

15 K

A. 5ml Y40; 1ml 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	191.

no ev. of selection here!  
o.k.

B. 1ml Y40; 5ml Y53.

	2	0	1	1
L	<del>7</del> 5	1	<del>3</del> 0	0
S	7	1	0	0
	7	2	0	0
	<del>21</del>	<del>5</del>	<del>2*</del>	<del>0</del>
	22	5	4	1

same plate! ~~also~~ check for viruses  
no S/plate!

do not use this expt. too small anyhow

15 min

-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

TL    Lac-R    Lac+R    Lac-S    Lac+S  
          2    0    3    2

BTL    2    0    0    1

480 x 481.

~~480.~~  
461

Repeat 451 for lac seg. test + phage-segregation.

Plate in  $T(0)$ ,  $T(B)$ ,  $T(B_1)$ .

1 plate - 480 in  $B_2$  to check on reversion of 14.

$B_1^- \gg \gg B_1^+$

++.	31/31	Dly+
	27/27	
	10/10	
	<u>32/32</u>	
	100/100.	

from B plates:	42/42
	20/20
	8/8
	<u>28/28</u>
	98/98

Totals of Dly+ seg.

461.	198
451	29
441	<u>70</u>
	<u>29.7</u>

- ∴
1. Linkage is very tight, perhaps requires a double X against interference
  2. Cytoplasmic inheritance
  3. Trickup in an reversion.

---

480/B → no colonies

Y80 x Y81.

462

March 20, 1947

Grow Y80 + Y81 in mixed culture, and plate out on EMB medium. Select Lac+ colonies and test for hly reaction to determine possibility of transformation.

plate too crowded. to be repeated.

Fermentation mutants.

March 22, 1947

Proc Y40T (mustard see Y40) and Y53T in YB P 22

spread diluted cultures of Y40 on EMB-lactose 1% P23  
of Y53 on EMB glycerol 2%

and incubate at 35°.

Y40-lactose. ca. 13,000 colonies.

no mucoid.

1. lac-

1. translucent pigmentation.

recover for test

Read P24. ↑

~~Y40~~  
~~mustard~~

Y87.

Lac- ✓  
V<sub>1</sub>R ✓  
V<sub>1</sub>S ✓  
V<sub>1</sub> ✓

as mutants.

Nutrition:

Y53-glycerol.

ca 20,000 colonies.

mucoid - 1 (??)

gly - 1? (mucoid?)

gly - probably contaminant.

Test by streaking  
on lac, V.

Selection, etc.

March 21, 1947

A. 440 x 453

B. 58-161 x 464.

Phase 4:9. lac- unreliable as they were scored on second transfer to lac-V plate for checking on phase

	lac-	lac+
T(0) rand.	20	5
	+30	+15
small	23	4
large	18	6
B <sub>1</sub> random	27	17
small	31	8
large	39	14

These data are too uncertain to be used.

168 54 corr. 198:69

B.	-R	+R	-S	+S.
SL	0	0	4	5
IS	2	0	5	1

T(0) rand	33	12
small	15	13
large	22	8

difference A + B p = .1

B <sub>1</sub> small	16	8
large	15	7

101 48

$$\frac{269 \quad 102}{371} = 72.5\%$$

Dung 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 resistant.

Phy / Sth 100 B.G. 100 turbid!  
 Sm 50 clear - some resistant, ca. 10<sup>3</sup>

Phy / Pro seems M.G. resistant also, to a certain ext.

Staph / M.G. 5 M.G. 5. irregular turbidity. some "resistant"  
 10 clear plate. ?  
 20 "  
 50 "  
 100 clear  
 B.G. 100 clear

Pen. 1 turbid.  
 5 spots clear = resist.  
 10 turbid  
 50 turbid  
 100 turbid.

Staph / Sm. 5 Sth 10 clear = resist. - to selectivity  
 20 clear =

Ser / Sth 100 Sm 5. clear - small resists.

Ser / Mg 50 B.G. 100 turbid.  
 Pen 100 turbid.

S20 / Bg 100 Mg 0.5 ureg. turb. S21 / Bg.  
 1 clear - fine in center. clear zone in center (not mixed?)  
 5 clear!  
 10 clear!

