Suppressor bacteria

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

Jan. 10, 1949.

Lactose adaptation in W-112 (Lac_i)

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 7/100 Na Pbuffer + BCP
1 ml 5% sugar. 2 hours reads

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

Checks by streaking out cells used.

Lac_i — produces lactase with butyl galactoside but not with lactose. Cf. Cohn'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 in 0.1%.

Lactose in W-108.

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

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

Harvest + test:

a) spot plate ONPG: B: +++ L: —

b). E .1ml 7/50 KP buffer pH7.0. 1ml cells (2x) 1ml 3% sugar.

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

Note adaptation to glucose! Cf. W327 which does not adapt on mea
With respect to lactose, W108 is like W112. Non-reactive but can form c

Rb inhibition of lactase

a) Add ONPG to enzyme-buffer. NaPH/100. 7.5 (RbClH/50) ONPG mg/100.

b) "enzyme ONPG".

			10^{-3} mol.	
a)	1	319A	—	510
	2	"	Rb	470
	3	395	—	680
	4	"	Rb	630
	5	394	—	310
	6	"	Rb	309
b)	7	319		660
	8	"	Rb	650.

∴ no appreciable inhibition!

Repeat comparing fresh solution of RbCl. 319H/2000 mg

—	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.	CoCl	Na	393		10		
4.	RbCl new	Na	316		28		
5.	—	K	239		—(45)		
6.	RbCl old	K	220		08		
7.	CoCl	K	182		24		
8.	RbCl new	K	100		58		

Rb inhibition

January 14, 1949.

	t/s	1/v.	Δ			Di		1/v corrected (+ 1/3).
NaP								
	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	188		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

