Lactase in non-adapted cells.

Oct 13 - 1949.

Harvest from T(m) ½ % sugar. K12. 24 hours.

Held 24h. after in H_2O; benzene

		Di		
Intact.	Lac .1	119		
	Mal .1	078		
	Glu .1	112		
Benzene	Lac .005	002		
	Mal .1	075		
	Glu .1	111		
Fresh intact	Lac .1	122	107 139	v. low activity

no (viscous) activity!

Washed cell adaptation

Oct. 15, 1949.

K12 harvested from aer. Y2 Glu. 5x. Conc + Wash

System: 1 ml cells, 1 ml M/10 NaP. 5 ml M/100 lac or glu. 10 AM – 1 PM. 3 hours.
1 ml supplement (YP broth) H_2O qs. 10 ml. Inc. in tubes 37°.

	Cells	Sugar M/200	Supp.	Di	D_{Dnpg}^{20}	R.A. (in.) cultur	R.A. (cen.) cells.
1	L	L	–	134	511		
2	L	G	–	138	671		
3	S	L	–	134	162		
4	S	G	–	~~148~~ 150	151		
5	"	–	YP	132	142		
6	"	S	YP	180	190		
7	"	L	YP	183	540		
8	"	aer! L+G M/500	YP	189	530		
9	"	L	YP	275	458		
10 O-G	G	(L)	–	128	128		
11 O-L	L	(L)	–	141	330		

conditions

8	S	L	YP	189	530
9	S	L+G aer.	YP	275	458

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

YP = yeast peptone broth. Use 1:10

Assay 8PM.

		Di	Dnpg
3	1.0 ml	106	120
7	.1 ml	008	184
10	1.0 ml	080	106
11	.1 ml	002	660
3	1 ml inact (10 hrs.)	147	130 13-14

