"Effective intracellular pH"

11/19/48.

To determine whether the intracellular buffering capacity might influence activity determinations, set up cells A) c̄ E coli K-12, O.D. λ420, = 1.00; B) do. + M/5000 ONP + c) ONP only in acetate buffer .04M, pH 4.0. Compare readings (in O.D.).

$A_1 - A_2$	.007	(error term).
$B_1 - A_1$	.124	
$B_2 - A_1$	.124	
$B_1 - A_2$	.138	
$B_2 - A_2$	.138	
C_1	.151	
C_2	.153.	

If anything, the apparent absorption by ONP was less c̄ the cells than without. This may be due to scattering.

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.

modified buffer?

no enzyme

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

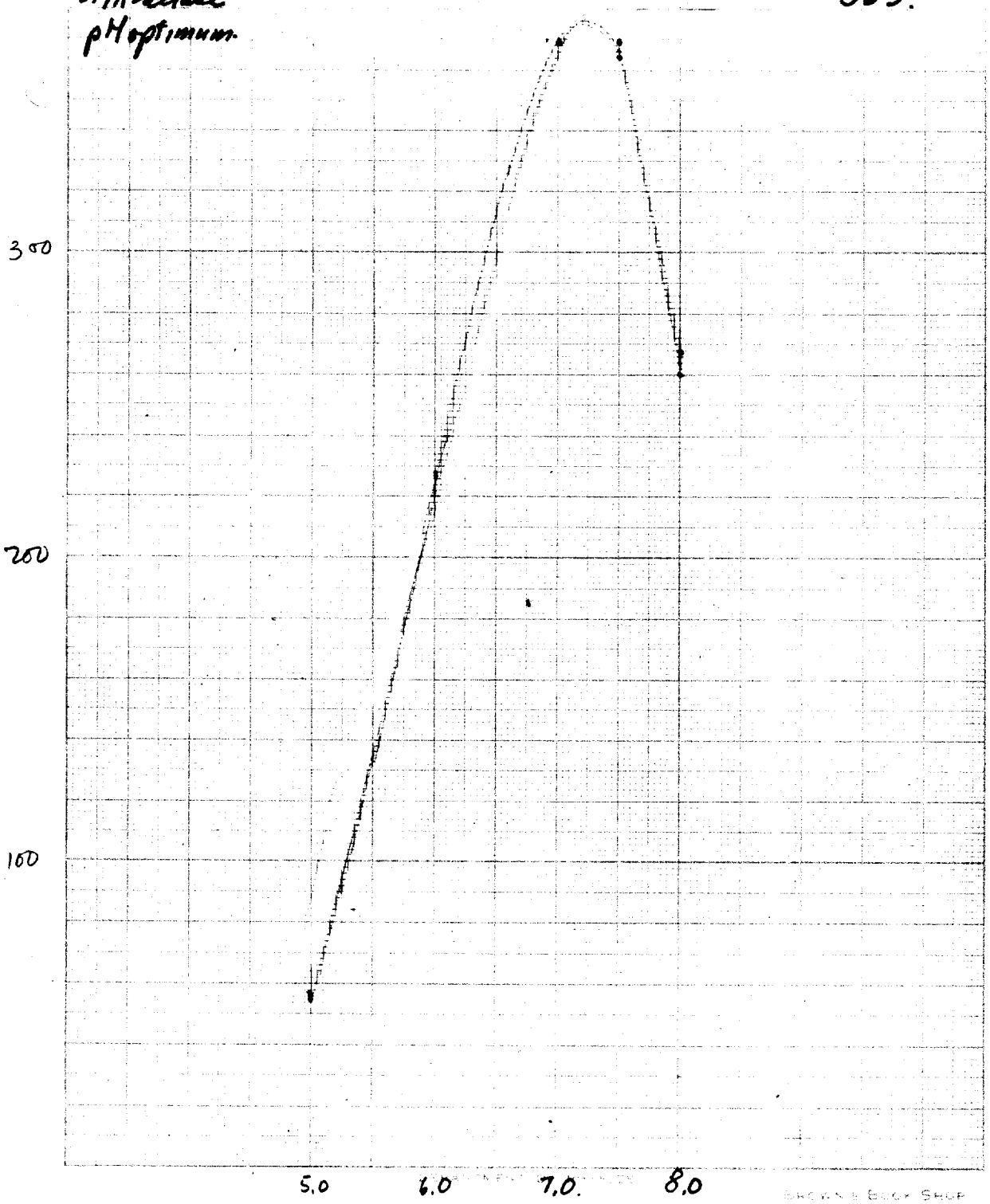
Na_2SO_4 M/50.

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

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

Repeat, using phosphate buffers only!

3/19A Lactase
pH optimum.



pH optimum - colilactase

11/18/48.

319A 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_4
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 42 glucose shaken overnight and resuspend in 40 ml M/5 Na P 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 tryp.
A = arginine
C = canavanine sulfate
T = tryptophane

Suppl.		Cell dens.
1. —	160	170
2. —	~~055~~ 190	192
~~3. 5MT 500γ~~		
~~4. 5MT 500γ~~		
~~5. T~~ "		
6. T "	060	172
7. C	140	179
8. C "	~~190~~ 171	~~177~~ 171
9. A "	190	~~178~~ 177
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
15. 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

Alfalfa seed emulsin galactosidase " " 381.

12/8/48.

