Hemizygosity tests: HZ42

May 14 ff 1952.

HZ42 is a culture giving weak lac+ (v?) colonies which may segregate very infrequently. In previous tests, Gal+ or Mal+ revertants were usually pure lac−.

3 Mal+ } from EMS. Apparently pure +. No signs of
4 Gal+ } segregation on lac. Gal+ are strange lac+. Held in incubator.
No signs of segregation.

Lactase Efficiency

4370 units split $\frac{17.45 - 5.91}{2.18}$ mg in 20 mins.

1 unit = 6×10^{-5} mg · per minute / ml

~~= .06 r/min.~~ ~~of 45 r/minute each.~~

1 unit = .6 r / minute

Lactase assay on lactose: (Barford)

752

May 18, 1950.

K12 harvested from 10 plates (= ca 400 ml) BNZ .3% Lactose, washed and resuspended in 15 ml H_2O. Store equal aliquots in water (A) and in water under benzene (B) (shake 3-4 hours). Overnight in refrigerator. Assay with onpg

		Di	Donpg	Acor	Assay stock susp:	mg/ml
1/10 dilutions from stock.	.1 ml A	(176)	301	123	123 u/ml	28.3
	.1 ml B.	110	++++		>	
	.01 ml.	011		437	4370 u/ml.	

LACTOSE ASSAY SYSTEM:

1 ml stock suspensions + 2 ml 10% lactose + 1 ml M/5 NaP buffer + 5 ml 10% $CaCO_3$ + 2 ml H_2O. Incubate 20 mins. Boil 2 minutes. Sediment and assay supernatants (1 ml)

	"Cells"		Assay
1.	A	no lactose	0
2.	A		4.84
3.	B	no lactose	0
4.	B		17.45
5	Noncells		5.91
6		Glucose 1 mg.	2.18

The boiling with $CaCO_3$ appears to cause appreciable lactose hydrolysis, but the increased hydrolysis of benzene-treated cells is quite apparent

←

Lactose activity measured on lactose

Preliminary

Substrate = 2% lactose. All experiments in M/100 NaP buffer. 7.5

a). 1 ml 744 extract (= 880 units/ml) + .6 ml 10% lactose + .15 ml NaP M/5 + 2.55 ml H_2O @ 37° 3 30 – 8 45

744 extract 880 u/ml

1 ml extract + .6 ml 10% lac + .2 ml NaP M/5 + 1.2 ml H_2O.

Ca. 5 hours incubation.

Add 4 ml Benford's Cu reagent to 1 and to .1 ml.

	N/100 ml KMnO4
Glucose 1 mg	2.47
lactose 10 mg	<< 1 drop
Assay .1 ml	5.67
Assay 1 ml	36.0

This is the equivalent of complete hydrolysis. Repeat with lower unitage; shorter time.

5/18/50. System as above: .1 ml extract .6 ml 10% lactose .2 ml NaP 2.1 ml H_2O to 3 ml volume.

Sample .1 ml from time to time into 5 ml Cu reagent.

9 25 0

1 15 .5 ml sample 3.65

Glucose 1 mg 2.43 Take 2.4 as standard.

" " (1 blank H_2O) 2.13

∴ sample contained $2 \times \frac{3.65}{2.4}$ mg "glucose"/ml = 3.04 mg/ml.

∴ 88 units enzyme · 230 minutes split $3 \times 3.04 \times 10^3 / 360$ μ moles lactose

1 unit = $\frac{9,120}{88 \times 230 \times 360}$ μM/min. = 1.25×10^{-9} moles/minute.

= .45 γ/minute

Trial Run.

Calibration of method.

(1)	1 mg glucose	3.35 − .63	2.72
(2)	10 mg glucose	27.5 × 20.10	33.8
(3)	10 mg lactose	[illegible] − 1.86	1.86
(4)	100 mg lactose	[illegible] 14.72	11.86

later: titrate with Setopolnic C, no phosphoric acid

Constitutive – lac, Recombinations from heterozygotes

May 22, 1950.

748 B1 and B2 – Each, 8 lac v colonies, streaks out on EMB lac. Note lac+ colonies ± + ± sheen. ~~Streak~~ Brush on DN2 Glu, test on onpg spot plates. npg tests not correlated with sheen, but some are constitutive; others not. (Possibly a negative correlation (sheen + npg –; sheen – npg +).)

		sheen	npg	Nutrition
B1 – 1		+	–	
		+	+	
	①	–	+	M –
		–	+	
2		+	+	
	②	+	+	TLB,
		+	+	
3		+	+	
		+	+	
	③	–	+	++
		–	–	
5		+	–	
		–	+	
6	④	–	+	++
		+	+	
		+	–	
7		+	+	
		+	–	
		+	–	
		+	–	
8		+	–	
		–	+	
		–	+	
	⑤	–	+	M –

		sheen	npg
B2 – 1		+	+
		+	–
		+	–
		–	+
		–	+
		–	+
2		+	–
		–	+
3		+	–
		+	–
		–	+
		–	+
4		–	+
		–	–
5		+	–
		+	–
6		+	–
		+	+
7		+	–
		+	–
		–	+
		–	+
8		+	–
		+	+
		–	+
		–	+

	sheen	+	–
p1	+	7	7
	–	8	1

B2	+	2	12
	–	11	1

Use 1, 2, 5 in crosses. Sec over

753-1 × Y10
753-2 × 58-161 } on EMS Lac
753-3 × Y10

Ca 250 prototrophs from each. All Lac+.

∴ None of these is Lac₁ − Cst+

Brush Lac − segregants on EMB Lac.

Repick and separate −, + revertants and pick to E DNZ Sln. Lac− components are all npg−.

B1: + camp.

	npg	
1	−	
2	−	1319, 1320
3	+	1
4	+	2
5	−	
6	+	3
7	+	4
8	−	
9	−	
10	+	5

B2

	npg	
1	±	
2	+	6
3	+	7
4	+	8
5	±	
6	+	9
7	−	
8	+	10
9	+	11
10	−	
11	+	12

Restreak Lac − which give onpg+ revertants.

Retest

1	−
2	+
3	+
4	−
5	−
6	+
7	~~+~~ −
8	−
9	−
10	−
11	+
12	−

Classification from brief growth on DNZ probably incorrect. On retests (aerated DNZ)

W1301 ++++
W1318 −
1319 −
1320 ±.

Recheck onbetose reaction.

Irradiation of H226

754-0

May 22, 1950.

Fresh D(Lac). Dilute 10^{-6}. A: control B: UV 20 secs.

0.1ml per plate.

P22

A. EMB Lac

V	−
170	18
162	19
332	37

EMB Mal

+	V	−
33	139	7
10	122	4
43	261	11

B EMB Mal

+	V	−
40	42	16
24	26	13
11	18	6
40	80	15
26	25	7
38	24	10
43	94	14
222	219	81
32	31	12

Many of the Mal + in A may be V

B, the Mal V were typically much
Mal − than in A.

EMS ~~Mal Lac~~

+	−
150	0
158	0

A. EMS Mal

+	−
118	1
39	2

May 23, 1950.

B EMB Lac
36-40 hours.

	V	−
	25	33
	29	52
	27	52
	38	58
	23	72
	35	73
6	187	340
	31	57 /68

EMS Mal

	+	−	S
	28	3	6
	24	20	16
	29	8	6
	26	12	9
	32	18	10
5	139	73	47 /
	28	15	9 / 52

Notice very high proportion of protoplasts in yield! Test by picking at random from EMB Lac.

5/24/50.

a) Pick Mal− from B and ~~streak~~ brush on ~~EMB~~ (EMB Mal) Lac ; Xyl; EMS Lac

b) Pick Lac v from EMB Lac (B) to water; spot on EMS Lac; EMB Mal for partial segregants.

c) Pick apparently pure Lac − from EMB Lac to water. Spot on EMS Lac. After 4 days, none of 40 spots was Lac+. 6 Lac− prototrophs. These colonies may be presumed to be pure

A25. a) ①. Pure Mal−. Spot from water scrap. 35 tests: EMB Mal, Lac; EMS Lac

1 Mal− Lac+ ; remainder are Lac−. Hold EMS plate. Streak out Mal− Lac+ on EMB Lac as 754a1

① Brushes from Mal− and Mal +/−. (From middle, no suspension). [LEFTHAND IS MAL− COMP. I

✓=growth
+=Lac+

Xyl EMB	Lac EMB	Lac EMS
−	−	
−	−	
−	−	
−	−	
−	−	
−	−	
+	−	
−	−	
−	✓	✓
+	+	−
−		−
+		−
+	+	−
+		−
−	+	−
−	+	−
−	+	−
−	✓	✓
+		−
−		−
−	+✓	−
−	+	−
−	−	

X		L B		L S	
−	✓	−	✓		+
−	—	−	−		
−	+	−	−	≠	
−	+	−	−+	✓—	✓—
−	−	−	−	✓—	✓—
−	+	−	✓		+−
−	✓	−	−		
−	+	−	+	≠	+
+	+	−	−	—	—
−	✓	−	−		
−	+✓	−	+	✓	+
−		−			✓
−		−			✓
+		−			
+		−			✓
−		−			
−		−			
−		−			
+		−			
−	+	−			754a2.
+		−			
−		−			
+		−			

Subtotal: 47 tests: 1 Mal− Lac v

Mal− : 35 Xyl− : 12 Xyl+

Total 2/82.

	Lac	Mal	Xyl	EMS Lac	Nutr
a1	✓	—	—	ng	M−
a2	✓	—	—	ng	M−

May 25, 1950

b) 120 Lac^{-} picked to Lac EMS; Mal EMB.
All Mal+(or v) except #'s: 8, 10, 17, 23, 59, 66, 67, 73, 82, 84, 88, 117 [12/120]
which are Mal−, predominantly. Save suspensions.
All formed colonies on EMS by 24 hours except:
~~5~~, ~~6~~, 11, 14, 20, 21, 22, 24, 30 32, 35, 36, 38, 40 43, 48, 49, 50 54, 55, 58, 60
61, 66, 68, 69, 70, 81, 82, 83, 87, 88 ~~91~~ 91, 93, 100 106-110, 113, 114, 116, ~~119~~ 120
Hold additional 24 hours.

Restreak Mal− suspensions on EMB Lac; Mal; EMS Lac.

c) EMS Lac : Hold EMB Mal: 29 app. pure +
6 app. pure −
6 clearly mixed.

