"Effective intracellular pH"

11/19/48.

To determine whether the intracellular buffering capacity might influence activity determinations, Set up cells A) 1 c E coli K-12, O.D. λ420, =1.00; B) do. +2M/5000 ONP + c) ONP only in acetate buffer .04M, pH4.0. Compare readings (as O.D.).

$A_1 - A_2$	.007	(enzyme).
$B_1 - A_1$	.124	If anything, the apparent absorption by
$B_2 - A_1$	.124	ONP was less in the cells than without
$B_1 - A_2$	.138	This may be due to scattering.
$B_2 - A_2$	.138	
C_1	.151	
C_2	.153.	

Lactase pH optimum

	Type	pH.	$D^{+}_{420.}$
1	A	4.0	009
2	A	5.0	011
3	A	5.5	024
4	P	5.0	028
5	P	6.0	193
6	P	7.0	190
7	P	7.5	166
8	P	8.0	186
9.	P	8.0	116.

maltin buffer?

no enzyme

acetate M/100 and K. phosphate buffer M/50 ~~at M/100~~

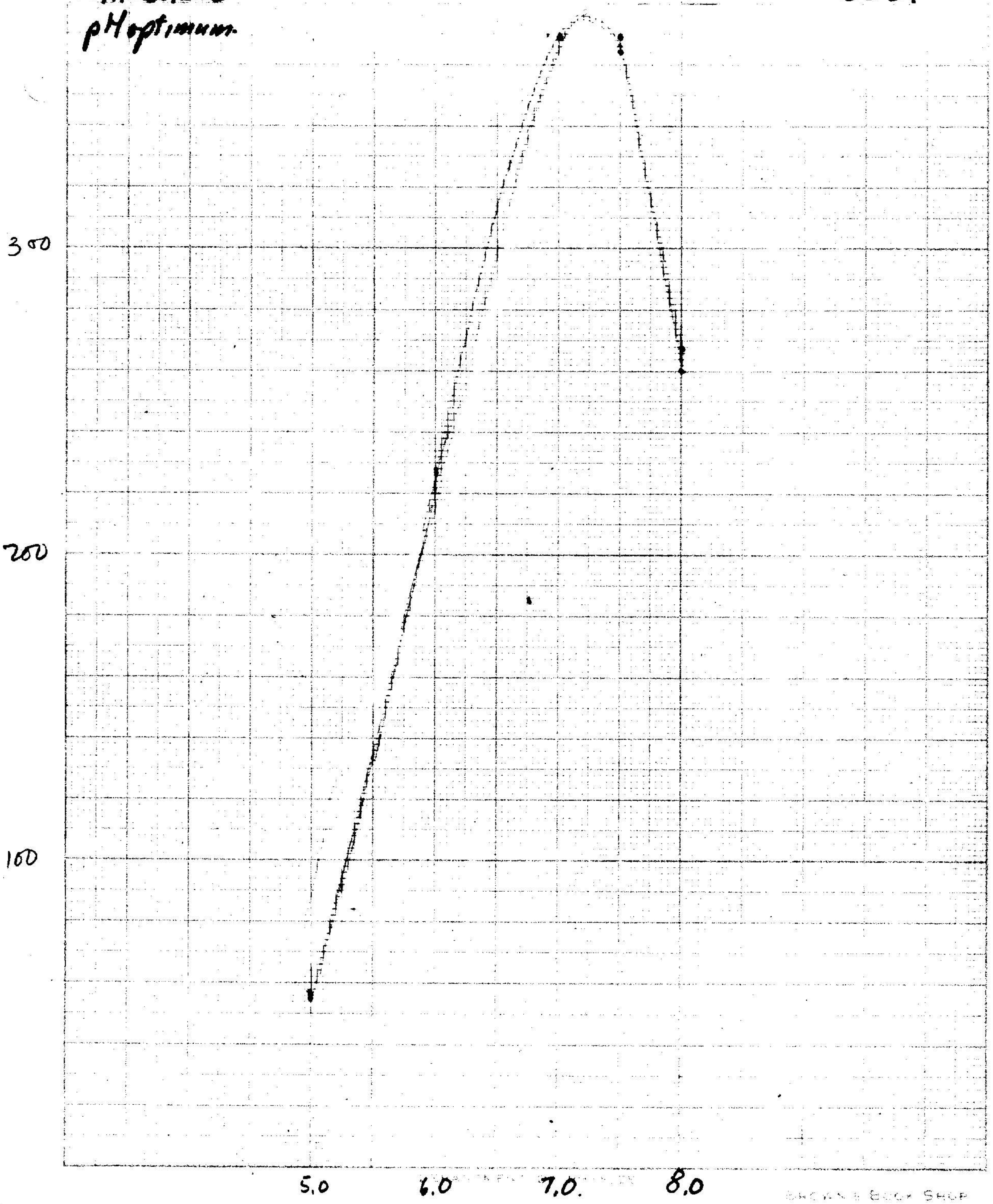
Na_2SO_4 M/50.

Make up to 9 ml ; at t add 1 ml Na_2CO_3 M/10 to alkalanize at M/10 Na_2CO_3

o NPG M/2000 219A 10^{-3} 20 min. pH > 10.

Repeat, using phosphate buffer only!

319A Lactase
pH optimum.



pH optimum - colitectase

11/18/48.

B19A Na_2SO_4 N/50 KP buffer M/50. ONPG M/2000. 20m 38°

Duplicate tubes. Add Na_2CO_3 M/10 at conclusion:

pH	D_t
5.0	057
5.0	055
6.0	.228
6.0	227
7.0	369
7.0	369
7.5	364
7.5	369
8.0	268
8.0	260

AA antagonists and adaptation.

11/27/48.

Harvest K-12 from 200 ml 1/2 glucose shaken overnight and resuspend in 40 ml 1/5 Na^{Ph} buffer 7.5.

Set up adaptation systems to 5 ml/tube:

2 ml cell suspension
2 ml lactose 4%
11:30 AM - supplement + H_2O qs 1 ml. / 5 ml.

MT = 5 methyl trypt.
A = arginine
C = canavanine sulfate
T = tryptophane

#	Suppl.	Cell dens.	
1.	—	160	170
2.	—	~~055~~ 190	192
~~3. 5MT 500r~~			
~~4. 5MT 500r~~			
~~5. T~~			
~~6. T~~		060	172
7. C		140	179
8. C		~~140~~ 171	~~177~~ 171
9. A	"	190	~~170~~ 171
10. A	"	199	161
11. T+5MT	gelatinous		
12. T+5MT	pH > 8-9		
13. C+A	Di = 178 381	A_{cn} =	
14. C+A		159	170
16. A+5MT			
16. C+T.			

No inhibition by canavanine

Resuspend in 4 ml and use 1 ml in 10 ml colorimeter tubes, in M/50 buffer. M/2000 ONPG. Matched against corresponding suspensions - ONPG. except #13

12/8/48.

