Crosses on low P media.

August 12, 1948.

W-251 × W-480 B-H-Ar-

T-L-B_1-Hal,-Lac,-V_1^R

Cross very heavily on a low phosphate EMS:

EMS – Phosphate

+ K_2HPO_4 M/500

+ Ethylenediamine citrate buffer pH 7.5 M/100. (= Medium 277).

Cf. EMS normal.

No colonies found at all, either on – P media or on EMS.

August 17, 1918.

Streaks out 262-J. as: (BHTLB added).

EMS: methyl sulfonate.

1. [EMS-P] + M/100 Phosph. buffer pH8.
2. " M/500 + citrate M/100
3. " M/1000 + " M/100.
4. [EMS] + ARSENATE M/1000
5. " " M/200
6. " " M/100
7. " " M/50.
8. " BARBITURATE M/500
9. " " M/100
10. " " M/50.
11. EMS + H.C. + BENZIMIDAZOLE M/1000 = 118 γ/ml
12. " " M/2000 =
13. " " M/5000.

a) Growth of + and -.

Y46 Y87.

1. limited. +, - not scored.
2.
3. Growth very poor.
4. Growth moderate; fermentation inhibited.
5. ditto
6. ditto
7. " , growth may be sl. inhibited.
8. mod. gr., no ferm.
9. Very. sm.
10. No growth.
11. Growth & ferm. OK.
12. OK.
13. OK.

b) I: Too soon to read.

o growth + - ferm.

N15: (A) Growth of Y46(+) and Y87(-).

1 G+ F ±
2
3 G ±
4 G ++± F −
5 Growth moderate. Considerable rate of fermentation.
6 G(++) F(±)
7 G(++) F(±).
8 G(+) F(+).
9 G(±)
10 G(±)
11 G(+++) F(++)
12 G(+++) F(++)
13. G(+++) F(+++).

(B). (3). G(++) F(++). V(0).
(2).
(1). G(+++) F(+++). + and − colonies, but no visible variegation!
4. G(++) F(++) + and − ", no visible variegation.
5. " " " "
6. G(++±) " +, ± some variegation?
7. " " " " !
8. G +++. F+++. variegation possible, but not easily read.
9. ++ +++ " "
10. +± ±
11. +++ +++ Variegation +++.
12. " " "
13. " " Like 8.

EMS does not show satisfactory working of variegation.

Do W-278 manipulations.

August 11, 1945.

(1). W-478 × Y-46 on Lac 5'.

(2) Plate with T6 on Lac EMB: 107 Resistants observed: all lac+.
Purify for W278/6 stock to use in crosses.
Plate c T5 : ca 100 resistants all lac+

89 tested; 1 - colony noted [279-1]. Pick + test for T6, resistance T7.
on Maltose EMS!
Mal−, $T1^R$ V_6^R V_7^R. ∴ contamination.

August 10 – 1948.

W478 x W480 on MalS + LacS.

(A). On MalEMS (noB,):

N12: 182 – : 16+] 198. Ca 12:1 (Cf 100:1 for standard)

1-15 FullMal+ 16 is sectored + and –. Test on LacS-T1 and S.O. on LacEMB.

(B). LacEMS (noB,) 15+ : 41–] 56.

(C). "Slow" or indefinite Mal+. Test for T1 on EMS-Lac and for Mal segn. on EMBMal

(A). Mal+:	LacEMS-T1	MalEMBLac.	∴ None of these are Lac segregating.
1	+ P	+	
2	– R	–	
3	– P	–	
4	– P	–	
5	– R	–	
6		–, +	
7	– R	–	
8	– P	–, +	
9	+ P	+	
10	– R	–	
11	– P	–	
12	+ P	+	
13	– R	–	
14	– R	–	
15	– R	–	
16	– R.	–	+ and – on Mal EMS.

(C). Mostly Lac –. ∴ 8 = +P. None segregating on MalEMB

(B).

August 13, 1948.

B. Lac+:

	Mal S	TI	Lac EMB.	
1	—	?	++	
2	-	P	++	
3	-	P	++	
4	-	R	++	
5	-	?	+,	V ?
6	+	P	++	
7	+	P	++	
8	—	P	++	
9	+	P	V,	—
10	÷	S	+	
maybe dupl. ← #11.	—	P	++	
12	-	R	++	
13	-	R.	+.	

Retest 6, 9, 10 and 11 from Mal S plate.

(A). Streak from Lac S to Mal EMB.

1-5 pure + 6 + and — No V. 7 – 16 All +. No Mal variegation!

(C). Ditto : 1. — 2 - 3 -, + 4. — 5-8 +, - No V.

9-11 +, - No V. 12. + 13-16 +, - No V. 17-18 +, - No V.

	Lac S	Lac EMB.	Mal EMB.	
(B). 5:	All +.	All +.	All —	No V.
9.	All + —		→	
10.	+ slower than other prototrophs.	Variegated.	All + ??* maybe variegated.	= W487.
11.	+.	+	—	

* colonies were possibly variegated, but could not be definitely scored. Streak out from Mal and from Lac on Lac + Mal + Cf.

Segregation of W481.

484
482

280b.

