Lactose adaptation: conditions
cell concentration.

~~Sept~~ Oct. 1, 1948.

Harvest cells of K-12 from 50 ml T(0) grown overnight c̄ shaking, to 10 ml ~~4/50 Phosphate Buffer (PB) 7.5.~~ T(0)-Sugar.

Adaptation system [redacted] 5 ml, containing 1 ml T(0) c̄ 5% Lactose + varying amounts of cells. A (no supplement). B. .1 ml hydrolysed casein 10%.

		Cells.	T(-)	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.	(2.9) 3 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 diluter suspensions
b. pronounced stimulation of " by hydrolysed, although cells were grown in T(0). This medium, therefore, offers no advantage.

Lactase: Phosphate assay.

Oct. 4, 1948.

~~5~~ 2 ml 219A + 2 ml 10% TCA. Remove sediment. Assay in indicated aliquots against 10^{-4} - 10^{-3} Phosph buffer as standards. In terms of original 219A. ~~Also~~ assay 1 ml of ~~1:500 dilution~~ of 219A in M/40 Na barbital buffer. Na barbital developed no visible color.

		D660
M/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 dialysed 4 hours against distilled water. Final volume, 13 ml. = 219C. Compare activity + response to Na. Enzymes at 1:1000

	D420.	Enzyme.	Na_2SO_4
1	095	C	0
2	140	C	N/50
3	171		
4	219		N/~~50~~ 1000
5	277		N/100
6	290		N/~~1000~~ 50
7	178		N/10,000.

· opt. effect of NaCl at M/50 or above; ~~[illegible]~~ at N/1000 or below!

Lactase kinetics.

Oct 4, 1948.

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

37°.

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

Stopwatch T	Times T.	A	B	C	D.
	0	004 vol	001	009 vol	007
	1:20			069	200 154
	3:30	048			
	4:00		083		
	4:30			225	
	5:00				310
	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	~~19~~		
	14 —		191		
	4 30			640	
	15 —				750
	5 30	172			
	16 —		213		
	16 30			710	
	17 —				815
	17 30	195			
	8 —		238		
	18 30			780	
	9 —				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
30		363		
			980	
31				1050
	334			
32		381		
			1000	1060
33				1060
	351			
34.		400		
35			1000	—
	370			
36		420		
			1030	
37				1080
	389			
38		440		
			1045	
39				—
40	404	459		—
41				1095
	421			
42		473		
			1050	
43	438			—
	438	490		
44		490		
45				

	A	B	C	D
45-				1100
46	451	509		
53. 47			1050	1095
~~38~~				
30				
53		560		
54.			1050	1100
30	520			
55.		579		
56	530			
57		+ + 590		
58	541			
59		~~609~~ 609		
60	560			
61.		? 611		
65		652		
	600.			
67				
69				
70		683		
71	630			
70		740.		
83.	700			
Sl.	1145	1250	1145	1250.
1:10	209	1:11 690	213	810.

evaporation may have interfered overnight.

Oct 5, 1948.

49 g. Stiff Sharples 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.

AG Remove debris. Left c̄ opalescent yellow green solution, 17 ml.

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

Di 420.

	1. B	.01	930	
	2	.001	341	
329.	3. C	.01	430	
	4.	.001	540	Probably [illegible]
		Inhibition by serum.		
319 A.	5.	—	780	
.002 ml.	6.	+ 476F4 1:10	599	Inhibition? doubtful
	7.	+ 471F5 1:10	710	
— enz.	8.	+ 476F4 1:10	023	but should use [illegible]
— enz.	9.	+ 471F5 1:10.	[illegible]	[illegible]

[illegible] Note high values here. Probably due to use of the buffer.

Reassay "C"

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

throw out!

Lactase activation and inhibition
Mici assays

(1 ml Suppl.) In NaP buffer.

				Dy 20.
	1. 329B	-2		45.3
	2. " B	-3		075
329A	3. 329C		—	082
	4.		NaCl M/50	081
10^{-3}	5.		KCl M/50	092
	6.		LiCl M/50	078
	~~7~~		~~Et(NH2)2~~	
	~~8~~			
	~~9~~ In KPO4			
.001	7. 319A		—	150
	8	Et DiNH2	M	017
	9		M/10	022
	10		M/50	049
	11.		M/100	082
NaP.	1 [redacted]			
	13 [redacted]			

Repeat Assays of C!

