"Spontaneous heterozygotes" II

Jan 4 ++. 1949.

1. W644 x W126	14 plates.	ca 100/plate.	16 picked.	2H
2. W660 x W45		26 picked.		2H.
3. W595 x W45		2 picked.		
4. W660 x W69		1 " . Wood yield.		
5. W595 x W17		No yield whatever.		

1: A1, 3 are heterozygous	12 others probably lac−	1, 2
2: ≠ #7, #12	2 prob. lac−.	3, 4
3. #1 H.		5.
4. — −		

Additional:

2): 8 tested All ++

3): Two tested Both slow +. (Gal- Lac +?)

4). 4 tests. 3 − 1 ++.

Test + purify as lac EMB, EMS.

1. Clearly lac heterozygous.

2. " "

3. May be lac heterozygous; colonies fade quickly.

4. ++.

5. Mixture of +, − colonies. Probably not heterozygous, but test sample of + colonies from EMS lac. ++

January 18, 1971.

streak out as indicated:

402, -1, 2, from mosaic colonies, on Lac, Gal EMB. (note W6YY may be suppressive for Gal-). H136, 137 Gal+. (maybe heterozygous for Lac, Lac+ Gal?)

3. from a "mosaic +", on all sugars: Lac, Mal, Xyl, Ar, Xyl, Man.

5. from Lac+ on EMB on Lac ++.

? H137 may have some Gal+ I

3. (4 cols, identical). H138 Lac, Lac 2.

Lac variable Gal+ (as reputed)
Xyl −
Mal −
Ar +
Man variable.

Note: on lactose, colonies are pinky peripherally −, show sectoring in centers ◔ etc. These colonies tend to fade: Almost full + on EMB.

On mannitol, almost all colonies are as under with well defined central region ◉; occasionally colonies show sectoring.

~~H136 + 137. have been streaked out as Lac EMB to provide segregants for fertility study.~~

irradiations. -

January 9, 1949.

6. W644 on maltose. This culture was supposed to be galactose negative. When irradiated, it showed many Mal slow. Reinvestigation shows that there are two components in W644

(1) Gal slow Mal − mucoid on galactose.
(2) Gal + Mal +

(2) W660 on galactose. 50 × 100 = 5000.
= W595 Gal + irradiated.

(3) W656. on arabinose 20 × 70 = 1400 3 mutants:
Ar. Xyl. Glu. Lac

W-667 1.
W-668 2.
W-669 3.

W670 1
671 2
672 3
673 4
674 5
675 6
676 7
677 8 +
678 9
~~679~~ Mucoid.

Sp. heterozygotes

Jan. 12, 1948.

(1) W45 x W660

(2) W182 x W660. not.

contaminated ⊂ Aerobacter.

26 "+" tested: None heterozygous. ca 1/3 —.

Segregation of H136, H137.

Jan. 13, 1949.

Streaking out of mosaic colonies of these cultures gives about 50% mosaics; 50% −. Full + is quite rare.

e.g. 25− : 21+. This is rather lower rate of segregation ~~that~~ than shown by previous H stocks.

Picked well isolated − colonies for characterization with T1 and nutritionally. Also picked possible Lac+ ~~one~~

H136 A.

	T1
1	R
	R
	R
11	S
	R
	R
	R
	R
	—
	R

B

	T1
2	R
	R
12	S
	R
13	S
	R
	R
	R
	R
	R

C

	T1
3	R
	R
	R
	R
14	S
	R
	R
	—
	R
	R

D.

	T1
4	R
	R
	R
15	S
	R
	R
16	S
	R
	R
	R

H137. A.

17	S
	—
5	R
	R
18	S

B

6	R
	R
	R
	R
	R

C

7	R
19	S
	R
	R
	R

D

8	R
	—
9	R
	R
	R

Total: 47R : 9S. Highly abnormal. (non-random).

Test ~~mut~~ nutrition of 9S and 9R cultures.

January 14, 1949.

	ALL $Lac_2 - V_1^R$
1	+
2	+
3	+
4	+
5	+
6	+
7	+
8	+
9	+
	$Lac_2 - V_1^S$
11	L
12	TL
13	MTL
14	TL
15	MTLB,
16	TL
17	TLB,
18	TLB,
19	TLB,

Kupa W - 721.

