

Lactase in non-adapted cells.

627

Oct 13 - 1949.

Harvest from T(m) 1/2% sugar. K12. 24 hours.  
Held 24h. after in H<sub>2</sub>O; benzene

Intact.

lac .1 Di 119  
Mal .1 078  
Glu .1 112

Benzene

lac .05 002  
Mal .1 075  
Glu .1 111

~~no (residual) activity!~~

Fresh  
insects

lac .1 122 <sup>107</sup> 139

v. low activity

Oct. 15, 1949.

K12 harvested from 42 tubes. 5% Conc + Wash  
 Suspension: 1ml cells, 1ml 4/10 Y.P. 5ml 4/100 loc. or glu. 10 AM - 1 PM. 3 hours.  
 1ml supplement (4P bath) 4/10 Y.P. 10ml. Inc. in tubes 37°.

	Cells	4/100 Sugar	Supp.	Di	Damp	R.A. (in- cubes)	R.A. (cup) cells.
1	L	L	-	134	511		
2	L	G	-	138	671		
3	G	L	-	134	162		
4	G	G	-	148	151		
5	"	-	YP	132	142		
6	"	G	YP	180	190		
7	"	L	YP	183	540		
8	"	L + G 4/100	YP	189	530		
9	"	L	YP	275	458		
10	0-G	(L)	-	128	128		
11	0-L	(L)	-	141	330		
continues							
8	G	L	YP	289	530		
9	G	L + G 4/100	YP	275	458		

Take samples of 0-G; 0-L; 3, 7 under benzene.

YP = yeast peptone bath. Use 1:10

Assay	8PM.	Di	Damp
3	10ml	106	120
7	1ml	008	184
10	1.0ml	080	106
11	1ml	002	160
3	1ml in 10 (10 hrs.)	117	130 <sup>13-14</sup>

