 < +S / −R

−S not indic.

+R	−R	+S	−S
9	7	1	11

B. 58-161, Y46, Y53

BM+S × TLB1 < +R / −S.

−R indicator.

+R	−S	+S	−R
20	34	5	40

Some mistake??

See 411 for repeat

3-way cross.

Y10

BM Lac+ V_1^R — Y40

TLB, Lac+ V_1^S

TLB, Lac− V_1^R → +++

Y64

- Lac+ V_1^R
- Lac+ V_1^S
- Lac− V_1^R
- <u>not</u> Lac− V_1^S

Y46

BM Lac+ V_1^S — 58-161

TLB, Lac+ V_1^R

TLB, Lac− V_1^S

Y53.

- Lac+ V_1^R
- Lac+ V_1^S
- Lac− V_1^S.
- <u>not.</u> Lac− V_1^R

<u>Controls:</u>

BM Lac+ V_1^R × TLB, Lac− V_1^S → all types, Lac+ V_1^S <u>rare</u>.

BM Lac+ V_1^S × TLB, Lac− V_1^R → all types, Lac+ V_1^R <u>rare</u>.

already done!

Dilution Effect 1:10

January 18, 1947.

½ ml each:

	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)

Y53:		
10^{0}	0	120
	-1	60
	-2	8
10^{-1}	0	120
	-1	23
	-2	16
10^{-2}	0	13
	-1	8
	-2	1

f(Y53)

Y40:		
10^{0}	0	120
	1	120
	2	13
10^{-1}	0	60
	1	23
	2	8
10^{-2}	0	8
	1	16
	2	1

Y53 + Y40		
	10^{0}	120
	10^{-1}	23
	10^{-2}	1

Mucoid segregation

January 17, 1947.

Y57 x Y68 (TLB, -lac - $V^R_{1,3,5}$ x BM-Muc)

No prototrophs!

See 404

of 387 for mucoid segr.

Y53M

Y67 x 58-161 is OK.

Y68 x Y53 is OK.

~~58~~-161M

Toxicity of benzene and removal.

January 19, 1947.

T P 19. Layer 1/2 ml benzene on 1 ml Y40 in water. Keep on desk.

do in H_2O.

N 20. 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 24h. Plate.

January 20, 1947.

A. P19. Inoc Y40, Y53 into YB + Tween ; A 20 transfer likewise; plate in T(0) agar + 1% Tween

A. 1%
B. .1%
C. .05%

no growth effect!

P19 Inoc Y40, Y53. into YB. etc.
Plate into T(0) agar +

B.

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 YB. Wash + irradiate 2 mins. Dil 1:100 in nutr. sal.

1
2 1/5
3
4 1/5 survivors...
5.

58-161 is evidently more sensitive than Y53. (which has had 1 further X-Ray + u.v. exposure).

~~Y64 x 68.~~
Segregations

January 22, 1947.

1. Y65 x 58-161 (Y10/1/7) (in 1:100 dil.)

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 c̄ ca 100 (no sectors). ([illegible] at [illegible] See 400

Try Y64 x Y65

BM +R × TLB, -S

See 385.

P21. Streak 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	(Replate 3-0 also.)
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]		
6-9	B	-S	-S[6,7] +S[8,9]	+S	?
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]		

Test samples of above: P: parental

above #			Nutr	Comment		
3-1	1	-R	B_1 ✓		B_1 -R	
3-2	2	~~+R~~	~~BMTL?~~	P (BM)	B_1 -S	— 3 tests BML definitely / 1 test BM
3-3	3	-S	B_1		[B_1 +S]	
3-5	4	+S	B_1 ?		[++ +S].	See 408.
3-7	5	-S	✗ 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 ?		B_1 -R	
15-3	22	-S	B_1 ✓	of 21		
15-4	23	+R	BM ✓	P.	B_1 -S	
15-9	24	+R	BM ✓	P.		
15-10	25.	-S	B_1 ✓	of 21.	(B_1-R)(B_1-S).	
3-4	31	-S	B_1			
3-6	32	+S	B_1			
3-8	33	-S	B_1			
3-10	34					

January 21, 1947.

250 ml eq. 24 hour cells of Y53 harvested from YB + washed.
~~autl~~ autolyze 24h. under toluene at rm temp.

P22. add Y40 cells + plate.

P24 - no colonies.

January 22, 1947.

Plate "B_1^-" colonies into T(0) agar + BMTL. Use plates which relatively few, isolated prototrophic colonies.

0 Y65 x 58-161

1 Y40 x 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	10^5		
2	−R	1000	8 + S	
3	−S; +R	1000	8 + S	
4	−S	200	3 + S	
5	−S	1000		
6	−S	500	~~7 + S~~ 7 + S	
7	+S	300	7 + S	
8	+S	300		
9	+R	10^6		
10 (labelled "into T(0)")	−S	200.	8 + S	
11	−R	200	10 + R	
12	−S (+R)	500	10 + R	
13	−R	50	2 − R	7 + R.
14	−R	500	8 + R	
15	+R (−R)	300	9 + R	
16	−R	20	1 − R	5 + R.
17	−R	10^6		
18	−S	200	10 + R	
19	−S	500.		
20	−R	200	9 + R.	

(Rows 1–10 marginal note: $\frac{-R}{TLB_1} \times \frac{+S}{BM}$; rows 11–20 marginal note: $\frac{-S}{BM} \times \frac{+R}{TLB_1}$)

Rows 1–10: +S = BM type; −R = TLB_1 type; +R = lac type. All +S. [illegible] (BM) Test for a lac [illegible] requirement.

Rows 11–20: +R = BM type; −S = TLB_1 type; +S = lac type; [−R = B_1 type]

How explain "10" − Reversion of B_1^-?? [illegible]

Recombination types - see 383

January 25, 1947.

Retest 405-2. (475) in plates. Dil to ca 100/ml + pour plates c̄

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) w. very light inocula of the un-purified cultures.~~

Streak out colonies of 407 on EMB-lactose. Test on:

B11+S ×-R	407-		BMB.	BMTL.	
1.	2 +S		+	+	P
2.	3 +S		+	+	
4.	4 +S +R	use a colony in line of main streak (maybe mutant from S to R).	+	+	
5.	6 +S do.		+	+	
6	7. +S		+	+	
B11+R ×+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 10, 11, 12, 16, 17 to slants + test further.

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, ~~40~~ 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) stored in coldroom.

a. Streak out on EMB agar. & b. plate remainder of colonies in T(0) agar.
Use well separated colonies

		Lac.	variability in x/10³		
Y40 + Y53 and A	1	—	0/10³	0/3	0-turbid
	2	—		0/3	
	3				colonies
	4	+		0/3	0
	5	+		0/3	0
	6	—		0/3	turbid
Y64 + 58-161 and B	11	—		0/3	turbid
	12	—		0/3	0
	13	—		0/3	colonies
	14	—		0/3	0
	15	—		0/3	ca 20 colonies!
	16.	—		0/3	0

b. Streak out 18 other colonies on EMB, looking for variation.

A	1	+	0/10³	
	2	—	0/3	
	3	—	0/3	
	4	—	0/3	
	5	—	0/3	
	6	±	[illegible]	ca ½ each.
	7	—	0/3	
	8	—	0/3	
	9	—	0/3	
B		—	0/3	
		—	0/3	
		—	0/3	
		+	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 (Ebeckia prevosio latum) 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
allelism

7. Y67 × Y69. in O rather few ($<10^{-8}$) colonies. all dup.
allelism.

1	Y53×68	n.g.
2	Y57×Y68	n.g.
3	Y64×Y68	n.g.
6	Y67×Y68	n.g.

(2, 3, 6) ∴ Y68 is n.g.

4	Y69 × Y53 (Y40M, Y53S)	OK.
5	Y40 × Y67 (Y40S × Y53M)	OK.
7	Y67 × Y69	OK, but poor.

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.

	Lac	V_1
B	–	S
C	–	S
	–	S
	–	S
	–	S
	–	S
D	–	S
	–	S
	–	S
	–	S
	–	S
	–	S
	–	S
	–	S

On basis of map theory:

	9		15		25		8		8	
B_1		BM		Lac		V		P		LT
	①		Ⓐ		Ⓑ ②		Ⓒ		③	

1. Prototrophs are ①③ types or ①③②[2]. In 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 revert + it would likewise be difficult to use only B_1 as the marker.

b) $B_1^- > B_1^+$, no influence on segregation.

$B^- > B^+$ ē following distribution (ca.)

−R	.30
+R	.50
+S	.15
−S	.05

BLT, B_1P, LT, should be readily recoverable.

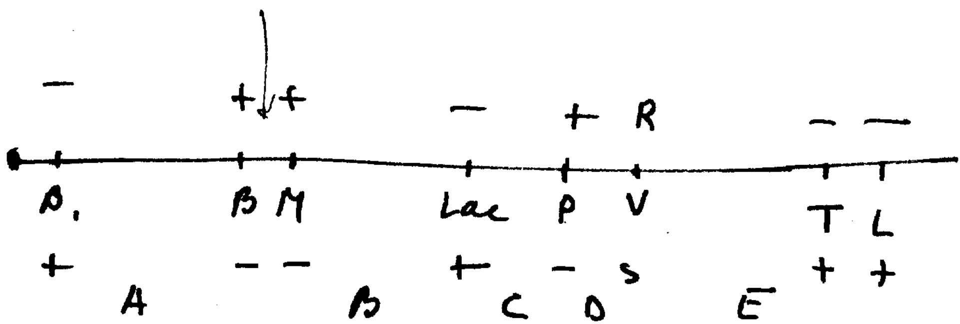
BP x TG

To demonstrate genetic as well as biochemical distinctness of prolineless (glut -) and prolineless (glut +)

58-5255 x 679-662   Rather small moi. (<10^8)

8 colonies found.

✓



B₁ M B

\+ − −

7 FEB

58-5255 x Y64.

1. Plate 5255 in O, Biotin alone for R test. O: no colonies. B- " "

2. Plate into O, B₁; B. $B_1 P$. P – too turbid } use more dilute inocula. BLT – very turbid, but several colonies recoverable

$B_1 >> O$. (5-10 x)

B ca 2-3 x O.

Prototrophs:	−R	−S	+R	+S	Total	Total −1
	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

$\chi^2_3 \; B/B_1 = 11.1$

of which 8.3 is +R, +S classes. ∴ B is certainly modifying the distr.

On basis of map B₁ 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 have better data on the frequency of B⁻/B⁺. This distribution suggests the map order:

B₁ B Lac P V TL.

$Lac^{R}_{-} \times Lac^{+}$ ~~¥6~~
Y76 × Y40.

7 FEB

B_1^- 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 c̄ 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 407 409

January 25, 1947.

~~Test types of 407 in BMTB, (-L) in very light inocula of the unpurified cultures.~~

Streak out colonies of 407 on EMB-lactose. Test on:

	407 —	BMB.	BMTL.	
1.	2 +S	+	+	P
2.	3 +S	+	+	
4.	4 +S +R use a colony in line of main streak (maybe mutant from S to R).	+	+	
5.	6 +S do.	+	+	
6.	7. +S	+	+	
7.	10. +S	+	+	
8.	11 +R	+	±	P

(Margin notes: 3111S ×-R; 6 FM1+R; 7 +-S)

Desoxyribonuclease and recombination.

7

Dissolve 10 mg DRNase (gift of Avery + McCarty) in 10 ml 2x coli minimal. Sterile filter — filtered well. Store in cold. Presume 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

				Av.
A.	.1 ml	Total 200γ/4 plates	17, 8, 19, 15	15 ✓
B	.5 ml ea.	2 mg/4 plates	6, 11, 12, 33	15.5 ✓
C	control		9, 7, 23, 13.	13. ✓

In this expt., DRNase has had no appreciable influence on recombination.

.5 mg   to 50γ [illegible]

2/10/47

BT/1 x B,L.

i.e.

	B_1	B_2 (42)	V (16)	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.

February 10, 1947

Y64 x Y10 x Y40. —

Yield very poor. Do not use ~~it~~ for testing.

cf. other experiments this date !!!! — minimal medium??

Jo February 10, 1947

Repeat part B and controls of 420. Y40 x Y53.

B: 1/2 ml cells + 1/2 ml DRNase separately + mix cells. Plate into minimal + B_1 agar. (1/2 ml of mixture).

B: 0 20
B_1 ca 150.

C: 0 2
B_1 8

controls did not do well here!
(agar base cloudy!)

Test various prototrophs - es against Y41, etc. for gene homology.

			No. prot. / 10^8
1.	Feb. 10 '47.	58-3214 x Y41.	0
2.	Feb. 13	6177	0
		3232	0
		6049	0
		6317	0
		5450	0
		5255	0, 0

679-440 x 5255 0

Test Roepke's mutants for recombination:

February 10, 1947.

		24h.	48h	
1.	Thr × bid	—	5	
2.	meth × arg	~~2000~~	$10^{3.5}$	
3.	lys × leuc	~~trub~~ —	0	
4.	prol × citr-uracil	~~trub~~ —	0.	
5.	~~[illegible]~~ meth × Y64.	—, —, —, —		later 3/4 plates!
* 6.	thr	—	0	558-228.
7.	ind	—	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!).	45
12.	prol	—	100	209-301
13.	citr-ur.	~~trub~~ —	0 (turbid)	823-304

no evidence of recombination:
threon + leuc seem to be most stable types in this series.

Inversions in Y53.

Febr. 1[illegible], 1947.

Treat cell suspension of Y53, $\overset{Y40}{\bar{c}}$ .01% HN2 (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, $\bar{c}$ Y40 + Y76 resp. in 0 + B_1 agar.

H15.

	0	B_1		0	B_1		0	B_1		0	B_1
1	10	100	11			21	20	100	31		
2	14	100	12			22	10	100	32		
3	25	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 $\bar{c}$ appr. controls.] 26? 0

Reversions.

Feb. 13, 1947.

Y76 x 58-161. in T(0) + T(B_1)

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.

Feb. 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 (circled) not well diffused; evidence of resistance in some regions.

Tyrocidin (in alcohol)

500γ turbid (ca 10^7 colonies) = 50γ/cc no inhibition.

200γ do.

100γ do.

∴ Tyrocidin neg.

B.G. OK at ca +1:100,000

Hg OK at ca 5 γ/ml.

Streptomycin OK at 5-10 u/ml.

February 17, 1947.

Repeat - [Used mustard treated cultures].
streak out Y40, Y53 on sugar-EMB media.

	Y40	Y53	
Lactose*	+++	-	* some - colonies? - pick + retest.
Maltose	+++	+++	
Mannitol	variable.	variable, predominantly -. later - pred +	
Glycerol	-	(+). variable, late +!	Note diff. Y53 + Y40.
Alcohol	±		
Sucrose	- to ±	- to ± (faint blue coloration not a + reaction).	
citrate	pH too low		

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		B: × Y53	
		O	B₁	O	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	~~Y40~~ 58-161! (no Y40 on).	100T	++T;	200,	++T
~~5~~.					