100 gms. alfalfa seed were allowed to germinate 2-3 days, then dried and ground.

Prep. A. 5 gms. meal were shaken 3h. c 10 ml H_2O. The debris was sedimented and supernatant diluted to ca 10 ml. (galacosid A).
Enzyme tests at pH 4.0 Na acetate buffer M/100 (after Veibel who showed optimum at 3.4). He finds Km for [illegible] galactoside as < 10^{-3}, which is limit of determination.

Assay preparation A; 20 min determinations.

.01 ml	ca 050
.10 ml	ca 500.
1 ml	>> 1.9

Inhibition by Rb + stim by Sodium. In M/100 Acetate buffer. Salts M/50 each. Terminate c M/1 Na_2CO_3 1 ml.

	salt	D_{420}.
1.	No enzyme	[illegible]
2.	—	167 ✓
3.	Na	248 ✓ (av. [illegible])
4.	Rb	196
5.	Na+Rb	212.

may be a chloride effect

1	—	220 ✓	Note appa stimulatory
2	NaCl	250	
3	Na_2SO_4	270	Na_2SO_4

Lactose : competition with ~~galactosidase~~ ONPG on ~~...~~ alfalfa lactase

12/9/48.

Run ONPG conc. series $\bar{c}$ various lactose concentrations.

10 ml   ~~.001~~ .05 ml ~~...A~~ 381A   add 1 ml Na_2CO_3 to terminate. in M/100 ~~...~~ NaAC pH 4.

20 m.

	ONPG M/	Lac M/	Di	Df	Δ	1/v
1	2000	00		182		
2	5000	00		123		
3	10 000	00		79		
4	20000	00		58		
11	2000	2000		171		
12	5000	"		131		
13	10000	"		82		
14	20000	"		53		
21	2000	1000		173		
22	5 "	"		120		
23	10 "	"		80		
24	20 "	"		59		
31	2 "	500		178		
32	5 "	"		116		
33	10 "	"		76		
34	20 "	"		53		

correct D: by ~~10/12~~ 9/11 for addition of enzyme and of substrate.

Alfalfa lactase is not appreciably bound by these concentrations of lactose. i.e. $K_L > 40\, K_{ONPG}$

Barley Seedling lactase

12/8/48.

Seedlings from Dr. Nancy Kent.

		D_1	D_2	Δ
A.	Grown on lactose, 6 seedlings, ca. 3cm long.	140	200	60
B.	sucrose, 3" short 13cm long	310	410	100

Ground in water in distilled water, 5ml. Without separation, test both samples ~~E~~ ONPG at pH 4 ea in ~~the~~ alfalfa system. Incubate at 37° 10:35 AM - 11 AM

∴ Barley lactase is constitutive

12/10/48. Qualitative tests on malt extract show no lactase activity.

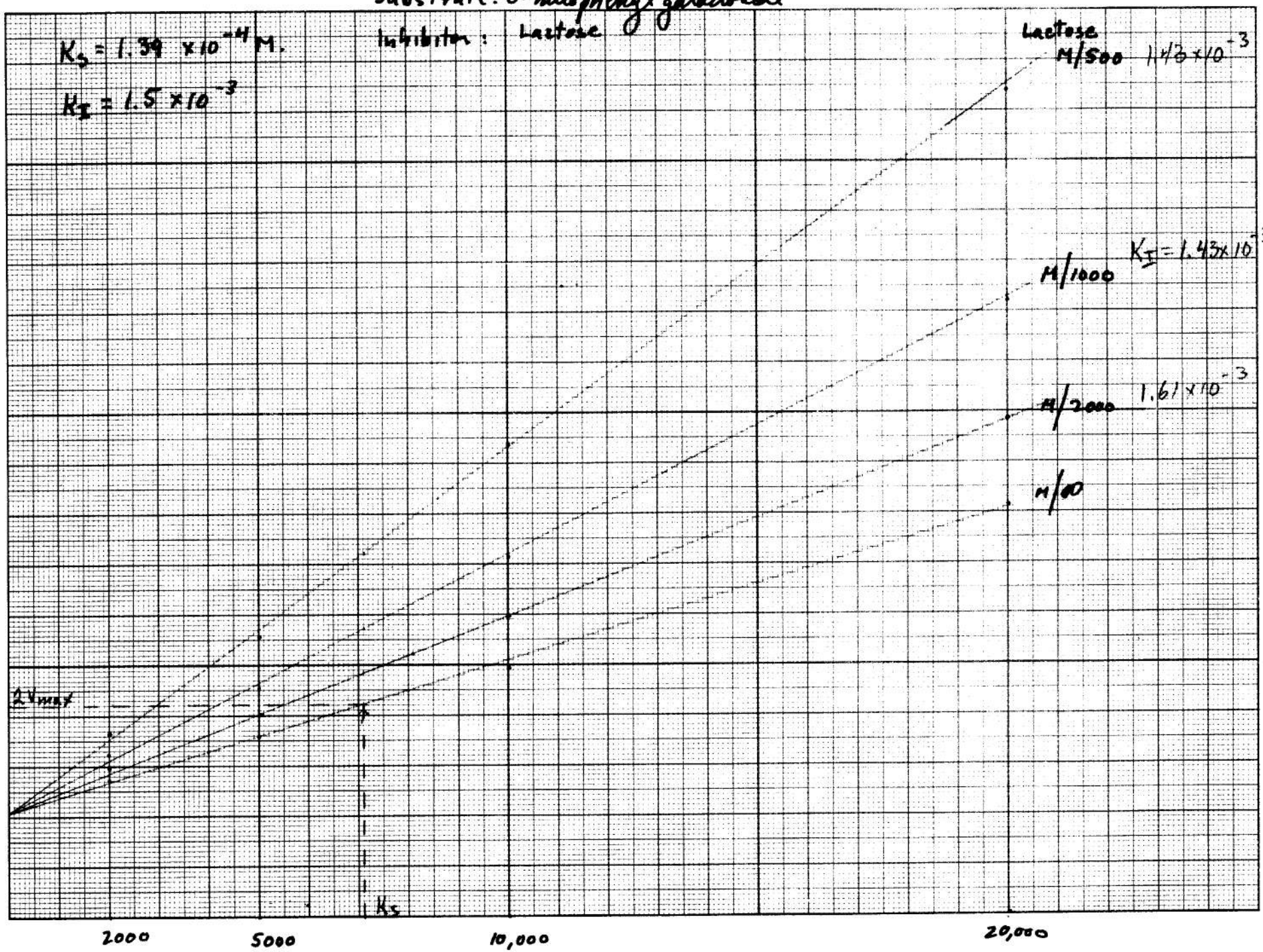
Competitive inhibition of coli lactase

December 10, 1948.