of 1:10  
of 10 tubes

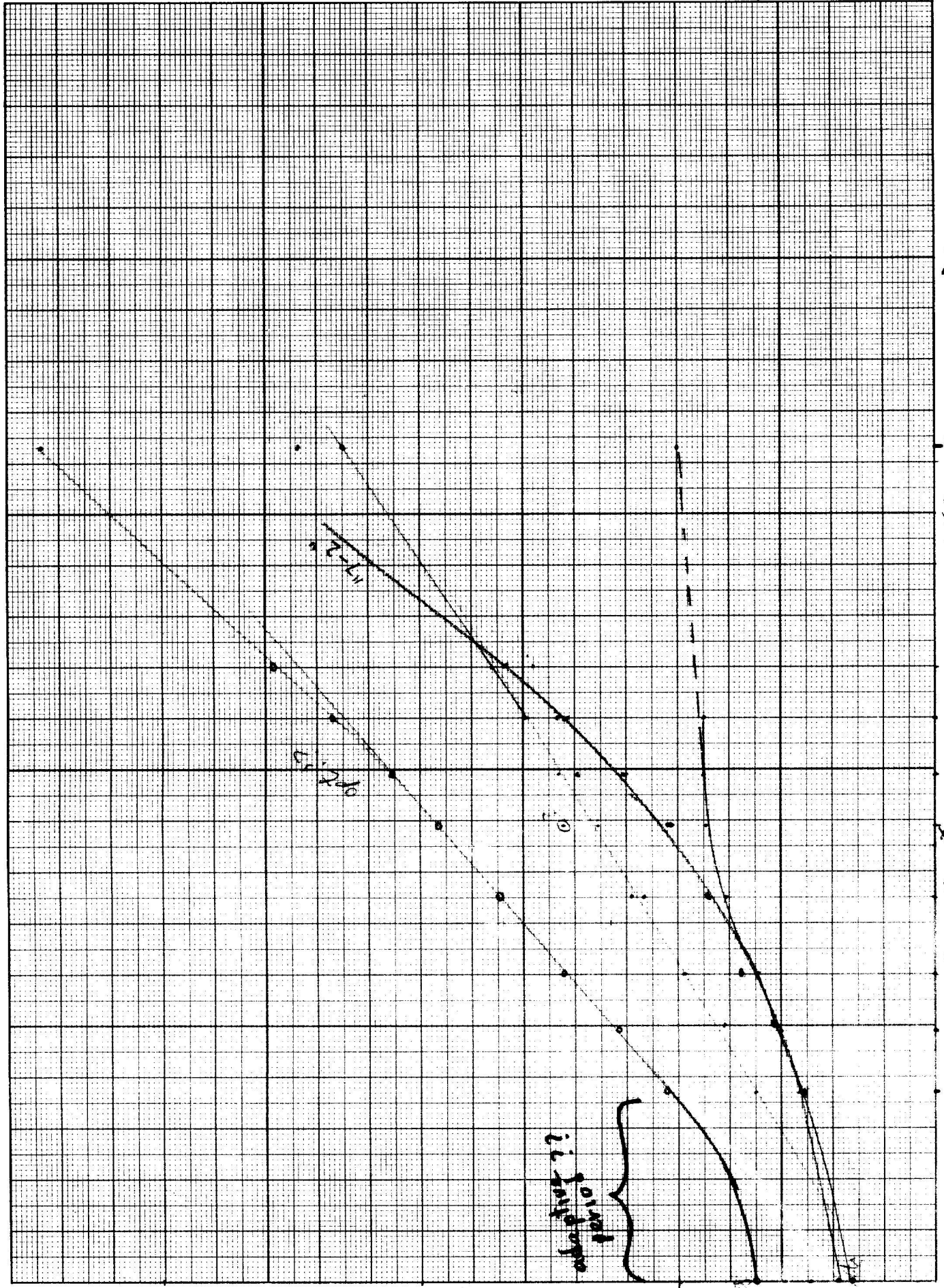
# Adaptation to onpg

628

		0	37	49						
	D <sub>i</sub>	↓ 10 <sup>47</sup>	11 <sup>24</sup>	11 <sup>36</sup>	11 <sup>47</sup>	12 <sup>02</sup>	12 <sup>12</sup>	12 <sup>16</sup>	12 <sup>26</sup>	12 <sup>37</sup>
1	019	040	040	042		038				
2	031	038	053	060	069	081	<del>083</del>	089	90	090
3	162	180	183	183	187	189				
4	006	032	031	030		032				
5	002	018	019	018		019				
6	005	009	012	012		013				
7	042	070	104	123	144	169	<del>180</del>	193	211	234
8	034	049	070	082	097	118	<del>123</del>	144	146	159
9	032	042	068	078	090	113	110	120	130	136
10	036	050	072	083	100	113	119	131	139	147
		10 <sup>40</sup>				↑				
					AZIDE M/100					
7-2		32	51	63	75	15 <sup>52</sup>	88	104	121	144

These cultures left in water bath to prewarm etc, but is only for 20-30 mins for temp equilibration. Cells should be added last.

	Cells	Supp.	ONPG N/			Δ
1	0	YP	1000	1247	1 <sup>30</sup>	10 <sup>40</sup> - 1 <sup>30</sup>
2	1	YP	0	090	040	0
3	1 (10x)	YP	0	0	100	62
4	1	-	1000		200	20
5	1	-	1000		034	02
6	1	-	4000		019	01
7	1	YP	1000	257	011	02
8	1	YP	1000	172	347	277
9	1	YP	4000	146	230	189
10	1	YP	2000	156	183	141
7-2				167	247	



Time in minutes

100 200 300 400 500 600 700 800 900 1000

is not a straight line

100

17.2

100

300

200

100

100

200

300

400

500

600

700

800

900

1000

October 17, 1949

Expt. B Use 2 x cells. Add cells at 7(0)