10 colonies from Lac EMB streaked out. 1 - (A) and 1+ (B) from each. B- not scored. Exc. where indicated

A.	1	B_1 - B+	B.	1	B_1 - B+
	2	TLB_1 -		2	B_1 - B+
	3	TLM -		3	B_1 - B+
	4	TLM -		4	B_1 - B+
	5	TL		5	B_1 - B+
	6	TLB_1		6	B_1 - B+
	7	TLM		7	B_1 - B+
	8	TLM		8	B_1 - B+
	9	TLB_1		9	B_1 - B+
	10	B_1 - B+		10	B_1 - B+

All segregants were Mal+ and ($T5^R$.) Remark: all B's show signs of sensitivity to T5, as does A10 and possibly A1. Not sharp!

W482 (6 pairs). All Mal - $T5^R$.

		40h.		
A	1.	TLM	TLM	
	2.		N.G.	
	3.	TLM	TLM	
	4.	TLM	TLM	
	5.	LM	✓	= W491
	6.	TLM	✓	= W492

B.		M		M	
		M		M	
		M		M	
		M		M	
	*	M'(+)		++	
		M.		M	Kupas W-[redacted]
					W-493

Mal $T5^S$

W484 6 pairs.

		24h.			40h.
A.	1	TLB_1	+	R	
	2	TLB_1(4)	+	R	TLB_1 *
	3	TL M	+	R	M *
	4	TLB_1(M)	+	R	TLB_1
	5	TLM	+	S	✓
	6	TM	+	R	M

	M	+	R	
	M	+	R	
Later++M *	TMB.	+	?	
*	++(TM)	+	R	
	M	+	R	
	M	+	R	

* Streaked and picked colonies.

Aug. 14 –

1-5 from Mal 6-10 from Lac. Segregating colonies to EMB.

	Lac	Mal.
1.	Mostly –, some + and V.	Mostly + and a diffuse "i"
2.	" "	" "
3.	Many + and –, also V.	All +
4.	Many – and + " V.	"
5.	Mostly –, +. " V.	All +.
6.	+ and – ; a few V.	All +.
7.	–, + ; " "	Mostly diffuse +, some sm. strong +
8.	–, + many V.	" "
9	–, + several V	All +
10.	–, +, "	All +.

Pick 10 – and + (A, B). and test on Mal EMB for phage rec. from Lac EMB.

Pick 10 Mal + & test on Lac for T5-K.

Aug. 12. 1948.

Droz. fresh 58 hrs into T(0). +.

	A 13	P 14.
O	O	±
B	+++	+++
M	±	+
T	±	−
MT	++	+++
L		±
ML	±	+++
B_1		−
MB_1	±	+++
TL	±	++±
TB_1	−	++
LB_1	−	+
MTL	++	+++
MTB_1	++	+++
TLB_1	±	++
$MTLB_1$	++	+++
MLB_1	±	+++

MT especially have considerable activity, possibly in excess of that shown separately.

August 16, 1948.

Prepare washed cultures of A 58-161 and B-W-1 from Pennassay B. Dilute to give A/B 1:1000 and B/A 1:1000. Inoc. .2ml each into tubes indicated. Assay for original content at 10^{-7} dilution, and add 3000 u Penicillin G / 10ml tube = 300u/ml: 2PM. 6:15PM, assay at dilutions equivalent to 10 x (A) and 100 x (-) original content, allowing for 90-99% total killing. Also, streak out each culture on Lac EMB

0: A/B	764 -: 1+	Total Count =	1.53×10^9			
0 B/A.	528+ : 3 -	" " =	1.08×10^9			
1. B/A T(m) BMTLB, Lac.	All+	T.C.^{9} =	.24	pS. =	~~.65~~ ~~.34~~	.65
2. B/A T(B)MTZB,).	All+		.25		.36	.64
3. B/A T(BM)	All+		.22		.30	.70
4. B/A T(TLB,)	All+		.30		.44	.56
5. B/A T(0).	All+		.35		.51	.49
6. A/B - (1)	All -		.6		.73	.27
7. " (2)	All -		.09			~~1.08~~
8. " (3)	All -		.7			.19 -
9. " (4)	All -		.7			.19 -
10. " (5).	All -		.09			~~1.08~~ -

Note: This run was made with cells grown overnight which had been washed and refrigerated in saline for several hours. The killing has been much less altogether than in Zinder's expts. It is likely that very fresh cells have to be ~~expts for A/B ...~~ used!

		−	+
A/B	0.	135	0
		169	0
sic.		156	1
		161	0
		143	0
		764	1

m = 153.

		+	−
B/A.	0.	135	2
		68	0
		107	0
		100	1
		118	0
		528.	3

m = 106

1. Crowded+ 7−

1A. 256+ 1−

2. Crowded+ 7−
A. 247+

3. Crowded+ 11−
A. 220+

4. Crowded+ 3−
A. 298+ 1−

5. Cr. + (A 349+) 14−

6. Cr − 0+
A. ca. 600 −

7. Cr (sm. cel-rect.) No+
A. 90 −

8. Cr (+) 0+
A. ca. 700 −

9 0+
9 (A) ca. 700 − 0+

10. ~~27~~
10A 89− 2+

Penicillin Radiation Runs
for glucose - mutants.

Aug. 16, 1948.