M+ > M-

T, L ca equal. (linkage to R.S).

Segregation of 11138

Streakout from segregating plate, grossly, to EMBLac.
Rather large proportion of Lac+ segregants, also Lac I.

Mal mutations.

Jan. 12, 1946

50 × ca 300 = 15,000 colonies. 487 / Mal EMB.

	W	Mal	Glu
1	679	slow	+
2	680	−	+ faded
3		S+	+
4	681	− s.c.	+
5		++	+
6	682	−	+
7	683	−	+
8	684	− ±	+
9	685	—	+
10		S	+
11	687	−	+
12	688	− s.c.	+
13	689	± −	+
14	690	±	+
15		+	+
16		++	+
17		+	+
18	690	—	+
19	691	−	+
20	692	−	+
21	693	−	+
22	694	−	+
23	695	−	+
24		+	+
25	696	—	+
26	697	±	+
27	698	−	−

segregations of sp. [illegible]

Jan. 16, 1919.

H138: (Lac; Man. variable). sp. W45 × W660

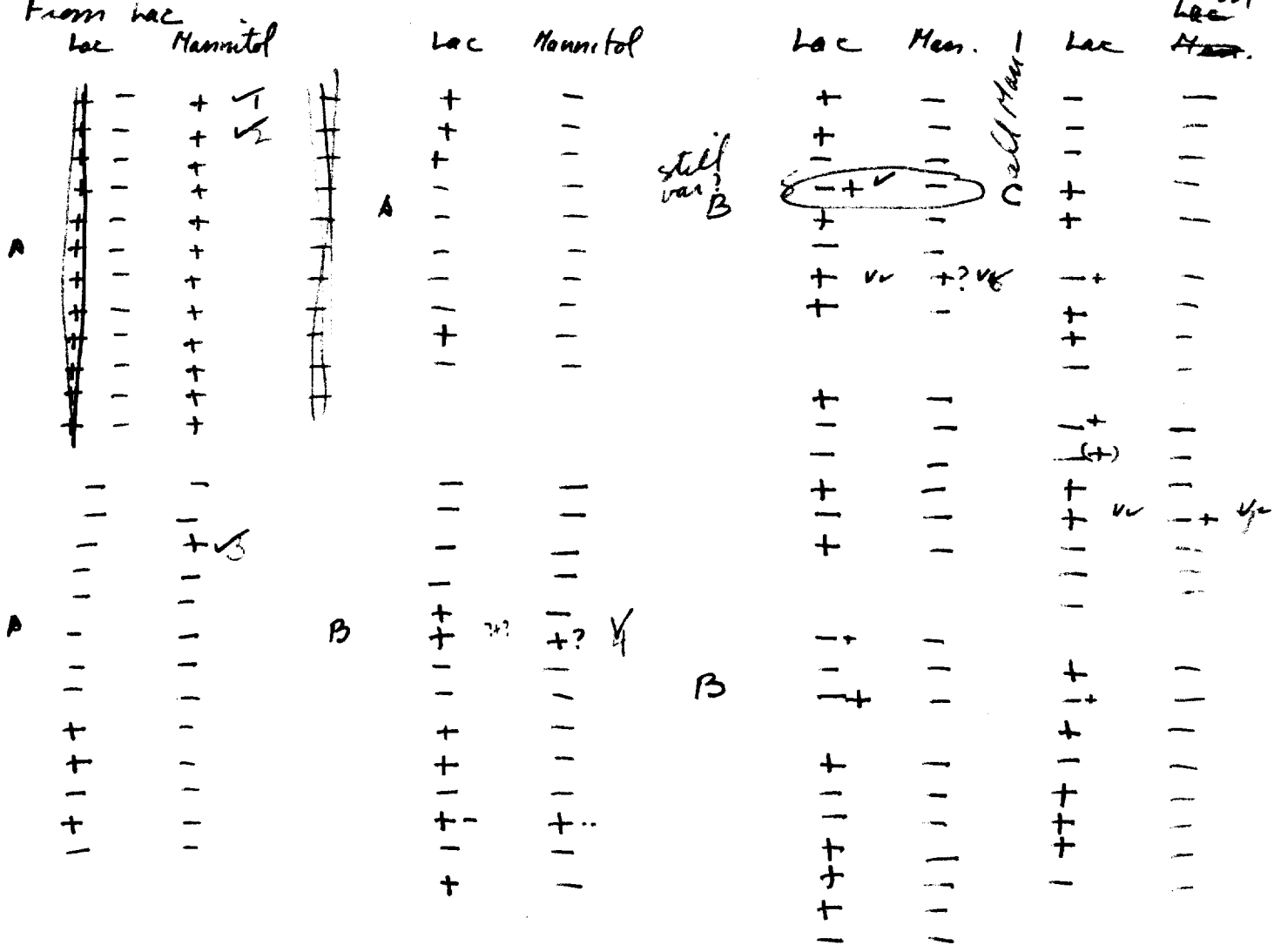
~~H134~~ H140: ~~(W45 × W660)~~. W478 × W595.

