Suppressor lactase

Jan. 9, 1948

Grow batches of W661 & 662 in S(Lac). Harvest and dry over P_2O_5.

#1 = W661 44g. wet paste 10g.

#2 = W662 62g. wet paste → 16.67g. dry cells

Lactase of W112.

Jan. 10, 1949.

Lactose adaptation in W-112 (Lac_1)

Grow W-112 in Y2 ½% sugar broth. 10 ml.

A. glucose

B. butyl galactoside

C. lactose.

Wash + resuspend in 4 ml H_2O.

1 ml cells
1 ml M/100 Na P buffer + BCP
1 ml 5% sugar. 2 hour reading

	—	glucose	lactose.
A	—	+++	—
B	—	+++	+++
C.	—	+++	—

Check by streaking out cells used.

Lac_1- produces lactase with butyl galactoside but not with lactose. Cf. Esther's expts. showing same result with nitrophenyl galactoside.

1/12. Grow W-112 in 2 × 50 ml Y2/Sugal. Harvest, wash + dry over P_2O_5. Yield 33 mg. dry cells. 1/13. very active on onpg.

Lactase in W-108.

Grow W-108 in 10ml Y2 Bugal ½% + Y2 Lac.

18h. Bugal actively fermented; heavy growth. More sparse; no ferm. in Y2 Lac.

Harvest + test:

a) spot plate ONPG: B: +++ L: —

b). c̄ .1ml M/50 KP buffer pH 7.0. 1ml cells (2x) 1ml 3% sugar.

—	108L	108B
Glu	—	+±
Gal	—	+++
Lac	—	++±

Note adaptation to glucose! Cf. W327 which does not adapt on maltose

With respect to lactose, W108 is like W112. Non-reactive but can form enzyme

Rb inhibition of Lactase

a) Add ONPG to enzyme-buffer. NaP M/100. 7.5 (RbCl M/50) ONPG M/200.

b) "enzyme ONPG ".

		10^{-3} ml.		
a)	1	319A	—	510
	2	"	Rb	470
	3	395	-	680
	4	"	Rb	630
	5	399	—	310
	6	"	Rb	309
b)	7	319		650
	8	"	Rb	650.

∴ no appreciable inhibition!

Repeat comparing fresh solution of RbCl. 319A/2000μl

—	289
old RbCl	268
new RbCl.	200

Rb inhibition of K-12 lactase.

1/15/49.

319A	10^{-3}.	Buffer	mid.	M/100	7.5.	Salts M/50.	ONPG M/2000
	Salt	Buffer			% inh.		
1.	—	Na	438		—		
2.	RbCl old	Na	409		07		
3.	CsCl	Na	393		10		
4.	RbCl new	Na	316		28		
5.	—	K	239		—(45)		
6.	RbCl old	K	220		08		
7.	CsCl	K	182		24		
8.	RbCl new	K	100		58		

Rb inhibition

January 14, 1949.

NaP	1/s	1/v.	Δ		Di	1/v corrected. (+ 1/3).
	1	27.2	368	388	20	
15m	2	30.4	329	340	11	
	5	39.4	254	255	1	
	10	~~31.1~~ 54.9	182	184	2	
	15	69.0	145	142	-3	
NaP+RbCl						
	1	31.1	322	339	17	
15m	2	36.9	271	280	9	
	5	52.1	192	198	6	
	10	76.9	130	[131] (~~104~~)	1	
	15	97.1	103	103	0	
KP						
	1	37.3	268	286	18	49.7
20m	2	43.7	229	242	13	58.3
	5	63.3	158	160	2	84.5
	10	90.1	111	111	0	120.
	15	~~87.7~~ 111.	90	87	-3	148
KP+RbCl						
	1	61.3	163	181	18	81.7
20m	2	87.7	114	121	7	117
	5		—	((42))	4	~~360~~
	10	270	37	37	0	360
	15	370	27	27	0	494