Mark gave EMS Lac+

d) As a) but from A

e) As b) but from A

f) 3 Mal− from A, EMS Mal. to EMB Lac, Mal, S Lac } See 756.

g) Mal− fetotypes from BT. Brush to EMS Lac

May 28, 1950.

b)

		Lac	Mal	EMS	Nutrition
1	8	✓	—	+	
2	10	✓	—	+	
3	17	✓	—	ng	L-
4	23	✓	—	+	
	59	✓	—	ng	TL-
	66	✓	—	ng	TLB₁-
	67	✓	—	+	
	73	✓	—	+	
	82	✓	—	—	
	84	✓	—	—	
	88	✓	—	ng +	M-
	117	✓	—	—	

Thus about 10% of the surviving Lac✓ colonies are Mal- Lac✓. From a) one infers that 2/82 ≐ ca 3% of the surviving Mal- colonies are Lac✓.

cf. d, e, f.

see 754b

See 756.

c) hold

d) Mal- from control plates. Also sectored colonies.
7 pure Mal-: 6 Lac- 1 Lac± (prototroph.) Restreak as 754d1. ✓Mal- Lac✓ prototroph
14 sectored colonies. Mal- fresh is Lac-.

e). Lac✓ from control plates: to EMB Mal, EMS Lac 89 tested.
3 are Mal-, prototroph. Restreak. 1 Mal+ (or✓) non-prototroph
754e 1-3 1 Mal- Lac✓ 2 Mal- Lac✓ 3 Mal✓ Lac✓ prototrophs. | 754e4 Lac- ✓Mal+,-,✓ nonprototroph. ≡ T-L-

f: Each of 3 is Mal- Lac✓ prototroph. Save 754f1.

h) Replate H226 ~~[illegible]~~ on EMB Mal 6 plates. Pick pure Mal- to Lac

g) 42 Mal-prototrophs from B. 19 are Lac+.
29 sectored pairs. Mal- component of 11 pairs is Lac+.
To be verified. Pick possible excepts, and their sibs, to water and spot on EMB Mal, ~~EMB~~ EMS Lac.

May 27, 1950.

h) 13 pure Mal− colonies : 2 lac ± ; 13 lac− Retest the +.
2 pairs Mal−/+ tested Lac of the recorded Mal−/+ were lac v, but also scored Mal+, and presumably Thal v.

g.

June 1, 1950

25 Lac+ av cultures reisolated as auxotrophs. Streak out on various sugars to determine further characteristics

	Lac	Mal	Xyl	Mtl		
1	v	v	—	—		
2	v	v	v	—		
3	v	v	—	v+ ?		
4	v	+ v?	—	—		
5	v	v	v	v	M	call it B12
6	~~+~~ —v	+	—	v		
7	~~+~~ v	v	+	v	FL	B13
8	v	—	v	v		
9	v	v	+	v		
10	+?	v	v+	v	M	
11	v ~~+?~~	v	v	—		
12	v	v	v+	v	M	
13	v	v+	+	+		
14	v	—	v	v		
15	+?	v	+	v+		
16	v+? ~~+~~	v	v	v	M	
17	—v	+	+	v		
18	o	o	o ~~+~~	o		
19	-v	+	~~+~~ v	—	L	
20	++ +?	—	+	+		
21	v	+	+	+v	M	B14
22	v	—	v	—		
23	v	v	v	v	M	
24	v	—	—	v		
25	v	+	v+	+		

See 756

Restreak v as possible use for outcrossing. Determine mutations.

June 2, 1950

Separate 7 Mal<u>sec</u> colonies of which Mal- component is lac<u>v</u>.

	EMBLac	EMBMal	EMSMal	EMSLac	
1 −	V	−		+	
+	V	V	++	+	
2 −	V	−		+	§ unstable - throws off Mal-lacv prototrophs.
+	V	V	++ −	+	
3 −	V	−		+	
+	V ⊙	++	++	+	
4 −	V V	−	−	+	
+	− −	V	++	−	
5 −	V	−		+	
+	V ⊙	V	− ?	+	
6 −	V	−		+	
+	V	V+	+, −	+	
7 −	−V	−		+	
+	−V	V+	++	+	

2, 6 Mal+ (EMS) may be segregating Mal− prototrophs (Lacv?)
Verify from single Mal+ colonies (EMS).

<u>3</u> may illustrate a segregation from Mal<u>v</u> into Mal+ and Mal− Lac<u>v</u>.
Verify from EMS Lac.

<u>4</u> seems to suggest another type of separation. Lac Malv ⇄ lacv Mal−, Lac− Malv

<u>5</u> may be confused: Mal− presumably was streaked on EMSMal, for Mal+.
Verify from EMS Lac. ✓

6/3/50 ✓ 3 → Mal+ lacv and Mal− lacv

4 → Malv Lac− and Mal− lacv

6/4/50 5 → pure Mal+

6 → each of 4 tests pure Mal+ prototrophs

2 → 1-10% Mal− prototrophs in each of 8 tests. Restreak; slant as 754g2; test Mal− on EMB Lac 4/10 were lacv.

A. Lac v ≒ ~~total~~ population ≒ prototrophs.

Auxotrophs = 1%

Mal- lac v = 2%

Mal- prototrophs = 2% (all lac v)

B. Lac v = 35.5% Prototrophs = ca 2/3? p Kill = ca 88/180 = 50%

	/Total	/ lac v	/prototrophs	/original total //	
Auxotroph lac v	7.4	21		3.7	
Mal- lac v	3.5	10		1.8	
Mal-	15.5			8	3.5
lac -	64.5			33	10
Mal- prot. lac v			12.7		

The predominant effect is to make haploids pure for Mal, lac. The residual lac v have a high proportion of perturbations. (should be checked on other characters).

754

B UV. Mal- (EMB) 81/522 = 15.5%

Lac- (EMB) 340/527 = 64.5% Lac v = 35.5%

Mal- (EMS) 73/259 = 28.2%

a) Mal- (EMB): 2/56 = 3.57% Mal- Lac v.

Total fraction = 3.57 x .155 = .55% intact Mal- Lac v.

b Lac v. 25/120 are auxotrophs: $\frac{25}{120} \times 35.5 = 7.4\%$ auxotroph

12/120 are Mal- Lac v = 3.5% Mal- Lac v.

c. Mal- (EMS). 19/42 are Mal- Lac v = $\frac{19}{42} \times 28.2 = 12.7\%$ of prototrophs are Mal- Lac v.

754

A. Control: Mal− = 11/315 = 3.5% EMB Mal

Lac − = 37/369 = 10.0% EMB Lac

EMS: Mal − = 3/157 = 1.9%

d 7 Mal− from EMB Mal : 1 Mal− Lac v.

∴ H226 suspension is ca .5% Mal− Lac v.

e. Lac v from EMB Lac 1/89 : 2 Mal− Lac v / Mal v Lac v non prototroph

This gives estimate of 2/89 = 2.2% Mal− Lac v.

1/89 = 1% auxotroph Lac v.

f. Mal− prototroph 3/3 is Mal− lac v. This gives estimate of ≅ 1.9% Mal− Lac v (prototroph).

Summary: controls. Take 2% as Mal− Lac v (prototroph)
1% as Lac v (auxotroph)

$$3080\ u/ml = V_{max}\ of\ \frac{1.25 \times 3080}{27} / mg.$$

Lactose activation in lactose

K-12 harvested from 6 x 40 ml DN2 .3% lactose agar. Wash and resuspend in + 10 ml H_2O. (A). 3 ml aliquot of (A) shaken with benzene 2 hours. Stored overnight in refrigerator.

a) onpg assay (before storage). in Na P M/30 7.5

RH/69.6 7.7 u/mg
RA 23.9 143 u/mg

	ml cells or extract	9 ml Di	20 min. Donpg	Δ cor	Assay
A	.01	129	221	090	A = 90 u./ml
B	.01	076	>910 6 mins.	corrected:	
B	.001	010	332	308	B = 3,080 u/ml.
	—	−004	011		

b) Manometric assay. Dilute 1.5 ml (A) with bicarbonate to 10 ml M/20. 2 ml cells; 0.1 ml 10% substrate in sidearms.

Flask	Subst.	3^{20} ↓	3^{25}	3^{32}	3^{40}	3^{50}	4^{00}	4^{15}	4^{27}	4^{37}
2A	Glu	26'	40	62'	91	124	157'	213	258	294'
3B	Lac	34'	41'	60'	87	118	147'	197'	239	270
6B	—	31'	36'	39	37	39	37	40	40	41
7A	ThBar.	— 24	29	29'	29'	30	27	29	32	31

Increments:

	T	TB−	A	B
		0	0	0
3^{32}	7	−	30	16'
3^{40}	15	0'	50'	45
3^{50}	25	2'	81'	74
4^{00}	35	0'	117	105
4^{15}	50	3'	169'	152
4^{27}	62	3'	214'	194
4^{37}	72	4'	250	224

A: 215 mm/hour k = 1.84 (glucose) 37°

B: 190 mm/hour. k = 1.78 (lactose) 37°

∴ A ≙ 17.7 μM CO_2/hr ≙ 1.27 mg glucose/hr.

B ≙ 15.1 μM CO_2/hr. ≙ 1.09 mg glucose/hr.

(Assuming 1 μM glucose ≙ 2.5 μM CO_2 – Stokes – J. Bact. Feb. 1949)

$Q_{Glu}^{CO_2}$ = .128

$Q_{Lac}^{CO_2}$ = .110

original suspension A) 33 ~~x 1 mg~~ 1γ/ml

33 x .3 = 9.9 mg / Warburg flask.

27 mg/ml original suspension

c) Lactose hydrolyses. System: 37° 10 minutes.

2% lactose 2.5 ml
Cells 1.0 ml
NaP 4/5 7.5 .5 ml.

Terminate Reaction by adding .5 ml 5% $ZnSO_4$. Clarify with .5 ml .15N $Ba(OH)_2$. Sediment and decant clear supernatant. Dilute 1:10 in water, and take 1 ml for assay, after Caputto, Leloir and Trucco, Enzymologia, 12:350 (1948). To read optical density, read the reduced Mo reagent at 1:10 dilution at 520 mμ in spectrophotometer. All readings of same single cuvette.