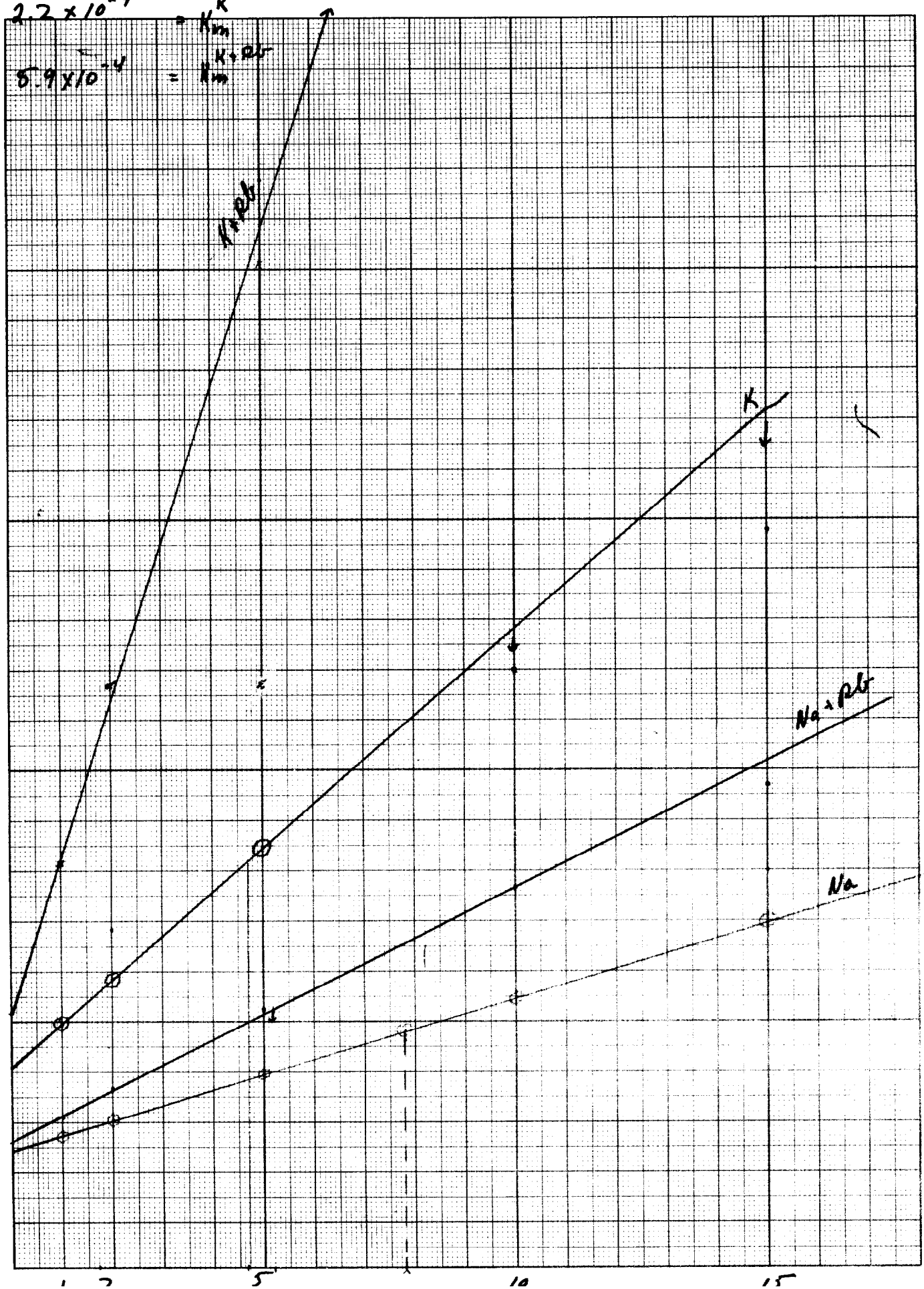
$1.28 \times 10^{-4} = K_m^{Na}$

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

$8.9 \times 10^{-4} = K_m^{K+Rb}$

$1.92 = K_m^{Na+Rb}$

411



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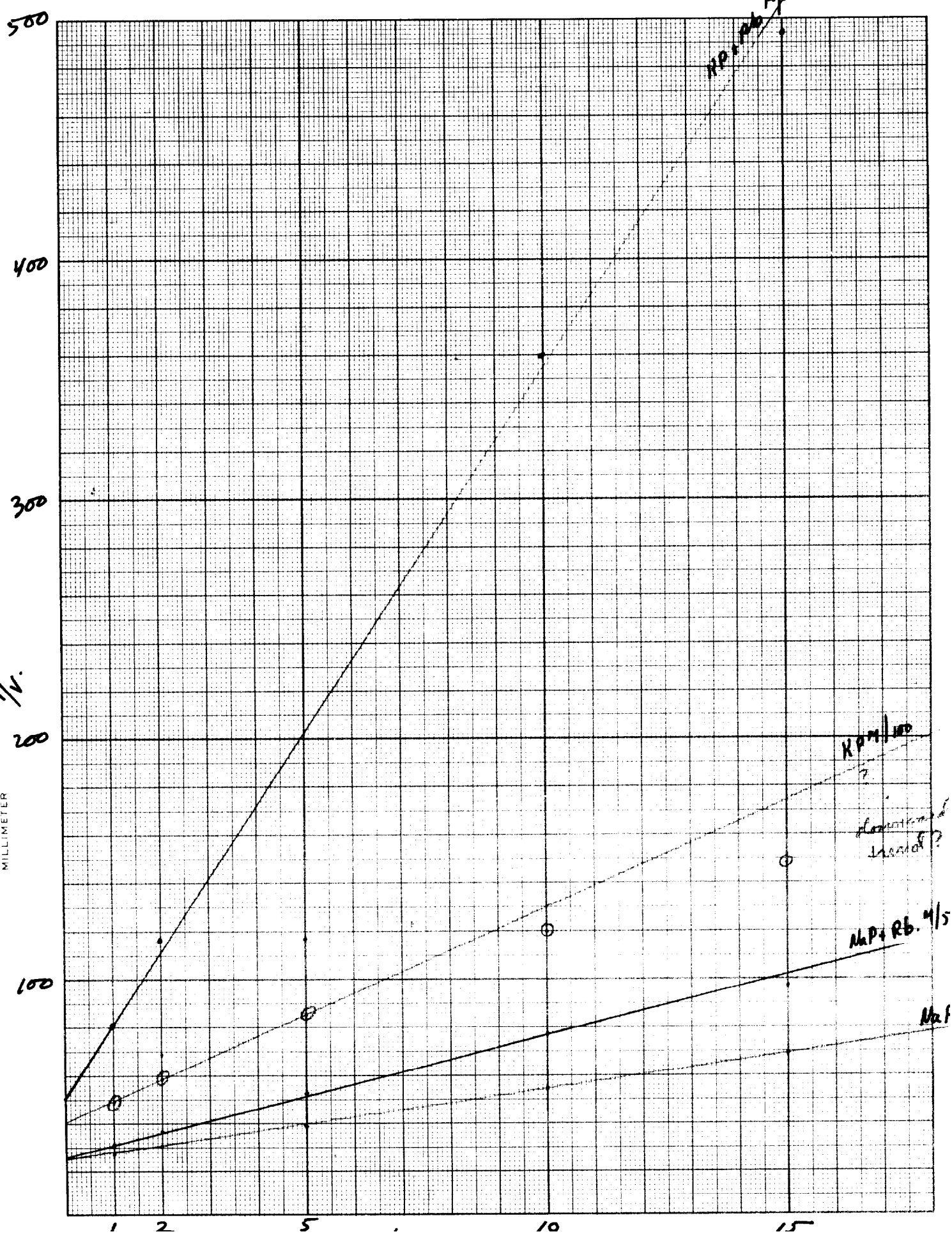