1 Sth 10 Pen 100 turbid.  
 Sm 10, 5 turbid 1 Sth - Sm 10, turbid.

Chlorocetiv acid acetamide.

March 22, 1947.

See Purfold ~~1911~~ 1911.

streak Y53 on NA + various conc. Monochloroacetate murex.  $\bar{c}$  NaOH conc. exp. as free acid.

r/pu ml

- 50 continuous growth
- 250 dense growth of streak  $\bar{c}$  ca 10 papilla of large size per streak.
- 500 Background growth less. do.
- 1000 = 1 " " very slight.

Pick papilla of 1 to new 1 CLA plate. P24.  
Isolated colony to  $\infty$  slant: Y88





broc into fermentation tubes P26. (Bailey in BP.)

12h.

	glucose 1%	glycerol 1%
Y53	A++ ++G	A± ±G
Y88	A++ -G	A± ±

see 468.

36h.

Y53	A++ /+ 	A+ +G 
Y88.	A++ 	A+ ±G 



Segregation of Mucoid Resistance.

March 26, 1947

677(0).

A 486 x 58-161

prototypes rare (2/7 plates!)

B 486 x 440.

A 8 mucoid 7 lac-  
all resistant. 1 lac+.

1 Smooth. lac- VRS  
check. ✓

~~[Mucoid different from  
resistance?]~~

B. 28 mucoid 8 lac+  
all resistant. 20 lac-

1 Smooth lac- VR

---

9 lac+ : 27 lac-

Smooth 2 lac- : 0 lac+.

Prepare Smith fermentation tubes + Nutri. Broth + various supplements as usual.

Formate includes 14/20 phosphate buffer.

12h.	control	formate 1/2%	F+gluc 1%	F+man 1%	gluc 1%	man. 1%	malt 1%	sucr 1%	glycerol 1%
Y53	-	+	+	+	++	+	+	±	±
Y88	-	+	±	±	++	-	±	-	±

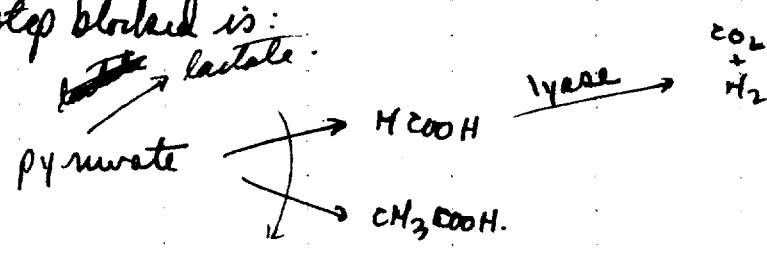
*H<sub>2</sub>:CO<sub>2</sub> ca 2:1*

	pyruvate 2%	lactate 1%	malate 1%	acetate 1%
Y53	++	+	-	-
Y88	±	-	-	-

second reading 72h.

Formic hydrogymlyase is apparently intact.

∴ stop blocked is: lactate.



Try: utilization of pyruvate in synthetic medium.

Carbon source utilization (T(0) - asparagine + TLB<sub>1</sub>).

Suppl:	1	2 pyr 2%	3 lact 2%	4 gluc 2%	5 mannitol 1%	6 acetate 1%	7 glycerol 1%	8 malate 1%	9 formate 1%
Y53:	-	+	+	±	++	?	+	++	+++
Y88:	-	+	+	±	++	±	+	++	+++

malate } were most eff. carbon sources  
mannitol } → major difference.

~~wild type: acid + gas form~~

	W.T.	Mutant.	
+	++	++	formate
+	++	+	glucose
+	++	-	pyruvate
±	±	±	glycerol
+	++	+	mannitol
+	++	±	maltose
±	±	±	sucrose
-	-	-	acetate etc.

} major differentiation

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 prod. ✓
	pyruvate	±	+	< 1/5 $H_2$ ✓
<del>Y40</del> Y88	pyruvate	-	-	← 1/5 $H_2$ ✓
	glucose	+	-	
	mannitol	+	+	< 1/5 $H_2$ ✓

March 28, 1947

not a good exp.

