Lactose adaptation: conditions
cell concentration.

~~Sept~~ Oct. 1, 1948.

Harvest cells of K-12 from 50 ml T(0) grown overnight ~ shaking, to 10 ml ~~1/50 Phosphate Buffer (PB) 7.5~~ T(0)-Sugar.

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

		Cells.	4(-)	D_{420}	D_{650}
A.	1.	.5 ml	3.5	244	095
	2.	1 ml	3	233	090
	3.	2 ml	2	218	103
	4.	3 (2.9) ml	1	201	100
B.	5	.5 ml	3.5	601	133
	6.	1 ml	3	582	128
	7.	2 ml	2	426	113.

Susp .1/10 ml D_{420} 078

Resuspend, after 3 hours, in 5 ml H_2O, except for 1 & 5, in 2.5 ml. To read activity at cell densities of ca .150, i.e. 1:50 dilutions of the original suspension, use in each colorimeter tube 1 ml of 1, 2, 5, & 6, directly, and 1:2 & 1:3 dilutions, respectively of the others.

Note) a. somewhat more rapid adaptation in dilute suspensions

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

Index: Phosphate assay. 321

Oct. 4, 1918.

2 ml 219A + 2 ml 10% TCA. Remove sediment. Assay in indicated aliquots against 10^{-4} - 10^{-3} Phosph. sulfuric standards. Extreme of original 219A. ~~Also~~ assay 1 ml of ~~1:500 dilution~~ of 219A in M/40 Na bacitzt buffer. Nabarbital developed no visible color.

		D_{660}
H/10^{-4}P	x 10	670
	x 3	230
	x 1	091
	0	040 particles
219A.	.5 ml	1170
	.1 ml	274
	.01 ml.	053 vis. < 10^{-4} Phosph.

Visually, .1 ml 219A corresponded to ca. 3×10^{-4} M Phosphate, i.e., 219A assays ca 3×10^{-3} M Phosphate. At 1:500 and 1:1000 dilutions, therefore, 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 sensibly absent and therefore unnecessary.

10 ml 219A dialyzed 4 hours against distilled water. Final volume, 13 ml. = 219C. Impair activity + response to Na. Expresses at 1:1000

	D_{470}.	Enzyme.	$NaCl$
1	095	C	0
2	140	C	N/50
3	171		
4	219		N/1000
5	277		N/100
6	290		N/50
7	178		N/10,000.

Opt. effect of NaCl at N/50 or above; ~~Activity~~ at N/1000 or below!

Lactase kinetics.

Oct 4, 1948.

Septum contain .001 + .005 ml 319A and 1, 5 ml M/200 ONPG
+ 1ml K_2HPO_4 buffer + 1ml N/50 Na_2SO_4 in 10 ml.

37°.

	E	S.
A.	.001	1
B	.001	5
C	.005	1
D.	.005	5.

Separated T	Times T.	A	B	C	D.
	0	004 ✓ .001		009 ✓ .007	
	1:20			069 200 154	
	3:30	048			
	4:00		083		
	4:30			225	
	5:00				320
	5:30	069			
	6:00		102		
	6:30			326	
	7:00				411
	7:30	089			
	8:00		128		
	:30			409	
	9:00				503
	9:30	110			
	10:00		148		
	:30			491	
	11:00				589
	1:30	130			
	12—		170		
	12 30			563	
	13—				670
	13 30	150	~~16~~		
	14—		191		
	14 30			640	
	15—				750
	15 30	172			
	16—		213		
	16 30			710	
	17—				815
	17 30	195			
	18—		238		
	18 30			780	
	19—				870
	19 30	212			

Mins+Secs.	A	B	C	D
+11 20		258		
30			825	
4				920
11 30	236			
12		280		
13			860	
	258			955
14		300		
			905	
15				995
	277.			
16		320		
			940	
17				1005
	298			
18		341		
			955	
29	~~316~~			1045
	316			
30		363		
			980	
31				1050
	334			
32		381		
			1000	1060
33				1060
	351			
34.		400		
			1000	
35				—
	370			
36		420		
			1030	
37				1080
	389			
38		440		
			1045	
39				—
	404			—
40		459		
41				1095
	421			
42		473		
			1050	
43	~~438~~			—
	438	~~490~~		
44		490		
			-	-
45				

	A	B	C	D
~~45~~				1100
	451			
~~46~~		509		
			1050	
53. ~~47~~				1095
~~38~~				

↓

~~49~~ 30				
~~53~~		560		
~~54.~~			1050	
				1100
~~55.~~ 30	520			
~~55.~~		579		
~~56~~	530			
~~57~~		+ 590		
~~58~~	541			
		~~609~~		
~~59~~		609		
60	560			
61.		? 611		
65		652		
	600.			
67				
69				
70		683		
71	630			
~~79~~		740.		
~~83.~~	700			
∅.	1145	1250	1145	1250.
1:10	209	1:11 690	213	810.

evaporation may have intruded overnight.