No inversion

Inhibitors

February 18, 1947.

per "20" ml plate NSA.

Y64 Hg 100γ "1 resistant colony. not too well diffused
"secondary colonies!!
Y46 do. clear. —

Y64 Streptomycin 50u turbid
100u. clear in parts. Not well distr.

Y7?

58-161. 50u. turbid
100u. as above.

Y64. Brilliant Green .5 ml 1:1000 clear! (1:40,000). pick up resistant
" Malachite Green 1:500 1 ml clear! (1:10,000). ca. large; 40 mm.
.5 ml " 1:20000
.1 ml (1:100,000). poorly diffused.
clear where dye is visible

Y46. Sodium Azide 10γ turbid
100γ turbid
1 mg. irregular clearing.

Y AG from glucose

Y64 "Spergon" (1/20) .1%. no inh.

Y64 tri-iodobenzoic ac. (1/20) 1% ".

58-161 } $HgCl_2$, 1 mg. autoclaved with 100 ml N.A. — not inhib.
Y64 }

" $BiCl_3$. 2 mg/100 ml. N.A. — ~~inhibits~~ only faintly inhib.
= 1:50,000. (need more!)

" Streptomycin 5u/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
" therm.	0
Y5 × therm	0.
Y5 × Y44	0
Y5 × 58-161.	0

no evidence for sexuality under these conditions. Try growing together!

Y40 x Y53.

~~Recombination~~

2/21/47

Mix Y40 + Y53 in water + add to agar, mixing. Add 5 ml aliquots ≗ ca. ½ ml ~~a~~. to various suppl. plates. (see 433 for note on medium).

5 ml.	0	12	B	11
		10		10
		10		4
		3		15
		11		10
		10.		
	B_1	>25 T.		
		" T.		
		" T.		
		" T.		

This expt. illustrates influence of conditions on detection of recombinants.

10 ml - $\bar{s}$ sublayer.

	0	—			
	0	—			
	0	—			
$\bar{c}$ sublayer.	0	5	B_1	T.	B. 12.
	0	4			

Tests for Inversion in B_1-B region.

2/21/47.

Cross mustard-treated (476) isolates of Y40, Y53 $\bar{c}$

Y53 x Y40 resp in O, B_1 medium resp.

Plate in medium lacking NH_4NO_3 (inadvertent omission).

		O	B_1
Y40T.	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 tube test tubes, Y40 x Y53 is app. inhibited by anaerobic conditions

Fermentation tests.
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. Streak out on fresh medium.
all M+.

Glycerol - slow utilization. — compare passages on glycerol
c̄ peurosis.

Sucrose - very slow but definite utilization

Fermentation mutants – enrichment cultures.

Feb. 25, 1947.

Inoc 50 ml NB + 2% sugar + Brom Cresol Purple A25. Y53.
symbols ind. acidity.

	A26	A27	A3	A4	A5	
glucose	+++					
glycerol	+	+++ Y81.				growth heavier than in glucose — no % black on EMB.
lactose	-	-	+	++	* +++	[illegible]
sucrose	-	-	-	-	-	all white colonies!

* growth inc. on standing at room tem.

Streak out some apparently Gly – (a) colonies from 434 + compare with atypical Gly ± — (b).

a) Y84. – Y80.

b) +

Compare a, b + gly + enrichment culture above.

	EMB	
Y80	–	No evidence of papillae.
(from glucose above) Y53 gly ±	+	
Y53 gly + enrich.	+ or ++	different from Y53?

on BCP –, medium is not changed in color, cells show slightly different 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 + Mal Green. 10^{-4}.
Streptomycin 5 u/ml

	M.G.	St.	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 removes effect of dye. No evidence of stimulation of previously streaked (Mg^s) culture. Probably due to pH change.

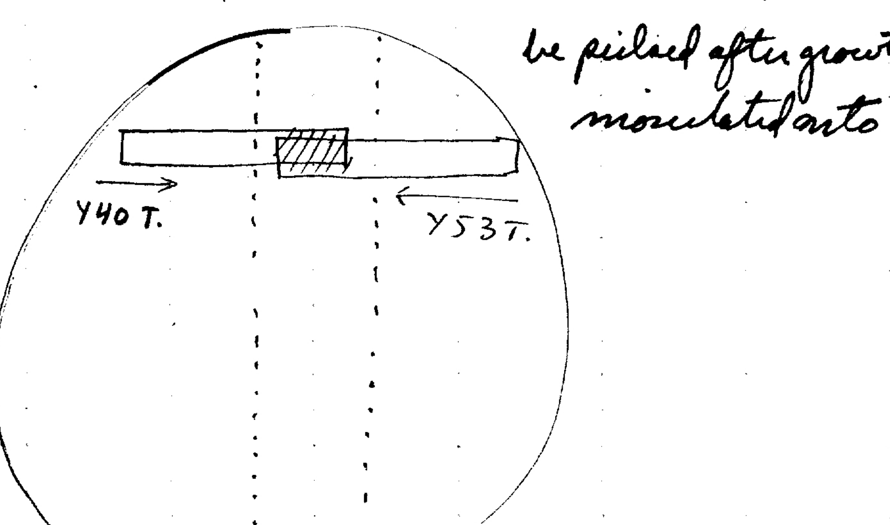
Inversion Detection

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 onto minimal.



P3. Test ~~combinations~~ by suspending growth from center in 1 ml. water, ~~and streaking~~ pouring on minimal plates. (i.e. 84 tests!) ca 10^9 inoculum.

Tests. log prototrophs

Test	log	Test	log	Test	log	Test	log
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+		
		19	1	32	1+		
7.	2	20	1.5	33	1		
8	1	21	1+	34	1		
9	1	22	1+	35	1		
10	1+	23	1+	36	1		
11.	1	24	1+	37	1		
12	1	25	2	38	1		

1-6 also tested by streaking 1 loopful on T(o) 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 trt.	Y40 × Y53T; Y53 × Y40T, by st. tech., ♂ + ♀,	20 tests.		0
433.	MN_2 treat.	do.	17	37.	0
437.	"	Y40T × Y53T. in ♂ only, X mayer; 2 × 42 =	84	121	0
508	X-ray	Y40T × Y53T. in ♂ only. mayer. 2 × 14 =	28.	149	0

Time of prototroph mutations.

Pour 440 × 453 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, Y68 } test for inversion.

Plate Y65 x Y40

Y68 x Y68.

in T(0) + T(B₁).

<u>no prototrophs at all!</u>

∴ not due to suppression of X in B₁-B4 region.

Segregation of Gly –

March 4, 1947 –

Y80 x Y81. cf. 435.

$B_1^- > B_1^+$ as usual. Pick + plate on EMB lac + EMB gly agar.

°					B_1			
Lac-R	Lac-S	Lac+ R	Lac+ S	Gly –	Lac-R	–S	+R	+S.
				Gly +.				

Gly segr.

Y80 x Y81.

A.

in min.

Gly	Lac	V
+	−	R
+	−	S
+	−	R
+	−	R S
+	−	R
+	−	S
+	+	S
+	−	S
+	−	S
+	−	R
+	−	R
+	−	S
+	−	R
+	−	S
+	−	R S
+		

B.

T(0)

Gly	Lac	V.
+	−	R
+	−	R
+	−	S
+	−	R
+	−	R
+	−	R
+	−	R
+	−	R

T(B_1)

Gly	Lac	V.
−	+	R
−	−	R R
−	−	
− ?	−	S
−	−	S
− ?	−	R
−	−	R
−	−	R
−	−	S
− ?	−	R

[scoring maybe inadequate]

when read again 4 hours later, series B had gotten considerably darker!

C.

B_1

Gly	Lac	V
−	−	R
−	−	S
−	−	R
−	−	R
−	−	S
−	−	S
−	+	R
−	−	R
−	−	S
−	−	R
−	−	R
−	+	R
−	−	R
−	−	S
−	−	S
+	−	R
−	−	S
−	−	R
−	−	R

D.

B_1

B_1

Gly	Lac	V
−	−	R
−	−	S
← [? +	− +	S R]
−	−	R
−	−	S
−	−	S
−	−	R
−	−	S
−	−	S
−	+	R
−	−	R
− ?	−	S
−	[illegible]	R
−	−	S
−	−	S
? −	+	R
? −	−	S

Note: B_1^+ : 25/25 ~~Lac+~~ Gly+ } close linkage? These results are difficult
$B_1^+ + B_1^-$: 44/46 ~~Lac+~~. to reconcile with the map!
+

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
all Gly +.					
B_1^-					
Gly −	2~~0~~	19	5	~~0~~	
Gly +	1	(1?)			

Faulty segregation of Lac + ?

This scoring of Gly, done at 2 da. is not borne out by readings at 3 days, when in both groups, all were Gly +, $\bar{c}$ some variation in intensity. (45)

Note: 24 Lac − : 1 Lac + in B_1^+ (95%!)
46 Lac − : 5 Lac + in B_1^-.

1) B_1^-/B_1^+

25	24	2	1	25
41	40	4	5	45
	64		6	70.

$\chi^2 = .04$
.02
.04
.03
.13

compare $\bar{c}$ standard.

18	24	7	1	25.
51 −	~~6~~	19 +	6	70

$\chi^2 = \frac{13^2}{51} + \frac{13^2}{19} = 3.3 + 8.9 = 12.2$

a) old data: 72% Lac −
b) new data 64% Lac −

$\chi^2 = \frac{36}{18} + \frac{36}{7} = 7 \quad p = <.008.$

deficiency of Lac +

Peculiar segregation may explain peculiarities of Gly segregation.

Caution:

if the equation is derived

Segregation of drug-resistances

~~Y79 x Y80~~

A Y77 x Y78. – in T(0) OK!

B Y53 x Y78 OK! ∓6 Lac– 1 Lac+.

C. Y40 x Y77 OK! no ∓

Streptomycin 5 u/ml
Malachite Gr. 100γ/ml

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

∓ peculiar deep 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+MR	Lac+MS	Lac–MR	Lac–MS
C.	2	1	8	2
total.	0	2	16	3
	2	3	24	5

Lac+S x Lac–R.

Scoring not certain

ind. cont.

notice of resistants. If valid, indicates linkage to B1.

should score on minimal plates to avoid selection for resistant contaminants.

Selection of recombinants with drugs.

P3. – mix YB cultures of Y77 (Mg^r) and Y78 (Str_5^r). 30°
also use mixture of nutr. 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	"	0	1 colony; ca 100 minute clearings, micro 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.

x Compare 4 (zeros) c̄ 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 x Y79. and plate in brilliant green. + streptothricin.]

Resistance steps; resistance to streptothricin.

March 4, 1947.

Recd. 127,000 u. streptothricin from Woodruff, Merck. non-sterile ampule.
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 on 5u streptothricin agar gives colonies but not so large as Y82.

Y83, Y84 OK on 10u agar.

See 452.

440 x 453. Mix cells in agar & pour 5 ml each plate.

A).

O:	B:	B_1.
11	12	124
15	11	136
9	14	
10	12	m = ~~10.~~ 130
15	9	more turbid.
60	13	
m = 12.0	6	
	12	
	m = 11.1	

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, V_1^R in O, B_1:

~~O: -R -S +R +S Σ; 24 11 9 0 44; B_1 22 15 14 0 51.~~

	-R	-S	+R	+S
O	17	10	9	0
B_1	16	12	13	0

Anyone 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 [Y40 x Y53 (T) by plate number].
(grown together - chance for selection)

Plate #		Lac - R	Lac + R	Lac - S	Lac + S.	Σ
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
* 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
14		6	1	0	1	8
40		1	1	2	0	4
21		2	1	1	0	4
7		6	3	2	1	12
23		3	2	0	0	5
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.

* V scoring not dependable - phage apparently very low titer.

(27, 22:) not signif. by itself

Lac - = 135/214 = 63%
compare c̄ 70% prev.

V^R = 190/214 = 85%
comp. c̄ .74 prev.

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

On map basis

B_1	−	+	R	+
	BM	Lac	V	TL
	+ a	− b	S c	−

<lac −   bvc <   >a

>V^R bva <.   <c

(<c. [illegible])

a = +R
b = −R
c = −S.

[−R would not be augmented by the diminution of c?.]

---

or, another interpretation, is that the previous strains were recruited for T or for L, c a longer distance in the interval V−(T or L)

∴ compare types c a 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~~ 5	0	~~1~~ 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 (27)	2	/ 214
	189	129	44	3	/ 365

cf. 445.
compare c̄ remainder of expt.
These samples agree.

$$\chi^2_2 = \frac{49}{83} + \frac{49}{106} + \frac{1}{53} + \frac{1}{76} + \frac{9}{20} + \frac{9}{27} = 1.86 \quad p = .4.$$

Homogeneity ??

.59 | 1.86
.46
.02
.01
.45
.33

analysis of

446 vs. 359 summarized.

$-R$	$+R$	$-S$	$+S$.	Σ
189	129	44^{63}	3	365
100	55	50^{36}	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 } (8.5) by this component. $p = .005$.

6 .23
  .33

12.7 $= \chi^2_3$

it is the difference in the frequency of $-S$ which differentiates the distributions.

8.5 -
4.2 $= \chi^2_2$ $p. = .04$

compare

194 - 189	124 129		318
95 - 100	60 55 ~~40~~		155
289	184		473

should be 194 : 124
95 : 60

$$\chi^2_1 = \frac{25}{189} + .25 + \frac{25}{124} + \frac{25}{60} \quad = \text{ca } 1.$$

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 ē Y40, Y53 stand. In previous test:

A	22:-	8:10:0:0
B	~~27~~:-25	9:6:0:0
C	~~25~~:-27	5:14:2:0

Lac, V.

D. Y40 x Y53.

		−R	+R	−S	+S
A		47	19	9	1
		44	20	7	1
B		26	12	16	0
			13	16	0
C		7	5	~~7~~	1
		80	36	32	2
D.	−	30	~~21~~ 22	19	0
	O_2	10	7	5	0
	N_2	6	5	~~7~~ 6	~~0~~
	M.B.	4	4	6	~~0~~
	S.H.	2	1	2	0
D. total:		52.	38	39	0

This is homogeneous with the previous tabulation: 100:55:50:4.

Total	132	74	71	2	279.

Compare A ē D..

47	19	9 + 1	0	76
52	38	39	0	129
99	57	49		205

$\chi^2 = \frac{100}{37} + \frac{100}{62} = 4.3$

$+ \frac{4}{21} + \frac{4}{36} = .2$

$+ \frac{64}{18} + \frac{64}{31} = 5.6$

$\chi^2_2 = 10.1$

$p = .007$

See over.

Compare R c̄ S.

58 66	18 10	76
98 90	39 31	129
156	49	205

$$\chi^2_1 = 64\left(\frac{1}{58} + \frac{1}{98} + \frac{1}{18} + \frac{1}{31}\right)$$

$= 64(.017 \quad .010 \quad .055 \quad .032)$

$= 64(.114)$

$= 7.3 \qquad p = .007.$

Therefore the total discrepancy is due to a difference in the proportion of V^R cultures. In practice, this means a deficiency of $\underline{-S}$ cultures in the new group.