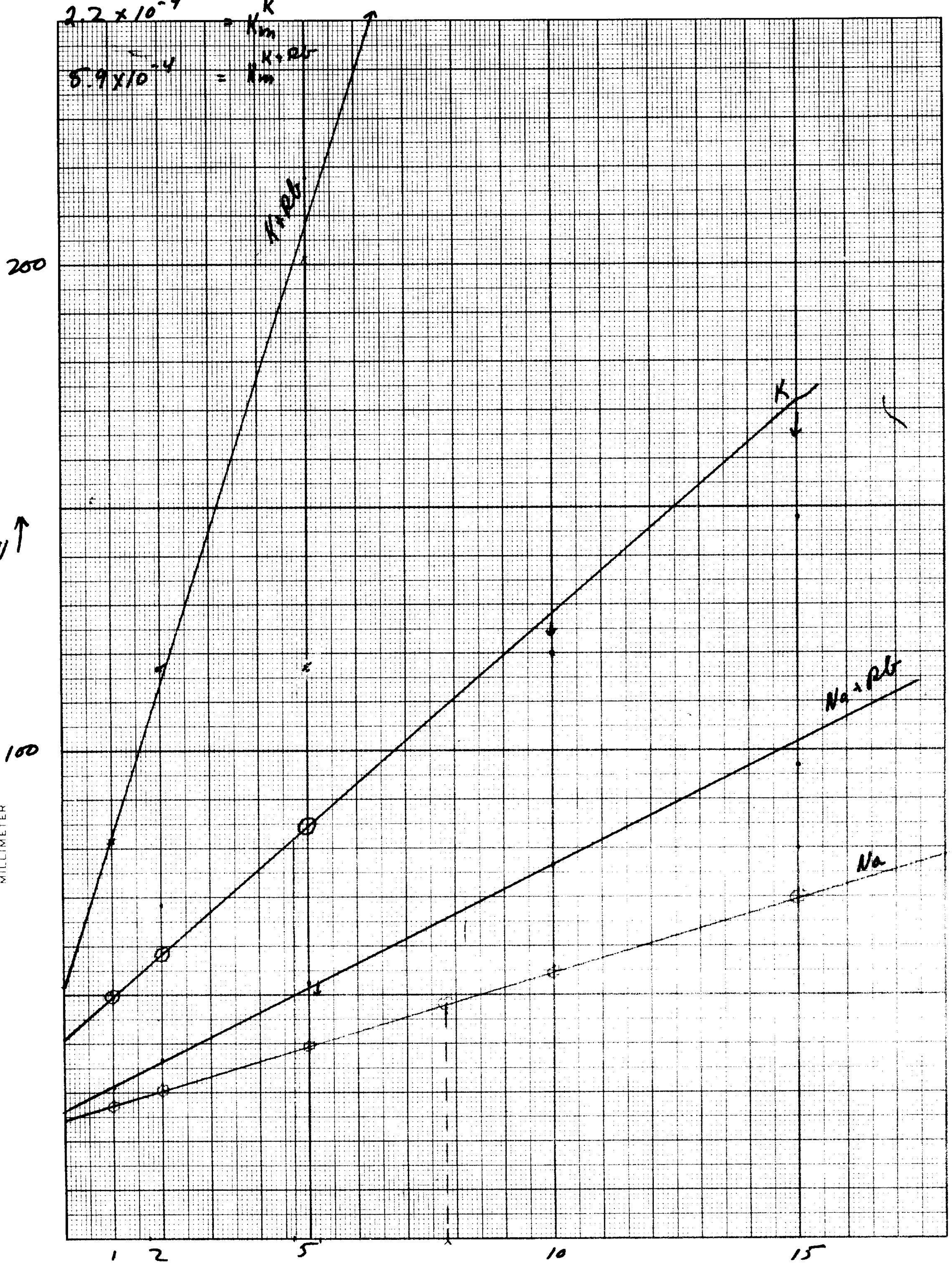
very good linear fit of Na data. K data may show some bending downwards

