β-phenyl galactoside.

Nov. 10, 1947.

Sample from E E Snell (2 grams).

Test in comparison with lactose + galactose at .05% in T(m).
Add necessary growth factors.

		galactose (A)		lactose (B)		β-φ galactoside. (C)		β-φ + galactose. (D)	
1	58-161	+	++ ✓	−	±	++	±	± −	+ + ++
2	Y87.	+	±± ✓	−	±	++	−	− ✓	+ + ++
3	ω-30.	−	−	−	−		−	−	− −
4	ω-35	++	++ ✓	±	±	++ ✓	−	− ✓	±± + ++
5	ω-36.	++	++ ✓	±	±	± ✓	−	− ✓	− − ✓
7	Y10	+	++ ++	++	++	+++	−	− +	+± ± + ++
8	Y53.	++	++ ✓	±	±	+ ✓	−	− ✓	++ ++ ✓
6	ω-2.	±	+ ++	++	++	++	−	− ±	+± ± ± ±

Readings at 20h., 24h., 36h.

φ-galactoside is not generally utilized and may be slightly inhibitory in galactose media. Cf Y10 however.

56 hours; 72h.

	gal	lac	β-φgal	β-φgal + gal.	
1	++	++	++ ✓	++	
2	++	++	− ✓	++	lac + cells present
3					
4	++	++	− ✓	++	
5	++	++	− ✓	++	
6	++	++	+± ✓	++	
7	++	++	++ ✓	++	
8	++	++	−	++	

Note that none of these cultures originally lac− have grown on β φ gal.

considerable pigment produced on galactose

Nov 15 1947

Inocula from 23 SP15. 0.1 ml/tube T(BMTLB1) base.

		A(Galactose .05%)	B (B-ØGalactoside)	C Galactose +Phenol .02%
TIME::::		SP16	SP16	SP16.
Inoculum				
1 (58 gal)	1a	+++	++	++
2 (161 lac)	1b	+++	++	++
3 (βΦ)	1c	+++	+++	++
4 (987 gal)	2a	+++	−	++
5 (lac)	2b	+++	++	++
6 (410 gal)	7a	+++	±	++
7 (lac)	7b	+++	++	++
8 (βΦ)	7c	+++	+++	+++
9 (gal)	8a	+++	−	++
10 (lac.)	8b	+++	++	++

?? Is utilization of β-Φgalactoside by wild type mutants?

		SP17
on gentiobiose	+	+
"α-Φ-galactoside"	+	++
2a on gentiobiose	+	+
"α-Φ-galactoside"	+	++

P17. Streak out on β-Φglucoside EMB:

1A; 1C., 1B.

6A; 6C.

A19. 1: all show a slow type of colony c̄ a few much darker suggestive of rapid utilization. 1B and 1C show these particularly. all streaks are papillated.

6: somewhat smeared. Two colony types also noted.

Need checking c̄ phenol + galactose.

Nov. 27, 1947

Test on EMB agar using heavy water suspensions of cells from YP agar slants, except W-28 and W-29 from galactose EMB agar.

48 hr. readings.

		W33	+++	W35	-		
		W37	++	W36	-		
		W38	++	Y70	++		
1. K12.	++	W41	++	W40	++	Y53	++
2 Y10	++	W28	++	W42	++	Y87	++
3 58-161	++	W29	++	W43	-	W30	++
4 W52	+++	W44	++	W45	-	W53	+
		W46	++	W48	-		
		W50	+±	W49	-		
		W51	++±	W-1	+++		

24 hrs. (A29) W52 + All others -

36 hrs. W52 +++ W-1, W33 ++, Y10 +, Y70, Y53 ± W53: -

48 hrs. 60 hrs. As above T.

There seems to be a graded spectrum of responses. Y52, W-1, W-51 and W-33 are distinctly the most positive reactors, especially W52. The "negative" types are all "sectorial" mutants derived from 58-161 and are Lac negative. Since their Lac+ counterpart is Bϕ+ a relationship is suggested! The only strain which is even relatively "Lac+/βϕ-" is W53. While Y53 is Lac-βϕ+.

Note: Lac+ Lac-

~~Lac~~βϕ+ Y10 Y53, W-1.

βϕ- W53 W45, -49.

Suggested Crosses. W53 × W-1 Lac+βϕ- × Lac-βϕ+, also Mal+/-

W49 × Y10 Lac-βϕ- × Lac+βϕ+.

Action of cell mixtures on lactose.
Trehalose / Maltose Cross adaptation, pulvis.

Dec. 10, 1947.

Prepare 10¹⁰ suspensions of

a. Y40 Lac+
b. W-1 Lac₁-
c. W-45 Lac₂-

Incu. in 37° water bath

Add 1 ml bacteria to 1 ml 4% lactose + dil. to 5 ml. Use Durham tube for gas, and BCP for acid production. Do mixtures in duplicate. + refers to acid production. (.1 ml M/10 buffer pH 1.0 added.) Set up. 3:45 P 9

		6 P 9	9 A 10 P 10
1.	a	–	+++, +++
2.	b.	–	–
3.	c.	–	–
4.	a+b	–	+++, +++
5.	a+c	–	+++, +++
6.	b+c.	–	–, –
a	glycose	+++	+++
c	glucose	++	+++

nothing. Mixtures of Lac₁- and Lac₂- therefore cannot ferment lactose.