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

Prep. ~~Exp~~ A. 5 gms. meal were shaken 3h. c 10 ml H_2O. The debris was sedimented and supernatant diluted to ca 10 ml. (galactosidase). Remarks at pH 4.0 Na acetate buffer M/100 (after Veibel who showed optimum at 3.4). He finds Km for cresol 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 c̄ M/1 Na_2CO_3 1 ml.

	salt	Dyzo.
1.	No enzyme	ambs
2.	—	167 ✓
3.	Na	248 ✓ (adv. substr. incr.)
4.	Rb	196
5.	Na+Rb	212.

may be a chloride effect

1	—	220 ✓	Note appr stimulation by
2	NaCl	250	
3	Na_2SO_4	270	Na_2SO_4

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

12/9/48.

Run ONPG conc. series ē various lactose concentrations.

10 ml ~~.001~~ .05 ml ~~341A~~ 381 add 1 ml Na_2CO_3 to terminate. in M/100 NaAc pH 4.

20 m.

	ONPG M/	Lac M/	D_i	D_f	Δ	1/v
1	2000	00		182		
2	5000	00		123		
3	10 000	00		79		
4	20 000	00		58		
11	2000	2000		171		
12	5000	"		131		
13	10 000	"		82		
14	20 000	"		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_i by ~~10/12~~ 9/11 for addition of enzyme and of substrate.

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

Barley Seedling Lactase

12/8/48.

Seedlings from Dr. Nancy Kent.

		Di	Df	Δ
A.	Grown on lactase, 6 seedlings, ca. 3 cm long.	140	200	60
B.	sucrose, 3 " shoot 13 cm long	310	410	100

Grind in mortar in distilled water, 5 ml. Without separation, test bnl samples ε ONPG at pH 4 as in ~~the~~ alfalfa system. Incubate at 37° 10:35 AM - 11 AM

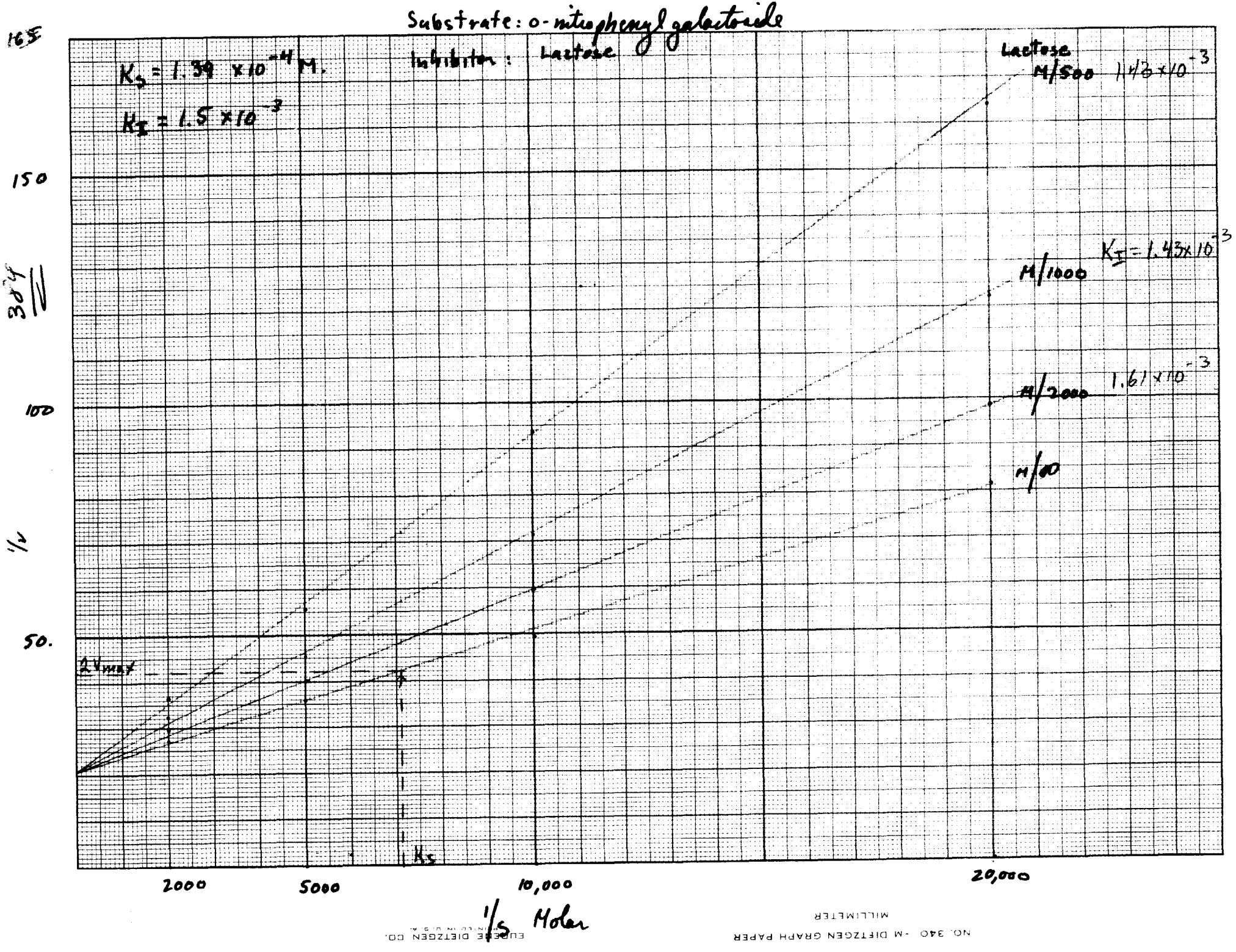
∴ Barley lactase is constitutive

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

December 10, 1948.

