Nov. 10, 1947.

Sample from E. coli 7 (2 grams).

Tests in comparison with lactose + galactose at 0.5% in T(a). Add necessary growth factors.

<table>
<thead>
<tr>
<th></th>
<th>galactose</th>
<th>lactose</th>
<th>B- galactoside</th>
<th>B- gal + gal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ ++</td>
<td>++</td>
<td>-</td>
<td>+ +</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+ +</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
<td>+ +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>++</td>
<td>+ +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>++</td>
<td>+ +</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Reactions at 20h, 24h, 36h.

B- galactoside is not generally utilized and may be slightly inhibitory in galactose media. Of 110 known.

<table>
<thead>
<tr>
<th></th>
<th>gal</th>
<th>lac</th>
<th>B- gal</th>
<th>B- gal + gal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+ +</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+ +</td>
</tr>
<tr>
<td>5</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>++</td>
<td>++</td>
<td>+ +</td>
<td>+ +</td>
</tr>
<tr>
<td>7</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note that none of these cultures originally sac- homologous on B-galactose.

Considerable pigment produced in galactose.
Nov 15 1947

Inocula from 23 Sp15. 0.1 ml/tube T(BMTLB1) base.

A (Galactose .05%)  B (β-β-galactoside)  C Galactose +Phenol .02%

<table>
<thead>
<tr>
<th>TIME</th>
<th>SP16</th>
<th>SP16</th>
<th>SP16</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>++</td>
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</tr>
<tr>
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</tr>
<tr>
<td>3</td>
<td>++</td>
<td>++</td>
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</tr>
<tr>
<td>4</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>6</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

?? Do utilization of β-β-galactoside by wild type mutant? ??

on gentiobiose + SP17
"α-β-galactoside" + ++

βα on gentiobiose + ++
"α-β-galactoside" + ++

P17. Strains act on α-β-glucoside EM13:

1A, 1C, 1D.
6A, 6C.

A19. 1: all show a slow type of colony with much slower suggestive of rapid utilization. 1B and 1C show these particularly.
all strains are papillated.

6: somewhat unrounded. Two colony types also noted.

Neutrocheating & phenol + galactose.
Nov. 27, 1947

Test on EMB agar using heavy water suspensions of cells from YP agar slants, except W-28 and W-29 from galactose EMB agar.

48 hrs. results:

| 1. K12 | ++ | W33 | +++ | W35 | - |
| 2. Y10 | ++ | W37 | ++ | W36 | - |
| 3. 58-161 | ++ | W38 | ++ | Y70 | ++ |
| 4. W52 | +++ | W41 | ++ | W40 | ++ |
| 24 hrs. (A2y) | W52 | ++ | W28 | ++ | W42 | ++ | Y53 | ++ |
| 36 hrs. | W52 | +++ | W43 | - | W30 | ++ |
| 48 hrs. | 60 hrs. As above. |

Time seems to be a gradient spectrum of responses. Y52, W-1, W51 and W-33 are distinctly the most positive variants, especially W52. The "negative" types are all "sectored" mutants derived from 58-161 and are lac negative. Since their lac + counterparts is βφ+ a relationship is suggested! The only strain which is even relatively "lac+/βφ-" is W53. While Y53 is lac-βφ+.

Note: lac+ lac-

βφ+ Y10 Y53, W-1.


Suggested crosses: W53 × W-1 lac+βφ- × lac-βφ+, also Malt + W45 × Y10 lac-βφ- × lac+βφ+.
For 2. Tableau, use culture of type 25 (matched strain control) 200 ml. 5% glucose at 37°C, 4 hours

3. a. b. c. d. e. f. g. h. i. j. k. l. m. n. o. p. q. r. s. t. u. v. w. x. y. z.

4. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

5. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

6. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

7. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

8. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

9. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

10. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

11. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

12. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

13. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

14. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

15. a + b + c + d + e + f + g + h + i + j + k + l + m + n + o + p + q + r + s + t + u + v + w + x + y + z.

Grow A-12 in T9O) plus 0.05% sugar 24 h. Harvest and concentrate to ca \(10^{10} \text{ /ml/}

Add 1 ml. cells to 1 ml 5% sugar, and in replicates add \(\text{NaN}_3\) to a final conc. of 
\(2 \times 10^{-3} \text{ M.}\) Add 0.1 ml M/10 phosphate buffer pH 7.0 and 0.05 ml BromCresolPurple .15%

Make up to 5 ml with water, cells added 2 P 16, incubate in 37°C water bath.

Readings at 2 h., 4 h., and 18 h., readings – unless indicated.
Cells grown: 24-48-186
4P17 6P17 10P18
Set up 2P17

A. Glucose\(\text{Tr}(0)+0.05\%\text{ sugar 18 hrs.}\)
B. Maltose\(\text{Harvest + concentrate}\)
C. Trehalose

\[
\begin{array}{c|c|c|c}
 & \text{Suc.} & + & \text{Azide} \\ 
M & + & A_2 & + & + \\ 
& + & + \\ 
T & + & A_2 & + & + \\ 
& + & + \\
\end{array}
\]

A cells did not adapt in 18 hrs. in presence of azide, either to trehalose or to maltose.

\[
\begin{array}{c|c|c|c}
 & + & + & + \\ 
& - & + & + \\ 
& - & + & + \\ 
& - & + & + \\
\end{array}
\]

B cells utilized maltose in the presence of azide, but did not adapt to trehalose.

\[
\begin{array}{c|c|c|c}
 & + & + & + \\ 
& - & - & + \\ 
& + & + & + \\ 
& + & - & + \\
\end{array}
\]

C cells utilized maltose as well as trehalose and glucose, even in presence of maltose.

\[
\begin{array}{c|c|c|c}
 & + & + & + \\ 
& + & + & + \\ 
& + & + & + \\ 
& + & + & + \\
\end{array}
\]

Azide in conc. of \(2 \times 10^{-3}\) M does inhibit fermentation to some extent but seems to block adaptation completely.

\[
\begin{array}{c|c|c|c}
 & + & + & + \\ 
& + & + & + \\ 
& - & + & + \\ 
& - & - & + \\
\end{array}
\]

Conc. trehalose and maltose cross-adapt, but only unilaterally, trehalose adaptation implying maltose adaptation, but not the converse.

\[
\begin{array}{c|c|c|c}
 & + & + & + \\ 
& + & + & + \\ 
& + & + & + \\ 
& + & + & + \\
\end{array}
\]

Query: Will malt-(Tref) cells utilize maltose if grown on trehalose?

Aside does seem to interfere with the fermentation as well as adaptation. 
In-adapted seem to be maltose-adapted but not vice versa.
Dec. 18, 1947.

Harvest K-12 from YP-.1%glucose broth. 16 hr. cultures. Conc. 50/20.

Tubes contain in 3 ml., 1% sugar, 1 ml cells, 0.1 ml Phosphate Buffer M/10 pH 7.0 and indicated conc. azide or DNP. Set up 12:20 PM.

<table>
<thead>
<tr>
<th>Glucose</th>
<th>3:40 PM</th>
<th>6:00 PM</th>
<th>9:40 PM</th>
<th>3:10 PM</th>
<th>5:00 PM</th>
<th>7:00 PM</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azide M/100 X</td>
<td>3:40 PM</td>
<td>6:00 PM</td>
<td>9:40 PM</td>
<td>3:10 PM</td>
<td>5:00 PM</td>
<td>7:00 PM</td>
<td>21.2</td>
</tr>
<tr>
<td>1. -</td>
<td>+++</td>
<td>✓</td>
<td>✓</td>
<td>4.50</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2. 1</td>
<td>++</td>
<td>✓</td>
<td>✓</td>
<td>5.79</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. .5</td>
<td>++</td>
<td>++</td>
<td>✓</td>
<td>5.57</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4. .1</td>
<td>+++</td>
<td>✓</td>
<td>✓</td>
<td>4.78</td>
<td>±</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>5. .05</td>
<td>+++</td>
<td>✓</td>
<td>✓</td>
<td>4.70</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>6. .01</td>
<td>+++</td>
<td>✓</td>
<td>✓</td>
<td>4.36</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

DNP 10^-4 M x

7 5  -  ✓  ✓  ✓  -  -

8 1  +  ✓  ✓  ✓  -  -

At 12:40, none changed.

DNP itself is an indicator. 10^-3 Azide does not appreciably inhibit fermentation, but it does permit slight adaptation:

\[
K = 6.2 \times 10^{-8}
\]

The pH of phosphate buffer is 7.24. \( pK = pK + \frac{(base)}{(acid)} \)

At the initial pH the ratio is ca. 1.6:1. There are altogether 10 uM phosphate. At pH 4.50, the ratio is 1:50. Thus down the pH, the more sensitive the pH is to slight additions of acid, i.e., all but 2% of the base is reacted, and about 66uM H⁺ have been produced (from 50mg = \( 1/2 \) mol = 167uM glucose). More buffer should be used in this system and an indicator used whose PI coincides the pH of phosphate, such as thymol blue.
Dec. 18, 1947.

W-24.

Grew on T (20) + 0.1% trehalose and glucose. Logarithmic growth.

Test for activity of glucose and maltose in yeast extract + Exp. 68.

Harvest some at 24h to 2ml. 50/2. Set Up SP 19.

Grown in broth:
- Glucose 2h: ++
- Trehalose 7p19: ++

Glucose: +++
Trehalose: +

W-1 is therefore capable of producing trehalose but not maltose.

Sporadically mutant (i.e. apparently Tc +), although W-21 is perhaps a little slower on trehalose.

Maltose is not simply an incidental activity of trehalase.

Harvest cells from 1% cultures in T(1/2) 36° into 1/10 ml. (4-12)

Set up tests with 1 ml cells, 1 ml 3% substrate, M/200.4% media and 0.1 ml M/10 phosphate. BCP indicates.

Substrates: G, glucose; L, lactose; M, β-methylgalactopyranoside; and B, N-Butyl-β-galactopyranoside. Ga, galactose.

Grown in/tested on:

<table>
<thead>
<tr>
<th></th>
<th>G/GA</th>
<th>G/G</th>
<th>G/L</th>
<th>G/M</th>
<th>G/B</th>
<th>L/G</th>
<th>L/L</th>
<th>L/M</th>
<th>L/B</th>
<th>L/Ga</th>
</tr>
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<tbody>
<tr>
<td>5 PM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10A15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(23h.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**W108: Test for maltase and lactase activity.**

**Utilization of C-sources**


Grow W-108, Y87, 56 and Y10 in YB broth overnight. Use $\frac{1}{2}$ ml inocula into 10 ml. indicator broth with 1% sugar.

<table>
<thead>
<tr>
<th></th>
<th>Maltose</th>
<th>lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>108</td>
<td></td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>87</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>87</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>56</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>56</td>
<td>±</td>
<td>+++</td>
</tr>
<tr>
<td>108;56</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>108;56</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>108;87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y10</td>
<td>+++</td>
<td>±</td>
</tr>
</tbody>
</table>

By P25 all +++ except W56/N.

Therefore, W108 cells do not produce maltase detectable by the utilization of the hexose components by symbiotic 56, and conversely with lactase and Y87.

Use small inocula from slant-suspensions. T(g) with .05% equiv. C-source.

**W-108: Vinc. P25.**

<table>
<thead>
<tr>
<th></th>
<th>N24</th>
<th>P25</th>
<th>P28</th>
</tr>
</thead>
</table>
| glucose|     |     | +++ | M-L | glucose muton.
| fructose(st. sep)|     |     | +++ | M-L+ |
| trehalose"       |     |     |     |     |
| sucrose          |     |     | ++  | M+L+ |
| maltose          |     |     |     |     |
| lactose          |     |     |     |     |
| Na lactate       | ++  | +++ |     |
| K gluconate      |     |     |     |

**Y-10 glucose**

<table>
<thead>
<tr>
<th></th>
<th>N24</th>
<th>P25</th>
<th>P28</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On 1% EMB plates:

<table>
<thead>
<tr>
<th></th>
<th>N24</th>
<th>P25</th>
</tr>
</thead>
<tbody>
<tr>
<td>K glucon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-arabinose</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>xylose</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>mannitol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lactose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>maltose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note for specific phenotype screening on glucose, maltose & lactose relations.**
Jan 26, 1948

Dissolve W108 + Y10 into 15 ml of 0.05% Bp gelat. + 0.5% H2O2. Incubate 36 hours & test for free phenol with diazo-sulfuric reagent. (Bp gel gives a strong color which, however, disappears in acid solution.) Compare with blanks, etc.

Test 1.

1. Blank
2. Blank medium (Bp gel)
3. pH 4.02%  ++  +++
4. W108 a  ±  +
5. W108 b  ++  +
6. Y10 c  ±  +
7. Y10 d  ++  +
8. Y10 phenomically  -  -

Not very nearly complete splitting by either Y10 or W108 under these conditions. Check out W108 on separate plate to ensure reproducibility. Some splitting is uncertain, ca. 10%. 
Cross-adaptation tests.

Jan 28-1, 1948


2. Glucose
   - Galactose
   - Gluconic acid d- and L-
   - D-xylose

3. Galactose
   - Glucose
   - Gluconic acid d- and L-
   - D-xylose

4. Gluconic acid
   - Galactose
   - Glucose
   - D- and L-acids
   - D-xylose

5. D- and L-arabinose
   - Glucose, galactose, gluconic acid
   - D- and L-acids
   - D-xylose

6. D-xylose
   - Glucose, galactose, gluconic acid
   - D- and L-acids
   - D-xylose
   - Not fermented

Observations:

1. Glucose and galactose are adapted. Also d-xylose and L-arabinose.
2. D-arabinose is not fermented.
4. The resting cell suspension of W108! metabolizes glucose!!! (Repeat).

Cells grown overnight and harvested from YP broth, 50 ml + 1% sugar. Concentrate to 7 ml. Use 1 ml cells, 1 ml agar buffer + 1% sugar.

Noted to be mostly glucose-responsiveness.

Cross-adaptation tests.

Grown in: 
- Tissue: Galactose, Glucanic, L-Arabinic
- HDP.

1. Y10 glucose
2. Galactose
3. Glucanic
4. L-Arabinic
5. W108
6. Glucose
7. + Galactose
8. Glucanic
9. + L-Arabinic
10. -
11. -
Design as above. Cells added 11:30 AM. Variable cell yields!

2 h. 3 h.

* streak out on maltose or glucose

1. Confirm cross-adaptation of galactose & L-arabinose
2. Glucose is adaptive. Glucanophore is forming in glucan-adapted cells.
\[ T(m) + 0.05\% \text{ C source.} \]

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>MDP</th>
<th>Glucose + MDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>W108</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Y10</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

24 hours.
January 31, 1948.

Grow cells of Y10 in 50 ml:

<table>
<thead>
<tr>
<th></th>
<th>glucose</th>
<th>galactose</th>
<th>lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+++</td>
<td>- ++</td>
<td>+++</td>
</tr>
<tr>
<td>B</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>C</td>
<td>+++</td>
<td>++±</td>
<td>+++</td>
</tr>
<tr>
<td>D</td>
<td>+++</td>
<td>(++)</td>
<td>+++</td>
</tr>
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<p>| | | | |</p>
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<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>D 1%</td>
<td></td>
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</tbody>
</table>

Yeast, 2 cm to 5 cm and
Test in corresponding 
medium with 
agar-buffer.

2 hours. 1h. Notice that lactose-adapted cells are also galactose-adapted, but galactose-adapted do not lactose-adapted... galactose is probably an intermediate in lactose utilization.

Adaptation is not completely inhibited by their concentrations of galactose (M/1000). Used (M/100) in future.
Harvest 2 batches (A, B) of A-108 grown in 50 ml. 1% YP-gluconate broth overnight. Test sample for genetic purity.

A. (10 AA) Conc. to 12 ml. Use 1 ml cells per tube, with ½ ml. 10% sugar and phosphate-indicator. (No azide!)

<table>
<thead>
<tr>
<th>Time</th>
<th>gna</th>
<th>gna/gl</th>
<th>gl</th>
<th>gal</th>
<th>gal/gl</th>
<th>Bu-gal</th>
<th>Dugal/glu</th>
<th>aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 AM</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 N</td>
<td>+++</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>130</td>
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<td></td>
</tr>
</tbody>
</table>

aa: 4 ml. cells + 1 ml. gal. + ½ ml. phosphate-indicator for adaptation, to galactose.

B. 11 AM as above. Conc to 10 ml. 1 ml. cells/tube

<table>
<thead>
<tr>
<th>Time</th>
<th>glu</th>
<th>glu</th>
<th>gna</th>
<th>gna-glu</th>
<th>gal</th>
<th>gal</th>
<th>gnagal</th>
<th>glgal</th>
<th>Megal</th>
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<tr>
<td>11:15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±</td>
<td>±</td>
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<tr>
<td>12 N</td>
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<td>±</td>
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<td>+++</td>
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<tr>
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<td>±</td>
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<td>±</td>
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</tbody>
</table>

C. (See page 2)

C. 130 PM. Tana blue, red, xyl, xyl+Acet.
February 13, 1948.

Harvest from 100 ml glucose broth, add to 7 ml. Use 1/2 ml fulin stock. 1/2 ml 10% sugar, 1 ml buffer indicator soln. ± 1/2 ml H2O.

Set up 9/15 A.M. Inc. 37°

<table>
<thead>
<tr>
<th>Time</th>
<th>Blue</th>
<th>Blue/1 ml Dabac</th>
<th>Blue/Sulf. aq.</th>
<th>Blue/Sulf. yel.</th>
<th>Blue/Sulf. T on.</th>
<th>+++++</th>
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<tr>
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<td>-</td>
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<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11:30</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>12:30</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2 P.M.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>5 P.M.</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

1:00 P.M. 3:00 P.M. All -

Medial  Sulf. Medial Sulf. Sulf. Medial

- -
- -
- ± ± ±
± +++ +++

Struck out on Sulfone plates:

Grow Y-10 & W-254 into YP 1% Lactose, 2x50 ml. each.
Y-10 & w-327 into YP 1% Maltose, do.

Harvest each, and concentrate in 10 ml volumes in sugar 0.5%, phosphate M/100.

At same time set up no-cells blanks.

Incubate at 37°C 9h-1P 16. Add 4 ml. Barfoed's reagent to clarify. Boil supernatants 10 mins. Cool. Add 1 drop dil. aerochol 0T to wet Cu₂O ppt, and sediment and wash in H₂O. Take up sediment in acid ferric solution and titrate against 0.0200 N permanganate.

<table>
<thead>
<tr>
<th>Sample</th>
<th>0.10</th>
<th>0.30</th>
<th>0.23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-10 Lac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-10 Mal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.327 Mal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.254 Lac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.40 Mal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.98 Lac</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.23 Lac</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There is therefore an almost equimolar accumulation of monose by 327, but none by 327 on lactose and maltose respectively.

The blanks contain 5 mg. sugar each. Note approximately 10% recovery of maltose, but negligible recovery of lactose.

Keep remainder of suspensions 1 and 4 for further characterization of the accumulated material.
Take 1 ml Exp. suspensions & controls of same carb. comp.
Clarify by 5 ml Cu solution, ppt., and boil; supernatant 10 min.
Sediment Cu, 0 ppt., wash + dry. 10 min. at 70°C.
Test for with N/100 KMnO4.

1. Glucose + Phosphate
2. Maltose + Phosphate
3. Y10 culture
4. W32-7
5. - Phosphate

22.60 - 12.71
23.55 - 22.60
23.55 - 1 drop. No glucose.
23.69 - 23.91. 1 drop. Maltose control.
23.91 - < 1 drop.

X. 20 g. of Sephadex paste W-25 Y ground with pyrex. Extract overnight in cold with 10% NaCl, 0.9% sodium bicarbonate, + dilute to ca. 100 ml.

3/28/48. Test extract on lactose cleavage by Bradford's method:

1 ml extract, 1 ml 5% lactose + make up to 3 ml.
Incubate 3 hr. at 37°. ± 0.01 N HCl to equal ca. 20 ml.

XL >17 cc. (Bradford method)
X 0.23 cc
L 1.18 cc

X + L (added reagent) 2.34 cc

V. High activity thus indicated.

Y. ca 10 g. Autolysate 48 hr. 37° under sterile. Remove volume + clarify. Make up to ca. 50 cc. Appreciate to yellow color, deeper than X.
Pool n-octylate + extract. Add vol. acetone & collect sediment. Wash in acetone. Dry. \( \rightarrow \) 1.6 gms. Acetone powder.
3/22:  
Work in cold.

1. 2 ml X + 8 ml acetone. Collect ppt + resuspend in 3 ml.

2. Do. 2 95% alcohol.

3. 5 ml X + 1.8 g Amides (AS) Collect ppt. Supernatant + Heavy ppt.

4. 

5. 5 ml Y as 2 (x3)

6. See 25. Add 1.9 g AS. Collect ppt. Resuspend. S + 
   Moderate ppt. Leaves v. pale cream solution.

7. See 55. Do.
   Leaves clear solution.

8. See 63. Add 1.9 g AS (to saturation + drop to pH 7.0) No ppt. Brown v. 
   Opalescent solution.


<table>
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<th>Alkali</th>
<th>Reaction</th>
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<td>.5 X</td>
<td>++</td>
<td>8.19</td>
<td>8</td>
</tr>
<tr>
<td>.1 X</td>
<td>++</td>
<td>7.83</td>
<td>5</td>
</tr>
<tr>
<td>.01 X</td>
<td>-</td>
<td>4.40</td>
<td>3</td>
</tr>
<tr>
<td>Y</td>
<td>++</td>
<td>5.84</td>
<td>8</td>
</tr>
<tr>
<td>1.</td>
<td>++</td>
<td>8.42</td>
<td>8</td>
</tr>
<tr>
<td>2.</td>
<td>++</td>
<td>7.20</td>
<td>7</td>
</tr>
<tr>
<td>3.</td>
<td>++</td>
<td>3.10</td>
<td>6</td>
</tr>
<tr>
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<td>+</td>
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<td>7</td>
</tr>
<tr>
<td>5.</td>
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<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>8.</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>9.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>++</td>
<td>8.78</td>
<td>-</td>
</tr>
<tr>
<td>X + Glucose</td>
<td>+++</td>
<td>8.39</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>0.13</td>
<td>Blank</td>
</tr>
</tbody>
</table>

Cuso4 color + ppt.
roughest.