		Buffer	Inst		
329C	1	Na	.001		116
	2.	Na	"		114
319A.	3	K	—		167
.001	4	K	EDA M/100		128
	5	K	" " + Na_2CO_4 M/50		260
	6	K	Na_2SO_4 M/50.		290
	7	Na	—		329
	8	"	NaF	M/10 1ml	006
	9	"	$CuSO_4$	M/10 .1ml	- 119
	10	"	$HgCl_2$	M/10 .1ml	- 412
	11		IAcONa	M/20 1ml	266
	12.		"	M/20 .5ml	286
	13.		"	" .1ml	335.

! Turbid D_i = 129
" D_i = 420

} v. sl inhibition.

may be protected from [illegible] by [illegible]

Mechanism of fluoride inhibition + ~~[illegible]~~ Km.

Oct. 5, 1948.

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

		Buffer.	D_{420}
1.	–	NaP	290
2.	NaF M/100	NaP	019
3.	NaPyroP M/50	–	042
4.	"	NaP	039
5.	NaBarb ⇄		230
6.	NaF M/100	NaBarb	222
7.	NaF M/500	NaP	183
8.	M/1000	NaP	291
9.	M $\times 10^{-4}$	NaP.	310.

	ONPG × M/2000	in NaP.	~ time
10	0.1	091	258
11	0.5	210	920
12	~1.0	254	1110

(8.5×10^{-5}).
Km may be estimated in the neighborhood of $5 \times 10^{-5} - 10^{-4}$ Linearity needs to be shown. Conc. of ONPG from 5×10^{-5} to 2×10^{-4} needs to be explored.

∴ fluoride inhibits only in presence of phosphate. M/100 needed for substantial inhibition. (Mg effect?)

Lactase. mechanism of fluoride inhibition

Requirement for Mg^{++}? Km.

Oct 7, 1948.

319A .001 ml in M/100 NaP buffer. [illegible] ONPG M/2000

Supp.		
1.	019!	Error?
2. NaF M/100	013	
3 " M/500	180	} Note aggravation.
4 " $MgSO_4$ M/200	132	
5 " M/100 $MnCl_2$ M/200		
6 " M/500 " "		
7 — " "		
8. — $MgSO_4$ M/200.	251	No marked stimulation!

.001 ml in M/50 NaP buffer. Vary amounts of M/2000 ONPG added. Measure q. 5 min.

	ONPG.	5 m	10 m	15 m	20 m.	0	30	$K_m (\times 10^{-5})$
11	1					000		
ng 12	1.5	038	065	098	123	002	178	8.3
13	2	049	079	111	149	007	210	7
14	5	077	124	173	227	015	323	5
15	10		141	203	262	017	381	—

↑ 094

90 slate

These data show a substantially linear decomposition of the galactoside in the interval studied, but taking V_{15} as V_{max}, one 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.