Set up as 383. .002 ml 319A. in M/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
$K_S = 1.39 \times 10^{-4}$ M.
$K_I = 1.5 \times 10^{-3}$
Inhibitor: Lactose
Lactose
M/500 1.43×10^{-3}
M/1000 $K_I = 1.43 \times 10^{-3}$
M/2000 1.61×10^{-3}
M/00
165
150
100
50.
ΔV_{max}
K_S
2000
5000
10,000
20,000
1/S Molar

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	~~Lac~~ 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.

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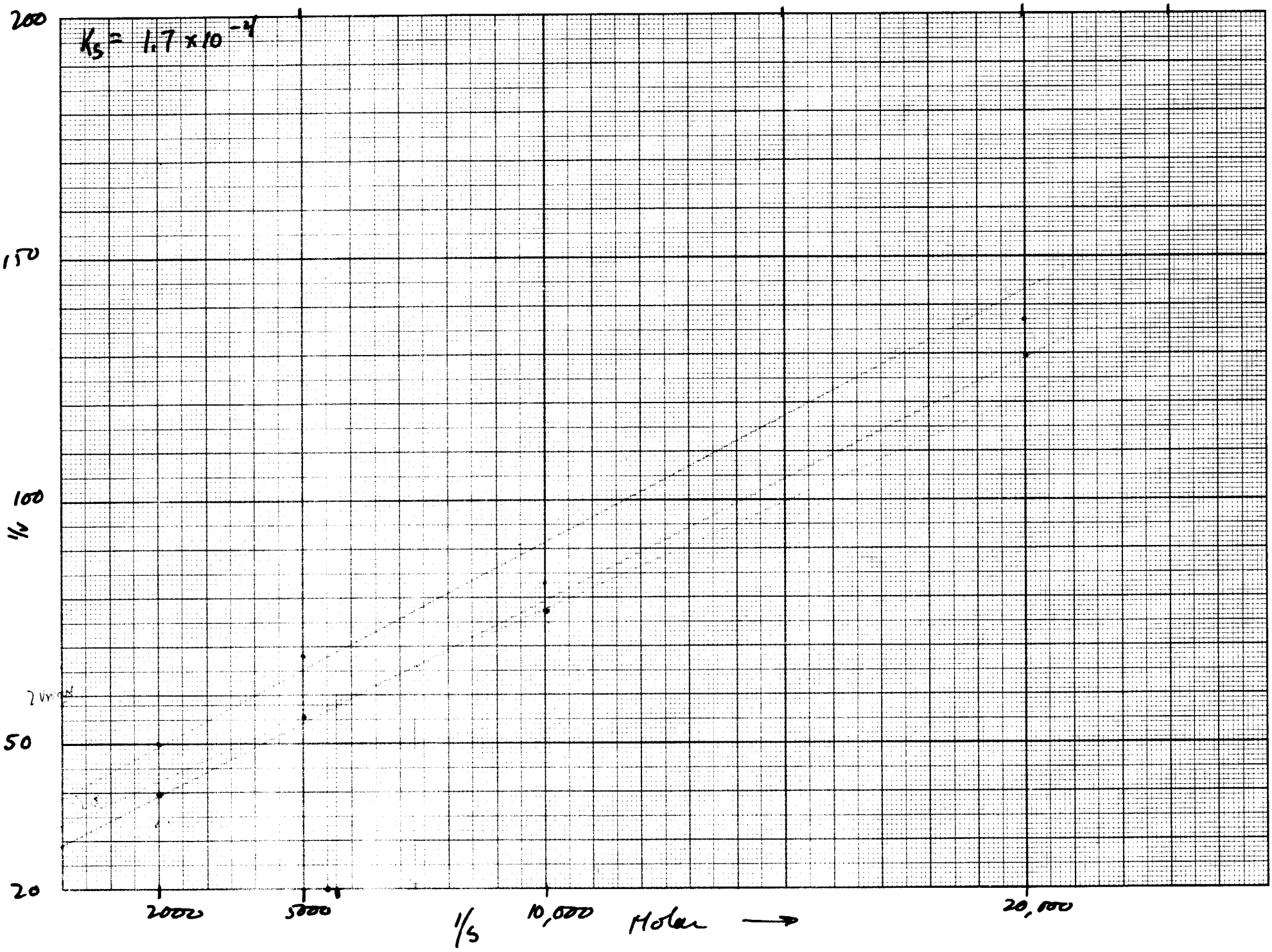


385.

Glucose inhibition of lactase.

