S51. ✓	O	glut	prol	orn	citr	arg.	E-arg.	Ho prol	~~arg~~ E	N
	+++	+++	−	++	±	~~++~~ −	−		+++	+++

later, all up-adapted(!?)

S55 ✓	O	trypt T+	cyst T+	meth T+	S-AcOH.
	−	++	+++	+++	+++

S52. ✓ NV E- all +++ exc. typt. ++.

S50. ✓ Biotin + N- all — BN, BE ++ !!

S57.	ENV	EN	NV	EV	E	N	V	O	
	—	~~++~~							
S58	ENV	EN	NV	EV	E	N	V	O	(may have adapted on slant (large + small colonies on V+B slant).
	+++	++	+	+	−	±	−	−	
S60	"	"	"	"	"	"	"	+++	

S56. ✓	O.	~~Vits.~~ Biotin	l-tyrosine	dl-tyrosine.	alcohol
	−	+++ (looks like a contam.)	+++		±

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

[chelamalcohol.]

	mg per 5cc
Blank	
l-tyrosine	0.10
l-phenylalanine	0.10
glycyl-l-tyrosine	0.16
l-tyrosyl glycine	0.15
l glutamyl-l-tyrosine	0.25
l-tyrosyl-l-tyrosine	0.20
glycyl dehydro phenyl alanine	1.5

filtered solutions

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

* old solutions.

Factors mutant: Y10 335

29 OCT 1946

washed suspension 10⁹

Inoculate Y10 1, 2, 5 units. SP 10/28.

Incr. 2 ml into 10 ml YB shake 11 H 29. Spread 5 min. ca 100 each on Endo-lactose + EMB-lactose plates. Incubate at 30°.

~~2~~ no mutants / 3000 on EMB.

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

Spread 2 units:

161 × ~~III~~ =

64 "

480 × ~~III~~ "

200

75 "

ca 200/plate average.

80

16,000

6 pink colonies which are not obviously contaminants.

Pick to complete for further identification.

1 Yeast — cont
2 morph. typical coliform.
3 came up v. slowly on YBagar
4
5 Yeast cont.
6 came up v. slowly on YBagar.

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

335-2:

T_1^S

requires T_1 L, B_1 ✓

Y 53

Activity on various sugars: K-12, Y53.

11/13/46

The strains were tested on EMB plates & sugars:

	glucose	sucrose	maltose	lactose	galactose	
K-12	+~	−±	+	+	+	
Y53.	+~	−±	+	−	+	& this is interesting!?

Salmonella crosses.

28 OCT 1946

Plate (washed) 24 hour cultures of:

S1 x S50. ○ typ.

S1 x S50 deleted. + T. Latent virus??? — no segm. (phage ??)

↓ → typical prototroph.? colonies

42 x 70
42 x 37
= 42 x 61 (T.) —

13 x 36
1 x 45
42 x 45.
61 x 70 -

Tryagain - many (++++)
do. v. small colonies

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

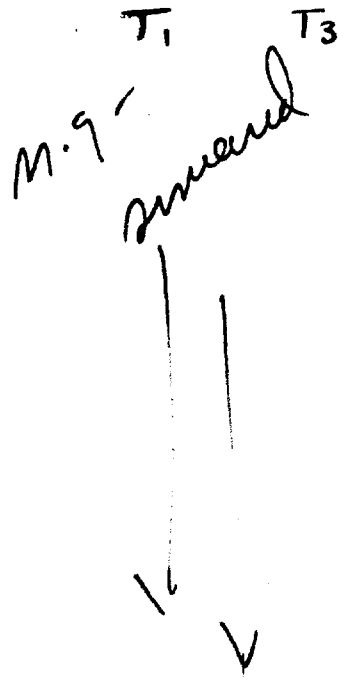
28 Oct 1946

Plate 24 hr. cultures:

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

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

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



(A) (1)

(B) (2)

⋮

28 OCT 1946

Plate 24 hour cultures of: mix-

	B⁰	B*	T*	L*
B⁻T⁻L⁺	*	*	*	*
B⁻T⁺L⁻	0	0		10
B⁺T⁻L⁻	0		0	400
3 strains:	0	100	1	0
	0			

* same minute colonies probably B⁻T⁺ or agar bacteria

- pick between colonies for new tests.

B⁻L⁻ × B⁻T⁻ apparently only B⁻ appeared in the mixture. (from B⁻T⁺L⁻ × B⁻L⁻L⁺)

must repeat + demonstrate recombinability of all three strains!

Try B⁻L⁻ × T⁻L⁻ etc.

But supports thesis of recombination.

Y10: revision.

339

28 OCT 1946

Plate washed Y10 in.

O

TL ca 100 colonies.