[H139 on Mann EMS.]

Streak out H138 + H140 on Lac EMB for starter inoculum.

Pick all isolated ~~colonies~~ colonies + or − from individual mosaics and score on other media.

H138. From Lac

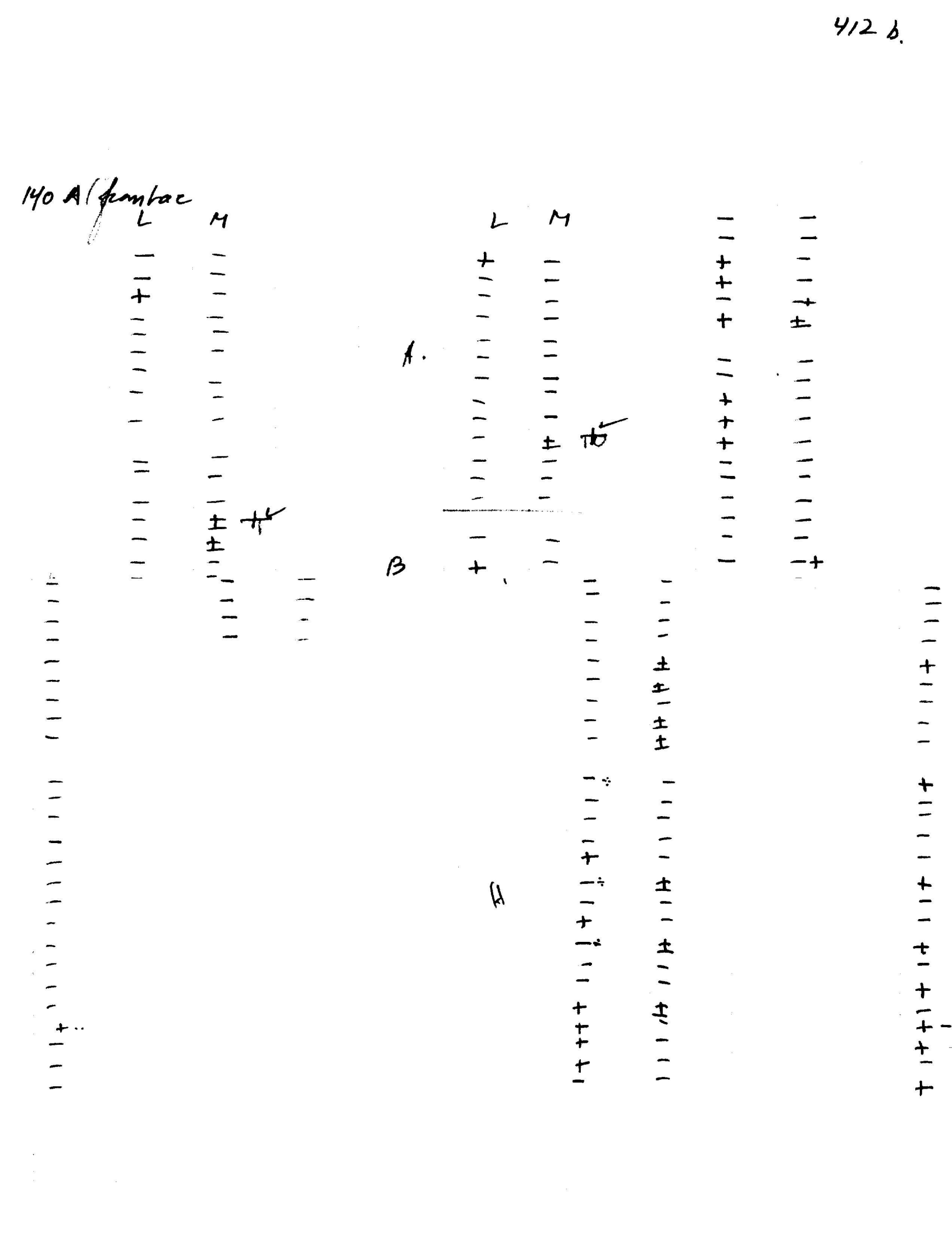


Segregation of nitrogen fixers

H138 (from Man)

	Lactose	Mannitol	L	M	L	M	L	M
D	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	+	−	−	−		
	−	−	−	−	−	−		
			−	−	−	−		
	−	−	−	−	−	−		
	−	−	−	−	+ (−+?)	−		
	−	−	−	−	−	−		
	−	−			−	−		
	−	−			−	−		
from M.								
E	+	−	+	−	−	−	+	−
	−	−	−	−	−	−	+ (−+?)	+ −
	−	−	−	−	+	−	−	−
	+	−	−	−	−	−	+	−
	+	−			−	−		
	−	−	−	−	+	−		
	−	−	−	−	−	−		
	−	−	−	−	−	−		
	−	−	+	−	−	−		
	−	−	−	−				
	−	−	−	−	−	−		
			−	−	+	−		
			−	−	−	−		
			−	−	−	−		
			−	−	−	−		
			−	−	−	−		
					−	−		

140 A (femtac



1/21. Tests on reisolated segregants.

	Lac	Mtl.
1	−	++
2	−	++
3	~~+~~ −	++
4	+?	V
5	V or +, −?	−
6	V	V
7	V	V
8	~~+~~ +, − V?	−
9	−	+
10	−	+

Picks from Mtl^{var} columns.
Rechecks on Lac.
" " "
" "

1/22 Rechecks

1
2
3
4 Lac variegated (not pure +)
5 Apparently Lac+ and Lac−. No definite Lac^{v}. Mtl for recheck.
6
7
8. 1, 2, 4. Lac −. 3 Lac$^{v?}$ and Lac −. Restreak Lac$^{v?}$ on EMB Lac + Mtl Lac+.

No partial segregations. High correlation in a colony. Suggests that sectoring may result from very few segregations per colony. Should try to find evidence for reversal of trend in +/− segregations!