		−R	+R	−S	+S.
A. 22		~~44~~	20	7	1
B. 25		25	13	16	0
C. 27		7	5	2	1
		~~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
	Σ.	52.	39	37	2
		69	38	31	2

. This is the "abnormal" distribution; 1 plate showed 13:14:0:0. (perhaps scoring of V^R was not proper.' Recheck!

4·17

Plate by plate

	−R	+R	−S	+S
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! Be careful of scoring V^s.

Interpretation of 447.

Compare $T^- \to T^+$ in 447 lines + standard.

March 7, 1947

Using cells from Exp. 447, plate the Y53 components at a <u>10^{-2}</u> dilution into LB_1 agar.

Y53	turbid	10 cols
(Y53-427)	clear	30 cols
25		10 "
22		1-2

There is no stable difference in segregation, but apparently due to scoring difficulties.

Plating medium for recombination

March 7, 1947

Factors: buffer: phosphate, citrate, acetate, phthalate. More alkaline.

R_H : thioglycollate; ascorbic ac.; pyruvate-lactate; O_2; Methylene Blue. Indophenol??

B_1 ? — Try on BM x TP.

T(0) — Y40 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 nevertheless.
4. Na thioglycollate 2 mg — .2 mg —
5. Ascorbic ac. 1% no colonies (pH 4-5) No turbidity
6. Methylene blue 100γ Turb. [illegible]
   10γ " "

The number of apparent colonies is correlated inversely c̄ 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. 453.

-also 441A - Lac blue - on lac → glycerol: white + black colonies + some c̄ blue haloes!

Y78 (58-161 Lac Sm^R) +++ gd. Gly. +++ - green sheen! count as Lac−

58-161 − (±??)

Y80 (58-161-V_1^R-Gly−) −

Note, Y80 on v. long incubation turns a faint violet color, à la sucrose

from previous expts + now

Y53	++
Y81	++
Y40	++
Y78	++

? relative amts.

Y80	−
58-161	±

58-161 is slow, but will score vs. Y80. see 453.

Y80 x Y81.

Gly. segregation:

March 9, 1947.

A. Repeat 440

See 451 A.

Y80 x Y81.

T(0).	16/16 Gly+	12/14 Lac- 86%
$T(B_1)$	13/13 Gly+.	56/72 Lac- 78%

Note, this denotes absolute linkage of Gly to BM. This is predictable either as the mapping:

neither Y80 x Y53 nor Y81 x Y40 seems to be disturbed! Suspect!

B Gly M

or

Inv.

B M Gly

Note (also) the segregation for Lac may be disturbed. Comparing c̄ accum. data for Y40 x Y53: (71% Lac-) $p = .06$

c̄ 440 $p < .025$

(This + 440) c̄ 71% $p = < .001$

440 c̄ 71% $p = .008$

B_1^+/B_1^- $p = .3$

Glycerol segregation.

March 9, 1947.

These plates were allowed to stand 3 das. before testing

B. Y80 x Y53. Test B_1^+ + (B_1^- + B_1^+) on Gly; Lac.

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 | Gly 16/16 + (not check)

B_1^+ 104 Lac− : 43 Lac+

B_1^- 28 : 13

132 : 56 188 70% Lac−

This experiment is not homogeneous with the earlier Y40 x Y53. nos.

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	44
	51.	40	27	1	119

→ stand $\chi_2^2 = 3.2$ $p = .2$ data not dissimilar

→ $\chi_2^2 = 3.5$. $p => .2$

119 of standard. $p = .06$

91 : 28 76% Lac−

Segregation of B-

March 9, 1947.

See 445

Y40 × Y53 in Biotin medium. Picks colonies to H_2O, + moi. loopful in T(0) + T(B). 50 isolates.

50 isolates. At 12 hours, 5 def. B⁻ (did not grow in either at first. 5)

Lac, V segr.

	Lac	V
1	+	R
2	+	R
3	−	R
4	−	R
5	−	R.

P13 – 5 grew on B, not 0. Earliest, 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.
卌 I	卌	0	1

∴ 0–S/12.

	6	5	0	1	12
A ([illegible])	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

Glycerol reactions, various strains.

March 11, 1947.

P11. Streak on a single Glycerol plate (EMB-2%) the following:

		A12	P12	A13	4P13	A14
1	K-12	−	+	++	+++	+++
2	58-161	−	−	−	− + at ends.	
3	Y40	−	−	±	± + at ends.	
4	Y10	−	−	±	+	
5	Y53	−	−	+	++	
6	Y46	−	−	+	++	
7	Y04	−	−	±	++	
8	Y78	−	±	++	++	
9	Y77	−	−	±	++	
! 10	Y80	−	±	±	± translucent, creamy shade, not opaque purple.	
11	Y81	−	−	+	~~+~~ ++	
12	Y~~8~~5	−	−	±	+~~+~~	
13	Y73	−	−	+	+	
14	Y74	−	−	+	+	
15	Y82	−	−	+	++	
16	Y83	+	++	+++	+++	
17	Y84	−	−	+	++	
18	Y79	−	−	+	++.	

4/11/47

On various plates, streak: Serratia marcescens 1
Salmonella 20 2
" 21 3
Phytomonas tumefaciens 4
Staph aureus 5

Incubate

γ or u./ml. 1 2 3 4 5

Malachite Green M.G. 1 ++ ✓ ++ ✓ — ++ ✓ — ± ++ — — ✓
10 + ++ ✓ ++ ✓ ++ ✓ — — ✓ — — ✓
50 — — — — — ± — — — —
— (— cols) ++ — — tiny cols.
100 — — ✓ — ± ✓ ± ++ ✓ — — ✓ — — ✓
+

Brilliant Green B.G. 100. — — ✓ — — ✓ — — ✓ — — ✓ — — ✓

Streptothricin Sth 1 ++[R] ✓ ✓ + — ✓ + ✓ ✓ + ++ ✓ ++[Y] ✓ ✓
5 + + + cols. — — ± ± papillae ± + ++ — colonies. ✓ OK.
10 — cols. ✓ good cols. — — — ✓ ± + ✓ —

Streptomycin Sm
1 — — + + papillae + + ± + ✓ — cols. ✓ grow large.
5 — — — — — — — ± — — ✓

Penicillin sic! 10 ++ ✓ — ✓ — ✓ — ✓ — ✓
sic! 100 ++ ✓[R] ++ ✓ ++ — ± ++ ++. ✓Y ✓

Control N.A. ++ ++[R] ++ ✓ ++ — ± ++ ✓ ++ ++[Y] —

Inoc 10P11. Readings 1st. 1130A12.
6P12
9A13

Concl. — [Approx. of selective conc.]:

	M.G.	B.G.	Sth		Sm		Pen
Serratia	10-50	— <100	10	10	<1.	—	10-100
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	

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 strains of 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 Sm5^R
	Proflavine	100.	Turbid.
		10	clear at 24 h.
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.
	Profl.	100	turbid
S20	Bg	100	clear, no Bg^R!
	Sm 1		turbid
	Sm 5		turbid; but some possibly Sm^R, v. small
	Profl 100		turbid.
S21	Bg 100		clear, no Bg^R.
	Sm 1		turbid.
	Sm 5		as S20. inhibition incomplete.
	Profl 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 1000 resist?
	Prof	100	clear! — ca 300? resistants

available:

~~Stamps~~: Phyto Sth 100
Sm 50
Prof 100

St: Sm 5
Sth 10
Mg 5

820 B.G.
Sth 10
Sm 5

S21 Bg
Sth 10
Sm 1

Senatey Sth 20
50
100 } color variation

Sm 5

Mg 50

4/11/47.

As above in N.A. and T(0).

photographs show up as papillae in T(0) streaks, 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.

n.g. Too irregular. NA mix + plate is more reliable.

"Syntrophic colonies?"

1 [illegible]

See 452 for sources.

Colonies from 440 x 453 on Biotin were picked to water + T(0), T(B) inoculated. Here reported are 5/50 which grew on T(B) only after 2-3 days. The T(0) and T(B) tubes were both streaked on Lac-V agar: (↓ no turbidity)

	T(0)	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(0) tubes on EMB lac to purify.

Test -(a) and +(b) colony of each on B, 0 medium.

	0	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 "[illegible]" 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.

← mucoid colonies. Y53M.

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.

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
1		17	1
27	12	30	1

} $\chi^2_3 = 5.14$ $p = .16$.

In large, +R > −S.

In small +R > −R.

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.

I 5 [illegible]

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 ev. of selection here! O.K.

B. 1 ml Y40 : 5 ml Y53.

	2	~~0~~	1	1
	~~7~~	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! ~~each~~ check for origins
no S/plate!

do not use this expt. no. too small anyhow

15 [illegible]

	−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.

~~450.~~ 461

Repeat 451 for Lac segr. test + for gly- segregation.
Plate in T(0), T(B), T(B_1).
1 plate – Y80 in B_{II} to check on reversion of M.

$B_1^- >>> B_1^+$

++. 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.

Exp	
461.	198
451	29
441	70
	29.7

∴ 1. Linkage is very tight, perhaps requires a double X against interference
2. Cytoplasmic inheritance
3. Trick up in an inversion.

Y80/B → no colonies

Y80 x Y81.

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. to be repeated.

Fermentation mutants.

March 22, 1947

Inoc Y40T (mustard see Y40) and Y53T in YB P 22

spread diluted (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. transducent pigmentation. Recover for test as mutants.

Read P24.

~~[illegible]~~

Y87. Lac - ✓
V_1 R ✓
V_T S ✓
Nutrition:

Y53 - glycerol. ca 20,000 colonies.

mucoid - 1 (2?)

gly - 1? (mucoid?)

gly - probably contaminant. Test by streaking on lac, V.

March 21, 1947

A. 440 x 453 B. 58-161 x 464.

phage n:g. Lac- unreliable as they were scored on second transfer to Lac-U plate for checking on phage

		Lac-	Lac+
T/(0)	rand.	20 +30	5 +15
	small	23	4
	large	18	6
B₁	random.	27	17 !
	small	31	8
	large	39	14
		168	54 corr: 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
B₁	small	16	8
	large	15	7
		101	48

269 102 / 371 = 72.5%

Drug resistance 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.

Phy/Sth 100 B.G. 100 turbid!
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 c̄ resists.
10 turbid
50 turbid.
100 turbid.

Staph/Sm. 5 Sth 10 clear c̄ resist. — [illegible]
~~20~~ clear s̄

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 zone in center. clear zone in center (not mixed?)
5 clear!
10 clear!

1Sth 10 Pen 100 turbid.
Sm 10,5 turbid 1Sth - turbid
Sm 10,5 turbid.

Chloroacetic acid resistance.

March 22, 1947.

See Penfold ~~19~~ 1911.

Streak Y53 on NA + various conc. Monochloroacetate neut. c NaOH
conc. expr. as free acid.
γ/per ml

50	continuous growth
250	dense growth of streak c ca 10 papillae of large size per streak.
500	Background growth less. do.
1000 = 1	" " very slight.

Pick papilla of 1 to new 1 CAA plate. P24.
Isolated colony to ss slant: Y88

Inoc into fermentation tubes P26. (BrThymol Bl.)

		glucose 1%		glycerol 1%.	
12h.	Y53	A++	++G	A±	±G
	Y88	A++	-G	A±	±
36h.	Y53	A++	/+	A+	+G
	Y88.	A++		A+	±G

see 468.

Segregation of Mucoid Resistance.

March 26, 1947
by T(O).

A Y86 × 58-161 — prototrophs rare (2/7 plates!)
B Y86 × Y40.

A. 8 mucoid. 7 Lac −
all resist. 1 Lac+.

1 Smooth. Lac − V ~~R~~ S ! ~~[Mucoid different from resistance?]~~
check. ✓

B. . 28 mucoid 8 Lac +
all resistant. 20 Lac −

1 Smooth Lac − V^R

---

9 Lac+ : 27 Lac − Smooth 2 Lac − : 0 Lac+.

Pyrex Smith fermentation tubes c̄ Nutr. Broth + various supplements as ind.
Formate includes M/20 phosphate buffer.

12h.

	control	formate ½%	F+gluc 1% (H_2:CO_2 ca 2:1)	F+man 1%	gluc 1%	man. 1%	malt 1%	sucr 1%	glycerol 1%
Y53	− ✓ −	+ + ++	++ +	+ ++	+ ++ ++	+ + ++	± ± ++	±? ±	− − ± ±
Y88.	− ✓ −	+ + ++	+ ± ±	+++g. +	++	− ++ −	(±) ± +±	− ± ±	− − − ± ±

	pyruvate 2%	lactate 1%	malate 1%	acetate 1%
Y53	++ + ++	− ✓	− − ✓	− − ✓ −
Y88	± − −	−	− −	− −

second reading 72h.

Formic hydrogenlyase is apparently intact.

∴ step blocked is:

pyruvate → lactate; pyruvate → HCOOH —lyase→ CO_2 + H_2; pyruvate → CH_3COOH.

Try: utilization of pyruvate in synthetic medium.

Carbon source utilization (T(0) − asparagine + TLB₁).

Suppl:	1 —	2 pyr 2%	3 lact 1%	4 gluc 2%	5 mannitol 1%	6 acetate 1%	7 glycerol 1%	8 malate 1%	9 formate 1%
Y53 :	−	+	+	+±	++	? +	+ ++	+++	−
Y88 :	−	+	+	+±	+ ++	? (±)	+ ++	+++	−

(±) → major difference.

malate } were most eff. carbon sources
mannitol }

~~wildtype : coli gas tion~~

W.T.		Mutant.		
	++		++	formate
+	++ +		−	glucose } major differentiation
+	++ −		−	pyruvate }
±	± ±		±	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 pop heard.
	pyruvate	±	+	< 1/5 H_2 ✓	
~~Y70~~ Y88	pyruvate	—	—	~~≪ 1/5~~ H_2 ✓	
	glucose	+	—		
	mannitol	+	+	< 1/5 H_2 ✓	

March 28, 1947

not a good expt.

Y77 x Y78. (≐ Y64 x 58-161)

	−R	−S	+R	+S.
O.	8	8	0	3
	3	1	0	0
	4	8	2	0
	15	17	2	3
B_1.	6	12	0	3
	21.	29	2	6

This distribution is entirely different from standard ~~Y46 x Y53~~.

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 mutr. req.

Test for M.G.^{R}; Sr^R:

	MG S	R	Sr S	R.
O: −R		16/16.		4/16.
−S	lost; mostly R.			
+R	S; R		s; S.	
+ S.	R; R; R		S; S; S.	

B_1
−R
−S
+R
+ S. ↘ ~~S; R; S~~ s; S; R. ~~ab~~.

small + sparse

not certain as at best only a dozen colonies appeared at site of streak.