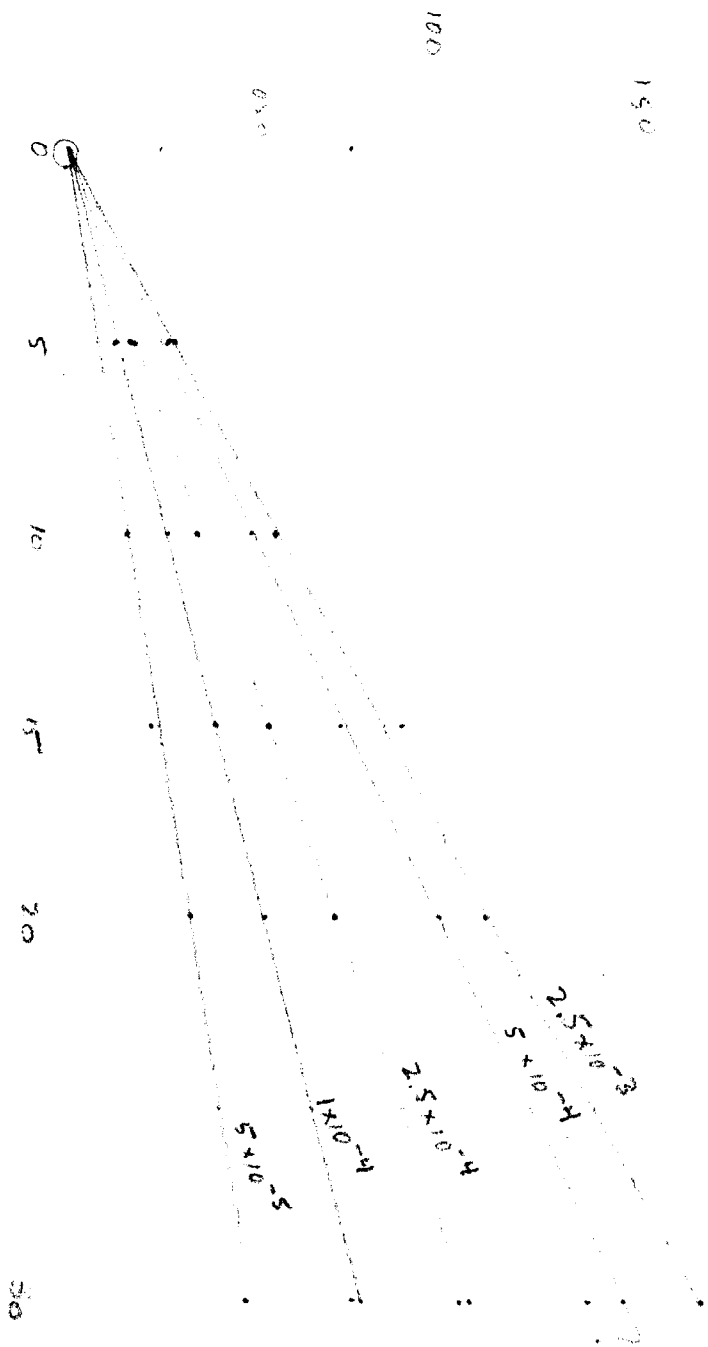
[illegible] 20 min. data

	V	1/V	1/S
①			
②	121	0083	13,300
③	142	00705	10,000
④	212	0047	4,000
⑤	245	0041	2,000

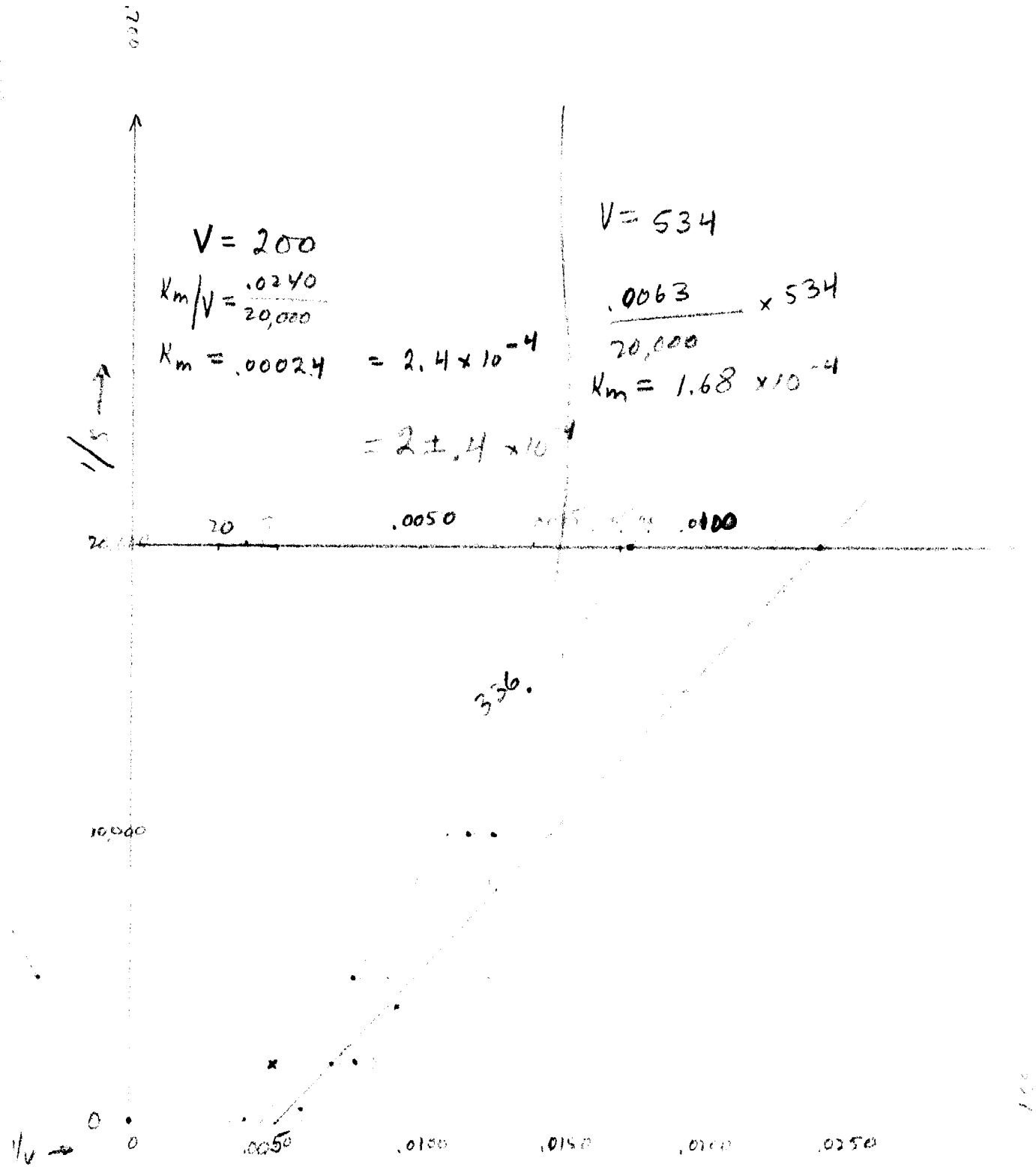
$\bar{V}$ = 315 0032

Careful extrapolation gives

K_m =



0
5
10
15
20
30
5×10⁻⁵
1×10⁻⁴
2.5×10⁻⁴
5×10⁻⁴
2.5×10⁻³



V = 200
Km/V = .0240 / 20,000
Km = .00024 = 2.4 × 10⁻⁴
= 2 ± .4 × 10⁻⁴
V = 534
.0063 / 20,000 × 534
Km = 1.68 × 10⁻⁴
1/s
20
.0050
.0100
336.
10,000
0
1/V
0
.0050
.0100
.0150
.0200
.0250

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.