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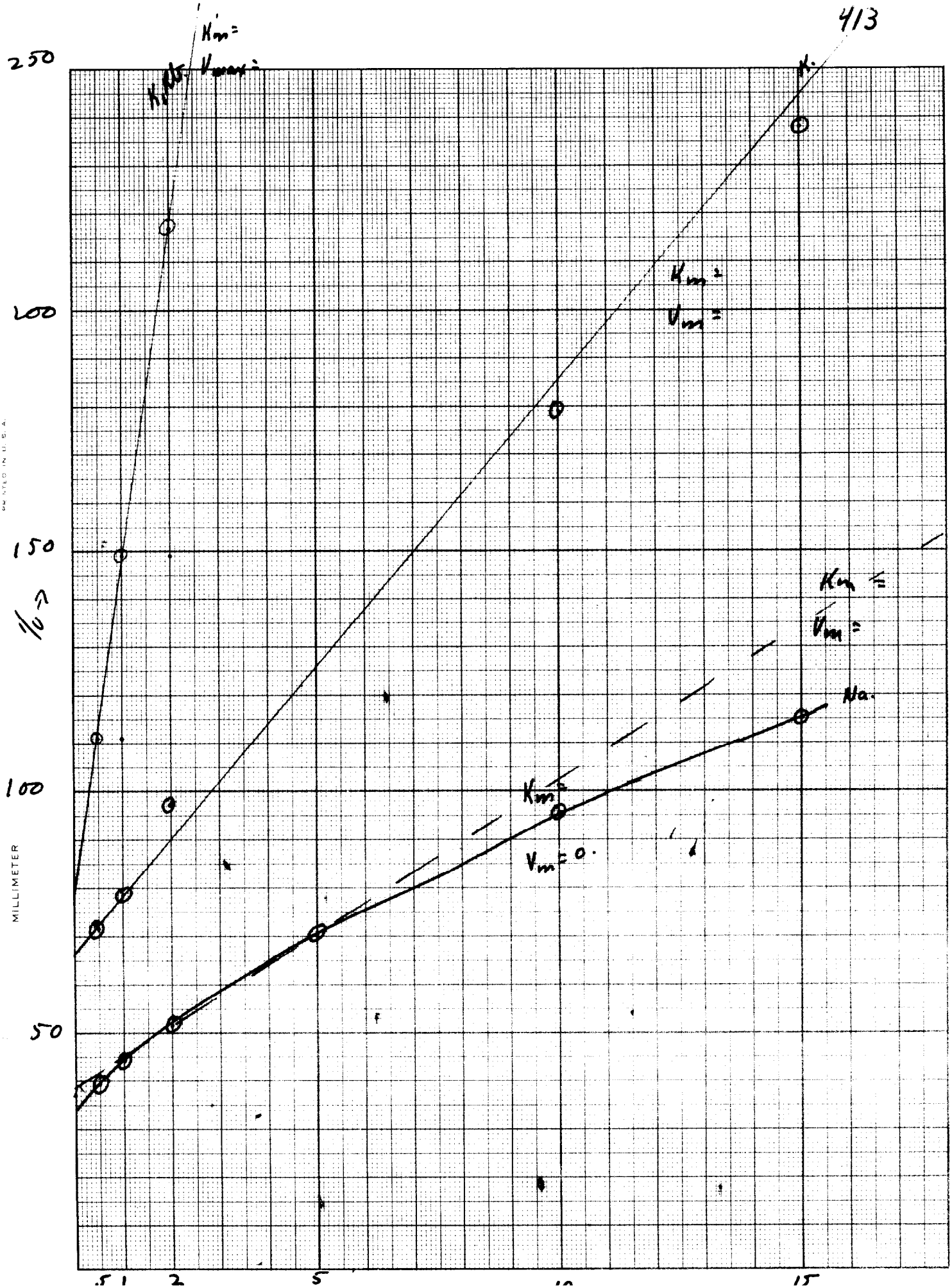
1/V.