Maltose deadication runs.

1/19/49.

W668. 40 plates EMB/Mal × ca 400 cols.
16 mutants

W700-715.

6/28/53 ~~Adaptation~~ W583

Cavalli's data 58-161 x W583

Gal \ Lac	+	−	
+	43	21	64
−	91	286	377
	134	307	441

[Need V_6 rather than Lac to map Gal.]

Assumed M Gal Lac

but too many doubles.

W478 x W583: Xyl, Lacv's isolated

330] Noted that Xylv were mostly "peculiar Lac−"

Gal doubtless provided by Lac ~~segregation~~ episteses.

330-2 (on EMS Lac) mostly Gal+ Mal− Lacv.

340: 58-161 x W583. close Lac, Gal correlation

351: Lac+ excess. ~~Ex~~

273
13

58-161 x W583

	Lac +	−	
Gal +	43	21	64
−	91	286	317
	134	307	441

Gal + Lac + if independent would be $\frac{64}{441} \times 134$ = about 20

Interaction in scoring? Use V_6.

	Mal +	−	
Gal +	8	53	61 Unlinked.
−	18	355	373
			434

	Xyl +	−
Sal +	6	
−		

Crosses for heterozygotes

1/18/49.

1. W677 × W478 ca 100/plate: No + probable.
2. W182 × W677 14 plates 44+ : 150-
3. W45 × W677 15 plates 58+ : 155- Probable not 1 mosaic noted

(1) In 20h., quite a few V noted. However, in 40h., someone not easily scored. (Medium rather dilute). 7 picked for recheck. 1-7

(2) 1? for recheck. Lac+ 8 ✓ Picked. "+" to Lac EMS + EMB.