Y77xY78. (= Y6Yx58-161)

O.	-R	-S	+R	+S.
	8	8	0	3
	3	1	0	0
	4	8	2	0
	<hr/>	<hr/>	<hr/>	<hr/>
	15	17	2	3
B <sub>1</sub> .	6	12	0	3
	<hr/>	<hr/>	<hr/>	<hr/>
	21.	29	2	6

This distribution is entirely different from standard Y46x453.

A:S = 23:35 = 40% (1-60%)  
 - : + = 50:8 = 86%.

Test for M.G.<sup>R</sup>; S<sup>R</sup>.

Probably a definite alteration of frequencies. Look for a lethal recombinant or for an additional unit. req.

O:	-R	MG S	R	S <sup>R</sup> S	h.
	+S	lost; mostly R.	16/16.	4/16.	
	+R	S; R		S; S.	
	+S.	R; R; R		S; S; S.	

B <sub>1</sub>	-R		
	-S		
	+R		
	+S.	S; R; S	at.
		S; S; R.	

not certain as at best only a dozen colonies appeared at site of stroke.

- have all MG<sup>R</sup>
- S are > MG<sup>R</sup>
- +R variable.
- +S

Formic Hydrogenlyase.

~~469~~  
470

March 27, 1947.

Proc Y53 heavily into Hefe. Broth + 2% glucose + 1/2% formate.  
Grow 12 hours. Wash cells once + suspend 100ml  $\approx$  in 5ml of  
buffered (6.8 M/10 phosphate) 1/2% formate, septically  $\bar{c}$  Smith tube.  
Gas production was measured within 1/2 hour. NaOH abs. ca 1/3 of gas.

Repeat  $\bar{c}$  K-12.

Proc. 1) glucose-formate-yeast & 2) 2% pyruvate broth heavily  $\bar{c}$   
K-12. 5P28.

Washed cells of 1) gave <sup>++</sup> gas on formate  
2) gave no gas on pyruvate.

# Segregation of $Cl_a^R$

March 28, 1947.

Y88 x Y40. on T(0).

Test 20 isolates.

	Lac V	Cl <sub>a</sub>	Dao-glucose	pyrEMB
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 ②	R	-	-
12	+R -	R	+	-
13	+R ③	R	-	-
14	-S ③	R ①	-	-
15	-R	R	-	-
✓ 16	-R	R	-	-
17	-R	S	+	+
18	+R	R	-	-
19	-R	R	-	-
20	-R	R	-	-

Many of the larger colonies in group 1 appear to be yellow suggesting possibility of contamination.

Repeat cross.

Y89  
 ② #14 seems OK however. Strains out on NA and Cl<sub>a</sub> for use in metabolic studies.  
 (OK.)

Isolate further, avoid "yellow" colonies.

all 3 sets show same no. of colonies on NA and on Cl<sub>a</sub>.

~~Red colonies from NA. to 10 starts as Y89-1, 2, 3.~~

Reverse Crosses

March 28, 1947.

A. 440 x 453 on minimal. B. 464 x 58-161

A. Plate large

	-R	+R	-S	+S
1.	6	1	2	0
2.	6	2	1	1
3	3	1	4	0
4	7	1	3	0
5	6	1	8	0
6	3	3	3	0
rank.	6	7	2	0
	37	16	23	1
	8	1	12	8

53:24 = 69% R/S.  
9:20 = 31%

B. small

	-R	+R	-S	+S.
2	3	4	5	0
3	0	5	0	0
3	0	1	1	0
6	3	1	0	0
3	3	1	3	0
3	1	4	4	0
	20	15	13	2

R:S = 35:15 = 70%

r.

Compare A <sub>2</sub>	Σ A <sub>s</sub> totals.			
.11	37	16	23	77
.18	20	15	13	50
	57	31	36	127
		39	3	

$$\chi^2_2 = .11$$

.11
.18
.153
.75
.04
.06
1.67

	R/S	+/-
A	.69	.22
B	.70	.35
r	.31	.31

$\chi^2 = 3.14$  p = .08  
OK. compare  
cumul. data -  
< .05 for fit

perfect fit

April 3, 1947.

Agent.	conc./20	conc. <sup>mg</sup> /ml	16 hours.	
			Y53	Y88
<u>Fluoroacetate</u>		.05	++	++
		2.5	++	++

intermediate  
conc. do.