Oct 5, 1948.

49 g. Stiff Shaplee paste K-12 harvested from 2 carboys D (Lac).

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

B. 17g. suspended in M/10 $NaPO_4$ buffer pH 7.5, shaken under toluene.

C. 30g. " " . Ground in Booth Green Mill 1 hour.

Remove debris + make to 100 ml. volume.

A6 Remove debris. Left a opalescent yellow green solution, 17 ml.

Assays. (in M/50 Na. Phosphate). ONPG M/2000. 20 m. 37°

D_i 420.

329. 1. B .01
2 .001
3. C .01
4. .001

Inhibition by serum.

319 A. 5. —
.002 ml. 6. + 476F41:10
7. + 471F51:10
−enz. 8. + 476F41:10
−enz. 9. + 471F51:10.

730
(341
430
540

780
599
710
023

Note high activity here. Probably due to use of the buffer.

Throwing "C"

(B): 45 units/ml (C) 820 u/ml?

throw out!

lactase activation and inhibition
Micro assays

(1 ml Suppl.) M Na P buffer.

				D_{420}
	1.	329B $^{-2}$		45.3
	2.	" B $^{-3}$		075
329A ~~10~~	3.	329 C	—	082
	4.		NaCl M/50	081
10^{-3}	5.		KCl M/50	092
	6.		LiCl M/50	078
	~~7~~		~~$Et(NH_2)_2$~~	

~~8~~ ~~9~~ M KPO_4

.001	7.	319 A	—	150
	8	Et DiNH$_2$	M	017
	9		M/10	022
	10		M/50	049
	11		M/100	082

Na P. ~~12~~ ~~13~~

Repeat Assays of C!

		Buffer	Amt			
329C	1	Na	.001			116
	2.	Na	"			114
319A.	3	K	—			167
.001	4	K	EDA M/100			128
	5	K	" " + Na_2SO_4 M/50			260
	6	K	Na_2SO_4 M/50.			290
↓	7	Na	—			329
	8	"	NaF	M/10	1 ml	006 !
	9	"	$CuSO_4$	M/10	.1 ml	− 119
	10	"	$HgCl_2$	M/10	.1 ml	− 412
	11		IAcONa	M/20	1 ml	266
	12.		"	M/20	.5 ml	286
	13.		"	"	.1 ml	335.

Twice D_i = 129
D_i = 420

} v. sl. inhibition

may be protected from inhibition by sugar too

Mechanism of fluoride inhibition
+ & Km.

Oct. 5, 1948.

P5. .001 ml 319A + indicated suppl. + M/2000 ONPG + M/50 ~~N~~ buffer. (total) @ pH 7.5

		Buffer.	Duzo
1.	—	NaP	290
2.	NaF M/100	NaP	019
3.	$Na_4P_2O_7$ M/50	—	042
4.	"	NaP	039
5.	NaBarb ⇌		230
6.	NaF M/~~100~~ 1000	NaBarb	222
7.	NaF M/500	NaP	183
8.	M/1000	NaP	291
9.	M $\times 10^{-4}$	NaP.	310.

	ONPG $\times$ M/2000	in NaP.		
10	0.1	091	258	
11	0.5	210	920	
12	$\times$1.0	254	1110	

α time

(8.5×10^{-5}).

K_m may be estimated in the neighborhood of $5 \times 10^{-5} - 10^{-4}$

Linearity needs to be shown. Conc. of ONPG from 2×10^{-5} to 2×10^{-4} must be explored.

∴ fluoride inhibits only in presence of phosphate. M/500 needed for substantial inhibition. (Mg effect?)

Lactase. Mechanism of fluoride inhibition

Requirement for Mg^{++}? Km.

Oct 7, 1948.

319A .001 ml in M/500 NaP buffer.

	Suppl.			
1.	—	019 !	ONPG M/2000	
2.	NaF M/100	013		
3	" M/500	180		
4	" $MgSO_4$ M/500	132		Note aggravation.
5	" M/100 $MnCl_2$ M/200			
6	" M/500 " "			
7	— " "			
8.	— $MgSO_4$ M/200.	251	Normal stimulation !	

.001 ml in M/50 NaP buffer. Vary amounts of M/2000 ONPG added.

Measure q. 5 min.

	ONPG.	5m	10m	15m	20m.	0	30	$K_m (\times 10^{-5})$
	11	1						
ng	12	1.5	038	065	094	123	178	8.3
	13	2	049	079	111	149	210	7
	14	5	077	124	173	201	323	5
	15	10		141		262	381	—

↑ 094
90 slope

These data show a substantially linear decomposition of the galactoside in the interval studied, but taking V_{15} as V_{max}, we can calculate the K_m indicated! Could this be due to the presence of an inhibitor in the system which is displaced by the galactoside (lactose?)