(3) 1? " " Lac+ and Lac-. Var? — 9. No var.

H (1) 1/81 Mostly +. 1 V? Restreak on Lac EMB. Lac Mal Gal Mal Xyl Not heterozygotes.

(2) 1/82 Mostly +. 1-. Wait for EMS-plated. not heterozygotes.

1/83	3	+, −, V	} Restreak.	✓	+	−?	+	+	+
1/84	4	+, −, V.		✓	−	−?	+	−	−
1/85	5	"		V	+	→	+	+	+
1/86	6	"		V	V?	→	+	−	+
1/87	7	+, − and V.		V	−	→	+	−	V

Additional 100 Lac+ colonies streaked from (1). 9 probable V. picked and restreaked.

H 142, 148 are vacant

Heterozygotes from W677 x W478. 4179.

		Lac	Xyl	Mtl	Gal	Mal	Ar
~~H142~~		~~V~~					
3	143	V	+	+	−s	+	+
4	144	V	−	−	−vs	−	+
5	145	V	+	+	−s	+	+
6	146	V	+	V?	−s	−	+
7	147	V	V	—	−s	−	+
~~+~~	~~148~~	~~V~~					

These are no more satisfactory than ~~H142~~ H-140 which has already been analysed to some extent. H144 and 147 are useful for getting Mtl+ recombinants of H139 (Lac, Xyl^{v} Mtl −)

See 4176 for additional heterozygotes in this series.

Heterozygotes from W478 × W677

	Lac	Xyl	Mtl	Mal	Arab	Gal.
1	V	V	V	+	+ V?	V
2	V	V	V?	+	"	V
3	V	—	—	—	±?	V
4	V	V	V	—	V?	V
5	V	—	—	—	±?	V-
6	V	V	V	—	V?	V
7	V	V	V	—	V	V
8	V	V?	—	—	V	V

Gal may be regularly variegated in these strains. May be associated with the Lac, -

Second observations:

		Lac	Xyl	Mtl	Mal	Arab	Gal	H	
H 167	1	V	V+	V	+	+V?)	V?	165	
168	2	V	V+(B)	V	+	(+V?	V?	166	
169	3 A	V -pur.	—	—	—	A V??	V	167	
170	4 A	V+pur.	AV-	V-	—	B V??	V -pur.	168	
171	5	V+	—	—	—	?	V?B	169	See 3/10/49. Pur+
172	6	V-	V+	V	—	?	V?B	170	
173	7	V+-	V?+pur.	V	—		VB	171	
174	8	V+	AV -pur.	—	—		V	172	

almost all gummy on galactose

(1) Restreak 1V colony from all arab + galac segregations.

(2) Retest all (4) segregations.

Jan 28., 1949

H168 is confirmed heterozygous for Lac, Xyl and Mtl.
The following ectets were made with Galactose and Arabinose.

H 165	(1).	Probably mixed	Gal / Gal^s	Arab. slow++
166	(2)	segregating for	Gal+ / Gal s	slow++
167	(3)	very clearly segregating		"
168	(4)	segregating	+/s	"
169	5)	Segregating	+/s	slow++
170	(6)	segregating		slow++, but some radiating colonies
171	(7)	Segregating	?	slow++
172	8)	Segregating	Gal+/s	slow++

check by streaking out a slow and + colony separately.
H168: heterozygous for four factors; use for crossover studies.

W589 Crosses.

1/20/49.

W589 (Luria's tryptophane-adenineless) is not fermentatively normal:

Slow on mannitol, galactose, Maltose – Lactose – Glucose ± → +.
+ after 2 days.

1/21

1. Cross with W477 (ILV, Lac–). × W589
2. Y-161 × W477. (Lysine-histidine).

Yield of ① very high in 48 hours. Sharp segr. +/–
② Less marked yield. Tests:

② : 40 + tested. 5 for retest. 1-5
Selection from "strong" + prototrophs.

Y : 28 + tested. 16 probable Lac⁻ 6-21; 26-29
Selection from "weak" prototrophs.