6.6 mg/ml

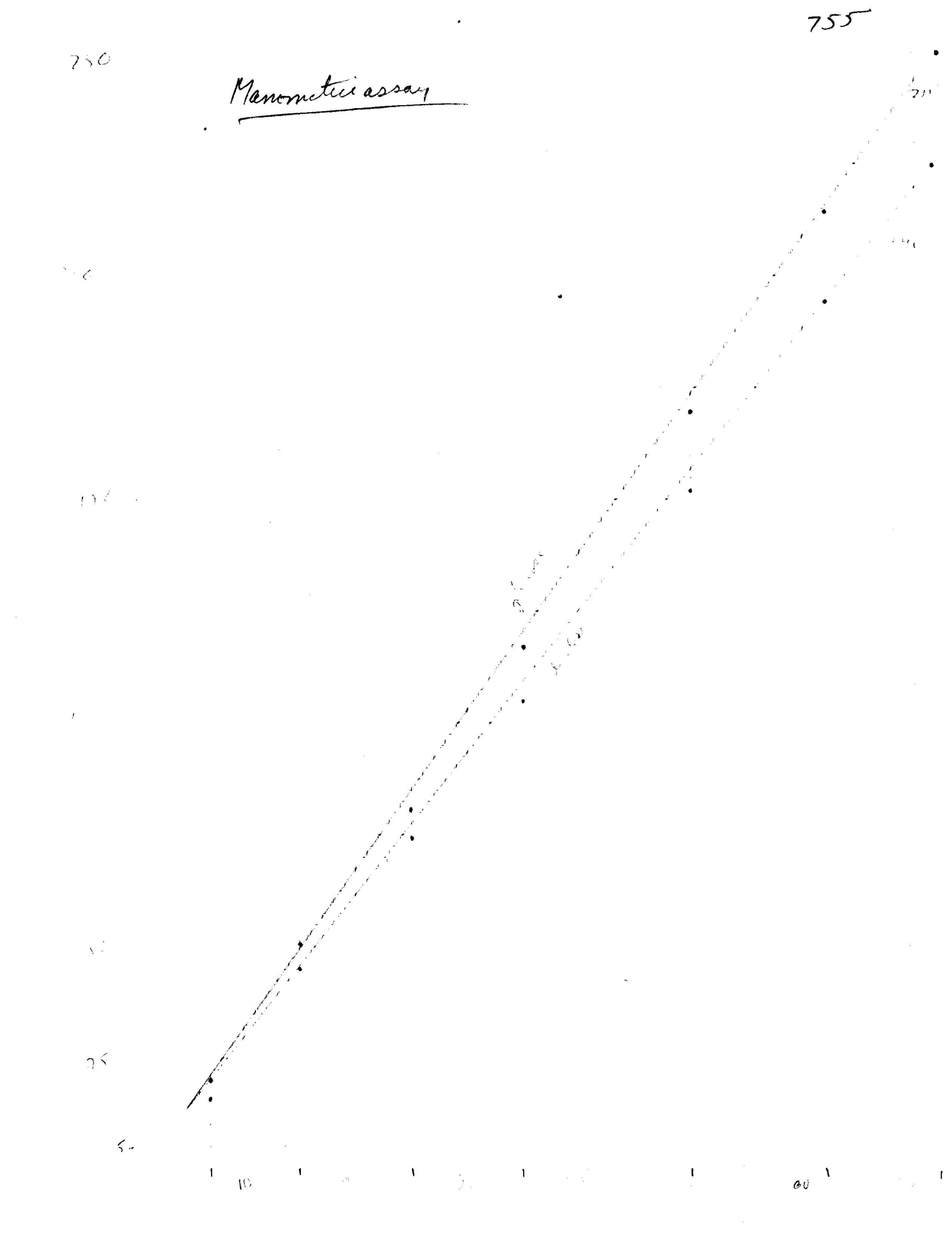
		Density
1.	A. No lactose	004
2.	B. No lactose	000
3.	A. lactose	002
4.	B. lactose	110
5.	— lactose	003 ~~110~~
6.	Glucose (1 ml 2% = 4 mg/ml).	107
7	Blank. + reagents.	003

Thus, 1 ml B in 5 ml liberated 4 mg glucose / ml in 10 minutes.

∴ 1 unit ≐ 20/3080 × 10 mg/min.
= .65 γ/min.

No accumulation of monose by A could be detected. It can be assumed to have hydrolysed ~~at the rate of 1.09/6 × 10/1.5 × 1/2 =~~ 121 γ/ml ($\frac{33 \text{ mg}}{5 \text{ ml}} \times \frac{10}{60} \times 110 \gamma/\text{mg}$), ignoring temperature correction. Activation of 4/.12 = 33 × re lactose. Activation re enzyme was 3080/90 = 34 ×

Manometric assay



Auxotrophic partial segregants.
FROM H226

May 29, 1950.

	#		Nutr.	Mal	Xyl	M4l	Agent
	H244	M	[redacted]	–	V	+	uv
T54	A1		M	–	–	–	uv
	A2		M	–	–	+	uv
	B3		L	–	V	V	uv
	B5		TL	–	+	–	uv
	B6		TL	–	V	–	uv
	B11		TLB,	–	–	–	uv
H245	E4		[redacted] TL	V	V	V	spont
H246	F1		[redacted]	–	–	–	spont.
	H244M+		M	V	V	+	

Attempt crosses of H245, H246.

[redacted]					
B12	M	V	V	V	uv
B13	TL	V	+	V	uv
B14	M	+	+	+	uv

Irradiation of H226

4 single colony isolations of H226 (A-D) used to start D(lac) cultures.
A-D are 10^{-7} cultures
A_x-D_x are UV 20 secs. at same dilutions. UV and EMS at 40 hrs. Others at 24.

A.

EMB Lac	v	−	EMB Mal	+v	−
	264	17		280	4
	291	24		258	2
	317	15			
	872	56 / 928		538	6

~~EMS Lac~~

EMS Mal

+	−
322	2
185	0!
184	0
691	2

EMB Lac	v	−	Mal	+v	−	EMS Mal	+	−
B.	311	12		215	2		321	0
	334	12		276	4		254	1
					4			
	645	24		491	6		575	1

C.

	217	8		260	5		248	1
ca ⅓ pl.	62	3		228	4		178	1
	202	17		274	2			
	481	28		762	13		426	2

D.

1	smeared or crowded - uncount.		(.2)	487	4		419	0
	236	23		283	2		131	0
	399	25		260	2			
	635	48						

Essentially homogeneous.

	EMPBlac		EMPMal			EMSMal		
A	V	−	~~2~~	V +	−	sec.	~~+~~ +	−
	88	76		201	29			
	143	97	(omit screened post.)	114	12	17	135	30
	80	93		260	25	24	161	26
	311	266						
B	1+ 70	70		199	14	8	101	23
	94	98						
	135	87						
	299	255						

Cx
Dx ditto.

June 2, 1950.

Pick lac⁻ survivors from $A_x - D_x$ to water suspensions. Get 120 on EMS ~~lac~~ Mal, Xyl, Mtl and EMS lac.

Stac	Mal	X	Mtl	Stac	Mal	Xyl	Mtl			Mtl			
1 +	V	+	V	11 +	+	+	V	21 +	V	+	V	31 +	V	+	V
2 +	(−)	+	V	+	+	+	V	+	+	+	(−)	+	V	V	V
3 +	V	V	V	+	V	+	V	+	+	+	V	+	V	+	V
4 +	V	V	V	+	+	+	V	+	+	+	V	+	V	+	V
5 +	V	(−)	V	+	+	+	V	(0)	+	(−)	V	+	V	+	V
6 +	+	+	V	+	V	+	V	+	+	+	(−)	+	Y	+	V
7 +	V	V	V	+	V	+	V	(0)	V	+	V	+	V	+	V
8 +	V	+	V	+	V	(−)	V	+	V	+	V	+	Y	+	V
9 +	+	+	V	(0)	V	V	V	+	V	+	V	+	V	V	V
10 +	+	+	V	20 (0)	+	V	V	30 +	V	+	V	40 +	V	V	V

41 +	V	+	V	51 0 +	+	+	V	61 +	V	+	V	71 +	V	+	V
2 0	(−)	+	+	+	V	+	V	+	V	+	V	+	V	+	V
3 +	+	+	V	+	V	+	V	(0)	V	+	+	+	V	+	V
4 +	+	+	+	+	V	+	V	+	V	V	V	−	+	+	V
5 +	+	(−)	V	(0)	V	+	V	−	+	V	V	−	V	V	V
6 (0)	+	V	V	(0)	V	V	V	+	V	V	V	(0)	+	V	V
7 +	+	V	V	+	V	V	V	+	V	+	V	+	V	V	V
8 +	+	V	V	(0)	V	+	V	+	+	+	V	+	+	+	V
9 +	V	V	V	(0)	+	+	V	+	V	+	V	+	V	V	V
50 +	V	V	V	60 (0)	V	+	V	70 +	+	+	V	80 +	V	(−)	V

81 +	+	+	V	91 +	+	V	V	101 +	+	+	V	111 +	+	V	V
2 +	V	+	V	(0)	V	+	V	+	V	+	V	+	V	+	V
3 +	+	+	V	(0)	+	+	V	+	+	+	V	+	+	+	V
4 +	+	+	V	+	V	+	V	+	+	+	V	−	V	+	V
5 +	V	+	V	(0+)	+	+	V	+	+	+	V	+	+	+	V
6 +	V	+	+	(0)	V	(−)	V	+	(−V)	+	V	+	+	+	V
7 (0)	V	+	V	(0)	V	V	V	+	V	+	V	+	V	V	V
8 ±	(−)	+	+	+	+	+	V	+	+	+	V	−	+	V	V
9 +	V	+	V	+	+	+	V	+	+	+	V	+	+		V
90 +	V	+	V	100 +	+	+	V	110 −	V	+	V	120 +	V		V

11 possible fermentative deviations.

19 additional auxotrophs. ~~check on EMS lac −~~.

30

See 759.

Almost — [illegible] still heterozygous
[illegible]
among [illegible]

Some [illegible] heterozygous are stabilized →
lethal mutations.

compare balanced lethals vs. unbalanced
50%

Zelle 6/5

M	Blac	Small	Large	Bunc
7	—	+	+↓	+↓
8	—	+	+	+
9	—	+	+	+
10	—	+	+	+
23	—	0	+	+
24	—	0	+	+
25	—	0	+	+
26	—	0	+	+
27	—	0	+	+
28	—	0	+	+
29	—	0	+	+
30	—	0	+	+

L				
23	+↓	+↓	+↓	+↓
26				
27				
30				
49				
50				
51				
52				
57				
58				
59				
60				

F				
6	+↓	+	+	+↓
11				
12				
15				
16				
17				
18				
19				
20				
21				
22				

G				
1	+↓	+↓	+↓	+↓
5				
6				
7				
8				
9				
10				

Note. sterile H_2O added to all empties

788

Zelle 6/5/50

C	Blac	Smal	Bvgl	BMH
7	− ?	+	+	+ ↓
9	+	+		
11	+	+		
14	+	+		
21	+	+		
35	+	+		
36	+	+		
37	+	+		
38	−	−	pict −	++ − ?
− 45	+	+	+	
46	+	+		
57	+	+		
52	+	+		
53	+	+		
54	+	+		
56	+	+		
58	+	+		
111	+	+		
112	+	+		+ ↑
B	+ ↓	+ ↓	+ ↓	+ ↓
7				
17				
18				
19				
21				
22				
23				
24				
25				
26				
59				
60				
61				

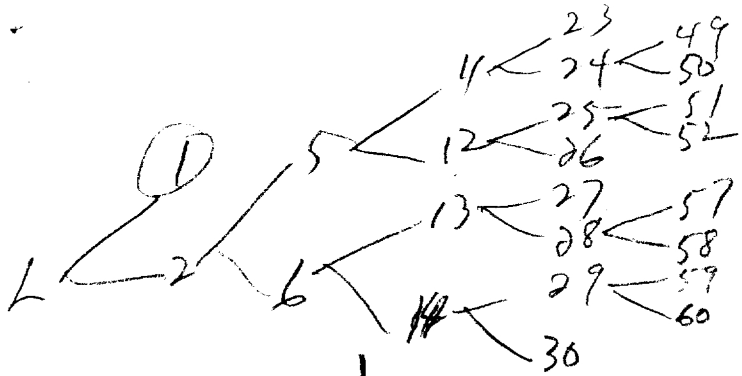
Zelle 615

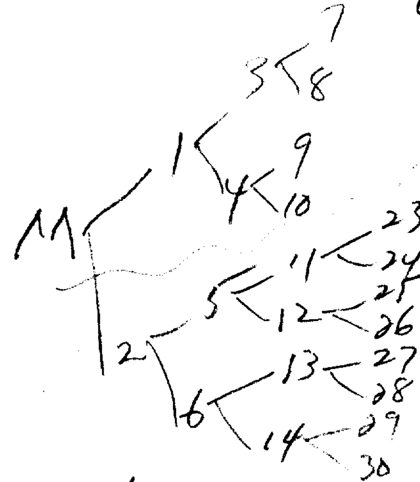
D	Blau	Mal		
7	++	++	++	++
9				
11				
17				
21				
27				
28				
29				
30				
37				
38				
46				
51				
52				
53				
54				
A	++	++	++	++
10				
15				
Empty 16				
18				
19				
20				
23				
24				
25				
26				
27				
28				
29				
empty 30				
35				
36				
H			++	++
7	+	++		
8	+	+		
12	—	0		
23	+	+		
24	+	+		
27	+	+		
28	+	+		
29	+	+		
30	+	+	-	

Zelle 6/5

	EMBLac	EMSMal	EMBXyl	EMBAte
F				
11	++	++	++	++
15				
16				
19				
20				
21				
22				
25				
28				
29				
30				
35				
36				
37				
38				
53				
54				
55				
J	++	++	++	++
7				
17				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
37				
38				
K	++	++	++	++
21				
23				
24				
25 (empty)				
26				
27				
28				
30				
45				
46				
59				
60				

6/5/50





Wh

Dear Josh:

Just to prove I haven't entirely forgotten about the stuff, I'm sending the above cultures. Other will be all for another two weeks as I'm off on another rover mission. I haven't had any time to think let alone write but I'll try to get at it when I get back to town.