1. Autolysate active
2. Acetone powder active
3. Comes down at pH saturation
4. Self
Fractionation of W-254 lactase.

1. Acetone Residue

2. Fraction 1 (1/4 sat.) sl. opalescent

3. F 2 (1/3 sat.) Clear

4. F 3 (1/4 sat.) Clear

5. F 4 (sat.) Clear

6. F 5 Residue after AS sat. V. opalescent.

Assay with 1/20 lactose, 1/2 hour 37°.
2/20  1.30 - 2.41  1.11
2/1   2.41 - 3.11  6.31
1/1   8.31 - 12.5  ++ H +
1/20  12.5 - 13.40  .81
R     13.40 - 15.70 2.30  Residue not uniformly distributed.
R/20  15.70 - 16.70.

Activity seems to be distributed among the "readable residue", the 1/4 AS and the 3/4 AS fractions. Continue to extract the residue + ppt with 1/2 AS. Pool 1/4 + 3/4 AS fractions with the extracted portion.

Pool Extractables from Decline because + 9 pt. with 1/4 AS. Resuspend in water and centrifuge 30 min at 2,000. Supernatant is very faintly turbid; individual ppt. (Sediment??)

Compare activities: Use 50 ml volumes initially. Assay 20 min at 40°C.

a) ml H/10 + 1 ml B 1/2 dilution
b) 9 ml ml H/10 1/2 dilution

Activity in soluble fractions after AS pptn.

Activity is much less than original conditions so close to substrate exhaustion.
When fraction B is added to AS 50%, these fractions are obtained:

C 1) Supernatant

C 2) Sedimentable residue after re-suspension, mi 1:10

C 3) Non-sedimentable residue

Assay 1/4 ml samples (mi 50 ml) and compare with whole AS

B. (2.5 ml) 10° may be too low!
Preparation of lectase : Batch 2.

Recover K-12 in 24°C. Plant 1% solution/30° under strong reaction.
After 24h: Harvest in Shaples (Watson).

Fraction 1. 31g paste. Add 100ml H2O, filter through, mix in blender + acetone type at 37°C #11/12/26.

Fraction 2. 42g paste. Add 100ml acetone, shake well, sediment + add fresh acetone. After dehydration, dry in desiccator + paraffin. > 15.4g "neatly dry" acetone powder.

Suspend 50g powder in 180ml H2O to extract.

Assay (as in 161.b) 1ml supernatant (20min, 40°C).
3.08 ml 0.02N KMnO4.

Extract with cold H2O 8x. Centrifuge at 4000x rpm 1hr.

Add 17.5g AS (1/2 mol) small gel. Redissolve in H2O. A

Test 1ml samples of each:

162-ya Normal 54.0
162-yb

10° may be too high for assay.

No activity!
P28. Clarify 48h. Autolysate (add a few ml CHCl₃ to take up volume and permit sedimentation of solvent). 120 ml autolysate. Almost entirely clear, light yellow-green solution.

Mix 20 ml sample. Work with the other 100 ml.

Add 35g AS. Collect pp 4.9, centrifuge in 50 ml H₂O. Pigment is left in supernatant.

Bacteria collected by centrifuge. 162-AP.

autolysate/sediment volume

centrifuge autolysate. → 162A.

S

50% AS.

P

100 ml (Residue in 50 ml). B.

Assay .1 ml .01 ml samples (in 100 ml basis) 20 m. 37°

A

C

] No visible Cu²⁺ pptn! [Were cells still adapted?]
[Dogless a factor?]
[Are products being metabolized?]

A29. Repeat using 1 ml, .01 ml. in M/100 Na Citrate buffer pH 7.3.
[Premise prepn. autolyzed in citrate 3.

No Activity.
March 29, 1948.

10 liters in 11-12 v. N2Case + Glucose, N2Case + lactose. (A) (B).
Acetate, 37°, 24 h. (Alb荳ed, antiferm). Collect in flasks.

Bottle A (A). Collect 5.3 g. cell paste from B. Drop A,B nemest)
AI. 10 g. put in 100 ml. ναθα-citrate + 1 ml. thym
BII. 4.3 g. put in 100 ml. 5% lactose ναθα-citrate buffer. 1½ h. Thromoseb, autolyze under 1% thym

Collect after 24 h. Store 1P3 in refrigerator.

B. became very cloudy on standing in refrigerator overnight. Renninin' this material dissolved. Drop 10 ml of each cloudy 
water = 163 B2. I add 1.9 g. Hm. In self. to remain color + cytarato 
fraction. ni-citrate

pp in. Resolved 163 - B2
sup. 163 - B2 from pp in cold
Activity 2 ml eng. + 1 ml 1% lactose, 30 mins, 37°

Glucose lactose Glucose in citrate
A ++
B 1.0 0.0 ++
B1 0.0 0.1 +
B2 1.0 0.1 +1
B3 1.0 0.1 +1

Probably fermentation in lactose 
with limited nitrogen seemed to 
de-adapt the culture. In future, 
add fresh lactose to whole medium 
before centrifuging.

Probable fermentation in lactose 
with limited nitrogen seemed to 
de-adapt the culture. In future, 
add fresh lactose to whole medium 
before centrifuging.
to B2, add 14g of salt.  Redissolve ppt with $\text{H}_2\text{O}$. 
March 29, 1948.

85 plates, Y10, 5 secs. Hamori U.V. ca. 147000. Incubate at 35° 11 A 29 -
x ca. 250 plates colonies.

= 20,000 tests.

Recovered W-340

Test at 45°.

April 1, 1948 + 25 plates, x 200 = 5000. - 25,000 total.

Test W-340 at 36° and 44°.

<table>
<thead>
<tr>
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<th>36°</th>
<th>44°</th>
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<tr>
<td>Glucose</td>
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<tr>
<td>malate</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>gluconic</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>maltose</td>
<td>+</td>
<td>slow</td>
</tr>
<tr>
<td>lactose</td>
<td>++</td>
<td>-</td>
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</tbody>
</table>

* factor at < 36.

At 44° this mutant is similar to W-108, but the lactose activity may be more resistant to 37° than the gluconicase.

April 6, 1948. As above. 100 plates x 300 = 30,000

No detected mutants at 45°.
Temperature maint. at 37 \degree C

W-340 grown GNA broth at 37\degree + 45\degree C, and lac YP at 37\degree C.

Cells harvested from 100ml GNA 37 / 5ml \textit{H}_2\text{O} = 2

\[37\degree + 45\degree = 5\]  

Cells from \textit{YP lac} = 1. (50ml into 2ml \textit{H}_2\text{O}).

Test at 37 \degree C + at 45\degree C.

Set up 11:35 AM. 1 hr. 5 mins. 37\degree 45\degree 5.

\begin{align*}
11. & \quad 1/\text{lac} + ++ \quad \pm +
\end{align*}

\begin{align*}
12. & \quad 2A/\text{Gna} \quad +++
\end{align*}

\begin{align*}
13. & \quad 2B/\text{Gna} \quad +++
\end{align*}

\begin{align*}
14. & \quad 2A/\text{lac} -
\end{align*}

\begin{align*}
15. & \quad 2A/\text{lac} -
\end{align*}

\begin{align*}
16. & \quad 2B/\text{lac} -
\end{align*}

\begin{align*}
17. & \quad 2B/\text{lac} -
\end{align*}

\begin{align*}
12/0 \text{ was } + + \text{ in 5 minutes. } 12x \text{ in 8-10.}
\end{align*}

\begin{align*}
13/0 \text{ ++ in 8 minutes.}
\end{align*}

15 \text{ mins.}

30 \text{ mins.}

No further adaptation in next 6 hours.
April 9, 1948.

Inoc. 50 ml each. K-12 cultures into 10 l. bottles (2) of synthetic medium (v. supra) with 1.5% lactose USP. Aerate at 37°C A9-410. Collect in Sharplies.

87 grams dry cell paste.

Suspend in 100 ml 14/10 ut. saline + 2 ml thymol + autolysate at 37°C. Stir until well dispersed and collect supernatant 10 A12. Cool in ice water 150 cc total.

Save 20 ml. whole autolysate. To remainder (cold), add 45 gms AS. + 1% During centrifugation, about 2/3 of this material was involved in an accident. The gross glass was removed + the supernatant recovered. The cup + broken glass were washed with 100 ml H2O, then 35 g. AS added. The ppt's collected were pooled and redissolved in 50 ml H2O. (A) Proceed with saliimetry of remaining 1/3, dissolved ppt. in 50 ml H2O (B).
<table>
<thead>
<tr>
<th>AO</th>
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<th>-0.01</th>
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<td>OB</td>
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<td>1.42</td>
<td>1.43</td>
<td>1.42</td>
<td>1.34</td>
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<tr>
<td>C20</td>
<td>1.38</td>
<td>1.39</td>
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<tr>
<td>C180</td>
<td>1.47</td>
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<td>1.38</td>
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</table>

No activity!

P180: 1.46

No activity!
April 27, 1948

Each tube is made to 9.5 cc. Cells harvested from V.P. glucose or V.P. lactose overnight.

Each tube contains

1 ml 50% lactose
1 ml cells
0.5 ml cyst. BCP media + 1 ml Phosphate Buffer 17/10
±1 mg valine ± 1 mg tyrosine ± 1 mg hydroxy aspartic ± 1 mg aspartic gram ml.

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<th>2</th>
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</table>

± ammonia added in 0.04 M HCl

By all appearances, valine did not inhibit adaptation, but the experiment is clearly of too long a duration. Hydroxy aspartic, as the other hand, seems to have been inhibitory to adaptation even in the presence of excess pantothenic. The clear interpretation of this experiment demands a better control of the adaptation process.

* + 5-V pantothenate.
April 29, 1948.

<table>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
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<tr>
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<td>+</td>
<td>+</td>
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<tr>
<td>10</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

value inhibits adaptation somewhat and is reversed by isoleucine.

Cells from 400 (in 4fl.) ml N3\% heat 100 - glucose, broth, diluted
ni 10ml. Each tube contains: set up 11:30 A.M.
1 ml cells
1 ml 5% lactose
1 ml Buff. indicator BCP.
1 ml addenda:

1. 
2. (Glucose 5%)
3. + glucose 5%
4. + 20% suc. 1%
5. + 3% case 1%
6. + 2LB.
7. + H2SO4 11%
8. + valine 1mg/ml
9. + isoleucin 0.5 mg/ml
10. + i.d.
May 3, 1948.

Add 1 drop inocula to BCP-fermentation broth, at indicated temperature:

<table>
<thead>
<tr>
<th></th>
<th>glucose</th>
<th>lactose</th>
<th>maltose</th>
<th>gluconic</th>
</tr>
</thead>
<tbody>
<tr>
<td>W-340</td>
<td>30°</td>
<td>++ ++</td>
<td>- + +</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>45°</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W-382</td>
<td>30°</td>
<td>++ ++</td>
<td>++ ++++</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>37°</td>
<td>- ✓</td>
<td>✓ + ✓</td>
<td>- -</td>
</tr>
<tr>
<td></td>
<td>45°</td>
<td>- ✓</td>
<td>✓ - ✓</td>
<td>- +</td>
</tr>
</tbody>
</table>

From 5P3.
First reading 8AV: 154. These are both temperature mutants.
W-340 inocula taken from distant.

From fruit Lee of W-382 on maltose, papilla pulped and starched out.
Halt colonies tested for 37°C but 37-5°C.

| lactose | 19+ 0- |
| glucose | 13+ 1- |

Uncertain on mixed.

Purify 1+ and 1- on maltose.
May 4, 1918.

Use 1 drop micro-cult from fresh gatbuth cultures & incubate fermentation with BCP tubercle indicated.

### Table

<table>
<thead>
<tr>
<th></th>
<th>32°</th>
<th>40°</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>lactose</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>maltose</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>galactose</td>
<td>+++</td>
<td>+++</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>58-161</th>
<th>+++</th>
<th>+++</th>
<th>++</th>
<th>+++</th>
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</thead>
<tbody>
<tr>
<td>W-108</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>W-34/0</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>W-382</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
</tbody>
</table>

Gen. 6 P.H.

1st. reading 9A5 = 15 h.
2nd. reading 9A6 = 39 h.
3rd. reading 9H1 = 63 h.

Note weakness of 58-161 on maltose.

All readings identical.

To
Critical temperature: Temperature mutants.

May 5, 1948.

W-340 and W-382 inoculated into BCP broth tubes at indicated temperatures:

30° Plus on glucose, lactose and maltose in 12 hours.
and galactose

32° Ditto. Inocula from gaa bráth .2 ml.

33-34° Ditto.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>30°</th>
<th>32°</th>
<th>33-34°</th>
</tr>
</thead>
<tbody>
<tr>
<td>W-340</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>W-382</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>58-161</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Temperature</th>
<th>9A6 16h.</th>
<th>Glu</th>
<th>Lac</th>
<th>Mal</th>
<th>Gal</th>
</tr>
</thead>
<tbody>
<tr>
<td>340</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>382</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>108</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>58-161</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

- 1R6

- At 36°, W-382 + lac + bldu -

947
May 6, 1948.

Harvest cells of W-287 from overnight cultures of YP-broth. 50 ml. / 3 ml suspensions.

A) maltose 1%  B) gluconate 1%

To 1 ml 5% substrate, add 1 ml cells and 1 ml .01 M Phosphate buffer plus BCP indicator. Incubate at 36°. Set up 11:15 A6.

\[
\begin{array}{cccc}
A & \text{glucose} & \text{maltose} & \text{gluconic} \\
\hline
\text{A} & \text{+} & \text{+} & \text{+} \\
\text{B} & \text{+} & \text{+] & \text{+} \\
\end{array}
\]

To 1 ml. B cells add 1cc gluconate and .5 ml 1% triphenyl-tetrazolium hydrochloride.

very deep red dry/5 min.

Histological Study:

\[
\begin{array}{ccc}
1 \text{min} (11.30) & \text{+++} \\
4 \text{min} (12.10) & \text{+++} \\
10 \text{min} (12.15) & \text{+++} \\
15 \text{min} (12.30) & \text{+++} \\
4 \text{H} & \text{3:30} \\
6 \text{PM} & \text{-}
\end{array}
\]

9A7. All tubes were +++
Show Y/10, W382 in yno 1/2 broth. Collect cells in 12mL and test at 34°C on glucose and glucosamine. Set up 11 AM.

<table>
<thead>
<tr>
<th>Time</th>
<th>Glucose</th>
<th>J3Na</th>
<th>W382</th>
<th>Glucos</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 AM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1115</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>1130</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Here we inserted W-58, W-340 and 58-161 into BCP tubes at $33^\circ + 40^\circ$ as indicated. 6 P.m. first reading 947:156.

<table>
<thead>
<tr>
<th></th>
<th>340</th>
<th>382</th>
<th>58-161</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Neutral</td>
<td>++</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Salts etc. may suffer a marked decrease.

---

947
234117
May 7, 1948.

Harvest K-12 from 16 hour cultures of YF sugar broth:

<table>
<thead>
<tr>
<th></th>
<th>a) galactose</th>
<th>b) glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>cell a</td>
<td>++</td>
<td>- ++</td>
</tr>
<tr>
<td>cell b</td>
<td>- ++</td>
<td>++ ++</td>
</tr>
<tr>
<td>cell c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10:45 (14.57).

Initial reading:

1st reading:

11:30

2nd reading:

12:00

See 105. (Adaptation in presence of anyid: \(\text{galactose} \times \text{galactose}\) + Cohen's letter

with Y10.

2- \(\text{galactose}\) and d- \(\text{galactose}\) adapted cells were reciprocally shortened adaptation times. The interconversion is not inhibited by \(\text{glycine}\).
May 7, 1948.

Prepare 8 ml cell suspensions from 50 ml YP broth cultures (YZ-sugar).

Cells: A: no sugar, B: glucose C: galactose D: lactose.
Substrates: 1 glucose, 2: galactose 3: lactose.

After harvesting, incubate cells without substrate or buffer at 33-34° for two hours. Then (1:30 P 7) add 1 ml 5% sugar and buffer-BCP.

W-340 Exactly as above.

Cells: A-glucose, B-galactose, C-lactose Substrates as above.

Glucogenesis is adapted at 34°, but is produced during galactose adaptation.

2 P.M. (20-30mm).
Summary of p. 195

Tested for stability at 40°.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Glucose</th>
<th>Galactose lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Lactose</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

W382 and W340 gave identical results.

1. Glucocyanase in glucose adapted cells is unstable at 40° in absence of substrate, but in galactose and lactose adapted cells is stable.
2. Glucocyanase is adapted at 34°.
3. Lactase is unstable at 40°.

Suggested:

Compare enzymes from Y10 and W-382 under otherwise comparable conditions. I.

Does substrate protect stability? I.
May 8, 1948.

Grow Y-10 and W-382 in 50 ml. batches YZ-sugar broth at 34°.

A. Glucose (2 flasks each)
B. Lactose (2 each)
C. Gluconic (1 each).

Dispense 1 ml. volumes to tubes with 1 ml. indicator buffer (with and without azide) at 40°.

At stated times add 1 ml. substrate and record time required to ferment.

Cells: A, B, C. Substrate: a, b, Azide +, —

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Aa+</th>
<th>Aa-</th>
<th>Ab+</th>
<th>Ab-</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Y-10 cells

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Glucose (a)</th>
<th>Lactose (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

W-382 cells

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Gluconic (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

To = 10:45 AM

15 = 10:45
30 = 10:45
45 = 10:45
60 = 10:45
120 = 10:45
180 = 10:45
cells disincubated 400' for minutes incubation before addition of substrate.

Trypt Referred to Luminal
May 15, 1918.

| W-42 | --- | --- | --- | --- | --- | --- |
| W-110 | --- | --- | --- | --- | --- | --- |
| W:305 | ++ | ++ | ++ | --- | --- | --- |
| Y-10 | ++ | ++ | ++ | --- | --- | --- |

0. N/16. **= 12h.**

1. **= 25h.**

2. **= 39h.**

W-42 is not temperature-responsive.

W-110 is --- at 30, + above 37.

W-305 is almost equally slow at all temperatures compared to Y-10, perhaps slower at 40° than at 37.
to some 12 lac broth, cells harvested in 10 ml H₂O. Sucrose 10% gel

diluted in 10 ml 1/50 citrate buffer pH 7.5 at 37°, OCP 1/5,000. 10 ml

Incubate 10 minis then boil.

1 Preliminary tests:

<table>
<thead>
<tr>
<th>cell</th>
<th>initial absorbance (ext)</th>
<th>1</th>
<th>1.5</th>
<th>3.4</th>
<th>4.4</th>
<th>6.1</th>
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<tbody>
<tr>
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<td>0.065</td>
<td>0.041</td>
<td>0.08</td>
<td>0.071</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.09</td>
<td>0.008</td>
<td>0.027</td>
<td>0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>0.007</td>
<td>0.004</td>
<td>0.023</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>0.0005</td>
<td>0.020</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correction = \( \frac{\lambda_{650}}{\lambda_{420}} \)

2 Final digest: \( \% \text{glyco} \)

<table>
<thead>
<tr>
<th>cell</th>
<th>( \lambda_{420} )</th>
<th>( \lambda_{650} )</th>
<th>( % \text{glyco} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.066</td>
<td>0.041</td>
<td>0.083</td>
</tr>
<tr>
<td>0.1</td>
<td>0.09</td>
<td>0.008</td>
<td>0.027</td>
</tr>
<tr>
<td>0.01</td>
<td>0.007</td>
<td>0.004</td>
<td>0.023</td>
</tr>
<tr>
<td>0.001</td>
<td>0.0005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Use amm. gelate. Vary citrate. 10 min tests 5 boiling.

Range 1-10 seems to be satisfactory. Boiling should be milder as it causes some 2-3% hydrolysis.

<table>
<thead>
<tr>
<th>cell</th>
<th>( \lambda_{420} )</th>
<th>( \lambda_{650} )</th>
<th>( \lambda_{420} )</th>
<th>( \lambda_{650} )</th>
<th>( \lambda_{corr} )</th>
<th>( \Delta )</th>
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<tbody>
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<td>0.041</td>
<td>0.083</td>
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<td></td>
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</tr>
<tr>
<td>0.2</td>
<td>0.09</td>
<td>0.008</td>
<td>0.027</td>
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</tr>
<tr>
<td>0.4</td>
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<td>0.004</td>
<td>0.023</td>
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<td></td>
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<tr>
<td>0.6</td>
<td>0.0005</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>0.8</td>
<td>0.0005</td>
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</tr>
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<td>1.0</td>
<td>0.0005</td>
<td></td>
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</table>

After 14 h

<table>
<thead>
<tr>
<th>cell</th>
<th>( \lambda_{420} )</th>
<th>( \lambda_{650} )</th>
<th>( \lambda_{corr} )</th>
<th>( \Delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.066</td>
<td>0.041</td>
<td>0.083</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.09</td>
<td>0.008</td>
<td>0.027</td>
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<tr>
<td>0.4</td>
<td>0.007</td>
<td>0.004</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>0.0005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.0005</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.0005</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \lambda_{420} = 0.750 \)

\( \lambda_{corr} = 0.525 \)
C   D
1  1.070
1  1.065
2  1.140
2  1.132
4  2.270
4  2.272
6 + 4.409
6  3.94
8  5.15
8  5.11
10 6.14
10 6.19

λ = 4.20
λ = 5.00

160 0.20
107

172 0.25
104

10 min in ATP system.
Inhibition by maltose.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
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<td>1</td>
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</tr>
<tr>
<td>2</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Inhibition by maltose and glucose.

**M/10**

Cells 0.5 ml + 9 ml sugar solution + 1 ml ONPG All in M/50 buffer.

1. Lac 40 ONPG
2. Lac ONPG
3. Glu "
4. Mal "
5. -- "

For readings at 370.

Repeat using sucrose + maltose.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
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<td>.014</td>
</tr>
<tr>
<td>Suc</td>
<td>2.39</td>
<td>.010</td>
</tr>
<tr>
<td>Mal</td>
<td>.083</td>
<td>.004</td>
</tr>
</tbody>
</table>

Note inhibition by maltose but not by sucrose.
Sept. 15, 1949

Inhibition of galactosidase by carbonyl reagent.