Overall: 21 Y /68+ or ca 34%.
an additional 40 are formed from the first group. On reexamination;

① 100 tested. 4 possible mosaics noted. 22-25
None of these is very sharp. Restreak on EMS, EMBLac etc.

Store 20 m EMS-Lac- T1 test plates
Work up 5 additional

In addition to routine restreaks, take 8 colonies and 1 gross streak of H149 (419-2-1) and streak out on EMB Lac.

H148 = 419-2-2 Lac EMB: Many +; occ. – and V.
H150 = 3 Mostly +; occ V and –
" 151 4 Mostly – occ V and +
" 152 5 " " "
153 6 ca = +, –

W589 x W477

1/25/49.

W589 x W477
Series (1). 22-25. EMB Lac:

22: Most colonies either large, rough spreading lac+ or small, smooth lac−, with some sweiss combinations. 1? still variegated colony. Not sharp.
48+: 10− 1 or 2 V. H 154

23: Majority −. 44−: 17+. 1 or 2 V. H 155

24: 20+: 11+ H 156

25: 54+: 27− H 157

None of these seems to be strictly mendelian. The colonies which are probably variegated are not very sharply defined, and some of them may represent the slow fermentation type of W589. At any rate these do seem to be heterozygotes. Wait for EMB plates to develop before proceeding.

A 25. Add'n'l 108 colonies picked and tested on Lac EMB.

W1977 x Y-161. 4196.

(H149 segregation, etc.).

Jan 25, 1949

Bind. colonies of "H149" had been streaked out on EMBlac.

1. Only + and V? or lighter colonies noted.
2. +, vague + or V, and a few −
3. + only. Probably miscored as mosaic.
4. Mostly "mosaic"; few, = + and −.
5. Mostly +, spreading. A few −
6. All +
7. All +
8. + = −. A few mosaic.

0 (gross streak). + sl. > −. Recover H149 to EMS from 8 to avoid possibility of losing this strain.

Corrigenda !

January 25, 1949.

A considerable part of the work done this day used contaminated tubes for suspending colonies, etc.

Following can be recovered from original plates:

(1) Resolution of H148-153 (Lac+/- from Y-161 x W477)
T.O. H149 [too much trouble]

(2) 417-7 (H147) from EMS Xyl plate.

(3) H138 M+ from EMS Mal.

Repeat: H144 on Mtl

January 23. 1949.

1. W126 × W701 Lac_4^- × Lac_1^- Mal- Gal- Ar-
2. W589 × W677 Tr^- Rd- × F_6^-.

Yields poor on ①. V. few + as expected. high on ②.

②. 100 picked P25. A26. No clearly segregating colonies.
streaked on Lac EMB (N2)

1 is picked. Show peculiar mottled appearance on Lac EMB (Is this another Gal-epistasis?)

After 36-40 hours, on Lac EMB, These colonies (7 of the 8) show definite sectoring, especially #6. Assign H159-164 to these cultures. Streak out Mosaics of H163 (#6).

②. Additional 100 picked P26. A27: 1 very questionable ±.
Streak out on EMB; EMS as 420-2-1 Not variegated.

∴ "2" has given no reasonable heterozygotes.

Jan 26, 1948.

S.O. H139 and H141 on EMS Mtl, Mal and EMB Lac to select reversions.

On EMS Mtl, H141 shows speed +, a few − colonies. At this time is Mtl ±.

To confirm, streak out on EMS Lac, EMB Lac + EMB Mtl

H139 OK.

P30. 16 papillae from H139 picked to Mtl EMS (or EMB). Later 4 more
P1 Restreaked on EMS Mtl; EMB Lac and EMB Mtl.

	Lac EMB
1	V
2	V
3	V
4	V
5	V
6	V
7	V
8	V
9	V
10	V
11	V
12	V
13	V
14	V
15	V
16	V
17	V
18	V(?)
19	V
20	V

All the cultures are obviously still lac V.

On mannitol, however, they show an indefinite reaction never fully +, rather gummy, and sometimes against a vaguely sectored background. Pick the most clearly sectored colony in each set and restreak on Mtl EMB.

On EMS Mtl, similarly, the colonies show an intermediate response. (This may be due to vigorous reduction of M.B.)

Segregation of M163

January 28, 1949

After 48 h.