TB1 1 dup colony, n.g. for pic bank.

LB1. (plated too heavily.) ca 20 colonies.

Time of recombination.

29 OCT 1946

add ± 1ml old Y10 and Y41 into ca. 4ml YB. at various incl. times. At terminal time, wash all cultures + plate; compare prototroph frequency. Plate .5ml agar. 30° ch.. also compare YB + 5% glucose, YB + 5% NaCl, YB + 10% Blood

24h.	1030P29.	1.	+++
12h.	10A30.	2	+++
10h.	12N30	3	+++
9h.	130P30	4	+++
7h.	330P30	5	+++
	Centrifuge 1030 P.		
2h.	11 P.	6	+++
1h.	1215A	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 + 2 hours + mix — hours + plate.

conditions:

glucose	++
NaCl	++ ± although growth was very much diminished!
Blood	++
" 1/10	−
control	++

Yeast – N.R.

29 OCT 1946

Sc 0.

S. cerevisiae haploid – (S.c.) on B(0) [Burkholder's + 5% glucose].
37° ; streaking preferable

V–

B_1 – stimulatory (carryover??)

pab-
Pyridoxine
pantothenate
inositol
biotin.
} required on this medium.

Cf. Fries (may contain B_6, inositol in the lactate.)

came up in 48 hours on 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 x S50.

342

1 NOV 1946

5 cultures each - S1, S50, S1 x S50. mix mainly P 1.

Plate N4 ½ ml = or .01 ml =.

S1 — dil

S50 — dil. no colonies.

S1 + S50 — dil. v. supra.

colonies appear which are the same in appear. number as those which occur in the S1 + S50 mixture. It is difficult to account for them on the basis of a double mutation!

S1 x S50. – phage 3 4/3

Plate 3 da cultures of S1 + of S50 as follows.
on nutrient salmie 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.

whereas area previously inspected, it showed no ~~zone~~ transmissable lysis. Therefore this hypothesis is probably not tenable !!

Recombination: Time of Occurrence

Y10 x Y40.

4 NOV 1946

Prepare fresh cultures for inocula. YP4.

Inoc 10 ml YB with 1 ml each inoc. at various times as indicated.
(Calculate from beginning of centrifuging) 28°

Read initial and final optical densities to measure amount of growth.

Transfer cultures to smaller tubes for centrifuging and washing.
Inoc. ~~1~~.5 ml each washed ~~cultured~~ into a minimal ~~a~~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 ~~Xd~~	Inoc.d. (1:6)	(cells) x10⁹	colonies	(R)/10⁹
* 1. 2h	1007	66¹			2882	1.60		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		53	2366	1.31	88²	1.24	18	15
4. .5	11:42			63	2007	1.11	(81³)	2.04	25	12
			1800	62¹	2059	1.14	87¹	1.32	14	11
5. 15m	11:57			64²	1905	1.05	91	.96	20	20
				64²	"	1.05	91¹	.94		
6* ~~8P1~~	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.

Washing commenced at:

#4 on 540 [illegible]
15: 77
73²

Centrifuging 12:24.

Readings at 12:10

* contains Neurospora cont; inhibited accord 1 colony call 344-1 Neur.

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 ~~Y47~~ Y40

Segregation of lactose fermentation.

5 NOV 1946

Prepare inocula P5 in modified YB broth (NYB): per liter-

Nutrient Broth	8
Yeast extract	1
R_2HPO_4	3
KH_2PO_4	1
NaCl	5
Lactose	10.

Plate after 4 hrs. growth.

a) ~~YB-glucose~~ NYB-glucose

b. NYB-lactose

c. NYB-no sugar.

all colonies are similar on lactose + bar on glucose plates!

1. Y53a x Y40a in NYB-glucose.
   Plate in: glucose- 187! v. variable!
   lactose 247! v. variable size.

2. Y53c x Y40c in NYB- glucose no colonies.
   lactose no colonies.

3. Y53a x Y40b in NYB- glucose
   lactose

4. Y53b x Y40b in NYB-lactose glucose
   lactose

5 Y53a x Y40a in NYB-lactose glucose
   lactose

6. Y53b x Y40c in NYB glucose no colonies
   lactose no colonies.

7 Y53b x Y40b in NYB. glucose 115 do.
   lactose 120. uniform size

	$L^+ T_1^R$	$L^+ T_1^S$	$L^- T_1^R$	$L^- T_1^S$
7 gluc.	4 ✓	–	6 ✓	–
lac lact.	3 ✓	1 ✓	1 ✓	2 ✓
4. gluc	3 ✓	1 ✓	4 ✓	2 ✓
lac lact	2	–	7	2
1 gluc	1	[illegible]	7	2
gluc lac	4 ✓		6 ✓	
	16	4	31	6

Test Lac^+ after replating on glucose medium.