$1.28 \times 10^{-4} = K_m^{Na}$

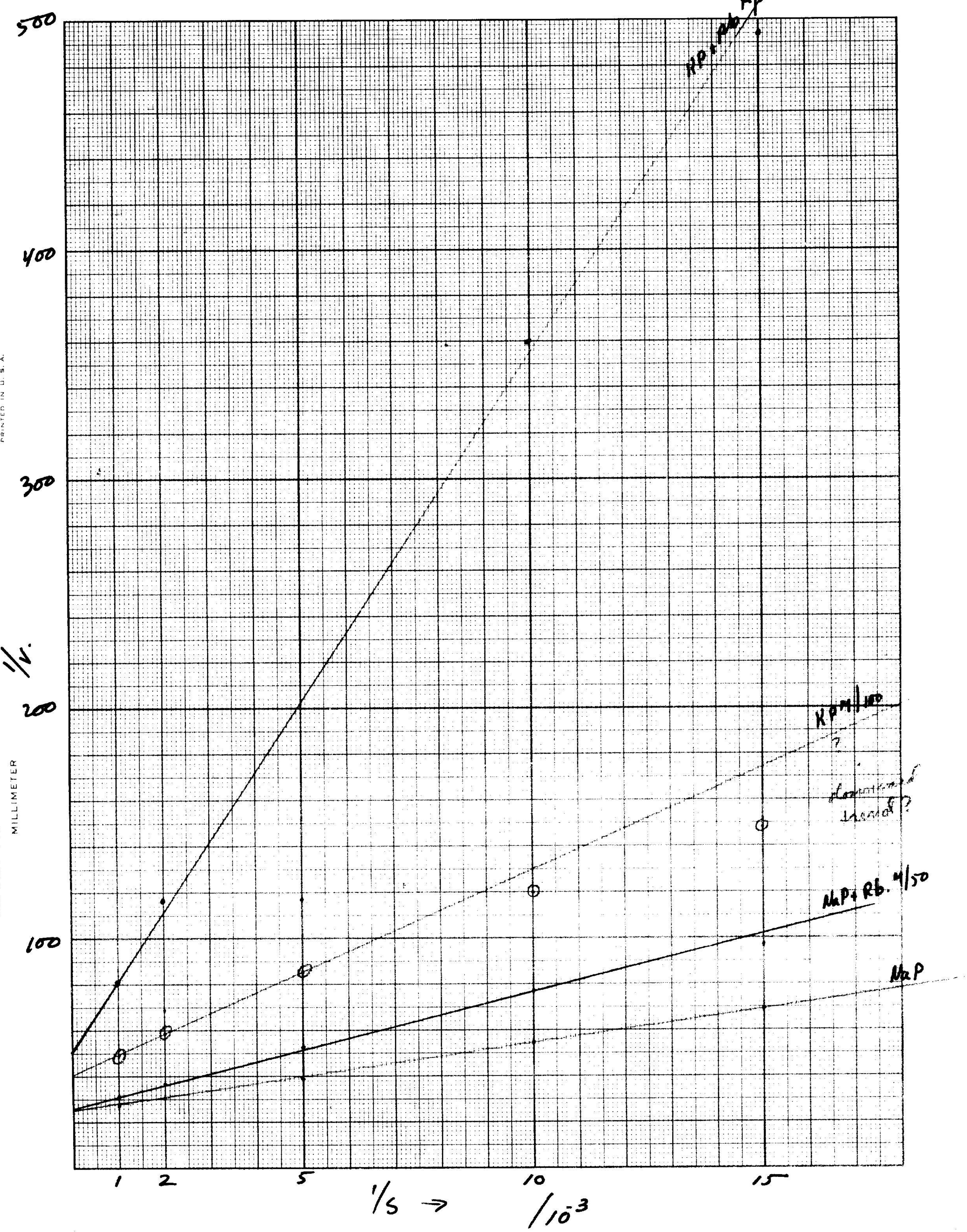
$2.2 \times 10^{-4} = K_m^{K}$

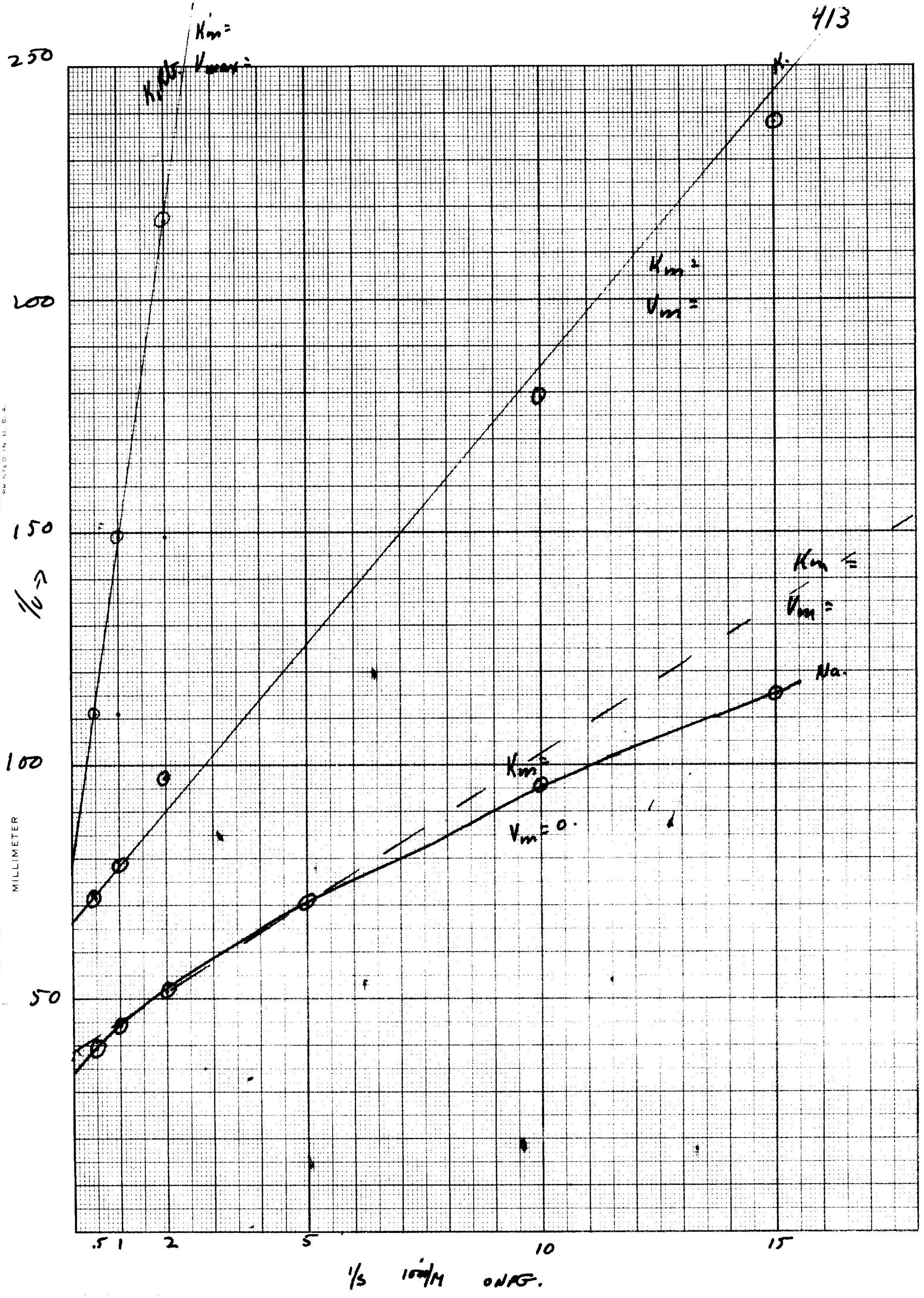
$5.9 \times 10^{-4} = K_m^{K+Rb}$

$1.92 = K_m^{Na+Rb}$



411


KP + Rb. M/50.
500
400
300
1/V.
200
KP M/100
?
contaminated
100
NaP + Rb. M/50
NaP
1
2
5
10
15
1/S →
/10⁻³