of 1:10 tubes

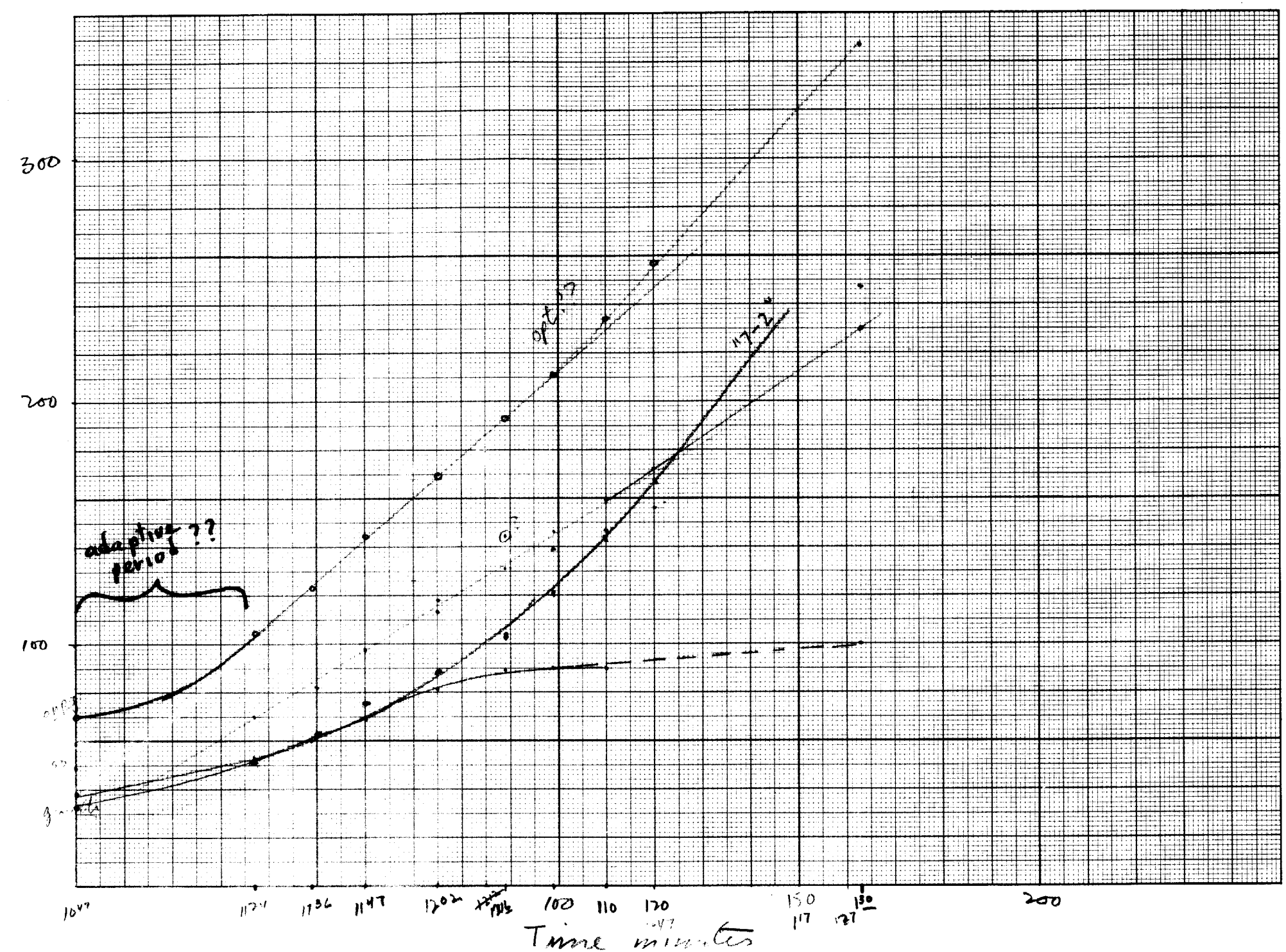
Adaptation to onpg

		0	37	49						
	Di	10^{47}	11^{24}	11^{36}	11^{47}	12^{02}	12^{12}	12^{16}	12^{26}	12^{37}
1	019	040	040	042		038				
2	031	038	053	060	069	081	~~083~~	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	~~180~~	193	211	234
8	034	049	070	082	097	118	~~123~~	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^{40}								
						AZIDE M/100				
7-2		32	51	63	75	11^{52} 88		104	121	144

These cells were left in water bath c̄ peptone etc, but is onpg for 20-30 mins for temp equilibration. Cells should be added last.

	Cells	Supp.	ONPG M/	12^{47}	1^{30}	Δ $10^{47}-1^{30}$
1	0	YP	1000		040	0
2	1	YP	0	090	100	62
3	1 (10x)	YP	0	0	200	20
4	1	–	1000		034	02
5	1	–	2000		019	01
6	1	–	4000		011	02
7	1	YP	1000	257	347	277
8	1	YP	2000	172	230	181
9	1	YP	4000	146	183	141
10	1	YP	2000	156		
			7-2	167	247	

NO. 340 -M DIETZGEN GRAPH PAPER
MILLIMETER
EUGENE DIETZGEN CO.
PRINTED IN U. S. A.