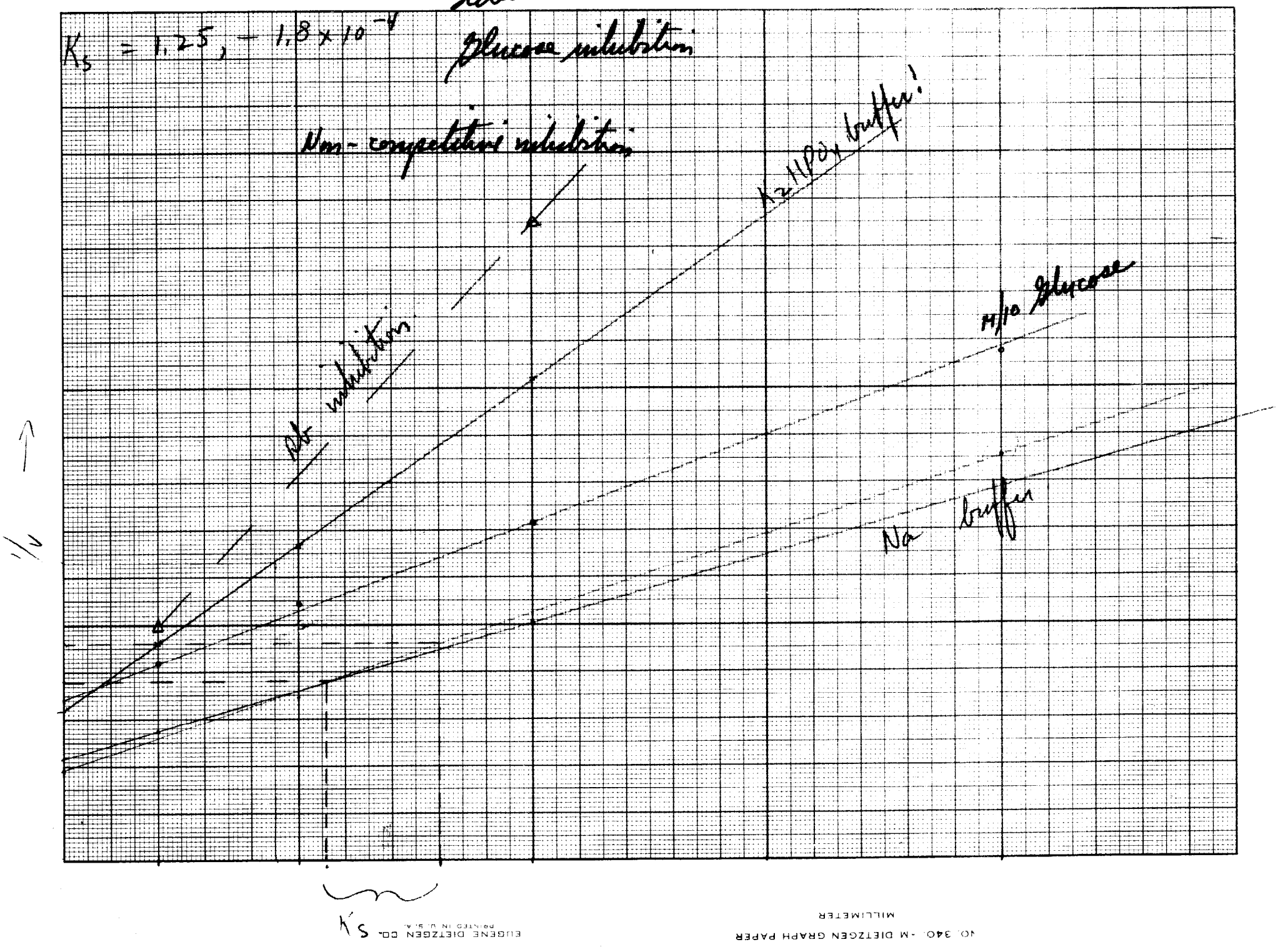
12/11/48.
As 385. .002 ml 10 Mins. Val 7.5 M/50.

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	[illegible]	
24	20	—	57	175.4	
31	2	M/50	[illegible]	142 × 1.4 [illegible] 50	1.4 [illegible]
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 Ks!! Rb may accentuate this response!

Substrate ONPG



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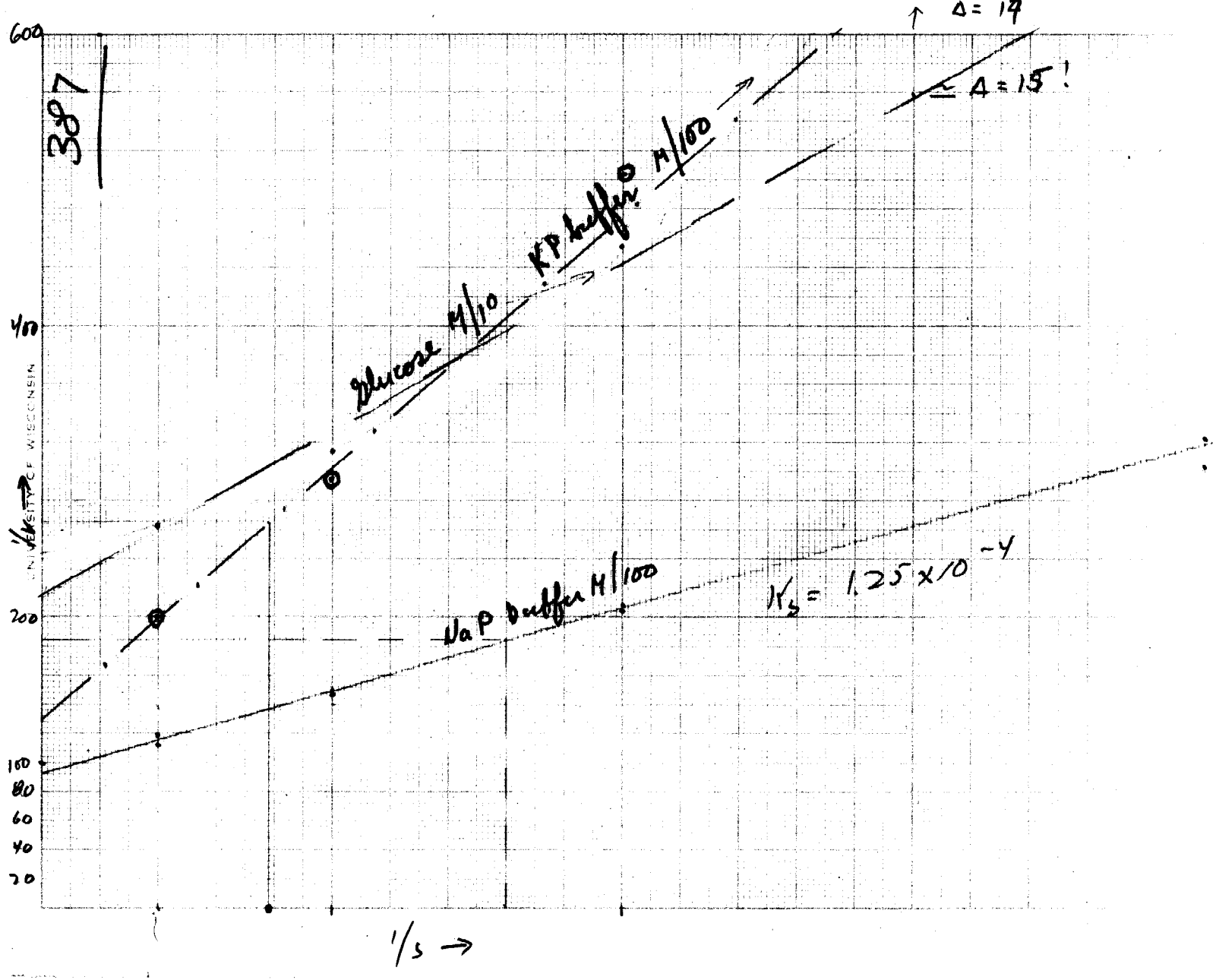