−R are all MG^R
−S are > MG^R
+R
+S variable.

Formic Hydrogenlyase.

March 27, 1947.

Inoc Y53 heavily into Nutr. Broth + 2% glucose + ½% formate. Grow 12 hours. Wash cells once + suspend 100 ml c̄ in 5 ml of buffered (6.8 M/10 phosphate) ½% formate, aseptically c̄ Smith tube. Gas production was reached within ½ hour. NaOH abs. ca ⅓ of gas.

Repeat c̄ K-12.

Inoc. 1) glucose-formate-broth & 2) 2% pyruvate broth heavily c̄ K-12 5P28.

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(0).

Test 20 isolates.

		Lac V	Cla	Gas-glucose	pyr EMB
	1	—R	S ✓	+	—
	2	—R	S	+	—
Y ✓	3	—R	R ✓	+	—
	4	—R	S	+	±
Y ✓	5	—R	R ✓	+	—
	6	—R	S	+	—
	7	—S · —R	R	+	—
	8	—R	R	—	—
	9	—R	R	—	—
Y ✓	10	—R	R	+	±
	11	+R ?	R		—
	12	+R —	R	+	—
	13	+R ?	R		—
	14	—S ③	R ①		—
	15	—R	R		—
✓	16	—R	R		—
	17	—R	S	+	± (OK.)
	18	+R	R		—
	19	—R	R		—
	20.	—R.	R.		—

Many of the large colonies in groups 1 appear to be yellow suggesting possibility of contamination.

Repeat cross.

② #14 seems OK however. Y89 | Streak 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 slants as Y89-1, 2, 3.~~

Reverse Crosses

March 28, 1947.

A. Y40 x Y53 B. Y64 x 58-161
on minimal.

A.

Plate large	α				small	β			
	−R	+R	−S	+S		−R	+R	−S	+S.
1.	6	1	2	0		2	4	5	0
2	6.	2	1	1		3	5	0	0
3	3	1	4	0		0	0	1	0
4	7	1	3	0		3	1	0	0
5	6	1	8	0		6.	3	0	2
6	3	3	3	0		3	1	3	0
rand.	6	7	2	0		3	1	4	0
	37	16	23	1		20	15	13	2

53:24 = 69% R/S. R:S = 35:15 = 70%

B. total	8	1	12	8

9 : 20 = 31%

r.

Compare A_L ē A_S. totals.

.11	.53		.04	
37	16	23	1.	77
.18	.75		.06	
20	15	13	2	50
57	31	36	3	127
		39		

$\chi^2_2 =$ .11 + .18 + .53 + .75 + .04 + .06 = 1.67

OK. compare

	R/S	+/−
α	.69	.22
~~β~~	~~.70~~	~~.35~~
r	.31	.31.

$\chi^2 = 3.14$ p = .08 ē cumul. data — < .07 for fit

perfect for reverse!

April 3, 1947.

Agent.	conc./20	conc mg/ml	Y53 (16 hours)	Y88
Fluoacetate		.05	++	++
		2.5	++	++
	intermediate conc. do.			
Chloroacet.		1.0	±	++
Cl hydrin.		10.0	++	++
iodoacetate	.1 mg	5γ	++	++
		50γ	—	—
		250γ	—	—
		500γ	—	—
		1mg	—	—
		2.5mg	—	—

showed no resistants

resistant colonies appeared profusely in 36 hours (Ia[R]). Y90.

a) mutational effect or manifestation
b) lethality of double mutant due to a metabolic cycle like.

Streak suspension 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 γ/ml)

~~2. Streak out Y90 on 50 γ/ml. Sa.~~

No growth on any plates by any of the cultures.

Acetate utilization
~~nucogenesis.~~

April 8, 1947.

Inoc. (lightly) T(m) + suppl. c̄ 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(o) + glucose 2% (gas tube)~~						
~~9.~~	~~T(o) + glucose 1% + y.e. .5% (gas 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 K-12 on various acetate, glycine concentrations. Inoc P11.

	T(m) +.	A13	P13
	Acetate.		
1	1%	—	—
2	.5%	±	+
3	.1%	+ -+	+± More opt. conc.
4	.01%	±	±
0	Glycine		
5	1%	—	
6	.5%	—	
7	.1%	—	
8	.01%	—	
0	0	—	

Nutrition of 679-~~183~~ 662

April 10, 1947.

γ/10 αKG	glut	etc.	[illegible]	12h	24h.	36h.		
1. 0	—				++	++	++	
2. 0	5γ				+	±	++	+
3 0	200γ				++	++	++	
4 5mg.	—				++	++	++	
5 5mg.	200γ				++	++	✓	
7 2.5mg					++	++	✓	
8 2.5mg		1mg alanine		±	++	++		
9 2.5mg		1mg alanine + 100γ B6		±	++	++	✓	
10. 5mg	5γ				+++	++		

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.

Colonies:	T +0	T+glut	T+αKGlut	Glut.	24h
1	—	+++	+++	—	✓
2	++	+++	+++	—	✓
3	—	+++	+++	—	✓
4	++	+++	+++	—	✓
5	+	+++	+++	—	✓ ++
6	++	+++	+++	—	✓
7	+++	+++	+++	—	✓
8	—	+++	+++	—	✓ +
9	—	+++	+++	—	✓
10	±	+++	+++	—	✓

no doubt of utilization of α-ketoglutaric

T- OK.

transfer 1 and 2 as T-G- a d T-GK resp.

April 10, 1947.

1.) m acetate 1%

	12h	24h	48h	72h.
K-12	=	±	++	++
Y89.	=	−	±	±

2) on glucose

K-12 A б

Y89 A ng.

Formic H-lyase Activator

April 11, 1947.

	per liter.
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.

= Formichydrogenlyase basal. = FH(0).

Use c̄ Durham tubes.

— "gas."

	12h. K-12		Y89.		36 ~~24h.~~ 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 (= Enzicase .5%) or in y. ex.
perhaps by way of formation of formic dehydrogenase.

Hydrogenlyase coenzyme.

April 13, 1947.

	K-12 12h. growth	gas	Y89 12h.		K-12 24h. gr	gas	Y89 gr	gas	K-12 36h.		Y89	
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. c̄ high activity of N2 Case & intermediate activity of acid-hydrolyzed casein.

NA, tryptophane have slight activity.
try individual NA. & more tryptophane

Oxidation tests.

Grow in Acetate 1% broth. Wash & adjust to ca. = densities.
1ml (≗5ml original) bacteria in M/10 phosphate + substrate.

	K-12	Y89
—	ca 150	ca 150
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	
contr?	(4)	~~Pyr~~ Pyr	ClAc	4:30.
	5	Form		< 4:20
	6	Form	Cl Ac	< 4:20

Carbon metabolism of 679-~~182~~ 662

	K-12	679-662	
1. T(Φ)(100γ/10ml).	—	—	
24h.			
2. T(Φ) + .1% glutamine	++	++	pellicular growth.

Quantitative response.

T(G) + glut	α-ketogl.	
0	0	+++
3γ ↓	do.	
1mg/10ml		

∴ this strain has fully reverted & data on utilization are fallacious.

Reisolate from lyophil & check rigorously. OK ✓

Using α-ketoglutaric in lieu of glutamine for growth OK.

Utilization of Acetate

24 APR 1947

Moc P24

T(m) + Acetate	Glucose	~~24h.~~ 36h. K-12	48h.		36h. Y89 ~~Y90~~		
.1%	—	+	+	✓	±	—	✓
.1%	1%	++	++	✓	+++	✓	✓
.2%	—	+	+±		±	—	
.2%	1%	++	++		+++	✓	
.5%	—	++ sic.	++		—	—	
.5%	1%	± "	++		++.	✓	

? ↑ Mutant is acetate –. Not inhibitory.

are acetate + glucose inhibitory when autoclaved together? cf. 475. In prev. expt. test was in phosphate buffer.

Glycine utilization data are needed

autoclave together.

	T(m) + Acetate	Glucose	Glycine.
	.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%	—

2? APR 1947

Prep. pour plates of NA + IA 50γ, 100γ/ml. etc. Streak thickly.

		ClA 1 mg/ml		ClA 2		IA 50 γ/ml.		NaN₃ 100
Y53	24h.	±	pap.	±	pap	–		–
Y40		±	"	±	pap	–	pap	–
Y88		+++		+++		–		–
Y53	36h.	✓		✓		–	do. → isolate for B-M-$V_1^R Ia^R$ (acid and gas on glucose!) test on 100γ/ml	Y90
Y40								
Y88						–	isolate for B-M-$V_1^R Cla^R$ (acid, gas ± on glucose.)	Y91

1 small colony on Y40 streak. (Too conc. NaN_3).

Lack of Ia^R from Y88 + Y53 may be due to the more recent origins of these isolates, c̄ a smaller chance of accumulating mutants.

Y53, Y40, Y88 60h. --pap. | 1 small, 1 large colony, – } some residual growth in very tiny colonies.

→ Y92 Az^R. note: zone of adjacent streak was v. strongly stimulated. pH effect likely.

Y93 = Y53 ~~Cla^R~~ Ia^R

No Ia^R from Cla^R?? Due to very heavily N24 — confluent growth. see 497.

Segregation of Cla^R.

Apr 4, 1947

Y40 x Y89.

Hold in icebox

Comp. 0; B, on Lac V segr.

T(0).

	Lac- V^R	Lac- V^S	Lac+ V^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 grow. No. is from sample tested.

T(B₁).

	20	7	9	0
	10	6	4	0
	30	13	13	0
Cla^R				
Cla^S				
Gas+	4			
Gas−	1			

Total 4/78 Sens. 2 − R
1 − S
1 + R.

Segregation of Cla^R

Y40 x Y88.

T(0) Pick from Lac-V tests to water. Streak on Cla (1-2 mg/ml)

1-	Lac-V +R	Cla
1		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

T(B1) 4-

1	-S	R
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

2-	Lac-V	
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	

5		
1	-R	(S) R
2	-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	

Total: 56/58 = S
+ 18/20
74/78 = S.
R = 5%

test this group in B1-T(0).

+	−	−	S	+	R	+	+		[illegible] S
B_1	B	M	Ca	Lac	V	T	L	…	Ca
−	+	+	R	−	S	−	−		[illegible] R

mostly R. ∴ R near [illegible] BM

~~R's are −S; −R~~

S's are −S; −R.

R between B, M?

Homogeneity of B_1^- : B_1^+
Y40 x Y53; Y64 x 58-161.

483

April 14, 1947

22 APR 1947

Use light mixtures Y40 x Y53. Add B_1.
Hold in cold room after 2 da.

ca 1 - 5 colonies / plate.

4/19 Streakout 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 Y88 ($Y53-Cla^R$)

Reverse Crosses —

28 [illegible] 1947

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(B₁)	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.

B. Y64 × 58-161.

		−R	−S	+R	+S.	
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(B₁) —						
	Σ	109	125	8	70	312.

See next page.

Expressed as percentages.

	−R	−S	+R	+S.	
A (0).	35	30	35	0	28
A (B₁)	42.3	26.6	28.8	2.3	173
B (0)	31.9	47.4	4.3	16.4	116
B (B₁)	34.9	39.0	2.5	22.4	312

See summaries of data

Y04 x 58-161 B
Y40 x Y53 A

April 20, 1947

In perurosis cypts, +R >> -S occasionally.

A :

-R	-S	+R	+S.	
7	9	3	0	
4	8	3	1	
7	5	4	2	
20	22	10	3	55

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	[illegible]1
2[illegible]		2	11	51 B

A

8	1	1	2	A(B(6))

1192
22
1214

484 ctd. Y64 × 58-161. T(B$_1$)

	−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
b.				
6.	11	6	6	1?
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.

(5b, 6:) Retest – phage n.g.?? 13−R : 6+R. appearance very poor!

N. tetrasperma

A Pw16 / Sub. + B. Sub

Spread spores (or perithecia) on agar surface.

2/26/47. Irradiate spores c̄ ca 20,000 r (courtesy of Pollard)

Isolated 2/27 Transfer spores to small corn meal agar slants + heat-activate.

Do. c̄ unirradiated controls.

First scoring —

					Σ fertile		
Control	100	80	37 + 16		53	28	35%
X 25000 r	100	69	9	7	16	51	= 76%
Sub/16 J.	47	34	69	23	5	6	
E.	21		25	4	0	1	
	= 70	49		27	5	7	
Xray J	50	14		6	2		
E	77	35		13	0	6	
	127	41		19			

X: 41/109; 2/41 ⊕

C: 40 / 70, 22/40 ⊕

D / d: 10 | 4 + 3 combo

C → 28
X → 51

C → 18
X → 13

April 15, 1947.

FH (2% glucose +). Mor 5P18

HC ½%	++	+
NZCase ½%	+++	+++
	growth 12h.	[illegible] h.
1 Glutamic ac.	++	++
2 Glycine	++	++
3 Serine	++	++
4 Aspartic ac.	+	±
5 Asparagine	+	++
6 Glutamine	+±	++
7 Proline	+	+
8 Hydroxyproline	++	++
9 Cysteine	++ H_2S [illegible]	++
10 Alanine	±	
11 Tyrosine	++	++
12. 0.	+	++

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 36-48h.

abandon momentarily. One could seek an antagonist of formate, however, which seems to be present in NZ Case.

Glutathione?

Phytomonas – Recombination.

April 1947

Strains: **B**. A6/Pro/MG. **A**. A6/Sth/Sm. Inoc separately and together after growth on ∞ slants, into carrot medium. – 4 wks. Sediment & wash pellicles.
1: carrot 2: slant. Inoc from carrot into YB liq: 3.

On 1AB/Nutr. Agar plate, ! "rough" colony noted. Failed to recover

A = Proflavine 100γ/ml.
B = Malachite Green 100γ/ml.

C = Streptomycin 50u/ml.
D = Streptothricin 100u/ml.

Read at 48 hrs.

