

Apr. 29, 1948.

	Dlu	Mal	Lac	Gal	Gma	Mgal.	T1	lac
182	++	+	-	+	+	-	S	
185	++	-	-	-	-	-	S	
187	<u>thin</u>	-	-	+	-	-	S	
188	-	-	-	+	-	-	S	
189	-	-	-	-P	-	-	S	*
218	+	+	++	+	-P	+	S	
239	-	-	-	-	-	-	S	
243	+	-	-	+	-	-	S	
245	-	-	+	++	-	-	S	
- 253	+	+	+	+	-	-	S	
319	-	-	-	+	-	-	S	
321.	++	++	++	++	+	□	S	3
- 108	- v.pop.	-v.p.	-v.p.	++	+	□	S	3

These are suspensions from fairly old cultures.

* v. few plaques.

B

47
72
74
76
83
87
~~108~~
~~110~~
~~112~~

S.O. 321. ~~anglucose~~ lac

245. on lac 1.6% gal, glucose. Plaque formed!

218. +

182 lac for 2 min.

185 lac

108 S.O.

Engelmann

[Try O.P. effects on types thin on glucose I.

Many are "thinner" on glucose than on disaccharides - e.g. 187, 218,

S.O. 249 on lactose 90%+. Purify also - for test as Lac₃.

243 on lactose. All colonies are slow ++. Broad streak is -. One (-) colony noted. Purify.

245 on lactose. - and very faint ± colonies predominate, with numerous papillae +.

S.O. - colony on lactose EMBS: all - colored.

Test:	Edu	Mal	Lac	Glu	Gal	Gal	
	108 pur	-	-	+++	++	-	W108
	245Lac-	many papillae	±	+	++	-	
184, 1-3.	243Lac+	-	±	+	±	- th.	W381
	249Lac-	-	-	+	++	-	
	243Lac-	-	±	+	±	- th.	W243
Bacteriae purified		W108 on Lac					

249 is comparable to W108 and may be Lac₃-. 243Lac+ may be a reagent poor. Call 243Lac- = W243 as recovered, and 249Lac+ = W381

Reconstitute all these stocks.

W185, tested not: Colonies small and slow on glucose. 95%+. Glu - nitro.

Mannose All +.

Sorbitol All -

Fuctose All +.

Recover

~~Recover~~ glu - and compare with + on extended series of sugars.

Reversions of W-245

1776.

May 5 + 1948.

Streak out 177a, W-245/Mal on Mal E 14B.

Pick 14 Mal+ colonies to Lac and Blu. at 37°.

a) All 14 are Lac++ Blu-

b) 3 Mal+ colonies Lac+ Blu-

1 Mal- colony Lac- Blu- apparent.

S.O. from a and b on maltose to purify. W397 + W398

Megal.

~~Megal.~~ Megal

K-12	+++	1	112	+++	+++
W-108	+	2	121	++	+++
243	+	3	276	-	-
260	-	4	283	-	-
261	+	5	286	-	-
A-267	++ (variable)	6	287	-	+++
269	+	7	313	++	-
270	+	8	316	-	-
277	-	9	317	-	++
280	+	10	122	+	++ (variable.)
284	-	"	132	-	-
285	-				
292	-				
B-297	++ var.				
298	+				
301	-				
307	++				
308	++				
C-312	+				
249	+				
257	-				
258	-				
319	-				
322	+				
321	+				
120	- n+				
R501	+++				
R5#	+++				
Y53	+++				
-Y10	+++				

* 312 + 302 were found filled with water! Some?
SO on glucose.

Glucose - mutation run

18°

April 28-30, 1948.

58-161R. 135 plates \times >100 scoreable colonies
= ca 15,000 total.

15 tiny colonies picked. None mutants.

No mutants from ca 6 other sectors.

Formate mutation Run.

Y10. Spiked on Glucose 1%, Formate .4% EMBS and irradiated as above. 46 plates \times 500/plate = 25,000 colonies.

Due to crowding it is not certain how efficient mutant recovery would be. Test some representative colonies.

Formate mutants.

180₂.

May 1, 1918.

Compare - (glucose EMB+) and + (-) colonies from formate-glucose EMB on

- + (a). Formate .5% Ncase & thalini .01% agar
- + (b) Formate - phosphate Ncase gas tubes.