Set up as 383. .002 ml 319A. mM/50 Na_2HPO_4 7.5. 10 mins. 37°

	(1/s) ONPG M/1000	Lac M/100	ΔD_{420}.	1/v
1	2	∞	369	27.1
2	5	"	279	35.9
3	10	"	203	49.3
4	20	"	123	81.3
11	2	20	340	29.4
12	5	"	250	40.0
13	10	"	169	59.2
14	20	"	102	98.0
21 ~~15~~	2	10	311	32.2
22 ~~16~~	5	"	221	45.2
23 17	10	"	140	71.5
24 18	20	"	82	122.0
31	2	5	274	36.5
32	5	"	180	55.5
33	10	"	107	93.5
34	20	"	61	164.0

Substrate: o-nitrophenyl galactoside



Kinetics of inhibition of coli lactase with glucose

Dec. 11, 1948.

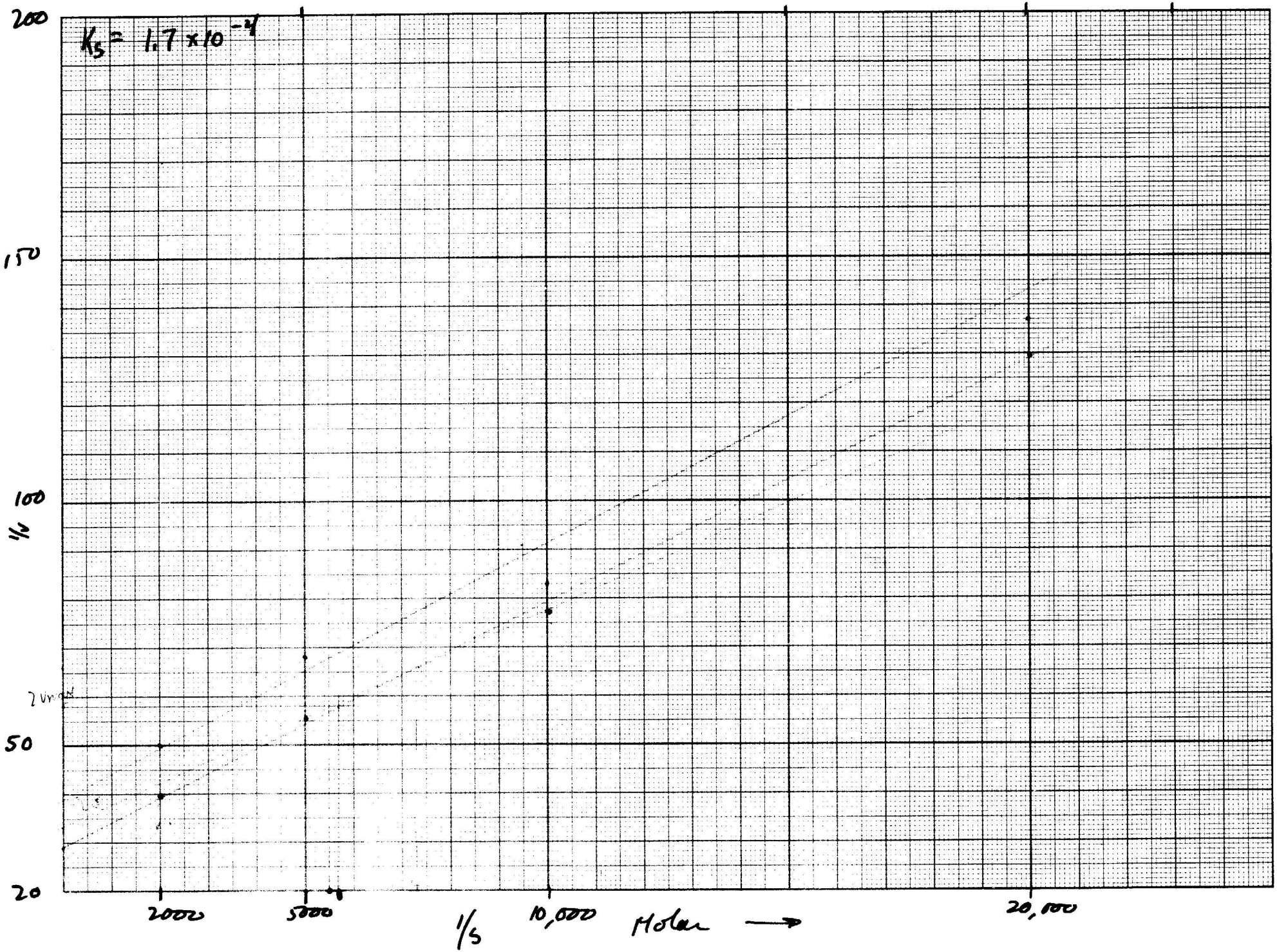
Set up parallel to 384. But use .001 ml enzyme; 20 mins.

	ONPG M/1000	Glu M/10		ΔD.	1/v	D_i	D_f
1	2	∞		252	39.7	10	262
2	5	"		180	~~44.0~~ 55.5	3	183
3	10	"		129	77.5	1	130
4	20	"		77	129.9	-2	75
11	2	20		244	41.0	10	254
12	5	"		173	57.8	5	178
13	10	"		127	78.7	1	128
14	20	"		78	128.2	0	~~75~~ 78
21	2	10		197	50.7	13	210
22	5	"		158	63.3	2	160
23	10	"		111	90.1	2	113
24	20	"		63	158.7	3	66
31	2	5		200	50.0	11	211
32	5	"		147	68.0	1	148
33	10	"		120	83.3	1	121
34	20	"		73	137	2	75
		RbCl M/50					
41	2	"		249	40.2	9	258
42	5	"		183	54.6	6	189
43	10	"		129	77.5	0	129
44	20	"		78	128.2	-2	76

RbCl is not measurably inhibitory with this concentration of (Na). Glucose at M/50 is only very slightly inhibitory, and not, as far as can be seen, competitively. Retest at M/10. The competitive reaction may be, conceivably, $2G + E \rightleftarrows EG_2$

K_s estimate here is 1.7×10^{-4}.

Note } Glucose here used soon after solution in H_2O; lactose in previous expts. had been standing a couple of days.


$K_s = 1.7 \times 10^{-4}$
200
150
100
1/s
50
20
2000
5000
10,000
20,000
1/s
Molar

Glucose inhibition of lactase.