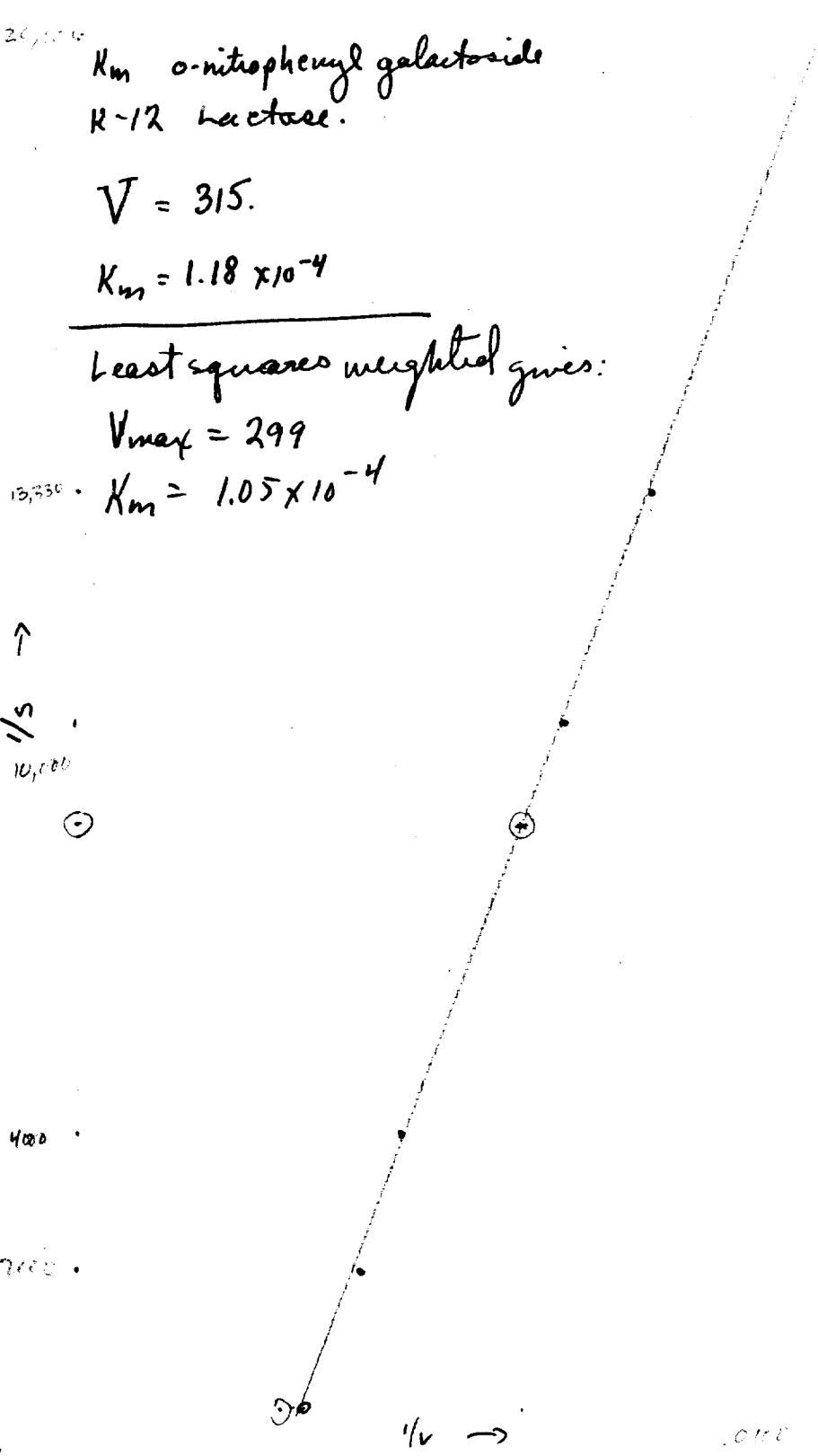
$V = 315.$

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

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)

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

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

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

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

$= .105$

0
5
10
15
20
30
10
5
2
1.5

Kinetics ; $Mg^{++} + F^-$ 334/2

Oct 8 1948.
.001 ml 319A / 10ml in colorimetric tube. in M/50 NaP buffer.

(1). Time series c̄ substrate dilution. D420.

ONPG x M/20,000.

		t_0	5M	10M	15M	20M	30M
0	50	051	080	10[illegible]	1[illegible]0	162	219
1	10	011	[illegible]7	060	[illegible]	110	149
2	5	009	027	[illegible]	063	[illegible]	114
3	2	000	017	027	[illegible]	053	
4	1	−003	010	014	[illegible]	031	046

(2). in M/100 NaP buffer.

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

Corrected values of (1).

	t_0	5	10	15	20	30	$v_{rel.}$	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	02400	20000