If I haven't already given you it, my new home address is:

30 Decatur St
Kensington, Maryland.

I hope the cultures don't all turn out to be haploids. I'm sorry about so many uncertain groups of 4 cells but they grew too fast for me, probably due to the horridly warm humid weather we've been having.

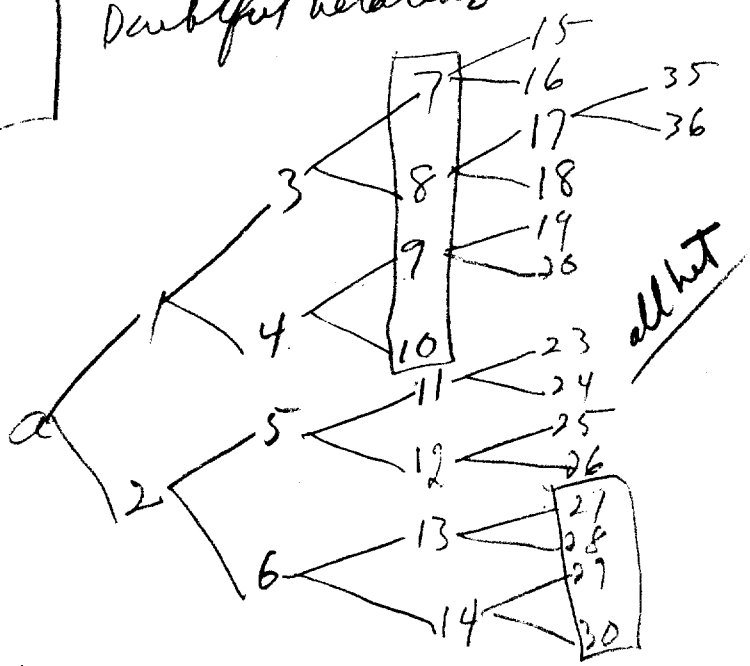
As ever

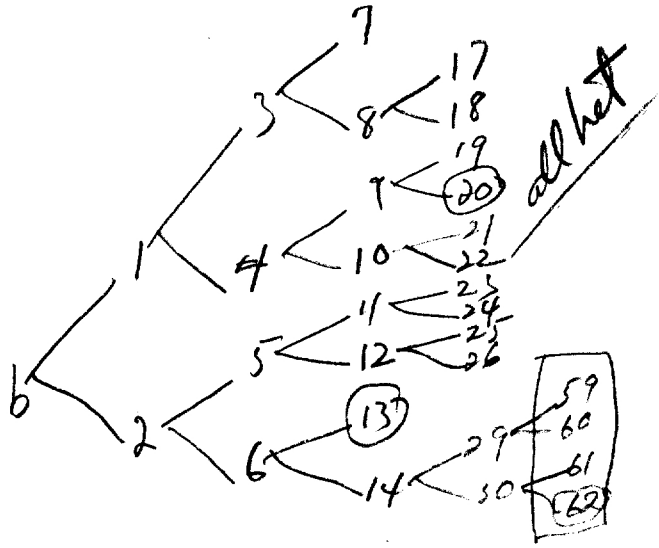
Max

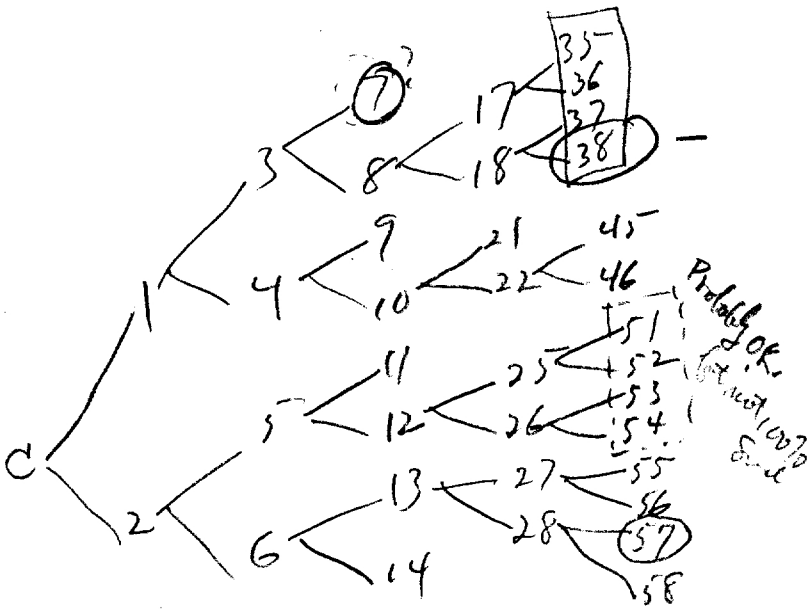
Cultures of 6-5-50.
Source H-226 - EMS colony in Davis synthetic media.

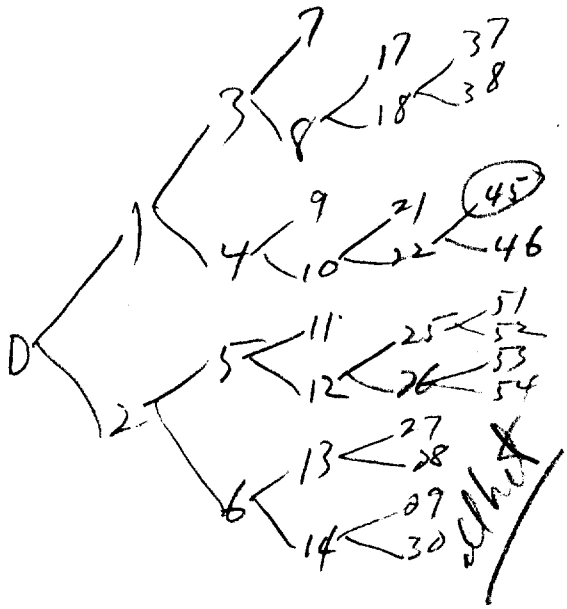
○ = didn't grow

▯ Doubtful colonies

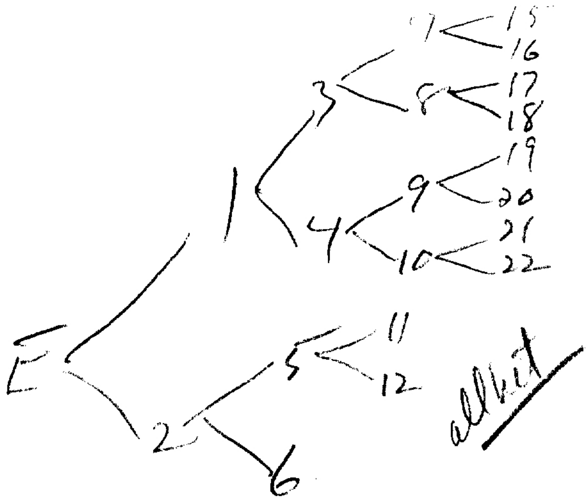


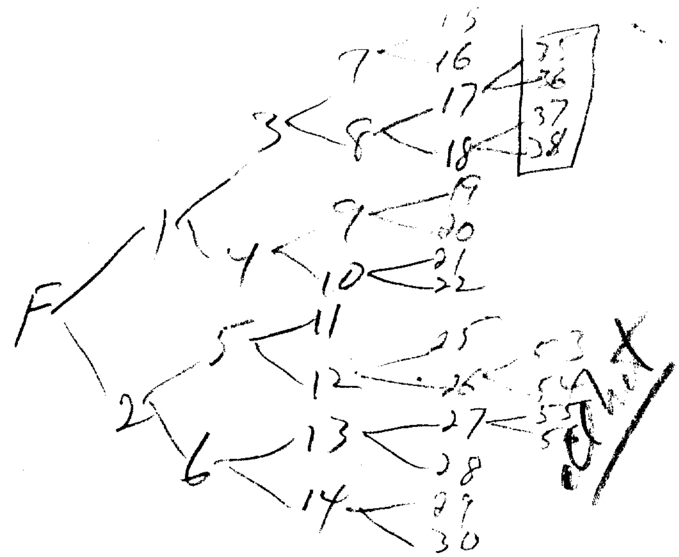




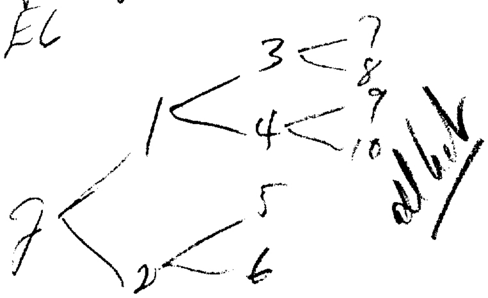


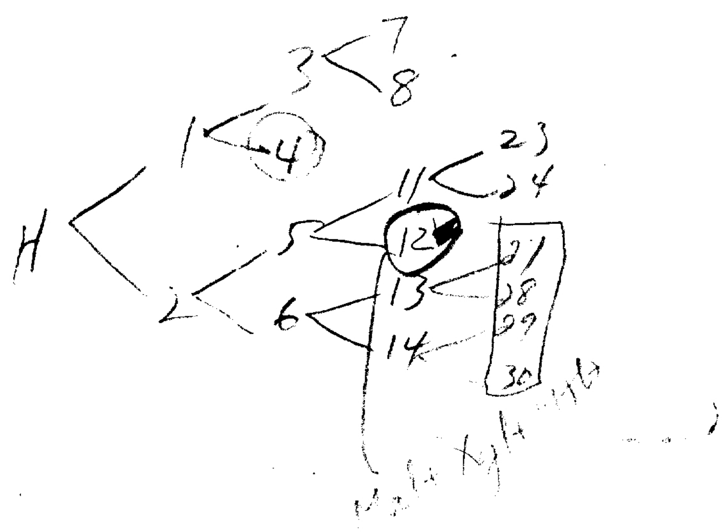
Picture
/ = 21
/ = 45
/ = 46
3 equal ?