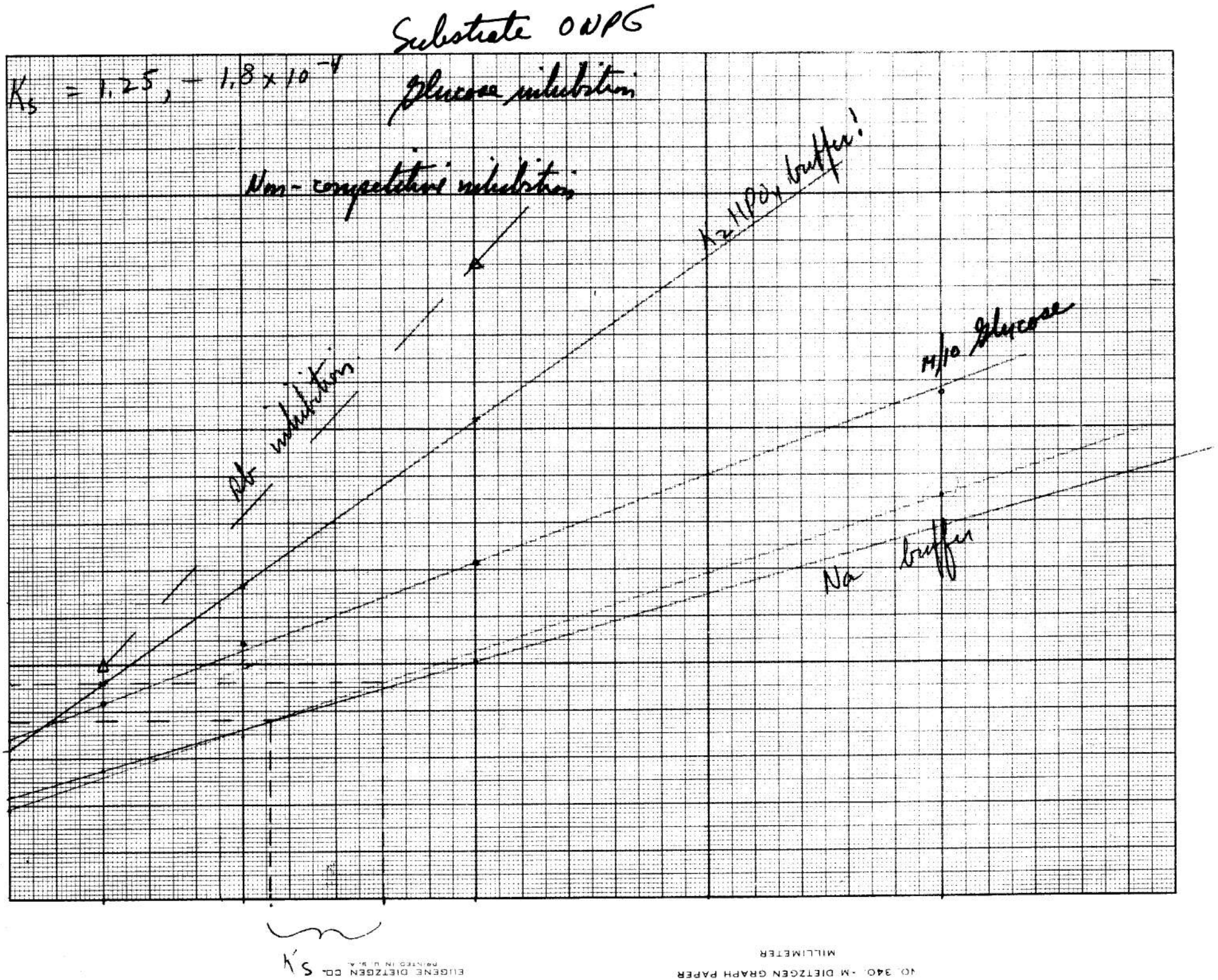
12/11/48.

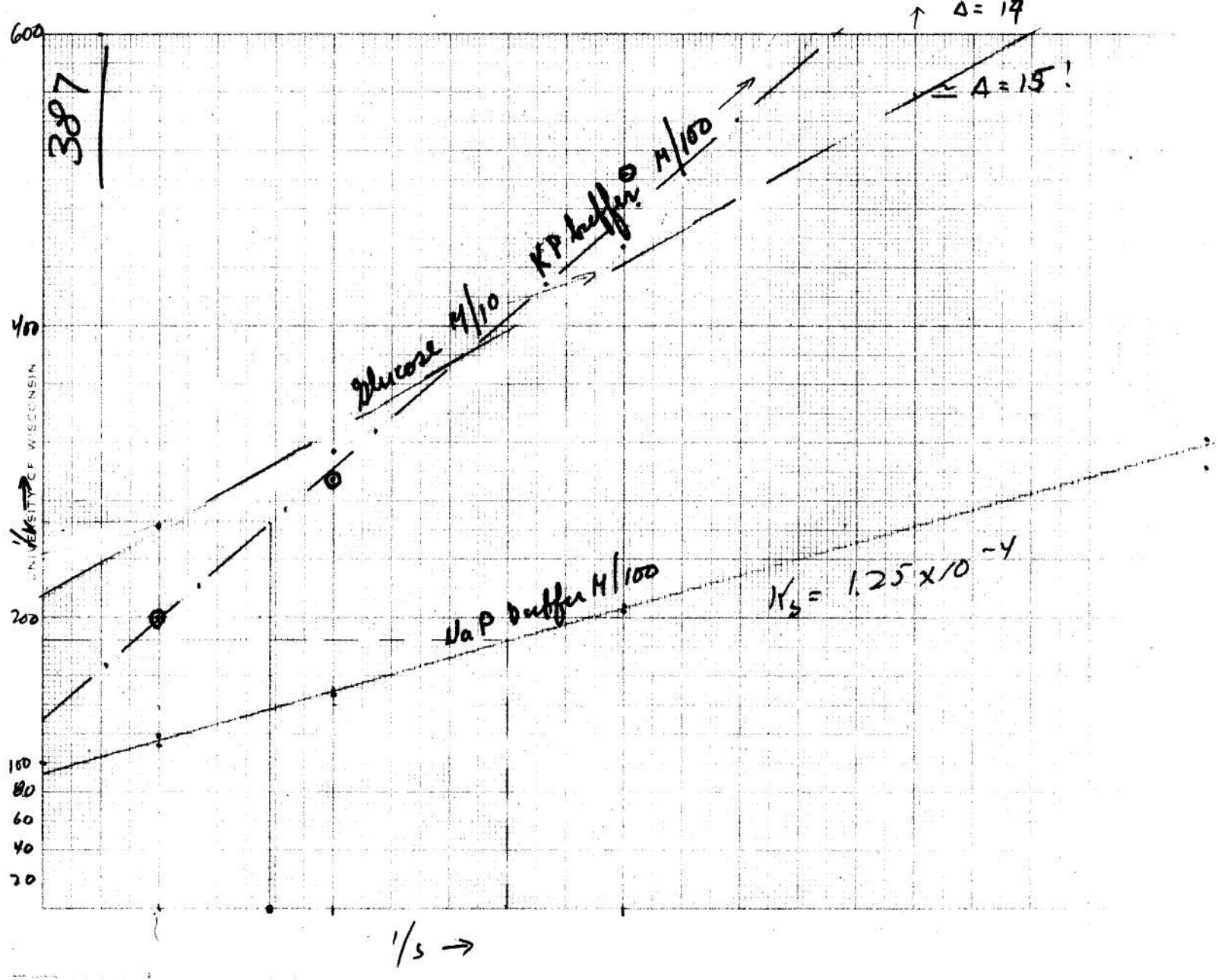
Ls 385. .002ml 10 Mins. Val 7.5 M/50.