K'm=
V'max=
K/Na.
K.
Km=
Vm=
Km =
Vm =
Na.
Km=
Vm= 0.
250
200
150
100
50
1/v →
.5
1
2
5
10
15
1/s 10⁻⁴/M ONPG.

V_{max} / K_m' 411 data 413.

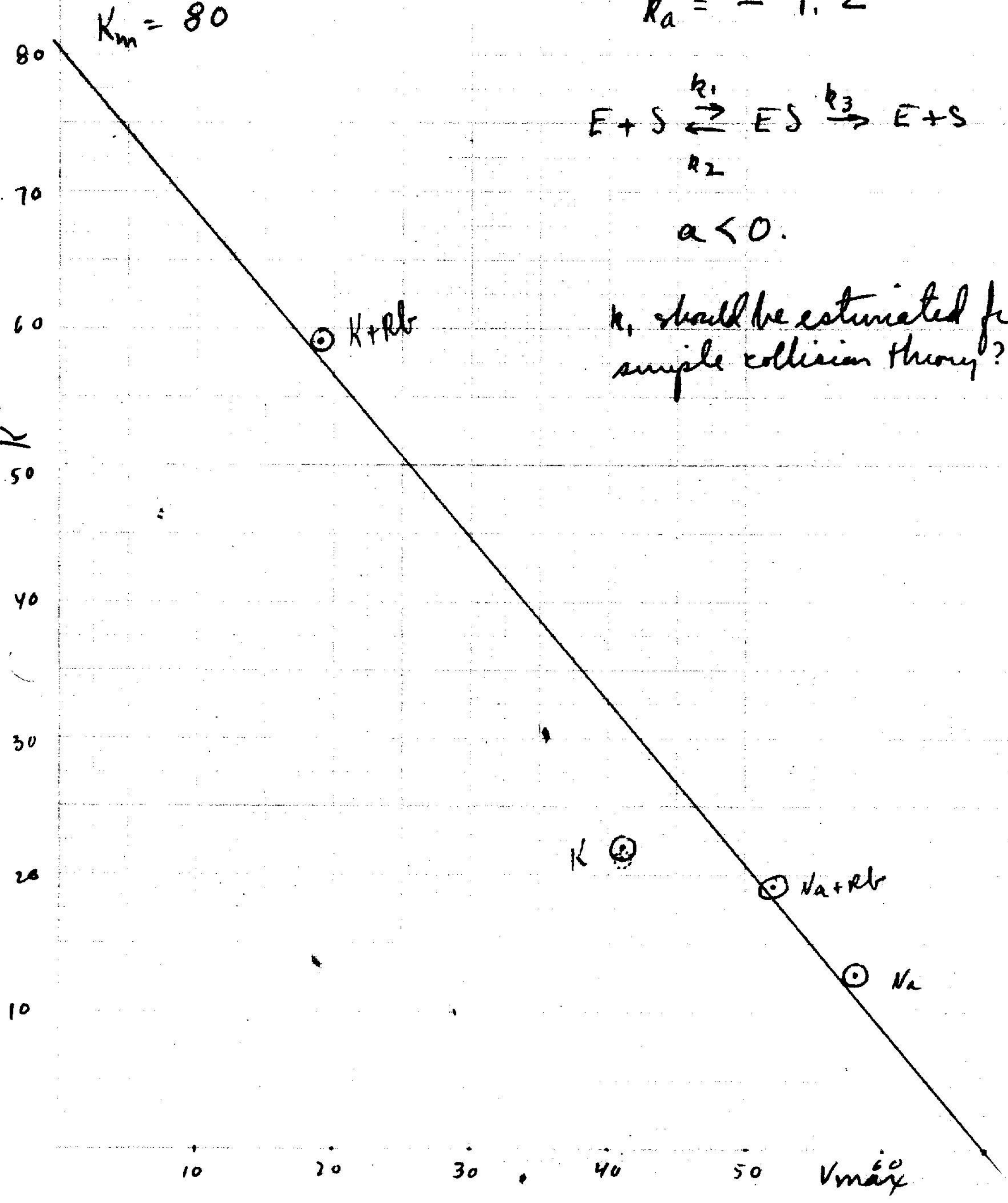
$$K_s' = K_m + k a V_{max}$$

$$k_a = \text{"} - 1.2 \text{"}$$

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + S$$

$a < 0.$

k_1 should be estimated from simple collision theory?



Rb inhibition

January 16, 1949.

If K_m' is apparent dissociation constant for $E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES, \xrightarrow{k_3} E + P$.