387
600
400
200
100
80
60
40
20
1/V →
1/s →
Δ = 14
Δ = 15 !
Glucose M/10
KP buffer M/100
NaP buffer M/100
1/Vs = 1.25 x 10 -4

K_s nitrophenyl galactoside as a function of salt concentration

December 13, 1948.

	ONPG. M/-1000.	Suppl.	Buffer.	1/V	l	F	Δ = "V"
			NaP ~~[illegible]~~ M/100				
1.	2			112	10	99	89
2.	5			147	2	70	68
3.	10			208	—	48	48
4.	20			303	-3	30	33
		Glucose M/10.	NaP M/100				
11.	2			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			244	-1	33	34
33	10			454	-3	19	22
34	20			1429	-3	10	7

"K_s 1.25×10⁻⁴" "V_{max}" = 109

V_{max} = 78

"K_s apparent" = 2.6×10⁻⁴

Glucose inhibition non-competitive, but may be related to substrate, as is more effective at ~~[illegible]~~ 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 mins

1. 319A. 2×10^{-3} ml. Precipitated [illegible] [illegible] 500
2. 319B. 10r 18
3. 319C 2×10^{-3} ml 70.
   ca 50 u/ml

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

		pH.	
(B) lactose	11	4	8%
	12	5	(")
	13	6	(")
	14.	7	(")
(A) glucose	21	4	97
	22	5	(")
	23	6	(")
	24.	7	(")

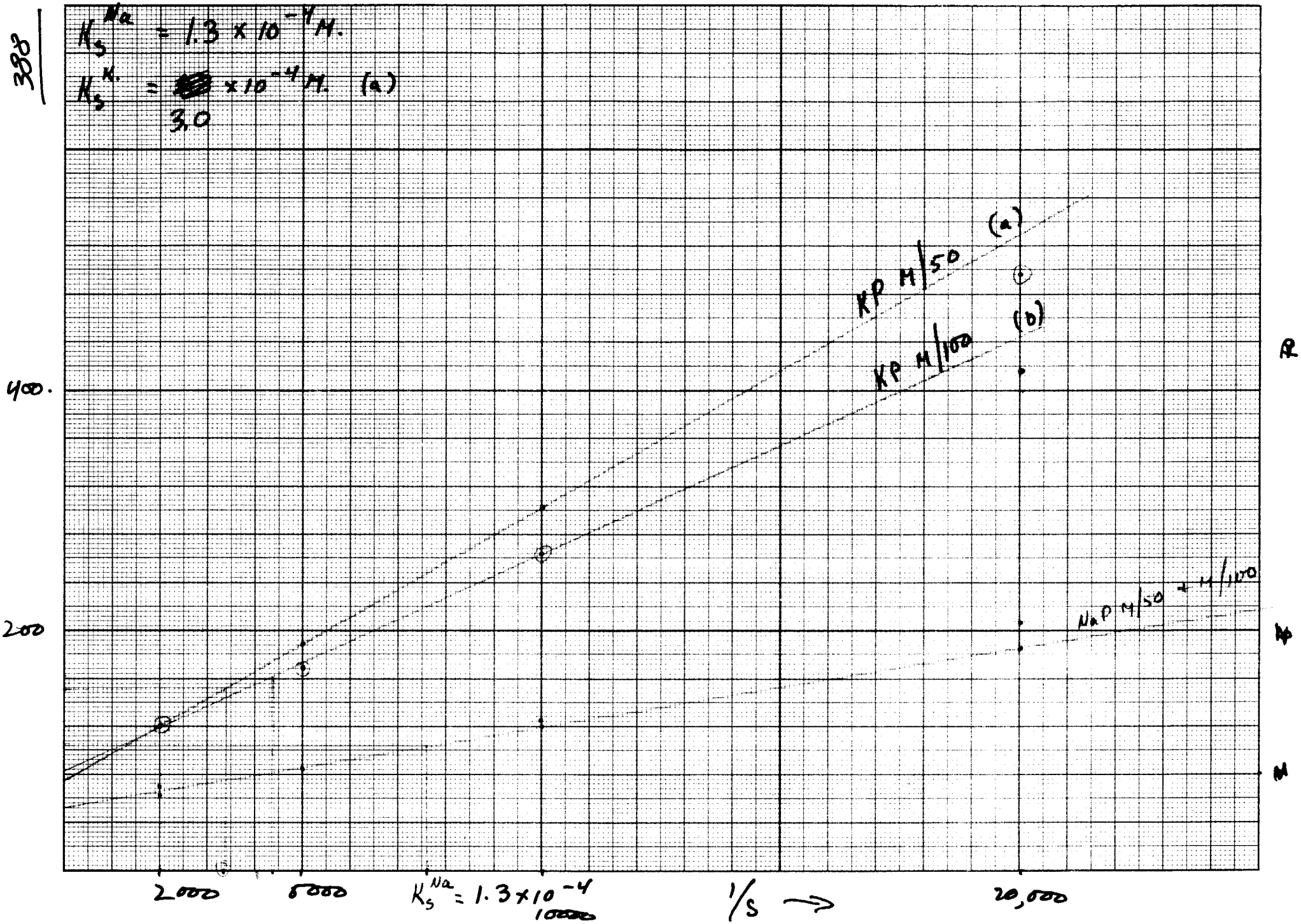
No activity noted in [illegible] ē these very dilute suspensions.