		1A ①	1B ②	1AB ③	2A ④	2B ⑤	2AB ⑥	3A	3B	3AB.
11	A	– –R ✓	+ ++ ✓	– (+) ++ ✓	– – ✓	– ++ ✓	– ++ ✓			+
12	B	– – ±R	± ++ ✓	– ++ ✓	– –	– – –2R	– – –2R			++
13	C	+ ++ ✓	– –R ✓	± ++ ✓	± ++ ✓	– – – ✓	± ++ ✓			++
14	D	+ ++ ✓	– – –1R ✓	± ++ ✓	± ++ ✓	– – ✓	± ++ ✓			++
5	AB	– –R ✓	– + (best pop.) ++	– ± ++	– –	– –	– – –			++
7	AC	– –R ✓	– –R	– –R	– –	– –	– –R	✓10		many R
8	AD	– –R	– – ±R ✓	– – ±R ✓	– – ✓	– – ✓	– – ✓			±R of 1B
⑨	BC	– – –1R	– – –	– – –	– – –	– – –	– – –			–
⑩	BD	– – –	– – –	– – –	– – –	– – –	– – –			–
6	CD	+ ++ Pap.	– –R ✓	± ++ ✓	– ++	– – ✓	– ++ ✓			+
1	ABC	– – ✓	– – ✓	– – ✓	– – ✓	– – ✓	– – ✓			–
2	ABD	– – ✓	– –	– –	– –	– –	– –			–
③	ACD	– –1R ✓	– –	– –1R	– –	– –	– –			–1R
4	BCD	– 1 R± ✓	– – ✓	– – R±	– – ✓	– – ✓	– – ✓			–
15	ABCD.	– – ✓	– – ✓	– – ✓	– – ✓	– – ✓	– – ✓			–
	NA.	+	+	+						

Inoc series ~~1+2~~ P 21; also inoc on NA plates for series 3.
1+2

Streak on plates in order:

Read at 1) 12h.
2) 48h. No evidence of recombination. Proflav + M.G. apparently interact. (see 5).
3) 60h.
Sm and Sr OK. (see CD). But 3 and 4 do not suggest recombination

R = individual resistant colonies (1–10)

Inoc 3AB N24.

Use ACD c̄ &B in combinations.

28 [illegible]

Y40 x Y88.

Compare c̄ 191A.

A. T(O)   B. T(B).

Streakout on ClA-minimal or ClA-biotin agar.

A. 1R/6S. = 1/7   S for R!

B. 1R/6S = 1/7.

2/16 = R.   need data on segregation c̄ B$^-$
Use selection of Cla^R on B plates, c̄ reverse cross to establish correlation.

#14/16 Res.

20 APR 1947

(A) Plate 440 × 488 on B_1-Acetate medium. This should check for B_1^- la[5] ~~[illegible]~~ segregants. By plating these colonies into SMT-la medium it should be possible to eliminate the parental types (B-M- req. la insensitivity; T-L-B_1- req B_1 requirement) and find any complementary segregant.

Growth is moth-eaten, suggesting phage! very strikingly.] See 489. 4-9.
probably not.

(B.) Plate 440 × 488 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 contg 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.

A		
1	all −	
2	all +	
3	+	
4	−	
5	−	
6	+	;(−)
7	−	
8	−	
9	+ −	((−))
10	−	
11	−	
12	−	
13	−	
14	−	
15	no cols.	

+ − 10/4

all phage homogeneous unless Lac reaction different. Test these intensively

B	
1	100+ : 1 −
2	100+ : 1 −
3	all −
4	"
5	"
6	"
7	all +
8	~~[illegible]~~
9	all −
10	all −
11	all +
12	all −
13	"
14	"
15	no colonies
16	all −
17	all −.

11 − / 5 +
~~12~~ −

T Lac.

See over.

Streak various plates c̄ 4 cols. each plate.

A) mostly +R Cla^R

~~R~~ Cla^R

1 - S. TLB,

parental.

B) mostly -R Cla^R (B_1)

1 + R Cla^S (B4)

no new combinations aside from main component in these colonies!

28 MAR 1950

A. Y40 x Y86. (to compare V^R loci)

B_1. (to reduce inoculum required). $B-M-Lac+V_{1A}^R Muc^- \times T-L-B_1-Lac-V_{1B}^R Muc^+$

(−S Sm.) $V^R M^+ Lac-$ 16 ∴ $V_1^R \neq V_{1B}^R$

$V^R M^+ Lac+$ 3

$V^R M- Lac-$ 1

488-1 $V^S M^- Lac-$ 1 This Recombination suggests that $V_{1A}^R \neq V_{1B}^R$

488-2 $V^R M- Lac+$ 1

22. V_{1B}^R = T5 S.

B. ~~Y5~~ 58-161 x Y86. (to test complexity of $Muc^+ V^R$). $Lac+V_{1A}^R M^- \times Lac-V_{1B}^R M^+$

" (to reduce inoculum required.)

−R Sm. ∴ $Muc^+ \neq V_{1B}^R$

$V^R M^+ Lac-$ 13

$V^R M^+ Lac+$ 3

$V^S M^- Lac-$ 1

488-3 $V^R M^- Lac-$ 1. (This recombination suggests that $Muc^+ \neq V_{1B}$.)

[resistant to all phages. Contam?] "diplococci"!

Compare the resistance patterns of Y88-1; 2; 3; Y40; Y86

Compare recombination values of Mucoid / Lac c Standard V_1^R/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%

∴ V_{Muc}^R is ~~[illegible]~~

Reversion of Ac^R.

24 [illegible]

Plate 487 into Acetate [.1%] - minimal.

Indefinite background growth. no definite colonies. probably Ac too low.

Allelism of V_1^R

April 23, 1947

allelism of V^R. Y40 x Y64. all resistant.

159/159

T(0). Lac- Lac+
4 1 (Mucoid)

T(B₁)

Lac-	Lac+	
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 Y54

Compare fit c accumulated data.

a).

	106	48 ✓	
	122	32	154
1222	1206	562 / 578	1784
	1328	610	1938

$\chi_1^2 = 8.44$

$\chi^2 = \frac{16^2}{}$ χ 4

= .25
2.42
5.32
.45
8.44

for Y40 x Y53

$\chi_1^2 = <1.$

119	122	35 32	154
540	537	156 159	696
	659	191	850

Linkage Relations of Cla.

24 APR 19[illegible]

Plate Y40 x Y88 into Biotin (B_1^-) - Acetate-agar [select for Ac^+ = Cla^s and compare segregation of B⁻ and B_1^- in the Cla^s class].

n.g. see 489

Mitchell - Westergaard medium.

24 APR 1947

1. Cf. 490. 1 drop of Y40 x Y64 mixture in B_1 suppl medium, to compare c̄ 490 for rates. (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 previously. Try s̄ buffer., s̄ 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
KH_2PO_4	1
$MgSO_4$	.1
trace + $CaCl_2$	

I (N Z Case 5, Yeast Extr 2.5) to 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 × ~~[illegible]~~ Y64 (B-M-V_1^RLac− × T-L-B_1-V_1^RLac−). for test of allelism.

B). 134 tests of prototrophs all Lac− ∴ loci are allelic.

A) Segregation:

	−R	−S	+R	+S.	
Plated mT(10)	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

	−R	−S	+R	+S	
mT(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 ($B-M-Cla^R V_1^R$ x $B_1-T-L-Lac-V_1^S Cla^S$)

Minimal plates too crowded.

B₁ - Streak on $B_1 \cdot$ Cla or ~~[illegible]~~ Cla NA. to classify resistants/susc.

4/56 ~~[illegible]~~ Resistant

Use plating on B·Cl Hagar for counting loci.

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	A2 50	+++	—			
Y92	A2 100.	++	—			
Y53	A2 50	++	—			
Y53	A2 100	— ++	—			
Y40	Ia 25	++	—			
Y93	Ia 25	+++	—			
Y40	Ia 50	—	++			Y97
Y93	Ia 50	+++	—			

need a buffered test medium probably.

	Bg	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 slants.

Note - terminology: unless otherwise stated, figures are γ/ml. Underlined figures are mg/ml.

Virus Resistance Pattern.

		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	—?	—	—	R	too light virus?
	Y68.	"R"	R	S	S	S	S	

This phage OK on other plates. Repeat! phage?

Y40 $V_1^R V_3^S V_5^S$. compare original description

K12 = S...

488-1 = S...

488-2 = V_1 R, 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
Y65	R	R
Y68.	S.	S

Unstable resistant?? Less mucoid on this plate

streak out

stock Y86 is predominantly mucoid; a few smooth colonies

this which streak is predominantly smooth; a few mucoid colonies.

April 25, 1947.

Add varying amounts of 30% Camphor in 95% Alcohol to plates of Nutr. agar to give following "concentrations" of camphor. Inoculate 3 days. Str. W53:

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 bacteria.

Streak out 6 on EMB to isolate clones and test for diploidization by the suppression of recessive mutations (e.g. Lac⁻?). Many smooth-mucoid colonies noted.

	Papillae on LA?
1	±
2	±
3	+++
4	—
5	—
6	++
7	+
8	-
9	-
10	++
11	+++
12	±
13	+++
14	±
15	±
16	+
17	++
18	+++
19	++
20	+
21	+
22	+
23	++.

Recover 4, 5, 8, 9 to test for polyploidy.

Utilization of Acetyl-Glycine

April 27, 1947

Sol 480.

		Acetate	~~Glycine~~ Glucose	Glycine ~~Glucose~~	Y89 (Y)		K-12 (K)		48h.-62h. Y89.		K-12	
A. .1%	1	✓			−		−	−	±	±	±	++
	2			✓	−		−	−	−		−	✓
	3	✓	✓	✓	+		−	−	+++		+++	
	4	✓			−		−	−				
	5	✓	✓	✓	++		−	−				
B .2%	1	✓			±		−	±	±	±	+	++
	2			✓		✓	±	✓	−	−	−	−
	3		✓	✓	++	++	±	++	+++	−	+++	−
	4	✓			−	✓	±	✓	±	±	+	++
	5	✓	✓	✓	++	++	−	✓	+++		+++	−
C .5%	1	✓	ACETATE		−		-		±		±	
	2		GLYCINE	✓	±		-		−		−	✓
	3		✓	✓	++		-		++		++	✓
	4	✓			±		-		±		±	
	5	✓	✓	✓	−		-		−		−	
D 1%	1	✓			−		−		−	±	±	++
	2			✓	−		−		−		−	✓
	3		✓	✓	++		+		++		++	
	4	✓			−		−		±	±	±	++
	5	✓	✓	✓	−		−		−	±	−	−
E	1.		✓		+	+++	+	+++				

F. Acetyl-Glycine .5% — + (Y), + (K)

2. " + glucose — +++ (Y), +++ (K)

both + in 11 hours
all others −.

Glucose 1% throughout

Readings at 24h;

Concl. Glycine is not utilized; not inhibitory
Acetylglycine is utilized by both
Acetate is not utilized by mutant comp[letely]/ mild

Staining in zone of lysis.

April 27, 1947.

Compare Y53 lysed by T1 on:

EMB - lactose :

- sucrose :

- blank. :

all show coloration in lytic zone margins, suggesting that it is mostly staining of debris.

Segregation of Az and Ia

April 28, 1947

A. Y90 x Y53 (Y40/Ia)

B. Y92 x Y53 (Y40/Az)

A.

T(0).

readings on complete are unreliable. Technique for tests on synthetic should be developed.

T(B1).

B:

Lac V1	R	S
−S.	29	2
−R	8	1
+R	12	1
	49	4

Use 100γ/ml NaN3 in T(0)+B1. Somewhat too concentr.

B1	BM	Lac	V	TL	Az
−	++	−	S	−−	S
+	−−	+	R	++	R

Mostly R. ∴ Az 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 (crowded??)
T(B₁) -

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; ~~...~~
34 Muc: 31 Smooth.

P 2 2: Streak out: A. Muc from 1. B. Mix pop. from 1.
A: "all" mucoid ~~B.~~
B: 19 Muc: 70 Smooth.

P 4 3 A - mucoid from 2A. B. Mix pop. from 2B.
all mucoid. ca 100:1 smooth: mucoid

A 6 4. A - mucoid from 3A. B Mix from 3B.
all mucoid. > 200:1 smooth: mucoid

P 7 5. B. (mix) from streak of 4. all mucoid

P 10 6 streak from mass streak of 5: ca 10 Mucoid: 1 smooth.

Selection and mutation of V_{mi}^{R}

May 15, 1947.

A15. 7. Streak from mass-streak of 6.
ca 45 : 20 M:Sm.

A17 8. Streak from mass-streak of 7.
ca 23 : 43 M: Sm.

P18 9. do. 9 : 21 M: Sm.

P20. 10. do. 19 : 48 M: Sm.

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. K-12	60h. Y89
1.	½%			— —	— —	—	—	+	±
2.	¼%	¼%		— +	± +	++	+±		
3.	¼%		¼%	— —	— —	—	±	++	±
4.		½%		± ++	± ++	++	++	+++	+++
5.		½%	½%	± +++	+ ++	++	++	+++	+++
6.			½%	— —	— —	—	—	—	—
7.			¼%	— —	— —	—	—	—	—
8.	Acetyl-Glycine ½%.			± ±	— ±	+	+	+	+
9.	0	0	0	— —	— —	—	—	—	—

Autoclave separately from medium. Adjust acetate to pH 6.8 c̄ 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 strictly aerobic or anaerobic conditions?)

84h:	K-12	Y89.
1. Ac.	++	+
3.	++	±
8	+	+.

! } eventually the bug does better on acetate than on Ac Gly!

diacetyl-diketopiperazine ½% neither K-12 nor Y89 showed any response!

Test of Camphor Treatments

May 1, 1947.

See 499.

Recover presumptive strains.

a) Streak again on ClA agar.
4,5,8 finally threw off many mutants
9 only 2.

b) Cross c̄ Y40.

c) streak out on EMB lactose
all isolates are Lac-V_1^s
but semi-mucoid character interferes with determination of resistance.

b. P2. ~~[struck out]~~ .1 ml mixtures into B_1, plain agar respectively.

1. 499-4   2. 499-5   3. 499-8   4. 499-9.   × Y40.

Discrepancies between O and B_1 plates are only ca. 3-fold rather than 10-fold.

1-(O).

		Smooth				Mucoid			
	[illegible].	+R	−R	+S	−S	+R	−R	+S	−S.
①	T(0)	1				2	9		1
	B_1:	11	5			9	22		3
②	T(0)	—							
	T(B_1)	14	32	1	11	typical segregation.			
③	T(0)	1					2	1	
	T(B_1)	13	1			1	21		
④	T(0)	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.

probably contam. c̄ T1. Start new stocks.

	① T-1	② T3A (neg.)	③ T3 B (OK)	④ T3 Batch 2 (OR.)	⑤ T5	⑥ T5 Batch 2
K-12	S	R	S	S	S (large + small plaques)	S
Y40 (from lyophil)	R	R	S	S	S	R
Y40 prev.	R	R	S	S	S	R
Y53	S	S	S	S	S (large + small)	S
Y64	R	S	S	S	S	R
Y87	R	R	S	S	S	R

It is phage stocks which have varied, not original cultures since Y40 = Y40 nov in all respects. T3A must be fallacious. T5 Batch 2 behaves like T_1 and is similar to previous responses. Could it be contaminated??

Phages need purification! Recheck T3A. Present indications favor the interpretation that the results of last fall were due to gross contamination of T3 and T5 c̄ T_1.

Program: Purify T5 - ~~..~~ 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.

T-5 from original culture (Demerec) was plated with K-12 but gave uniform lysis. several resistants appeared; test these with T1, etc. Use this to reinitiate T5 stocks.

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 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

these are isolates

from 506 - ⑤ which, previously, lysed Y40.

May 2, 1947.

~~Plate~~ Pour ca 10^9/.1ml on middle of NA plates + irradiate at ca 2500r/min 20min ≙ 50,000 r. Wt target 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 protots.
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. It may provide a source of Cla^R cells.