$K_m' = K_m + \frac{k_3}{k_1}$. Now $k_3 = k V_{max}$. Conceivably, all the effects of alkali metal substitution could be explained as effects on k_3, of which there are undoubtedly some since V_{max} is affected.

~~$\frac{1}{v} = \frac{1}{s} \frac{1}{V_{max}}\left(\frac{K_s}{s} - 1\right) =$~~ If this could be applied here,

$K_m' = K_m + a V_{max}$.

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

M/100 buffer. Salt M/50. 1/Substrate ONPG 1000/M.

Buffer	1/s	Salt	1/v	Δ	Di	Df
Na	.5	–	39.1	256	40	296
	1	–	44.2	226	24	250
	2	–	51.5	194	13	207
	5	–	70.9	141	11	152
	10	–	95.2	105	4	109
	15	–	115	87	3	90
K	.5	–	71.9	139	32	171
	1	–	78.7	127	19	146
	2	–	97.1	103	6	109
	5	–		–	2	36 spilled
	10	–	179	56	-3	51
	15	–	238	42	-3	39
K	.5	Rb	111	90	36	126
	1	"	149	67	19	86
	2	"	217	46	8	54
	5	"	370	27	6	33
	10	"	714	14	-1	13
	15	"	833	12	0	12

The enzyme dilutions + other preps stood at room temperature at ease for several hours. This may acc't for the v.c. variations seen.

Note: $\bar{P} \doteq$.

Note M/5000 ONP $\doteq$.

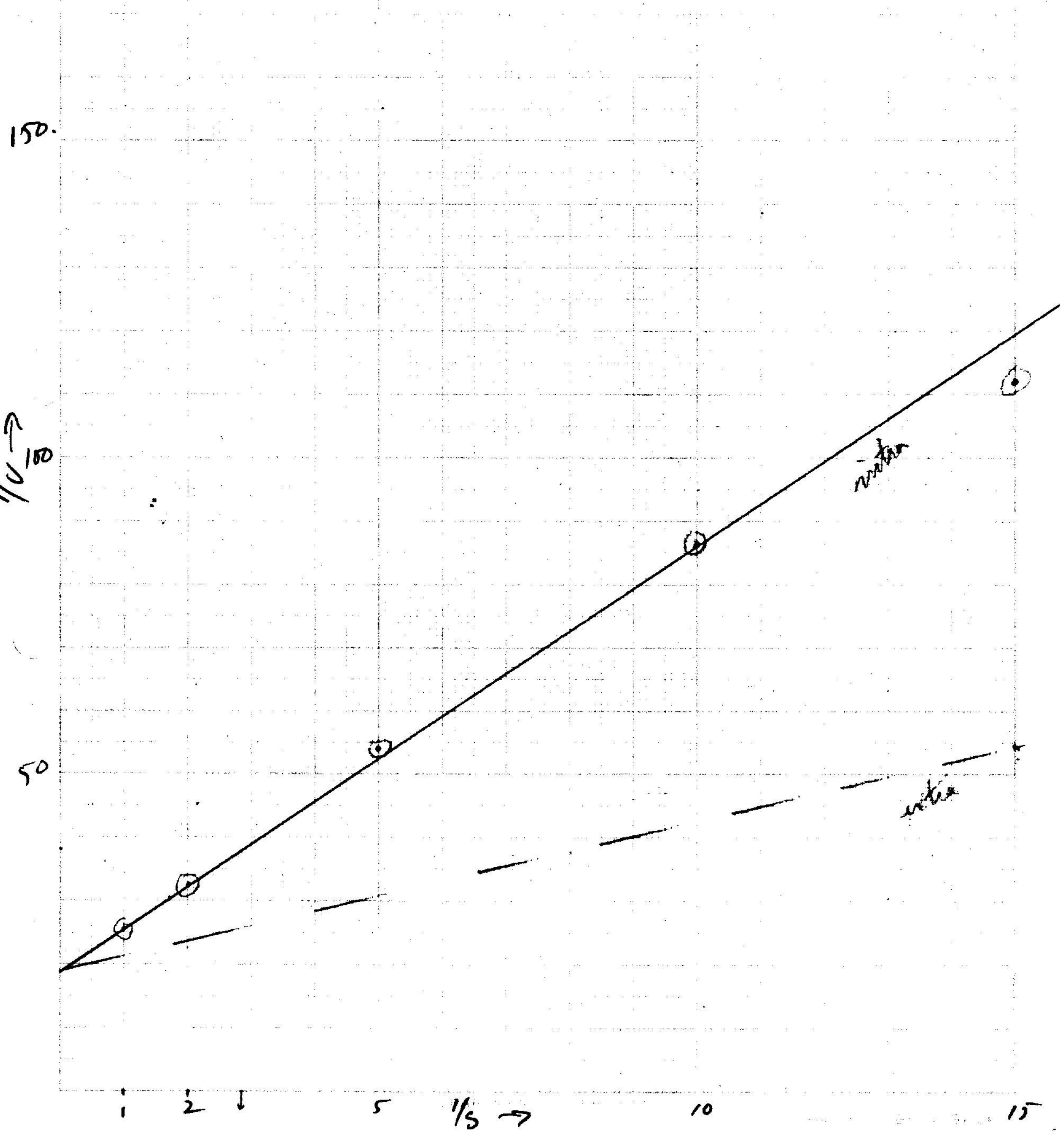
415

$K_m = 4.5 \times 10^{-4}$ K

$V_{max} = 527$

Kinetics of intracellular galactosidase.