There is an insufficient discrepancy between 11,12 and 14,15 i.e. the former are too high, or the latter too low.

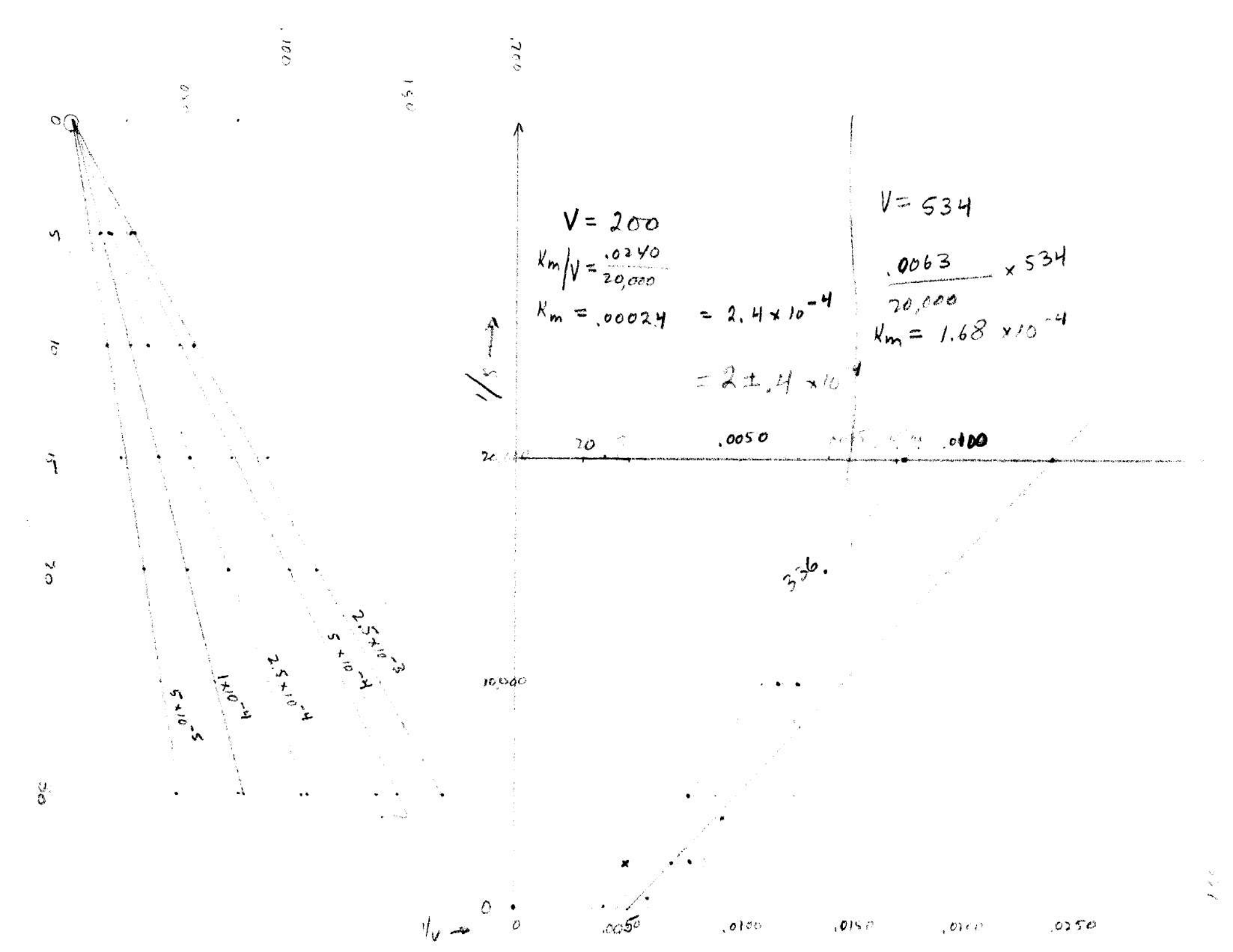
Careful extrapolation gives

K_m =

Last 20 min. data

	V	1/V	1/S
①			
②	121	.0003	13,300
③	141	.00010	10,000
④	212	.00011	4,000
⑤	215	.00011	2,000

$\bar{V}$ = 315 ± 32


V = 200
$K_m/V = \frac{.0240}{20,000}$
$K_m = .00024 = 2.4 \times 10^{-4}$
$= 2 \pm .4 \times 10^{-4}$
V = 534
$\frac{.0063}{20,000} \times 534$
$K_m = 1.68 \times 10^{-4}$
1/S
1/V
.0050
.0100
10,000
356.
5×10^{-5}
1×10^{-4}
2.5×10^{-4}
5×10^{-4}
2.5×10^{-3}

In 3 determinations, Km was 1.4
1.5
1.18 $\times 10^{-4}$.