Chloroacet. 1.0 ± ++

chl<sup>o</sup> leucine. 10.0 ++ ++

iodoacetate .1 mg 5v ++ ++

50v - -

250v - -

500v - -

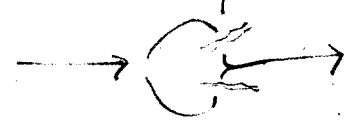
1mg - -

2.5mg - -

showed no resistants

resistant colonies appeared profusely  
in 36 hours (2a<sup>R</sup>). 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: Ia-resistance.

474.

April 7, 1947.

1. Streak Y53, Y88, Y90 on Ia plates (~~50~~, 100, 250 v/ml)
2. ~~Streak out Y90 on 50 v/ml. Ia.~~

No growth on any plates by any of the cultures.

Acetate utilization  
aerogenesis.

April 8, 1947.

Proc. (lightly) T(m) + suppl. c k 12.

	24h	36h.	48h.	72h.	84h.
1. Acetate 1%	—	—	—	+	+
2. Acetate + <del>glycine</del> + malate .01%	—	—	—	+	+
3. Malate .01%	—	±	+	✓	+
4. Glycine 1%	—	—	—	✓	—
5. Glycine 1% + malate .01%	—	—	—	✓	—
6. Malate 1%	±	++	✓	✓	++
7. Glycine 1% + malate 1%	—	—	—	—	—
glyc inhib					
<del>8. T(m) + glycine 2% (gestate)</del>					
<del>9. T(m) + glycine 2% + y. ca. .5% (gestate)</del>					
8. Glycine 1/2% + acetate 1/2%	—	—	—	✓	—
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. Proc P 11.

	T(m) +	A13	P13
Acetate			
1	1%	—	—
2	.5%	±	+
3	.1%	++	±
4	.01%	±	±
More opt. conc.			
Glycine			
5	1%	—	—
6	.5%	—	—
7	.1%	—	—
8	.01%	—	—
0	0	—	—

April 10, 1947.

r/10	αKG	glut	etc. (see 11)	12h	24h.	36h.
1.	0	—			++	++ ++
2.	0	5r			+ ±	++ +
3.	0	200r			++	++ ++
4.	5mg.	—			++	++ ++
5.	5mg.	200r			++	++ ✓
7.	2.5 mg				++	++ ✓
8.	2.5 mg		1mg threonine	±	++	++
9.	2.5 mg		1mg alanine + 100r Bc	±	++	++ ✓
10.	5mg	5r			+++	++

adaptation?

Proc. 679-183 into T(0) + threonine + indicated supplements to determine if block of this glutamic acid mutant.

indicates strongly the utilization of α-keto-glutaric acid by this mutant.

Test 10 single-colony isolates of 679-662.

Wks.	T +0	T+glut	T+αKG	glut.	24h
1	—	+++	+++	—	✓
2	++	+++	+++	—	✓
3	—	+++	+++	—	✓
4	++	+++	+++	—	✓
5	+	+++	+++	—	✓ ++
6	++	+++	+++	—	✓
7	+++	+++	+++	—	✓
8	—	+++	+++	—	✓ +
9	—	+++	+++	—	✓
10	±	+++	+++	—	✓

no. doubt of utilization of α-keto-glutaric

T- OK.

transfer land 2 as T-G- a d T-GK imp.

Y89; K-12.

477.

April 10, 1947.

1.) on acetate 1%  
K-12      12h      24h      48h      72h.  
Y89.      =      ±      ++      ++  
                 -      -      ±      ±

2.) on glucose  
K-12      A B  
Y89      A ng.

# Formic H-lyase Activator

478.

July 11, 1947.

per liter.

KH <sub>2</sub> Cl	5
Na <sub>2</sub> SO <sub>4</sub>	2
K <sub>2</sub> HPO <sub>4</sub>	3
KH <sub>2</sub> PO <sub>4</sub>	1
Malic acid	5g.
NaOH	3g.
Trace elements	
MgSO <sub>4</sub>	.2g.

= Formic hydrogenlyase basal. = FH(0).

Use E Durham tubes.