EHB.	(a)	(b)	(c) EMB formate
1. 1-	-	++	-
2. 1-	-	-	-
3. 1-	-	-	-
4. 1+	-	-	-
5. 1+	-	-	-
6. 2-	-	-	-
7. 2+	-	-	-
8. 3-	-	-	-
9. 3+	-	-	-
10. 4-	-	-	-
11. 5- (lump?)	-	-	-
12. 6-	-	-	-
13. 6+	-	-	-
14. 7-	-	-	-
15. 7+	-	-	-
16. 8-	-	-	-

All cultures produce voluminous gas from formate broth

a) cannot be scored due to diffusion of alkalies through agar.

* Streak out 1, 4, 6, 7, 8, 9 12, 13 + 14, 15 on glucose EMB. Indistinguishable!

Test streaks on formate glucose agar.

* + = to 1 mm. 1/2" dia.

Transfer (b) to nutrient agar slant as W-385

For fumci

Test H-12 on:

24h.

48h.

1. EMB - 2% Naglyceophosphate · 5H₂O. Large - colonies. ✓
2. 1% Peetti acid, neutralized NaOH. N.S. Agar very soft. ✓
3. Hydrolyzed casein (HC) agar. Moderate colonies.
4. HC - succinate - Chlorophenolendolphenol. Moderate colonies.
Agar was decolorized after autoclaving. Shows diffuse recoloration around colony groups.
entire plate decolorized
colonies greenish gray
colonization p. 20 of 21
5. HC + succinate Cli " v. slight lightening around colony mass
recoloration after U.V.
6. HC - NaCl. No growth. Spontaneous coloration in agar overlying lit.
7. HC - Indigo sulfate .01% Decolorized on autoclaving & agar
+ See } Moderate colonies; no recoloration.
- See }
8. HC - starch Iodine.
+ See. } Color discharged on pyrolysis (I₂) induced.
- See } large, slightly brownish tan ap. colonies.
9. Sorbitol 1% ++ Not quite so intense + as a glucose
but unquestionably strong +.
10. Sorb. 5% + Galactose 5% +++ late reaction Nonhydrolyzation
11. (tactitol)
12. Galactose .5%. +++ / ✓

K-12"; W-145; growth on synth. medium.

183

April 30, 1948.

Inoc W-145 lightly into T(m) T_{LB}, BM + .1%

	24h.	72h.
1. Glucamate	-	+++
2. Glucose	+++	++
3. Lactose	±	++
4. Maltose	+	+±

Bacillus further and examine for
sp. reactivities. S.O. P3 on homologous
medium.

58-161 into.

1. Na glyceophosphate .5H ₂ O	0.2%	24h. +++	S.O. Sp plate
2. Pectic acid; neutr. NaOH.			Faint + on EMB
EMB.	58-161	-	72h. + +
	Y10	-	+ +

P3.

→ S.O. 1, 3 and 4 on homologous EMB agar.

1. No acid production; colonies very substantial

3. Numerous + colonies. Pick to new EMB

4. Maltose - all -

3/4 colonies all -. Purify on lactose EMB.

W-391

April 29, 1948.

V10 1 drop, etc. (Haworth lamp 5 secs.) on glucose EMB.

Most of 52 plates were heavily contaminated.

Select some likely colonies from 20 best cont. plates; ca 500 selectable colonies

3 Glucose - streak across T1. All V^S. = 19,000.

	Glu	Gal	Lac	Mal	Gma
1. W-382	- *	+++	+++	+ pap.	+++
2. W-383	-	±	±	-	++
3. W-384	-	++	-	-	++

-382. Why papillae only on maltose? This appears to be the desired Glucose-specific mutant, for crosses with Gal -.

* produces acid strongly when left out at room temperature 2-3 hours!
(compare 340).

~~Streak out 382 and 340 on each of five glu plates. Incubate overnight at 37°.~~ See 185

5-3-42

Strains out to form colonies of: (on EMB 1%:).

	Rhamnose	Glucose	Sorbitol	Fructose	Mannitol	Mannose	Galactose	D-Glucitol	Mannurac	Xyl	Nr
1. 254 *	-	++	++	+	v	++	++	++	v	+	-
										+ and -	
2. 108	-	++	-	-	-	-	-	v	-	+	-
										-	++
3. 185 b + mch inb	-	++	-	inb	-	++	-	-	-	-	inb mch
4. 185 b -	+	-	-	-	AbP.	-	P	-	P	-	AbP.
5. 249	-	+	-	*	-	-	-	-	-	-	AbP.
				V							
6. 351	-	++	++	v	++	v	++	v	++	v	Ab:P.
7. 361	-	++	++	v	++	v	++	v	++	v	-
8. 58-161	+	++	++	v	++	v	++	v	++	v	-
Y10 -	<i>mannitol</i>										
	<i>Ab some X water</i>										

p = papillar, presumably reversion.