$\frac{4.1}{3} = 1.4 \times 10^{-4}$

K_m o-nitrophenyl galactoside
K-12 Lactose.

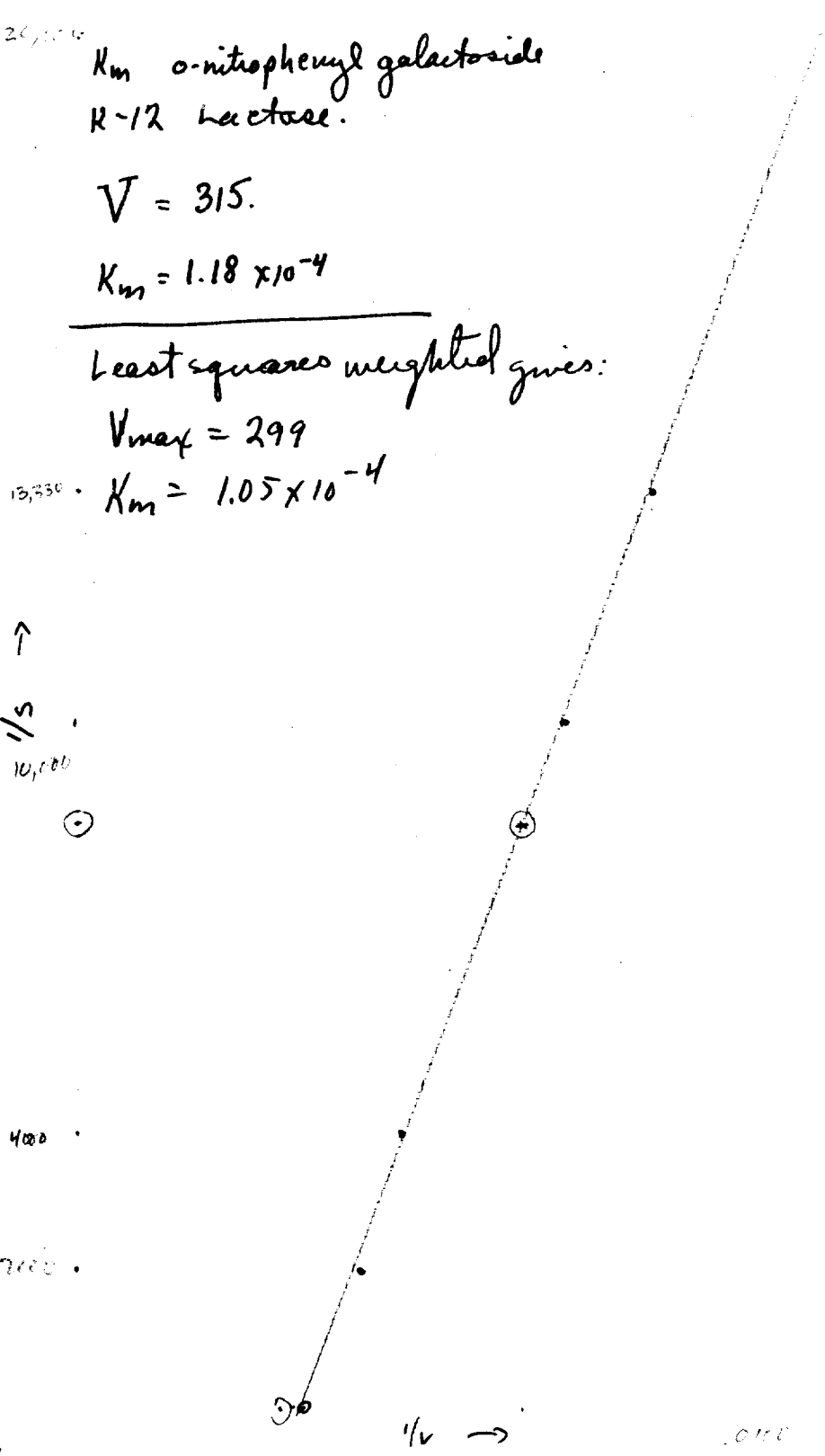
$V = 315.$

$K_m = 1.18 \times 10^{-4}$

Least squares weighted gives:

$V_{max} = 299$

$K_m = 1.05 \times 10^{-4}$



Analysis of 3rd data by weighted least squares

3/29/49

T	V	V^3	V^4	$V^3 T$	T^2	$V^4 T^2$	$V^4 T$
13.30	1.21	1.77	2.14	23	176.9	378.57	28.46
10.00	1.42	2.86	4.07	29	100	407.00	40.70
4.00	2.12	9.53	20.20	38	16	323.20	80.80
2.00	2.45	14.70	36.03	29	4	144.12	72.06
Σ		28.81	62.44	119.66		1252.89	222.02