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)



K_s / salt concentration

12/13/48. 319A 10^{-3} ml. 40 mins.

Buffer pH 1.5 as indicated.

	ONPG 1020/M		1/V	1	2	4
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	87	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
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 pup'n 319A 12/12/48 to prevent 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

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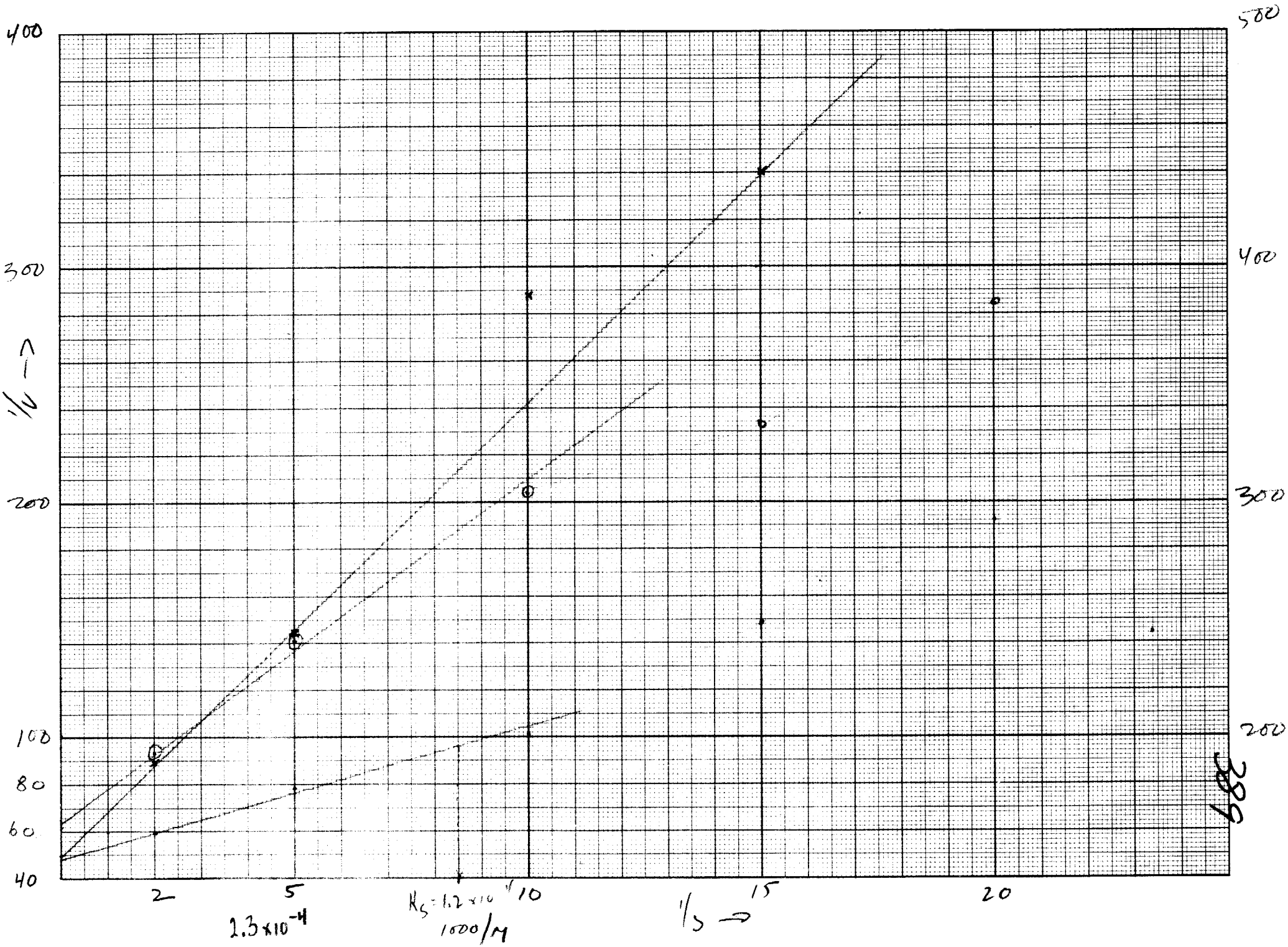


Influence of metal ions on K_s (ONPG).

Dec. 14, 1948.