— gas. //

	12h.	36h.	72h.	96h.
K-12	—	—	—	—
Y89	—	—	—	—
1210	—	—	—	—
1211	—	—	—	—
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	+	+	+	+
6	—	—	—	—
7	+	±	+	+
8	+	+	+	—
9	—	±	+	+
10	+	+	+	+
11	—	—	—	—

1. FH(0).
2. FH - glucose 2%
3. FH - formate 1/2%
4. FH - glucose 2% + formate 1/2%
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 1/2% + y.ex.
11. T(0)

to glucose formate

Formate is inhibitory, reversed somewhat by something in HC or in y.ex. perhaps by way of formation of formic dehydrogenase. = glucose .5%

# Hydrogenase coenzyme

April 13, 1947.

	H-12		V89		K-12		Y89		K-12		Y89	
	12h.		12h.		24h.		24h.		36h.		36h.	
	growth	gas	+	+	+	+	+	+	+	+	+	+
1 FH 10) + glucose.	-	-	-	+	-	+	-	+	++	-	+	-
2 + formate	-	-	-	-	-	±	-	±	-	±	-	-
3 + glucose + y. ex.	++	+	++	+	++	++	+	+	✓	✓	✓	✓
4 + formate + y. ex.	+	±	+	±	++	++	++	++	✓	✓	✓	✓
5 + glucose + N2 case	++	±	++	-	++	++	+	+	✓	✓	✓	✓
6 + formate + N2 case	+	±	+	±	++	++	++	++	✓	✓	✓	✓
7 + glucose + HC	+	-	+	-	++	++	+	+	±	±	++	-
8 + formate + HC	-	-	-	-	-	-	-	-	-	±	-	±
9 + glucose + EAA	±	-	±	-	++	++	-	++	++	±	±	-
10 + formate + EAA	±	-	±	-	+	+	-	+	+	±	±	±
11 + glucose + NAA	±	✓	±	✓	++	++	+	++	++	+	+	-
12 + formate + NAA	-	✓	-	✓	-	-	-	-	-	±	-	±
13 + glucose + EA + NA + vits.	-	+	-	+	±	±	+	±	±	±	++	-
14. glucose + tryptophane.	±	±	±	±	++	±	±	++	++	±	±	-
15 formate.	-	+	-	±	-	-	-	-	-	-	-	-

Intercept 1

Compare poor activities of NA. ± high activity - N2 case & intermediate activity of acid-hydrolyzed casein.

NA, tryptophane have slight activity.  
by individual NA. & more tryptophane

Oxidation tests.

479a

Grow in Acetate 1% broth. Wash & adjust to ca. = density.  
1ml ( $\approx$  5ml original) bacteria in 1/10 phosphate + substrate.

<p>— Acetate .1% Pyruvate .2%</p>	<p>K-12 489 ca 150 ca 150 ca 10 min ca 35 m (not complete) ca 2 min ca 3 min.</p>
---	---

4:05.

- |        |   |                      |       |        |
|--------|---|----------------------|-------|--------|
| K-12   | 1 | —                    | ClAc. |        |
|        | 2 | Ac                   | —     |        |
|        | 3 | Ac                   | ClAc  |        |
| conts? | ④ | <del>Ac</del><br>Pyr | ClAc  | 4:30.  |
|        | 5 | Form                 | ClAc  | < 4:20 |
|        | 6 | Form                 | ClAc  | < 4:20 |

K-12 679-662

- |  |    |    |                    |
|--|----|----|--------------------|
| 1. T( $\alpha$ ) (100r/10ml).                | —  | —  |                    |
| 24h.   |    |    |                    |
| 2. T( $\Phi$ ) + <u>.1%</u> of glutamic acid | ++ | ++ | pellicular growth. |

Quantitative response.

T(G) + glut

$\alpha$ -keto gl.

0

0

+++

3r



do.

1mg / 10ml

$\therefore$  this strain has fully reverted & data on utilization are fallacious.

Reisolate from lyophil & check rigorously. OK ✓

use of  $\alpha$  keto glutamic acid instead of glutamic acid for growth OK.



# Utilization of Acetate

480

24 APR 1947

T(m) + Acetate Glucose		36h. K-12	48h.	36h. 489	490		
DSCP24	.1% —	+	+	✓	±	—	✓
	.1% 1%	++	++	✓	+++	✓	✓
	.2% —	+	+±		±	—	
	.2% 1%	++	++		+++	✓	
	.5% —	++	++		—	—	
	.5% 1%	±	++		++	✓	

? ↑ Mutant is acetate-. Not inhibitory.

are acetate + glucose inhibitory when autoclaved together? Cf. 475. In prev. rept. Acet was in phosphate buffer.