% inhibition

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>20%</th>
<th>80%</th>
<th>83%</th>
<th>15%</th>
<th>56%</th>
<th>85%</th>
<th>55%</th>
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<tbody>
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<td></td>
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<td></td>
</tr>
<tr>
<td>BL-1</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<td>0</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>W74</td>
<td>87</td>
<td></td>
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</tr>
<tr>
<td>W33</td>
<td>82</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Y10</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4/50 citrate buffer 7.5
20m, 37° M/10(c.o.) sugars
Sept. 15, 1948.

Due to paucity of material, the following tests were done in 1.0 ml volumes. 0.1 mm was dissolved in 0.01 ml tetrachlor suspension in buffer as above, then 1 ml 1/500 CMC was added after Kemp equilibration. Color read as + or -

- HCl
- KCl
- NaCl
- NaBr
- CaCl
- CaBr

+ Original color makes this reading doubtful.
Sept 17, 1978

<table>
<thead>
<tr>
<th></th>
<th>λ 420</th>
<th>λ 650-680</th>
<th>λ 690</th>
<th>λ 720</th>
<th>zn unw water %121</th>
<th>zn unw water %119</th>
<th>zn unw expt opt color</th>
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<tbody>
<tr>
<td>glucose</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>lactate</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>lact+acid 1/100</td>
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<tr>
<td>lact+Na2</td>
<td></td>
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<tr>
<td>lact</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>lact ATP</td>
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</tr>
<tr>
<td>lact + ADP</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>water 2000</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Centrinate cells from Y2 lac(+) and Y2 Blu(-) 5:1**

Adaptation system: 2 ml cells: 2 ml 4% sugar + H5 buffer + 1 mlk (supplement if any). Centrifuge once + resuspend in 4 ml of test of 0.1% NaC150 + H5 buffer + centrifuge above, 1 ml: 90% NaC150 + buffer.

SMT: Acet apparently inhibits adaptation, benzimidazole does not at this concentration.

Con 100 ml H.12 from Y2 tube to 20 ml (5:1)

Add 2 ml cells to 7 ml sugar 1% in H/S-buff. pH 7.0. Add H2O to
suppl. to 5 ml volume 1/30 A18. Incubate 5, shaking at 37°.

<table>
<thead>
<tr>
<th></th>
<th>Sugar Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactose</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>Peptone, 1%</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>Glucose</td>
</tr>
<tr>
<td>6</td>
<td>Glucose + Folin, 1/2</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
</tr>
<tr>
<td>8</td>
<td>Hydrolyzed Casein 1%</td>
</tr>
</tbody>
</table>

ONPT, as above, but use total volume
of 9 ml, greater than 10, and use 1/10 of cells
phorosy.

Head tubes a) against water suspensions
of same cells, and b) the latter antigen
water, all at 420.

<table>
<thead>
<tr>
<th></th>
<th>A (activity)</th>
<th>B (cells)</th>
<th>R.A.</th>
<th>% L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.160</td>
<td>.207</td>
<td>.77</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>.499</td>
<td>.329</td>
<td>.79</td>
<td>233</td>
</tr>
<tr>
<td>4</td>
<td>.551</td>
<td>.274</td>
<td>.78</td>
<td>231</td>
</tr>
<tr>
<td>5</td>
<td>.1022</td>
<td>.200</td>
<td>.11</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>.201</td>
<td>.230</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>.008</td>
<td>.000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>.519</td>
<td>.039</td>
<td>1.55</td>
<td>202</td>
</tr>
</tbody>
</table>

V. Ext., Peptone + H.C are definitely stimulatory to adap-
tation.
Sept. 20, 1948

System as above (except 2x for anaerobic tests.)
All flasks contain lactose 1%. Deleterious activity

1. - Suppl. 03

2. Lec 35

3. " Glucose/10g 07

4. " (W.H.) 50y 1ml 1% 32

5. " " 1, glucose 11

6. " apragin 43

7. " TL 75

8. " 4, anaerobic 28

9. " 5, anaerobic 21

10. " 4, Vit. 1ml 33

11. " 2, Ane. 125

12. H.C. 120
Sept. 11, 1948.

Effects of amino acids on adaptation.

K-12 harvested from Y-2. Else as above.

Added supplements as 1 mg. in 1 ml.
| A | B | C | D | Percent
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>242</td>
<td>228</td>
<td>-</td>
<td>69</td>
</tr>
<tr>
<td>Loc</td>
<td>280</td>
<td>218</td>
<td>-</td>
<td>71</td>
</tr>
<tr>
<td>Hagal</td>
<td>246</td>
<td>231</td>
<td>++</td>
<td>73</td>
</tr>
<tr>
<td>Hagal</td>
<td>310</td>
<td>310</td>
<td>++</td>
<td>76</td>
</tr>
<tr>
<td>CNP</td>
<td>710</td>
<td>219</td>
<td>-</td>
<td>78</td>
</tr>
</tbody>
</table>

A: D146G, gain, change, V916G
B: D146G + substrate, change, 100
c
C: D146G, (n, n, n)
D: b/c = A
E: b/c = related activity

| A | B | C | D | E % can
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A12</td>
<td>1</td>
<td>171</td>
<td>277</td>
<td>202</td>
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<tr>
<td>A3</td>
<td>2</td>
<td>370</td>
<td>320</td>
<td>216</td>
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<tr>
<td>AN</td>
<td>3</td>
<td>555</td>
<td>535</td>
<td>224</td>
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<tr>
<td>AN</td>
<td>4</td>
<td>273</td>
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<td>246</td>
</tr>
<tr>
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<td>224</td>
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<td>236</td>
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<tr>
<td>AN</td>
<td>8</td>
<td>290</td>
<td>268</td>
<td>232</td>
</tr>
<tr>
<td>AN</td>
<td>9</td>
<td>270</td>
<td>268</td>
<td>207</td>
</tr>
<tr>
<td>AN</td>
<td>10</td>
<td>256</td>
<td>260</td>
<td>232</td>
</tr>
<tr>
<td>AN</td>
<td>11</td>
<td>276</td>
<td>366</td>
<td>222</td>
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<td>12</td>
<td>238</td>
<td>409</td>
<td>214</td>
</tr>
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<td>AN</td>
<td>13</td>
<td>241</td>
<td>383</td>
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<tr>
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<td>14</td>
<td>211</td>
<td>374</td>
<td>226</td>
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<tr>
<td>AN</td>
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<tr>
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<td>376</td>
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<td>AN</td>
<td>17</td>
<td>314</td>
<td>548</td>
<td>312</td>
</tr>
<tr>
<td>AN</td>
<td>18</td>
<td>245</td>
<td>172</td>
<td>237</td>
</tr>
</tbody>
</table>

Only arginine and methionine showed significant stimulatory effect for N-12 adaptation.

5ml system for adaptations above. All sec. K-12.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C = A - B</th>
<th>D (E+C)</th>
<th>E (D</th>
<th>% of FAC(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>225</td>
<td>305</td>
<td>205</td>
<td>100</td>
<td>56</td>
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<tr>
<td>2</td>
<td>H</td>
<td>310</td>
<td>70</td>
<td>279</td>
<td>421</td>
<td>151</td>
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<tr>
<td>3</td>
<td>AA</td>
<td>296</td>
<td>650</td>
<td>266</td>
<td>384</td>
<td>144</td>
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<tr>
<td>4</td>
<td>S</td>
<td>371</td>
<td>520</td>
<td>244</td>
<td>276</td>
<td>118</td>
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<tr>
<td>5</td>
<td>AA - Al</td>
<td>229</td>
<td>309</td>
<td>206</td>
<td>103</td>
<td>(50)</td>
</tr>
<tr>
<td>6</td>
<td>A</td>
<td>249</td>
<td>449</td>
<td>222</td>
<td>257</td>
<td>116</td>
</tr>
<tr>
<td>7</td>
<td>A</td>
<td>251</td>
<td>520</td>
<td>233</td>
<td>187</td>
<td>80</td>
</tr>
<tr>
<td>8</td>
<td>A</td>
<td>241</td>
<td>449</td>
<td>217</td>
<td>212</td>
<td>98</td>
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<td>9</td>
<td>A</td>
<td>256</td>
<td>460</td>
<td>225</td>
<td>235</td>
<td>104</td>
</tr>
<tr>
<td>10</td>
<td>Arg + Meth</td>
<td>237</td>
<td>371</td>
<td>215</td>
<td>156</td>
<td>72</td>
</tr>
</tbody>
</table>

2ml each AA group in 4-9.

5ml ca: 10.

1ml HCl 10% 2.

1ml 10%/HCl 3.

K-12 grown 24 hours in Synthetic + lactose 1%, 10Kites.

2.5 g. cell paste recovered. 202.4 g. + 10 cc. 7.5 phosphate buffer shaken 14 hrs. under toluene. Remove debris & collect supernatant in ca 30 cc buffer. Deep yellow-green fluorescence ca 1 ml./gram protein.

(a) Ca 1 g. washed in acetone and dried at room temperature. Considerable loss by spattering yellow-green calculation only of final product.

See 316

See 325 for assay.
Sept. 25, 1948

K-17 grown in 200 ml Y2 lactose. Harvest to 5 cc 7.5%buffer and lyse in acetone and saline 24 hr + 48 hr.

A. 24 hr. 1 ml withdrawn, debrisi sedimented, supernatant diluted to 4 ml.

B. 48 hr. remainder (4/5) removed, et. dilute to 16 ml

each ml corresponds to 10 ml original culture & should have an activity of ca. 10 x bacterial suspensions (i.e. 0.5 ml should give a 100% hydrolysis of 10 ml 11/5000 on 20 min. 20°C). i.e., calculating 29.1 liter, corresponds to 20 mg/ml

See 3/16
Sept. 27, 1948.

N-12 grown 36 hours in 10 liters S(bac). 9.4 liters of supernatant were removed leaving 31 grams wet sheared paste. Make up to ca. 45 ml with PBS buffer pH 7.5 and grind 75 minutes in Booth-Dean mill. Combine all fluxe & washings. Transpose, opalescent supernatant to obtained in ca. 100 ml, i.e. 0.31 grams/ml.

10 ml sample of culture was taken. Resuspend in 10 ml, measure turbidity at 1:20 dilution.

Effluent A of .100 ml in D2O.

<table>
<thead>
<tr>
<th>Assays</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Act. /ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.08</td>
<td>2.90</td>
<td>2.83</td>
<td>14.315A</td>
<td>1.2 ml</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>2.05</td>
<td>2.05</td>
<td>1.0 B</td>
<td>1.2 ml</td>
</tr>
<tr>
<td>3</td>
<td>0.07</td>
<td>2.40</td>
<td>2.51</td>
<td>2.5</td>
<td>314A 0.1 ml</td>
</tr>
<tr>
<td>4</td>
<td>0.01</td>
<td>0.43</td>
<td>0.42</td>
<td>0.40</td>
<td>0.41 ml</td>
</tr>
<tr>
<td>5</td>
<td>0.10</td>
<td>0.20</td>
<td>0.11</td>
<td>0.90</td>
<td>1.00 ml</td>
</tr>
<tr>
<td>6</td>
<td>0.32</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>316A 0.1 ml</td>
</tr>
<tr>
<td>7</td>
<td>0.02</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>8</td>
<td>0.00</td>
<td>2.90</td>
<td>2.90</td>
<td>2.900</td>
<td>0.01</td>
</tr>
<tr>
<td>9</td>
<td>0.60</td>
<td>1.90</td>
<td>1.60</td>
<td>1.900</td>
<td>(4.5)</td>
</tr>
<tr>
<td>10</td>
<td>0.79</td>
<td>8.80</td>
<td>0.80</td>
<td>(1.00)</td>
<td>0.2 ml</td>
</tr>
</tbody>
</table>

For non-restractive (nono) hydrolyzate.

In prep. 3/4, 1 ml being = culture medium / liter / 20 000 cells.

and .001 ml should be equivalent to 1 ml culture product, i.e. very

likely. Therefore a large proportion of the cellular activity is present
in extract. Hydrolysis is readily effected with 0.1 percent 40% HI.
Sept 28, 1948.

K-12 grown on 100 ml T(0) glucose + de- + H.C. (0.1 ml/100) shaken 16 hours. Adjust densities:

A
1/10 dilution: 259
HC: 319

Dilute the (0) culture to 50 ml H2O; the HC culture in 24.6 ml H2O to adjust initial densities.

The adaptation system consists of 3 ml cells + 3 ml T(0) + glucose + 8 ml buffer. Run per standard procedure. Compositions:

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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</thead>
<tbody>
<tr>
<td>1. T0</td>
<td>.201</td>
<td>.196</td>
<td>.200</td>
<td>.184</td>
<td>.171</td>
</tr>
<tr>
<td>2. T0</td>
<td>.248</td>
<td>.260</td>
<td>.177</td>
<td>.187</td>
<td>.188</td>
</tr>
<tr>
<td>3. T0</td>
<td>.256</td>
<td>.260</td>
<td>.188</td>
<td>.189</td>
<td>.189</td>
</tr>
<tr>
<td>4. H.C</td>
<td>.720</td>
<td>.730</td>
<td>.175</td>
<td>.170</td>
<td>.170</td>
</tr>
<tr>
<td>5. H.C</td>
<td>.710</td>
<td>.731</td>
<td>.170</td>
<td>.170</td>
<td>.170</td>
</tr>
<tr>
<td>6. H.C</td>
<td>.745</td>
<td>.641</td>
<td>.169</td>
<td>.175</td>
<td>.170</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>5.85</td>
<td>6.49</td>
<td>5.85</td>
<td>5.85</td>
<td>5.85</td>
</tr>
</tbody>
</table>

Negligible activity of unadapted culture and of B series.
Sept 28, 1948.

(NE) W478 x W583 on lac B1.

20 colonies inoculated
lac EMB: All +.
Sept. 28-9, 1948.

Original extract (3/6) consisted of 2900 u/ml in 100°C on 2.5 x 10⁵ units altogether. To fractionate remove 50 ml and dilute e 50 ml H₂O (1.5 x 10⁵ units; 1500 u/ml). 

"3/6" is fraction 0. Add on itself in 4 aliquots of 17.5 g. each in ice bath to give 1/4 saturation. Take up aliquots in 10 ml 4/50 PO₄.


1 (1/4sat) 5.00 12 0 19
2 (1/4sat) 5.00 39 0 55
3 (2/3sat) 5.00 19 0 22
4 (sat) 10.00 01 0 15
5 Superml. 1.00 06 0 15

Assay the equivalent of 0.1 and 0.01 ml of the original fraction 0.

1 ml 15/5000 NPG in 4/50 PO₄ buffer.

Enzyme activity is definitely not quite linear. Fractions have higher total activity than the nominal "comps.

Pool fractions 2 + 3 (40 ml) and add 34 gase 48 (3/4sat).

Take up on 1 ml 4/50 citrate buffer 10.0 0 114

P30. To remaining 50 ml (1.5 x 10⁵ units) add 250 ml calcium acetate, litostand, and filter off 330 mg dry powder. 3/1943. This should have an activity of about 5000 u/ml. Take up 10 mg in 10 ml phosphate buffer.
Sept. 29
Lactase preparation 379A is suspended in M/50 citrate buffer, pH 7.5 (Ethylene diamine - citric acid) = (EDC buffer), and should have a potency ca. [100/20] x (0.5 ± 0.23) x 10³ u/ml. = 4000 u/ml.

A. 0.001 ml in citrate and in phosphate buffer M/50, pH 7.5.
Triplicate series.

<table>
<thead>
<tr>
<th>EDC</th>
<th>Phosph</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.371</td>
</tr>
<tr>
<td>2</td>
<td>0.381</td>
</tr>
<tr>
<td>3</td>
<td>0.390</td>
</tr>
<tr>
<td>11</td>
<td>EDC = 0.12</td>
</tr>
<tr>
<td>12</td>
<td>EDC = 0.13</td>
</tr>
<tr>
<td>13</td>
<td>EDC = 0.12</td>
</tr>
</tbody>
</table>

ONPH/5000ml

| 21 | EDC | 64.0 |
| 22 | EDC | 64.0 |
| 31 | PO4 | 75.0 |
| 32 | PO4 | 7.45 |

41. (7 min later.)
EDC + PO4 = 0

may reduce to inulin by citrate.
Sept 30, 1948.

K-12 in A) T/10,

shake overnight.

1:1000 dilution.

A 119
B 114
C 0.52
D 0.50

Adaptation system: 5 ml. Shaken at 37°. 10^3 A - 1^3 C

A 1 176 220
2 239 331
3 162 218
4 160 291

B 1 169 215
2 167 206
3 186 226
4 174 272

C 1 150 281
2 190 310
3 226 589
4 219 778

T(C) cells did not adapt!! T(AA) cells were stimulated by T(10).

+ further by amino acids.
Sept. 30, 1948.

EDC

A. Phosphate vs. citrate. System is, aqueous, 10 ml and 1/2000 in 0.01 M phosphate 0.01 M lactate 3/19A used for test.

1. 1 ml M/5 Phosphate 222
2. 1 ml M/5 Citrate 021
3. 1 ml each 022

All contain 1 ml Phosphate Buffer

A 189
B 012
C 190

The inhibition is clearly due to the ethylene-diamine component of the EDC buffer!

Oct. 1. Test 0.002 ml of 3/19A in the following buffer, each at 4/50 pH 7.5

1. Phosphate 310
2. Glycophosphate 488
3. " + Phosph. 477
4. Galactol 513
5. " + " 494

On N/M 5000 in 4/50
1. Phosphate 694
2. Galactol 645
3. Glycophosphate 725
To test influence of NaCl add 1ml of 1/5 NaCl, KCl, and Na₂SO₄, respectively, to a phosphate buffer system as above. 3198 mol mL⁻¹ phosphate 4/50+.

1. - 2.75
2. NaCl 3.95
3. KCl 2.59

4/50. Repeat

1. 3.17
2. NaCl 5.12
3. Na₂SO₄ 5.92
4. KCl 2.98
5. LiCl 2.18
6. NH₄Cl 2.30
7. (NH₄)₂SO₄ 2.52
8. MgSO₄ 2.57

NaCl concentration series:

1. - 3.18 0.1
2. 0.1 4.05
3. 0.5 3.88
4. 1.0
5. 5.0

Inhibitory.
Sept 30, 1948.

17 g. wet paste K-12 harvested from 20 liters (low yield) S(lae)

Add ca 50cc cold acetone to dehydrate, filter, and decr. the residue. Assay sample of cells for activity.

Duo. A 621 B also, other assays

225

314B. 1 mg 1150
       .1 mg  oo  379
       .01 mg oo  046

319B. 1 mg 1070
       .1 mg oo  960
       .01 mg oo  193

ca. 35 u/mg.

c. 190 u/mg.

3.2 gmmr dry powder obtained: lactase 325 A
Harvest cells of K-12 from 50 ml T(0) grown overnight in shaking to 10 ml 450 phosphate buffer (PB) 7.5, T(0) sugar.

Adaptation system: 5 ml containing 1 ml T(0) ± 5% lactose + varying amounts of cells. A (no supplement): B. 1 ml hydrolyzed casein 10%.

<table>
<thead>
<tr>
<th>Cells</th>
<th>4 (c)</th>
<th>D420</th>
<th>D550</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 0.5 ml</td>
<td>3.5</td>
<td>244</td>
<td>095</td>
</tr>
<tr>
<td>2. 1 ml</td>
<td>3</td>
<td>233</td>
<td>090</td>
</tr>
<tr>
<td>3. 2 ml</td>
<td>2</td>
<td>218</td>
<td>103</td>
</tr>
<tr>
<td>4. 3 ml</td>
<td>1</td>
<td>201</td>
<td>100</td>
</tr>
</tbody>
</table>

C. 5. 5 ml | 3.5 | 601 | 133 |
| 6. 1 ml | 3 | 582 | 128 |
| 7. 2 ml | 2 | 426 | 113. |

Susp 1/10, 078

Re-suspend, after 3 hours, in 5 ml H_2O, except for 1 c 5, in 2.5 ml.
To inoculate in dilution series, i.e., 1:50 dilutions of the original suspension, use each, colorimetric tube 1 ml of 1, 2, 5, 10, 20, and 1:2 or 1:3 dilutions, respectively of the others.

Note: 1. somewhat more rapid adaptation in dilute suspensions

2. pronounced stimulation of " by hydrolyzed, although cells were grown in T(0). This medium, therefore, offers no advantage.

2 ml 219A + 2 ml 10% TCA. Remove sediment. Assay in
indicated aliquots against 10^{-4} - 10^{-3} M Phosphate buffer standards.
Intensities of original 219A. Also assay 1 ml of 1:500 dilution
of 219A in 0.01 M Na bicarbonate buffer.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>10^{-4}</th>
<th>10^{-3}</th>
<th>219A</th>
<th>NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>x 10</td>
<td>470</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x 3</td>
<td>230</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x 1</td>
<td>091</td>
<td>040</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

219A: 1.5 ml 1170

0.1 ml 274
0.01 ml 053

Visual: 1 ml 219A corresponded to ca. 3 x 10^{-4} M Phosphate, i.e.,
219A assay was 3 x 10^{-3} M Phosphate. At 1:500 and 1:2000 dilutions,
phosphate, there will be much less than 10^{-4} M Phosphate, in fact
will be 10^{-5} M except for possible contamination of reagents.
Phosphate is present about and therefore unnecessary.

10 ml 219A dialyzed 4 hours against distilled water. Final volume, 13 ml.

= 219C, Inhibit activity of response to NO.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>C</th>
<th>1/50</th>
<th>1/1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>095</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>111</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>219</td>
<td></td>
<td>1/1000</td>
</tr>
<tr>
<td>5</td>
<td>277</td>
<td>1/100</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>290</td>
<td>1/1000</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>178</td>
<td>1/10,000</td>
<td></td>
</tr>
</tbody>
</table>

Opt. effect of NO at N/50 or above; inhibition at N/1000 or below.