1.) Inoculate from EMBlac to Purassay. Dilute and spread on various sugar media (Lac, Mal, Gal, Ar EMB. Two sets, A + B.

2.) Streak out single variegated colonies from EMB Lac to same

(1) P31 A. Lac EMB: 1) 58− 1+ 9± /68.
2) 76− 1+ 9± /86
3) 76− 2+ 14± /92
210− 4+ 32± /246 Σ

Lac − 1/two kinds, one pinkish; one blueish.

Mal	105+	4−			Corrected for heterozygotes.
	109+	6−			
	214+	10−	/ 224.	195	
Gal	151+	17−			
	96+	5−			
	247+	22−	/ 269	234	
Arab	122+	6−			
	73+	2−			
	195+	8−	/ 203	177	

Lac EMB	75−	1+	11±	Mold. tests for M−(Rb) on all media.
	90−	0+	8±	
~~Vacuoles~~	145	1+	19± /165	
Gal	107+	5−		
	104+	6−		

In series A, Lac plates show 87.0% segregation. Of the segregants there was: 1.87% Lac+; 5.13% Mal−; 9.4% Gal−; 4.5% Ar−.
In series B, there was 88.5% segregation.

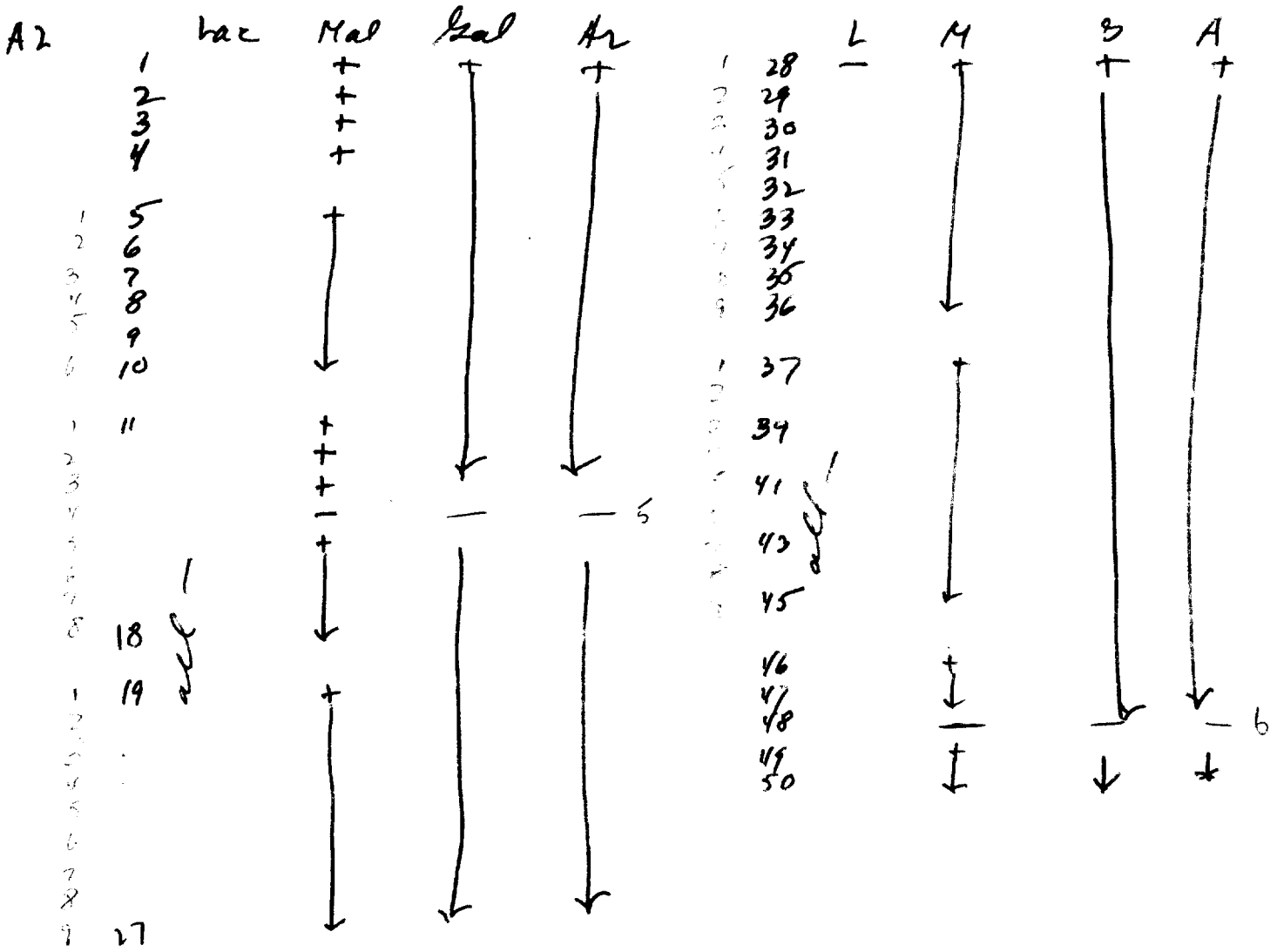
Pick Lac- at random and test:

A1.

	Lac	Mal	Gal	Ar	
1	−	+	+	+	
2	−	+	+	+	
3	−	+	+	+	
4	−	+	+	+	
5	−	−	−	−	1
6		+	+	+	
7			+		
8			+		
9			+		
10			+		
11			+		
12			+		
13			+		
14					
15					
16					
18					
17					
20					
21					
22		−	−	−	2
23		+			
24					
25					
26					
27					
28					
29					
30					
31		−	−	−	3
32		+	+	+	
33		+	+	+	
34		+	+	+	
35					
36					
37					
38					
39					
40					
41					

	Lac	Mal	Gal	Ar	
42		+	+	+	
43					
44					
45					
46					
47					
48		−?	+	+	4
49					
50					

42YAA


A2
Lac
Mal
Gal
Ar
L
M
B
A
1
2
3
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11
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19
27
28
29
30
31
32
33
34
36
37
39
41
43
45
46
47
48
49
50
all 1
all 1
5
6

[illegible] throughout

All – hue tested are glucose-negative.

∴ W701 carries such a factor, probably introduced as Hal–.

Albert alone to invert + test inversions in Xyl and Ar.

	Lac	Mal	Gal	Ar.	
Bl.	−	+	+	+	46 tests.
except.					
Row B-6	−	−	−	−	
E 1	−	−	−	−	
G 2	−	−	−	−	
A4	−	(−?)	+	+	1, 1, 2
B2	−	+	+	+	49 tests.
except.					
A2	−	−	−	−	
A3	−	−	−	−	
B4	−	−	−	−	
B8	−	−	−	−	
C2	−	−	−	−	
E4	−	−	−	−	
E8	−	−	−	−	
B4	+ input	+	+	+	

Total: 195 tests of Lac−. 15 were Mal-Gal-Ar−
180 Mal+ Gal+ Ar+
2 were possibly Mal slow.
Retest these as 424-1 and 2
Recheck some of the ---- on glucose. There may be an epistatic−

Feb. 1, 1949.

H163B. Ar-: 11 tested. 10 are Lac- Gal- Ar- Mal- V_1^R

[#8 is Lac ± Gal + Ar + Mal +. Streaks out on Lac ± ' S
omit. Still Lac vary.

Nutritional tests on 1-7, 9-11.

February 1, 1911.

H 163A lac+ : 1-4
B 5
A Mal- 6-14
B 15-19 No 20
A Gal- 21-36
A Ar- 37-44
B " 45-46.

	Lac	Mal	Gal	Ar
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	—	—	—	—
7				
8				
9		—	—	
10				
11				
12				
13	all others —			
14				
15		—	—	
16				
17				
18				
19	—	—	—	—
—				

	Lac	Mal	Gal	Ar
21				
22				
23			—	—
24				
25				
26				
27				
28				
29				
30				
31				
32				
33			—	—
34				
35				
36				
37				
38				
39				
40				

W589 crosses.

425

January 28, 1949

(1) Y10 x W589
(2) W477 x "
(3) W677 x "

P30-31. (1): + colonies only. 20 picked for retest, all ++ Lac.
(2). 100 picked + streaked on EMB Lac Hold 2 for retest.
(3) " " All ++

425-1-2 on EMB Lac for retest.