Adaptation takes some hours under these conditions. (No extra N)

Dec. 11.

For ~~Tre~~ Trehalose, use culture of Exp. 25 and compare i glucose adapted from same culture. (Controls are inadequate.) Set up 4:15 P 11.

	Grown in	Testing
A	glucose.	glucose
B	"	maltose
C	Trehalose	glucose
D	"	maltose

TREHALOSE***MALTOSE CROSS-ADAPTATION EXPERIMENT.

Dec. 16, 1947.

Grow K-12 in T(0) plus .05% sugar 24 h. Harvest and concentrate to ca 10^{10} /ml/

Add 1 ml. cells to 1 ml 5% sugar, and in replicates add NaN_3 to a final conc. of 2 x 10^{-3} M. Add 0.1 ml M/10 phosphate buffer pH 7.0 and ,05 ml BromCresolPurple .15%

Make up to 5 ml with water, cells added 2 P 16, incubate in 37° water bath.

Readings at 2 h., 4 h., and 18 h., Readings - unless indicated.

Cellogramm:

	2h.	4h.	18h.
	4P17	6P17	10A18

Set up. 2P17

A. Glucose
B. Maltose
C. Trehalose

} T(0) + .05% sugar 18 hours. Harvest + concentrate.

A. Gluc.		+++	+++
" + Azide		±	+++
M		−	+++
" + Az		−	−
Tr		−	+++
" + Az		−	−
B. G		+++	+++
" + Az		−	+++
M		++	+++
" + Az		−	+++
Tr		−	+++
" + Az		−	−
C G	+++	+++	+++
G + Az		−	+++
M		++	+++
M + Az		−	+++
Tr	±	++	+++
Tr + Az		−	+++

A cells did not adapt in 18 hrs. in presence of azide, either to trehalose or to maltose.

B cells utilized maltose in the presence of azide, but did not adapt to trehalose.

C cells utilized maltose as well as trehalose and glucose, even in presence of maltose.

Azide in conc. of 2 x 10^{-3} M does inhibit fermentation to some extent but seems to block adaptation completely.

Conc. trehalose and maltose cross-adapt, but only unilaterally, trehalpse adaptation implying maltose adaptation, but not the converse.

Query: Will Malt-(Tre-) cells utilize maltose if grown on trehalose?

Azide does seem to interfere with the fermentation as well as adaptation.
Tr-adapted seem to be maltose adapted but not vice versa

The inhibition of lactose-adaptation by Azide.

Dec. 18, 1947.

Harvest K-12 from YP-.1%glucose broth. 16 hr. cultures. Conc. 50/20.

Tubes contain in 3 ml. , : 1% sugar, 1 ml cells, .1ml Phosphate Buffer M/10 pH 7.0 and indicated conc. azide or DNP Set up 12:20 PM

	Glucose Azide M/100 X	(3:20) 3:40PM.	6:00PM	21.h. 9A20.	Lactose 3:40	6:00PM.	7:00PM	21.h.-	(pH.)
1.	—	+++	✓	✓ 4.50	-	+	++	+++	4.62
2.	1	++	✓	✓ 5.79	-	—	—	-	6.28
3.	.5	+±	++	✓ 5.57	-	—	-	+	5.95
4.	.1	+++	✓	✓ 4.78	-	±	+	++±	5.48
5.	.05	+++	✓	✓ 4.70	-	+	++	+++	5.18 ~~4.78~~
6	.01	+++	✓	✓ 4.36	-	+	++	+++	5.01 ~~5.18~~
	DNP 10^{-4}M x								
7	5	—	✓	✓	-		—		
8	1	++	✓	✓	-		—		
	original solution								7.36

At 12:40, none changed.

DNP itself is an indicator. 10^{-3} Azide does not appreciably inhibit fermentation. but it does permit slight adaptation.

The pK ($K = 6.2 \times 10^{-8}$) of phosphate buffer is 7.21. $pH = pK + \frac{(base)}{(acid)}$

At the initial pH the ratio is ca. 1.6 : 1 There are altogether 10μM phosphate. At pH 4.50, the ratio is 1:50 The lower the pH, the more sensitive the pH is to slight additions of acid. i.e. all but 2% of the base is neutral., and about 6μM H^+ have been produced (from 30mg = $= \frac{1}{6}$ mM = 167μM glucose). More buffer should be used in this system and an indicator used whose pK is near the pK of phosphate. such as bromthymol blue.

On the maltase activity of trehalase.

Dec. 18, 1947.

Grow W-34. in T(TRC) + .1% trehalose and glucose. No growth (±) on maltose.
Test for activity on glucose and maltose in system like Exp. 65.
Harvest 50 ml & conc. to 2 ml. 50/2. Set Up. 5P19.