In A, the difference 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, suppl.

This experiment is not entirely valid because the plates ~~less turbid~~.

		ca 40		
B	ca 40 · turbid.	3	see 0.	see Cla 500
	ca 50 · turbid	5		
		12		40
		19		12
C	12	0		0
P3:	25	17		24

colonies. A & B B, have large numbers of isolable c

...lonies. Test A to determine % sensitive col

g. demonstration of ...s. Use B, plates.

later, more colonies appear in B probably n... middrint ... ratio

Cla segregation.

See 496.

See 509

A. 488 x 440.

T(o) 17/17 Res.

B₁ 18/18 Res.

35/35 Res.

B.	B4-Cla		Lac	V	TL
−	+	R	−	S	−
+	−	S	+	R	+.

453 x 491.

T(o)

T(B₁) 74/76 ~~Res~~ Sens.
2(2) ~~sens~~ Res.

∴ Reverse cross is supported.

Summary.

A. 491 x 453

496. 4/56 T(B₁)

509 ~~74/76~~.
2/76. T(B₁)

6/132
4.5%

440 x 488

486 14/16 < 1/7 T(o), 1/7 T(B₁)

35/35 T(o); T(B₁).

49/51

4%.

~~Str~~ Camphor

May 3, 1947.

Streak Y40, Y87, Y64, Y10 on 10 Camphor/NA.

May 5 - streak out on EMB

May 7 - Isolated colonies. Test by streaking heavily on Cla 2 agar. Following showed no papillae + were recovered:

Recovered streak out on EMB.

Y40	2 / 15
Y87	~~1~~/18
Y10	~~10~~ 9/9
Y64	1/8
Y53	0/8

Phage resistance patterns.

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. need better stocks.

T5 many smooth resistants. 30 tested on T1 all resistant.
present stock. from Demerec' 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 (virus 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 NA agar +

	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/Mg on Mg + BG   same as above!

Y79/Bg on BG.

Identification of "T5".

Enumerate stocks.

[wait for indicator stocks from Demerec.]

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)

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 x Y91 on B₁ Cla agar. Pick on second day &
TLB₁− 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-B₁ and B₁ plates. The "~~resistants~~ susceptibles" formed tiny colonies early, ē evident frequent ~~adapted~~ mutation! Need higher conc. of Cla??

May 15, 1947.

1). Streak 58-161 c̄ T1.

2) Streak 58-161 alone.

No mucoids developed!!

~~[illegible]~~: Mucoids sometimes develop on old plates on lactose agar. Look into this!

May 16, 1947.

Test isolates from 510 by following crosses. on T(0).

A. Y10$_n$/Cam × Y87/Cam. T-L-B$_1$-B+M+Lac+V^s ×
...

on T(0) 1
2
3
4

B. Y40$_n$/Cam × Y88 B+M+Lac+$Cla^s V^R_x$

1
2

401).

-R	-S	+R	+S.		Cla	
5	2	2	0 /	9.	all R.	normal seg.

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 streaks 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.

∴ $V_{1,5}^R$; V_6^R; $V_{3,4,6,7}^R$ are available!

$Y10/_1$ Cam x Y87/Cam.

+R	+S	-R	-S.
3	6	8	0
9		8.	

	Lac +	Lac -	
Y10/3.	15.	7.	
Y10/4.	22	6	
	17	1	
	39.	7	/46. ?
Y10/2.	22.	5	

Is $Y10_4$ aberrant?

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 whole!
T1, T4.	"	<u>1 surviving colony.</u> ?	* Some nibbling! mostly OK.
T1 T6	"	<u>1 surviving colony?</u>	* small colonies.
T3, T4	"	Many R.	[illegible]
T3, T5	"	0 - R; <u>many tiny</u>	* OK. 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 colonies???	* v. small colonies. see (1, 6.)

* streak out.

Segregation of V_6^R

A B

Y94, Y95 × Y40. $(T-L-B_1-Lac-V_1^S V_6^R \times B-M-Lac+V_1^R V_6^S)$

O, B_1.

Y94-O.

Lac	T6	T1
+	R	R ✓
+	S	R
+	R	R
−	R	R
−	R	S ✓
+	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	T6	T1
−	R	S ✓
−	R	S ✓
−	R	R ✓
−	R	R ✓
−	R	S ✓
−	R	S ✓
−	R	R ✓
+	S	R ✓
+	S	R ✓
−	R	R ✓
−	R	S ✓
−	R	R ✓
+	R	R ✓
−	R	R ✓
−	R	S ✓
−	R	R ✓
−	R	R ✓
−	R	R ✓
+	S	R ✓
−	R	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.	

B-M V_6 Lac

Cum:

Lac	6R	6S	T1
−	44	2	R
−	28	0	S
+	9	20	R
+	1	2	S

82 74

See 528

Y95 x Y40.

T(0)

lac	T6	T1
−	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 ✓

T(·B1)

lac	T6	T1
+	S	R ✓
+	S	R ✓
−	R	S ✓
+	S	R ✓
−	R	S
−	R	S
+	S	R ✓

lac	T1	$\forall_6^R$	V_6^S
−	R		
−	S		
+	R		
+	S.		

Phage effect

Y40 x Y53

May 19, 1947.

(T1)

A. Add phage to Y53. Adsorb 10 mins. Mix $\bar{c}$ Y40 and wash

good yield of colonies!!

B. Mix Y53 + Y40. ~~Let stand ... hour~~. Let stand in H_2O overnight.

Add phage before final wash + plate

C. Mix as above, no phage.

(Repeat)

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 (struck out):~~

Lac	V_1	V_6
−	S	R
−	R	R
−	R	R
−	R	R
−	S	R
−	R	R
−	R	R
−	S	R
−	R	R
+	R	S
−	S	R
−	R	R
−	R	S
−	R	S
+	S	R

Lac	V_6	V_1
−	R	S
−	R	R
−	R	R
−	R	R
−	R	S
−	R	R
−	R	R
−	R	S
−	R	R
+	S	R
−	S	R
−	R	R
−	R	S
−	R	S
+	S	R
−	R	R
−	R	S
−	R	R
+	S	S
− +	S	R
−	R	R
−	R	R
− +	S	R
−	R	R

T(B_1)

Lac	V_6	V_1
+	R	R
−	R	R
−	R	S
−	R	S
−	R	R
−	R	R
−	R	R
−	R	S
−	R	R
−	R	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 streaks - all rather ~~smooth~~ mucoid - all resistant n.g.

Tests on complex resistants.

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 MHC.	R	S	R	R	R	
5,6.	S	S	S	S	S.	!

Do not use these mutants further; their origins 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	
Y86.	Y53/1M.	R	S	S	R	R	R	
Y53/3 ??		R	R	R	R	R	R	probably contam.
Y53/3 old??		R	R	R	R	R	R	

Phage-mutants.

Mix Y53 + phages in a tube + plate on EMB.

16,1 No survivors. . See Y99.

16,5 2 v. mucoid colonies; a few tiny ones.

13,5 Numerous mostly mucoid.

13,6

11,4 Very numerous colonies. (test) on 1,4. (V. considerable growth before lysis!) Probably invalid.

14,5 several mucoid colonies; occ. smooth.

11,3 several "mucoid" colonies. Test on T1,3. Mostly very mucoid + nibbled. 1 streak 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. 5/18 = T_5^R. Pick both var. pick Y100. = $T_1^R T_5^S$.

Y40/6 Test for T_3^R. smooth are susceptible; mucoids generally resistant. isolate one as Y101

Y53/1,4. Mostly nibbled. Keep one $T_1^R T_4^R$ 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.

~~T534~~		T1	T3	T4	T5	T6	T7
	Y40/6	R	R?	S	R	R	R.
"Y53/(1,6)	Y99	R	S	S	S	S	R!
Y53/3 6S!	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

T4S! T3R!

T7R! stock??

Papillation of the L- reversion.

May 20, 1947.

To 20 ml plates of T(0) + excess B1 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	micr. colonies.	→ do.	✓	no change!
2	"	Pin points	✓	
5	"	— "do.	✓	
10	Bare visible pinpoints	v. tiny	(1 colony)	
20	> " "	tiny but visible	(1 colony)	
50	> " "	v. small; fairly uniform (Neurospora cont noted)		
100	tiny colonies.	small colonies. No papillae. fair myc. #		
1 mg.	v. small colonies. papillae in gross streak?	Good sized (1-2 mm.) Some variations. no obvious papillation.		

range for further tests. (50 – 1 mg.)

Linearity Y26 x Y87

May 19, 1947.

ϕ-C- B+M+ X ϕ+B-M- Lac - V_1^R

a) Y26 grew poorly in YB. [OK $\bar{c}$ cystine supplement or $\bar{c}$ "Proteose 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 $\bar{c}$ ϕ - C.

	Lac	V
C, ϕ+	−	R
C, ϕ−	+	S

↑ +S ↑ −S ↑ −R.

↑___↑ +R.

523 B.

−R	−S	+R	+S.
9	1	1	1

May 21, 1947

A. Y26 × Y53. B. Y26 × Y87.

B. - No colonies in 9 T(0) plates
2 colonies in 4 T(B) plates!

A T(0). 75. T(B₁) 184. Compare with previous estimations of 1:1 T(0) : T(B₁).

~~B-~~ ϕ-C- × T-L-B₁-Lac- all V_1^s.

T(0) 6/51 Lac +. T(B₁) ~~14~~ 14/43 Lac +.

(9) 14	(34) 29		43
(11) 6	(42) 46		51
20	75		94

The 43 tests are divisible into 2 parts:

a) 75/184 × 43 = 17.5 ≐ B_1^+ ≐ 2 Lac - ; 15 + Lac +
25.5 ≐ B_1^- ≐ 12 Lac - ; 13 + Lac +

∴ the B_1^- may have ca. ½ Lac- ½ Lac +.
i.e. This may not be precisely true.........

$\chi_1^2 = 25(.091 + .11 + .03 + .02)$
$= 25(.09)$
.11
.03
.02
(.25)
$= 6.2$ $p = .01+$

B₁ — 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 da. 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.

	Pap.	No pap.	
Y40"Z"	8	8	(too heavily streaked)
	14	0.	
	12	0	
	2	0	
Y10 (pure recent)	5+5	0	
	11	11.	(too heavy!
58-161 C.	0	12.	(too heavy).

all these eventually gave papillae

May 24, 1947.

A. Y26 × ~~Y…~~ ~~Y…~~ ~~Y…~~ × Y87

B. Y26 × Y64.

No colonies in 4 minimal plates.

ca. 50 colonies/plate c̄ biotin.

Reversion of Y87 to M+ not checked.

Test c̄ V1; Lac.

∴ compare | Lac | V

ΦC +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!

Φ	M+C−	+	S
Φ	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

indic colonies; phage n.g.

Total, reversely Lac segregation

compare 524: (n.g.).

			compare 524	
T(0) :	29− : 31+	48% Lac−	45− : 6+	!
T(B1) :	70− : 15+	82% Lac−	29− : 14+	

Segregation of Lac, V_1, V_{15}

May 24, 1947

Y40 x Y99 [B-M-V_1^R x T-L-B_1-Lac-V_{1T}^R].

T(0) too turbid; auto-inhibitory

T(B_1): rather turbid, but colonies can be picked.

T(0).

	Lac	T1		T5	V_{15}	V_{1T}
	−	R		R	R	−
	−	R		R	R	−
	−	R		S	S	R
	−	R		R	R	−
T(B_1) →	+	R		R	R	−
	−	R		R	R	−
	−	R		R	R	−
	+	R		R	R	−
	+	R		S	S	R
	−	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		R	R	−
	+	R		R	R	−
	−	R		R	R	−
	−	R		R	R	−
	+	R		R	R	−
	−	S	*	S	S	S
	+	R		R	R	−
	+	R		R	R	−
	−	S	*	S	S	S
	−	R		R	R	−
	+	R		R	R	−
	−	S	*	S	S	S
	−	R		S	S	R
	−	R		R	R	−
	−	R		R	R	−
	+	R		R	R	−
	+	S	*	S	S	S
	−	S		S	S	S
	−	R		R	R	−
	+	R		R	R	−
	−	R		R	R	−
	+	R		R	R	−
	+	R		R	R	−
	−	S		S	S	S

	T1	T5	V_{15}	V_{1T}
−	R	R	R	−
+	R	S	S	R
+	R	R	R	R
−	R	R	R	R.

	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.

	Lac	V_6	V_1
T(0)	+	S	R
	−	R	S
	−	R	R
	+	S	S
	−	R	S
	−	R	S
	−	R	R
	−	R	R
	−	R	R
	−	R	S
	−	R	R

See 518.

	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

	Lac	V_6	V_1
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
	−	R	S
	−	R	R
	−	R	R
	−	R	S
	−	R	R
	−	R	R
	+	S	R
	−	R	R
	−	R	R
	−	R	R
	−	R	S
	−	R R	R
	−	R	S
	−	R	S
	−	R	S
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	−	R	R
	−	R	R
	−	R	S
	−	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 c̄ underlayer of T(B₁)+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%	Unduly spread: 54+ ; —
1% .	16, 7
1.5% .	5, 2
2% .	32, 27
2.5%.	29?, 10.
¾%.	54, 36

needs repeating.
¾% is lowest suitable concentration.

Acenapthene.

May 17 - 1947.

Streak NA plates c̄ Y40; 58-161, and invert over acenapthene crystals. Incubate 5 days 37°. No marked inhibition noted; no papillation. Streak out on EMB. A23.

P24. Marked size dimorphism noted.

Test colonies on Cla for papillation.

Streak out large + small colonies and Y40 standard: dimorphism breeds 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 or ± 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(B1).

T(0).

Columns: Lac, T6, T3, T1

- Lac: −, −, −, +, −, +, −, −, +, −, −, −, −, −, −, +, −, −, +, +, −, +, −, −, −, −
- T6: R (↓ throughout)
- T3: R (↓ throughout)
- T1: S, R, R, R, R, R, S, S, R, R, R, R, R, R, S, R, R, S, R, R, S, R, R, R, R

T(B1).

Lac	T6	T3	T1
	all R →		
	all R →		
	all R		
−	~~[illegible]~~		R.

(probably selective picking of $T_3^R T_1^R$ types.)

Y26 x Y87.

May 27, 1947

mm: ~~[illegible]~~ Y87 x Y64. (the [illegible] [illegible] h.g.)

[40 tests.]

Segregation of V_{1T}

May 27, 1947.

Y100 x Y40

See below for Y100 x ~~Y40L~~.
x 58-161L.

Batol - tested, erroneously, on T6, T3, T1.

Phages n.g.? - all R.

Y26 × Y64

874/377.
377
1251

(This Y64 stock is substantially L)
$m B_1 | -B_1$, ca 690:302: 2.25
Lac, V_1.

Compare 524
184:75 ~ 2.42

$\chi_1^2 = < 1$.