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V_{max} / K'_m 411 Leta 413.

$$K'_s = K_m + k_a V_{max}$$

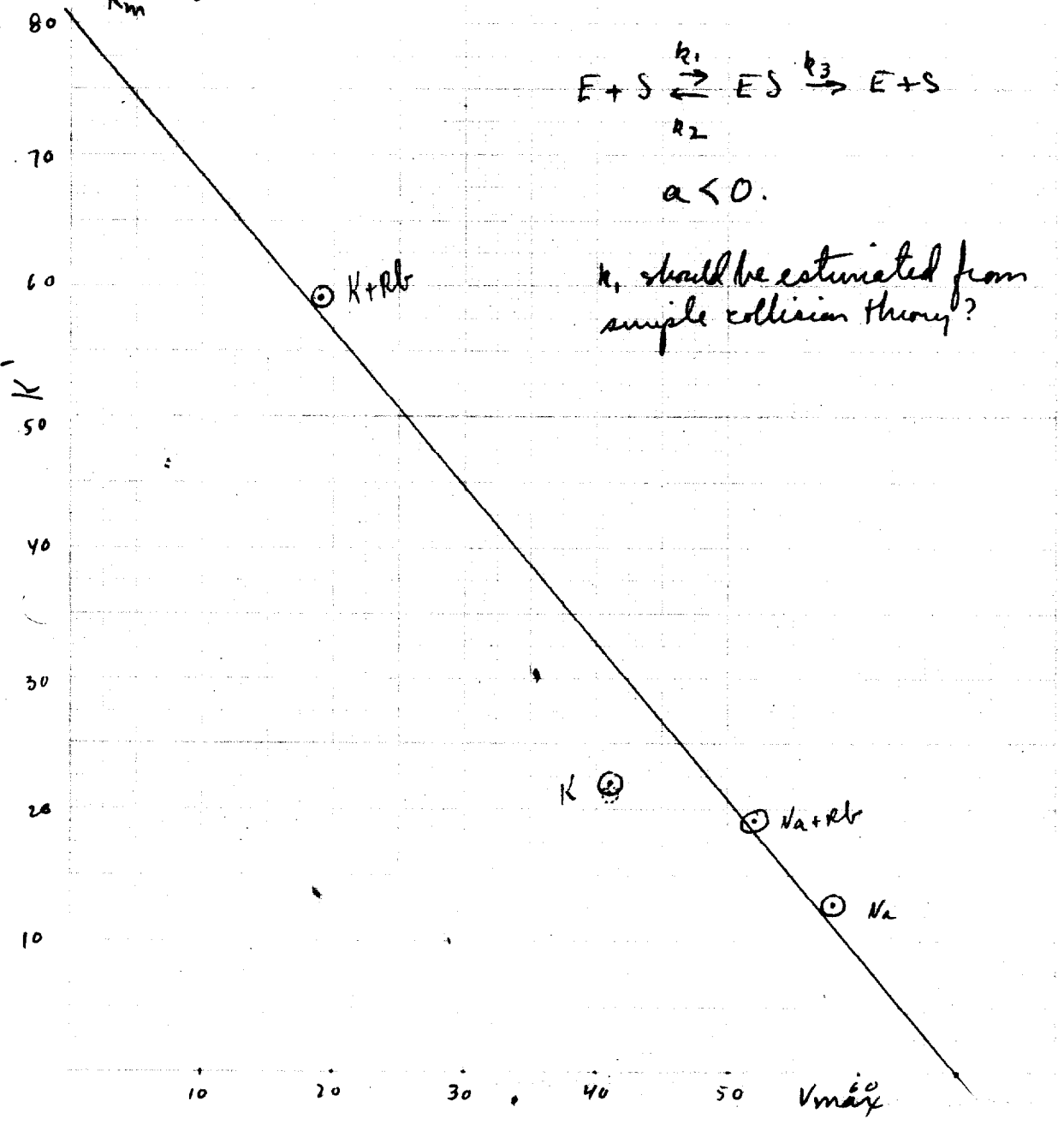
k_a = " − 1.2 "