215  
Cells  
added

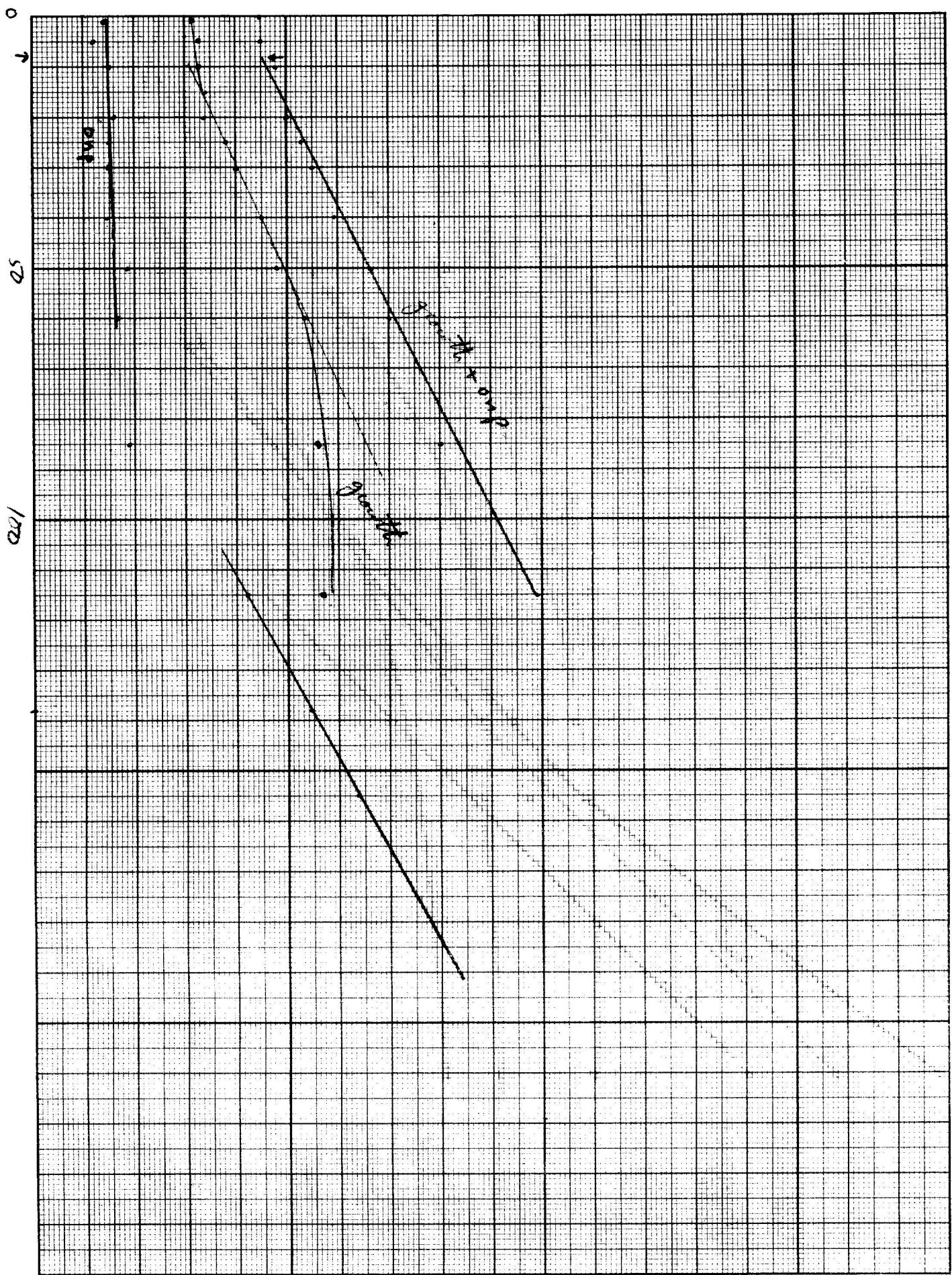
	Do	2 <sup>26</sup> M	2 <sup>20</sup> (5)	2 <sup>25</sup> (10)	2 <sup>35</sup> (20)	2 <sup>40</sup> (25)	2 <sup>45</sup> (30)	2 <sup>55</sup> (40)	3 <sup>05</sup> (50)	3 <sup>15</sup> (60)
1	050	091	090	096	100	106	110	119	133	140
2	049	090	090	094	101	106	109	121	133	145
3	047	093	093	097	100	107	110	121	132	140
4	051	094	095	096	092	092	096	097	097	100
5	017	063	066	066	068	076	080	090	096	107
1-5		0	-1	5	9	15	19	28	42	49
		0	3	3	5	13	17	27	33	44
		28	24	30	32	30	30	29	37	33

Correction: - . 1 x 50 (leave 45 + (63-17) 46 = 91

	<del>230</del>	3 <sup>20</sup> (75)	4 <sup>10</sup> (115)	4 <sup>30</sup> (130)	4 <sup>50</sup> (155)
1		150	198	229	253
2		150	191	231	260
3		157	190		250
4		102	121		131
5		112	114	120	126
1-5		38	84	109	127

no delay at 1?





630

6

Adaptation kinetics: cell density effect.

October 18, 1949.

	10 ml volumetric tube.	1 ml Y2.			1-3 ml cells (1:100, 5x from Y2 plus, K-12)			
	N.P.	a) conpg		b) conpg				
	$\frac{D_i}{A_{45}}$	$\frac{10^{05}}$	$\frac{10^{20}}$	$\frac{10^{35}}$	$\frac{10^{50}}$	$\frac{12^{05}}$	$\frac{12^{20}}$	$\frac{115}{260}$
growth + conp.	1a 080	083	090	99	110	180	200	260
	2a 090	098	110	124	139	215	225	288
	3a 103	112	129	146	157	240	257	327
growth	1 050	053	063	070	080	109	115	120
	2 060	069	080	095	106	128	128	131
	3 078	090	106	121	126	146	149	149
	0	20	35	50	65	140	155	210
A = onp	1 30	30	27	29	30	71	85	140
	2 30	29	36	29	33	87	97	157
	3 25	22	23	25	31	94	108	178

Barium: = 010. Subtract 040 from 1-3

October 18, 1949.

Inoculate 3ml of 632 suspension  
into a) + mpg (1-2)  
b) - mpg (3-4)

1  
2  
3  
4



Galactosidase in Sanett

November 21, 1949.