* S.C. on homologous medium, 16

Lac₃ Crosses

May 4, 1948.

Cross the following on EMS-Lac-B₁.

1. W-108 x W-249 (A conc. susp) T-L-B₁-Lac₃ x B-M-Lac_X
 2. W-108 x Y-40 x B-M V₁^r V₁
 3. W-249 x Y-46 x T-L-B₁-V₁^r

P7.

① Yield very poor.

By plate.

	+	-	to retest
	0	1	
	0	1	
	0	0	
	0	0	
	0	1	
A.	0	1	
A.	0	5	
A.	0	3	
A	0	4	
	0	2	
	0	0	
	0	3	
	0	1	
A	0	2	
	0	1	
	0	0	
	0	2	
	0	0	
A	0	3	
A	0	4	
	0	2	
	0	1	
	0	0	
	0	38	
	0	1	

After several days incubation, some lac+ 's came up. Since these may represent crossovers, do not use these plates.

(2)

	+	-
2	31	
1	25	
6	34	
2	52	
2	30	
4	50	
0	32	

$$\begin{array}{r} 17 \quad 254 \\ \hline 281 \end{array} = 6.7\% \text{ Lac}_3 +.$$

$T-L-B_1-Lac_3-B+M+$ } x
 $T+L+B_1+Lac_3+B-M-$ }

Lac_3 is fairly closely linked to $B14$. (very near Lac_2)

Phage tests (on glucose plates).

$Lac+$: $6^R \quad 2^S \quad | \quad 8 \text{ All Blue +}$

$Lac-:$ $\left(\begin{matrix} 48^R \\ 51 \end{matrix} \right) \quad \left(\begin{matrix} 13^S \\ 12 \end{matrix} \right) \quad \left(\begin{matrix} 61 \\ 63 \end{matrix} \right) \quad \left[\begin{matrix} \text{All Blue -} \\ \% V^R = 80\% \end{matrix} \right]$

$99 \quad 25 \quad | \quad 124.$

(3). Very poor yield and rather dense background.

$$\begin{array}{r} 0 \quad 1 \\ 0 \quad | \\ 0 \quad 0 \\ 2 \quad 0 \\ 0 \quad 0 \\ 1 \quad 0 \\ \hline 4. \quad 3 \end{array}$$

May 3, 1948.

$$100 \text{ plates DluEMB} \times 250/\text{plate} = 25,000.$$

17 tiny colonies streaked whole on glucose

3 - (1-3)

14 other possibles S.O. on glucose.

	4	0	nucoid
	5	0	+
	6	0	+
(4)	7	0	+
(5)	8	0	-
	9	0	-
	10	0	+
	11	0	+
	12	0	+
(6)	13	0	+
(7)	14	0	slow?
	15	0	- sm. cl.
	16	0	+
	17	0	+

1, 2, 4, 5 and 7 are T, S, and probably mutants.

3 is a yellow charrogen } almost certainly contaminants.
6 a pink charrogen }

W-

1.	386	-	-	+ slow	+	++
2.	387	±	±	+	±	++
3.	388	-	-	- th.	- th. +	-
4.	389	Charr. +	+	++	+++	+++
5.	390	Charr. +	++	++	+++	+++
6.	391	Charr. +	+++	-	±	-

→ specifically bac +

May 5, 1948.

1. 108 × 58-161 + glucose ± B,
2. 249 × 108 + glucose B,
3. 382 × 249 + glucose, lactose
4. 382 × 58-161 glucose, lactose.

P7:

1 - B₁.

+	-
0	48
19	177
16	133
35	300
	335

To be paged
cont'd

+ B₁

21	163	184
56	463	519

Some colonies are darkened
but probably not +

Second
P10:

2. Yield negligible (ca^{<1} peptate)

3. (glucose) Yield negligible - all-
lactose. All look "+" after prolonged incubation. Score on glucose, T₁.

4. Glucose - measurable - no yield
lactose - all turned +.

192a

Tetragolur.

May 7, 1948.

- ①. Make up varying concentrations of triphenyl tetragolur chloride in nutrient agar and autoclave. Sterile 7100_r plates.

Per ml:

- 1mg. Medium faint pink; all colonies intense deep red
150r Medium sl. tinged; isolated colonies deeply red with traces magenta.
50r As above. Medium less tinged
30r As above for isolated colonies; confluent growth colorless
10r Color more limited in colonies and sl. less intense.