Glyceric utilization data are needed

autoclave together.

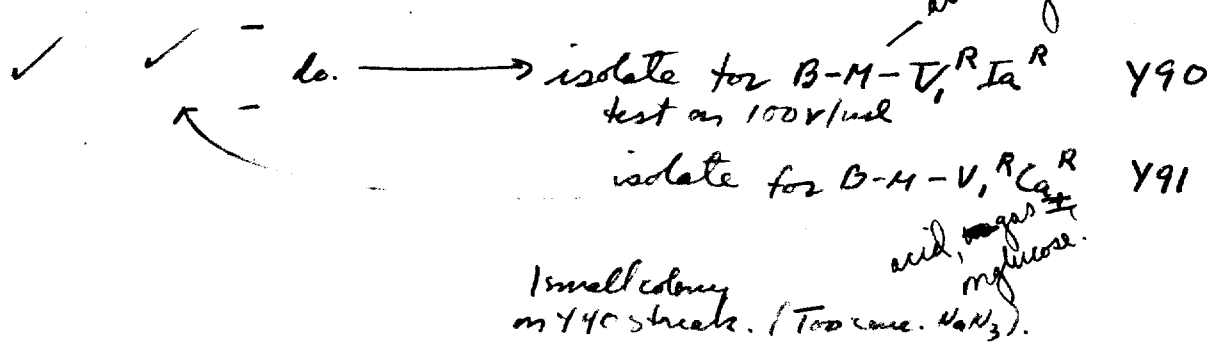
T(m) + Acetate	Glucose	Glyceric.
1	.1%	—
2	—	.1%
3	—	.1%
4	.1%	—
5	.1%	.1%
6	.2%	—
7	—	.2%
8	—	.2%
9	.2%	—
10	.2%	.2%
11	.5%	—
12	—	.5%
	.5%	.5%
	.5%	.5%
	.5%	.5%
	.5%	.5%
	1%	—
	—	1%
	1%	—

22 APR 1947

Pepsin plates of NA + IA 50v, 100v/ml. etc. Streak thickly.

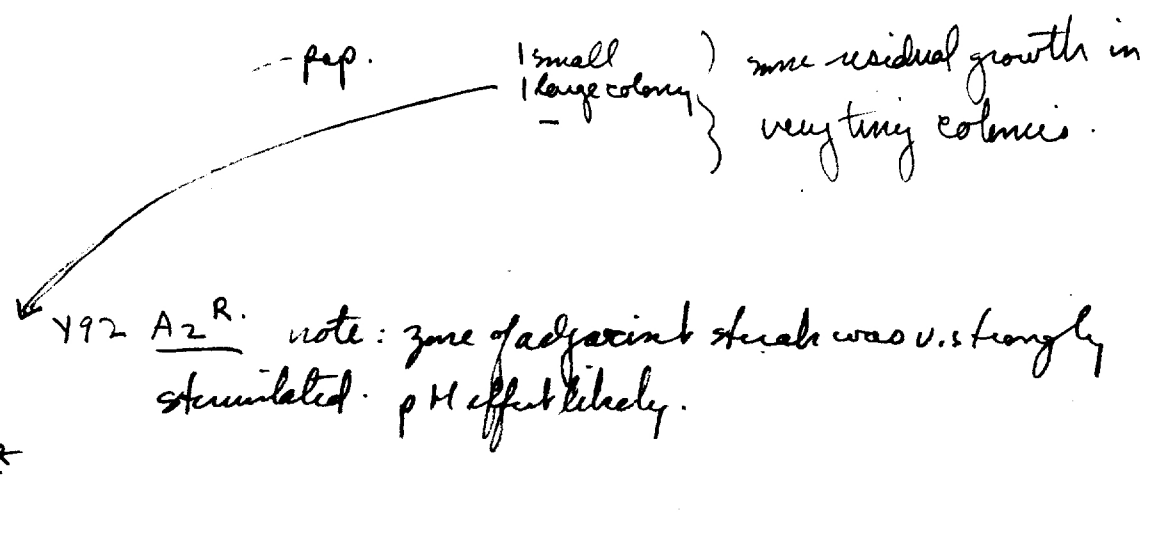
	CLA $\perp$	CLA $\geq$ <sup>mg/ml</sup>	IA	50 <sup>v/ml.</sup>	NaN <sub>3</sub> 100
Y53	±	pap.	±	pap	-
Y40	±	pap.	±	pap	-
Y88	+++	+++	-	pap	-

Y53 }  
Y40 } 36h.  
Y88 }



Lack of Ia<sup>R</sup> from Y88 + Y53 may be due to the more recent origin of these isolates, i.e. a smaller chance of accumulating mutants.