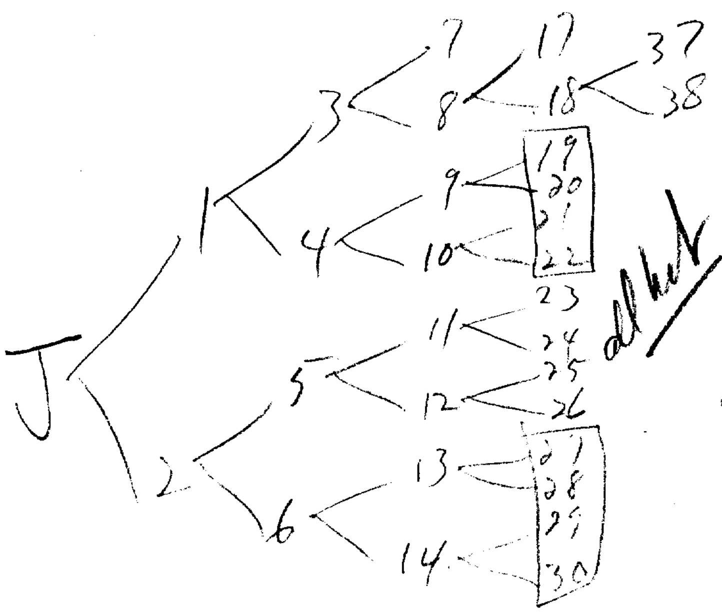


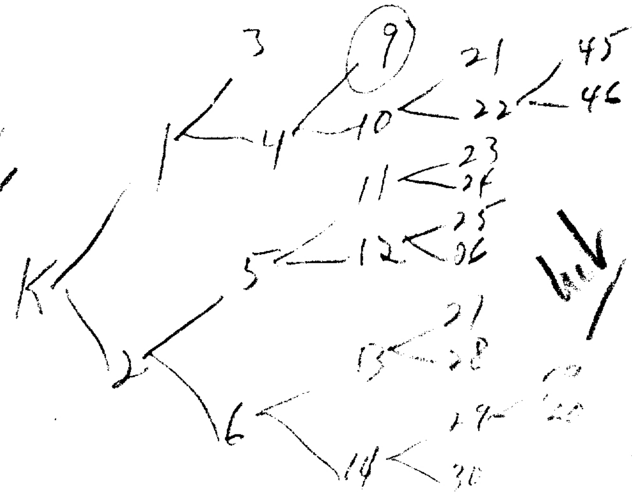
E 6 [illegible] E 13, E 14 were [illegible] by the middle, & left E13 or E6





G [illegible]
[illegible] cells, i.e. larger than usual.





	A	B
Q_{CO_2}	40.0 ul/hour mg^{-1}	34.2 /hour mg^{-1}
=	1.18 uM^{CO_2}/hr/mg	1.52 /hr/mg
=	.71 uM^{gluose}/hr/mg	.61 uM^{glu}/hr/mg
=		

Original suspension (A) had $\frac{1}{.01} \times \frac{129}{.9} \times .23$ mg/ml bacteria.

$= 33$ mg/ml.

Each vessel therefore contains $33 \times 2 \times .15 = 9.9$ mg.

$\therefore Q^{lactose} = 1.09$ mg $/ 9.9$ mg / hour

$= .11$ mg / mg / hour.

		glucose	lactose
$Q^{CO_2}_{CO_2}$	= +	396 ul	338
$Q^{CO_2}_{CO_2}$	=	40 ul/hr	34 ul/hr
	=	uM 17.8 /hr	15.2 /hr.
	=	7.1 uM glucose/hr.	6.1 uM glu(lac)/hr
	=	1.27 mg glucose/mg/hour	

MR Zelle: single cell isolations H226

Summary of exceptions:

		Sib
H12	Lac- auxotr.	23-24
C38	Lac-Mal-Xyl-Mtl- prototroph.	35-37. ✓C7
M 1	prototroph } Lac-	—
M 2	auxotroph } Lac-	

Check samples of each pedigree for heterozygosity

			Lac	Mal	Xyl	Mtl
A	1	A 10	✓	✓	✓	✓
	2	B 7	✓	✓	✓	✓
	3	D 7	✓	✓	✓+	✓
	4	E 6	✓	✓	✓	✓
B	1	F [redacted] 11	✓	✓	✓	✓
	2	G 5	✓	✓	✓	✓
	3	J 7	✓	✓	✓	✓
	4	K 3 28	✓	✓	✓+	✓
	5	L 23	−✓	+✓?	✓	✓
M		7	—	✓	✓	✓ } prototroph. Revertials on ERS Mal.
		9	—	✓	✓	✓
		23	—	++	++	++
		30	—	++	++	++
C		35	✓	✓	✓	✓
		36	✓	✓	✓	✓
		37	−✓	−✓	−✓	−✓
		38	✓	✓	✓	✓
		7	−−✓	✓	✓	✓
H		12	−	+	+	+
		23	✓	✓	✓	✓
		24	−,✓	+ (=?)	+,(−)	+(−)✓

∴ H12 is haploid segregant.

C: none heterozygous!

M 1 prototroph partial segregant

M 2 auxotroph segregant.

No information!

Irradiation of H226

June 12, 1950.

Exp. 757 interrupted owing to "Virus Seminar".

Repeat. A = H226 10^{-7} B = H226 10^{-7} UV 20 sec. Plate on EMB lac, and pick Lac v. Spot on EMS lac EMB Mal EMB Xyl

A). 100 suspensions.

nogr. on # 2, 29, 43. Remaining 97 are all Mal+ Xyl+ (v presumed).
" " on EMS: #49 Residuals: Nogrowth

B). 99 ~~100~~ suspensions.

nogr: 36, 44, 56, 91

Xyl − 28, 31, 37, 83

Mal − 37, 39?, 43 83, 89.

Auxotr: ~~37, 39?, 4~~ 6, 9, 10, 13, 17, 18, 25, 26, 27, 29, 32, 34, 35, 39, 43, ~~##~~ 46, 48, 49
Lac-pr.: 5, 12, 30, 45, 57, 63, 72, 74 51, 52, 53, 59, 61, 62, 65, 66, 67, 69, 70, 75, 78, 79, 80, 81, 84, 86, 87, 89, 91, 95, 96, ~~100~~

Exceptions: 7 Fermentative (Mal- or Xyl-)

Residuals on EMB Lac. Examine for non-segregating + colonies.

Lac − 95 None found.
ng ~~100~~ 49 52

Replate lac v for confirmation of EMS character.

This test takes into account only those cases in which no Mal+(e.g.) is produced by the colony. A closer test would involve the fractions of Mal− and Mal **S** which are partial segregants

Retest single lacV colonies

Mal: − : 6, 16,17 22 24 37 40 42 43 51 52 53 56
63 64 71 83 89 90 92 98

Xyl − 4 7 14 18 19 25 28 31 37 38 42 43 58
63 64 68 75 83 92 97

MR − 4 7 14 18 20 24 25 30 36 37 42 43 51 52 58
63 64 68 72 80 83 84

DISCREPANT TYPES. lacV:

Mal	MR	Xyl	
−	−	−	~~37~~ ~~24 42~~ 42, 43, 63, 64, 83
* −	+	+	16, 17, 22, 40, 53, 56, 71, 89, 90, 98
+	−	−	4, 7, 14, 18, 25, 58, 68,
+	−	+	72, 80
+	+	−	~~*~~19, 28, 31, 38
−	−	+	~~24, 42~~, ~~37~~ 24, ~~*~~37, 51, 52,
−	+	−	92

Prototrophs	1	11	21	31	41	51	61	71	81	91
1	·	+	+	+	+	·	·	+	(+)	·
2	+	·	·	·	+	·	+	·	+	+
3	·	·	+	+	·	·	·	(+)	·	+
4	+	+	·	−	·	+	+	*	−	(+)
5	+	+	−	·	−+	·	*	(+)	+	·
6	+	·	−	+	·	+	*	+	−+	·
7	·	·	·	+	+	+	+	+	·	+
8	·	·	+	+	+	·	+	+	·	+
9	+	+	+	+·	+	·	*	+	·	·
10	·	+	(+)	·	+	+	·	·	+	

	Mal	Xyl	Mtl	Nutrition
4	+	−	−	+
6	−	+	+	+
7	+	−	−	−
14	+	−	−	+
16	−	+	+	−
17	−	+	+	−
18	+	−	−	−
19	+	−	+	+
20	+	+	−	+
22	−	+	+	−
24	−	+	−	−
25	+	−	−	+ (±)
28	+	−	+	+
30	+	+	−	(+)
31	+	−	+	+
36	+	+	−	+
37	−	−	−	+
38	+	−	+	+
40	−	+	+	−
42	−	−	−	+
43	−	−	−	−
51	−	+	−	−
52	−	+	−	−
53	−	+	+	−
56	−	+	+	+
58	+	−	−	−
63	−	−	−	−
64	−	−	−	+
68	+	−	−	+
71	−	+	+	+
72	+	+	−	−
75	+	−	+	+
80	+	+	−	−
83	−	−	−	−
84	+	+	−	−
89	−	+	+	−
90	−	+	+	+
92	−	−	+	+
97	+	−	+	+
98	−	+	+	+

18 −
22 +

Total aberrants (no overlaps)
are 40 fermentative (18 aux + 22 prot)
+ 19 aux (sum +);
59% detected changes!

S^R x Prototroph Method

September 21, 1950.

Inoculate 8 PM 9/20 from young aerated cultures in Penassay. Grow overnight + wash.

Aerated.

1 K-12
2 K-12 + W-1177
~~3 K-12 + W-1276~~
~~4~~ 5 W-1276
5 W-1177

Unaer. (less time)
6 K-12
7 K-12 + W-1177
8 W-1177
~~9. W-1276 + W-1177~~
= [illegible]

Results:

9/22 1 ~~4~~, 5: 3 plates each. No colonies

2: ca 1000/plate

9/23: 1 colony on 5. (K-12 S^R mutant) Check unselected inocula.

See 763

9/23 6; 8 2 plates each. No colonies

7 ca 400/plate.

Test single colonies on EMB lac

760-2 Either found, on EMB lac

	+	−	?
	42	46	88
	57	41	98
	109	87	196

χ^2 =

Conclusion: (11/12/50): $S^R X^+$ selection is a reliable method for detecting recombination.

Pseudomonas fluorescens

Preliminary and penicillin rem.

Sept. 21, 1950.

P. fluorescens. A3.12 received from R. Stanier.

Grow at 30 ±. Aerated cultures gave heavy growth overnight in Penassay or in D(glucose). However D— also supported growth, presumably due to citrate utilization.

a) Test "PF" base: per liter with addn. of 0.1% substrates.

K_2HPO_4	4
KH_2PO_4	4
$MgSO_4$	.5
NH_4NO_3	2

n.g. with benzoate or glucose.

Throw out!

b Dilute cells from dense Penassay culture 1:20. Add varying units penicillin and aerate. 230 PM.

Pen.	4 30	6 30
0	++++	++++
50/ml	"	++++
100	"	+++
500	"(+++?)	++ lysed
1000	++ lysed? (granular deposit).	