should be 1/[illegible] [illegible] 69.
too high

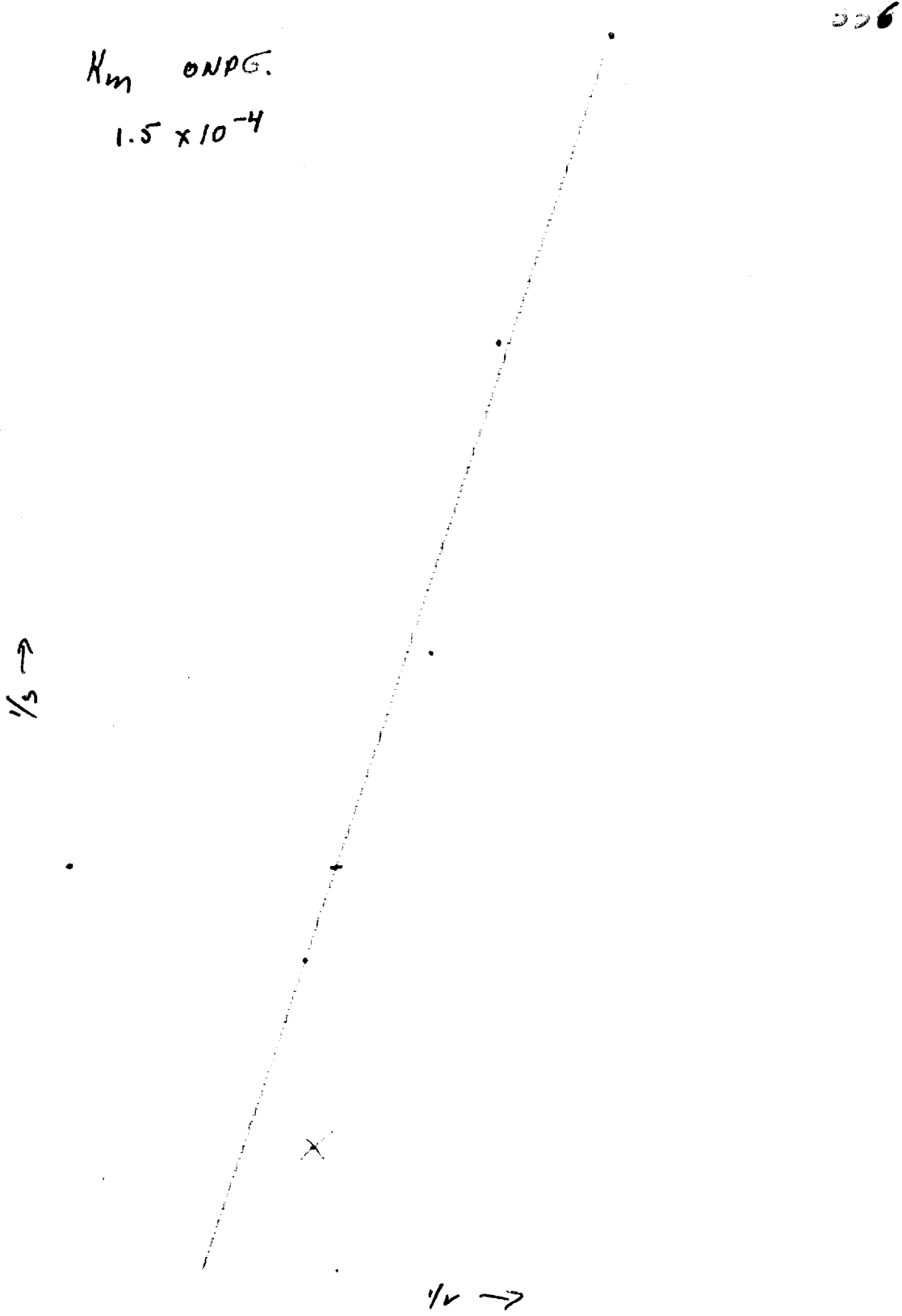
Km is estimated at 2.4×10^{-4}

V at 200/30m.

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

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	$D_0^{V/20}$	$D_{10}^{V/20}$			1/s	1/v
1.	1.00	000	115	115		20000	.0001
2.	1.33	002	116	114		15000	0001
3.	2.00	001	180	179		10,000	0058
4.	4.00	001	272	271		5000	0038
5.	10.00.	026	281	255		2000	0009

Visible discrepancy / inactivity = 3/34.

② In M/100 NaP buffer. + M/50 salts.