NaP buffer pH7.5 M/100.



Intracellular galactosidase

Jan. 17-18, 1949.

Harvest K12 from 100ml Y2 Lactose broth. Resuspend in ca 20 ml.

Preliminary assay: 10 mins in NaP M/100 7.5

	Di	Df.
.1ml	91	280
.5ml.	452	1100+.

Ca 40u/ml. Relative activity$_{20M}$ 400.

Use 1ml 1:10 bacterial suspension. Add to prepared system + to control.

a) pH optimum. Use M/100 KP buffer M/50 NaCl. ONPG M/2000 unless stated.

	pH	Δ		h.
1.	5.0	322	329	007
2.	6.0	374	381	007
3.	7.0	380	390	010
4.	7.5	371	380	009
5.	8.0	326	339	013

b) K, Na, Rb effects. M/5000 ONPG.

6.	K buffer M/100.	185	191	006
7.	" + Rb M/50	163	169	006
⑧	Na Buffer.	181	183	002

c) Kinetics. Na buffer M/100. 7.5

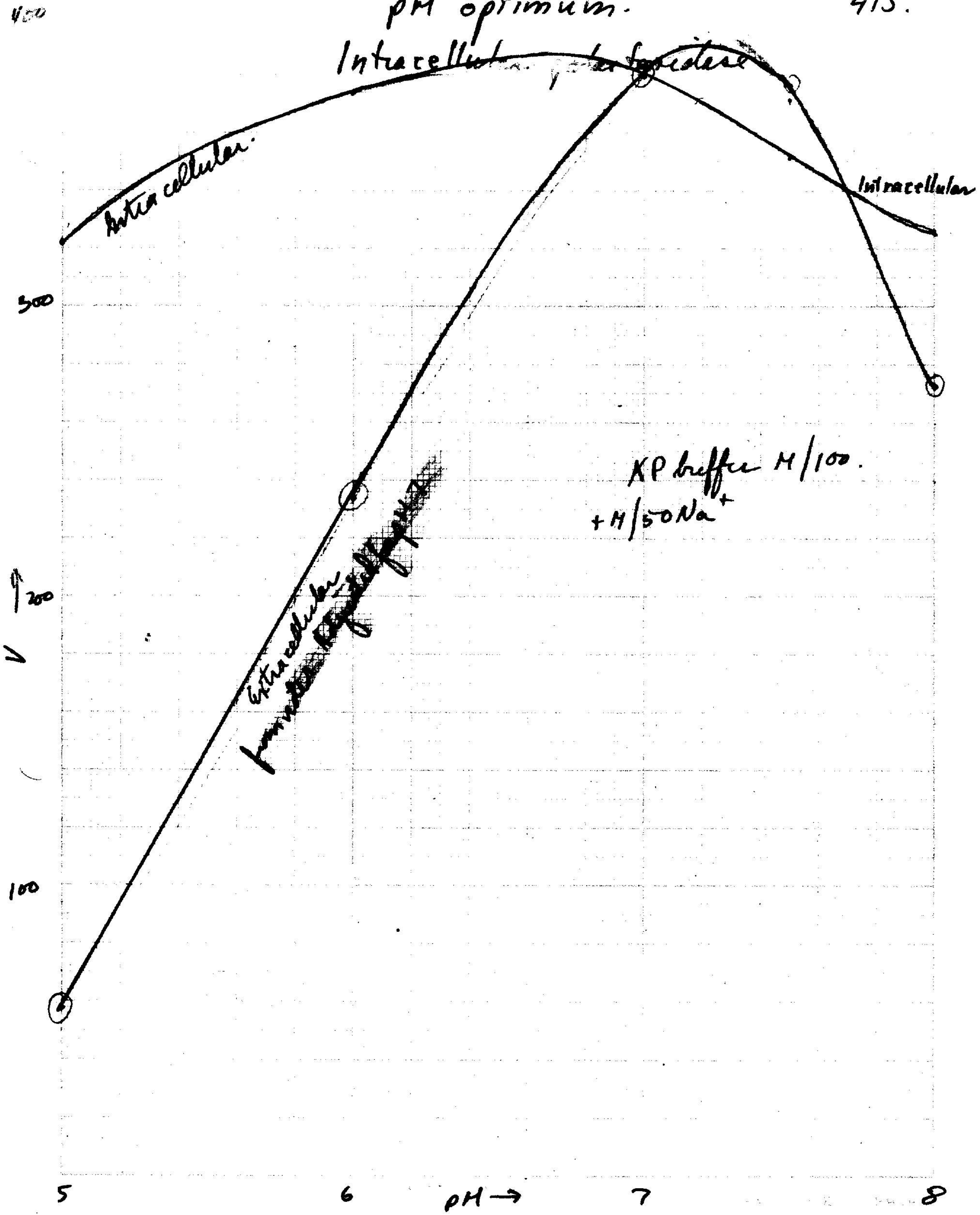
Vmax = 527
Km = 4.5x10^{-4}

	1/ONPG 1000/M	1/S			
11	1	25.4	393	411	018
12	2	32.2	310	318	008
⑬	5	54.0	185	188	003
14	10	86.2	116	117	001
15	15	112	089	090	001

Need time curve

	-007	
-alla	090	
+alla	054	50

pH optimum.