$r = \frac{222.02}{62.44} = 3.56$ $\quad 2r = 7.11$

$r^2 = 12.64$

$a = \frac{28.87}{62.44} = .462$

$$b = \frac{119.66 - 3.56(28.87)}{1252.89 - 7.11(222.02) + 12.64(62.44)}$$

(102.77; 1578.56; 789.24)

$$= \frac{16.89}{480.46}$$

$b \doteq .035 = \frac{K_s}{V_{max}}$

$V_{max} = a - br = .462 - .128$

$1/V = .334$ $\quad V_{max} = 2.99$ $\quad K_s = (.035)(2.99)$

$= .105$

10
5
2
1.5
0
5
10
15
20
30

Kinetics ; $Mg^{++} + F^-$ 534

Oct 8 1948.
.001 ml 319A / Wood in colorimeter tube. in M/50 NaP buffer.

(1). Time series ± substrate deletion. D_2O.

ONPG x M/10,000.

		to	5M	10M	15M	20M	30M
0	50	051	080	10[illegible]	1[illegible]0	162	219
1	10	011	0[illegible]7	0[illegible]0	0[illegible]7	110	149
2	5	[illegible]09	027	[illegible]0[illegible]	06[illegible]	[illegible]71	114
3	2	000	017	027	0[illegible]	053	
4	1	−003	01[illegible]	014	[illegible]	031	046

(2). in M/100 NaP buffer.

	Suppl.	D.
1	—	155
2	NaF M/100	013
3	NaF M/500	035
4	" + $MgSO_4$ M/200	017
5	"	164

Corrected values of (1).

	to	5	10	15	20	30	V_{max}	1/v	1/s
50	—	029	055	089	111	168	168	.00595	400
10	—	026	049	073	099	138	147	.00704	2000
5	—	018	035	054	072	105	107	.00935	4000
2	—	017	027	040	053	76	79	.01265	10000
1	—	013	017	028	034	049	49	.02040	20000

Km is estimated at 2.4×10^{-4}

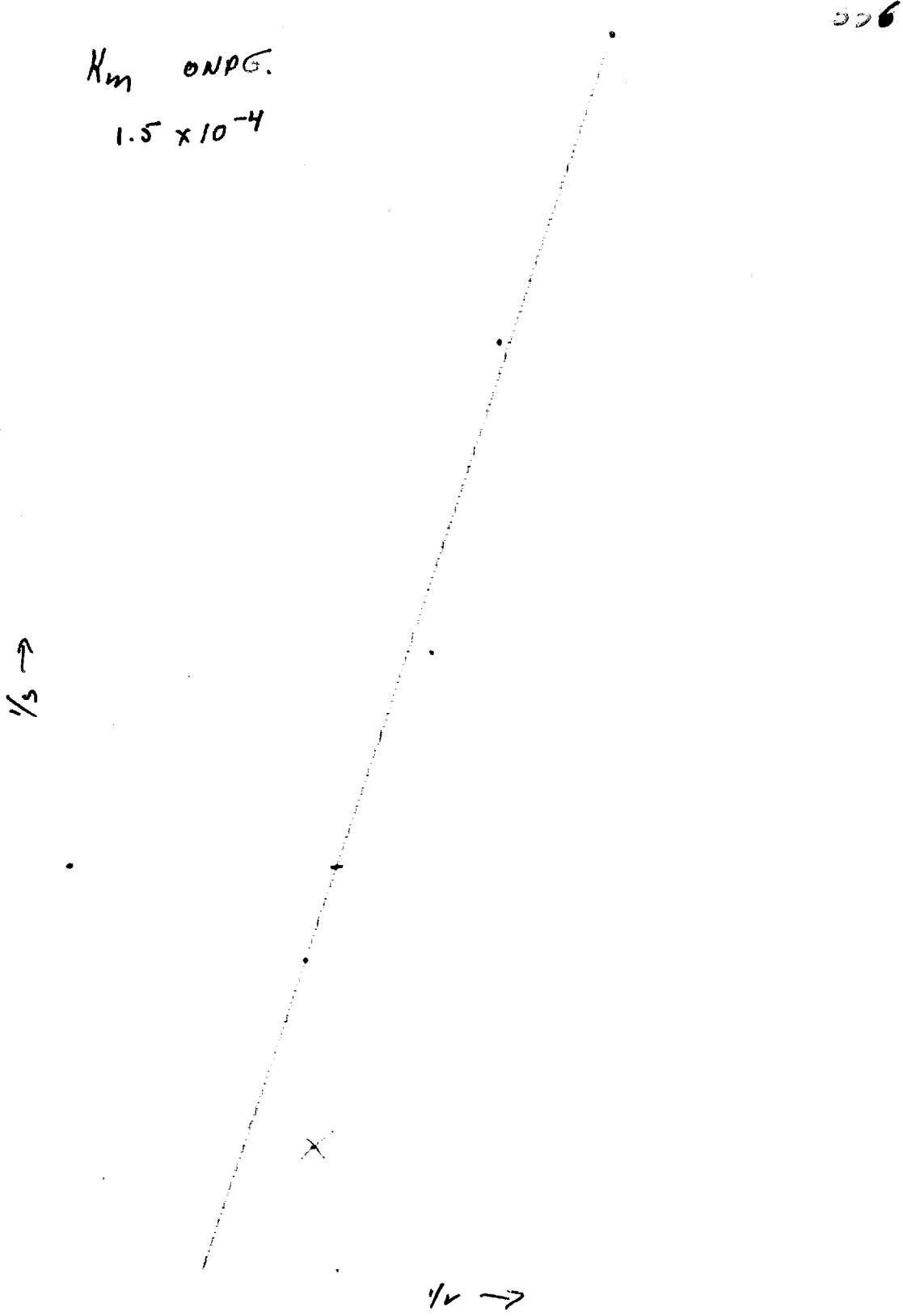
V at 200/30m.