∴ 500 – 1000 units/ml will lyse Pseudomonas fluorescens.

9/22/50. [20-40-60 sec. doses. Dilute in Penassay 1145]

Wash 48 hr. aerated D(Glu) culture and resuspend in H_2O.

UV at 50cm 10ml samples in Petri dish. Dil. ½ml/10 and dilute from this as 10^0 for viable counts

UV sec.	Count
0	5.3×10^7; 4.8×10^7 – 5×10^7
20	5.6×10^6
40	5.5×10^6
60	10^7

See over for conclusions

Auxanograms – P. fluorescens

Culture Responses

		(old symbols)	new symbols · TEST	
1	Yx, HC, A1(?), A5	A5	A4	HISTIDINE (±)
2	Yx HC A3	A3	A2	A2. (comping.)
3	Yx HC		A2	
4	Yx. HC very slight		A2.	
5	A3	A3	A2	A2
6	Yx Vits?	Vit?	V	not VITS.
7	Yx HC A4 A5	A4	A3	ϕalan.
		A5	A4	HIST (±)
8	A3	A3	A2	A2
9	A4	A4	A3	TRYPT. HC ng
10	A4		A4	
11	Yx HC	A4	A3	TRYPT. HC ng
12	A4			

Further tests

					PF
1				HISTIDINE	1
2	IV	✓			2
3	IV	✓			
4	IV	✓			
5	IV	✓			
6	Rundown		9/30 A2		
7				ϕalanine histidine	3
8					
9	IV	✓			
10				TRYPT.	4
11	A4		TRY HIST.		
12				TRYPT.	

10/1 Throw out all but PF – x stocks.

Notes:

PF-1. Growth on histidine is slow and limited compared to A4.

761-8. After 48 hours, growth on glutamate exceeded histidine. Try hist + glut and hist, glut + vits; + gna.

PF-2 Growth on A2 ~~slightly~~ considerably faster, than on isoleucine-valine. (balance?)

PF-3 and PF-4 probably preferred as mutants for further work

Pseudomonas – penicillinum

Sept. 23, 1950.

P.F. A 3.12 irradiated 60 sec. Grown overnight in aerated Penassay. Remoculate into Penassay 9^00 AM for young growth.

2 PM wash and resuspend in D(2Glu). Rm. temperature

2^20 aerate A) .5/10 dilution

B) .05 x .2 = .01/10 dilution

2^40 add 1000 u penicillin per tube. (1000 u/ml)

6^15. A strongly lysed cf. non glu – non pen. control

Plate A, B, in D(0); NSA.

9/24 1PM. Comparison of D(0) with NSA impractical owing to failure of subsurface colonies.

A2 → ca 30 surface colonies

B1 → ca 3-4.

Pick from each to water. Test on NSA; D(glu 0) agar.

A: 30 tests. 11 did not grow on D(0).

B: 20 tests 2 did not grow.

lower dilution into penicillin is not more effective. Possibly a longer interval should be used.

Pick presumed mutants to nutrient agar for ~~pres~~ preservation.

Further colonies tested

A 20/27 + 13/19 = 33/46 Store on NSA plate.

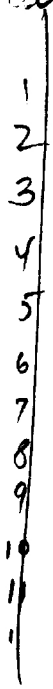
B 4/10

Pantagrams.

Positions 1-10 on periphery
A-D in center

A Yx	1 A12
B HZ	2 A3
C YNA	3 AV
D V.t.	4 A5
	5 A6

Picture



Responses

Test remaining stocks of mutants on D-agar + gal, tupt, ~~his~~ IV, hist + glut. to screen hitherto undetected mutants. Hold only those which show ± response.

	D -- agar
PF 0	++
1	++
2	+
3	++
4	++

Keep ~~14~~/36 as distinctive mutants. Some ± growth not retained.

Test on plates & random supplements. Allgrow on EMB.

		HC	V,T	YNA	A1	A2	A3	A4	A5	FINAL ± TEST IN LIQUID
PF 0		+	++	++	+	+	+	+	+	
	1	+	−	−	−	−	−	+	−	
	2	+	−	−	−	+	−	−	−	
	3	+	−	−	−	−	+	−	−	
	(4)	+	++	++	+	+	+	+	+	
PF #6	1 A2	+	−	−	−	+	−	−	−	LEUC
	~~2 XX~~	+	± +	+	±	+	± +	+	+	
	3 A2	+	−	−	−	+	−	−	−	LEUC
7	4 YNA	−	−	+	−	−	−	−	−	GUAN OR AA AD±
	5 HC	+	−	−	−	−	−	−	−	
8	6 A1	±	−	−	±	−	−	−	−	ARG AD, GU OR wk±
	7 YNA	−	−	+	−	−	−	−	−	
9	8 A1	+	−	−	+	−	−	−	−	METH GUAN OR
	9 A1	+	−	−	+	−	−	−	−	
	10 A1	+	−	−	+	−	−	−	−	
	11 A1	+	−	−	+	−	−	−	−	
	12 YNA	−	−	+	−	−	−	−	−	PUR
	13 YNA	−	−	+	−	−	−	−	−	PUR
	14 ??	−	−	−	−	−	−	−	−	

ERROR? CROSSFEED?

4 YNA
2 A2
5 A1
1 XX
1 HC

(see over)

PF5 = PF3 S^R (surface plating - ca 100u/ml effective).

Still to check:

5: AA OK Single add. allo. Single om: (−3, −2) } no growth

9 } ——— Meth
10 } A1 Meth.
11 } ——— Meth

14 Yx. ⟶ YNA + V gavrg.

PF 1

Hist + A1235 ⟶ +++ other AA stim.

− Hist (AA) ⟶ −

Hist + V +

Hist +

" + Isleut +

" + YNA +±

PF 4 now prototrophic. T. O.

5 ϕal + A2 (L, Isol, IV n.g.)

14 YNA + B12 !! Purine n.g. for YNA

H-1 F2 self. 762

September 23, 1950.

Lac+Mal+ Lac,-Mal,-

W478 × W1178.

2- : 100+

in EMS Lac.

9/25 Pick 100 lac+ colonies and streak on EMB lactose.

All lac++

9/26. 28 addnl. Lac+ 2: Lac v. (762-1, 21.

9/29 ← EMS Lac → Restreak 4 single lac+ from EMS to EMS, EMS lac + Mal.

1. all Mal++, lac v. ~~in EMS lac.~~

2. " " "

Conclusions:

This cross yields too many Mal+ (lac+ and lac v) to be very efficient in testing for Mal- lac v hemizygosity. Remeow with other combinations of markers. See W1325 × W588 (767)

E. coli outcrosses

September 23, 1950.

See 760. (W1177 × K-12).

Proc. from overnight cultures W1177 + various prototrophic E. coli isolated by S. Shapiro from chickens. into Permassay 9 AM

Wash + plate mixtures on D(SM) agar.

	#	
1	803	
2	115	
3	110	
~~4~~	~~111~~	~~3 plates each.~~
5	109	
6	105	

no X+ S^R noted

X+ prototroph
X− auxotroph

" " " 7, 8 nogr.

Carbenicillin, using 2 drops from mixed Permassay culture, overnight, as inoculum, without washing, on D(SM) plates.

W-1177 + Culture #

S^R prototrophs

K-12 3 plates: ca 50/plate

	Aq. Bact. #		
7	866	31	111
8	114	32	820
9	7	33	114
11	10	34	
12	23	35	
13	834	36	816
14	841	37	106
15	865	38	845
16	863	39	838
17		40	120
18	828		
19		111	
20	833		
21	827		
22			
23			
24	2 plates 830		
25	825		
26			
27	839		
28			
29	818		
30			

other cultures, 1 plate only.

No X+ S^R found in any of these test crosses

E. coli outcrosses.

9/27/50. Plate mixtures of indicated strains + W1176 on DSM without washing:

W 1115
1113
1114
1117
W1045
W1176
} 2 plates (2-3 drops culture)

W1258 5 plates. No X+ S^R.

No X+ S^R colonies except for

1) 1176 → pure lac−

1) 1115. → pure lac−

Pick and streak out on EMB Lac.

9/29/50. Concentrate mixed cultures above from 10 ml YE to ca. 1 ml H_2O and plate .2 ml per plate. W1113, W1114, W1117 gave pellets not readily redispersed. Disperse these as far as possible P30.

B.

(CONCENTRATED SUSPENSIONS)

10/1/50. B: K-12 x gave 10^3 X+ S^R per plate

	1115	1 (2 plates)
	1176	2 (2 plates)
	1045	ca 50 /plate (2 plates)
10/2/50	1258	0
	1113	0
	1114	0
	1117	0

10/3/50. Plate 12 plates, conc. W1117 — no S^R X+.

Stock is also pure − in lac, Mal EMB.

760-5 is mixed on EMB Mal: must be a mixed culture, or somewhere. Not a W1177 X+ lac+ Mal+ !!

Marker tests on various prototrophs

Tests on EMB Lac, Mal, etc:

	Culture	Lac	Mal	Xyl	Gal	Sucr.
1	W 1177	−	−	−	−	+
2	W 1176	+	+	+	+	+
3	W 1115	+	+	+	+	+
4	W 1045	+	+	+	+	−
5	760-5	+	− +	−	−	−
6	K-12	+	+	+	+	
7	K-12 × 1177	+	−	−	−	
8	"	+	+	+	−	
9	"	+	−	−	−	
10	"	* −	−	−	−	
11	1115 × PUR.	−	−	−	−	−
2	1176 × PUR.	−	−	−	−	−
3	1176 ×	+	+	+	+	+
4	1176 ×	+	+	+	+	+
5	1045 ×	+	+	+	+	
6	"	+	+	+	+	
7	"	+	+	+	+	
8	"	+	+	+	+	
9	"	+	+	+	+	
20	"	+	+	+	+	
11a		−				−
21-30	1045 ×	all +	all +	all	+	all +
31-42	direct from colonies in suspension.					++

! Repeat. See 760

Re: K-12 see 6!

1045× very likely S^R mutants. W1115×, 1176× may be either recombinants or very peculiar types like 760-5 which demands explanation.

Inoculate Y2 with stock W-1177. Inoculate heavily into DSM.

H248 : 3 papillae on Mal EMS. (hemizyg. test)
all bac - segr. pure Malt pat in teat.

E coli outcrosses

10/8/50 + prev.

W1177 ~~plate~~ cultured with

1 W1176
2 W1115
3 W1258 in YE broth 24 hours. Wash + plate very heavily (ca 10^9/plate DSM).

Controls: W1177, etc. alone: no colonies

1. ca 200/plate
2. ca 1000/plate
3. 0 colonies. (10 plates)

These colonies on test proved to resemble their prototroph parent: Lac+ Sucr± and Streptomycin-sensitive!!

10/~~9~~/50. Repeat with fresh cultures:

Sh24x 5 plates ca ~~4~~/
Sh24c 2 plates ca ~~15~~/

W1045x 3 plates 1
c 2 plates 0

Brush ~~Stroke~~ on EMB Lac, Mal. Result very peculiar:
all slow on Mal. Sh24x; 1045x are Lac+, -
Sh24c Lac+.

Sh24c Lac+ Chief prototrophy! — none prototrophic!
Sh24x (1-8): Lac- S^R
W1045x Lac- S^R

probably S^D mutations.