1mg. level shows slight initial growth inhibition

Lac 3 mapping. May 10, 1948

- ① W-108 x Y40. in lac and glc EMS (NF).
 ② W-249 x Y46
 ③ W-108 x W-249.

3:

	-	+
24	0	
55	0	
9	0	
10	0	
31	0	
L 67	0	
L 32	0	
L 24	0	
L 22	0	
L 25	0	
L 11	0	
L 31	0	
L 26	0	
L 31	0	
L 41	0	
L 24	0	
L 16	0	
L 17	0	
5	0	
<hr/>		
Lac:	191	0.
Glu:	310	0

~~Folded~~ 01 0
 5D1.

Both are probably lac₃ - .

(2)

Plates v. unsatisfactory. Overgrown or noxious sample plates
readable, esp. lactose. + -

18	2
2	1
16	4
3	2
3	0
4	0
4	1
3	0
7	0
7	2

67 12 789.

This count unsatisfactory except to indicate more + than -.

(1)

Lac.

-	+
53	8
45	13
24	3
39	10
14	5
44	16
29	3
31	4
35	8
42	11
42	9
39	8
75	7

all scored (-) on glucose,
probably due to unsatisfactory
of medium. Test by streaking
to fresh glu EMB.

512 105 617 = 17% Lac+. 83% Lac-

Test Lac+ on Glu, T1:

R	S
22	2
17	2
13	1

= 13% among Lac+

Test Lac- on Glu, T1

52 8 60.

Test Lac- segregants on T₁ (Is it or isn't it MS?)

R	S	
15	5	20
14	6	20
14	6	20
13	7	20
56	24	80 ✓

30%^s among Lac-

The distribution is then:

m.d. (calculated from $\overline{15}$).

-R	.58	I	.67
-S	.25	II	.26
+R	.15	III	.16
+S.	.022	IV	
1.00		109	[cf 80 as previous estimates.]

This gives a total for the V. segregation of 73% R; or 23% crossing

Y40. $\frac{-}{-} + R + +$

108 $\frac{++}{Ia.} - \frac{S}{II} \frac{III}{--}$

over in regis III which agrees
very well with preceding data
(v. thesis table 6) giving 27%.

Estimating x from these data:

$$\begin{array}{lcl}
 \text{such } a^2 = .022 \times .15 / .58 \times .25 & = & .0238 \\
 " b = .022 \times .58 / .15 \times .25 & = & .340 \\
 " c = .022 \times .25 / .15 \times .58 & = & .064
 \end{array}
 \quad \begin{array}{r}
 \sqrt{154} \\
 .\underline{\underline{1}}\underline{\underline{5}}\underline{\underline{4}} \\
 .16
 \end{array}
 \quad \begin{array}{r}
 a \\
 .\underline{\underline{5}}\underline{\underline{8}}\underline{\underline{3}} \\
 .67
 \end{array}
 \quad \begin{array}{r}
 .253 \\
 .26
 \end{array}
 \quad \begin{array}{r}
 \\ \\
 \\ \\
 \hline 1.08
 \end{array}$$

May 17, 1948.

1. 108 x y40 On Lac- and on Gna EMS'
2. W-67 X Y46 On Lac
3. W-126 X Y40. On Lac

1: gna: Yield ≤ 10 / plate. Test on glucose EMS: T1.

$-R$	$-S$	$+R$	$+S$
22	8	1	1
			32.

Lac-	Lac+	Lac- : $6^R : 1^S$	all beta +
9	0		
20	0		
24	1		
1	0		
5	0		
4	0		
2	1		
13	1		
4	1		
3	0		
3	0		
10	2		
8	0		
6	0		
6	1		
4	0		
3	0		
3	0		
2	0		
<hr/>		137.	5.1% -

The distribution is:

$-R$	$-S$	$+R$	$+S$
.684	.275	.044	.007

Total V^R segregants:
28.2% ~~S~~ S

(2).

$$\begin{array}{r}
 + \\
 3 \\
 1 \\
 1 \\
 2 \\
 1 \\
 1 \\
 1 \\
 3 \\
 3 \\
 3 \\
 1 \\
 4 \\
 4 \\
 1 \\
 3 \\
 2 \\
 1 \\
 \hline
 32 \quad 0.
 \end{array}$$

W-67 x 4.46.

B-M-Lacy - x T-L-B, - V₁^R.

R	S
29	2 31.