$K_m = 80$

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

$a < 0$.

k_1 should be estimated from simple collision theory?



Rb inhibition

January 16, 1949.

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

$K_m' = K_m + \frac{k_3}{k_1}$. 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.

~~$\frac{1}{V} = \frac{1}{V_{max}}\left(\frac{K_s}{S} - 1\right)$~~ 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
	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 pups stood at room temperature ~~at room~~ for several hours. This may acc't for the exc. variation seen.

Note. 5 ÷

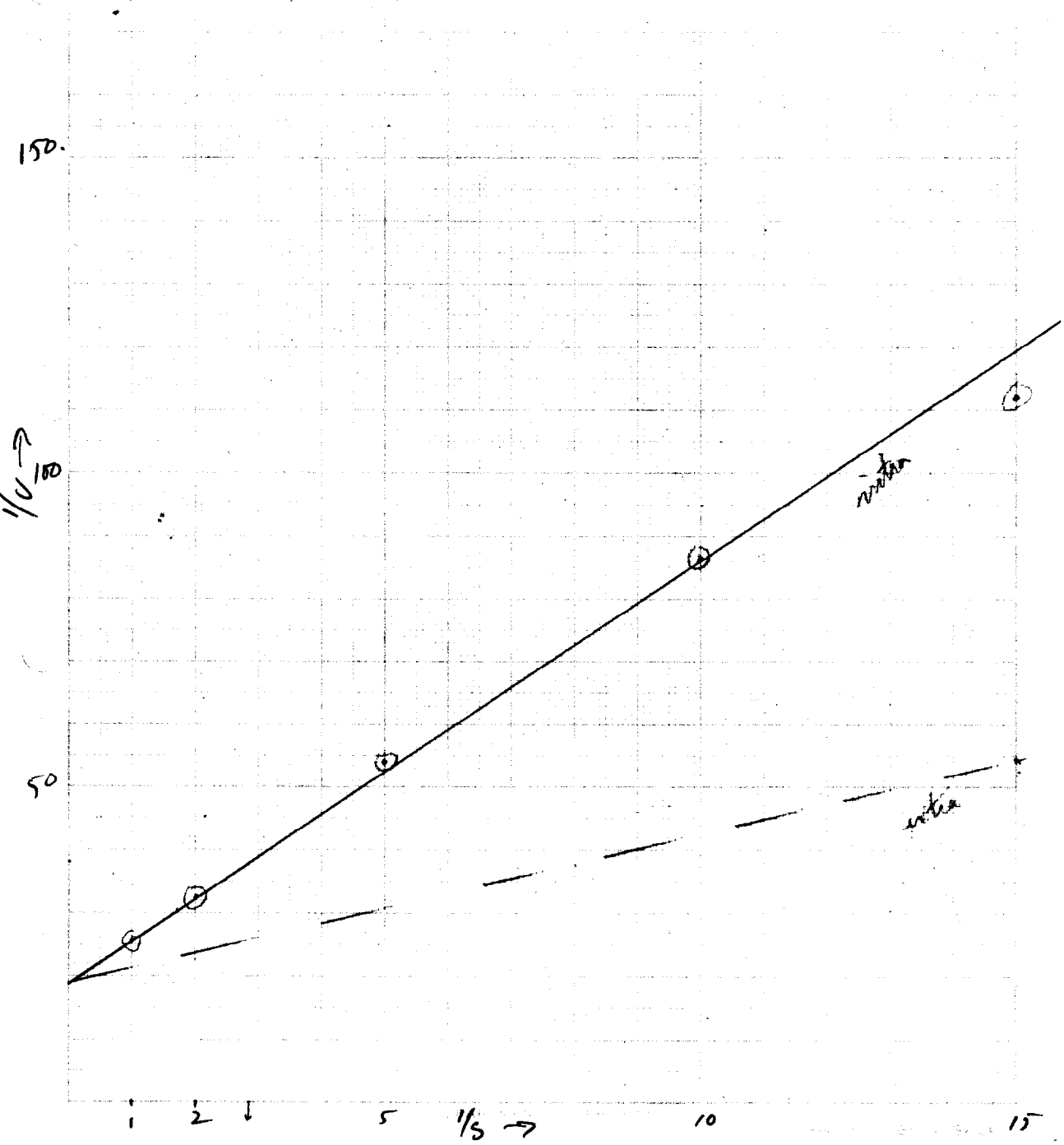
Note M/[illegible] [illegible] ÷