Reagents contain 0.01 + 0.05 ml 319 A and 1.5 ml M/2000 N/405
2 ml KCl buffer + 1 ml N/50 Na_2SO_4
37°C, 10 ml.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.04</td>
<td>0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>1:30</td>
<td>0.48</td>
<td>0.83</td>
<td>2.25</td>
</tr>
<tr>
<td>2:30</td>
<td>0.69</td>
<td>1.02</td>
<td>3.26</td>
</tr>
<tr>
<td>3:30</td>
<td>0.89</td>
<td>1.88</td>
<td>4.09</td>
</tr>
<tr>
<td>4:00</td>
<td>1.10</td>
<td>1.40</td>
<td>4.91</td>
</tr>
<tr>
<td>4:30</td>
<td>1.30</td>
<td>1.70</td>
<td>5.63</td>
</tr>
<tr>
<td>5:00</td>
<td>1.50</td>
<td>1.91</td>
<td>6.40</td>
</tr>
<tr>
<td>5:30</td>
<td>1.72</td>
<td>2.13</td>
<td>7.10</td>
</tr>
<tr>
<td>6:00</td>
<td>1.95</td>
<td>2.38</td>
<td>7.80</td>
</tr>
<tr>
<td>6:30</td>
<td>2.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ampules: Time: T T
45-  1100
46  509  1050  1095
57    

53  560  1050  1100
54  520  579
55  530
56  590
58  541
59  609  609
60  560
61  611
65  600  652
67
69  630  683
70  740
83  100

1:10  115  1250  1145  1250
209  1:490  213  810.

Evaporation may have interfered overnight.
Oct 5, 1948.

49 g. Stiff Shapley paste R-12 harvested from 2 carboys 

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

A. 2g. suspended in cold acetone, dehydrated + dried. Yield:

B. 17g. suspended in 4/10 NaPO₄ buffer pH 7.5, realem under toluene.

C. 30g. " " Ground in Booth Green Hill Hours.

Remove debris and water to 100 ml. volume.

Add more debris. Left 2 opalescent yellow gum solution, 17ml.

Assays. (in 4/50 NaPO₄ buffer).

ONPG 4/2000. 20m. 37º

Di 420.

439.

Inhibition by serum.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

Note: high readings but probably due to use of the buffer

(1) 45 units / ml (2) 820 u / ml?

Throw out!
1. 1.329 B
2. 0.329 C
3. 10⁻³
4. NaCl M/50
5. Na₂M/50
6. KCl M/50
7. KPO₄
8. 0.001
9. 0.01
10. 0.1
11. 0.5

Nap.
Repeat assays of C!

319 A

Buffy

1. Na
2. Na
3. K
4. K
5. K
6. Na
7. "
8. "
9. "
10. M/10
11. M/10
12. M/10
13. M/10

D₁ = 129
D₂ = 420

(totol)

0.01M NaP + indicated reag. + H/1000 ONPG + H/50 buffer, pH 7.5

1. Buffer, DNP 290
2. NaF M/100 NaP 0.19
3. NaF M/50 — 0.12
4. " NaP 0.39
5. NaB 23.0
6. NaF M/100 NaB 22.2
7. NaF M/500 NaP 183
8. H/1000 NaP 291
9. M/10 NaP 310

ONPG in NaP

<table>
<thead>
<tr>
<th></th>
<th>X H/1000</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.1</td>
<td>0.91</td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
<td>0.92</td>
</tr>
<tr>
<td>12</td>
<td>1.0</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Nature

Km may be estimated in the neighborhood of 5 x 10^{-5} - 10^{-4}

Linearities need to be shown. Care of ONPG from 5 x 10^{-5} to 3 x 10^{-4} to be explored.

Fluoride inhibits only in presence of phosphate. NaF needed for substantial inhibition. (Mg effect?)

319 A .001ml ni M/100 NaP buffer.  
Sugg.  
1.  
2 U F M/100  
3 " M/200  
4 " HgSO₄ M/500  
18.0  
5 " M/1000M/4, M/500  
12.5  
6 " M/500  
7 "  
8 " HgSO₄ M/200  
251  

.001ml in M/50 NaP buffer.  Vary amounts of M/2000 ONPG added. 
ONPG: 5m 10m 15m 20m 0 30 

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Activity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.5</td>
<td>0.38</td>
</tr>
<tr>
<td>2</td>
<td>0.59</td>
</tr>
<tr>
<td>2.5</td>
<td>0.79</td>
</tr>
<tr>
<td>3</td>
<td>1.11</td>
</tr>
<tr>
<td>3.5</td>
<td>1.49</td>
</tr>
<tr>
<td>4</td>
<td>1.85</td>
</tr>
<tr>
<td>4.5</td>
<td>2.10</td>
</tr>
<tr>
<td>5</td>
<td>2.32</td>
</tr>
<tr>
<td>5.5</td>
<td>2.62</td>
</tr>
</tbody>
</table>

These data allow a substantially linear decomposition of the galactosidase in the 
intestinal studied, but being Vmax as V10, we can calculate the Km, indicat 
ed! Could this be due to the presence of an inhibitor in the system 
which is displaced by the galactoside? (Lecture?)

There is an insufficient discrepancy between 11.2 and 14.5, i.e. the former too high or 
the latter too low.

1st to 5th min data: 

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Activity (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
</tr>
<tr>
<td>2.5</td>
<td>0.40</td>
</tr>
</tbody>
</table>

V = 315 ± 32
As determined, the value is 1.4

\[ \text{value} = 1.5 \]

\[ 1.18 \times 10^{-5} \]

\[ \frac{1.1}{3} = 1.4 \times 10^{-4} \]
Kₘ, o-nitrophenyl galactoside
K⁺, lactose.

V = 315.

Kₘ = 1.18 \times 10^{-4}

Least squares weighted gives:

V_{max} = 299

Kₘ = 1.05 \times 10^{-4}
<table>
<thead>
<tr>
<th>T</th>
<th>V</th>
<th>V^3</th>
<th>V^4</th>
<th>V^3 \Gamma</th>
<th>\Gamma^2</th>
<th>V^4 \Gamma^2</th>
<th>V^4 T</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.30</td>
<td>1.21</td>
<td>1.77</td>
<td>2.14</td>
<td>23</td>
<td>176.9</td>
<td>378.57</td>
<td>28.46</td>
</tr>
<tr>
<td>10.00</td>
<td>1.22</td>
<td>2.86</td>
<td>4.07</td>
<td>29</td>
<td>100</td>
<td>407.00</td>
<td>40.70</td>
</tr>
<tr>
<td>4.00</td>
<td>2.12</td>
<td>9.33</td>
<td>70.20</td>
<td>38</td>
<td>16</td>
<td>323.20</td>
<td>80.80</td>
</tr>
<tr>
<td>2.00</td>
<td>2.45</td>
<td>14.70</td>
<td>36.03</td>
<td>29</td>
<td>4</td>
<td>144.12</td>
<td>72.06</td>
</tr>
</tbody>
</table>

\[
\bar{V} = \frac{28.81 + 62.44 + 119.66}{3} = 52.89
\]

\[
\bar{V} = \frac{222.02}{62.44} = 3.56
\]

\[
\bar{V} = 7.11
\]

\[
\bar{V}^2 = 12.64
\]

\[
a = \frac{28.81}{62.44} = 0.462
\]

\[
b = \frac{119.66 - 3.56(28.87)}{1252.89 - 7.11(222.02) + 12.64(62.44)} = \frac{16.89}{480.16}
\]

\[
b = 0.035 = \frac{K_S}{V_{max}}
\]

\[
V_{max} = a - bn = 0.462 - 0.128
\]

\[
\sqrt{V} = 0.334 \quad V_{max} = 2.99 \quad K_S = (0.035)(2.99)
\]

\[
K_S = 0.105
\]
Kinetics: $Mg^{++} + F^-$

Oct 8 1948.

- 0.01 ml 3/19A / 10ml of colorimetric tube. in 4/50 NaP buffer.

1. Tune series to substrate depletion.

<table>
<thead>
<tr>
<th>t₀</th>
<th>5M</th>
<th>15M</th>
<th>15N</th>
<th>25M</th>
<th>25N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>0.51</td>
<td>0.80</td>
<td>1.04</td>
<td>1.23</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>0.11</td>
<td>0.77</td>
<td>0.97</td>
<td>1.10</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0.27</td>
<td>0.65</td>
<td>0.80</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>-0.3</td>
<td>0.10</td>
<td>0.18</td>
<td>0.21</td>
<td>0.21</td>
</tr>
</tbody>
</table>

2. Add M/100 NaP buffer.

<table>
<thead>
<tr>
<th>1</th>
<th>10</th>
<th>0.13</th>
<th>0.19</th>
<th>0.14</th>
<th>0.09</th>
<th>0.09</th>
</tr>
</thead>
</table>

Corrected values of (1):

<table>
<thead>
<tr>
<th>t₀</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>30</th>
<th>V_max</th>
<th>1/v</th>
<th>1/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.29</td>
<td>0.55</td>
<td>0.89</td>
<td>1.11</td>
<td>1.68</td>
<td>168</td>
<td>0.00575</td>
<td>400</td>
</tr>
<tr>
<td>10</td>
<td>0.26</td>
<td>0.49</td>
<td>0.73</td>
<td>0.99</td>
<td>1.38</td>
<td>1.37</td>
<td>0.00704</td>
<td>2000</td>
</tr>
<tr>
<td>5</td>
<td>0.18</td>
<td>0.35</td>
<td>0.54</td>
<td>0.72</td>
<td>1.05</td>
<td>1.07</td>
<td>0.00935</td>
<td>10000</td>
</tr>
<tr>
<td>2</td>
<td>0.17</td>
<td>0.27</td>
<td>0.40</td>
<td>0.53</td>
<td>0.76</td>
<td>0.79</td>
<td>0.1267</td>
<td>10000</td>
</tr>
<tr>
<td>1</td>
<td>0.13</td>
<td>0.17</td>
<td>0.28</td>
<td>0.34</td>
<td>0.49</td>
<td>0.49</td>
<td>0.2100</td>
<td>10000</td>
</tr>
</tbody>
</table>

$K_m$ is estimated at $2.4 \times 10^{-4}$

$V$ at 200/300 m.

Flakes should be distributed as: 1, 1.4, 2.
Oct 9, 1948.

1. In H/50 NaP buffer. Read after 20 mins. only. 0.0015 ml 3/94.

<table>
<thead>
<tr>
<th></th>
<th>0.00</th>
<th>0.00</th>
<th>0.00</th>
<th>0.00</th>
<th>0.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>0.02</td>
<td>1.15</td>
<td>1.15</td>
<td>0.0015</td>
</tr>
<tr>
<td>2</td>
<td>1.33</td>
<td>0.02</td>
<td>1.16</td>
<td>1.14</td>
<td>0.0015</td>
</tr>
<tr>
<td>3</td>
<td>2.00</td>
<td>0.01</td>
<td>1.80</td>
<td>1.15</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>4.00</td>
<td>0.01</td>
<td>2.92</td>
<td>2.33</td>
<td>0.0088</td>
</tr>
<tr>
<td>5</td>
<td>10.00</td>
<td>0.26</td>
<td>2.81</td>
<td>2.55</td>
<td>0.0088</td>
</tr>
</tbody>
</table>

Mole fraction of NaP buffer: molarity = 354.

2. In H/100 NaP buffer. + H/50 salt.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>NaCl</td>
</tr>
<tr>
<td>13</td>
<td>KCl</td>
</tr>
<tr>
<td>14</td>
<td>LiCl</td>
</tr>
<tr>
<td>15</td>
<td>RbCl</td>
</tr>
<tr>
<td>16</td>
<td>CaCl</td>
</tr>
</tbody>
</table>

Rb is the only antiparostic rei (cf. other alkali metal derivatives).
K-12 Lactase.

$K_m (o$-nitrophenyl-$\alpha$-lactoside) = 7.4 \times 10^{-4}$

$V = 272.$
1. *L. bulgaricus* from E.E. Smell. Grow 1 tub overnight in 

\[ \text{N\text{a}Cl} 1\% \] 
\[ \text{Yeast} 0.5\% \] 
\[ \text{Lactose} 20\% \] 
\[ \text{Tween 80} 0.1\% \] 
\[ \text{NaAcetate} 0.1\% \] 

LB medium. Heavy growth noted!

Wash and concentrate 1:5. Use 1:10 on 0.054/2000 pH 1.5

\[ \text{Diphosphate buffer} \ H/50 \] 
\[ \text{pH} 371 \] 
\[ \text{830} \]

2. NaAcetate 
\[ H/50 \] 
\[ 393 \] 
\[ 770 \]

\[ \text{pH} \] 
\[ 11.0 \] 
\[ 12.33 \] 
\[ 13.20 \] 
\[ 14.40 \] 
\[ 15.00 \] 

\[ 0.003 \] 
\[ 0.005 \] 
\[ 0.007 \] 
\[ 0.009 \] 
\[ 0.012 \] 

\[ \text{H} 1.00 \] 
\[ \text{H} 1.33 \] 
\[ \text{H} 2.00 \] 
\[ \text{H} 4.00 \] 
\[ \text{H} 10.00 \]

\[ \sqrt{V} \] 

\[ \text{N} = 0.031 \]


1. — 

2. NaCl 

3. KCl 

4. Na+KCl

175

Questions:

1. Does K+ unequivocally inhibit
49 g. wet shaggy paste collected
grown 24 h. in 12 liters LB-lactone broth
5 aeration.

a) 4g. in 1/100 NaP buffer for autolysis. 6/10 ml (v. little activity)

b) 20g. in cold acetone for acetone powder. → 5.0 g dry powder.

c) 25g. ground in 1/100 NaP in B. homi Hill for extraction. → 45 ml
10/16/48.

33°C .05 ml. of buffer, NaCl  M/50
Buffer pH
1. Kp 6 080
2. Kp 7 092
3. Kp 8 059
4. Kp 7.5 097
5. Kp 7.5 210!

6. No

Tests in 9.0 ml. Add 2 ml. HCl to develop color and stop reaction.
M Na₂CO₃

Need repeat? Repeat above & add 5 ml. M/5 NaCl
<table>
<thead>
<tr>
<th></th>
<th>D&lt;sub&gt;20&lt;/sub&gt;</th>
<th>C&lt;sub&gt;2010&lt;/sub&gt;</th>
<th>A&lt;sub&gt;20&lt;/sub&gt;</th>
<th>D&lt;sub&gt;10&lt;/sub&gt;</th>
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<tr>
<td>1</td>
<td>C&lt;sub&gt;0&lt;/sub&gt; 0.1 ml</td>
<td>0.2</td>
<td>0.05</td>
<td>2.93</td>
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<tr>
<td>2</td>
<td>+ Ethylenediamine HCl 4/10</td>
<td>0.1</td>
<td>0.09</td>
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<td>+ Ethanolamine HCl 4/10</td>
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<td>+ Ethylene Glycol 4/10</td>
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<tr>
<td>5</td>
<td>+ RbCl 7/50</td>
<td>0</td>
<td>0.36</td>
<td>0.50</td>
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<tr>
<td>6</td>
<td>+ KCl 7/50</td>
<td>-0.1</td>
<td>2.89</td>
<td>2.85</td>
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<td>7</td>
<td>+ RbCl+KCl 7/50</td>
<td>0</td>
<td>1.76</td>
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<p>| | | | | |</p>
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<tr>
<td>5</td>
<td>L. delgancii, Cell suspension</td>
<td>22</td>
<td>32%</td>
<td>123</td>
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<td>6</td>
<td>Dextrose powder 1 mg.</td>
<td>320</td>
<td>36%</td>
<td>0.76</td>
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<td>7</td>
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<td>0.1 mg</td>
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<td>0.45</td>
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<td>8</td>
<td>Extract 338C 1 ml</td>
<td>15</td>
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<td>9</td>
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<td>0.1 ml</td>
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<td>10</td>
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<td>0.01 ml</td>
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<td>0</td>
<td>0.3</td>
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<td>12</td>
<td></td>
<td>10&lt;sup&gt;-4&lt;/sup&gt; ml</td>
<td>-0.2</td>
<td>0.21</td>
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</table>

All tests in M/100 NaCL, pH 7.5 ± 0.1/2000x onp. 37°. 20 min. λ = 910.
This may not be the opt. pH for delgancii.

Note: Intense stimulation by glycol! Reversal of NaCl inhibition c R.
Relatively low activity of cells of glycol extract for purity of extract.
October 17, 1948.

0.01 ml 3/9A. NaP buffer M/100. Alcohol... M/10. 

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Conc.</th>
<th>Notes</th>
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<tbody>
<tr>
<td>1.</td>
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<tr>
<td>2.</td>
<td>p-CH</td>
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<td>i-PrOH</td>
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<td>BuOH</td>
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<td>Dioxan</td>
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<td>MeOH</td>
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<tr>
<td>11.</td>
<td>Et&lt;CH</td>
<td>0.8</td>
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</tr>
<tr>
<td>12.</td>
<td>Et&lt;CH&lt;CH</td>
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<tr>
<td>13.</td>
<td>Na&lt;CH3</td>
<td>0.8</td>
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</tr>
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<td>14.</td>
<td>K3PO4</td>
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<tr>
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</tr>
<tr>
<td>16.</td>
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</tr>
<tr>
<td>21.</td>
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<td>25.</td>
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</tr>
<tr>
<td>26.</td>
<td>K2HPO4</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

0.05 ml 0.338C. K2HPO4 M/50. pH 7.5. pH 7.0. Con. 1.1. pH 7.5. 10% alcohol. 10% alcohol.

Note: A 18. CF, ONP 0. and o-PhOH. ONP ca. M/2500. Water buffer. etc.

1-PhOH. 1.68
2-PhOH. 1.65

PhOH at 0 dilution, 1/10 M/10 does not influence absorption of ONP.

pH adjustment and buffer solutions.
October 15, 1948.

38°C .10 ml/tube. 9 ml pH 7.5 stop 90% Na₂CO₃. Mo mourn buffer, 1/100. Add Na₂PO₄ buffer additional 1/50 when called.

Buffer:

<p>| | | |</p>
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<thead>
<tr>
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<tbody>
<tr>
<td>1</td>
<td>Na₂PO₄</td>
<td>1.0</td>
</tr>
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<td>2</td>
<td>NaH₂PO₄</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>&quot; + Na₂PO₄</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>ET4Na₂HPO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>&quot; + Na₂PO₄</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>Na₃H₂P₂O₇</td>
<td>0.8</td>
</tr>
<tr>
<td>7</td>
<td>&quot; + Na₂PO₄</td>
<td>1.0</td>
</tr>
<tr>
<td>8</td>
<td>Na₂H₂PO₄</td>
<td>1.5</td>
</tr>
</tbody>
</table>

A) 1st tubes: B) 2nd tube: C) 3rd tube: D) 4th tube: E) 5th tube: F) 6th tube: G) 7th tube: H) 8th tube:

Mg, K+ are stimulatory.
Subject: Increase in 50% utilization of the \[ \frac{1}{1} \] line.

According to the 50% increase.

1. Increase in production from 150% to 300%.
2. Increase in sales from 20 to 30 units.

<table>
<thead>
<tr>
<th>Date</th>
<th>Production</th>
<th>Sales</th>
</tr>
</thead>
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<tr>
<td>1/1/15</td>
<td>150%</td>
<td>20 units</td>
</tr>
<tr>
<td>1/5/15</td>
<td>200%</td>
<td>25 units</td>
</tr>
<tr>
<td>1/10/15</td>
<td>250%</td>
<td>30 units</td>
</tr>
<tr>
<td>1/15/15</td>
<td>300%</td>
<td>35 units</td>
</tr>
</tbody>
</table>

Note: The increase in production has led to a significant rise in sales, indicating a positive trend.

Km.

3/28
Oct 23, 1948

Adapt *L. bulgaricus* (mill) to glucose by successive passage in LB glucose broth. Compare original and adapted cultures in other sugars: (24h)

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<thead>
<tr>
<th></th>
<th>(Bar)</th>
<th>(Ehe)</th>
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<tr>
<td>Blu</td>
<td>-</td>
<td>++++</td>
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<tr>
<td>Lur</td>
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<tr>
<td>Mil</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Gal</td>
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<td>+</td>
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<tr>
<td>Suc</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Xyl</td>
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**Date 20, 1918**

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</tbody>
</table>

* -colonia and zone V. slow +

These readings point to the necessity of resubulating H stocks from stock cultures before proceeding.
To determine whether the intracellular buffering capacity might influence activity determination, set up cells A) E. coli K12, 0.0

\( \mu \) M HCl, 1.00; B) do. + 5\( \mu \)l 5000 ONP + C) only in acetate

buffer 0.044, pH 7.0. Compare readings (in 0.0).

\[ A_1 - A_2 = 0.007 \quad \text{(error term)} \]

\[ B_1 - A_1 = 0.124 \]

\[ B_2 - A_1 = 0.124 \]

\[ B_1 - A_2 = 0.138 \]

\[ B_2 - A_2 = 0.138 \]

\[ C_1 = 0.151 \]

\[ C_2 = 0.153 \]

Of anything, the apparent absorption by

ONP was less in the cells than without

This may be due to scattering.
<table>
<thead>
<tr>
<th>Type</th>
<th>pH</th>
<th>$D^+_{\text{H}_2O}$</th>
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</thead>
<tbody>
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<td>2</td>
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<td>8</td>
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</tr>
</tbody>
</table>

9. without $8.0$ M/100 acetate and phosphate buffer M/50.

Make up to 9 ml; at t add 1 ml M/100 CO$_2$, to alkalinize at M/100 Na$_2$CO$_3$
and M/100 2H$_2$O$_2$ 2H$^+$ 10$^{-3}$ 20 min. pH > 10.

Repeat, using phosphate buffer only!
**PH optimum - colloids**


3.19A: Na$_2$CO$_3$ N60 KPBuff 1:50. 04/15/48. 70m, 380 Duplicate tubes. Add Na$_2$CO$_3$ 4/10 of conclusion.

<table>
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<td>260</td>
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</table>

Hamest K-12 from 200 ml Y2 glucose shaken overnight and
resuspended in 40 ml 1/5 Na-phosphate 7.5.