400
Intracellular. β-fructosidase
Extracellular.
Intracellular
300
KP buffer M/100.
+M/50Na$^+$
Extracellular
[illegible]
V ↑ 200
100
5
6
pH →
7
8

4/18

Adaptation of ML; K-12 on galactose

		Δ1	Δ2	Corrected	[12:45AM]	20m.		315PM	20m. R.A.	3h. R.A.
MLm.	gal	20	301	180	200	200	⁞	481	11	22
	lac	308	—	180	200	488		1100+	171	
	glu	—	126	95.5	105	84 ?		121	<0	18
K·	gal	60	424	116	129	176		540	52	49
	lac	228	—	111	123	339		970	206	—
	glu	002.	30	117	130	119		147	~~<1~~ <2	3.4
blanks —										

Glucose cells may have grown and/or begun to adapt.
~~Relative activity.~~

Galactose therefore has ca. 14x activity

In ML Lac/Gal = 16

K-12 Lac/Gal = 4

Mutant adaptation to galactose.

1/22/49.

Harvest cells from 10ml Y2 - 1% sugar broth and resuspend in 2ml. Butyl Galactoside ½%. Tubes c̄ BCP indicator. Also checks constitution on EMB Lac plates.

	Lac	✓	Gal	✓	~~Glu~~ Bug.	✓	~~Bugal.~~ Glu		✓
							BCP		
K-12	114 680 514		150 298 120	[illegible]	147 1000 590	[illegible]	++ 120	~~1000~~ 131 −010	[illegible]
W108	102 518	+	105 150	+	126 1100	+	÷ 85	112	✓
W45	110 122	✓	140 146	✓	83 120	✓	− 140	150	✓
W112	106 160 (30)	✓	117 196 ㊾	✓	210 870 310	✓	++ 123	134 −	✓
W255	127 1050 800+	✓	89 386 305	✓	93 930 1000	✓	− 86	104 −6	✓
Substrate:	33								

ONPG readings:
1:30P- initial in —
final in —
A.A. —

For K-12 with Lac as 100%
Bugal. 115%
Galactose 22%

✓ is check on plates.

Note: Adaptation of K-12 to Galactose < Butyl galactoside.
Moderate adaptation to galactose of W112, but marked in W255.

Response of W-108 may be due to presence of + cells. Even 108/Glu had 1%+

Adaptation to related substrates

Harvest K-12 from 1% sugar Y2 broths 10 ml quants

	D_i	5:20PM	cor. D_i	Δ	Δ/D_i cor =R.A. 20M.	2hrs om. 7:15PM D_f	Δ'	R.A.'
✓ Glucose	141	135	139	-004	—	147	008	
✓ Galactose	187	250	180	+ 70	39	810	630	—
✓ Lactose	153	470	150	320	213	1150	1000	—
(Na) Mucate	320	318	300	018	(006)	490	190	009
✓ (Na) Galactonate	180	191	174	017	(010)	285	111	010
Na Lactobionate	180	348	174	174	100	940	766	-
Dulcitol	~~144~~ 83	97	87	010	(011)	155	68	011
✓ l-Arabinose	104	101	106	-005	—	116	010	
Substrate blanks		012	—			013	—	

✓ were evolving gas during growth. Growth on mucate was very heavy. Growth on dulcitol was very light.

Very slight responses are shown by galactonate and dulcitol.

Calculating lactose as 100:

Lactobionate	58 %	Not utilized by intact cells.
Galactose	23 %	
Dulcitol	4 %	
Galactarate	3 %	
Mucate	3 %	

Absorption spectrum
of E. coli + formazan.
(tetrazolium)