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

$V_{max} = 527$

Kinetics of intracellular galactosidase.

Na P buffer pH 7.5 M/100.



Jan. 17-18, 1949.

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

Preliminary assay: 10 mins in NaP M/100 7.5

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

Ca 40u/ml. Relative activity 20M. 4

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

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

	pH	Δ		kr.
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.

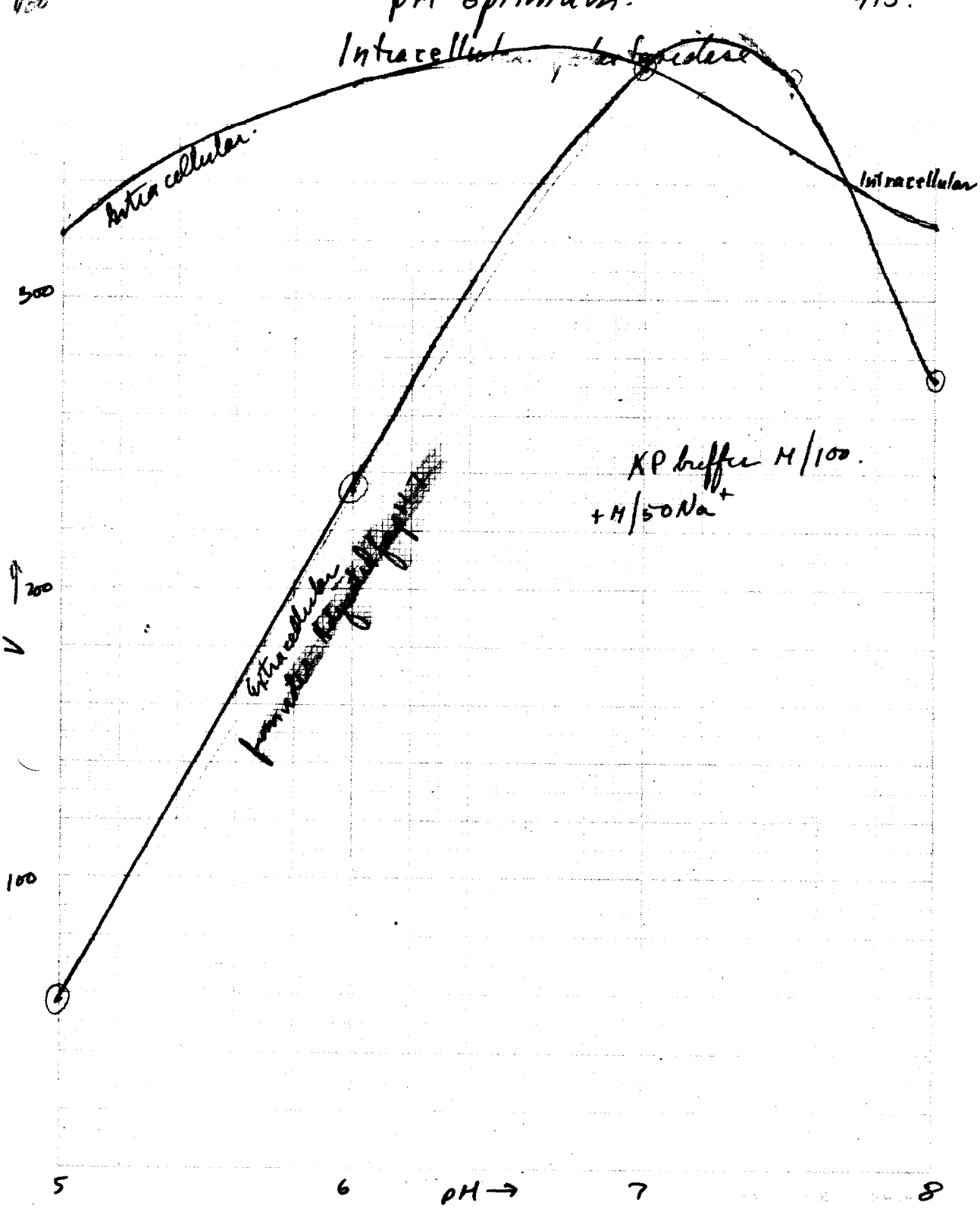
6.	K buffer M/100.	185	191	006
7.	" + Rb M/50	163	169	006
8.	Na Buffer.	181	183	002