T(0).

-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	3
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. ✓

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 BH synth. all S.

On EMB, Lac+ S do not show a green 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). Ac 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 · Co^s × Y53. [Co^L] B) Y40 · Co^L × Y53. [Co^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 selections:

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	15 18 / 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).

8	L 9/11
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. χ^2 = < 1.

A) 118 122 46^{50} / 168 p = ...

222 / 95 317

May 3~~0~~, 1947

A. 58 161L × Y64L.

B. 58 161 S × Y64L

C. 58-161 S × Y64S.

[D.] 58-161L × Y64 S.

streaked out 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(B_1)).

B : B' do. lower frequency. of prototrophs. May due to suspensions.

C very few recombinants. (S×S)

D. same. [Y64S n.g. for recombination ???].

[illegible]

June 3, 1947.

Y100 x 58-161. [Y53-V_{1T}^R x 58-161 V_{1T}^S].

plates of V-test too dry for good test. n.g.

T(o).

-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		-R	-R	
-S		-R	-R	
+S		+R	-R	
-S		+R	-R	
+R		+R	-R	
-R		-R	-R	
-R		-R	-R	
			-R	
			-R	
			-R	
			-R	

pick off.

6S:3R.

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 c 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 intimate an intercalary location.

T(B_1).

June 13, 1947.

	-R	-S	+R	+S.
T(o).	9	7	1	4
	2	3	2	1
T(B_1).	1	8	0	2
	2	9		5

Total:

-R	-S	+R	+S
14	22	3	12

S = 36/51 = 70%

R = 30% → or

B_1	BM	Lac	V_{15}^R	V_{1T}	TL	V_{1T}^S
	−	+		↑	+	
	+	−			−	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 against phages ∴ no reversions

Segregation of V_6, V_1

Y103 × ~~Y10~~ ~~[illegible]~~ ~~Y10~~ BM Lac⁻ $V_1^R V_6^R$ × TL B_1
× Y46.

Y103 × Y10

no T_6 sensitives seen; phage probably n.g. (see tests m$C_1$$C_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
	0	0	4	6
	~~0~~ 1	0	0	4
	4	0	3	7
	2	0	5	2
	2	0	3	2
	13	1	26	32
	19	1	36	49.

OK.

Sex activity of sonates.

Prepare Y40, Y53 suspensions as usual in ~~Y53~~ saline. Treat in sonic 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) turbid ++

V_2^R.

June 4, 1947.

T2R - stock received from Luria. titre = 10^9 when made.

plate covered c̄ colonies

Cla - filtrate

Plate 58-161 on NA + Cla:

		12h.	24h.
1.	0		✓
2.	200 γ/ml	no inhibition	✓
3	400	colonies somewhat smaller; no papillation	v. sl. inhibition:
→ 4	600	marked inhibition; no papillae co colonies pin point	large and small colonies.
5	800.	very marked inhibition. 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 γ/ml probably optimal. Use mutagens??

June 6, 1947

Rec.d from A. Boivin, his strains C_1S and C_2S, recorded as Y105, Y106 respectively.

a) test on Cla-agar: papillae found; streaked to purify and test for aerogenesis.

b) tested on T(0) - grows well both on liquid + solid T(0).

c) Lac - both strongly Lac+

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 new ones?

Test Cla^R types on glucose fermentation tubes. (pick 5 colonies / plate streaked c a Cla^R papilla)

		gas →				
105 (C_1)	A	+	+	+	+	+
	B	−	−	−	−	−
106 (C_2)	A	−	−	−	−	−
	B	−	−	−	−	−
	C	+	+	+	+	+

Y106 = $C_1 \cdot Cla^R \cdot S$

Y108 = C_2 $Cla^R \cdot S$

Y109 = C_2 $Cla^R \cdot S$.

e) Sucrose: no definite fermentation by either. Both show slight papillae in region of second streak. When these are streaked,

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 ml in 10 ml H_2O. Treat sonically for 2 hours at 0°C. Sediment debris + emulsify supernatant with benzene overnight. Remove sediment + excess benzene; remove benzene in vacuo. Should leave a sterile preparation. —
add 1:10 to YB tubes for assay.

b). Inoc Y107, 108, 109 into glucose-gas tubes + 1:1 filtrate.

24h.	107	—
	108	—
	109	— no acid

c) Inoc 107, 108, 109 into YB + filtrate. grow 24h. 6h. Use this to inoculate heavily gas tubes:

	107	—
(Transformation?!)	108	—
	109	±

d. Streak out 109 (da); 109/Fb (db); 109/Fc (dc); 109/Fc/test (dd) on Cla plates to detect sensitive colonies.

ca 20% sens. [illegible] sensitive [illegible]!

e. Sucrose — all sucrose — on second transfer on EMB plates.

June 13, 1947.

		gas
Y109. in glucose broth	[illegible]	−
		−
		−
		−
Y109 in YB.		−
then glucose		−
Y109 in YB + 1:1 filtrate of Y105		[illegible]
		−
Y109 in YB + 1:10 extract of Y105		+
		+
		±
1:10 extract Y105		− no acid.

See previous test for +.

∴ It appears very likely that a transformation has been effected from Ac^- to Ac^+.

Lactose mutants of Y106.

June 11, 1947.

Irradiate a washed suspension of Y106 in saline : uv 4 mins.

Inoc 1 ml into YB; incubate 24 hours. Plate out on EMB lactose.

ca 500 x 58 = ca 30,000 colonies examined.

No clearcut, 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 cols: all disperse 10/10.

b) small cols. 23 clumped 17 disperse. discard disperse

and inoc. 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 disperse growth on second in large tubes.

4 good roughs 546-1, 2, 3, 4

Treat washed suspension of Y106 with 4.5% Na desoxycholate (DX) for 3 hours at room temp. Slight lysis observed. Wash & inoculate into 10 ml YB broth + 1 mg. desoxyribonuclease. Incubate overnight and plate for mutant detection p 21.

A23 – look over for preliminary small colonies. Overlay with: agar 1.5%, NZcase 2%; Y. Extr. 1% 11A23. The colonies of this form are extremely uniform, and less than .1% of the original sample are abnormally small, e.g. / • norm. <.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. To small YB.

Grew:
○
×
Δ.
Test on T(0)

Spread treated suspension on EMB lactose. 82 × 100 cols. = **8000** examined

others grew on minimal on second test or not E. coli by L. R.

		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
Louis Rodriguez

Later: 133 lysine, 138 leucine, 139 histidine } from 118 ∴ arginine −

141, 142, 143 : arginine from 121 ∴ cystine.

[June 20]

Chemical test for uronide:
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 +±

DX- alcohol procedure.

June 23, 1947.

36 hour culture of Y105 : 600 ml YB in 1 liter flask.
Shake at 30°.

Sediment + suspend in 20 ml 4.5% DX. Add benzene and shake at 25° from 1230P23 to .

P23. Sediment and remove debris + ~~[illegible]~~ benzene phase by filtration.

~~add 10 vols. 100% Alcohol.~~ Collect sediment in a sterile tube.

Sedimentation required ca 5 hours, Supernatant collected.
due to thick emulsion. Possibly pH too low

some DX in solution.

Upon addition of alcohol, a thick fibrous ppt. S_1 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 pptn attempt, add a few drops of NaOH to prevent pptn of NaDX $\bar{c}$ alcohol.

Test R_1; R_2 on Y109. no gas + produced.
Sterility - OK.

maltose.

(Repeat, omitting DX)

June 26, 1947.

Y109 in YB + 545 extract.

tests on glucose tubes.

sterility	—	
Y109	— ;	—
Y109 + TP	+	+

transformation OK. Streak out on Cla.

Preparation of TP: alcohol procedure.
Autolyse 500 cc Y105 in 15 ml NaCl .9% + 1 ml benzene at 30°.
Sediment + separate extract.
550-X1 aliquot centrifuged free 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~~ suspend in 100% alcohol for 5 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.) inoc. c 109 in error. ±

all were inoc c X_L and 109!

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 500 ml of Y105 by alcohol ppt'n. method, after toluene autolysis 3½ hours. Add ext 10 in YB.

		8 hours test
1.	109	+++
2.	109 + X	+++
3.	109 + X	+++
4.	109 + X + DNase	+++
5.	109 + X + DNase	++
6.	X.	not sterile? — now 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. · Recheck:

inhibitor.

N-379

5/8/46.

Blend 368-7 (43-3 mycelium in exc. leuc.) [illegible], 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

			Inoc.	P11.	P13	P22
3	extr. + F(o)		—	—	±	±
4	" "		10A	+	+++	++++
5	" "		43-3	—	±	±
6.	" "	+ leuc	—	+	++	++
7	" "	"	10A	++	+++	++++
8.	" "	"	43-3.	++	++	++

No evidence of inhibition.

Production of Neurospora. 5/14/46.

In 20 l. Pyrex carboy, 10 l. of a Fries $\bar{c}$ 2% glucose,
5 γ/l desthiobiotin. Neuro $\bar{c}$ 847

cotton pack
gux bar
fixing wire
LIP

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.

5/14/46. 349-A5 x 25a.

Isolate ascospores, 1/perithecium. 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	~~++~~ + —	—	
15	n.g.		
16	—	+	++
17	+	+	++
18	ng		
19	+	+	++
20	ng		

Isogenic N- stocks
Isolations.

N382

5/28/46 SY7 x 360-6. (S series.) 4/30 protograph

A. 1 ng
2 "
3
4 ✓
a ✓ 5
6 ng
7
8 "
9
10. "
11.

G(o)
SY7 x 1633a (70-26) 4/30 pab p
21 —
22 +
23 +
✓ 24 —
25 —
26 +
27 n.g
28 ~~ng~~ +
29 n.g +
30 n.g.

SY7 x 378-3 5/10 inositol
a 31 —
a 32 — Store
A ✓ 33 +
34 —
35 +
36 +
37 +
38 ng
39 ng
40. ng

3740 la x SY7
↓
378-3 x SY7
↓
382-32

N-582
a

nic a × pnt A
4540 5531.

dated 4/28. ~~Isolate~~:

5/14: ✓
infertile.

	0	nic	pnt	nic-pnt
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				

none germinated. Repeat.

4/27 – 5/14. 366-3 × 25a. (Lc-4637 + [illegible]-15300).
21 isolates 2/peri. not oriented here. Only 6 germs!

#41 is giant spore

		Color	F(o)
	21	+	++
~~n.g.~~	22	– (pf)	"
	23	+	"
	24	+	"
	25	+	"
	26	± (dil)	"

none so far!

multiple mutants

4540a × 370-12 (4540-5531 At. dil)

5/28/46		G (nic)	G (pnt)	G (pnt-nic)	Color	Sex
	1	n.g.				
~~[illegible]~~	2 ✓	—	—	+	±	~~A~~
	3 ✓	—	—	+	+	a
?	4 ✓	—	—	—	—	
A ✓	5 ✓	—	—	+	—	A
	6 ✓	—	—	+	+	a
	7 n.g.					
	8 "					
	9 "					
	10 "					
	11 "					
	12 "					
	13 "					
	14 "					
	21					
	22					
	23					
B	24 a.					
	25					
	26 W					
	27					
	28					
	29.					

most spores are not colored.
Gp. A. select for deep pigment.
Gp. B. " " light " .

5531 A × 33757a.

5/28/46.		pnt	lc — pnt-lc.	Sex.
	31			
✓	32			
	33			
Random	34 ✓			
	35			
	36			
	37			
	41 ✓	+	—	
Mode.	42 ✓	+	—	
	43 ✓	+	—	
	44 ✓	+	+	
??	45 ✓	+	+	
	46 ✓	+	+	
	47 ✓	+	+	
	48 ✓			
	51 n.g.			
	52 n.g.			
	53	+	—	
Mode	54 n.g.			
	55	+	+	
	56	+	+	
	57	—	+	

1633a x SY7A.
58-6 x SY7.

N-383a

4/30-5/14.

5/14. -
all spores
colorless.

~~366-3 x 33757a.~~

G(0). 58-6 x SY7. Need more isolates.

5/14:
several peri.
3spored p.
several p.

12 peri.
4 other spores.

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.

358·6 × 847A

5/28/46 G(o).

Random - from spore print.	1	+
	2	+
	3	+
	4	+
	5	+
	6	+
29 coil.	7	+
	8	+
	9	+
18 y.g. 1st mating	10	+
	11	+
	13	+

~~[illegible]~~.

all morph. OK. compare neurospora gen-eration.

Expt. 371. Nuclear origin of protoperithecia

I isolate perithecia as free as possible of extraneous spores. 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	+		+
x	6	+		–
5531-37401	7	+		–
	8	+		–
	9	+		–
	10	–		–
	11	+		–
	12	+	–	–
	13	[illegible]	+	–
	14	+		–
	15	[illegible]	–	–
	16	+	–	–
	17	+	[illegible]	+
	18	+		–
	19	+		–
	20	+		–
	21	+		–
	22	[illegible]	ng	3+
	23	[illegible]	ng	18 –
371-12	31	+		
do.	32	+		+
	33	+	–	
	34	+		+
	35	+		–
	36	±		+
	37	±		–
	38	+		–
	39	+		–
	40	+		–
	41	+		–
	42	+		–

		Color		F(o)	F(prot)
371-15	51	+	+	–	+
	52	+	+	–	+
p: 5531-	53	±	+	–	+
37401	54	+	+	–	+
x	55	±	+	–	+
5531-15300	56	+	+	–	+
+	57	+	–+	–	+
37401	58	±	+	–	+
heteroc.	59	+	+	–	+
	60	+	+	–	+
	61	+	+	–	+
	62	+	+	±	→ slow ++. ??
371-16	71	+		–	+
	72	+	–	–	+
5531-37401	73	+		–	+
x	74	+		–	+
5531-15300	75	+	–	–	+
and	76	+		–	+
37401	77	+		–	+
mix.	78	+		–	+
conidia	79	+		–	+
at fert. 12.	80	+		–	+
	81	+		–	+
	82	–		–	+
	83	+		–	+

Why should prot. conidia be more effective?

Nuclear origins, etc.

6/1/46

Inoc. F(0) plates c̄ 4545a + 37401a. for heterocaryon formation

P1.

P3. No growth. Need starter?

P4. Repeat c̄ fresh cultures.

P6 – no heterocaryotic growth!!! see 389. Compare

L_1 and L_2 ; $L_1^- + L_2^+$. leucine inhibition

N 387

6/1/46. 25° Proc 4P.

Use 43-3 as L_2^- 368-22 as L_2^+. 10A as L_1^+ L_1^- = Lc-4637A.

5Pl.

Proc N plates c the following in pairs, c control plate in Aq. all OK.

1. L_2^-✓ + L_1^+✓

prev. rule.

2. L_2^-✓ + L_1^-✓

3. L_2^+✓ + L_1^+✓

4. L_2^+✓ + L_1^-✓

5 L_2^+✓ + L_2^-✓

N 388.