Compare 0 and M/10 Glucose at various concentrations.

	ONPG	Glu		1/V	
1	2	—	365	27.4	
2	5	—	290	34.5	✓
3	10	—	197	50.8	
4	20	—	117	85.5	
11	2	M/10	239	41.8	✓
12	5	"	184	54.3	
13	10	"	140	71.4	
14	20	"	93	107.5	
		RbCl			KP 7.5 M/100
21	2	—	218	45.9	
22	5	—	150	66.7	✓
23	10	—	98	102.0	
24	20	—	57	175.4	
31	2	M/50		142 × 1.4	
32	5	"			
33	10	"		74	135.1
34.	20	"		40	250

If these data are acceptable, glucose may be a non-competitive inhibitor, especially at these high concentrations M/10. It may also be noted that low buffer concentration, i.e., K_2HPO_4 buffer, affects not only Vmax, quite appreciably, but also the K_S!! Rb may accentuate this response!


Substrate ONPG
$K_s = 1.25, -1.8 \times 10^{-4}$
glucose inhibition
Un-competitive inhibition
K₂HPO₄ buffer
1/10 glucose
No buffer
1/v
1/s
K's


3887
600
400
200
100
80
60
40
20
1/Velocity →
1/s →
Δ = 14
Δ = 15 !
Glucose M/10 KP buffer M/100
Vap buffer M/100
$K_2 = 1.25 \times 10^{-4}$

December 13, 1948.

		ONPG. Suppl. M/-1000.	Buffer: NaP M/800	1/V	δ	F	Δ = V
1.	2	"	"	112	10	99	89
2.	5		K_S 1.25×10⁻²	147	2	70	68
3.	10		V_{max} = 109	208	—	48	48
4.	20			303	-3	30	33
			Glucose NaP M/800				
11.	2		M/10.	263	19	57	38
12.	5			333	8	38	30
13.	10			454	7	29	22
14.	20			714	3	17	14
			NaP M/100				
21	2			119	10	94	84
22	5			151	4	70	66
23	10			204	-3	46	49
24	20			323	0	31	31
			KP M/~~100~~50				
31	2			200	7	57	50
32	5		V_{max} = 78	244	-1	33	34
33	10		" K_S apparent" = 2.6×10⁻²	454	-3	19	22
34	20			1429	-3	10	7

Glucose inhibition non-competitive, but may be related to substrate, as is more effective at lowest substrate concentrations.

These preps. tested at too low a level of enzyme activity.

Coli lactase: Summary assays

Yeast

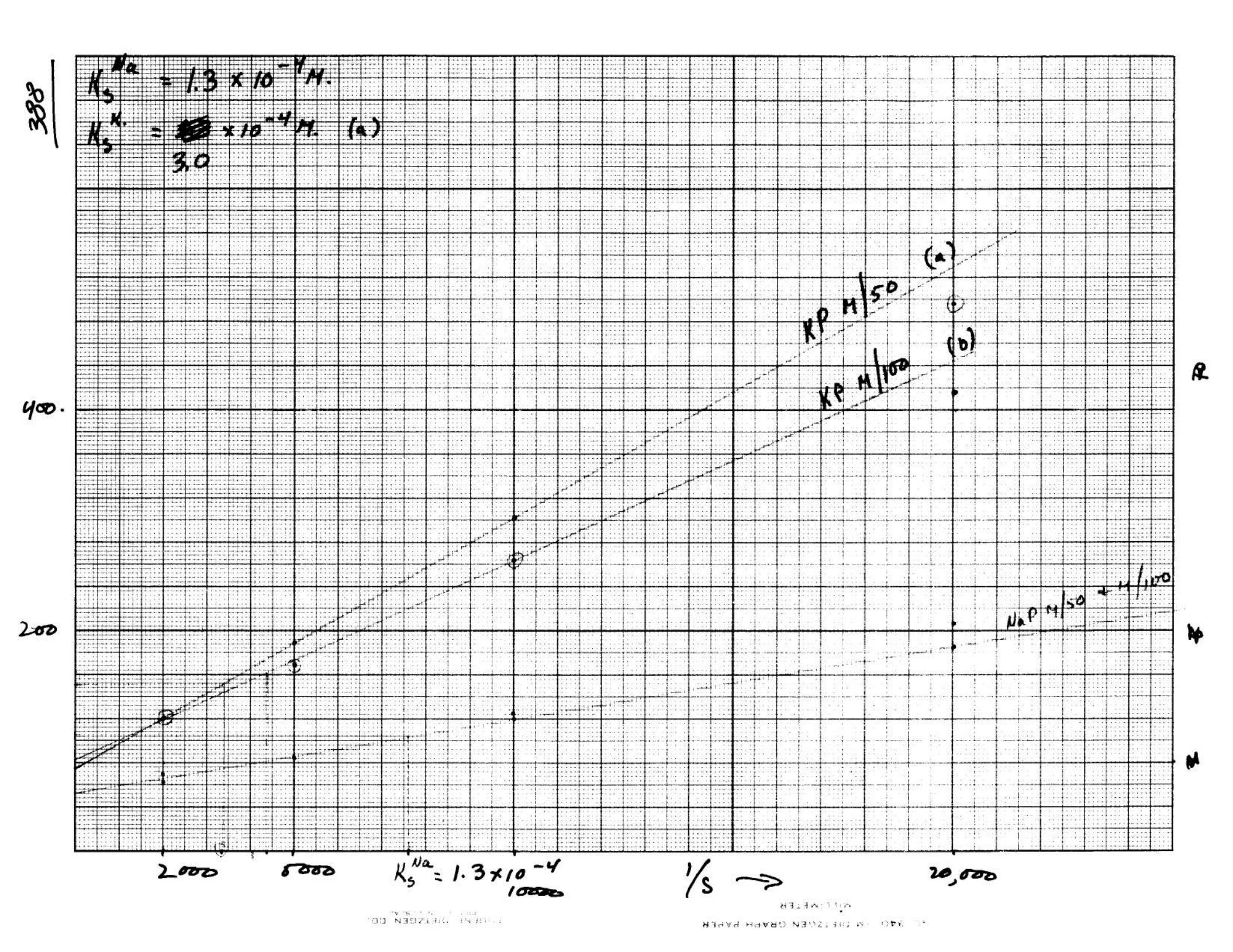
ONPG M/2000. NaP M/50. 15 min

1.	319A.	2×10^{-3} ml.	Proceeds rapidly! [illegible]	500
2.	319B.	10γ		1.8
3.	319C	2×10^{-3} ml ca 500 u/ml		70.

Torula lactosa, cells harvested from 1% Y. ext. 2% Sugar broth.

		pH.		
(B) lactose	11	4	84	Not actually valid since these are dilute suspensions.
	12	5	(")	
	13	6	(")	
	14.	7		
(D). glucose	21	4	97	
	22	5	(")	
	23	6	(")	
	24.	7	(")	

Cell density indicated by light absorption.