Irradiate 4 ml 58-161 suspension 5 secs. in small gem dish under Harrowia lamp. Recover 3 ml and inoculate 1 ml each in ½ gms. (2 used).

A 17. Wash thoroughly. ~~HA~~ N17. Inoc. ½ ml into

1 A. T(BM). 2 B. T(m) Glucose + B4T1B1. 2 C. T(m) Lac + B4T1B1.

2 D. T(BM). Add 300 u/ml Penicillin G and shake for 4 hours.

Plate out on Lac + Glu EMB at cumulative dilutions of
2.5×10^{-7} (5), .. $\times 10^{-6}$ (4), .. $\times 10^{-5}$ (3), $\times 10^{-4}$ (2).

A. (5). 149, otherwise. $pS \doteq .3$

B. (5) 78, 57, 69, 92, 22, 81. (2) smeared. $m = \frac{399}{6} = 66$ $pS \doteq .58$

C. (5). 94, 88, .. $pS \doteq .43$

D. (5) 296. 2 smeared. $pS \doteq 0.$
4
3
2 Smeared.

N2 assisted

Do not attempt to assay for biochemical mutants. Fermentation mutants were looked for on the (3) (4) and (5) dilutions.

Aug. 16, 1948.
W-478 x 480 on var. medig. see 64.

NB. — (A). Lac EMS·B. 40 + cols. } Nonvariegated 1: · ≥ 1. on Lac EMB.
(B). Lac EMS. 40 + cols. }
(C). Mal EMS B, 32 sectored colonies, relatively isolated, picked to water and streaked on Mal S.
(D) Mal EMS·B, 40 "pure" Mal+ streaked out on Mal EMB

A). All pure + c̄ occasional –. No variegation.

B. Not accurately readable A19. ~~37 + 38 maybe heterozygous~~ A20 No variegated cols.

(C. On Mal S. ~~xxx~~ (17-20 also inadvertently on Lac EMB (No var.)

(D). 40 tested. No varieg. possibly excepting #15. Retest.

1. Mostly + 1 – .
2. " "
3. All +
4. + and –
5. +, –
6. All +
7. +, –
8. –, +
9. +, –
~~10. +, –~~ poor growth.
11. Mostly + 1 –
12. " "
13. +, – unisolable
14. +, –
15. +, –
16. All +
17. All +
18. +, –
19. – unisol.
20. +, –
21. +, –
22 All –
23 +, –
24. +, –
25. +, – unisol.
26. –
27. +, – unisol.
28. +, 1 –
29. +, –
30. +, –
31. +, –
32 + –

C. Tests of purified Mal+ and Mal- prototrophs on Lac EMB. Lac recorded.

	Mal+	Mal-
1.	—	—
2.	—	—
3.	+	
4.	—	—
5.	—	—
6.	+	
7.	—	—
8.	—	—
9.	—	—
10.		+
11.	+	—
12	—	—
13.	—	
14.	+	+
15.	—	—
16.	+	+
17	—	
18.	—	+
19	—	—
20	—	—
21	—	—
22		+
23	—	—
24	—	—
25	—	—
26		* —
27	+	
28	—	+
29	+	—
30	+	—
31	—	+
32	—	—

Totals

	Mal-	Mal+	
Lac-	20	21	41
Lac+	7	8	15
	27	29	56

Cumulative:

Mal+ ↓ Mal- →	Lac-	Lac+
Lac-	15	1
Lac+	3	2

Lac and Malac[M+ M-] independent.

	— —	— +	+ —	++	
F =	15	1	3	2	21
Exp =	12	3+	3+	1+	

(1) W478 × W480.

(2). 58-161 × W480.

A.) Lac B, B) Lac (o) C). Mal (o).

1A. 108 + colonies picked and streaked out on Lac EMB. ~~#~~ 109 is a Lac sectored colony.
#72, 88, #30, #12, #56 appear possibly heterozygous. ~~Picks~~ Restreak on Lac EMS, EMB to check.

1B. 14 possible "+" colonies. 1. ++ 2. − 3. ++ 4. −
5. − 6. ++ 7. ++ 8. ++
9. ++ 10. Var? 11. ++ 12. ++
13. ++ 14. ++.

1C. 44 picked; not readable P23. P24 No Var.

2A. 70 picked. No variegated.

2B. 18 picked. "

2C. 37 picked not readable P24. No Var. Streak snaps. on Lac EMS; pick 4 colonies & S.O. on Lac EMB to test heterozygosis.

72A1 None hetero.
88A1 #1 hetero. #3 is not.
30A1 4+: all varieg. 4 Lac −.
12A1 4+. ~~Mostly~~ not. het. 5 hetero. 6,7 Lac −.
56A1 4+ none hetero → 1− W503
10B1. All 4 hetero. → W502.

5-8 on bac S to select + papillae.
see 287.

72 and 56 have to be tested again;

88-1 (Lac±) = W494 88-3 (Lac+) = W495 12-5 = W498 12-1 = W499 12 7 = W500
30-1 " = W496 -5 (Lac−) = W497 10B1-1 = W501

Aug. 28, 1948.

Redrock 56 × 72. 10 cds. each from EMS bac' plates, s.o. on EMS.

No variegation seen.: Not heterozygotic.

