Jan. 9, 1947.

Graduate 5 ml standard suspension in water of 1/10.

Plate out 0.5 ml samples.

<table>
<thead>
<tr>
<th>Time</th>
<th>Plate Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>4/6</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>120</td>
<td>0</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
</tr>
</tbody>
</table>

11/9/47 PH. Repeat.
Jan 8, 1947.

As 75, duplicate Y10.

Maltose: 36 plates x 200 = 7,200 cols.

1. ○ + and -; rest uncleared. W102 - W103
2. ○ faint. All - Picke up W95.
3. ○ + and - + W97 - W96.
4. ○ + and - - W98 + W99.
5. ○ + and -; rest uncleared. W104 - W105 + W106
6. ○ + and -

Rae: 36 plates x = 7,200 cols.

1. ○ + and - W108 - W109+
2. ○ Incubate
3. ○ + and ± (○). W110 ± W111 + See 197.
4. ○ All +
5. ○ + and - W112 - W113 +

[Cross-test here].
Jan 9, 1948.

**Lactose analogues**

<table>
<thead>
<tr>
<th></th>
<th>b-De-galact</th>
<th>b-N-butyl gala.</th>
<th>O-Cresyl-b-galact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y10</td>
<td>++</td>
<td>++</td>
<td>± papillate similar to</td>
</tr>
<tr>
<td>Y53</td>
<td>± slow</td>
<td>++</td>
<td>±</td>
</tr>
<tr>
<td>Y35</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Y45</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The β-N-butyl galactoside gives the most straight forward differentiation, as far noted.

**Sucrose & Melibiose & Raffinose**.

<table>
<thead>
<tr>
<th></th>
<th>ref 3%</th>
<th>Melibiose &amp; fil.</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raff. +</td>
<td>±</td>
<td>slow ++</td>
<td>-</td>
</tr>
<tr>
<td>Raff. -</td>
<td>±</td>
<td>slow +</td>
<td>-</td>
</tr>
<tr>
<td>Y40</td>
<td>±</td>
<td>slow +</td>
<td>-</td>
</tr>
</tbody>
</table>

Melibiose activity should be enhanced before attempting test on raffinose.

Fructose sterile filtered.

Y40     +++
W-1     +++
January 4, 1948.

Inoculate YP broths with following:

Y53 (Lac<sup>-</sup>) and:

Cross each on three plates.

A8. (perception, +)

<table>
<thead>
<tr>
<th>No.</th>
<th>Strain</th>
<th>Growth</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>W-30</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>W-35</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>W-40</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>W-42</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>W-43</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>W-44</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>W-45</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>W-47</td>
<td>+</td>
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<tr>
<td>9</td>
<td>W-48</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>W-65</td>
<td>-</td>
</tr>
</tbody>
</table>

Harvest and mix cells. Plate dilute on Edi-Lac(B1).

None seem to be allelic with Y53 Lac.-

a) W35, W45 1/2 - 1/3 lac+ recombinants

b) W40, W42, W43, W48, W65 1% lac+ recombinants.

c) Y53 (Y87?). Original data in Y87 were more limited than these.

Screen out all lac- and Mal- mutants for recheck!
January 8, 1948

Prepare inocula overnight in YP broth.

Y40 10 AM add 2-3 ml to YP-maltose (A,B) and YP-glucose (C,D) broths.

Incubate W-l similarly in YP for five hours to 2 PM. Cultures of Y-40 are actively producing gas at this time. Was and cross samples of A,B,C,D, with W-l. Plate on synthetic EM-Maltose(B₁). Count sectores as x.