Y53 }  
Y40 } 60h.  
Y88 }



No Ia<sup>R</sup> from Ca<sup>R</sup>?? Inc very nearby N24 - confluent growth. see 497.

# Segregation of Cla<sup>R</sup>.

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Y40 x Y89.

Hold in icebox

Comp. 0; B, on bac U segt.

T(0).

	Lac - V <sup>R</sup>	Lac - V <sup>S</sup>	Lac + V <sup>R</sup>	Lac + V <sup>S</sup>
cla <sup>R</sup>	24	9	24	2
cla <sup>S</sup>				
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. of from sample tested.

T(B<sub>1</sub>).

	20	7	9	0
	10	6	4	0
	30	13	13	0
cla <sup>R</sup>				
cla <sup>S</sup>				
Gas +	4			
Gas -	1			

Total 4/78 Sus. 2 - R  
1 - S  
1 + R.

# Segregation of $\text{Cla}^R$

Y40 x Y88.

T(10) Pick from lac-v tests to water. streak on Cla (1-2 ng/ml)

1-	Lac-v	+	R	Cla
1	+	R	R	R
2	+	R	R	R
3	-	S	R	R
4	-	S	S	R
5	+	R	R	R
6	-	S	R	R
7	-	R	R	R
8	-	R	R	R
9	-	S	R	R
10	-	S	R	R
11	-	R	R	R
12	+	S	R	R
13	-	R	R	R
14	+	R	R	R
15	+	R	R	R
16	+	R	R	R
17	+	R	R	R
18	-	R	R	R
19	+	R	R	R

2-	Lac-v	+	R
1	+	R	R
2	-	R	R
3	-	R	R
4	+	R	R
5	-	R	R
6	-	S	R
7	+	R	R
8	-	S	R
9	+	R	R
10	+	R	R
11	+	R	R
12	-	R	R
13	-	S	R
14	+	R	R
15	+	R	R
16	-	R	R
17	-	R	R
18	+	R	R
19	+	R	R
20	+	R	R
21	-	R	R
22	-	R (s?)	R
23	-	R	R

$$\begin{aligned} \text{Total: } & 56/58 = S \\ & + 18/20 \\ \hline & 74/78 = S. \\ & R = 5\% \end{aligned}$$



T(B<sub>1</sub>)  
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

test this group  
in B<sub>1</sub>-T(0).

1	-	R	S
2	-	R	R
3	+	R	R
4	-	R	R
5	-	R	R
6	-	S (R)	R
7	+	R	R
8	+	R	R
9	-	R	R
10	-	S	R
11	-	R	R
12	-	R	R
13	-	S	R
14	-	S	R
15	-	R	R
16	+	R	R
17	-	R	R
18	-	R	R
19	-	R	R
20	+	R	R

▲											● S
+	-	-	S	+	R	+	+				● S
B <sub>1</sub>	B	M	Ca	Lae	V	T	L	...		Ca	
-	+	+	R	-	S	-	-				● R

mostly R. ∴ R near ~~BM~~ BM

~~R's are -S, -R~~

S's are -S, -R.

R between B, M?

Homogeneity of  $B_1^-$ :  $B_1^+$   
Y40 x Y53; Y6Y x 5P-161.

~~483~~  
483

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Use liquid mixtures Y40 x Y53. Add  $B_1^-$ .  
Hold in cold room after 2da.  
ca 1-5 colonies / plate.

4/19 Struck out on EMB Lact

1. all lac-
2. " 6/6
3. "
4. "
5. "
6. "

6/6 const lac-

Should use  $B_1^+$  on  $B_1^-$  plates.

Repeat with Y40 x Y88 (Y53- $Cl_a^R$ )