c) Kinetics. Na buffer M/100. 7.5 V_{max} = 527, K_m = 4.5×10^{-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 — alla 090

pH optimum.


Intracellular
Intracellular
Intracellular
XP buffer M/100.
+M/50 Na+
400
300
200
100
V
5
6
pH →
7
8

Adaptation of ML; K-12 on galactose

		Δ_1	Δ_2	[12:45 AM] Corrected:		20 m.		315 PM	20 m. R.A.	37. R.A.
Mhm.	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-12	gal	60	424	~~116~~ ≡	129	176		540	52	49
	lac	~~128~~	—	111	123	339		970	206	—
	glu	002.	30	117	130	119		147	~~<2~~ <2	3.4

blanks —

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

Galactose therefore has ca. 14× activity

for ML Lac/Gal = 16

K-12 Lac/Gal = 4

Mutant adaptations to galactose.

1/22/49.

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

	Lac	✓	Gal	✓	~~Glu~~ Bug.	✓	~~Bugal.~~ Glu	✓
K-12	114 680 514		150 298 120		147 1000 590		BCP ++ 120 ~~1000~~ 131 -010	
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 —
R.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

	Di	5:20 PM Di cor.	Df	Δ	Δ/Di cor = R.A.	7:15 PM Df	Δ'	R.A.
✓ Glucose	141	135	139	-004		147	008	
✓ Galactose	187	250	180	+070	39	810	630	—
✓ Lactose	153	470	150	320	213	1150	1000	—
(Na) Mucate	320	318	300	018	(006)	490	190	00
✓ (Na) Galactonate	180	191	174	017	(010)	285	111	01
Na Lactobionate	180	348	174	174	100	940	766	—
Dulcitol	~~144~~ 83	97	87	010	(011)	155	68	01
✓ 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 c
Galactose	23 %	
Dulcitol	4 %	
Galactonate	3 %	
Mucate	3 %	