Set up adaptation systems to 5 ml/tube:

- 2 ml cell suspension
- 2 ml lactose 40%
- 1 ml H2O 85% 1 ml. 5 ml.

**Suppl.**

<table>
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<th>Suppl.</th>
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<th>2</th>
<th>3</th>
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</tbody>
</table>

**pH 7.9**

12. T+5MT + phlorizin

13. C+A Di = 178 381 A_0.5 =

14. C+A

15. A+5MT

16. C + T

No inhibition by carboxamine

Resuspended in 9 ml and resuspended in 10 ml column tubes in 1/5 Na-phosphate.

H 2000000 NO. Matched against corresponding suspensions in ONPS.

100gms. alfalfa and wine allowed to germinate 2-3 days, then dried and ground.

trig. 1. 5gms. wine diluted 26. c molal HCl. The reds were deduced with red copper anode solution 10% NaOH soln.

inocule at pH 4.0 Na acetate buffer (after Nadel who allowed optimum at 3.4). He found Km for red gallicide as <10⁻³, which is 1/10 of cellulase.

Assay preparation A; 20mm acetate buffer.

<table>
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<tr>
<th>vol (ml)</th>
<th>conc (M)</th>
</tr>
</thead>
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<td>0.50</td>
</tr>
<tr>
<td>0.1</td>
<td>0.50</td>
</tr>
<tr>
<td>1.0</td>
<td>&gt; 1.9</td>
</tr>
</tbody>
</table>

Inoculation by 1st of stirring by saloin. to M/100 Na acetate buffer.

with M/50 each. 

1. NaCl         0.37
2. NaNO₃        248 (avg. 204.84)
3. Na₂SO₄       196
4. Na₂HPO₄      212

may be a chloride effect

1. -
2. NaCl       22.7
3. Na₂SO₄     2.50
4. Na₂HPO₄     270

Note: assay somewhat irregular.
12/1/48.

Run ONPG assay with various lactose concentrations.

10 ml  0.01 M Na₂HPO₄  add 1 ml of 0.03 to terminate, min 4/100.

<table>
<thead>
<tr>
<th>ONPG</th>
<th>L/G</th>
<th>m/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>0.05</td>
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<tr>
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<td>24</td>
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<tr>
<td>31</td>
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<tr>
<td>32</td>
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<tr>
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<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>34</td>
<td>0.05</td>
<td>0</td>
</tr>
</tbody>
</table>

0:  D₄  A  1/10

Correct 0: by 42% for addition of enzyme and of substrate.

Alpha lactase is not appreciably bound by these concentrations of lactose, i.e. $R_L > 40R_{40,000}$. 

lactose: competition with ONPG-modified lactose.

Seedlings from Dr. Nancy Kent.

A. Pinenia lactea: 6 seedlings, ca. 3 cm long. 14/10. 700 60

B. succul: 3" short 13 cm long. 310. 4/10 100

Determined as Pinenia lactea in distilled water, 5 ml. Without separation,
filter and samples EONIPG at pH 7.1 in alfalfa system.
Inoculate at > 10.85447 - 11/13/48

Daily lactase is constitutive.

12/10/48. Qualitative tests in malt extract show no lactase activity.
December 10, 1948.

Set up as 383. 1002 ml 319A. mM/50 NaOH 7.5. 10 mmol. 32°

<table>
<thead>
<tr>
<th>(1/5)</th>
<th>1/420, 1/1</th>
<th>OD420</th>
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<tbody>
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<td>ONPG</td>
<td>M/1000</td>
<td>M/100</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>369</td>
</tr>
<tr>
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<td>5</td>
<td>279</td>
</tr>
<tr>
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<td>2</td>
<td>311</td>
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<td>22/15</td>
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<td>221</td>
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<td>10</td>
<td>140</td>
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<td>24/15</td>
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<td>2</td>
<td>274</td>
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<td>180</td>
</tr>
<tr>
<td>33</td>
<td>10</td>
<td>107</td>
</tr>
<tr>
<td>34</td>
<td>20</td>
<td>61</td>
</tr>
</tbody>
</table>
Substrate: o-nitrophenyl galactoside

$I = \frac{1.5 \times 10^{-3}}{M}$

$K_s = 1.5 \times 10^{-3}$

$Lactose$

$M/5000 \quad 1.45 \times 10^{-3}$

$M/1000 \quad 1.61 \times 10^{-3}$

$M/2000$

$M/10$

$K_i = 1.5 \times 10^{-3}$

$\frac{1}{S}$ Molar

No. 340 - M. DIETZGEN GRAPH PAPER

EXPIRED

11/5 1967
Dec. 11, 1948.

Setting parallel to 384. BUTUCAE 0.01 ml enzyme; 20 min.

<table>
<thead>
<tr>
<th>H/1000</th>
<th>H/10000</th>
<th>Δ D</th>
<th>Yv</th>
<th>D+</th>
<th>D-</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>00</td>
<td>252</td>
<td>39.7</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>180</td>
<td>44.555</td>
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<td>183</td>
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<td>10</td>
<td>129</td>
<td>77.5</td>
<td>1</td>
<td>130</td>
</tr>
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<td>77</td>
<td>124.9</td>
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<td>20</td>
<td>244</td>
<td>41.0</td>
<td>10</td>
<td>254</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>173</td>
<td>57.8</td>
<td>5</td>
<td>178</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>127</td>
<td>78.7</td>
<td>1</td>
<td>128</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>78</td>
<td>128.2</td>
<td>0</td>
<td>78</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>197</td>
<td>50.7</td>
<td>13</td>
<td>210</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>158</td>
<td>63.3</td>
<td>2</td>
<td>160</td>
</tr>
<tr>
<td>11</td>
<td>10</td>
<td>111</td>
<td>90.1</td>
<td>2</td>
<td>113</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>63</td>
<td>158.7</td>
<td>3</td>
<td>66</td>
</tr>
</tbody>
</table>

AbCl is not measurably inhibitory with this concentration of Wa.

Glucose at H/50 is only very slightly inhibitory, and not, as far as can be
seen, competitively. Abstain at H/10. The competitive reaction may
be, conceivably, 2G + E ⇌ E52

H5 estimate here is 1.7 x 10^-4.

Note: Glucose has crystallized after standing at room temperature in
previous tests. Had them standing a couple of days.
Glucose inhibition of lactase.


Compare 0 and 1/10 glucose concentrations.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Concentration</th>
<th>Activity (units)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>365</td>
</tr>
<tr>
<td>1½</td>
<td>0</td>
<td>280</td>
</tr>
<tr>
<td>2</td>
<td>1/10</td>
<td>197</td>
</tr>
<tr>
<td>3½</td>
<td>1/10</td>
<td>117</td>
</tr>
<tr>
<td>5</td>
<td>1/10</td>
<td>237</td>
</tr>
<tr>
<td>7½</td>
<td>1/10</td>
<td>184</td>
</tr>
<tr>
<td>10</td>
<td>1/10</td>
<td>140</td>
</tr>
<tr>
<td>14</td>
<td>1/10</td>
<td>93</td>
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<td>23</td>
<td>1/100</td>
<td>128</td>
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<td>57</td>
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<td>31</td>
<td>1/50</td>
<td>142</td>
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<tr>
<td>32</td>
<td>1/50</td>
<td>142</td>
</tr>
<tr>
<td>33</td>
<td>1/50</td>
<td>74</td>
</tr>
</tbody>
</table>

Additional data are acceptable. Glucose may be a non-competitive inhibitor, especially at these high concentrations 1/10. It may also be noted that low buffer concentration, i.e., K₂HPO₄ buffer, affects not only Vmax, quite appreciably, but also the K_s. It may accentuate this response!
Substrate ONPG

$K_s = 1.25, = 1.8 \times 10^{-4}$

Glucose inhibition

Non-competitive inhibition

NaCl + buffer

H+ + glucose

Na buffer
December 13, 1948.

<table>
<thead>
<tr>
<th>ONPG.</th>
<th>Buffer</th>
<th>Nap</th>
<th>Vmax</th>
<th>Δv</th>
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<tbody>
<tr>
<td>1. 2</td>
<td></td>
<td></td>
<td>112</td>
<td>89</td>
</tr>
<tr>
<td>2. 5</td>
<td></td>
<td></td>
<td>147</td>
<td>68</td>
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<tr>
<td>3. 10</td>
<td></td>
<td></td>
<td>208</td>
<td>48</td>
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<tr>
<td>4. 20</td>
<td></td>
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<td>303</td>
<td>33</td>
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</table>

Glucose, Nap M/1000

<p>| | | | | |</p>
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<thead>
<tr>
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<th></th>
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<td>5</td>
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<td>14</td>
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<tr>
<td>2</td>
<td>19</td>
<td>5</td>
<td>714</td>
<td>14</td>
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</tbody>
</table>

Nap M/100

<p>| | | | | |</p>
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<thead>
<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10</td>
<td>4</td>
<td>151</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>151</td>
<td>33</td>
</tr>
</tbody>
</table>

Kp M/500

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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</thead>
<tbody>
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<td>3</td>
<td>7</td>
<td>3</td>
<td>304</td>
<td>23</td>
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<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>304</td>
<td>23</td>
</tr>
</tbody>
</table>

Glucose utilization non-competitive, further related to substrate, as is more effective at low lowest substrate concentrations.

These peps. tested at too low a level of enzyme activity.
E coli lactase: Summary assays

ONPG M/2000, NaN H/50. 15mm o.d.

1. 3/9A. 2 x 10^{-3} ml
2. 3/9B. 10 ml
3. 3/9C. 2 x 10^{-3} ml

Touza lactose, cells harvested from 1% yeast, 2% sugar broth.

pH
lactose 11 5 12 6 14 7
glucose 21 4 22 5 23 6 24 7

Cell density indicated by light absorption.

{-ina
$K_s^{0.4} = 1.3 \times 10^{-4} N$

$K_s^{0.4} = 3.0 \times 10^{-4} N \ (a)$
12/13/48... 319 A $10^{-3}$ nA/40 mm.

<table>
<thead>
<tr>
<th>ONPG 100ug/ml</th>
<th>Buffer pH 4.5 as indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/5</td>
<td>1/10</td>
</tr>
<tr>
<td>NaPn/50</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
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<tr>
<td>4</td>
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<td>15</td>
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<tr>
<td>16</td>
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<td>17</td>
<td>67</td>
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<tr>
<td>34</td>
<td>152</td>
</tr>
</tbody>
</table>

Note: volume added to enzyme prep in 319 A 12/12/48 to prevent gross contamination. About 50% loss of activity seems to have occurred.

K and Na definitely alter the Ks primarily. K may also have an effect on V.

<table>
<thead>
<tr>
<th>ONPG 1000/M.</th>
<th>NaP M/50.</th>
<th>(A/2^{1/2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>179</td>
<td>162</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
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<td>25</td>
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<table>
<thead>
<tr>
<th>KP M/50.</th>
<th></th>
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<tbody>
<tr>
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<td>179</td>
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<td>12</td>
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<tr>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NaP M/50 + Glucose M/10.</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>19</td>
<td>120</td>
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<tr>
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<td>21</td>
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<td>22</td>
<td>37</td>
</tr>
<tr>
<td>23</td>
<td>26</td>
</tr>
</tbody>
</table>

Townsend's study in the macerating reaction was conducted at 30 minutes for 1-3, 11-13, and at 80 minutes for 4-10.

Glucose also causes an alteration of slope!

These data msg enzyme assay low.

Used 388: 314A deleted 1:2:5.

K-12 grown in 500 cc Y26ac flasks, harvested into 2
12 liter carboys S(Lac). Yield: 110 grams streptolase paste.

Grind ca 35 g. in NaP04 0.1 M buffer, pH 7.5 (buffer) prepared anaerobically.

As original paste in frozen.

As grinding proceeded, noted increasing waxy - pink color.

Yield, about 60 ml yellow from opalescent supernatant with
pinkish fluorescence.

Assay for lactase. Test 0.01 ml and 0.001 ml at 4/2000 0.1 M pH 7.5 Na
12/21/48.

1). Assay prep 319A + 390A. NaPhosphate 7.5 200mM.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>319</th>
<th>131</th>
<th>390</th>
<th>390</th>
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<tbody>
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<td>16^-2</td>
<td>14</td>
<td>13.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 x 10^-3</td>
<td>11.0</td>
<td></td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td>10^-3</td>
<td>359</td>
<td>0.38</td>
<td>0.38</td>
<td></td>
</tr>
</tbody>
</table>

Study basic values in the next tube.

Tubes 1+2: 100mL enzyme + buffer, incubated 90 minutes in an ice bath.

3+4: **Add** Na buffer just like we add to substrate.

Note: enzyme was irreversibly activated, as prolonged incubation of tube 3 gave no color.

319A lactase is irreversibly activated by dilution in distilled water (controls).
$K_s = 1.3 \times 10^{-4}$

$K_h^f = 1.1 \times 10^{-4}$

$K_{rh} = 2.2 \times 10^{-4}$
$K_5 = 1.4 \times 10^{-4}$

$K_5 = 1.4 \times 10^{-4}$

$K_{50} = 1.5 \times 10^{-4}$

3/14/48 10^-3 ml M/100 buffin

<table>
<thead>
<tr>
<th></th>
<th>NaP</th>
<th>NPS/1000 ml</th>
<th>V</th>
<th>A</th>
<th>D1</th>
<th>D2</th>
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<tbody>
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<td>148</td>
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<td>145</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>88.5</td>
<td></td>
<td>113</td>
<td>-6</td>
<td>107</td>
</tr>
</tbody>
</table>

| 11 | KP  | 45.2        |   | 221| 18  | 189|
| 12 |     | 52.6        |   | 190| 9.9 | 199|
| 13 |     | 78.7        |   | 127| 3   | 130|
| 14 |     | 115         |   | 87  | 8.1 | 87  |
| 15 |     | 143         |   | 70  | -3  | 67  |
| 16 |     | 196         |   | 51  | -2  | 49  |

| 21 | KP+RBC | 57.5       |   | 194| 18  | 212|
| 22 | M/50   | 60.2       |   | 166| 12  | 178|
| 23 |       | 98.0       |   | 102| 2   | 104|
| 24 |       | 147        |   | 68  | 6.8 | 52  |
| 25 |       | 192        |   | 52  | -7  | 38  |

K₁₅ = 1.4 x 10⁻⁴
Kₛ = 2.2 x 10⁻⁹

In this system, substrate and buffer are mixed up, enzyme is frozen, dialyzed before Cunningham's rin at 0. Ch. 337 in which expected much more marked effects (separate salts) are seen.

Grow 1 culture of K-12 in S(Lac) new formula. 24 hr. Harvest A29. 
Yield 56gms. Decant 20g. (moist) over P2O5 in a desiccator. Remove 35g, add aspirin K2HP2O7/50 pH 7.5 buffer and grind 80 mins. Remove debris. Supernatant, about 27ml.

Drycell yield 4.47 (ca. 22%).

A). Extract (2/3) 24/21 = 1.3 g/ml assay.

B). Suspended blue reagent in 10ml 100g/l NaCl. Stirred 2 hrs

C. Cell 50 mg/ml with cells

D). Hemoglobin cell suspension in 1/50 NaP 7.5 "120 mins"

<table>
<thead>
<tr>
<th>Di</th>
<th>C</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 ml</td>
<td>0.03</td>
<td>241</td>
</tr>
<tr>
<td>0.01 ml</td>
<td>0.06</td>
<td>71</td>
</tr>
<tr>
<td>0.11 ml</td>
<td>0.02</td>
<td>59</td>
</tr>
<tr>
<td>0.2 ml</td>
<td>0.82</td>
<td>113</td>
</tr>
</tbody>
</table>

B. Quick test is only about 1/3 as efficient as C. Initial extraction using cells.

1/3/49.

Separate flocculate from papers 399-A and 395-A.
Originally assayed 2400 and 2900 w/mt negat.

1. 13  385 Ppt.  
2. 497  
3. 20  399 P  
4. 210  S.
I - 112 lecture
Time series.

314.10-3 ml.
Initial system: KP 7.5 M/100. At t = 0 add enzyme. Add additional supplements as indicated.

<table>
<thead>
<tr>
<th>Sup.</th>
<th>time</th>
<th>time</th>
<th>Sup.</th>
<th>time</th>
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<tr>
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</tr>
<tr>
<td>3</td>
<td>45</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Add substrate to initiate assay at 45 min.

Significant activity was noted. No demonstrable time effect can be noted. How, then, account for the different response to K noted now and previously?
**Suppresor lactose**

**Jan. 9, 1942**

<table>
<thead>
<tr>
<th>Test: lab work</th>
<th>W61 &amp; 662 in 5 lit.</th>
<th>Extract and dry over P</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 = W611</td>
<td>4.1 g. wet paste</td>
<td>10 g.</td>
</tr>
<tr>
<td>#2 = W612</td>
<td>6.7 g. wet paste</td>
<td>16.67 g. dry cells</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>lactose adaptation in W-112 (lac-)</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>A</td>
<td>glucose</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Wash and resuspend in 4 ml H2O.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incubate 1 ml 5% lactose, 1 ml 5% sugar, 1 ml 0.1M buffer + BCP.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Check by staining red cells red.

lac- produces lactase with butyl glycolate but not with lactate.
If Cohn's reagents show same results with nitrophenyl lactate.

1/2. Grow W-112 in 2 x 50 ml Y2 broth. Harvest, wash, and dry over P2O5. Yield 33 mg dry cells. 1/3. Preparation on pg.
Lactose in W-108.

<table>
<thead>
<tr>
<th></th>
<th>10ml Y+2</th>
<th>Beige 1/10 + 1/2 rac.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harvest + Yeast:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Spot Plate O.V.S.</td>
<td>B: +++</td>
<td>L: -</td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) E. 1 ml 4/50 K/NaF pH 7.0</td>
<td>100B</td>
<td>100B</td>
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<tr>
<td>Bpe</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Bald</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Lact</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

Note adaptation to glucose! cf. W327 which does not adapt here with regard to lactose, W108 is like W112. Non-reactive but ferment.
<table>
<thead>
<tr>
<th>a)</th>
<th>b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add 0.05 to enzyme-buff.</td>
<td>NaPO₄/100; 7.5 (RBCM/50) only ²³⁰²⁰⁰.</td>
</tr>
<tr>
<td>(c)</td>
<td>(c)</td>
</tr>
<tr>
<td>1</td>
<td>319A</td>
</tr>
<tr>
<td>2</td>
<td>10⁻² ml.</td>
</tr>
<tr>
<td>3</td>
<td>680</td>
</tr>
<tr>
<td>4</td>
<td>630</td>
</tr>
<tr>
<td>5</td>
<td>310</td>
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<td>6</td>
<td>309</td>
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<tr>
<td>7</td>
<td>319</td>
</tr>
<tr>
<td>8</td>
<td>RBC</td>
</tr>
<tr>
<td>9</td>
<td>650</td>
</tr>
</tbody>
</table>

no appreciable inhibition!

Repeat comparing fresh solution of RBCP.

- 289
- 268
- 200

3/7/87 2000
<table>
<thead>
<tr>
<th>Date</th>
<th>319A</th>
<th>Buffa</th>
<th>M/100</th>
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<th></th>
<th></th>
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<tbody>
<tr>
<td>1/15/49</td>
<td>10⁻³</td>
<td></td>
<td></td>
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<tr>
<td>1.</td>
<td>Salt</td>
<td>Buffer Na⁺</td>
<td>438</td>
<td>% inh.</td>
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<td>-</td>
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<tr>
<td>2.</td>
<td>Cl⁻Cl Na⁺</td>
<td>409</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>3.</td>
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<td>393</td>
<td>10</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>4.</td>
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<td>316</td>
<td>28</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>5.</td>
<td>K⁺⁺</td>
<td>K⁺⁺</td>
<td>239</td>
<td>- (45)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Cl⁻Cl K⁺⁺</td>
<td>220</td>
<td>0.8</td>
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<td>-</td>
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</tr>
<tr>
<td>7.</td>
<td>Cl⁻Cl K⁺⁺</td>
<td>182</td>
<td>24</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>8.</td>
<td>Cl⁻Cl K⁺⁺</td>
<td>100</td>
<td>58</td>
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</tbody>
</table>
### January 14, 1948

<table>
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<td>NaP</td>
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<tr>
<td>15m</td>
<td>1</td>
<td>37.7</td>
<td>90</td>
<td>111</td>
</tr>
<tr>
<td>2</td>
<td>43.7</td>
<td>158</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>63.3</td>
<td>268</td>
<td>229</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>90.1</td>
<td>286</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>87.7</td>
<td>286</td>
<td>194</td>
<td></td>
</tr>
</tbody>
</table>

|     |     |     |     |     |
|-----|-----|-----|-----|
| 15m | 1   | 31.1| 32.2| 329 |
| 2   | 36.9| 271 | 280 | 0   |
| 5   | 52.1| 192 | 198 |
| 10  | 76.9| 130 | 103 |
| 15  | 17.1| 103 |

| K+  |     |     |     |     |
|-----|-----|-----|-----|
| 7am | 1   | 61.3| 163 | 181 |
| 2   | 87.7| 114 | 121 |
| 5   |     |     |     | 181 |
| 10  | 270 | 37  | 37  |
| 15  | 370 | 27  |

| K+  |     |     |     |     |
|-----|-----|-----|-----|
| 20m | 1   | 61.3| 163 | 181 |
| 5   | 87.7| 114 | 121 |
| 10  | 270 | 37  | 37  |
| 15  | 370 | 27  |

**Note:**
- NaP data may show some trend downwards.
- K data may show some trend downwards.

**Graph:**
- Suggest linear fit of Na data.
- Suggest linear fit of K data.
$1.28 \times 10^{-4} = K_m$

$2.2 \times 10^{-4} = K_m$

$5.9 \times 10^{-4} = K_m$
\[ K'_5 = K_m + k_a V_{\text{max}} \]
\[ k_a = -1.2 \]

\[ E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + S \]
\[ k_1 \]
\[ \alpha < 0. \]

$k_1$ should be estimated from simple collision theory.
January 16, 1969.