Mal reversions of W482.

Aug 20+, 1948.

Several attempts were made to recover Mal+ papillae from W482 which, still segregated for Lac, to determine whether Mal heterozygotes could be obtained. A series of + colonies was picked from

W482 in T(m) Maltose → Mal EMS. to EMB/Mal and EMB/Lac.

of 8 colonies, #1, 6, & 8 were probably segregating for Lac, and all the others probably so. It could not be clearly ascertained whether there were any Mal- colonies or sectors. Transfer to T/O slants.

Isolary each, vaugated, from Lac EMB of 286-1, 6, +8 streaked out on Lac and on Mal EMB. On Lac, predominantly + and - ± some ± colonies. On Mal, exclusively Mal+, suggesting heteroploidy between the Mal and Lac loci.

Rep (8) as W-504.

Lac-mutations of ~~co~~ co-zygotes. ~~Lac~~ 287

Aug. 26, 1948 ff.
Sep. 285.→

30A1 (5-8) were streaked on EMS lac' N27 many papillae noted.
4 picked from each colony & s.o. on lac EMB + EMS as 287-1-1....
2 -
3 -
4 - ....

12A1-(6,7) showed no marked papillae at this time on EMS although beautifully papillate on EMB.

---

(1): 1. + and - Var? 2. +, - 3. +, - 4 +, -

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

(3) 3, +, - Var? 1-2, ~~3~~ 4 +, - (4) 1-4 +, -.

Restreak + colonies from EMS of 1-1 and 3-3

No variegation.

Aug. 30, 1948.

Résumé: see 275.

(1). 104 Lac- is M- but a Lac+ papilla was M-T-B_1-. This segregant is, conceivably, M- Lac- T+ B_1+ / M- Lac- T- B_1- , and in the course of purification of the papillae, a new segregant may have been obtained.

(2). 110 Lac- is TS_S; 110 Lac+ TS_R.

Streak out 275- 104 Lac- (= 288-1) and 110 Lac- (= 288-2.) on [illegible] from NA slants.

a). Lac EMB. b) Lac EMS c̄ methionine.

Test nutr. 5 cultures each of -1 and -2 from EMB Lac plates.

	-1	-2.
1.	BMT	MTL
2.	BMTB,	MTL
3.	M-	----
4.	(BMTLB,?)	(B)MTL
5.	"	MTL

[illegible]: When heavy inocula were taken to EMS Lac, M, -2 gave no growth whatever while (1) gave rather scattered colonies. If the original M- cultures had been heterozygous, they are now thoroughly segregated. ~~However 288-1-3 (or 288-3) may still be useful. Transfer it from -L to a T(Meth) slant.~~ Terminate Expt!

Aug. 30, 1948.

A. Y87 x W255 Gal S + B_1.

B. W488 x W480 Lac S — B1 are Lac- on EMS!

C. W488 x W255 Gal leucine? ~~Lac S~~ Gal S

D. W491 x W255, add leucine to mixtures! Gal S

A. (B_1) 194 - : 17 +] 211 = 8% Gal +
(0) 71 - : 6 +] 77 = " "
∴ should be between BM and V_6, left of

check Gal+ for Lac, T1. ~~Could~~ were not accurately scored for Lac on Lac S. Gal +/- may interfere?

	Lac -R	-S	+R	+S
A0 Gal+ :	3	0	2	1
Gal -	6	4	0	0
A_{B_1} Gal +	15	2	0	1
Gal -	16	4	0	0

Total for Gal and V_1.

	R	S
+	20	4
-	22	8

B. 105 + prototrophs picked by DG and S.O. on Lac EMB, saving suspensions.
The following were definitely segregating for Lac: (9) 6 Mal - : 3 Mal +

	1 colony.	Mixture.
7	Mal -	—
32	Mal -	—
51	Mal -	—
52	Mal -	—
56	Mal +	+, -?
73	Mal -	—
78	Mal -	—
94	Mal +	+
100.	Mal +	+
70.	Mal -	—

scored, not 4th V on Lac EV (probably was Lac + V)

#70 and ~~three~~ uncertain at first reading.

S.O. 56 on Mal EMS.

C. 44 Gal + S.O. on Gal EMB. All pure +.

D. 11 Gal + S.O. On Gal EMB " ".

E. 40 - cultures streaked out on EMS Lac.

See 293

Sept. 4, 1948.

289 cultures SO Lac EMS. Picks 4 + cultures from each (+ only found) and a) SO Lac EMB b) Streak to Mal EMS.

NB. 56 maybe Mal+/Mal−

94 maybe $V_6^{R/S}$

± = Variegated.

	EMSlac 1	2	3	4.	Mal EMS .1	2	3	4	
7.	±	±	±	±	−	−	−	−	W5 [illegible]
32	±	±	±	±	−	−	−	−	W5 [illegible]
51	±	±	±	±	−	−	−	−	W5 [illegible]
52	±	±	±	±	−	−	−	−	W5 [illegible]
56	+	+	+	+	+	+	−	+	None variegated!
77	±	±	±	±	−	−	−	−	W5 [illegible]
78	±	±	±	±	−	−	−	−	W5 [illegible]
94			±	±	+	+	+	+	W5 [illegible]
100	±	±	±	±	+	+	+	+	5 [illegible]

~~70.~~ ~~Hetero.~~

70.	+	+ cols!	+	−	−	−	−	−
	+ −	+ ...	+	− +	−	−	−	−
	+	±, +, −			−	−		

W5 [illegible]

70 segregates much less frequently than the typical heterozygotes!