(3).

$$\begin{array}{r}
 + \\
 1 \\
 0 \\
 7 \\
 6 \\
 1 \\
 1 \\
 0 \\
 2 \\
 2 \\
 4 \\
 1 \\
 4 \\
 1 \\
 1 \\
 16 \\
 2 \\
 1 \\
 \hline
 54 \quad 118
 \end{array}$$

Lac-	R	S	-R	,47
	11	4	+R	,27
	11	6	-S	,22
	22	10 32	+S.	,04
Lac+	8	3	+ > rather high, otherwise agrees with sign. of Lac-1.	
	19	0		
	20	3		
	47	6 53		

$$\overline{54 \quad 118} \quad | 172 \quad 31\%+ \quad (\text{Maybe excessive})$$

On these plates, - colonies were much smaller than + possibly distorting ratios.

W-108 X Y-40.

p/187: 17 \neq : 254- on lactose. ie 6.7% Lac₃ \neq Among \neq , 6 V₁^R : 2 V₁^S.

- 99 : 25

80% R.

/191: 56 \neq : 463- i.e. 13% Lac₃ \neq For argument of
Lac- segregation,

$$\chi^2_2 = 22.2$$

$$p = < .001$$

/198: 105 \neq : 512- 17% Lac- \neq Among \neq , 52R:8S

13% S.

Among - 56R:24S

70% R among Lac-. → cf 187.

$$\chi^2_1 = 2.83$$

$$p = .09$$

for fit of V, R.