388
$K_s^{Na} = 1.3 \times 10^{-4} M.$
$K_s^{K} = 3.0 \times 10^{-4} M.$ (a)
400.
200
KP M/50
(a)
KP M/100
(b)
NaP M/50 & M/100
R
Rp
M
2000
5000
$K_s^{Na} = 1.3 \times 10^{-4}$
10000
1/s →
20,000

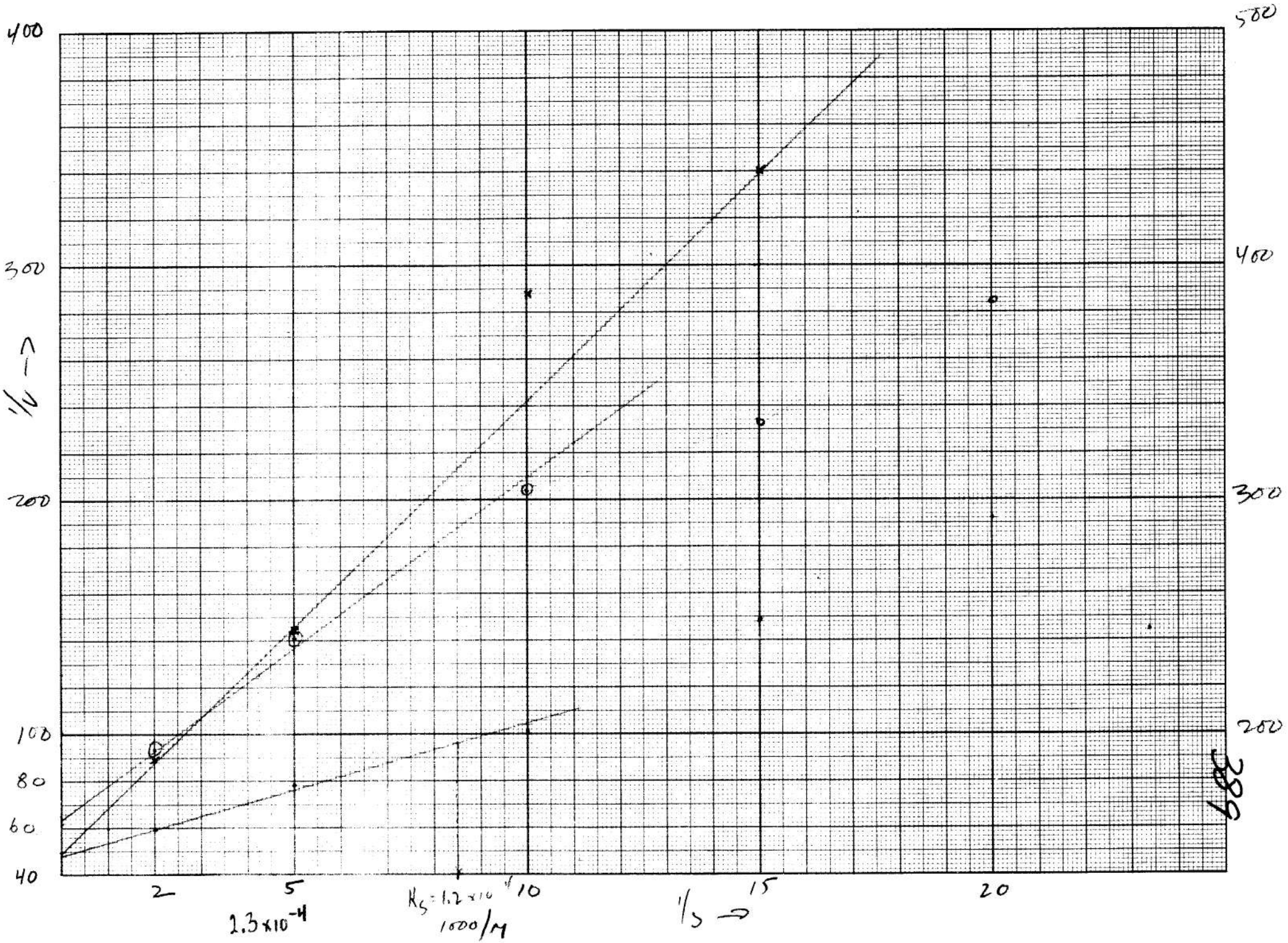
K_s / salt concentration

12/13/48. 319A 10^{-3} ml. 40 mins. Buffer pH 7.5 as indicated.

	ONPG 1000/M		1/v	I	+	Δ	
1.	2	NaPM/50	62.9	13	172	159	
2	5		84.7	4	122	118	
3	10		120.	0	83	83	
4	20		185	-3	51	54	
11	2	NaP M/100	64.9	18	172	154	
12	5		83.3	9	129	120	
13	10		125.	7	81	80	
14	20		208	3	51	48	
21	2	KP M/50	120	10	93	83	
22	5		185	10	64	54	
23	10		303	6	39	33	
24	20		417	0	24	24	(about 6 of 7.4 pH)
31	2	KP M/100	117	15	100	85	
32	5		169	3	62	59	
33	10		263	0	38	38	
34	20		476	0	21	21	

Note: Toluene added to enzyme pip's in 319A 12/12/48 to forestall gross contamination. About 50% loss of activity seems to have occurred.

K and Na definitely alter the K_s primarily. K may also have an effect on V_s



Influence of metal ions on K_s (ONPG).

Dec. 14, 1948.

	ONPG 1000/M.		1/V
	1.	2	59.5
20 min	2.	5	78.1
	3.	10	101
41 min	4.	15	149
	5.	20	192
	11.	2	93.5
20 min	12.	5	141.
	13.	10	204.
41 min	14.	15	333
	15.	20	385
	21	2	189
	22	5	345
41 min	23	10	488
	24	15	540
	25	20	769

		I	F (40 mins!)	A	A/2 Final
NaP M/50.		11	179	168	
		0	~~128~~	128	
		1	100	99	
		0	134	134	67
		-3	101	104	52
KP M/50.		10	117	107	
		0	71	71	
		1	50	49	
		0	60	60	30
		-2	50	52	26
NaP M/50 + Glucose M/10.		14	120	106	53
		6	64	58	29
		0	41	41	20.5
		0	37	37	18.5
		0	26	26	13

-1--3 20 min
-4,5 40 min

To even out some of the inaccuracies, caution was stopped at 40 mins for 1-3, 11-13 and at 80(+1) minutes for other tubes.

Glucose also causes an alteration of slope!

These data NVG enzyme prep low assay!

Used 388: 319A diluted 1:2.5

E. coli lactase.

12/17/48.

K-12 grown in 500cc Y2Lac flasks, inoculated into 2 12 liter carboys S(Lac). Yield: 110 grams Sharples paste.

Grind ca 35g. in $NaPO_4$ M/100 pH 7.5 buffer; Preserve remainder as original paste in freezer.