5 colonies on EMSlac picked to Mal EMS + scored as + and −.
1-15 Mal− and 21-36 Mal + SO Lac EMB to find any heterozygotes.

~~94 colonies (incl. 1-4) picked (1-9) and tested for V_6^R / T5 on Lac EMS [ref. 293]~~

None of these 31 colonies show Lac heterozygosis. When streaked out on Mal EMS, 56 showed + colonies and + uncertain. Test these on Mal EMB. → Mal−.

Original Slant of 56 S.O. on Lac EMB shows pure Lac + and a single (1:7100) Lac − colony. Maybe "70" type!

A number of Gal- cultures were tested for Lac+ on Lac S.
21 + cultures picked + S.O. on Lac EMB.
19 were pure lac +. 2 were predominantly - but may have heterozygous components. (# = 11 + 22). Repeat tests on EMB and EMS Lac (L) with these suspensions.

Single colonies picked and tested for T5, T6 resistance on EMB, EMS Lac. All were T6^S exc. indicated.

	TS	EMB	EMS.	EMS papillae to EMB.	EMB cols. A	Nutr.	B	A	B	...
1	R		R	+	+	+				
2	R		R	+	+	+				
* (3)	R		S	+	±	+	S	S	S...	
4	..	too thin		+	+	+				
5	S		S	+	+	=				
6	R+	too thin		Lac+	=	=	Pure Mal+			*
7	S		S	+	+	+	↓			
8	R?		S	+	+	+	Both pure+ and ± prototrophs			
9	R		R	T6^R; +	+	+	1-6, 8± 7+ = w581			
10	R		R	+	+	+	= ws80			
11	R		R?	+	+	±				
12	R		R	+	+	+				
(13)	R		S	+	+	+	S	S	S...	
14	R		TT	+	+	+				
15	R		R	+ *	+	+				
16	R		—	—	+	+				
17	R		TT	+	+	+				
(18)	R		S	+	+	−	R	R	(6R 1S.)	= = *
(19)	R		S	+	+	+				
20	R		R	+	+	+				
21	R		R	+	+	+				↓
22	R		R	+	±	+				
23	R		R	+	+	+				
24	R		R	+	+	+				See
(25)	R		S	+ (v?)	+	+	S	S	S...	294
26	R		TT	+	+	+				
27	R		TT	+	+	+				
28	R		R	+ (v?)	+	+				
29	R		R	+	+	+				
30	R		—	—						
31	R		—	+ ?	+	±				
32	—		—	—	+	+				
33	R		R	+	+	+				
34	R		R	+	+	+				
(35)	R		S	+	+	+	S	S	S...	
(36)	R		S	+	+	+	S	S	S...	
37	R		R	+	+	+				
(38)	R		S	+	+	+	S	S	S...	
39	R		R	+	+	+				
(40)	4 cannd.		S	+	+	+	S	S	S...	

Streaked out from EMB ♀ testers to Lac EMS to obtain segregants. ⊙ should be checked exhaustively for ♀^{R} segregation. ♀ includes reteste

Results

Sept. 5, 1948

Papillae picked from EMS streaks of 289E and S.O. on LacEMS + EMB.
#'s: 6, 11, 13, 14, 16, 17, 19, 20, 27, 30, 31, 32 — could give no papillae. Mold plates.
See 293 for tabulated results.

Sept 2, 1948.

	O	V	AA	VAA.	V(-AA) series.	
SY19.	+++	+++	+++	+++	+++	prototrophs

SY58 — + -AA series. -4, -6 + others — 36h. -1 + ~~others~~ faint ± AA only +.

					HC	HCV
SY71.	—	+++	++	+++	+	+++

SY70. — Cyst +++ M +++ Homocystine + Na_2S M/1000 +++ PARATHIOTROPH.

	O	B_1	Thiazole	Pyrimi.	Py+Th.	
SY36.	—	+++	+++	—	+++	Thiazoleless!

SY56. none grown as yet. B Tyr B Tyr [illegible]
36 hours — — ++ ++ — +++ [illegible]

SY71. Vitamin Series. Single ~~additions~~ omissions — B_1 ~~shows some diminution?~~
AA series, " group " .

SY56. HC, V, HCV, AA, AAV, and reinoculate series above.

SY58. HC, V, HCV, AA, AAV.

SY56: — on V, +++ on others. AA stronger response than tyrosine.

		AA	-12	-3	-4	-5	-6	
SY71	-AA.	+++	+	±	+	+	±	(Promising response!)

	HC	V	HCV	AA	AAV	
SY58.	+	—	+++	±	++	Vit. apparently required.

SY71. -B_1 shows slight diminution. Test vs. Vits.
In amino acid set, AA is higher than any single gr. omission.
Test omission series from AA 3 and 6.