Points should be distributed as: 1, 1.4, 2,

[illegible] 89.

K_m ONPG.

1.5×10^{-4}



Kinetics; metal inhibition

Oct 9, 1948.

① In M/50 NaP buffer. Read after 20 mins. only. .0015 ml 3/9A.

	ml M/20000	D_0^{V20}	D_{10}^{V20}		α	1/s	1/v
1.	1.00	000	115	115		20000	.0001
2.	1.33	002	146	144		15000	0001
3.	2.00	001	180	179		10,000	0038
4.	4.00	001	272	271		5000	0035
5.	10.00.	026	281	255		2000	0039

Visible discrepancy / inactivity = 3/34.

② In M/100 NaP buffer. + M/50 salts.

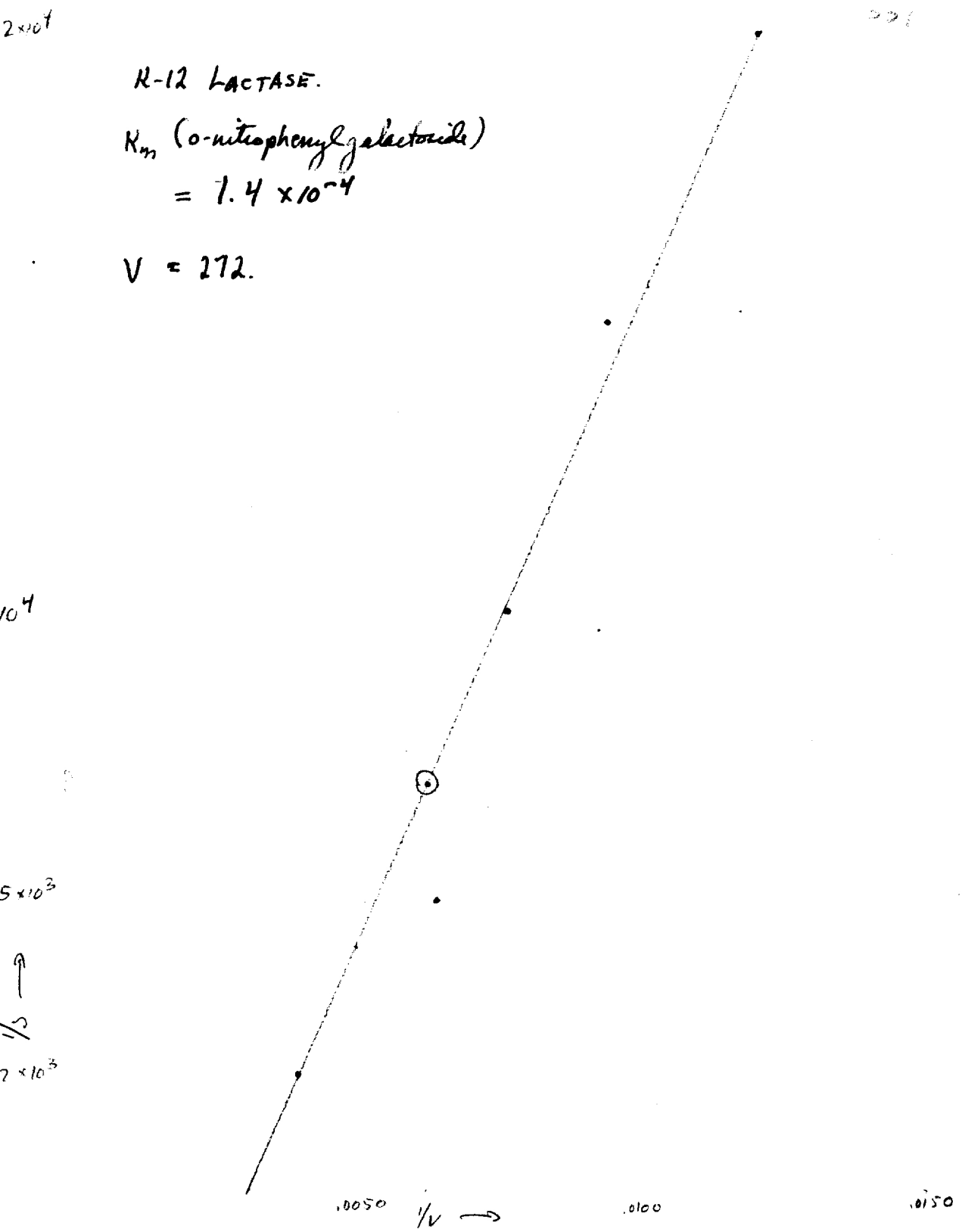
11.	—	3[illegible]
12.	NaCl	351
13.	KCl	316
14.	LiCl	305
15.	RbCl	087
16.	CaCl	302