	L^+	L^-	
R	16	31	47
S	4	6	10
	20	37	57

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

35% L^+

20% S 17%

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

7+4:

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

4	6
3	7
7	13

The presence of asparagine in the plating medium might be responsible for loss of fermentative ability and segregation of the gene. Must the media contain no other carbon source?? Grow cells on synthetic medium, 4% on glucose; 1% on lactose; mate in presence of lactose only 3 growth; plate in lactose minimal (- asparagine) and in glucose and compare numbers which appear; also test Lac^+ for dissimilation.

Salmonella Crosses

S42 x S45; S61 x S70.

8 NOV 1946

In YB broth IIA 5 Crosses YB 4 P V.

— dil.

S42
S45 v. numerous colonies.

S42 + S45 turbid dil

S61 turbid. numerous
S70 — v. minute colonies.

× turbid
no colonies.

Dissimilation of lactose fermenters

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

all as bfore. (4)

Non-Genetic, Nutritional Adaptation

Disproportionate segregations can be interpreted on the basis of the transfer of ~~cytoplasmagenes~~ 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 variation have the most numerous and efficient cytogenes; on complete medium, there will be no selection at this level; if anything, it will be for efficiency at later synthetic steps (e.g. protein synthases). This might be revealed by comparing cells grown on synthetic and on supplemented medium with respect to the lag which they exhibit when transfered, after washing, to minimal medium. Briefly, can cells become <u>adapted</u> to minimal medium? (in the adaptive enzyme sense, as opposed to gene mutation.)

For media use - coli (o) + 1% glucose; as supplement, add vitamins, hydrolyzed yeast nucleic acid, and hydrolyzed casein 0.3%

Wash cells in coli (o) + glucose or asparagine or NH4 source.
i.e. in phosphate buffer
Na_2SO_4
NaCl
trace of
$MgSO_4$; CaCl

Nutritional adaptation

11/7/46

a. Large inoculum from stock b. Shaken at 37° for 15 hours. resuspend in H₂O. wash. 3 ml.
b 1 ml from a in same medium. (930#8) Shake 37° hr.

1. 2	Lactose	adapted	a.	into Lactose ferm.
3 4	Glucose	adapted	a	into L/G
5 6	x	adapted	a	into x 0
7 8	0	adapted	a	into x 0.

11 etc. is as above ± b.

3'' b

L/L	L/G	G/L	G/G	0/0	0/0	0/0	0/0		
1	2	3	4	5	6	7	8	Time ~~hours~~	
88	88³	92³	92	96	98	91		0	1045A
~~89~~ 89¹	89	92³	91	95¹	97³	91²	93²	30m.	~~1115A~~ 1115A
89³	89²	93³	90	92²	97³	90¹	94¹	2h.	1245A
90¹	90¹	88	76²	94³	97²	59¹	61¹	4 1/4	3P
90³	90²	75¹	67³	94³	98	38³	39¹	5 1/2	420P
90¹	91	62²	54¹	94³	98	34¹	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	80¹	80	80¹	80

Grow Y54 + Y41 in YB 1½ days. Plate in T(0) P 9.

P12 – numerous yellow + white colonies (cent??) —
check for T_1^R, Lac$^+$.

Segregation of Lac⁻; Evidence on transformation.

18 NOV 1946

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

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

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

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

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

4. ✓ Y53 × Y4D.
   TLB, Lac⁻ T_1^S BM Lac⁺ T_1^R as usual. in glucose

   do not use, of course.

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

N.B.: If mixing of factors can take place (i.e. transformation) +++ should be found which are Lac⁺ T_1^R (such as are found in cross 4.)

Conditions for Rx: 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/inoc.
830P. wash 9P plate .5 ml ± 0 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
to grow separately +
mix after washing = no growth!
use relatively young inocula.

Also, inoculate 0, 5% separate cultures, 1ml. washed 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. nils.
0	—	—
1	—	—
2	++	—
3	++	—
4	++	—
5	++	—
6	+++	+
7	+++	+
8	+++	+
9	++++	++
10	++++	++

Sex conditions

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

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

Note: they were more dense in the H_2O than in NaCl
$(\frac{c_1}{c_2})^2$ = ca 4. which might account for the results.

5. Mix 1 ml sep. culture ≈ 5 ml agar + pour successively. 0.

Next use younger cultures. Use combination of effectiveness of NaCl in increasing rate.