If $K_m$ is apparent dissociation constant for $E+S \xrightleftharpoons{K_2 \over K_1} ES, \xrightarrow{K_3} E+S$,

$$K_m' = K_m + \frac{K_3}{K_1}.$$  Now $K_3 = k V_{max}$. Consequently, all the effects of allosteric metal substitution could be explained as effects on $K_m'$, of which there are undoubtedly some since $V_{max}$ is affected.

$$\frac{1}{v} = \frac{1}{V_{max}} \left( \frac{K_s}{s - 1} \right).$$

Of this could be applied here.

But data given show a negative sense, so that this interpretation can securely apply. It must be concluded that there is a "true" effect on $K_m'$.

Table:

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Sack</th>
<th>$\frac{1}{v}$</th>
<th>$\frac{1}{s}$</th>
<th>$\Delta$</th>
<th>$\frac{1}{V_{max}}$</th>
<th>$\frac{1}{s - 1}$</th>
<th>$\frac{1}{V_{max}}$</th>
<th>$\frac{1}{s - 1}$</th>
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<tbody>
<tr>
<td>M/10 DPN buffer</td>
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<td>39.1</td>
<td>256</td>
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<td>29.6</td>
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</tr>
<tr>
<td></td>
<td>1/2</td>
<td>44.2</td>
<td>226</td>
<td>24</td>
<td>250</td>
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<td></td>
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<td>51.5</td>
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<td>13</td>
<td>70.7</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>1/10</td>
<td>70.9</td>
<td>141</td>
<td>11</td>
<td>152</td>
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<td></td>
<td>1/15</td>
<td>95.2</td>
<td>105</td>
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<td>152</td>
<td></td>
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<td>3</td>
<td>90</td>
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<td>12</td>
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</tbody>
</table>

The enzyme dilutions are kept at room temperature at least for several hours. This may account for the erratic variations.

Note: Varies.
$K_m = 4.5 \times 10^{-4}$

$V_{max} = 5.27$

Kinetics of intracellular galactosidase.

Hepes buffer pH 7.5 $M/100$.

Harvest K12 from 100 ml Y2 lactose broth. Resuspend in 20 ml.
Pre-incubate 10 mins in NaP 4/100 7.5
1 ml 430
10 ml 452

Use 1 ml 1:10 bacterial suspensions; add to prepared system and to control K2.

a) pH optimum.

<table>
<thead>
<tr>
<th>pH</th>
<th>A</th>
<th>222</th>
<th>329</th>
<th>007</th>
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<td>8.0</td>
<td>326</td>
<td>339</td>
<td>013</td>
<td></td>
</tr>
</tbody>
</table>

b) Na, K: effects. M/5000 0.1Pb.

   185 191 006
   163 169 006
   181 183 002

K kinetics. Na buffer 4/100 7.5

<table>
<thead>
<tr>
<th>[NPb] (M)</th>
<th>Vmax (A)</th>
<th>Km (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
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<tr>
<td>0.2</td>
<td>310</td>
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<tr>
<td>0.3</td>
<td>185</td>
<td>4.5 x 10^{-4}</td>
</tr>
<tr>
<td>0.4</td>
<td>116</td>
<td>4.5 x 10^{-4}</td>
</tr>
<tr>
<td>0.5</td>
<td>089</td>
<td>4.5 x 10^{-4}</td>
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</table>

All 090
Adaptation of ML: K-12 on galactose

<table>
<thead>
<tr>
<th>Trait</th>
<th>Δ1</th>
<th>Δ2</th>
<th>Protein</th>
<th>2 M</th>
<th>20 M</th>
<th>315 PM</th>
<th>RA</th>
<th>RA</th>
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<tr>
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<td>301</td>
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<td>180</td>
<td>200</td>
<td></td>
<td>481</td>
<td>11 22</td>
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<td>180</td>
<td>200</td>
<td>488</td>
<td>1100+</td>
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<td>glu</td>
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<table>
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<td>117</td>
<td>130</td>
<td>119</td>
<td>147</td>
<td>4.2</td>
</tr>
</tbody>
</table>

Glucose cells may have begun to adopt the activity.

Galactose therefore has ca. 14x activity

ML Lac/I gal = 16
K-12 Lac/I gal = 4
Hansen cells from 10 and 22 -1% sugar broth and one drop
Arabinose extract 7%. Tubes are pH indicator. Also check counts in 8CP agar plates.

<table>
<thead>
<tr>
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<th>290</th>
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<th>1000</th>
<th>590</th>
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</thead>
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<tr>
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<td>114</td>
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<td>W45</td>
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<td>106</td>
<td>160</td>
<td>117</td>
<td>196</td>
<td>210</td>
<td>870</td>
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<td>W255</td>
<td>127</td>
<td>1050</td>
<td>89</td>
<td>386</td>
<td>93</td>
<td>930</td>
</tr>
</tbody>
</table>

ONPG readings:

- Initial m -
- Final m -
- R.A. -

For K-12 with lac at 100%
- Log. 115%
- Selection 22%

Note: Adaptation of K-12 to Selection < Bestial galactoside.
Moderate adaptation to galactose of W112, but remained in W255.

Response of W-108 may be due to presence of + cells. Cures 100/200

The check plates.
### Adaptation to Related Substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>ΔD/D₀</th>
<th>ΔD/D₀</th>
<th>ΔD/D₀</th>
<th>ΔD/D₀</th>
<th>ΔD/D₀</th>
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</thead>
<tbody>
<tr>
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<td>4</td>
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</tr>
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<td>Mucate</td>
<td>33.0</td>
<td>318</td>
<td>300</td>
<td>4</td>
<td>0.01</td>
</tr>
<tr>
<td>D-Lactate</td>
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<td>191</td>
<td>174</td>
<td>4</td>
<td>0.01</td>
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<td>248</td>
<td>174</td>
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<td>0.12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

### Growth Observations

- Growth on arabinose was very light.
- Very slight responses were shown by galactonate and didecetol.

### Calculating Lactose as

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Calculated</th>
</tr>
</thead>
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<tr>
<td>Hactarinate</td>
<td>58%</td>
</tr>
<tr>
<td>Lactose</td>
<td>23%</td>
</tr>
<tr>
<td>Didecetol</td>
<td>4%</td>
</tr>
<tr>
<td>Galactonate</td>
<td>3%</td>
</tr>
<tr>
<td>Mucate</td>
<td>3%</td>
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</tbody>
</table>
Absorption spectrum of E. coli + formycin.

<table>
<thead>
<tr>
<th>λ</th>
<th>I</th>
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<tbody>
<tr>
<td>400</td>
<td>491</td>
</tr>
<tr>
<td>450</td>
<td>430</td>
</tr>
<tr>
<td>500</td>
<td>533</td>
</tr>
<tr>
<td>555</td>
<td>581</td>
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<tr>
<td>600</td>
<td>581</td>
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<tr>
<td>650</td>
<td>455</td>
</tr>
<tr>
<td>700</td>
<td>390</td>
</tr>
<tr>
<td>750</td>
<td>340</td>
</tr>
<tr>
<td>800</td>
<td>310</td>
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<tr>
<td>900</td>
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<td>580</td>
<td>600</td>
</tr>
<tr>
<td>585</td>
<td>598</td>
</tr>
<tr>
<td>570</td>
<td>590</td>
</tr>
</tbody>
</table>

Jan 25, 1949.

Read 11-12 glucose in glucose buffer with 0.02% trypsin, and study absorption spectrum. Peak at λ = 590 Å but not very sharp.
Feb. 28, 1944.

Harvest cells from Y2 lac (L) and Y2 glu.
Test 1 ml cells a 1 ml 5% sugar + 1 ml 4/100 buffer + 130 K.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>L/lac</th>
<th>L/glu</th>
<th>H/lac</th>
<th>H/glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>H</td>
</tr>
<tr>
<td>20</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>H</td>
</tr>
<tr>
<td>30</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>H</td>
</tr>
<tr>
<td>60</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

This organism, adapted to lactose, clearly produces
ferments lactose much more rapidly than glucose or
galactose.
3/1/49.

Harvest cells from 72 leu and 72 blu, dilute etc. +

\[ \text{leu} \quad D_420 \quad 300 \quad 270 \quad 280 \quad \text{R.A.} \quad 4\]

\[ \text{blu} \quad 436 \quad \rightarrow 1000 \quad > 300 \]

W815 produces a defective zelastonease! (although it cannot utilize galactose as rapidly as leucine.)
Harvest cells from 1 L of \( \text{W815} \) incubated \( \frac{1}{2} \)-ac 24 h. Wash and disperse \( \frac{3}{5} \) S. Yield \( 42 \text{ mg} \). Test for lactose fermentation and compare with \( K-12 \) freshly peparred in same way (yield \( 360 \text{ mg} \)).

3/4/49. Prepare 1% suspension of dried cells in water.

Add 1cc cells, 1cc \( \frac{1}{100} \text{MgSO}_4 \) 7.0, 1cc substrate and niculate at 37°C for 30 minutes.

K  lac  +++
K  lactose +
W  lac  --
W  lactose --

Apparantly, the fermentation of lactose in \( \text{W815} \) does not tolerate dyes as does that of \( K-12 \).

Use 1/2 quantity of 1%, \( \text{Glu-1-P} \), stand at 3:15 PM.
**Carbohydrate utilization by W815.**

4/2/49

Compare carbohydrate utilization by cell suspensions harvested from 20 hour bacY2 broth, unremoved of W710 and W815.

Add 10 mg sugar to 1 ml cell suspension, and 1 ml buffer BCP.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>0m</th>
<th>15m</th>
<th>5m</th>
<th>10m</th>
<th>15m</th>
<th>20m</th>
<th>25</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>D-fructose</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>D-glucose</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>L-galactose</td>
<td></td>
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<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

- Lactose is fermented much more quickly than galactose (ca. 3x).

- Does glucose accumulate from lactose? If W235 and W815 grow on lactose, also W10821L and Y.

- Entry: does galactose permeate the cell? Use inhibitor of galactosidase.
Competitive inhibition of galactosidase

Extract 399 dry cells, dilute 1% azo-scenes extract 1:200 and use 1:100 aliquots.
NaP buffer pH 7.5 M/50.

<table>
<thead>
<tr>
<th>ONPG M/1</th>
<th>Di</th>
<th>De</th>
<th>Dco2</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>020</td>
<td>307</td>
<td>289</td>
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<td>2</td>
<td>200</td>
<td>010</td>
<td>263</td>
<td>254</td>
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<td>3</td>
<td>600</td>
<td>002</td>
<td>193</td>
<td>191</td>
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<td>4</td>
<td>1000</td>
<td>-- 003</td>
<td>121</td>
<td>132</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>Lac M/500</td>
<td>020</td>
<td>261</td>
</tr>
<tr>
<td>6</td>
<td>200</td>
<td>013</td>
<td>221</td>
<td>209</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
<td>003</td>
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<td>119</td>
</tr>
<tr>
<td>8</td>
<td>1000</td>
<td>-- 001</td>
<td>68</td>
<td>69</td>
</tr>
<tr>
<td>9</td>
<td>Lba M/500</td>
<td>021</td>
<td>328</td>
<td>319</td>
</tr>
<tr>
<td>10</td>
<td>013</td>
<td>281</td>
<td>269</td>
<td>37.2</td>
</tr>
<tr>
<td></td>
<td>05</td>
<td>2.04</td>
<td>2.08</td>
<td>49.0</td>
</tr>
<tr>
<td>11</td>
<td>003</td>
<td>147</td>
<td>144</td>
<td>69.4</td>
</tr>
</tbody>
</table>

Lba = lactobionate; Ca-replaced by Na; azo-scenes, benzin and Mg, soy.
Make substrate 1 ml. Add/ml: 1mg pyridoxal, pH 7.0, 36°C.
<p>| | | | | |</p>
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<tr>
<td>3. lac</td>
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<td></td>
</tr>
<tr>
<td>4. Azide+lac</td>
<td>190</td>
<td>570</td>
<td></td>
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</tbody>
</table>

**Di.**

**Def.**
<table>
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<td>27</td>
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<td>343</td>
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<td>102</td>
<td>58</td>
<td>71</td>
<td>172</td>
<td>170</td>
</tr>
</tbody>
</table>

**Appearent Km:**

- Blach: 1.22
- Megalasteride: 1.35
- Bugelasteride: 5.9
- Lecteol: 1.82
<table>
<thead>
<tr>
<th></th>
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<th>1/v</th>
<th>1/\text{vadj}</th>
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<td>019</td>
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</tr>
<tr>
<td>2</td>
<td>3%</td>
<td>009</td>
<td>3.11</td>
<td>32.9</td>
</tr>
<tr>
<td>3</td>
<td>5%</td>
<td>003</td>
<td>221</td>
<td>219</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>002</td>
<td>149</td>
<td>147</td>
</tr>
<tr>
<td>+1</td>
<td>1%</td>
<td>025</td>
<td>300</td>
<td>280</td>
</tr>
<tr>
<td>2</td>
<td>3%</td>
<td>012</td>
<td>240</td>
<td>230</td>
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<td>152</td>
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<td>010</td>
<td>260</td>
<td>272</td>
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<td>3</td>
<td></td>
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<td>188</td>
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<td></td>
<td>002</td>
<td>090</td>
<td>088</td>
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<td>023</td>
<td>450</td>
<td>432</td>
</tr>
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<td>2</td>
<td>3%</td>
<td>016</td>
<td>273</td>
<td>260</td>
</tr>
<tr>
<td></td>
<td></td>
<td>005</td>
<td>166</td>
<td>142</td>
</tr>
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<td></td>
<td></td>
<td>004</td>
<td>92</td>
<td>89</td>
</tr>
<tr>
<td>101-2</td>
<td>1%</td>
<td>014</td>
<td>339</td>
<td>324</td>
</tr>
<tr>
<td>2</td>
<td>3%</td>
<td>006</td>
<td>280</td>
<td>269</td>
</tr>
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<td></td>
<td></td>
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<td>06</td>
<td>080</td>
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<td></td>
<td>023</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td></td>
<td>022</td>
<td>220</td>
<td></td>
</tr>
</tbody>
</table>

\[ K_m = 7.7 \times 10^{-4} \]
Kgalactose: $2.1 \times 10^{-2}$
Kmethylgalactoside: $1.25 \times 10^{-2} - 1.7 \times 10^{-2}$
Kbutylgalactoside: $5.1 \times 10^{-3}$
Klactitol: $5.1 \times 10^{-3}$
Klactose (50%) $1.2 \times 10^{-3}$ - 1.5
Koros: $1.3 - 1.5 \times 10^{-3}$

See 38V

$R = 7.7 \times 10^{-4}$
$R' = 2.7 \times 10^{-4}$
$R'' = 2.17 \times 10^{-4}$
$R''' = 1.49 \times 10^{-4}$

$\frac{m}{10,000}$
\( \frac{1}{S} \)
These determinations show no unusual deviations and are in agreement with 524.
4/5/49.

Grow K-12 overnight in 200ml Y2. 4.4% NaCl and 0.5% distilled water. 10% dry over PbO.

Yield: 85mg dry cells.

Intricate and extract 40mg/10ml H2O for extract 5.06A.

Extract potency: 600u/ml.
4/5/49.

<table>
<thead>
<tr>
<th>Process</th>
<th>Reagents</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-12.</td>
<td>2 x 50 ml</td>
<td>1/2 H2O1%</td>
<td>Harvest and dry on 3-05. Yield: 2.9 mg.</td>
</tr>
</tbody>
</table>
Kinetics of cellular lactase

4/6/44

K-12 harvested from Y21100 5X. Then 2 ml in 10
NaP buffer M/100 pH 7.5

<table>
<thead>
<tr>
<th>ONPG Adg.</th>
<th>Ext. Ext.</th>
<th>V+0.5</th>
<th>V</th>
<th>1/V</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1000</td>
<td>346</td>
<td>370-358</td>
<td>338</td>
<td>2.49</td>
</tr>
<tr>
<td>2000</td>
<td>293</td>
<td>299-291</td>
<td>281</td>
<td>1.92</td>
</tr>
<tr>
<td>5000</td>
<td>220-222</td>
<td>221</td>
<td>219</td>
<td>1.30</td>
</tr>
<tr>
<td>10,000</td>
<td>167-178</td>
<td>173</td>
<td>172</td>
<td>0.83</td>
</tr>
<tr>
<td>12,000</td>
<td>260</td>
<td>240</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(stirred vigorously)

\( V_{\text{max}} = 322 \)
\( K_m = 3.2 \times 10^{-4} \)

Stirring does not stimulate enzyme action!

Km is, at least, twice that of isolated enzyme.
### Enzyme Activity from Lactase and Lactose-Grown Cells

**Temperature Coefficients at Enzyme Extraction**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Condition</th>
<th>Enzyme Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>1.2 at 37°</td>
<td>22</td>
<td>161</td>
</tr>
<tr>
<td>2.4</td>
<td>2.5 at 20°</td>
<td>2.5</td>
<td>307</td>
</tr>
<tr>
<td>2.5</td>
<td>2.5 at 20°</td>
<td>2.5</td>
<td>307</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature</th>
<th>1°C</th>
<th>2°C</th>
<th>3°C</th>
<th>4°C</th>
<th>10°C</th>
<th>15°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1.3</td>
<td>1.4</td>
<td>1.5</td>
<td>1.6</td>
<td>1.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

### Enzyme Calculation

\[
Q_{10} = 2.5 / 1.5 = 1.6
\]

### Notes

- \( Q_{10} \) cell is higher than \( Q_{10} \) extract at their high substrate concentration.

---

### Additional Information

- **Strain**: K-12 + W-349 grown on lactose, tested in lactose + leucine.
- **Media**: M-12 + M-lact + 5 mmol leucine + 50 mmol lactose.
- **Temperature**: 2.5°C at 20:30.
- **Date**: 4/17/49.

---
<table>
<thead>
<tr>
<th>Date</th>
<th>N, P, M/100</th>
<th>Temperature</th>
<th>ppm</th>
<th>g/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/7/49</td>
<td>7.5</td>
<td>37</td>
<td>203</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td>0.20</td>
<td>4.21</td>
<td>462</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>3.19</td>
<td>406</td>
<td>21.6</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>2.03</td>
<td>319</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>1.97</td>
<td>208</td>
<td>48.1</td>
</tr>
<tr>
<td>5/6</td>
<td>1.20</td>
<td>4.31</td>
<td>406</td>
<td>26.6</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>3.46</td>
<td>430</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>2.30</td>
<td>97</td>
<td>103.1</td>
</tr>
<tr>
<td>1:150</td>
<td>1.20</td>
<td>4.81</td>
<td>208</td>
<td>48.1</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>3.29</td>
<td>275</td>
<td>36.5</td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>2.04</td>
<td>70</td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>1.31</td>
<td>24</td>
<td>30.4</td>
</tr>
</tbody>
</table>

Data n.g. - experiments not repeated.
Test for induction period in cellular catalysis of ONPG.

| Time (min) | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 120 | 140 | 160 | 180 | 200 | 220 | 240 | 270 | 300 | 330 | 360 | 400 | 450 | 500 |
|------------|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 134        | 131| 60 | 270| 271| 90 | 282| 70 |
| 121 (mixing)| 137| 147| 146| 148| 149| 159| 162| 167| 170| 177| 180| 184| 190| 197| 202| 209| 212| 219| 225| 231| 239| 245| 252| 257|


Concentration: -12.4 for dilution of cells. +10 for substrate. -311 +131 given initial.
April 10, 1949

W349 lactose

W349 was treated as lactitol + B-H. Mixture 2 x 100 ml
1/2 lactose and acetate 24 hours. Wash + dry over 130°
Yield: 672 mg.
<table>
<thead>
<tr>
<th>Dilution</th>
<th>1 mL/tube</th>
<th>VAP</th>
<th>Date</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/100</td>
<td>D0</td>
<td>25</td>
<td>4/15/75</td>
<td></td>
</tr>
<tr>
<td>1/10</td>
<td>D1</td>
<td>56</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>1/1000</td>
<td>D2</td>
<td>80</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>1/1001</td>
<td>D3</td>
<td>111</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>1/10001</td>
<td>D4</td>
<td>142</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>1/10000</td>
<td>D5</td>
<td>173</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>1/100000</td>
<td>D6</td>
<td>196</td>
<td>5/6</td>
<td></td>
</tr>
<tr>
<td>1/1000000</td>
<td>D7</td>
<td>242</td>
<td>5/6</td>
<td></td>
</tr>
</tbody>
</table>

Substrate: 0.16 from all - 0.025 on VAS.

Note: VAS refers to the variable of interest in the experiment.


![Image](https://example.com/image.png)

**Date:** 4/29/48

**Procedure:**

1. Grow K-12 shaken overnight in 1/100% lactic acid 5% meth and compare with lac 1/100 adapted cells, etc.

<table>
<thead>
<tr>
<th></th>
<th>7:00 AM Di</th>
<th>7:00 PM Di</th>
</tr>
</thead>
<tbody>
<tr>
<td>tac</td>
<td>119</td>
<td>800</td>
</tr>
<tr>
<td>bar</td>
<td>106</td>
<td>126</td>
</tr>
<tr>
<td>str(y2)</td>
<td>157</td>
<td>172</td>
</tr>
<tr>
<td>NSB</td>
<td>162</td>
<td>170</td>
</tr>
</tbody>
</table>

These tubes were made up from Stokoe's purified lactobionate buffer. Each tube was preincubated for 30 minutes in KNO3 0.005 molar. Apparent increase of 1/2-4 probably artefacts, reversible color.

The second tubes were also adapted to NNO3 after then, progressive color in culture, can 50, probably adaptation.

**Effect of acid on pH sensitivity.**

<table>
<thead>
<tr>
<th>pH</th>
<th>Acid</th>
<th>Di</th>
<th>Df</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>-</td>
<td>54</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>+</td>
<td>60</td>
<td>361</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>-</td>
<td>53</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>+</td>
<td>51</td>
<td>145</td>
<td></td>
</tr>
</tbody>
</table>

Sic! Adequate stimulation cells.

Should use KNO3 to eliminate Na effect.

Test galactosidase activity of washed suspensions. ONPG M/2000; NaP M/50 7.5 20 mins. 37°C.