Cells.			Di	Dampg
+	1ml	1:100	034	134
-	1ml	1:80	030	177
Thymol treated				
+	1ml	1:500	-004	027
-	1ml	1:100	-008	156

54/1  
K-12 X

Intact cells.		$\frac{9 \text{ ml}}{\text{Di}}$	$\frac{11 \text{ ml}}{20 \text{ M. (Gumm stroffer)}}$ Dongg	R.A.
.05	Lac	120	370	ca 370 (Low!)
.1	Mal	120	100	
.1	blu	147	118	
.1	Suc	176	158	

b2.Hd.(6h.)

.005	Lac	011	013	
.01	Mal	012	013	
.1	blu	183	80	
.05	Suc	080	067	-vis.
-	on pg	-009	003	

A + / Lac  
 B + /  $\beta$  gal  
 C - / Lac  
 D - /  $\beta$  gal

Case. 50/10.

1 ml. samples under benzene 11 AM - 8 PM.  
 for X series.

cells.

		D <sub>i</sub>	D <sub>omp</sub>
A	.02	020	379
B	.1	089	072
C	.05	060	481
D	.1	090	076

Extr.

A	.005	-010	447
B	.1	050	064
C	.05	059	690
D	.1	049	047

11/23 Part + / 42 Mal.

as above. Harvest 10<sup>30</sup> AM.

Assay 11<sup>30</sup> AM.

Also take aliquots for "activation"

E	.1	199	35+ minio 340	R.A ca 50
---	----	-----	------------------	--------------

	D <sub>i</sub>	D <sub>f</sub>	Assay 4 PM Δ <sub>minors</sub>
A	207	303	96
B	114	460	346
C	123	520	397
D	122	530	408

1 ml samples:

Note activation of gal'ase is treatment other than incubating cells in water!!

Compare clarity of K-12; Dant +.

643

Nov. 24/1949.

Harvest K-12; Dant from 42 Mel 50:10.

Doxy. 1ml samples immediately. Also store

1ml samples at 37° 12 hr

	Di	Doxy.
K	198	269
S.	<del>181</del> 181	309
O	-003	047

Reassay 7<sup>30</sup> 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 (Thym.)	150	670
O	003	

Note greater fragility in water of Dant.

12/8/49

Harvest K-12 from 30 hr. unshaken cultures in Davis minimal medium (new).

Conc. 50:10.

Intact cells 0.2 ml per tube ea

	$D_i$	$D_{onpg}$	$\Delta_{cor}$	R.A.
Lac	044	521	473	1070
Mal	059	064	003	—
Glu	053	057	001	—

R.A.

Extr. (Bz tment)

Lac	.01	-003	103	098	1960/
Mal	.2	053	057	001	
Glu	.4	108	097	-008	

"Activation"  
4.1

0	-004	004	008
---	------	-----	-----

$$\Delta_{cor} = -(D_i)(0.9) + 008 + D_{onpg}$$

Dactosidase: cells from synthetic medium 648

12/8/49

Harvest K-12 from 48hr. Shaken succ } Davis minimal  
 Cone 50:10 24hr. " sugar }

Start cells: 0.1ml / tube ; Extr. acidified (Benzene 5 hours)

	RA		u/mg
Lac	141	498	12.0
Mal	101	100	0
Glu	111	111	0
Suc	084	091	0.4

Lac	005	-001	439	20x	297	
Mal	.2	127	140		<del>0.8</del>	0.3
Glu	.2	142	155		<del>0.8</del>	0.3
Suc	.2	086	144		1.7	



Conditions of adaptation

048a.

12/12/49

Prepare tubes of D(0) + maltose + supplements as indicated

1. —	—	—	} all +++
2. Peptone .1%	+	A12	
3. Peptone .5%	++	-Cy	} Superadaptation?
✓ 4. Y. Cstr. .1%	++	-M	
✓ 5. Ac. hyd. casein .1%	±	-ly	
✓ 6. " " .5%	±	-Ar	
<del>7. YNA</del>		7 Cy	
<del>8. YNA</del>		8 Ar	
✓ 9. Vits	—	9 M	
✓ 10. YNA + YNA.	—	10 ly	
✓ 11. AA. = .1% Casein	+	11 S <sub>2</sub> O <sub>3</sub>	
✓ 12. A12	+++		
✓ 13. A3	—		
✓ 14. A4	+		
✓ 15. A5	—		
✓ 16. A6	+		
17. B12	—		
(after aut.) 18. Live Cstr. sterile filtered.			

Response to A12 was outstanding!

1 ml samples of culture under O<sub>2</sub> at 2-4 hours.  
add 4 ml 1/10 NaP buffer, 1/2 ml 1/2000 orgg.  
read qualitatively after 20m.