SS8: AA − V series: — V_4 is ±. others +. nicotinic
— V_{11} — **Vit K!**

AA + V	++
AA	—
V	—
0	±

V −AA series:

−12	—	(cyst, meth.) arg, lys.
−3	—	val, isol, leuc.
−4	++	
−5	+	
−6	++	

tran pew: [illegible] is in 12)

Next set: AA, +nic, +K, +nic+K.
Vits

SY71 1/3.

A3	+
A6	±
A36	+
−L	+
−H	+
−Al	+
−Gly	+
−Sl	+
−IV	++
−V	+

Leucine

0	±
B_1	+±
Pyr	±
Tr	±
Pyr+Tr	±

B_1 Try together & separately.

0, L, B, L+B,

SY56. B+Tyr.+ 18h.

0	—
AA	+++
−12	+
BAA−Tyr.	+

others +++.

Tyrosine and a component of #12 may be needed for optimal growth.

Try single omissions + additions with B + supplement!

SY36. B_1 only.

296b.

	O	BT	BT+ A12	+C	+M	+ Arg	+Lys.	- C + A12	M	Arg	Lys.
S56.	—	—	+++	++	±	—	—	—	++±	++	+++

∴ Cystine is required by S56 for prompt growth.

	O	B,	L	LB,	
SY71	—	+++	++	+++	Thiamin!

	O	AA	AA·nic	AA·K	AA·nic+K	AA Vits.	A235 V	A23 V	A25	A35
SY58	—	+±	+±	—	++	++	+++	+	—	—

A25 V		A35		A23	
L	+	C	—	H	—
I	—	M	—	T	—
IV	—			S	—
				P	—

Complex AA requirements.

[nic required in presence of K!]

SY36. 24h. B, +++ others...

48h. " , T2 +++, Ly+T2 +++, Ly —. vv Thiazole

cf. S. dublin.

Sept. 6, 1948.

SY71.	0	B_1	T2	Pyr.	T+Pyr.	l-leucine.	
	—	+++	+++	—	+++	++	
	—	+++	+++	—	++	++...	

SY36 0 B_1 l-leucine
no growth

SY56	0	BT	BTCys.	TCys.	BCys.	$BTN_{a_2}S$	
	—	++	++	+++	—	—	

B, Tyrosine. Cystine replaces biotin and is stimulatory.

SY58.	0	AA	AAVits.	AAV−K.	AAmic	AAnic+R	AA·K.		Vitamins stimulatory.
	—	± ++	++ +++	+±	+	++	+		
	Vits +:	A'235	A'25	A'25+L	A'235−L	A135	A135+M	A135+C	A235+Arg	A235+Lys
		++	—	++	—	—	+	+	++	—
		+++	++	+	+++	—	++	+—	++	+—

	A123	A123−H	123−T	123−Gly	123−Pr.	Arginine, SH, Leucine, (glutamic)
	±+	++	++	++	++	
SY37	0	B_1	T2	Pyr,	P+T	[redacted]. (vits.?)

no growth

	TL	TLB_1	TLPyr	TL T2	TL Pyr T2
Y53.	+	+++	+—	+++	+++

Thiazole

Y8h

SY58 rather indefinite vitamin requirement: nicotinic.
" " AA requirement. (leucine?, cystine, arginine,

SY71. Thiazole or leucine. Purify!

Sept. 1 ±, 1948.

Streak v. heavy inocula of the following on Mal EMS (+B₁ assumed). After several days incubation

W482 Very poor growth c̄ numerous papillae. Maybe contam.
W483. No growth!
9/2 W494 Moderate growth; papillae becoming apparent!
W496. " " " " ". 1 good + in heavy streak!
W498. No growth!

A). Pick papillae from W482 and W496 to 1) [redacted]' and 2) Mal EMB. and 3) Mal EMS'. 482 papillae Mal −, probably not coli.

Additional W-496 papillae noted.

B) Streak out cultures of W482, W483, W494, 496 and 498 and 501. on Mal and Lac EMS'. 501 only Grew. Probably B_1 deficient.

291-1. On Mal EMB, apparently only Mal + and Mal −.
Mal EMS.' + and − colonies. Pick 10+ to water + streak on Lac EMB + Mal EMB

Mal EMB.	All +	+; −?	++	++	+;	+;	++	+,	++	++
Lac EMB	−!	−!	−	−	−	−	−	−	−	−
	1	2	3	4	5	6	7	8	9	10

Mal plate rather defective. Retest all except 1, 2.

−2 +3. 2 cols each picked to Mal EMB, pure Mal + but Lac − −
−4 2 cols. #2 maybe Mal +/− ? Recheck. No. Lac −
∴ These papillae are probably segregants, no longer Lac +! Hold 291-1 as such for further study.

September 4, 1948.

1. W491 x W255 on EMS Lac (Lefue., Gal.

{ 2. Y87 x W-1 on EMB Lac, Mal
{ 3. " " low P (see 270 etc)

{ 4. W488 x W480 on " Lac, Mal
{ 5. " " low P.

A6. Yield of 2 and 4 much higher on Mal than on Lac (added, ? or real phen ???)
only 4 Lac+ noted on several plates of (4).