3PM 6/2/46

Isolate 387 hyphal tips to mid. medium: ∞ is solid; o is liq.

Incubate at 30°.

	1.	1 to ∞ Color	P4 G(o) - color.
L_1^+	11		+
L_1^-	12		+
L_2^-	13		+
	14		+
	15		+
	16		+
		1 to o	
	11	++	—
	12	"	—
	13	"	—
	14	"	—
	15	+	—
	16	++	—
		2 to ∞	
-	21		—
L_2^-	22		—
L_1^-	23		—
	24		—
	25		—
	26		—
		3 to ∞	
	31		—
	2		—
L_1^+	3		
L_2^+	4		—
	5		—
	6		—
		3 to o	
No	41	+	=
pigm!	2	+	=
	3	+	=
	4	+	=
		4 to ∞	
-	51	—	+
L_1	2	—	+
L_2^+	3	—	+
	4	—	+
	5	—	+
	6	—	
		4 to o	
	61	++	—
	62	"	—
	63	"	—
	64	"	±
	65	"	±
	66	"	—

	5 to ∞	Color.	G(o) - color.
	71	±	+
	72	—	+
	73	±	+
	74	±	+
	75	±	+
L_2^+	76	±	+
	5 to o		
L_1	81	++	—
	82	—	
	83	++	—
	84	"	+
	85	"	++
	86.	"	++
	77		+

The color hue is quite deep. Unless a further mutation of 15300 is considered, this stock is highly suspicious. Again should be repeated.

See:

No inhibitions demonstrated.

6/4/41. Pour F(0) plate c̄ 94-4 + 37401a. Use edges of plate for controls.

P6 - no growth!

Repeat P10.

- initial hyphae, but no extended growth!

In vitro activity: synthesis of pantothenic acid by Neurospora. N 391

6/5/46.

50 ml Fries +   Neur 5471 1030A5.

1. –

2. 1mg β-alanine + 1mg pantolactone.

Harvest: 11P11. (6½ days).

A Medium

B. Mycelium in 10cc H_2O. Then steam boil, and remove mycelium for digestion.

Use medium 50% in assay; extract 0.10 ml/50 Fries.

Neur 5531. 12N12.

1. F(0) 50ml.
2. 1A.
3 1B
4 2A
5 2B.

no demonstrable response in 2 days.

33757 - Selection vs. dominance. N-392

9 JUN 194?

In previous experiments, color markers were used; here another biochemical mutant type is employed: 33757-4540 + other single mutants.

All F(0) plates + 1mg dl leuc./10 ml: P10:

361-6 is 33757-4540 A.

" P10.   Isolate 4P11.

α 361-6 + 5531 A.

β 361-6 + 16117 A.

γ. 361-6 + 1633-15300 A.

To F(lc) small hyphae.

	1230 A13	1130 A13
γ 1	–	–
2	–	–
3	–	–
4		–
5	–	–
6	–	–
11	–	–
x 12	–	–
β 13	–	–
14	–	–
15	+	++
16	–	–
21	–	–
α 22	–	–
23	–	–
24	–	–
25	–	–
26.	–	–

To F(0).

	1230 A13	
51	–	
52	–	
53	–	✓
54	–	
55	–	
56	–	
61	–	–
62	–	–
63	–	±
64	–	–
65	–	–
66	–	±
71	–	–
72	–	–
73	–	–
74	–	–
75	–	–
76	–	–

To N00 slants.

α	81	+++
	82	+++
	83	+++
β	91	+
	92	+
	93	+
γ	41	+++
	42	+++
	43	+++

15, 62, 66 to N00 large slants.

A24. test on: F(0) F(lc) F(lc nic) F(nic)

15
62
66

Selection vs. Dominance

N-393.

Test 392 on le, nic, etc.

There may have been deficient leucine in the medium.

		le		nic	le-nic
	81	+	(±?)	+	+
	82	+	(±?)	+	+
check.	83.	−		+	+
	91	+		+	+
	92	+		+	+
	93	+		+	+
	41.			+	+
	42			+	+

Interspecific heterocaryons. N-394.

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 c̄ mycelium + inoculate F(o) plate:
11. a few hyphae grew out and covered plate. Conidia spottily white + colored in various areas.

Syntrophism? or unstable heterocaryon?

Heterocaryon transformation
nic x pant.

N-395

14 JUN 1946

1. Inoc F(0) c̄ 5531A + 4540A. 1130P14.

3P15. Isolate hyphae to F(0) ~~2m~~ 1 ml tubes.

1
2
3
4
5.

1A16. Inoc pot-cornmeal c̄ 383-3

Chlamydomonas newportii.

A rain pond on 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 inoc. into Moore's/5.

P22 - some green growth noted - a few filaments & some sediment

A24 - micr exam. showed numerous flagellates. Plan to streak out when culture is heavier.

P1 - streak out on Moore's/5 agar. + inoc. liq. c culture

Endomyces - Killing & Mutants

36. hour culture in F(0). Shaken at 30°.

Irradiate in quartz tube 2 mins., etc. Inoc. coli CM plates

6P26

7P27: small colonies c̄ hyphal radiations.

1. Control. dil. 1:25,000 (1); 1: 1,250,000 — (29) — 36,000,000.
   1:10 dens. = σ = 89 ; d = 0.51 d_{orig} = 505 G_{or}. 31
   limit of d = 71,000
2. Irrad. 2 min. 1 ml
   1:50
   1:2500   (11)   275,000   S = .0076
   1:125000
   1:625000   p Survival = 2.1
3. ✓ 1 ml to coli CM. 6P26. Dilute to $(10^{-6}$ approx.) 100/ml and spread over coli CM plates. d = 1340 ≐ 95×10^6. 2P28.
   etc.

Complete
1. Spread 1cc over
2. surface.
3. Embed in ca 5cc
4. surface agar.
4. [illegible] cover
5. as coli procedure.

Too heavy.

Fairly uniform. Liquid on surface e.g. spreading growth. and a poor plate.

F(0).
6. Spread 1cc over
7. surface
8. Embed in surface agar
9.
10. Embed + cover lightly
11. heavily.

11   4 colonies. P1.   Large c̄ complete 1P2.

2A30.

11   not up yet.   Nothing came up

to 38°.   7 colonies!

pS = − log survival   This should be ∝ dose.
= log (1/1 − killing)

A 2

Schizosaccharomyces octosporus (SO). 6/1

1. Vitamin Requirements.

1. T(o) + Biotin, Thiamin, Riboflavin, niacin, pnt, inositol.

1.

2. – biotin

3. – thiamin

4. – riboflavin

5. – nic

6. – pnt

7 – inos.

8 – pab

9 – folic

10. – B_6

M.G.

		6/6
6/5.	1 coli ?	+++
F)	2 HC+V	±
	3 HC	±
	4. V	±.

unknown factors?

Anyhow, octosporus is practically not satisfactory for mutant production as it diploidizes very readily. Use S. pombe which requires biotin, nic, pnt + inositol. Compare S. versatilis which does not req. pnt

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, inos.

		pq.
1. Fries + vits.	+++	++++
2. " + 4 vits.	+	+++
3 - pnt	-	++
4 - nic	-	—
5 - inos.	-	—
6. T - + vits.	+	+++

1230A7. Inoc coli ∞. 37° sh. - growth only fair worse than F

Use 10 vits. Omit 1 of 1.

1A9 inoc.

1 - B_1 ~~(Biotin in Fries.)~~
2 - B_2
3
4
5
6
7
8
9
10

etc.

50 ml 11. F(vits) 37° shaken
12. " 37° s shaking.

See A5.

Vitamin Requirements
S. pombe.

A-5

9 JUN 1946

9 JUN 1946 Compare A-1. 37°.

Fries + vitamin supplement: 10 B vits. — ind. F conts. desthio biot.

	11P9	10A10
1 - B_1	+	++
2 - B_2	+	++
3 - pant	+	++
4 - nic	—	—
5 - ~~pab~~ folic	+	++
6 - B_6	—	+±
7 - ~~folic~~ pant	-	+
8 - chol	+	++
9 - inos.	—	—
10. - Biotin	+	++

(pnt) nic inos ~~[struck]~~ — pnt nic inos ~~[struck]~~ OK.

Compare shaking & unshaken cultures. 37°.

11 F-vits	sh.	+
12. "	unsh.	—
13 colise	sh.	+
14. "	unsh.	—

Growth is poorer than in tubes.
Relatively anaerobic conditions

~~Vitamin Req.~~ A 10.

~~Fries:~~
~~21 pnt, inos, nic, folic (biotin)~~
~~22 — pnt~~
~~23 — inos~~
~~24 — folic~~

For colony type, plate A4-2 into F(P) = Fries + pombe vits
σ 1:10 = 78^2 d = 1051 (45,000,000) dil. 10^6.

P13 — colonies finally noted. Resemble bacterial colonies.
~~Moratum?? too old.~~

PAB - [illegible] E 2

8/20/46.

[illegible]

I PAB

γ/cm T(?) — % Transmission 36 hrs

	other	0	.001	.003	.01	.03	.1	.3	1
Y1	T L	76.0 / 100	[illegible]	[illegible]	[illegible]	[illegible]	100	[illegible]	[illegible]
Y44	H	100	[illegible]	99.2	[illegible]	[illegible]	90.1		
L 815-[illegible]	0	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]		
L [illegible]-762	0	[illegible]	96.2	[illegible]	52	93	[illegible]		
L 1161-46[illegible]	0		94	95	[illegible]	[illegible]	[illegible]		

II Glut[illegible] acid Y44 (#[illegible])

	0	1.0	[illegible]	[illegible]	[illegible]	[illegible]
blank Y44						

III [illegible] #22

IV [illegible] #11 Y[illegible]0

blank 95	0	.001	.003	.01	.03	.1
	98	91.3	92.4	[illegible]	93	90
	100	87.5	88	93	91	93

(82)

[illegible] #9

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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 Harris
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 Axl

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 (IV? V? i..)	monophasic m^ 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	"	X8 --SH

ACETOBACTER M.A. 11 series:

A 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
1 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: B6
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.
-15300A,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
-39401 "-nic
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
"-37401A,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
~~XXXXX 25 x x~~
Abbot 4 + A
A" 12 + A
Chilton + a
SY. + A,a
E 5297 a
977 R L a

N. sit: Sands PC A
" .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 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 ph 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
84886 hist
85880 "
86842 "
89172 "
96730 "
97054 "

Absidia glauca (Giles)

I
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	
7112	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? (i-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	"-?
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
" xr 15
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122
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83
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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	Y10	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; probably 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	[illegible] 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^r (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.] X
102	521	Y53	sel	(T1+T4)	resist T1,T4...

Aneutrophic Salmonellas

	S		type	requirements	
L	1	✓	para A	methionine tryptophane.	
	4	✓	cholera suis	~~trypt.~~ 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	~~tryptophane,~~. BIOTIN;	
L	52	–	para B		
L	55	✓	cholerae suis	~~Biotin~~; (~~stim cystine~~) TRYPTOPHANE; cystine (adapts)	
L	56	✓	cholerae suis	tyrosine, OR BIOTIN!	
	57	–	typhi suis		
	58	–	abortus ovis		
	59		sendai	——— slow prototroph.	
	60		sendai	——— do.	
	61 – 69.		typhi-murium	SH METHIONINE.	
	51	✓	para B	PROLINE	
L	70		typhi murium	SH.	u.-v. mutant of S20.

also S20 = typhi-murium

Salmonella stocks

	S	type	serotype		nutrition	
	1	para A	I, II, XII	a	−	+ * methionine, tryptophane
P+S	2	para B	I, IV, IX	b, 1, 2	+	✓
	3	choleraesuis	VI VII	c.. 1, 5	±	
	4	"			−	methionine.
	5	enteritidis	I, IX, XII	g, m	++	✓
	6	"			++	✓
	7	oranienburg	VI VII	m, t	++	✓
	8	montevideo	VI VII	g, m, s	++	✓
	9	newport	VI, VIII	e, h..	++	✓
	10	"			++	✓
	11	typhimurium	I, IV, V	i, .. 1, 2, 3	++	✓
B16	12	pullorum			−	E leucine, cystine ~~isoleucine, histidine~~ (meth.) LIX n.g.
143	13	"			−	EN leucine, (arg, meth.) cystine
138	14	"	IX, XII		−	EN leucine, arginine, methionine, (~~histidine~~) cyst.
W17	15	"			−	E leucine, ~~isoleucine, meth., hist.~~ ✓ ok on leuc.
WhR	16	"			−	EN leucine, cystine
Rettger.	17	"			−	EN leucine (~~arg, meth.~~) cystine (slow on L C)
P.R.Ed.	18	abortus bovis	IV, XXVII	b.. enx	++	
	19	"	"	"	++	
	20	typhimurium	IV, V	, i ... 1, 2, 3	++	monophasic - phase 1
	21	"	"		++	monophasic - phase 2.

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* + = 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~~	++	
~~20~~	415	kentucky	++		S84 open.
~~21~~	421	typhi-murium		++	
~~22~~	3490	typhi murium	++		
~~23~~	3542	typhi murium	++		
~~24~~	422	abortus equi	++		
~~25~~	426	newport	++		
~~26~~	3491	newport		++	
~~27~~	429	london	++		
~~28~~	547	urbana	++		
~~29~~	1681	budapest	++ R.		
~~30~~	1916	inverness	++		
~~31~~	1918	adelaide	++		
~~32~~	3486	montevideo	∓S	++	
~~33~~	3539	panama	++		
~~34~~	3573	paratyphi	∓S	++	
35	3576	paratyphi		++	
36	3045	gallinarum →		− +late	Thiamine
37	1684	dublin →		−	Thiamine. motile ✓
38	3481	newport	∓S	++	
	Yale:				
39		typhimur	++		
40	28	"	++		
41	27	"	++		
42	32	~~"~~ para A.	−		TRYPTOPHANE type corrected by Edward
43	25	enteritidis	++		
44	23	"	++	+late	
45	24	"	−		~~ARGININE~~. ORNITHINE
46		"	++		
47	34	para A	++		
48	33	"	++ R		
49	18	"	++ R.		
50	17	"	−		
51	19	para B	~~[illegible]~~ ± ✓		
52	20	"	−		
53	21	"	++		
54	22	"	++		
55	31	cholerae suis		−	
56	30			−	Tyrosine;

[stim. but not ess.] ∓ cyst or meth (repl. SH)

Virus Strains

T-1. From Demerec.

a. See 230 6/10/46. Found contaminated 7/14.
b. Recover again from Demerec's suspension. Incubate c̄ 879-183 4h., centrifuge and filter. Titer on Y9 = 16×10^6 Small + large plaques.

c. Recover from lyophilization - excellent viability.

Substrains

679-680—A TL from Ryan, for better mutants (triples) 4/15/46.
Y10A Reisolate Y10 and test. (Separate from threonine reversions).

Y-Y0 = S. cerevisiae — haploid clone from Lindegren —
Requires pab, pnt, biotin. Use B(a).