As grinding proceeded, noted increasing ivory-pink color. Yield, about 60 ml yellow brown opalescent supernatant with a pinkish fluorescence.

Assay for lactase. Test .01 ml and .001 ml ē 4/2000 ONPG pH 7.5 Na

E. coli lactase.

12/21/48.

A). Assay prep 319A + 390A. Na Phuffer 7.5 20 mins.

	319	390.
10^{-2}	++ 1310	290
5×10^{-3}	1100	149
10^{-3}	359	038

Study heat inactivation in Na buffer.

Tubes 1+2. 10^{-2} mC prep + buffer, incubated 90 mins. before adding substrate.

3+4. " add Na buffer just before adding substrate.

2: 189
4: 15.

Note: inactivation was irreversible, as prolonged incubation of tube 3 gave no color!

∴ 319A lactase is irreversibly inactivated by dilution in distilled water (and incubation)

December 14, 1948.

3.9A. 10^{-3} diluted some before using.

Succ 0 — Na P. M/50 pH 7.5

10 — K P " "

20 — K P " " + KCl M/50.

	ONPG	1/V	Di	Df	Δ	
0	M/1000	29.1	20	363	343	
1	2000	32.5	12	320	308	$K_s = 1.3 \times 10^{-4}$
2	5000	41.5	0	241	241	
3	10000	58.1	−4	168	172	V_{max} =
4	15000	70.4	−3	139	142	
5	20000	98.0	−2	100	102	
10	1000	51.0	23	219	196	
11	2	58.5	11	182	171	
12	5	83.3	1	121	120	
13	10	126.6	−4	75	79	
14	15	149	−3	64	67	
15	20	208	−8	40	48	
20	1000	53.5	20	207	187	
21	2	64.5	10	165	155	
22	5	97.0	0	103	103	
23	10	154	−1	64	65	
24	15	192	−2	50	52	
25	20	244	−8	33	41	