October 17, 1949

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

2^{15} Cells added

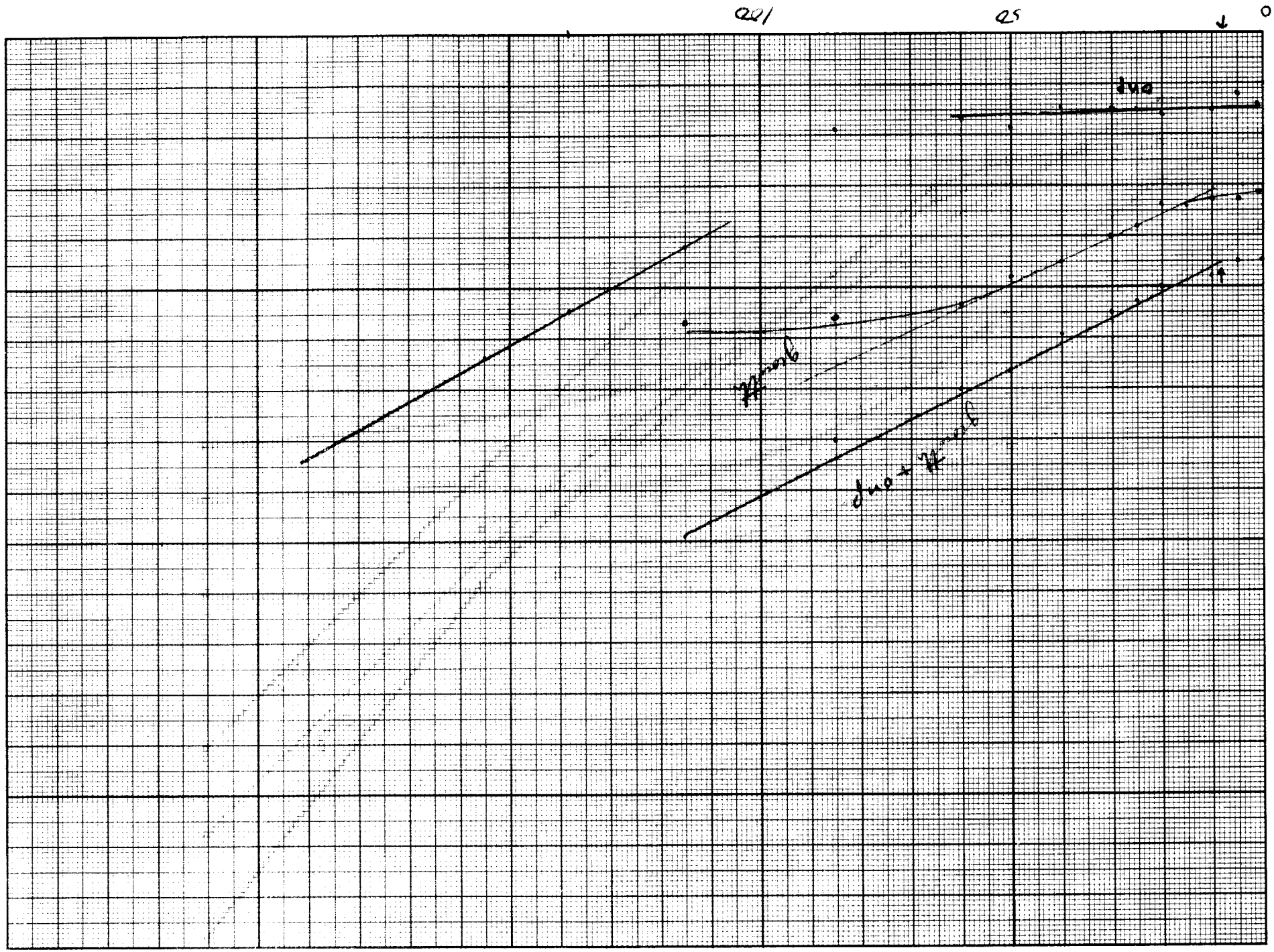
	Do	2^{16} 1 M	2^{20} (5)	2^{25} (10)	2^{35} (20)	2^{40} (25)	2^{45} (30)	2^{55} (40)	3^{05} (50)	3^{15} (60)
1	050	091	090	096	100	106	110	119	133	140
2	049	090	090	094	100	106	109	121	133	145
3	047	093	093	097	100	107	110	121	132	140
4	051	094	095	096	092	092	096	097	097	100
5	017	063	066	066	068	076	080	090	096	107
1		0	-1	5	9	15	19	28	42	49
5		0	3	3	5	13	17	27	33	44
1-5		28	24	30	32	30	30	29	37	33

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

		3^{30} (75)	4^{10} (115)	4^{33} (135)	4^{50} (155)
1	~~3^{30}~~	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 adapt!?

NO 340 -M DIETZGEN GRAPH PAPER
MILLIMETER
EUGENE DIETZGEN CO.
PRINTED IN U. S. A.



Adaptation kinetics: cell density effect.

October 18, 1949.