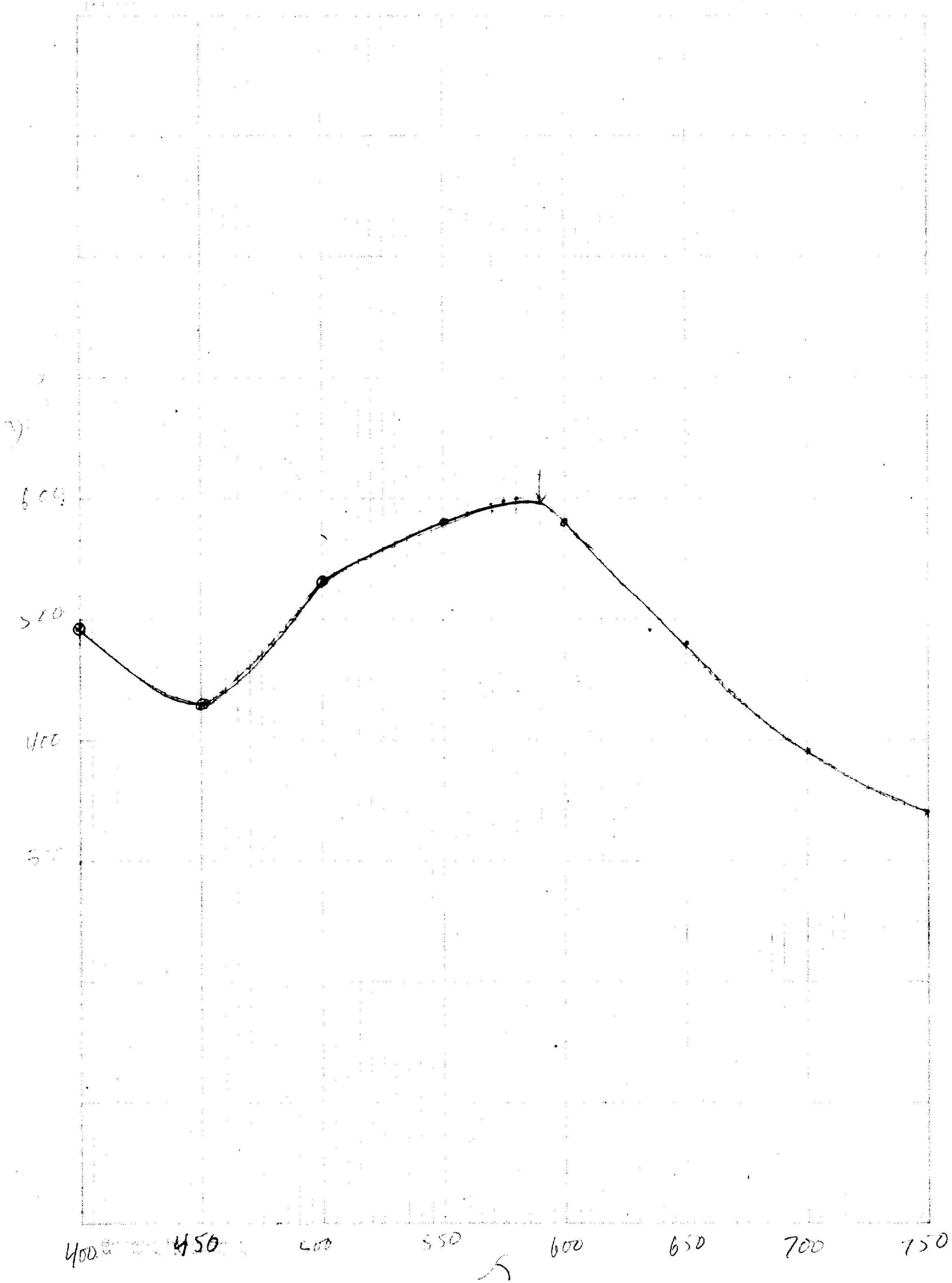
#23.

λ	d
400	491
450	430
500	533
550	581
600	581
650	450
700	390
750	340
800	310
[illegible]	
560	589
575	599
590	597
580	600
585	598
570	590

Jan 25, 1949.

Reach 11-12/ Glucose in glucose buffer with 0.02% triphenyl tetrazolium, and study absorption spectrum. Peak at λ = 5800 Å but not very sharp.

4523



Sugar utilization by W815.

446a.

Feb. 28, 1949.

Harvest cells from Y2 Lac (L) and Y2 Glu.

Test 1ml cells c̄ 1ml 5% sugar + 1ml M/100 buffer + BCP.

Time (m).	L/Lac	L/Glu	L/Gal	G/Lac	G/Glu
15	+	−	±	−	−
20	+++	±	+	±	±
45	+++	±	+	±	±
60	++++	±	+	±	±

This organism, adapted to lactose, clearly ~~produces~~ ferments lactose much more rapidly than glucose or galactose.

Galactosidase in W815.

446b.

3/1/49.

Harvest cells from 42 Lac and 42 Glu. Dilute, etc. + test c̄ M/2000 ONPG KP 7.5 M/50.

	D_i^{420}	corr.	D_f	R.A.
Glu	300	270	280	< 4
Lac.	436	—	>>1000.	> 300

∴ W815 produces an adaptive galactosidase! (although it cannot utilize ~~galactose~~ hexoses as rapidly as lactose!)

Lactose of W815 of K-12.

3/2/49.

Harvest cells from 1l. W815 in aerated Y2-Lac 24h. Wash and dry over P_2O_5. Yield 442 mg. Test for lactose fermentation and compare with K-12 freshly prepared in same way. (yield 360mg).

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

Add 1cc cells, 1cc $M/100\ KPO_4$ 7.0, 1cc 5% substrate and incubate at 37°.

10:45

	Substr	30m.	4^h30	
K	Lac	+++	✓	Glu-1-P
K	Glu+Gal	+++	✓	
W	Lac	—	—	Glu-1-P
W	Glu+Gal	—	—	

Apparently, the fermentation of lactose in W815 does not tolerate drying as does that of K-12.

Use ½ quantities + 1% Glu-1-P, start at 3:15PM