<table>
<thead>
<tr>
<th></th>
<th>D₁</th>
<th>D₂</th>
<th>R.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-12</td>
<td>229</td>
<td>880</td>
<td>300</td>
</tr>
<tr>
<td>Y-53</td>
<td>222</td>
<td>222</td>
<td>008</td>
</tr>
</tbody>
</table>

This prep. of lactobionate certainly elicits a very active galactosidase, but not from Lac⁻/⁻.

The cells harvested fermented glucose, lactose very very slowly.

2. Inhibitions. Make up tubes with .01 ml 399A lactase, M/1000 ONPG, NaP as above.

To 3, 4 add M/100 Lba.

<table>
<thead>
<tr>
<th></th>
<th>D₁</th>
<th>D₂</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>002</td>
<td>251</td>
<td>250</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>252</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>010</td>
<td>169</td>
<td>155</td>
</tr>
<tr>
<td>4</td>
<td>014</td>
<td>164</td>
<td></td>
</tr>
</tbody>
</table>

Taking $K_{onpg}$ as $1.3 \times 10^{-4}$, $K_{lba}$ can be calculated:

$$K_I = \frac{1}{V_0} \left( \frac{1}{V_i} - \frac{1}{V_0} \right)$$

$$= \frac{M}{100} \sum \left( \frac{V_0}{V_i} \left( 1 - \frac{1}{K_5 + S} \right) \right)$$

$$= \frac{1}{100} \left( \frac{10^4}{4.5} \left( 1 - \frac{1}{10^{-4}} \right) \right)$$

$$= \frac{10^4}{2.3 \times 10^{-2}} = 8.3 \times 10^{-3} \quad \text{(circulated lactobionate)}$$

3. Impose dried cells from 180 ml aerated Y-12-Lba. Yield 160mg. Well aerated culture was very dense.
\[ \frac{K_T}{T} = \frac{V_i k_S}{(V_0 - V_i)(1 + k_S)} = \frac{359 \times 1.3}{(41)(2.3)} = \frac{367}{23} \times \frac{1.3}{2.3} \times \frac{1}{200} \]

\[ = 4.9 \times 10^{-2} \]

Use \( m = 4.7 \times 10^{-2} \)
4/20/49

<table>
<thead>
<tr>
<th>Lactose (26%)</th>
<th>Stole</th>
<th>Make-up</th>
<th>Y2:</th>
<th>A</th>
<th>R.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M/50</td>
<td>0.41</td>
<td>4.32</td>
<td>4.50</td>
<td>1,000</td>
<td></td>
</tr>
<tr>
<td>2. M/100</td>
<td>0.44</td>
<td>5.70</td>
<td>5.50</td>
<td>7.100</td>
<td></td>
</tr>
<tr>
<td>3. M/500</td>
<td>0.56</td>
<td>3.95</td>
<td>3.50</td>
<td>6.50</td>
<td></td>
</tr>
<tr>
<td>4. M/1,000</td>
<td>0.53</td>
<td>4.77</td>
<td>4.30</td>
<td>9.00</td>
<td></td>
</tr>
<tr>
<td>5. M/10,000</td>
<td>0.45</td>
<td>1.20</td>
<td>1.20</td>
<td>1.70</td>
<td></td>
</tr>
<tr>
<td>6. M/100,000</td>
<td>0.48</td>
<td>0.77</td>
<td>0.35</td>
<td>0.75</td>
<td></td>
</tr>
</tbody>
</table>

Harvest K-12 grown overnight in Y2 + each of above case (10 ml/10 ml). Cent. at 5x; use 1 ml/10 ml sterile assaying for galactomannase.

Retest adaptation to lactose (10%) and Leucosid B (M/40 x 1/2).

| I2 + | 0.87 | 1.39 | 6.0 | 7.5 |
| I2   | 0.63 | 0.97 | 4.0 | 5.5 |

The cut-off of adaptive response appears to be much lower than for combination of the enzymes.

The response to lactohemate is undoubtedly due to lactose-mannypyridine. If M/40 lactohemate is used, an impurity of 10% will give M/400, in the range of effective response.
<table>
<thead>
<tr>
<th>Di</th>
<th>De</th>
<th>Vea</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-3</td>
<td>048</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>083</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>124</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>159</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>198</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>253</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>274</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>321</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>337</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>406</td>
</tr>
</tbody>
</table>

NapH \( \geq 7.5 \)

H₂SO₄ 0.085 399 \( 10^{-2} \sim 10^{-3} \)
<table>
<thead>
<tr>
<th>Date</th>
<th>Ki2</th>
<th>Y2-Lac</th>
<th>Di</th>
<th>Df</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/23/49</td>
<td>090</td>
<td>090</td>
<td>790</td>
<td>349</td>
</tr>
<tr>
<td></td>
<td>090</td>
<td>120</td>
<td>087</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99</td>
<td></td>
<td>529</td>
<td></td>
</tr>
</tbody>
</table>

Note: tremendous activity of MuGK 12!
**Comparison of lac- and Bngal.**

4/24/44

<table>
<thead>
<tr>
<th></th>
<th>lac N/50</th>
<th>N/500</th>
<th>lac N/500</th>
<th>lac N/500</th>
<th>Bngal N/500 and later N/500</th>
</tr>
</thead>
<tbody>
<tr>
<td>A = K-12</td>
<td>70</td>
<td>73</td>
<td>291</td>
<td>208</td>
<td>297</td>
</tr>
<tr>
<td>B = Y70</td>
<td>110</td>
<td>109</td>
<td>223</td>
<td>114</td>
<td>104</td>
</tr>
<tr>
<td>C = W112</td>
<td>87</td>
<td>83</td>
<td>470</td>
<td>367</td>
<td>478</td>
</tr>
</tbody>
</table>

**Di cells**: Di con | Δ | Δ/Di | R.A. 20 min.

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>117</td>
<td>115</td>
<td>140</td>
<td>025</td>
<td>021</td>
</tr>
<tr>
<td>2</td>
<td>111</td>
<td>110</td>
<td>120</td>
<td>010</td>
<td>009</td>
</tr>
<tr>
<td>3</td>
<td>113</td>
<td>112</td>
<td>178</td>
<td>064</td>
<td>057</td>
</tr>
</tbody>
</table>

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90</td>
<td>91</td>
<td>127</td>
<td>036</td>
<td>040</td>
</tr>
<tr>
<td>2</td>
<td>113</td>
<td>112</td>
<td>127</td>
<td>015</td>
<td>013</td>
</tr>
<tr>
<td>3</td>
<td>88</td>
<td>89</td>
<td>239</td>
<td>150</td>
<td>171</td>
</tr>
</tbody>
</table>

These cells are phaenous, and they frequently revert!

Compare earlier data while showing wider discrepancy.

[CF 421. — last column J.]

**EML 194 (Y10 for K-12)**

Much greater differentials.

Compare Y10 (K) and W112 (lac-).
**April 25.**

<table>
<thead>
<tr>
<th>Without shelving</th>
<th>20mm data</th>
<th>8/20</th>
<th>12/20</th>
<th>8/30</th>
<th>12/30</th>
<th>8/40</th>
<th>12/40</th>
<th>8/50</th>
<th>12/50</th>
<th>8/60</th>
<th>12/60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y10, Dac 4/50</td>
<td>152</td>
<td>174</td>
<td>112</td>
<td>070</td>
<td>056</td>
<td>01</td>
<td>086</td>
<td>119</td>
<td>043</td>
<td>013</td>
<td>011</td>
</tr>
<tr>
<td>Y12, Dac 4/30</td>
<td>108</td>
<td>119</td>
<td>097</td>
<td>130</td>
<td>080</td>
<td>086</td>
<td>119</td>
<td>120</td>
<td>262</td>
<td>139</td>
<td>021</td>
</tr>
</tbody>
</table>

**Shelving:**

<table>
<thead>
<tr>
<th>Y10</th>
<th>12</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y11</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

These data can be used:

<table>
<thead>
<tr>
<th>Y10 Late</th>
<th>Y12 Late</th>
<th>Y12 Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>380</td>
<td>330</td>
<td>014</td>
</tr>
</tbody>
</table>
5/6/49

Y10 after 3 transfers in NSB, grown overnight stationary

lac Y2

renover (6hr) NSB.

increase upon adaptation is 61x

i.e., unadapted cells have activity ca. 1.6% of adapted!

Theo may be nine point adaptation.
<table>
<thead>
<tr>
<th>t</th>
<th>D1</th>
<th>D4</th>
<th>Acm</th>
<th>R.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>104</td>
<td>97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>101</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>086</td>
<td>097</td>
<td></td>
<td></td>
</tr>
<tr>
<td>170</td>
<td>079</td>
<td>090</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No adaptation found
### Adaptation Kinetics

<table>
<thead>
<tr>
<th></th>
<th>(A)</th>
<th>(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5/26/49</td>
<td></td>
</tr>
<tr>
<td>Y10</td>
<td>amylase 1 ml</td>
<td>1% tris-HCl buffer 1 ml</td>
</tr>
<tr>
<td>T0</td>
<td>2:35 PM</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>0</td>
<td>D+</td>
</tr>
</tbody>
</table>

#### A

<table>
<thead>
<tr>
<th>Time</th>
<th>3 PM</th>
<th>3:35 PM</th>
<th>5 PM</th>
<th>7 PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>121</td>
<td>130</td>
<td>117</td>
<td>109</td>
</tr>
<tr>
<td>16</td>
<td>132</td>
<td>148</td>
<td>132</td>
<td>134</td>
</tr>
</tbody>
</table>

#### B

<table>
<thead>
<tr>
<th>Time</th>
<th>3 PM</th>
<th>3:35 PM</th>
<th>5 PM</th>
<th>7 PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>128</td>
<td>130</td>
<td>118</td>
<td>118</td>
</tr>
<tr>
<td>16</td>
<td>133</td>
<td>148</td>
<td>147</td>
<td>133</td>
</tr>
</tbody>
</table>

---

Verte adaptatio.
Harvest K-12 from standing culture in Y 2 broth. Add 0.10 x
11.0. Add 0.01 ml NaCl 7.5, 1 ml 2% lactose, 1 ml cells and 1 ml supp.

Total 3 ml samples to 9 ml ONPG test system.

A). No supplement.
B). Peptide 1 ml 2%.

<table>
<thead>
<tr>
<th>T</th>
<th>DI</th>
<th>DF</th>
<th>A(con)</th>
<th>R.A.</th>
<th>DI</th>
<th>DF</th>
<th>A</th>
<th>R.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.0</td>
<td>061</td>
<td>071</td>
<td>-005</td>
<td>005</td>
<td>064</td>
<td>087</td>
<td>008</td>
<td>012</td>
</tr>
<tr>
<td>5.0</td>
<td>056</td>
<td>077</td>
<td>+005</td>
<td>009</td>
<td>067</td>
<td>098</td>
<td>038</td>
<td>087</td>
</tr>
<tr>
<td>7.0</td>
<td>048</td>
<td>098</td>
<td>+004</td>
<td>071</td>
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<td>8.0</td>
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<td>780</td>
<td>670</td>
<td>680</td>
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</tr>
</tbody>
</table>

Desadaptation.

Harvest K12 freshly grown on Y 2 lec. 840 pm.

Sample (from E): 8 min.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>062</td>
<td>267</td>
<td>300</td>
</tr>
<tr>
<td>062</td>
<td>260</td>
<td>260</td>
</tr>
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</table>

Appreciable desadaptation!

C should be counted for inhibition by 0.1% lactose.
July 6, 1949

Yeast K12 from 50 ml Y2 broth overnight, ca. ca. 10x.
System (4 ml)
1 ml cells, 1 ml buffer, 1 ml 7% sugar, 1 ml peptone water

A. ab
B. ab - d
C. ab glucose
D. ab glucose d

Buffer: saline peptone glucose (final concentration 2.2 x 10^{-3} M)
peptone + glucose.

Assay in M/100 saline M/50 NaCl buffer, 2 ml samples (d = 0.5)

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
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<tr>
<td>Di</td>
<td>0.50</td>
<td>0.50</td>
<td>0.48</td>
<td>0.49</td>
</tr>
<tr>
<td>Df</td>
<td>143</td>
<td>181</td>
<td>118</td>
<td></td>
</tr>
</tbody>
</table>

Does glucose equilibrate for utiliza into cell?

145 PM

A 0.38  552
B 0.49  226
C 0.46  380
D 0.80  234

Note augmented activity of cells incubated in buffer.
Sediment these tubes and examine supernatant.
5 ml supernatant ca 120. Most activity is still in cells!
Storage Effects on galactose isomerase

7/14/49

32 ham cells from 212 ace

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>059</td>
<td>059</td>
</tr>
<tr>
<td>B</td>
<td>061</td>
<td>061</td>
</tr>
<tr>
<td>C</td>
<td>056</td>
<td>056</td>
</tr>
<tr>
<td>D</td>
<td>060</td>
<td>060</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>Df: 10ml</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>472</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>930</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>241</td>
<td></td>
</tr>
</tbody>
</table>

A) Note: Circular area of buffer treated cells over water treated. Buffer was 0.1M Na, pH 7.5

7/14/49.

Ph.

Harvest 10 hr. cells from Y2 bac.

Dilute equal volume 1) with 0.1 ml samples assayed.
2) NaP 4/5 pH 7.5 c) NaP de.
3) NaCl 4/5 d) sucrose 4/5

D: H2O 0.24 2.74

10 min.

a 0.05 158.
b 0.02 >750 [5 min].
c 0.10 >750 [5 min].
d 0.66 0.10

e 0.11 375

Phosphate buffer, which also permit lysis, are most effective in augmenting activity. Ph effect? concentration? Measure pH's.

Verify lysis by UV absorption of supernatant.
Streptococcus A and B contain ca. 1.5 and 2.2 mg/mL respectively. For an 0.1 mg, use \( \frac{1}{15} \) mL for A and \( \frac{1}{22} \) mL for B.

Assay 0.05 mL each.

A

B

Blank

<table>
<thead>
<tr>
<th>018</th>
<th>18.4</th>
<th>155</th>
</tr>
</thead>
<tbody>
<tr>
<td>030</td>
<td>430 (5 min)</td>
<td>390 x 4</td>
</tr>
<tr>
<td>001</td>
<td>014</td>
<td></td>
</tr>
</tbody>
</table>

-0.13 for substrate, +10% for dilution.

\( B \) 0.11 mg had activity of \( \frac{20}{5} \times 4 = 16 \text{ u.} \) \( \Rightarrow 150 \text{ u/mL} = \text{ full activity of the cells dried.} \)

\( A \) 0.075 mg had 1.5 u. \( \Rightarrow 20 \text{ u/mL} \) full activity, not augmented.

Differences between treated and untreated cells persist on drying.

?? Can inactive, cell-free or dried preparations be activated?
Sediment A and B. Resuspend sediment in 5 ml H$_2$O (x 1) and keep supernatant (x 2). B2 is much more paler than A1.

Same samples, also mix A1, A2 etc. 1:1 = NaP H/5.

Incubate 3$^2$C $\rightarrow$ 5$^2$C

Test 1 ml samples A1, A2 and AIP, A2P.

<table>
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<tr>
<td></td>
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<td>040</td>
<td>155</td>
<td>68</td>
<td>68</td>
<td>016</td>
<td>140</td>
<td>030</td>
<td>305</td>
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<td>530</td>
<td></td>
<td>90</td>
<td>180</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td>470</td>
<td></td>
<td>305</td>
<td>260</td>
</tr>
</tbody>
</table>
1) Y10 and Y70 grown in lactose. Incubate 1:1 with water, buffer, M/10. Assay.

2) K-12 grown in lactose. Incubate 1:1 with water, buffer, etc.

K-12 glucose [KGS]

1:1:1 lactose, 2%, water, M/100 buffer, M/100kgf.

<table>
<thead>
<tr>
<th></th>
<th>D:</th>
<th>062</th>
<th>041</th>
<th>negl.</th>
<th>10m</th>
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<tr>
<td></td>
<td>032</td>
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</tr>
<tr>
<td></td>
<td>027</td>
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</table>

<table>
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<tr>
<th></th>
<th>KL-O</th>
<th>139</th>
<th>37.1</th>
<th>64m</th>
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<tbody>
<tr>
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<td>KL-P</td>
<td>111</td>
<td>520</td>
<td>64m</td>
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<tr>
<td></td>
<td>L-P</td>
<td>078</td>
<td>1150</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>095</td>
<td>119</td>
<td>10m</td>
</tr>
<tr>
<td></td>
<td></td>
<td>072</td>
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<tr>
<td></td>
<td>Y70-O</td>
<td>113</td>
<td>negl.</td>
<td></td>
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<td></td>
<td>Y70-P</td>
<td>076</td>
<td>152</td>
<td>9H</td>
</tr>
</tbody>
</table>
August 8, 1949.

Lactobacillus extract 2%. Activity ca 1200 u/ml.

<table>
<thead>
<tr>
<th>Coombe</th>
<th>m/50 NaP</th>
<th>7.5 M/1000 0.9%</th>
<th>Staph e NaCl</th>
</tr>
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<tbody>
<tr>
<td>Acetone</td>
<td>conc 20%</td>
<td>sol 119</td>
<td></td>
</tr>
<tr>
<td>Hamitol</td>
<td>m/10</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Solitot</td>
<td>m/10</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>ProOH</td>
<td>m/100</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m/10</td>
<td>134</td>
<td></td>
</tr>
<tr>
<td></td>
<td>m/1</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5%</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

Optimal concentration.

Reduced Hamitol and ProOH concentrations. Also of 24% which showed lesser effect.

8/8/49 100 ml, as above

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProOH</td>
<td>EtOH</td>
<td>Hamitol</td>
<td>Acetone</td>
<td>Hamitol</td>
<td>ProOH</td>
</tr>
<tr>
<td>m/10</td>
<td>m/10</td>
<td>m/10</td>
<td>m/10</td>
<td>m/10</td>
<td>m/10</td>
</tr>
</tbody>
</table>
Summary of lactose activation

September 9, ff., 1941.

2. L. aced K12 Y2 lac washed and concentrated to 30 ml.
   Aliquots of 15 ml ea. mixed with 1) 15 ml H2O; 2) 15 ml NaPO4.
   and incubated 1 hour at 30°. After removal of 1 ml, 29 ml
   samples were dried, and subsequently found to yield 1.642 and .560 g.
   respectively after washing, or 22.1 and 19.3 mg/ml respectively.

   Assays of A and B before and after drying were (U./mg.)
<table>
<thead>
<tr>
<th>Wet</th>
<th>Dried</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.1</td>
</tr>
<tr>
<td>B</td>
<td>44.5</td>
</tr>
</tbody>
</table>

After benzene treatment, an activity of 157 U./mg was recovered.

2. Can dried cells be further activated? Relate these activities to
   pH characteristics of activated cells. No responses.
<table>
<thead>
<tr>
<th></th>
<th>Assay aliquots of A and B</th>
<th>( \frac{1}{10} = 0.01 \text{ ml} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Div. ( 1 )</td>
<td>Div. ( 2 )</td>
</tr>
<tr>
<td>A</td>
<td>0.9</td>
<td>193</td>
</tr>
<tr>
<td>B</td>
<td>0.8</td>
<td>329</td>
</tr>
<tr>
<td></td>
<td>( 1 \text{ ml} A = \frac{642 \text{ mg}}{29 \text{ ml}} = 22.1 \text{ mg} ), assuming complete recovery.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3% 0.67</td>
<td>4% 0.98</td>
</tr>
<tr>
<td></td>
<td>4% 3.0 ( 1 \frac{3}{4} )</td>
<td>5% 2.7( \frac{3}{4} )</td>
</tr>
<tr>
<td></td>
<td>= ( 3500 \mu \text{I/mL} A )</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1 \text{ ml} samples of A, B suspensions have activities of ( 13; 860 \mu \text{I} ) respectively, ( \div (13, 860) \mu \text{I/ML} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total: ( \text{mg/day} \times \text{I/ML} )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A ( 3280 )</td>
<td>B ( 29800 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 \text{ ml} samples of ( 1% ) suspensions of crude cells for comparison.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Div. ( T )</td>
<td>Div. ( F ) ( g / \text{mg} )</td>
</tr>
<tr>
<td>A</td>
<td>0.04 ( 560 )</td>
<td>0.05 ( 2880 )</td>
</tr>
<tr>
<td>B</td>
<td>0.14 ( 380 )</td>
<td>0.05 ( 1144 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Benzene: ( 157 )</td>
<td></td>
</tr>
</tbody>
</table>
September 9, 1949

Harvest and wash K-12 from 2L. aeration 37° C. 2L. 7% 1°/₀.

Suspend in 25 ml. Remove (5 ml), and separate 15 ml portions of remainder: A) +15 ml. H₂O  B) +15 ml. Na₃P 4/₅ pH 7.5. Incubate in stopped flasks at 30° C. 850 to 1000, for subsequent dry cell preparation. At 25° C. remove 1 ml aliquots, and sediment + dry remainder.

Dilute 1/100; 1/50 = 1/2000 for assays.

A) assay in dil (H₃0₀) and con dil (H₄/₁₀) buffer. Do latter in colorimeter.

BLA cell 0.025 cc. volume.
H/₅ buffer Na₃P 8.5 ml.
Cell (add at 70°) 0.5 ml.

Time: 2.05 2.20 2.39 2.42 2.50 2.53 3.05
20 s 60 s 180 240 577 7 11 35 42 0.36 0.37 0.37 0.39 0.42 0.47 0.52 0.57 0.59 0.61
Kinetics of activation
NaP buffer 17 M 30°.

Activation ratio: \( \frac{62}{18} = 4.5 \)
<table>
<thead>
<tr>
<th>Time</th>
<th>7-7B</th>
<th>6-8B</th>
<th>9-7B</th>
<th>10-10B</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:35</td>
<td>167</td>
<td>371</td>
<td>86.7</td>
<td>37.8</td>
</tr>
<tr>
<td>3:40</td>
<td>148+3</td>
<td>95+5</td>
<td>58+7</td>
<td>79.8</td>
</tr>
<tr>
<td>3:45</td>
<td>147+4</td>
<td>93+7</td>
<td>57+1</td>
<td>77.8</td>
</tr>
<tr>
<td>3:50</td>
<td>150+7</td>
<td>106+7</td>
<td>70+7</td>
<td>84+4</td>
</tr>
<tr>
<td>3:55</td>
<td>149+8</td>
<td>108+9</td>
<td>70+7</td>
<td>84+4</td>
</tr>
<tr>
<td>4:00</td>
<td>147+7</td>
<td>107+9</td>
<td>70+7</td>
<td>84+4</td>
</tr>
<tr>
<td>4:10</td>
<td>149+7</td>
<td>104+7</td>
<td>69+1</td>
<td>72+4</td>
</tr>
<tr>
<td>4:20</td>
<td>153+7</td>
<td>104+7</td>
<td>68+1</td>
<td>72+4</td>
</tr>
</tbody>
</table>

Manometric rates on "activated" cells.