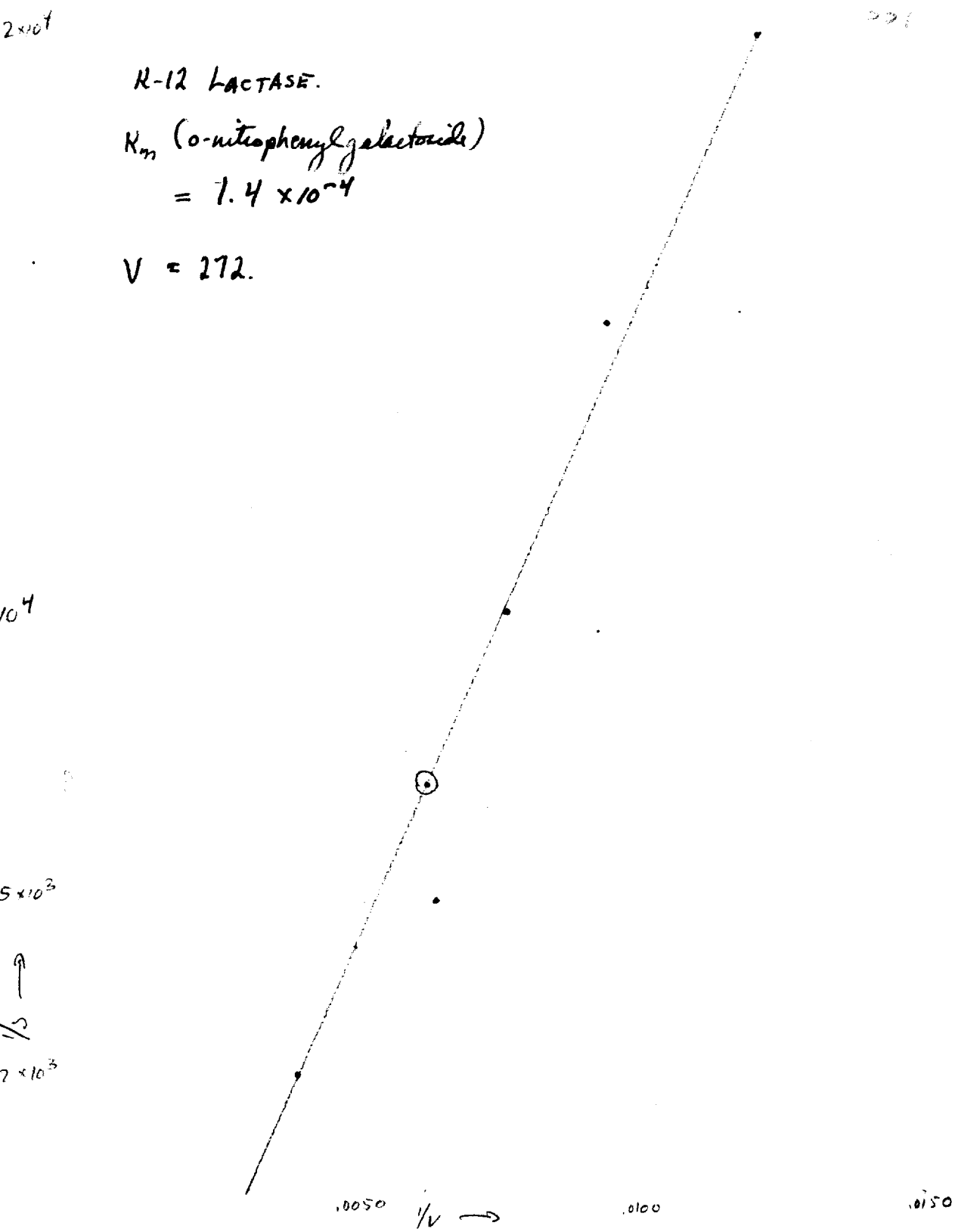
11. — 3[illegible]
12. NaCl 351
13. KCl 316
14. LiCl 305
15. RbCl 087
16. CaCl 302

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

R-12 LACTASE.

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

V = 272.



Lactase of L. bulgaricus.
Km Coli Lactase.

337

10/12/48.

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

N2Case	1%	LB medium.
Y Extr.	.5%	
Lactose	2%	
Tween 80	0.1%	
Na Acetate	0.1%	

heavy growth noted!

Wash and concentrate 1:5. Use 1:10 c ONPGM/2000 pH 1.5

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

[illegible]	N	[illegible]	[illegible]
84	.0119	252	1
107	.0093	328	
132	.0076	472	
157	.0064	830	
252	.0040	1200	

Repeat 334. [illegible]

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

272. 1/V = .0031

V =

d (less composition)
1200
1200
172
575

(3). M/100 Na P buffer. Salts M/50.