	ONPG 1000/M.		1/V		I	F (40 mins!)	A	A/2 (Final)
				NaP M/50.				
20 min	1.	2	59.5		11	179	168	
	2.	5	78.1		0	128	128	
	3.	10	101		1	100	99	
41 mins	4.	15	149		0	134	134	67
	5.	20	192		-3	101	104	52
				KP M/50.				
	11.	2	93.5		10	117	107	
20 mins	12.	5	141.		0	71	71	
	13.	10	204.		1	50	49	
41 mins	14.	15	333		0	60	60	30
	15.	20	385		-2	50	52	26
				NaP M/50 + Glucose M/10.				
	21	2	189		14	120	106	53
	22	5	345		6	64	58	29
41 mins	23	10	488		0	41	41	20.5
	24	15	540		0	37	37	18.5
	25	20	769		0	26	26	13

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

To iron out some of the inaccuracies, reaction 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 n/g 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 .01ml and .001ml ē 4/2000 ONPG pH 7.5 Na

E. coli lactase.

12/21/48.

A). Assay pups 319A + 390A. NaPbuffer 7.5 20 mins.

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

Study time factor in Na [illegible].

Tubes 1+2. 10^{-3} ml enzyme + buffer, incubated 90 [illegible] before adding substrate.

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

2: 189 } Note: inactivation was irreversible, as
4: 15. } prolonged incubation of tube 3 gave no color!

∴ 319A lactase is irreversibly inactivated by dilution in distilled water (and [illegible]

December 14, 1948.

319A. 10^{-3} diluted some before using.

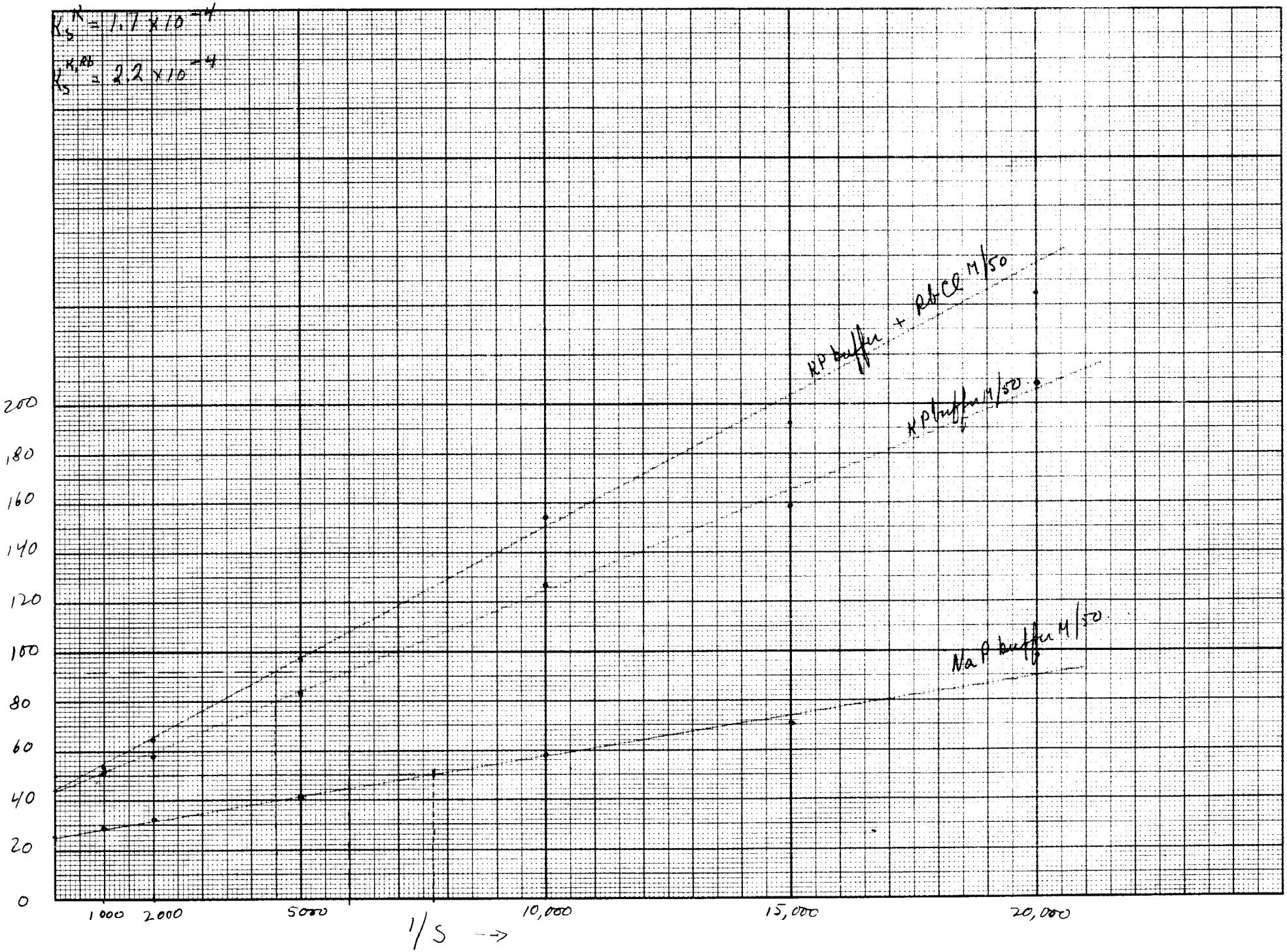
Series 0 - NaP. M/50 pH 7.5

10 - KP

20 - KP " " + RbCl 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	$V_{max} = \frac{1}{25}$	= 400.	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	$V_{max} = \frac{1}{43}$	= 232.	1	121	120	
13	10	126.6			-4	75	79	
14	15	149			-3	64	67	
15	20	208	= 58%		-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	