Ca. 50% inactivation of buffer-treated cells.
<table>
<thead>
<tr>
<th></th>
<th>9A</th>
<th>2A</th>
<th>4A</th>
<th>8A</th>
<th>10A</th>
<th>740A</th>
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<td>12</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>2.10</td>
<td>5, 18</td>
<td>7</td>
<td>12</td>
<td>15</td>
<td>13</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>2.17</td>
<td>14</td>
<td>11</td>
<td>12</td>
<td>09</td>
<td>14</td>
<td>0</td>
<td>03</td>
</tr>
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<td>2.28</td>
<td>13</td>
<td>13</td>
<td>09</td>
<td>19</td>
<td>4</td>
<td>18</td>
<td>0</td>
</tr>
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<td>10</td>
<td>18</td>
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<tr>
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<td>35</td>
<td>14</td>
<td>11</td>
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<td>72</td>
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<td>49</td>
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<tr>
<td>20</td>
<td>38</td>
<td>19</td>
<td>08</td>
<td>1</td>
<td>92</td>
<td>77</td>
<td>62</td>
</tr>
<tr>
<td>25</td>
<td>51</td>
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<td>56</td>
<td>50</td>
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<td>3</td>
<td>126166</td>
<td>7754</td>
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<td>60</td>
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<td>21</td>
<td>133</td>
<td>06</td>
<td>9</td>
<td>43</td>
</tr>
</tbody>
</table>

**K12**

- T. d. p. n. glucose or maltose (D, M)
- T. d. p. n. dextrose 10%: = f. n.
- NaHCO₃ 1/20
- Na₂CO₃ 1/1000
- Mg 1/100
- 4A M, g
- 2A H, m
- 8A H, m
- 10A M, isomaltose
- 7A H -

Isomaltose not utilized by maltose - adapted K-12!
Some autogenous
$\alpha$- or $\alpha$-removal
indicated (ideally perfect).
<table>
<thead>
<tr>
<th>T/T</th>
<th>T/gel</th>
<th>T/mel</th>
<th>T/ar</th>
<th>S/gel</th>
<th>S/ar</th>
<th>2/1</th>
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<td>40</td>
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<td>14-4</td>
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<td>9</td>
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<td>4</td>
<td>15</td>
<td>9</td>
</tr>
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<td>21-2</td>
<td>66</td>
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<td>7</td>
<td>15</td>
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<td>18-1</td>
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<td>168</td>
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<td>26-7</td>
<td>12-1</td>
<td>22</td>
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<td>15</td>
<td>15</td>
</tr>
<tr>
<td>32-14</td>
<td>35-16</td>
<td>13-1</td>
<td>27</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

K12 grown over in 12 D turbid, 5% (T) or Dextrose 1% (D).

Test m. molasses, glucose, fructose, and arabinose.

Cells 5x, 2mil in NaHCO3 1/20 NaP 0.1/1000 solid CO2 3-20.

1ml 10% sugar 30º C.

Fructose // molasses. Need autoferment. control.

Note rapid adaptation to arabinose (30 minutes)
All grown on trehalose
<table>
<thead>
<tr>
<th></th>
<th>Glucose 4</th>
<th>Maltose 8</th>
<th>Totalize 10</th>
<th>Theme 149</th>
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<tbody>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>08</td>
<td>10</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>38 + 18</td>
<td>29 + 20</td>
<td>21 + 5</td>
<td>149 - 0</td>
</tr>
<tr>
<td></td>
<td>54 + 15</td>
<td>43 + 13</td>
<td>22 0</td>
<td>150 - 1</td>
</tr>
<tr>
<td></td>
<td>79 + 26</td>
<td>71 + 27</td>
<td>21 0</td>
<td>149 + 1</td>
</tr>
<tr>
<td>940</td>
<td>97 + 16</td>
<td>91 + 18</td>
<td>23 0</td>
<td>151 - 2</td>
</tr>
<tr>
<td>9 + 15</td>
<td>118 + 23</td>
<td>116 + 27</td>
<td>25 + 3</td>
<td>150 + 1</td>
</tr>
<tr>
<td>950</td>
<td>137 + 9</td>
<td>140 + 26</td>
<td>27 + 2</td>
<td>150 - 1</td>
</tr>
<tr>
<td>1005</td>
<td></td>
<td></td>
<td>32 + 3</td>
<td>152 - 2</td>
</tr>
<tr>
<td>1102</td>
<td></td>
<td></td>
<td>60 + 26</td>
<td>154 - 2</td>
</tr>
<tr>
<td>1107</td>
<td></td>
<td></td>
<td>61 + 1</td>
<td>154</td>
</tr>
</tbody>
</table>

Ym. 99 98 109 04

Hour 26
September 9, 1949

**K12 lac. 10 mg gal in one sidearm, 10 mg glucose in 2d.**
2 ml diluted cells from exp. 3, in NaHCO₃-NaPO₄/100 /CO₂ 3%.

<table>
<thead>
<tr>
<th></th>
<th>19A</th>
<th>7A</th>
<th>3YA</th>
<th>4A</th>
<th>2A</th>
<th>5</th>
<th>10A</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>940</td>
<td>151</td>
<td>150+1</td>
<td>152-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>945</td>
<td>153</td>
<td>152+1</td>
<td>152-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Glyceraldehyde**

<table>
<thead>
<tr>
<th></th>
<th>58 46</th>
<th>61 45</th>
<th>83 53</th>
<th>98 95</th>
</tr>
</thead>
<tbody>
<tr>
<td>952</td>
<td>151</td>
<td>150+1</td>
<td>152-1</td>
<td></td>
</tr>
</tbody>
</table>

**Glu**

<table>
<thead>
<tr>
<th></th>
<th>84 88</th>
<th>74 119 91</th>
<th>149 148</th>
</tr>
</thead>
<tbody>
<tr>
<td>900</td>
<td>149+2</td>
<td>149+2</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>112-96</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Volume</td>
<td>( \text{cm}^3 \text{ at } 25^\circ \text{C} )</td>
<td>1 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>--------</td>
<td>------------------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>21.12</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>19.51</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20.19</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>19.97</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>18.20</td>
<td>1.62</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>18.43</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>18.99</td>
<td>1.67</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>19.02</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>18.44</td>
<td>1.70</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>19.60</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>18.86</td>
<td>1.68</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>19.61</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>18.26</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>14</td>
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</tr>
</tbody>
</table>

A

Subtract

Subtract

0.89

178
<table>
<thead>
<tr>
<th>Date</th>
<th>Lactose</th>
<th>Maltose</th>
<th>Lactose Adaptation</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 14, 1947</td>
<td>44%</td>
<td>45%</td>
<td></td>
</tr>
<tr>
<td>2/11</td>
<td>10A</td>
<td>11A</td>
<td></td>
</tr>
<tr>
<td>16/11</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>27/11</td>
<td>32</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>7/12</td>
<td>28</td>
<td>16/12</td>
<td></td>
</tr>
<tr>
<td>18/12</td>
<td>20</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>24/12</td>
<td>82</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>2/13</td>
<td>76</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>19/13</td>
<td>76</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>20/13</td>
<td>76</td>
<td>76</td>
<td></td>
</tr>
</tbody>
</table>

* Note: K12 grown on maltose, aerated.
September 23, 1949

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>L 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H2</td>
<td>L 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>L 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H4</td>
<td>L 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cell density: L 19.9
M 13.3

Cell inoculated from 3:30 PM
in indicated supplement
1 ml cells
12 ml 1% sucrose
.1 ml K buffer pH 7.0 M/5.
(q) + .1 ml MgSO4 M/5.

1 ml saline and 1 ml
showing decrement of activity
when incubated with lactose or glucose.
September 24, 1969.

1) Tubes each receive 1 ml benzene/tube.
2) Stir tubes and add 1 ml benzene/tube.
3) Add 9 ml H2O to 1-5. (No #2 in vial.)
4) Assay and samples:
   - Tube
   - 10 mL
   - 8 am
   - 100 mic.
   - 157
   - 173
   - 163
   - 172
   - 205

Too cute to be used in present stage of development.
September 24, 1941.

Harvest K-12 from Y2 Mal and Y2 lac.

Add NaP 7.5 to 4.5 0. Add cells + 1 ml supplement

incubate from 12:00 to 3:00 PM = 3 hours. 37°

Add 1 ml D-glucose to activate.

40 pg/ml 2000 in 0.1 ml NaP 7.5 37°.

Cell density (before: 7/6: 1:1)

<table>
<thead>
<tr>
<th>Suppl.</th>
<th>Di 10 m</th>
<th>D (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y2</td>
<td>0.24</td>
<td>3.87</td>
</tr>
<tr>
<td>Y2 lac</td>
<td>0.27</td>
<td>5.90</td>
</tr>
<tr>
<td>lac 1%</td>
<td>0.28</td>
<td>2.17</td>
</tr>
<tr>
<td>H20</td>
<td>0.24</td>
<td>2.36</td>
</tr>
<tr>
<td>lac 2% + (NH4)2SO4 + 0.1 ml</td>
<td>0.22</td>
<td>2.64</td>
</tr>
<tr>
<td>(NH4)2SO4 + 1.1 ml + lac 2%</td>
<td>0.22</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.11</td>
</tr>
</tbody>
</table>

?? Na2CO3 1 ml added
<table>
<thead>
<tr>
<th>Culture</th>
<th>Start Date</th>
<th>End Date</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>W251A/lac</td>
<td>201</td>
<td>222</td>
<td></td>
</tr>
<tr>
<td>T1SA2</td>
<td>223</td>
<td>241</td>
<td></td>
</tr>
<tr>
<td>C4A9</td>
<td>224</td>
<td>239</td>
<td></td>
</tr>
<tr>
<td>C2A9</td>
<td>241</td>
<td>239</td>
<td></td>
</tr>
<tr>
<td>1SA1</td>
<td>224</td>
<td>241</td>
<td></td>
</tr>
<tr>
<td>1A12</td>
<td>201</td>
<td>222</td>
<td></td>
</tr>
</tbody>
</table>

*Note: The data is not clear and may need further clarification.*

Harvest K-12 from 12 hour aer.Y2—50 ml. Concentrate to 5 ml (10x).

Leave water suspension on table top 10A—730 pm.

1 ml adjusted in 10i.m. 750 pm, (90 minutes).

Treated samples per standard ON/6

<table>
<thead>
<tr>
<th>Untreated samples:</th>
<th>(1 ml/10)</th>
<th>R.A/mkl</th>
<th>R.A/mkl/10,000/10³</th>
</tr>
</thead>
<tbody>
<tr>
<td>K/Lac</td>
<td>250</td>
<td>800</td>
<td>(12 min.)</td>
</tr>
<tr>
<td>K/HeLa</td>
<td>307</td>
<td>475</td>
<td>19</td>
</tr>
<tr>
<td>K/Ide</td>
<td>118</td>
<td>119</td>
<td>0.2 [1]</td>
</tr>
</tbody>
</table>

Treated

(0.01 ml) K/Lac 017 540 (7 min.) 1.5 x 10³ 0.58 100
(0.01 ml) K/HeLa 027 380 0.36 x 10³ 0.12 21
(0.01 ml) K/Ide 070 269 0.2 x 10³ 0.02 3

Activation of ca \( \frac{1500}{94} = 16 \times \) fairly consistent here, but 17 hrs. may not provide maximal activation with benzene.

Lactose is present in glucose and especially in maltose-adapted cells.
Oct. 1, 1944.

**Harvest K-12**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>A.</th>
<th>B.</th>
<th>C.</th>
<th>D.</th>
<th>E.</th>
<th>F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
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<td>10</td>
<td>10</td>
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<td>5</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

---

**Note superiority of octyl alcohol activation.**

Octyl alcohol > Benzyl alcohol

**Test Oct 1: Thymol for partition of 20,000 at pH 7.5.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Octyl Alcohol</th>
<th>Thymol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octyl Alcohol</td>
<td>1.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Thymol</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Comp. 10**: Negligible change from untreated.
Research 1, 3, 4p2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B (a)</th>
<th>C (B2)</th>
<th>Reaction</th>
<th>1/2 hour test</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>318</td>
<td>131</td>
<td>200</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>306</td>
<td>544</td>
<td>054</td>
<td>054</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>082</td>
<td>069</td>
<td>067</td>
<td>067</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>073</td>
<td>053</td>
<td>061</td>
<td>107</td>
<td></td>
</tr>
</tbody>
</table>

Time may have been insufficient for complete activation! Thymol seems to act most rapidly. I try phenol, others in 0.4F.
Effect of shaking

<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a) 1ml unsh. d) 5ml sh. b) 5ml unsh. c) 1ml sh. d) 5ml sh.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (MINS)</th>
<th>Di</th>
<th>Donpy</th>
<th>318</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>089</td>
<td>023</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (HRS)</th>
<th>ABCD</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>A</td>
<td>149</td>
</tr>
<tr>
<td>30</td>
<td>B</td>
<td>518</td>
</tr>
<tr>
<td>0.5</td>
<td>C</td>
<td>460</td>
</tr>
<tr>
<td>0</td>
<td>D</td>
<td>490</td>
</tr>
</tbody>
</table>

173x2 = 346
201x2 = 402 (1)

16% > above!

2 hour optimum for unshaken cultures.

30% 540 - 840 test + compare.

Trypsol

<table>
<thead>
<tr>
<th>Time (HRS)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h</td>
<td>279</td>
<td>168</td>
<td>178</td>
<td>162</td>
</tr>
<tr>
<td>24h (over)</td>
<td>369</td>
<td>269</td>
<td>025</td>
<td>289</td>
</tr>
</tbody>
</table>

Reheat overnight.
October 5, 1949:

I) W112 harvested from Y2-Lac, Y2 Mal, K-12 lac. as above.

<table>
<thead>
<tr>
<th>Chromat. cells, 0/1ml</th>
<th>Di.</th>
<th>Don py</th>
</tr>
</thead>
<tbody>
<tr>
<td>K/L</td>
<td>131</td>
<td>7.10</td>
</tr>
<tr>
<td>W/L</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>W/H</td>
<td>124</td>
<td>-</td>
</tr>
</tbody>
</table>

2) Benzene 24 hours 0.06 5.90 (12.5 min; NaOAc)

| K/L                  | 2.61 |
| W/L                  | 0.073|
| W/H                  | 0.042|


<table>
<thead>
<tr>
<th>Intact: K/L</th>
<th>130</th>
<th>5.20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>129</td>
<td>1.51</td>
</tr>
<tr>
<td></td>
<td>204</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-0.07</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Engine: K/L</td>
<td>4.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Engine: K/L + 0.01</td>
<td>4.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>
K12 harvested from yeast - peptide (VP)/sugar. Search/10ml.

<table>
<thead>
<tr>
<th></th>
<th>Lac</th>
<th>Rph</th>
<th>Donup</th>
<th>40f</th>
<th>18f</th>
<th>16f</th>
<th>40f</th>
<th>18f</th>
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</thead>
<tbody>
<tr>
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<td>104</td>
<td>212</td>
<td>17f</td>
<td>17f</td>
<td>141</td>
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</table>

R.A. 134 2 4
R.A./Di 8 10
R.A./Lac 180 10 12

Activation 20 x 196 x 3/4. 57 x !!

u/mg 6.4 0.1 0.2

R.A. 231 8 10
R.A./Di 23 4
K nitro lgalee i1 intact cells.

Date: 7, 1949.

K12 harvested from L acY2

Kompq and K lac. Di Donpg

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Graph cells: \( y_{\text{max}} = \frac{1}{25} = \frac{400}{5} \)

\( K_{\text{ompq}} = \frac{M}{2000} = 5 \times 10^{-4} \text{ M} \)

\( K_{\text{lac}} = [\text{lac}] = 2 \times 10^{-3} \)

Note: Extracts + cells of (N) = (10^{-4})

cell \( \times 1.3 \)

\( \text{ompq} \) cell \( \times 14 \)

\( \text{lac} \) cell \( \times 14 \)

i.e., transport hole to lac

\(< \) ompq. But still note that the 1/3 ratio is not extrapolated to the full max for lac. Possibility of bending needs to be considered.
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<td>Harvest from T (m) 1/2 % sugar, K12. 24 hours.</td>
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<th>Activity</th>
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<td>10⁻⁹</td>
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<td>Activity</td>
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Lactase in non-adapted cells.

K12 bacterial strain: V2 & 4. Conc + Wash

System: 1 ml cells, 1 ml 1% NaP, 5 ml 0.1% Lactalbum, 10A - 4 - 1PM. 3 hours.
1 ml supplement (VPHH) 14.5 gr. 10 ml. Br. in tubes 37°.

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1 2 3 4 5 6 7 8 9

0-6 0-L

Assay 8PM.

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Take 3 samples of 0-6, 0-L, 3, 7 under biopsy.
YP = yeast-peptone broth. Use 1:10
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Correction: 45 + 63 - 17 = 91
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<th>4/100 m.k.</th>
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<th>1-3 ml cells (1:100)</th>
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Bacteria = 0.01. Subtract 0.040 from 1-3.
October 18, 1942.

Dissolve 3 ml of 632 suspension into a) + mpg (1-2)

b) - mpg (3-4)
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<td>- 1 ml 1:80</td>
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<th>Dil</th>
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<td>- 1 ml 1:160</td>
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Infect.allo.

\[ \frac{\text{ml}}{\text{Di}} \]

\[ \frac{\text{hml}}{24 \text{h} (6 \text{mm} \text{shuffl})} \]

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R.A. ca 370 (lacr. !)

02. Hdl. (6h.)

\[ \text{onpg} \]

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003
A + 1 lac
B + 1 Lec
C - 1 lac
D - 1 Lec

Circa. 50/10.

... and samples under benzene 11.411 - 8 PM.
for X series.

Cells

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Etn.

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<td>067</td>
</tr>
<tr>
<td>C</td>
<td>.05</td>
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<td>090</td>
</tr>
<tr>
<td>D</td>
<td>.1</td>
<td>049</td>
<td>077</td>
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</tbody>
</table>

11/23 Harvey / 12 Hac. as above. Harvest 1000 A.
Assay 1100 A
Also take aliquots for estimation.

E .1 199 35 + mino 340 ca 50

Note: activator of gal'ase is treatment with then incubating cells min.!!
Nov. 24/1979.

Harvest K-12; Bact from Y2501b 60:10.

Pour 1 ml samples immediately. Also store
1 ml samples at 37° 12 hrs.

K 198 269
B 181 209
O 003 017

Reassay 7 PM

1 K (Ref.) 204 260
2 K (Inc.) 190 277
3 K (Thymol) 159 228

4 B (Ref.) 201 297
5 B (Inc.) 154 520
6 B (Thymol) 150 670
O 003

Note great fragility in water of Zenett.
Harvest K-12 from 30 hr. unshaken cultures in Davis minimal medium (new).

Conc. 30:10.

Intact cells 0.2 ml per tube ea

<table>
<thead>
<tr>
<th></th>
<th>D_1</th>
<th>Donny</th>
<th>G:1000</th>
<th>Acet</th>
<th>A:A</th>
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<tbody>
<tr>
<td>Lac</td>
<td>044</td>
<td>521</td>
<td>0</td>
<td>0.73</td>
<td>1070</td>
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<tr>
<td>Mal</td>
<td>059</td>
<td>064</td>
<td>0</td>
<td>0.03</td>
<td></td>
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<tr>
<td>Glu</td>
<td>053</td>
<td>057</td>
<td>0</td>
<td>0.1</td>
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Extr. (Bz ttment)

<table>
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<th></th>
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<th>1960/</th>
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<tr>
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<td>.01</td>
<td>-003</td>
<td>103</td>
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<tr>
<td>Mal</td>
<td>.2</td>
<td>053</td>
<td>057</td>
<td>0.01</td>
<td>4.01</td>
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<tr>
<td>Glu</td>
<td>.4</td>
<td>108</td>
<td>097</td>
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D_acet = -(D_1 (0.9) + 0.08 + Donny)
Date: 12/4/49

Harvest K-12 from 48 hr. shaken succ or Davis minimal

Tone 50:10

Dissociated cells: 0.1 ml/tube; 24 hr. ascidicada (Bernard Starns)

<table>
<thead>
<tr>
<th>RA</th>
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<td>Ltr</td>
<td>14/1, 4/98</td>
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<tr>
<td>Mal</td>
<td>1/0, 1/80</td>
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<tr>
<td>glu</td>
<td>1/1, 1/11</td>
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<tr>
<td>suc</td>
<td>0/8, 0/91</td>
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</table>

<table>
<thead>
<tr>
<th>RA</th>
<th>29/7</th>
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</thead>
<tbody>
<tr>
<td>Ltr</td>
<td>0/0, 1/39</td>
</tr>
<tr>
<td>Mal</td>
<td>1/2, 1/10</td>
</tr>
<tr>
<td>glu</td>
<td>1/5, 0/15</td>
</tr>
<tr>
<td>suc</td>
<td>0/8, 1/40</td>
</tr>
</tbody>
</table>
I/12/49

Surface tubes of D(o) + maltose + supplements as indicated.

1. 
2. Peptone .1%
3. Peptone .5%
4. Y. Ctr. .1%
5. Bc. hyd. casein .1%
6. "" .5%
7. ++
8. 
9. Vits
10. AVA + YNA.
11. A.A. = .1% casein
12. A12
13. A3
14. A4
15. A5
16. A6
17. B12.
18. Liver etc.

Response to A12 was outstanding!

1 ml samples of culture under Aco at 2-4 hours.
Add 4 ml 1/10 NaPhosphate, ½ ml 9/200 org.
Read qualitatively after 20m.