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

te 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 → 45 ml

L bulgaricus lactase, pH opt. 239

10/16/48.

338C .05 ml. of buffers, Na/K M/50. ONPG M/2000.
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 ~~[illegible]~~ enzyme tests in 9.0 ml. Add .5 ml ~~[illegible]~~ ~~[illegible]~~ to develop color and stop reaction.
M Na_2CO_3

NaCl needed! Repeat above c̄ addition of 5 ml M/5 NaCl.

Lactase; Coli & L. bulgaricus.

October 15, 1948.

10 PM		Di^{420}	D_{20m}^{420}	Di^{2m}	Δ.	
1	Coli 319A. .001 ml —	002	295		293.	
2	+ Ethylene diamine HCl M/10	010	029		020.	
3	+ Ethanolamine HSO_4 M/10	040	130		094.	
4	+ Ethylene Glycol M/10.	-001	378!		379.	
21	+ Rb Cl 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	324		123	
6	Acetone powder 1 mg.	320	364		076	$= \frac{1}{5000} \times 20g = \frac{1}{250}g$.
7	" .1 mg	040	055		019	
	Extract 338C 1 ml	182	1250		—	
9	" .1 ml	022	361		341	
10	" .01 ml	010	030		024	$= \frac{1}{4500} \times 20g$
11	" .001 ml	0	022		022	probably ONPG!
12	" 10^{-4} ml	-002	021		023	

All tests in M/100 NaP. pH 7.5 c̄ 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 c̄ K.
Relatively low activity of cells of L.B. may acct. for poverty of extract.

Solvents and Coli lactase.

October 17, 1948.

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

1.	—	341 – 410	
2.	RbCl	089	
3.	Ethylmeglycol	423	
4.	" + RbCl	190	No marked displacement of Rb-
11	EtOH	400 – [illegible]	
12	PrOH	469	
13	iPrOH	396	
14	Pr<(OH)$_2$	390	
15	BuOH	450	
16	Dioxan	300	
17	MeOH	441	
18	Et<$^{Cl}_{OH}$	157	
19.	Pr<(OH)$_3$	449	

.05 ml 338C KP buffer M/[redacted]50. [illegible] M/50. [illegible] +2 [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 [illegible]
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 E and S nPrOH. ONP ca. M/25000. NaP buffer etc

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

? Will PrOH + enzyme + ONP hydrolase lead to color [illegible]?
? Absorption spectrum of products of PrOH.

L. bulgaricus lactase

October 18, 1948.

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

	Buffer.		+ 1 ml Na_2CO_3, M/1
1.	NaP	110	120
2.	NaBarb	~~175~~ 116	160
3.	" + NaP	170	188
4.	Et SNH_2·Cl	020	
5	" + NaP	025	
6.	NaGlycroP	080	070
7.	" + NaP.	109.	110
8.	NaP + $MgSO_4$.	175	

A) No activity B) Repeat with .05 ml enzyme per tube (no [illegible]).

Mg, PO_4 are stimulatory.

Lactose - ONPG competition

Km.

October ~~10~~ 26 1948. — 10/28/48.

NaP M/50 pH 7.5. 319A 10^{-3} cc. 70 m. 37° . . . .

		ONPG	Lac.	D_{450}	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	~~[illegible]~~ 060		028	018	600
	4.	"	M/50	009		024	015	
(2 ml)	5.	M/1000	0	028		123	095	
(+ 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.	~~[illegible]~~ 290	360	070	

~~9. [illegible]~~

~~10. [illegible] M/10.~~

~~no color developed c̄ 10^{-3} ml. Reason~~

Add enzyme to system at 30s intervals

Serum shows ca 50% inhibition at dilution of .1/10

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	−	−	+, −	−	− *	
15	89	− (+ papill.)	−	++ ; (−)	++	++	
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 +	−	− (few +)	−		
23	97	− *	−	V	−	− (⊙±)	
24	98	V	−	+	++	++	
25	99	V bullseye + sectord.	−	+	++	++	

* − colonies and some v. slow +

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