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



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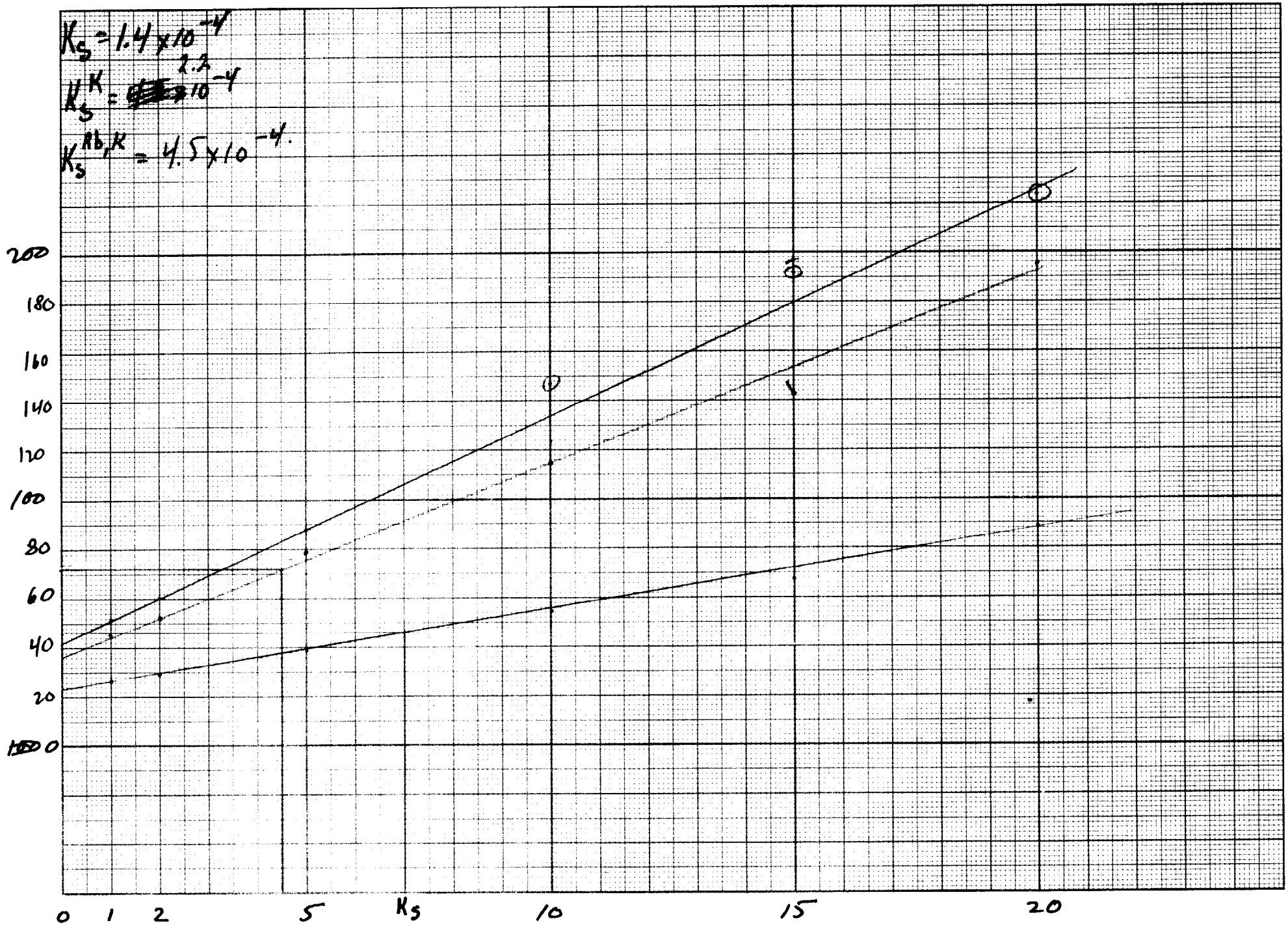

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Dec. 28, 1948.

B19H 10^{-3} ml M/100 buffer:

		NPG/1000M	1/V	Δ	Di	Df
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

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

In this expts., substrate + buffer are made up; enzyme is freshly diluted before dumping it in at F_0. Cf. 339 in which sb excited much more marked effects (enzyme & salts, substrate added later)

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 (≙) (36/27) = 1.3 g/ml Assay:

B). Suspend 100 mg dry cells in 10 ml M/50 NaP. Shake 2 hours. Remove, sediment + resuspend it in 10 ml. (C).
≙ 50 mg/ml wet cells

D). Assay original cell suspension in M/50 NaP 7.5 (−12.5 min.)

	Di	Dr	Δ
A. .001 ml	-003	241	243
B. .001 ml	006	71 77	77
C. .001 ml	~~082~~ -001	82 59	59
D. ~~[illegible]~~ 0.2 ml	082	113	31

B .01 ml ∴ Wet crushing is only about 1/5 as efficient as
C .01 ml extracting dry cells.

~~[illegible]~~ 1/3. Note heavy ppt. in 399A. Kept refrigerated. Separate ppt and redissolve in H_2O. assay. Ditto 395.

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.

Im - activation of K-12 lactase
Time Series.

319A 10^{-3} ml.

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

	Sup.	time.	Sup.	time	Sup.	time	D_f
1.	RbCl	0					121
2.	"	15					134
3.	"	30					140
4	"	45					137.
6.	RbCl	~~NaCl~~ 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. Drift noted may be non-specific

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

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

optical density comparison of E. coli & pyrex glass.

λ	D (A).	B.	42 broth dex
400	.69	.93	1.38
420	.68	.91	1.43
450	.67+	.87	1.23
500	.65	.83	0.74
550	.65	.79	0.35
600	.64	.75	.22
650	.62	.70	.14
700	.60	.66	.11