$V_{max} = \frac{1}{25} = 400.$

$V_{max} = \frac{1}{43} = 232.$

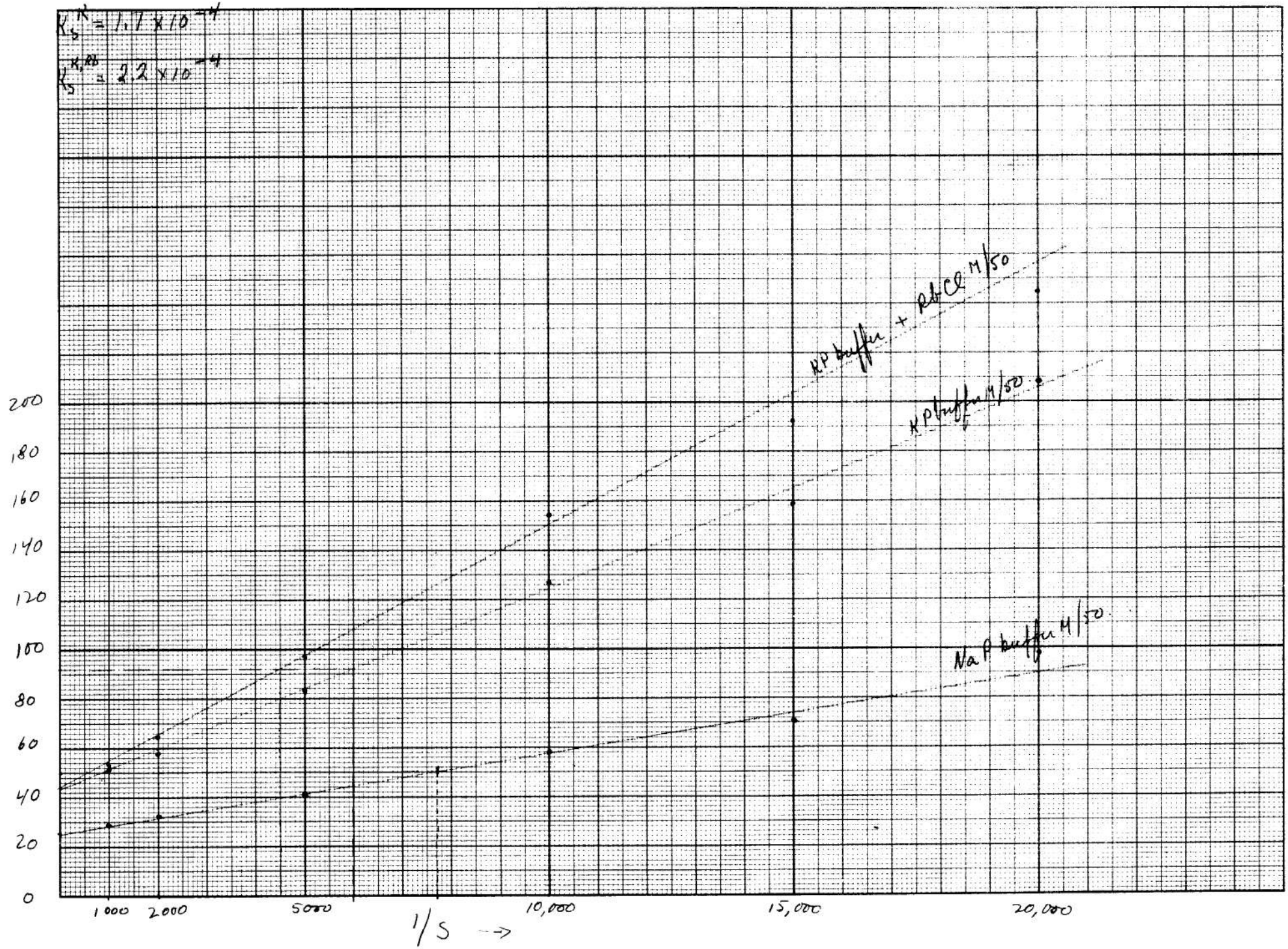
$= 58\%$

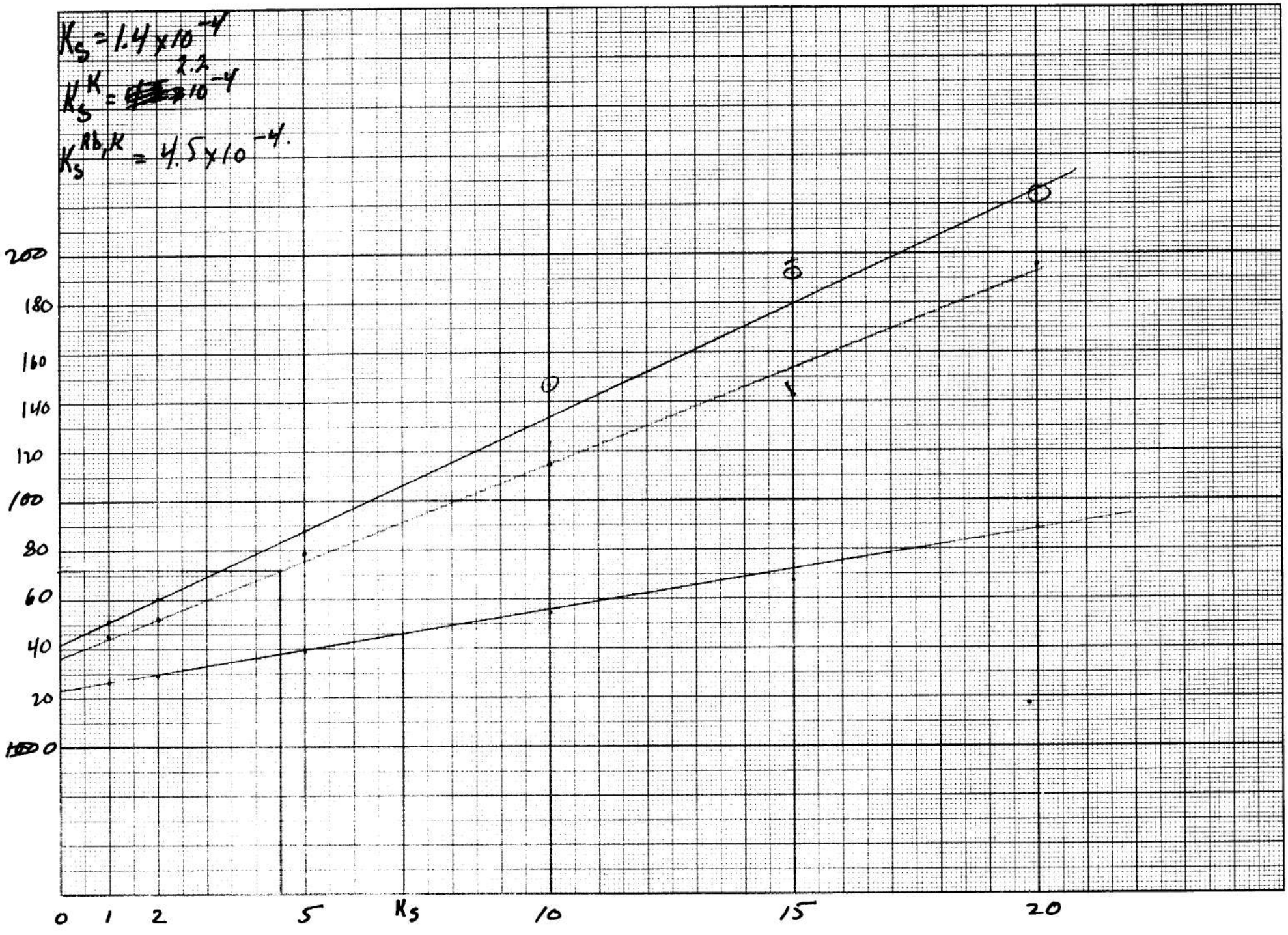
$K_S^{Na} = 1.3 \times 10^{-4}$

392

$K_S^{K} = 1.7 \times 10^{-4}$

$K_S^{K,PB} = 2.2 \times 10^{-4}$





Dec. 28, 1948.

3.19H 10^{-3} ml M/100 buffer:

		NPG/1000M	1/v	Δ	D_i	D_f
1	NaP	1	26.4	378	23	401
2	"	2	28.9	346	13	359
3	"	5	39.1	256	2	258
4	"	10	54.6	183	1	184
5	"	15	67.6	148	−3	145
6	"	20	88.5	113	−6	107
11	KP	1	45.2	221	18	239
12	"	2	52.6	190	9	199
13	"	5	78.7	127	3	130
14	"	10	115	87	—	87
15	"	15	143	70	−3	67
16	"	20	196	51	−2	49
21	KP+RbCl	1	51.5	194	18	212
22	M/50	2	60.2	166	12	178
23	"	5	98.0	102	2	104
24	"	10	147	68	—	68
25	"	15	192	52	—	52
26	"	20.	222	45	−7	38

$K_S^{Na.} = 1.4 \times 10^{-4}$ $\quad K_S^{K} = 2.2 \times 10^{-4}$.

In this expts., substrate + buffer are mixed up; enzyme is pipetted diluted before dumping it in at To. Cf. 339 in which pipetted undiluted enzyme masked effects (enzyme salts, unstable at dil. conc.)

K-12 Lactase.

12/29/48.

Grow 1 carboy of K-12 in S(Lac) new formula. 24h. Harvest A29. Yield 56gms. Desiccate 20g. (moist) over P_2O_5 in a desiccator. Remainder 35g., add a few ml K_2HPO_4 M/50 pH 7.5 buffer and grind 80 mins. Remove debris. Supernatant, about 27 ml.

Dry cell yield 4.47 (ca. 22%).

A). Extract (≏ 34/27) = 1.3 g/ml assay:

B). Suspend 100 mg dry cells in 10 ml M/50 NaP. Shake 2 hours. Remove cells + assay extract in ml. (C).
≏ 50 mg/ml wet cells

D). Assay original cell suspension in M/50 Na P 7.5 (−12.0° n.s.)

	Di		Δ
A..001 ml	-003	241	243
B..01 ml	[illegible]	71 ; 77	77
C..01 ml	~~.082~~ .082	59	59
D. ~~0.2 ml~~ 0.2 ml	082	113	31

B .01/pk ∴ Wet crush is only about ⅓ as efficient as
C .01/pk extracting dry cells.

~~Ref~~ 1/3. Note heavy ppt. in 399A. Kept refrigerated. Separate ppt and redissolve in H.O. assay. Ditto 395.

A-1 Lactates.

1/3/49.

Separate flocculate from preps. 399A and 395A.
originally assayed. 2400 and 2900 u/ml respectively.

1.	13	395 Ppt.
2.	497	Supernatant.
3.	20	399 P
4.	210	S.

Ions - activation of K-12 lactase
Time Series.

400

3194 10^{-3} ml.

Initial system KP 7.5 M/100. At t = 0 add enzyme. Add additional supplements at time indicated. RbCl 1/50 NaCl 1/50.

	Sup.	time	Sup.	time	Sup.	time	D_f
1.	RbCl	0					121
2.	"	15					134
3.	"	30					140
4	"	45					137.
~~5.~~							
6.	RbCl	~~0~~ 0	NaCl	0			192
7.	"	0	NaCl	45			192

Add substrate to initiate assay at 45 min.

No ~~demonstrable~~ appreciable time effect can be noted. Differential maybe non-specific

How, then, account for the different response to K noted now and previously?

optical density comparison of E. coli + pyrex glass.

Pyrex standard (A); bacterial susp. (B).

λ	D (A).	B.	42 broth dur
400	.69	.93	1.38
420	.68	.91	1.43
450	.67+	.87	1.23
500	.65	.83	0.74
550	.65	.79	0.38
600	.64	.75	.22
650	.62	.70	.14
700	.60	.66	.11