Rb is the only antagonistic ion (cf. ~~Ethanol~~ ethylene diamine)!

K-12 LACTASE.

K_m (o-nitrophenylgalactoside)
$= 1.4 \times 10^{-4}$

V = 272.



lactase of L. bulgaricus.
Km coli lactase.

10/12/48.

L. bulgaricus from E E Snell. Grow 1 tube overnight in

N2Case	1%
Yextr.	.5%
Lactose	2%
Tween 80	0.1%
Na Acetate	0.1%

} LB medium. heavy growth noted!

Wash and concentrate 1:5. Use 1:10 ε ONPG M/2000 pH 7.5

		Di	Df
1. Na Phosphate buffer	M/50	371	830
2. Na Barbital	" M/50.	393	770

84	.0119	752	1
107	.0093	328	
132	.0076	472	
157	.0064	830	
252	.0040	1200	

272. 1/V = 0031

11	1.0	006	090
12	1.33	0	107
13	2.0	005	137
14	4.0	003	160
15.	10.0	009	261

√ (heavy precipitate)

1200
1200
172
575

③. M/100 NaP buffer. Salts M/50.

1 —	250
2. NaCl	258
3. RbCl	101
4. Na + RbCl.	163

to the enzyme?

Does Rb+ irreversibly inactivate

L. bulgaricus lactose.

tare 79. wt. 128

49 g. wet Staples paste collected grown 24h. in 12 litres LB-lactose broth 5 aeration.

A) 4g. in M/100 NaP buffer for autolysis. Ca 10ml (v. little activity)

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

C) 25g ground in M/100 NaP in B-Gun Mill for extraction. → 115 ml

L bulgaricus lactase pH opt.

10/16/48.

338C .05 ml. of buffers, Na/K M/50. ONPG M/2000
Buffer pH 6 minute readings.

	Buffer	pH	
1.	KP	6	080
2.	KP	7	042
3	KP	8	059
4.	KP	7.5	097
5.	~~KP~~	7.5	210!

6. No ~~enzyme~~ tests in 9.0 ml. Add .5 ml ~~M/3~~ ~~HCl~~ to develop color and stop reaction. M Na_2CO_3

NaCl needed! Repeat above + addition of 5 ml M/5 NaCl

Lactase; Coli & L. bulgaricus.

October 15, 1948.

10 ml		D_i^{420}	$D_{20m.}$	$\Delta^{m.}$	Δ.
1	Coli 319A. .001 ml	—	002	295	293.
2	+ Ethylene diamine · HCl M/10		010	029	020.
3	+ Ethanolamine · H_2SO_4 M/10		040	130	094.
4	+ Ethylene Glycol M/10.		−001	378!	379.
21	+ RbCl M/50		0	050	050.
22	+ KCl M/50		−001	284	285.
23	+ RbCl + KCl. M/50 ea.		0	126	126.
5	L. bulgaricus. Cell suspension;		220	321	123
6	Acetone powder 1 mg.		320	364	076	$= \frac{1}{500} \times 20g = \frac{1}{25}g$.
7	" .1 mg		040	035	019
	Extract 338C 1 ml		150	1250	—
9	" .1 ml		002	361	341
10	" .01 ml		010	034	024	$= \frac{1}{4500} \times 2g$
11	" .001 ml		0	001	022	probably ONPG!
12	" 10^{-4} ml		−002	021	023	probably ONPG!

All tests in M/100 Na P. pH 7.5 ε M/20,000 ONPG. 37°. 20m. λ = 420.
This may not be the opt. pH for bulgaricus.

Note: Intense stimulation by glycol!, Reversal of Rb inhibition ε K.
Relatively low activity of cells (Lc) may aect. by poverty of extract.

October 18, 1948.