<table>
<thead>
<tr>
<th></th>
<th>M⁺</th>
<th>M⁻</th>
<th>%⁺</th>
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<tr>
<td>A</td>
<td>4</td>
<td>110</td>
<td>0</td>
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<td>3</td>
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<td>88</td>
<td>0</td>
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<td>6</td>
<td>113</td>
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<td></td>
<td>3</td>
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<td>0</td>
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<td>9</td>
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<td>177</td>
<td>0</td>
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<td></td>
<td>12</td>
<td>388</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>67</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.99%</td>
</tr>
</tbody>
</table>

B:

|   | 0   | 68  | 0   |
|   | 1   | 179 | 0   |
|   | 12  | 438 | 2   |
|   | 7   | 236 | 2   |
|   | 4   | 384 | 2   |
|   | 1   | 70  | 2   |
|   | 10  | 237 | 2   |
|   | 4   | 135 | 2   |
| 46 | 202  | 2.18% |

Conclusion: No effect of preadaptation.
\begin{align*}
\begin{array}{cccc}
8 & 3 & 5 & 0 \\
8 & 2 & 9 & 1 \\
8 & 5 & 6 & 0 \\
6 & 3 & 9 & 0 \\
9 & 5 & 2 & 0 \\
11 & 2 & 6 & 0 \\
\end{array}
\end{align*}
<table>
<thead>
<tr>
<th>C1</th>
<th>(C1)</th>
<th>H-</th>
<th>M+</th>
<th>S</th>
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<td></td>
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<tr>
<td>2.</td>
<td>2</td>
<td>47</td>
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<tr>
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<td>3</td>
<td>135</td>
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<tr>
<td>4.</td>
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<td>98</td>
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</tr>
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<td>2</td>
<td>71</td>
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<td></td>
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<tr>
<td>8.</td>
<td>2</td>
<td>1019</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>1019</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>1019</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>total: 1041</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D: (G2).</th>
<th>11</th>
<th>269</th>
<th>3</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>8</td>
<td>213</td>
<td>3</td>
</tr>
<tr>
<td>2.</td>
<td>3</td>
<td>108</td>
<td>1</td>
</tr>
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<td>3.</td>
<td>14</td>
<td>357</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>5</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td></td>
<td>total: 1153</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

41 1112 1153

63 2131 2194 2.871% Mal+

\[ L = 16 \left( \frac{1}{63} + \frac{1}{91} + \frac{1}{3} + \cdots \right) \]

\[ = .5 \]

Comparison:

\[ \text{Mean: Mal}^+ = \frac{154}{5765} = 0.027\% \]
Jan 12, 1948

Irradiate .1 ml per plate (LacEMB) 9 secs. under Hanovia.

71 plates x ca. 30 colonies or 2000 colonies.

3 suspicious colonies streaked out:

1: [Signature]

2: [Signature]  NO MUTANTS

3:

Plate mixtures on Lactose-EmbB:

Y87(Lac1−)  W-45(Lac2−)  see 81
(1000)  ++

W108  +++

W-112  + ±

Plate mixtures on Maltose-EmbB:

a  b

W56(Mal1−)  W-60(Mal2−)

W-1  (7/100)  +

W95  ? ±

W-96  ±, +

W98  ±(1/1000)  ±

W100  ±, −

W102  ±

W104  ±

W106  ±

++  =  1:100

+++  =  majority, no streaks

Parents checked:  p: papillation, no heavy streaks.

Y53  − p
W45  − p
W108  − p
Y87  − p
W-12  − p
W102  − p
W52  − Np
W98  − p
W96  − p
W95  ± p
W102  ± p

W78  slow− but utilizes

W10  − Np

W20  slow− utilizes

10  slow + p

W71  ± p
Jan 10 ff 1948

Test strains indicated on T(m) plus .05% substrate.

A. Inulin
   W-55
   Pl2 (48h)  -

B. "Bacterial Dextran"
   Lot L-10 from
   K.P. Link  -

Inoc. Pl2

C. "Soluble Starch"
   as above,

   A14  ±
   A17  ±

425

WS 5 flora+ seems to accumulate a red-staining "dextran" from
Amylopectin and soluble starch, but utilizes amylose completely.
"Saccharifying amylase??"
Cross available B-11 - lac minusfactors with T23, lac, and lac tester, Y3 and W-108

A  W-112
W-112

B  W-108

Y87.0

W31 n.c., N.C., col. n.c., col.
W35
W40
W42
W43
W45
W48
W55
W67 + + intermediates?
W72 n.c., + intermediate.
W74+
W76
W83
W87

+ +
+ +
+ +
+ +
+ +
+ +
+ +
+ +
+ +
+ +
+ +
+ +
+  + also intermediates??

n.c., + 2-m color (prey plate).

n.c., n.c.