	+	−
~~2M~~	~~6 18~~	~~33~~
4 M	6 + 1[s] = 7	33
2 M	9	95

3). 5 M plates. 14 − 2 + 1 sectored. → pure M+. 5 additional M+ colonies PT: 3-5 + 1, 2 recheck (thorough Mal EMS.
4 L plates. 4 − 0 +

5) L 3 plates 3 −
M 5 plates 1 − V. poor yields.

4L. 4 colo. + NoV. on Lac EMB

4M. + cols. 5-14 = 10. on Mal EMB. 9, 10 −, (5-8)(11-14) + No Var

4M − colo. 15-40 = 26 [# 25-28 on Gal by error All +. I. All −
−4
22 tests.

---

1. Gal. 1-20 All pure +.
Lac 21 − 80. 35 −
No apparent hetero zygotes.
S.O. on EMS Lac to recheck.

{ 26?
{ (57?)
{ 61?
{ 67

Gal + 60 additional colonies (by DG) All + and −; no variegated.

LacT.

Sept. 4, 1948.

289B cultures tested on EMS, EMB. Ψ.

	EMB T5	EMB T6	EMS T5	EMS T6.	
1	~~RS~~ R	RS	S	S	
32	~~RS~~ R	RS	S	S	
51	R	RS	S	S	
52	R	RS	S	S	
Mal+/Mal-? 56	R	R	S sl.	R	Mal+ and Mal-?
77	R	RS	S	S	
78	R	RS	S	S	
94	R	RS	S	R, S	pure Mal+ ← may have two components.
100	R	RS	S	S	← pure Mal+!
70.	R.	RS	S	S	

56. S.O. on Mal S to separate possible components.

94: cols 1-9 tested on Lac EMS/TS. Cf. "10" from Lac EMS.

W-528 1-9 are TS^S $T6^S$; 10 is ^{R,R} and more strongly Lac + than three others

Sept. 7, 1948.

289E-5, 6, 18 & 25+28 merit further study as possible heterozygotes (for nutritional, 4, or "lac" mutable") characters. Pressure on T6 streaks and streakout on EMS Lac for further study.

25, 28 are contaminated. 289E6 is lac+, clearly heterozygotic = WS64

18 S.O. EMS. a) "F" papillae noted twice S.O. on EMS, EMB Lac.

b) Test 10 - colonies ea. 4. T5.
All were T5 sensitive both on EMS and EMB.

a) ↓ EMS (1-8) on EMB. 7 and 8 are + and - 1-6 all +.

N11. ↳ EMS, EMB. 8 showed all + on EMB.

7 +, and - ". Test individual ± colonies from EMS. -7. All ++. (on EMS, some are -?)

P12. F5: Pick 8 + colonies of 289F-5 from lac EMS to lac EMB All but 6 were all + (exc. for likely contamination in one plate). F5-6 had appreciable numbers of + and -! Recover from EMS streaks and s.o. on EMS, EMB lac.

6 - on EMS lac. 4 + colonies tested on EMB gave all +

E2 reports that purified prototrophs did not segregate to genetically deficient types.

b)

Sept. 6+, 1918.

P5. S.O. W530, 531 to a) Lac EMB b) EMS.

P6. a) Numerous + colonies, or occasional - colonies and colonies with -sectors at edges only. Pick 4 apparently pure + from each → to EMB.

P7. W530: each of 4 showed + and -, no evident sectoring

W531: mostly +. - very occasional.

From W530 sets, pick 4+ and 4- cols (+- in alternating series) for initial testing:

+	1	TMB,	
-	2	MTLB,	
+	3	TB,	
-	4	TMLB,	
+	5	TB,	
-	6	TMLB,	
+	7	TLB,	Lac+!
-	8	TLB,	Lac+!

(Where is T+?)

b). Pure +. S.O. W530 as EMS and EMB, 4 cols. to carry through purification

9/9/48.

Inoculate heavy suspensions of following into T(m) Mal + Glu. and on EMS Lac + Mal.

		EMS Lac	EMS Mal.
X	482	n.g.	n.g.
X	483	n.g.	n.g.
1	522	+	+
2	523	"	"
3	524	"	"
4	525	"	"
5	526	"	"
6	527	"	"
7	530	"	"
8	531	"	"

At intervals, streak on Mal EMS to recover papillae.

9/10. 526 shows papilla. S.O. Mal EMS to purify; Mal EMB. 2 cols from EMS: pure Lac+, pure Mal+!

(Keep on T/O) as 296-1

9/14. Papillae from:

		to Mal EMS	Mal EMB.	Lac EMB.
522	-1	1 partially isolated +	2 likely Segregated +	Mostly Var
	-2	Mostly −; 2 real +		" "
	3.			
523		Mostly −; + ?	Pure −; +	
524		Mostly +	Pure +; −	
526	1	+, −	+, −	
	2	+, −	+, −	
530		+, −	+, −	

9/16. Take "2" well isolated + cols from each of above EMS (exc. 522-3) and S.O. on Mal + Lac EMB.

522	-1	Mal Var.	Lac mostly −, Var.
	-2 A	"	
	B		Lac Var.
	-3	Mal+, Var?	+, − & Var.
523		Mal+	Var
524		Mal Var?	Var
526	1	Mal Var?	Var
	2	Mal+	Var
530		+, incr. Var.	+, −, Var, not striking

9/18. Take suspensions to T(o) agar of

↳	ao.
522-2B	296-2
-〃	-〃
523	296-3
524	296-4
526-1	296-5
530	296-6

Possibly segregating colonies were taken from these EMS Mal plates to the same agars.