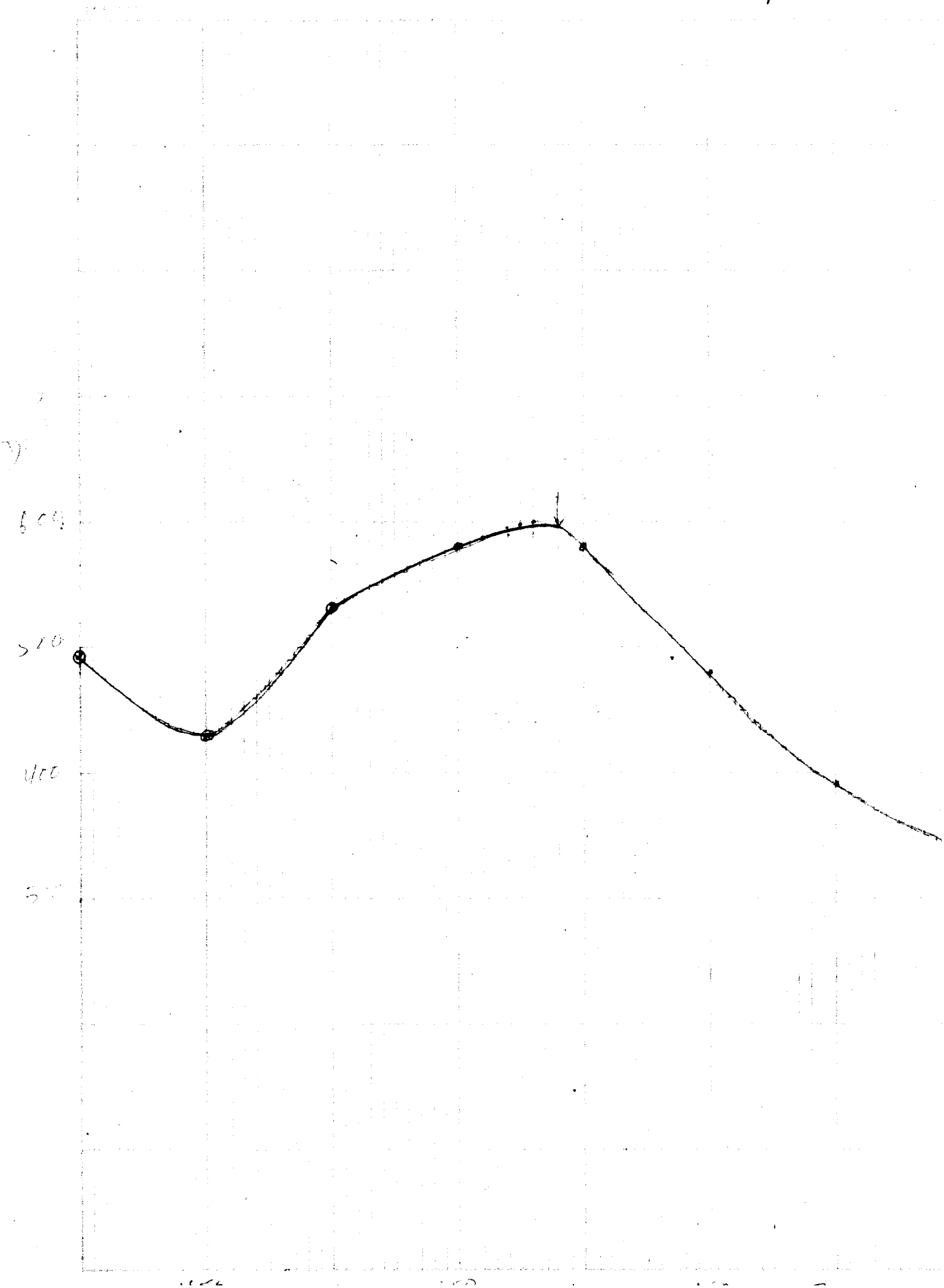
Absorption spectrum of E. coli + formazan. (tetrazolium)

4 23.

1	d
400	491
450	430
500	533
550	581
600	551
650	450
700	390
750	340
800	310
560	589
575	599
590	597
(580)	600
585	598
570	590

Jan 25, 1949.

Read 11-12 / Glucose in glucose buffer with 0.02% tetraphenyl tetrazolium, and study absorption spectrum. Peak at $\lambda = 5800$ Å but not very sharp.



Sugar utilization by W815.

Feb. 28, 1949.

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

Test 1 ml cells + 1 ml 5% sugar + 1 ml 4/100 buffer + BCP.

Time (m).	L/Lac	L/Glu	L/Gal	G/Lac	G/Glu
15	+	−	∓	−	−
20	+++	±	+	±	±
~~30~~ 25	+++	±	+	±	±
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. + ket = M/2000 ONPG KP 7.5 M/50.

	$D_{\frac{1}{2}}^{420}$	corr.	D+	R.A.
Glu	300	270	280	< [redacted]
Lac.	436	—	>> 1000.	> 300

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

Lactase of W815 cf. K-12.

3/2/49.

Harvest cells from 1 l. W815 aerated Y2 - Lac 24 h. 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 360 mg).

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

Add 1 cc cells, 1 cc M/100 PO_4 7.0, 1 cc 5% substrate and incubate at

10:45

	Substr	30 m.	4 h 30	
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 dyes as does that of K-12.

Use 1/2 quantities + 1% Glu-1-P_1, start at 3:15 PM