199. ~~130~~ : 130- : 7+ 5.1% Lac₃ \neq

Among + 6R:1S

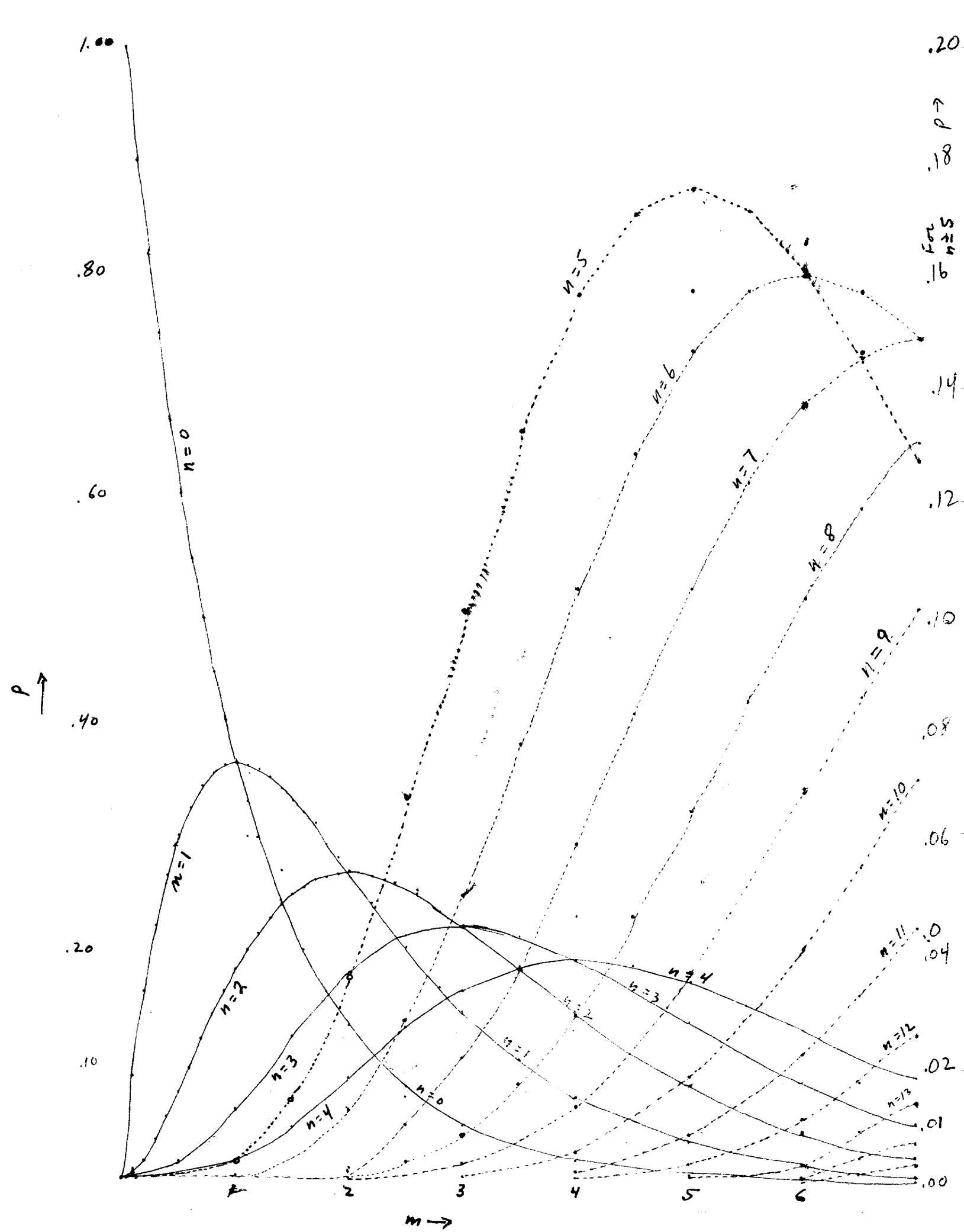
Among - 82R:33S

71% R.

199 (transf. from galactonate EHS).

	-	30	22	R	S	
	+	2.	1		8	= 73% R.

All agree on Lac- = R.
 Lac+ = Lac+ +
 on totaly tests for R.
 $\text{O} = 344 \text{ } 5/2/198$



	<i>coli</i>	<i>coli</i>	<i>coli</i>	<i>Acidovibrio salm</i>	<i>Typhim.</i>
Maltose	+			+	+
Saccharose	+			+	-
Galactose					
Cellobiose	+	some -			
Sucrose	±			+	-
Fruktose	+				
Raffinose	±				
Salicin	±			+	+
Amygdalin	+			+	-

C₂+C₃

(Compound) E.coli E.coli E.coli Aerobacter salmonella E.typhii

Glyceroldehyde + + +

Dihydroxyacetone + +

Glycerol + - + - +

$\text{CH}_3-\underset{\backslash \text{O}}{\text{CH}}-\text{CH}_2$ - -

$\text{CH}_3-\text{CH(OH)}-\text{CH}_2\text{OH}$ +

$\text{H}_2\overset{\text{O}}{\underset{\backslash}{\text{C}}}=\text{CH}_2$ - -

$\text{HOH}_2\text{C}-\text{CH}_2\text{OH}$ - +

c 4.

E. coli *coli* *coli* *Aerobacter* *salmonicola* *E. typhii*

erythritol - - - - -

Adonitol - +

C5

K-12

	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>Aerobac</i>	<i>Selma</i>	<i>Typh</i>	<u><i>li</i></u>
D-Arabinoose	+			+	+		
L-Arabinoose	+	+		+	+		-
D-Ribose	+			+	+		+
L-Ribose	+			+	+		-
D-Lyxose	+			+	+		-
D-Xylose	+	+		+	+		-
L-Rhamnose	+	K-12: -		+	+		-
arabinose ac.	-	+		-	+	-	+
xylose ac.	+			*		+	
α -methylarabinose	-			+	-	-	
β -methyl xylose	-			-	-	-	
α -methylmannose	-			-			
D-erythritol	-			+		-	

	<u>C₆ + deoxy.</u>	<u>R+R</u>	<u>R-12</u>	<u>(acet. diamine + glycinamide)</u>	<u>Acetoin</u>	<u>Salicin</u>	<u>Typtol</u>
glucose	+		+	+	+	+	+
mannose	+		+	+	+	+	+
galactose	+		+	+	+	-	-
sorbitol	+		+	+	+	+	+
dulcitol	±		-	-	±	±	-
inositol	-	+		+	-	-	-
mannitol	+		+	+	+	+	+

d-glucuronic	+			+	+	+	+
L-galacturonic	+			-	+	-	-
muconic	±			±	±	-	-
d-saccharic	+			+	±	-	-
glucosaminic	+			+	+	-	-
d-mannuronic	+			+	-	-	-
glycuronic	+			+	+	-	-
d-methyl glucoside	-	+	chloral	+	-	-	-
see over.		occ. form d.					
B-methylglucoside	+ ✓	+		+ -	+		
d-methylgalactoside	+ -	+	-	+ -	?		
B-methylgalact.	+ ✓	+	-	+ -	.		
tetramethyl glucoside	-	.	.	-			
3methyl glucose	-			-			
d-methylmannoside	-			-			
B-methylfructoside	-			-			

$\alpha \phi$ glucoside	<i>coli</i>	-
$\beta \phi$ glucoside		-
$\alpha \phi$ galactoside		-
$\beta \phi$ galactoside		+ (lectose adap.)