+ 2B' 1-4.

5222A'. 1-4 Two types of colony are seen. ① is smaller and more intensely stained, with a sheen; ② is large, and much less darkly +. No clear-cut mixtures are seen.

523' } Same as above; possible — noted ± 523.
524' }

572-1 4 + colonies: all pure Mal+, Lac −

low phosphate and segregation.

Sept. 7, 1948.

Cross on Lac EMS – P. + is standard Lac S

1. 487 × W-1
2. W488 × W480

(3)
(4).

+). ~~2.4~~) Low yields, 1-5 cols/plate. Higher on Mal than L!

4M. 5 Mal+ from 6 plates. 1-5 S.O. on homologous EMS + EMS

4L. 5 Lac+ " 5 plates 6-10.

-P. 2) Yields low.

2M. 11 plates. 2 Mal+ 11-12 " "

2L 5 plates. 2 Lac+ 13-14

1) 1-10/plate. Mal better. → 15-30 = 16 Mal+.

1M

1L. No Lac+.

3) Yields same as 1. Pick none.

	EMB	EMS
1	–, +	
2	–, +	
3	++	
4	++	
5	+, –	
6	–	
7	++	
8	++	
9	++	
10	++	
11	++	
12	+, –	
13	++	
14	++	
15	++	
16	++	
17	++	
18	+, –	
19	++	
20	++	
21	++	
22	++, –	
23	++	
24	++	
25	++	
26	++	
27	++, –	
28	++	
29	++, –	
30	++	

Sept. 13, 1948

W480 × W488 as lac EMS + Mal EMS Take + prototrophs to homologous medium to purify.

101-110 10 Mal+ from EMS → all pure +
100 lac+ " to EMB.

PM. 1-20 All pure +.

A IS 21-100 Following are heterozygotes, showing +, − and sectored colonies.

	for lac.	A (Mal)	B Mal EMS	
✓ 24 (W480 type)	= H25	—	—	
✓ 31	26	—	—	
32	27	—	++; few −	*
✓ 35	28	++	—	*
✓ 36	29	++	++; few −	*
✓ 43	30	++	" 1 −	*
✓ 61	31	—	—	
✓ 67	32	—	—	
✓ 73	33	++	++	
✓ 76	34	++	++	
✓ 86	35	++	++.	

Pure Mal sep.

Take the single colony suspensions to T(0) slants under W- numbers. Take up gross streaks from EMS and streak to EMS Mal to look for complementary types (B)

A ⇒ and to EMB Mal

* 32, 35, 36, 43 show discrepancy. Take heavy streaks to T(0) as 298 −, and attempt to separate Mal+ and − prototrophs for separation into complementary types, if such.

See

Heterozygote Test Crosses

9/14/48

(1) W471 × W21. } Lac EMS.

(2) W466 × W33 } → only 12 colonies altogether from ①. All pure +.

to Lac EMS. 100 tested from 2. High yield of heterozygotes apparent.

		1st EMS.	→ Lac EMB	H−	Gal EMS.
1	5?		?		−
2	7 ✓		H	36	−
3	9		H	37	−
4	12?	pure +			−
5	16?		H	38	++
6	18?		H (530type)	39	−
7	22?		+		+, −
8	24?		+		−
9	29 ✓ +, - prot.		H	40	−
10	34 ✓		H 530type?	41	−
11	36?		H	42	−
12	37		H	43	−
13	38		H	44	−
14	39		+		−
15	40		H	45	−
16	413		H (530type)	46	−
17	42 ✓		H	47	++
18	46 ✓		H	48	++
19	48??		+?		−
20	65		H	49	−

The above are candidates for further scanning: Streak out retained water suspensions on Lac EMS, to Lac EMB + Gal EMS.

9/21. Retest colonies of 5, 12, 22, 24, 39, 48.

299-29 (−). to ~~Lac EMS to pick together~~ T'd) for further study.

4 addnl. colo. tested:

5	++
12	++
22	+ some mut?
24	++
34	++
48	+, some mut?

Amino Acid Mixes
NK & JL

mixture ½ [illegible]

A. Non-Essentials: per 50 ml H_2O

	Glycine	5	mg.	10
dl	Alanine	19		38
l	Proline	87		174
-	HoProline	2		4
l	Glutamic	271	(.HCl)	542
dl	Aspartic	60		120
dl	Serine	58		116
l	Tyrosine	66		132
l	Cystine	4		8

B. Essentials

l	Argihine	46	.Hcl	92
dl	Lysine	79	.HCl	158
dl	Tryptophane	12		24
dl	Phenylalanine	39		78
l	leucine	50		100
dl	Valine	79		158
l	Histidine	32	.HCL.H_2O	64
dl	Methionine	33		66
dl	Threonine	40		80
dl	Isoleucine	50		100

Note: Gelatin differs from Casein:

a) No tryptophane

b) Much more glycine and hydroxyproline

c) No tyrosine?

Sept. 12, 1948.

(DG) W566 - uv 7 secs. -

20 plates X ca 300 colonies → 6,000 scored