Suspend cells from plants. Spread on lac EMB (ca. 10^9, 000 100/ml) and primate 15 sec. under short's lamps. As sugars caption.

Run n.g. Evidently wrong cells (mixture lac + / lac - ) were used for radiation.

Grow 1.2 l. W94 in N2ase 1%, glucose 75% (ster. sep.)
and K2HPO4 + KH2PO4 (3:1) 0.4%. 1.5 gallon Pernix carry
24 hr. at 37° with aeration.

Collect 539 g. paste in Sterapaks. Resuspend in 1.0% NaCl 2 liters
and recover 399 g. washed paste.

Mix paste with parts pyridine and 0.7% NaCl for 1 hr. (pH 7-
7.2) in 0.01 M NaCl (1 ml each). Sediment glass and debris and collect supernatant juice.

Add 2 vol. alcohol and store in refrigerator. To 100 cc portion:

(A) Ternamidone (40 ml.) add 1/3 vol. chloroform + 
3/10 vol. H3PO4

Mix and store.

(B) Decant and reject supernatant from A. Sediment and redisolve
in 50 ml 0.1M NaCl. Add 2 vol. 95% alcohol via sterile flask.
Repeat. → 3.9 gms. de- med. paste.

(C) Reject gelled CHCl3 0.1M NaCl. Sediment and decant supernatant.
Retreat with CHCl3 overnight. Repeat twice.
The bulk of extract A. in 95% alcohol.

Suspend 1 gm paste A. in 20 ml NaCl. Add 5 ml aliquots to sterile test tubes and add 10 ml alcohol to each (use acetone for B. V.). Allow to stand for 10 minutes, sediment and replace alcohol with sterile saline. These will contain 1 gm paste/40 ml saline.

Sol.

B. Third "swagging" → almost clear, opalescent. j. g. liquid. Remove from residual CHCl₃ and ppt. with alcohol 2:1 as above. Sediment and wash with 75% alc. to remove exc. CHCl₃. Reseeped sediment in 10 ml H₂O, add 5 x alcohol. Ppt. fibrous. Lift out with glass rod and resepnd in 1 M NaCl → clear but as. opalescent solution. Helpful.


Note. 1 tube of B. pptd with 2 vols. alcohol. No fibrous ppt. formed, suggesting depolymerisation.
January 19, 1948.

Add 1 ml. 90% B, resp. to 10 ml YB broth tubes 15 cc. Each.

Use 3 for fluidity tests, inoculate each of the other three with YBl. Culture Y138. Add 3 tubes of C virus for no. treatment controls.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1</td>
</tr>
<tr>
<td>2</td>
<td>A2</td>
</tr>
<tr>
<td>3</td>
<td>A3</td>
</tr>
<tr>
<td>4</td>
<td>A4+</td>
</tr>
<tr>
<td>5</td>
<td>A5+</td>
</tr>
<tr>
<td>6</td>
<td>B1</td>
</tr>
<tr>
<td>7</td>
<td>B2</td>
</tr>
<tr>
<td>8</td>
<td>B3</td>
</tr>
<tr>
<td>9</td>
<td>B4+</td>
</tr>
<tr>
<td>10</td>
<td>B5+</td>
</tr>
<tr>
<td>11</td>
<td>C1</td>
</tr>
<tr>
<td>12</td>
<td>C2</td>
</tr>
<tr>
<td>13</td>
<td>C3</td>
</tr>
</tbody>
</table>

All Mal +
all Mal +. (A plaque plaque?)