.001 ml 319A. NaP buffer M/100. Alcohols ... M/10. [illegible]

1.	—	341 – 410	
2.	RbCl	089	
3.	Ethyleneglycol	423	
4.	" + RbCl	190	No marked displacement of Rb [illegible]
11	EtOH	400 – [illegible]	
12	PrOH	469	
13	i PrOH	395	
14	Pr<(OH)$_2$	390	
15	BuOH	450	
16	Dioxan	300	
17	MeOH	441	
18	Et<$^{Cl}_{OH}$	151	
19.	Pr≪(OH)$_3$	449	

.05 ml 338C KP buffer M/[redacted]50. Salts M/50. [illegible] +200 Na_2CO_3 [illegible]

21	–	pH 7.5	257	
22	NaCl M/50	"	390	Na (or Cl) required
23	"	8.0	079	
24.	"	7.0	590	← pH optimum between
25.	"	6.0	410.	6 and 7.
26	338A 1 ml in NaP M/50. 7.5.		032.	Inactive.

Note stimulatory effects of primary alcohols, especially n-propyl and n-butyl alcohol, and inhibition by chloroethanol.

A 18. Cf. ONP c and s nPrOH. ONP ca. M/25000. NaP buffer etc.

1. nPrOH.	168	PrOH at dilution of M/10 does not influence absorption of ONP.
2. –	165.	

? Will PrOH + enzyme + ONP-galactoside lead to color [illegible] ?
? [illegible] of products c PrOH.

October 18, 1948.

338C .01 ml / tube. 9 ml. pH 7.5 Stop R$_4$ c̄ Na_2CO_3.
In various buffers, M/100. Add ~~[illegible]~~ Na_2PO_4 buffer additional M/[illegible] where called for.
NaCl M/50 in all tubes.

	Buffer.		+ 1 ml $MgSO_4$ 4/1
1.	NaP	110	120
2.	NaBarb	~~175~~ 116	160
3.	" + NaP	170	188
4.	Et$_3$NH$_2$·Cl	020	
5	" + NaP	025	
6.	NaGlyceroP	080	070
7.	" + NaP.	109.	110
8.	NaP + $MgSO_4$.	175	

A) No activity B) Repeat with .05 ml enzyme [illegible]

Mg, PO_4 are stimulatory.

Lactase - ONPG competition

K_m.

October 26 1948. — 10/28/48.

NaP M/50 pH 7.6. 3MA 10^{-3} cc. Tom. 37° ...

		ONPG	Lac.	$D_{t=0}$	D_i	D_f	Δ	1/V
(.5 ml)	1.	M/4000	0	009		163	154	65
	2.	"	M/1000	007		082	075	133
	3.	"	M/100	~~010~~ 010		028	018	600
	4.	"	M/50	009		024	015	
(2 ml)	5.	M/1000	0	028		123	095	
(1 ml)	6.	"	M/1000	030		170	140	71.5
	7.	"	M/100	030		118	088	134
	8.	"	M/50	032		078	046	
	9.	M/4000	0	+ .1 ml antiserum.		290	360	070
	9	~~M/4000~~	~~K9~~	~~0~~		~~H~~	~~H~~	~~H~~
	10.	~~H~~	~~H~~	~~H~~ M/10.		~~H~~	~~H~~	~~H~~

~~No color from 10^{-3} ml. Reagent~~

Add enzyme to system at 30s intervals

Serum shows ca 50% inhibition at dilution of .1/1.

L. bulgaricus adaptation.

Oct 23, 1948

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

	(Lac)	(Glu)
Glu	−	+++
Lac	++	+
Mal	−	+
Sal	−	+
Suc	−	−
Xyl	−	−

Retests on ferm. variability

Oct 20, 1948

	H	Lac	Mal	Xyl	Gal	Arab.	Notes.
1.	56	V		V	++		
2	57	V		++	++		
3	58	++		++, −	++		
4	59	++		V	++		
5	60	+		++ (V?)	++		
6	61						
7	62	V		++ (−)	++		
8	63	V		V	++		
9	64	V		V	++		
10	65						
11	85	− ±	−	V	−	−; +P	
12	86	− ±	−	V	−	−; +P	
13	87	−	−	*; −	−	++ *	
14	88	− (+ papill.)	−	+; −	−	− *	
15	89	−	−	++; (−)	++	++	
16	90	++	+	++; (−)	++	++	
17	91	− ? V±	−	V	−	slow +	
18	92	− ? V±	−	V	−	slow +	
19	93	−	−	V	− −	+, −, ±	
20	94	−; slow ++	−	V	− −	+, −, ± , −, ±	
	95	+, − (V)	−	V	−		
22	96	Slow +	−	− (two +)	−		
23	97	− *	−	V	−	− (⊙ ±)	
24	98	V	−	+	++	++	
25	99	V (bulging + sectors)	−	+	++	++	

* − colonies and some v. slow +

These readings point to the necessity of resolating H stocks from stock cultures before proceeding.