W1177 x

1	Gaunt +	4.	W1113
2	W1281 (Coli Lisbonne - Cavier)	5	W1115
3.	ML (Monod - Lwoff mutable)	6	W1176

1.	5 ⊗ ; 2 control plates	0	
2.	5 ⊗ 2 " "	0.	
3.	5 ⊗	16	#1-16
	2 c	0	
4	2 ⊗	0	
5	2 ⊗	1	#17
6	2 ⊗	15	#18-32
	1 c	3	#32-34.

Pick all colonies to water. Spot on DSM for preservation.

Streak

1-16 (ML x 1177) : all Lac - mutable; Malt (~~numerous~~ S^R mutations ??)

Peculiar sectoring noted in ~~the~~ colonies on EMB Mal

17 mucoid on water on EMB lac. mottling of fluid streak.

SD ? { 18-31 weak growth on EMB lac Lac -

32 mg. on EMB lac

DSM plate check.

#1-17 show peculiar mottling of colonies on EMB Mal.
Resemble (from #3), and ML itself. However, ML is definitely a weak MH+; 763-1-16 are all strong MH+. (May be effect of S^R mutation)
Clones:
#3 resembles ML in variegation on EMB Mal, from strong to weak Mal+.
Not unique.
#1-16 also resemble ML in Lac^{-mut}; Mal+; Xyl+, (V_1^R) and must be regarded as S^R mutations in absence of evidence for recombination of any other markers.

763f- #17 appears to be Lac+ Xyl- MH- V_1^R. v. Poor growth on EMB xylose!
From W1115 x W1177.

Compare 1. W1115
2. W1177
3. #17
4. 1+2
on various media.

EMB:	Glu	Lac	Suc	Mal	MH
1	+	−	+	+	+
2	+	−	+ −	−	−
3	wk.+	wk+	+	wk+	+ wk
4	+	+, −	+, −	+ −	+, −

#17 is a weak fermenter, but may be merely an S^D or S^R type mutant from W1115. It does not provide evidence for recombination.

S^R/S^S heterozygotes

Sept. 26, 1950

9/29 W67 x W1177 heavily plated on EMS lac 7 plates x ca 200/. = 1400 2 (lac+) prototrophs. Restreak on EMB lac, EMS lac, EMB Mal, DSM.

#1. Mal−; gives a few papillae on D(SM). lac v. grows poorly on EMS lac.

#2. Mal−; grows well on EMS lac. large colonies on ~~D~~ DSM. streak out several colonies of each on EMB lac for segregants for S^S/S^R test.

---

6 EMS lac+ colonies to ~~EMS~~ EMS Lac (SM)

3 " lac ± . (noted on streaks of 764-2)

all S^R

ca 30 Xyl− S^R (taken from EMB lac segregants).

This culture H248 is evidently pure S^R Mal− Xyl− (coupling of S^R to Mal− is not unexpected). It may be useful in physical comparisons with K-12.

B Repeat W67 x W1177, v. heavy parental inocula, on EMS lac. After 3 hrs., ca 1700 prototrophs. 10 possible lac+ picked for test: streak on EMB lac; spot on EMS lac; smush v. streptomycin on EMS Mal

	lac	EMS	Mal	S				
1	−			R				
2	++			R				
3	−			S				
4	−			R				
* 5	V			R	1	Pure Mal+	Xyl+	S^R
6				R				
* 7	V			R	2	"	−	
8	++			R				
* 9	V			R	3	"	−	
* 10	V			R	4	Pure	−	Xyl− S^R

None of these is segregating S^R/S^S. Repeat cross again (to look for Mal v)

→

to S^R dominance?

764C

W67 x W1177

10/11/50.

Cross 67 x 1177, 10/9/50 EMS Lac.

C Colonies examined A12. 14 plates ca 100/ = 1400.

3 colonies picked: (2) very doubtful). Test on EMB Mal
#1 + 2 Lac v Mal −; Mal + S^R. EMS Lac (S14).
#3 Lac − Mal + S^S

D1 ① W67 + W1177 + ladded volume x 2 = 900 to

② Separate mtl washed.
concentrate each from 20 ml combined volume to ca. 1.5.
(Spread .1 ml each on EMS lac (± camphor))
Yields in 1 and 2 ca same. (① may be not more than 2 x ②).
2?? Lac + in 19 x 50 prototrophs
1 (Acenaphthene) Lac − Mal + S^S
2 (Camphor). Lac + (pure) Mal − S^R

2da → Neither is Lac v.

3da. P15. 2 additional possible lac +
(15. - camphor ② Lac v Mal −
7. - acenaphth. ① Lac − Mal + Gal v Mtl − Xyl −. Gives many lac − proto (partial / ~~trophy~~ segregation?)

P17: P## (camphor; colchicine; controls)

(E) very poor yield.
20 plates x ca. 25 / plate = 500. No lac v.

(F) 10/19 10 plates. Parental inocula derived from cultures exposed to camphor on EMBagar for 60 hours.] Ca 150/plate. 12 Lac + segs. (ca 10%!) (Reincubated more)
Is this due to character of parents?
4 additional lac + 10/22. (14-17)

No suitable S^R/S^S heterozygote recovered.

10/22/50.

F's streaked out on EMBlac (poor batch!), EMSlac; EMBMal.

	Mal	lac	
1	+ ✓	✱	✱ v? ✓
2	—	✱	v?
3	—		v?
4	—		v?
5	—		v?
6	+ ✓		✱ ✓
7	—		V
8	—		V
9	(✓ mucoid)		V mucoid
10	—		V
11	—		V
12	+ ?		V ✓
13	—		V
14	—		V
15	—		V
16	+		V
17	—		V

Reticule 1, 6, 9, 12 for Mal, lac V.

Save F1 as example of Mal+ lac V.

all Mal − lac V were strongly mucoid on EMSlac Mal+ were not.

See 771 D

9/26/50 ff.

A) W478 2 plates × 300. (600) 1 Mal− (sectorial). Purify and slant as W−1323 (uv 7 secs.)

W466 2 " 0.

10/1/50 W466 14 plates, 150 (2000) 1 sectorial → 1 Mal− } 1324; 4 −? → 1 Mal− } 1325; → 1 Mal slow 1326

10/3/50. W1326 Appears + in thick streak portions. May be highly unstable.

765-4 Many "−" colonies have + wedges. Restreak these

All Glu ++.

−+ sectors give a profusion of Mal+ colonies.

Restreak apparent pure "−" and "+" [*] These remain pure.

However, from original plating [*], a Mal v line was established.

Hold in refrigerator.

10/6/50. After 3 days, Mal− colonies show + papillae and sectors. Picks to test for segregation.

10/7 → all streaks show: Mal v (D) and Mal− ; a few Mal+ (? E). → W1327

? W466 → = → −/+ → ±/+ ; → = mut → ∓

Hypothesis

A * Streak on EMB Mal as 765-4A. Put on NSA slant (gives mixture).

B * Re " " " 765-4B. Pure Mal+. This turns out pure −

C = W1327

D = W1329

E = W1328

Unstable Mal+ ⇄ Mal−

↓

Stable Mal+

Pseudomonas: double mutant penicillin-res. 766

Sept 30, 1950

PF-3 = ϕ alanine
PF-4 = tryptophane

Aerate overnight at room temperature. Harvest and resuspend in saline. Irradiate 9 ml samples in Petri dish, 40 secs.
Reinoculate 1 ml samples into Yz, aerate 10 AM —

n.g. Agglutinated too heavily in saline

10/4. Repeat, washing in H_2O. with PF 3.
Irradiate 40 secs. Inoc. in Yz, grow overnight
Regrow 1:1 inoculum 4 hours. Wash, treat with 1000 u pen. for 4 hours in D (ϕ-trypt). Regrind, & aerate.
Plate out on EMB lac. Platings of 10^{-3} unpickable.
about 60 clones of PF 3 / tested: no mutants. (Poor lysis)

No useful result

~~9~~ 10/4/50.

1. W1323 × W1177 Mal EMS. Mal×
2. W1324 × W1177 " Mal, ??
3. W1325 × W1177 " Mal, ✓
4. W1324 × W588 Lac EMS
5. W1325 × W588 Lac EMS.

All crosses gave good yield. Parents checked for purity: o×.

1. gave Mal+, ca 2%. 16+/433 total one plate
   5/260 total. " "
2. gave Mal− only. o+/ca 1000 (2 plates) Mal,
   Later: 1+ noted. Confirmed on EMS Mal. Crossover or Recomb??
3. gave Mal− only o+/ca 1200 (3 plates) Mal,
4. 30−: 18+
   31−: 8+
   39−: 22+
   100−: 48+ / 148.
   Instandard cross, excess lac + expected.
5. 119−: 30+
   91−: 32+
   210−: 62+ / 272

Pick lac+ for test as lac ✓. Spot to EMS Mal. Select "lighter" Lac+
4:1-11
5:1-12

Test these crosses in EMS Mal

10/7/50

#4	Lac	EMB Mal	EMS
1	V	—	—
2	V	—	—
3	+	+	+
4	+	+	+v?
5	+	+	+ ?
6	+	+	+v?
7	v ?	—	—
8	v ?	+	+
9	V	—	—
10	V	—	—
11	+ ?	+	+
12			

13–44: lacV
lac+ : 22, 35, 34?
?? :

All Mal+ on EMS except 14, 22, 31, 35, 46, 47

Restreak likely lacv on EMS Lac for purification

	Mal	767-
1	—	1
2	+	
3	—	2
4	—	3
a 5	+	
6	—	4
7	+	
8	—	5
9	—	6

	767-	
2=H2?4	7	
3	8	→ Mal + lac v [illegible]
4	9	Mal+ lac v
b 5	10	Mal+ lac−, no test.
6	11	

Reversions:
2: (3) pure Mal+ lac v (?); +(1) — Mal Δ/− → Mal Δ/+.
3: (1) pure Mal+ Lac+: no test

Conclude: 767-9 (rstests) and 767-2 are clearly Mal-/Δ (hemizygous)
767-8

Het microsses.

767b

10/7/52

#5	Lac	B Mal	S
1	V	−	
2	V	−	
3	V	+v?	
4	V	+v?	
5?		+	
6?		+	
7?		−	
8?		+	
9		−v?	
10		+v?	
11		+	
12		+	

13–36 lac: V 13 17 19 24 36

\+

? 14 21

Mal −: 21 22 24 29 30 35

Reciprocals likely lac V on EMS lac for purification; ∴ Mal EMS for Mal+ rev.

H253	1	Mal+
H254	2	−
	3	−
	4/5	−
	6	−
	7	Mal+
	8	Mal+

[1 M+L+ (rodent)].

4 Mal+ → 3 Lac V; Mal+s. ; 1 lac − Mal+. 3 rodt: Mal−/Δ

Het microsses → Mal−/Δ

PF crosses.

10/9/50.

Cross streak, incubate 48 hours EMB lac. Pick growth from intersection and spread with loop on D(0). Record # colo. 48 hrs.