Segregation of Lac⁻

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

		T1 ✓	T2 ✓	T3	T4 ✓	T5 ✓	T6 ✓	T7	φ-C
Y54 ~~S R φ~~									
Y40 T T B, Lac:									
1	Y54	S	S ±	S ✓		S	S	S	S
2	Y40	R	S +	R		R	S	S	
3	Y53	S	S ±	S		S	S ?	S	S all lac -
4	58-161	S	S	S		S	S	S	
5 —	Y10/1	R	S	R ?		R	S	S	
6	" BM/3"	R	S	R	S	R	S	S	
7	" Y10/3" —	S.	S	S	S	S	S	S	

5) is Y10/1,3,5 ~~mixture~~

plaques v. small !! OK.

Throw out these "K-11/3", "Y10/3"

T1 = (K/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 same as said on 1st set of Petri's.

58-161/3 ca 10^3 smeared. do. " " " Petri's

Y10/1/3 smeared — called T gram (-) 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	T1	1	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
A		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 (struck through)	S.	R		R		S
		11	R	S↓	R		R		S
		12	R	S			R		S
		13	R	S			R		S
		14	R	S			R		S
F		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	4	5	6	7
Y10/1	T7.	1	R↓	not readable	R↓		R	not readable;	resistant as a whole
		2					R	not free of phage	but entire streak has plaques
mucoid		3					R		
		4							
		5							
		6	do.	do.	do.		do.		
		7							
		8							
		9							
		10.							

Parent.	Phage.			T1	2	3	4	5	6	7
Y53	T3									
		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
		7	S	R	S?	R		R		R
M		8	S	R	S?	R		R		R
		9	S	R		R		R		R.

should be checked!

Phage resistance groups

		T1	T2	3	5	6	7	
Y53 T7	1			R	R		R	
mucoid	2			↓ with eaten through	R		R	
	3				R		R	
	4				R		R	
E	5				R		R	
	6				R		R	
	7	S	? do. S	later mucoid	S	no plaques (To mucoid) S?	R	less mucoid.
	8				R		R	
	9	?			R		R.	
	10	!			R.			

all with fuzzy edge (not smooth eaten)

Test isolate for strokes.

G	1	Y10/1	R	S	R	R	S	S	
	2	Y53	S	S	S	S	S	S	
	3	Y55	S	S	S	S	S	S	
Y53/3	4	Y57	S	S	(S) ÷ R)	S ÷ R	?	S	mucoid.
Y53/7	5	Y58	S	S	S	S	S	S	! - compare E.
Y10/1/7	6	Y59	R	S	R	R	S	R	(mucoid).
~~mu~~	~~7~~	~~Y60~~	~~R~~	~~S~~	~~R~~	~~R~~	~~S~~	~~R~~	
Y497	8	Y61	R	?	R	R	S	R	
58-161/3	9	Y62	S	S ?	S !	S	S	R.	

plaques v. small.

The 13 resistants seem to become sensitive very rapidly when removed from phage!

	1	R	S?	R	R	? S	S↓
	2		" ?	R	R		
F=Y53/1	3	↓	" ?	R	R	↓	
	4		" ?	R	R		
	5		" ?	R	R		
	6		" ?	R	R		
	7		S	R	R		
	8		S	R	R		
	9		S	R	R		
	10		S	R	R		

all are 1,3,5

Phage resistance patterns.

Conclusions.

A. } all (smooth) T_1^R are also T_3^R T_5^R T_2^S T_6?
B. } S mucoid T_1^R are resistant to all phages, incl. T_2, T_7..
F. }

C. On fresh isolation, Y101 ; Y531 give T_7^R which contain phage, and
E. which are plaque-infected, suggesting either reverse mutations, or loss of resistance by bacteria.

On streaking out, Y53/7 has given rise to $Y53T_7^S$. (reversion?)

Y401/7 ; Y101/7 → $Y53T_7^R$ OK.

D. Y53/3 shows equivocal resistance to T3; T5 after purification. KJ.
1,3,5,7. appear only clear obt. on immediate streaking.

G.

Sex: conditions.

a) 36 hour known cultures: Y53, Y40.

b) prepare fresh cultures from these 1130 A18.- compare when mixed in H_2O (after washing) for 1 hour. (see 352). Prepare the b) mixtures in 1:5 dilutions also. c.

a). 1ml eq. each in 1ml total

b.) do.

c.) as above + 4 ml. H_2O.

Plate comparable nos. of cells known.

d 1:5 — a. 75
b. 91
1.5×10^9 R = 1.4

∴ final cultures will all have
1) all

2) post [illegible] in H_2O is not favorable.

3) dilution effect is questionable.

	time 0		time 1 hr.	
	.5	1.0	.5	1.0
old A	3	6		20
new B	90		22	
mixed c		6		

(ca 10^{-7}).

Test for segregations:

T_1^R lac −	19
T_1^R lac +	2
T_1^S lac −	3
T_1^B lac +	0