10 ml colorimeter tube. 1 ml Y2. 1-3 ml cells (1:100, 5 x from Y2 glu, K-12)

M/100 NaP. M/1000 onpg. a) $\dot{c}$ onpg b) $\dot{s}$ onpg

		Di 9^{45}	10^{05}	10^{20}	10^{35}	10^{50}	12^{05}	12^{20}	1^{15}
growth + onp.	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

Bacteria$_i$ = 010. Subtract 040 from 1-3

Adaptation kinetics: cellage effect

October 18, 1949.

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

b - mpg (3-4)

1
2
3
4

Galactosidase in Garrett

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

641

K-12 X

		9 ml Di	11 ml 20M. (6 min stroffer) Donpg		R.A.	
Intact cells.						
.05	Lac	120	370		ca 370	(Low!)
.1	Mal	120	100			
.1	Glu	147	118			
.1	Suc	176	158			
B2. Hd. (6h.)						
.005	Lac	011	013			
.01	Mal	012	013			
.1	Glu	183	80			
.05	Suc	080	067	✓vis.		
—	onpg	-009	003			

Gal'ase in Garrett

642

A + / Lac
B + / Glu
C − / Lac
D − / Glu

Carc. 50/10.

1 ml. samples under benzene 11 AM − 8 PM. for x series.

Cells.

		Di	Donpg
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 Garr + / 42 Mal. as above. Harvest 10^{30} AM.
Assay 11^{30} AM. Also take aliquots for "activation"

			35+ mins	R.A
E	.1	199	340	ca 50

Assay 4 PM .1 ml samples:

	Di	Df	Δ uncor
A	207	303	96
B	114	460	346
C	123	520	397
D	122	530	408

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

Compare stability of K-12; Garrett +.

Nov. 24/1949.

Harvest K-12; Gar + from 42 Mal 50 : 10.

Assay 1 ml samples immediately. Also store

1 ml samples at 37° 12 15 to

	Di	Donpg.
K	198	269
G.	~~159~~ 181	309
O	-003	047

Reassay 7 50 PM

1	K (Refr.)	204	260
2	K (inc.)	190	277
3	K (thymol)	159	228
4	G (Refr)	201	297
5	G (inc)	154	520
6	G (thym.	150	670
	O	003	

Note greater fragility in water of Garrett.

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}		Δ_{cor}	R.A.	R.A.'
Lac	044	5210		473	1070	
Mal	059	064		003	—	
Glu	053	057		001	—	

Extr. (Bz ttment)

							"Activation"
Lac	.01	-003	103		098	1960/	4.1
Mal	.2	053	057		001		
Glu	.4	108	097		−008	—	
0		-004	004	008			

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

Galactosidase: cells from synthetic medium 648

12/9/49

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

Conc 50:10

Intact cells: 0.1 ml / tube; Extr. as indicated (Benzyme 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		~~0.5~~	0.3
Glu .2	142	155		~~0.8~~	0.3
Suc .2	086	144		1.7	

Conditions of adaptation

12/12/41

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

1. —	—		—	} all +++
2. Peptone .1%	+		A12	
3. Peptone .5%	++		-Cy	
✓ 4. Y. Extr. .1%	++		-M	Supraadaptation ?
✓ 5. Ac. hyd. casein .1%	±		-Hy	
✓ 6. " " .5%	+±		-Ar	
~~7. NAA~~		7	Cy	
~~8. CAA~~		8	Ar	
✓ 9. Vits	—	9	M	
✓ 10. RNA + YNA.	—	10	Hy	
✓ 11. AA. ≐ .1% Casein	+	11	$S_2O_3^=$	
✓ 12. A12	+++			
— 13 A3	—			
✓ 14 A4	+			
✓ 15 A5	—			
✓ 16 A6	+			
17 B12	—			
(after aut.) 18 Liver Extr. sterile filtered.				

Response to A12 was outstanding !

1 ml samples of culture under OcOH 2-4 hours.
Add 4 ml M/10 NaPbuffer; ½ ml M/200 orpg.
Read qualitatively after 20 m.