PF ×	1	3	6	7	8	9
1	(0)	0	0	0	0	0
3	0	(0)	0	2	0	0
6	5	0	(0)	0	4	2
7	0	1 mm	15 ±	(0) mm	0	0
8	0	0	1	1	(0) ×	3
9	1	1±	0	0	3	(0)

Note that parental controls are all negative. 6 × 7 is the most promising. (also 8 × 9)

10/13/50. Inoculate Y± from EMB Brushes. Grow still 24 hrs; aerate 24 hours. Wash + conc. ca 5×. Plate. 1 ml per plate D(0).

PF		
6	1-2/pl	
7	100/plate	Diffuse growth.
8		
9	1-2/pl.	
6 × 7	2-3/plate	diffuse or 10^3/plate
8 × 9		

No evidence of crossing! Put addnl. markers into PF 6, 9 and attempt these. (e.g. S^R)

Inconclusive. Used additional markers.

10/9/50.

Streak W1327 and 1328 on EMB Lac for reversions. Pick papillae for examination as lac V.

W1327

	Lac	− Mal
1		↓
2		
3		
4		
5		
6		
7		
8		

all almost pure + no obvious variation

W1328/9

1		−, V
2	little.	V, −
3		V, −
4		−, V
5		V, −
6		V −
7		V −
8		V, −

This suggests a mutable Mal allel rather than segregation. For 1327, select a lac+ and streak out to allow lac analysis; Mal reversions.

B) 1329: Pick a Mal V from each and restreak on Lac; Mal.

c) Pick any possible lac V from ~~EB~~ reversion plates and restreak.

"W1328" No pure + obtained! Study other reversions of W1327

c1 1 possible lac ±. Restreak single colony → pure lac+.

B: #3, 5, 8 are pure lac+, Mal V. Others happen to be Lac − Mal V
No pure Mal+ seen. Resegregated lac+ Mal V as W-

D. Papillae from W1327. Streak out individually: are there any Mal++?

W1327 reversions. 769D

8 Mal+ reversions from single Mal− colonies tested for purity. Some − components

	Mal
1	+ −
2	++
3	++
4	+ −
5	v
6	v
7	++
8	++

Restreak − and + components of 2, 3, 4, 7, 8.

Confirm: stable + ✓.

10/19 Repick associated − and + to determine whether a single − type gives both Mal+ and Mal+^{m}. Transfer + to slants as 7 & 8 etc.

D7 is distinctly more powerful Mal+ than the others.
D2− is most papillate

Reversions from 769 D:

2	almost all variegated.	
3	occ. Mal v.	Some Mal++, Mal+?
7	mostly Mal v	" " " ?
8	occ. Mal v.	

∴ all Mal− give Mal v reversions; some Mal+ ??

P 10/21 Restreak single Mal+ or v colonies from above.

2	varying degrees of instability
3	2 stable +; 1 stable ±*; 1 variegated +
7	2 stable +; 1 unstable ++*; 1 variegated +*
8	1 unstable very weak +.* 3 variegated ++

Repick interesting types *

10/21. Cross D7+ with Y10 for "suppressor" to 1 possible − / ca 1000 protoclones (& plates)

↳ streak out: mucoid Mal+ (not Mal+)

∴ W1327 probably differs in only region from K12

10/7/50.

Irradiate PF0. (30 sec.) Incubate in D(0) glucose.

A 8: Inoc. into T(mand) to preadapt 3 - 4 hours (heavy inoculum 1:1)

Wash and reinoculate 1:10 into T(mand). After 5 mins add penicillin to 1000 u/ml. After 5 hrs & overnight spread on EMB Lac. Test on EMB Lac; T(mand)agar

P10 55 tests : no mutants

Addnl test

P11 (Rhoplis Fin). 50 tests " ". No mutants.

ca 50 addnl. tests : " "

Query: Are methods suitable for C-source : 1. Penicillin selection
2. Agar growth tests.

Check 1 by reconstruction with Mand- strains
2. by T(m) agar (- mandelate).

EMB lac used solely for convenience & for identification

10/18/50

a. W1303 × W1178
b. W1303 × W1177

10/20 c W1304 × W1303

10/21 d W1304 × W67
e W1304 × W478

10/21 a. very infitile: ca 5-10 small colonies/plate. Reincubate. 8 plates
Meanwhile 2 lac+ seen. Picke to EMSlac, streak on EMBlac, Mal. (lac v ? + ? / Mal −)

b 11 plates ca 40/plate (fruit crop). New small colonies appearing: reinc.
No lac+ seen.

10/22 a. About 2% lac+. Ca 100/pl. 13 lac+ picked.

(b.) ca 200/plate. No lac+. Striking colony dimorphism 0°0
2000/0 apparently both lac−.
Picke to EMBlac to verify lac− of large colonies.

(c) ca 150/pl., small colonies. 14/pl. No+ Reincubate No+. 2000/0

(d) 12 plates, ca. 20/pl. No+. Reinc. 1 unlikely +. No+ 250/0

e ~~Good yield~~.

+	−
~~58~~	21
48	19
3	3
11	7
28	32
148	82
+	−

Repeat:

10/23 b 10 × 50/ 1 mucoid lac? pure lac+ Mal+. An Mal EMS, ca 10% Mal+. 500
c 10 × 150 1 lac+: ~~[illegible]~~ " 2+ : 8− 1500
Mal− lac v 771-C1 See over ↑
d 11 × 50 0+. 550
5 × 0 − Repst at 42° 12 hours after plating. Then 37.

771 C → see C1

Repeat W1303 – 1304 crosses.

10/27. large scale

$b^{\pm}$	1303 × W1177	2 × 30ml → 5ml	.1/plate	Shake mixtures at 37
$c^{\pm}$	303 × W1304.	2 × 20ml → 5ml	.1/plate.	11:10 – 2:10

b1 repeat 40° 2:30 → ||A>B (B) — 6:30 (A)

↓ b2 37° At 6:45 PM, expose to ~~(seconds)~~ UV 50cm distance

10/30. b. Very low yield. 768/36 plates. Many small colonies.

b1A Ca. 10-15/plate (3 plates)
B 0. 1 plates

b2.

2sec	109	(small colo.)
5"	146	var. size
10"	47	" "
15"	181	" "
20"	98	" " many small.

1-4 from b; 5 - b1A; 6 = b2-2 7,8 : b2-5 9 b2-10
10,11,12 b2-15
13,14 b2-20.

c. 37 plates very low yield c: 15–38

[28–38 from one plate. Destroyed feature may = possible excess accumulation of MB.]

None + except: 2, 6, 7, 8, 10, 11, 12.

Relabel 1–7, 771B

Results of W1303x crosses

10/23/50.
W1303 x W1178.

a.	EMBlac	Mal	EMSlac —	
1	v	—	+	
~~2~~	~~—~~	~~—~~	~~—~~	
3	v	—	+	
4	v	—, + ?	v. poor growth – + ?	✓ Lac v ~~+~~ ~~mottled~~ Mal —
5	+, v?	—, +	v p.g.	
6	v	—, +	v. p. g	
~~7~~	~~—~~	~~—~~	~~v. p. g. mucoid~~	
8	v	— mottled	v. p. g.	
9	v	+ mottled	+ + single cols.	Lac v Mal + mottled *
10	v	+ –	— + → reisolate	Lac v Mal —
11	v	+ –	— + → reisolate	Lac v Mal —

AR1 ~~12~~ 2
AR2 ~~13~~ 7

verification needed from EMSlac.

Restreak where possible.

* Restreak. Pure Mal+ but mottled (modifiers?)
No Mal v.

✓ Reisolated cultures

	Lac	Mal
1	v	— (see footnote)
2	v	—
3	v	—
4/5	v	—
5	+	—
6	v	—
7	v	—
8	v	—
9	v	+
10	v	—
11	v	—

771B

	Lac	Mal	SM EMS	SM EMB
1	+	—	R	R
2	v ?	—	R	R
3	+ ?	—	R	R
4	v	—	R	R
5	v	v	S ... (S R)	R
6	v	—	R	R
7	+ ?	+	R	R

Xgl 1HR ...

Segregating S^R/S^S

Streak Mal v colonies from 5.

Many Mal − and Mal v. Few or No Mal +. Repick possible +.

20 Mal − : S^R

Reisolated cultures, after purification

	Lac	Mal
1	+	—
2	v	—
3	+	—
4	v+ ?	—
H257 5	v	v
6	v	—
7	+	+

Check #4 ✓ Lac v. But very stable.

c1 see 771. Mal++ Lac^{v} extreme "bullseye" type ⊙

Revtreak from center of ⊙

95% Lac−

· Pure Mal+ Sal+ Xyl − Mtl −

EMS Lac 48h: colonies are variegated on EMS Lac.

Revtreak on EMS Lac; EMB Lac. Reversion EMS Mal.

− Picks from ⊙ center → almost all Lac − (some pink, some blue). Very few ⊙

→ ca 10% Lac+ on EMS! Extremely unstable Store on # D Lac

Attempt to find Mal+ revertants still Lac^{v}.

771C1-1 : all M+ Lac −.

2-5 also.

This enterprise is very difficult owing to the extreme instability of 771C1.

10/25/50.

e. 16 lac+ streaked out on EMBlac. Restreak[12] on EMSlac likely lac-v.
3 additional 24 (small colo.). Take 4 as likely lac-v.

	Mal		Lac	Mal.
1	—		√	√
2	—	nogr.		...
3	—		√	√
4	++		√	√
5	—			+
6	++			+
7	—			+
8	+ −		√	√ —
9	—		√	√
10	— +		√	√ —
11	—		√	√
12	+ −			+ —
13	—		√	√
14	++	no. growth.		...
15	++			+
16	−			+

10/28. Repick EMSlac+ colonies to EMSLac; EMSMal (if Mal- or mixed) and EMBL

10/19/50.

.0001 ml 5% Na tellurite in ~~bag~~ phage plate W67 culture added.
$= 50 \times 10^{-4} / 25 = 2 \times 10^{-4}$ mg/ml = .2 r/ml
inhibited W67, with colonial survivors. Resistant survivors:
When cross-streaked, showed no greater resistance than W67 per se.
2r/ml plates and higher remain sterile.
Prepare DNG with
.2
.5
1r / ml Tellurite.
W-1177 poured with ±1 r/ml tellurite shows considerable turbidity, no definite resistants in 24h.

Streaking W67 and "W67/Te" (from above) on surface of .2, .5, 1r plates:

Te,	W67	~~67/Te~~ 67/Te
.2	Heavy background. freq. outgrowths	lighter background. large coal colonies
.5	light background. a few res. outgr.	Marvii bckg. many large outgrowths
1r	v. light background; a few papillae	light bkg many large colonies *

10/26. Streak W67 and W67Te, on DNZD + 1, 5, 10 r Te/ml. *pictured

24h.

	67±	Te,
1	—	+++
5	—	+ darkening
10	—	±

Medium grossly contaminated throughout experiments

W1034 × W1177

October 25, 1950.

W1034 × W1177 EMS Lac.

Yields (one. noc.) ca 30/plate. Ca. 98% Lac+

Picks 50 Lac+ and streaks on EMB lac.

49 → pure lac+ streakings 1 → Lac+ colonies / colony ⊗ Rejected.

Repeat 11/5 —

+	−
4	6
2	3
3	4
5	9.

No unusual appearance this time. Note low ratio, however!

f. 590