All Mal +

No colonies
Some mening - Not coli.

All Mal +
All "

cont, Not coli.

All Mal +
"".

Study out all tubes on Mal and EM15.

Test on Y138.
<table>
<thead>
<tr>
<th></th>
<th>O</th>
<th>A</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
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</tr>
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<tr>
<td>13-1</td>
<td></td>
<td>0</td>
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</tr>
</tbody>
</table>

There is no evidence from this experiment of transformation of the A or L loci either by the crude extracts or by the fibrous material of B.

Replate cells in series 1 with A + L again.

Redo work for incomplete plates as soon as possible.

Late.
Jan 23, 1948.

370. Yield: 17 g. Sharple's paste (1/3 cerevisiae).
Suspend in 170 ml. NaCl (physiological) x blend 0.2 ml volume.
Let stand 4 hours, sediment + ppt. supernatant 2 1/2 vols 95% alc.
V. little sediment formed. Separate + store in 70% alcohol. 📊
Jan 27, 1948

Streak out the following "sureains" of W108 on the medium, as indicated, to purify:

From Glucose. EMB plates of 93. - to lactose & maltose.

Test 31 "sureains" on glucose plates on lactose and on maltose.

All 31 glucose-sureains are also lactose & maltose +.

plates M1, M2, M2, M2

From Lact + Malt EMB. Streak-out to lact + malt + mann.

+ Mann.
10 Malt + and Lact +
6 Lact + and Malt +.

From 93 broth. 108 H / M & L resp.

From 93 T(m). Maltose 108 H (Tm) / M & L resp.

All sureains are non-specific for glucose, maltose & lactose.

No. tested:

- Glucose 31
- Lactose 6
- Maltose 13
- Mannitol 41

54 tested altogether.
Characterization of W-108

January 28, 1948.

\[ T(m^+) + 0.05\% \text{ WO}_3^{+} \text{ (autoclaved together)} \] \% w/o.

- glucose: -
- d-glucose: -
- d-glucose 6-phosphate: ++
  + glucose: +++

The HDP was prepared from the scleran as salt product by adding excess oxalate and neutralizing with NaOH. The solution contains excess oxalate, which is evidently not inhibitory considering the control. Insoluble oxalates, the HDP solution turns quite yellow, so that breakdown must be suspected. Repeat retest using filter study of HDP.

Test substrate X-19 on HDP. Add to \( T(m^+) \) wvo:

A29, A2

- glucose: -
- fructose: -
- HDP: ++
Jan 29, 1948.

S. dublin I XV g,r ; - 4hbl. B, X

S. paratyphi A. I II XII a ; - Ar + D, + Meth - Tryp -

on asparagine minimal medium.

Mix sep. 1 together into VP broth. O 51 O 537 O 51 + 537

(A) Plate undwashed samples of 16 hr. cultures on asparagine T (m) minimal.

1. 51 12 cols.
2. 537 10 - 20 cols.
3. X ca. 10 - 20 cols.
4. 51 + 537 3 col. 10 - 20 col. 100 col. 100 col.


(4) may represent a cross. Addn't differentiating characteristics needed to eliminate 51 reverseri.
Jan. 21, 1948.

Test 93. W108: \( \text{glu}^+ \) and \( \text{tre}^+ \) on glucose & trehalose EMB.

1. \( \text{glu}^+ \): On EMB, all cultures are as thin as on glucose + trehalose. 
2. \( \text{tre}^+ \): In T(m), both grow quickly on glucose, fairly quickly on trehalose, T + better.

Strains from glucose filament to EMB glucose.

(1) = 24 hours.

Take 99-1, inoculate, as W-117.

W-117 is better on aerobes oxidation of glucose or else a slow fermenter.

Compare on glucose and on K gluconate:

**W117:**
- **EMB:**
  - glucose: + weak +
  - maltose: +
  - lactose: -
  - K glucos: +++

Use these colonies for pure W-117.