Growth ↓ Activation →	24. 7P19	Glucose 9A20	Maltose	
Glucose	+++	+++	–	–
Trehalose.	+±	+++	–	–

W-1 is therefore capable of producing trehalase but not maltase.
So far, all Mal- mutants are apparently Tre+, although W-21 is perhaps a little slow on trehalose.

Maltase is not simply an incidental activity of trehalase.

Cross-adaptation of galactosides

Jan. 14, 1948.

Harvest cells from .1% cultures in T(m) 36h. into 4/ml. (K-12)

Set up tests with 1 ml cells, 1 ml 3% substrate, M/200 Azide and .1 ml M/10 phosphate. BCP indicator.

Substrates: G, glucose; L, lactose; M, b-methylgalactopyranoside; and B, N-Butyl-b-galactopyranoside. , Ga, galactose.

Grown in/tested on: Set up 11A, 37°.

	G/GA	G/G	G/L	G/M	G/B	L/G	L/L	L/M	L/B	L/Ga
5PM	—	±	—	—	—	—	—	—	—	—
10A 15. (23h.)	—	+++	—	—	—	+±	++	+	—	±

	M/G	M/L	M/M	M/B	B/G	B/L	B/M	B/B
5PM	±	+	±	—	±	+	—	—
10A15 (23h.)	+++	+++	+++	±	+++	+++	+	±

Tested → / Grown ↓	Glucose	Lactose	Butyl-gal.	Methyl-gal.	Galactose
Glucose	+++	—	—	—	—
Lactose	+±	+±	—	+	±
Butyl--	+++	+++	±	+	
Methyl--	+++	+++	±	+++	

Cells probably too old for rapid adaptation. Lactose cells in exper[illegible] conditions.

In future, use mixture of BCP and BTB or most marked contrasts.

Use 2 BTB : 1 BCP. Cells may be too old.

Concl. ① M adapted are L adapted. ② L adapted are M adapted

③ B is poorly utilized under these conditions! ④ Galactozymase is adaptive

⑤

W108: Test for maltase and lactase activity.
Utilization of C-sources

Jan. 23, 1948.

Grow W-108, Y87, W56 and Y10 in YB broth overnight. Use ½ ml inocula into 10 ml. indicator broth with 1% sugar.

	Maltose			lactose			
108	—	—	—	—	—	±	adapted
108	—	—	—	—	—	±	
87	+++	✓	✓	—	—	—	much earlier
87	+++	✓	✓	—	—	—	
56	±	✓	✓	+++	+++	✓	here than on
56	±	✓	✓	+++	✓	✓	
108;56	±	✓	✓				maltose.
108;56	±	✓	✓				
108;87				—	—	—	
108;87				—	—	—	
Y10	+++	✓	✓	+++	✓	✓	
Y10	+++	✓	✓	+++	✓	✓	

By P25 all +++ except W56/M..
Therefore, W108 cells do not produce maltase detectable by the utilization of the hexose components by symbiotic W56, and conversely with lactose and Y87.

Use small inocula from slant-suspensions. T(m) with .05% equiv. C-source.

W-108: Inoc. P23.

	N24.	P25	P28	
glucose	—	±	+++	→ M-L- Streak out on glu + trehalose.
fructose(st sep)	—	—	+++	→ M+L+
trehalose "	—	—	+++	→ M-L-.
sucrose	—	—	—	
maltose	—	++	+++	→ M+L+
lactose	—	—	—	
Na lactate	++	+++	✓	
K gluconate	+++	+++	✓	
Y-10 glucose	+++	+++.		
	W108		Y10	

On 1% EMB plates:

	N24.	P25	
K glucon	++	+++	+++
glucose	—	— many mutants	+++
l-arabinose	+++	✓	+++
xylose	+++	✓	+++
mannitol	—	occ. mu.	++
lactose	—	"	+++
maltose	—	"	+++

Look for specific phenotypic mutations on glucose, maltose & lactose selections

W108: Test for β-galactosidase activity

Jan 26, 1948

Inoc heavily W108 + Y10 into T (m) + .05% βΦgalac. & .05% K gluconate. Incubate 36 hours & test for free phenol with diazo-sulfanilic reagent. (βΦgal gives a strong color which, however, disappears in acid solution!). Compare with blanks, etc.:

	Test 1.	
1. Blank	−	−
2. Blank medium (βΦgal)	−	−
3. ΦOH .02%	++++	++++
4. 108 a	±	+
5. 108 b	++	+
6. Y10 c	±	+
7. Y10 d	++	+
8. Y10/gluconic only.	−	−

Not even nearly complete splitting by either Y10 or W108 under these conditions. Streak out 108 on lactose plate to assure non-reversion.

Some splitting is autom- ca. 10%.

Cross adaptation tests.

Jan 28-9, 1948

		a Glucose	b Galactose	c Gluconic	d d-arab	e l-arab	f d-xyl.
Y10 A	Glucose	++ +++ ✓	± ± +	± ± +	− − −	− − +	− − −
Y10 B	Galactose	++ +++ ✓	++ +++ ✓	− − +	− − −	++ +++ +++	− − −
W108! C	Gluconic ac.	+++ +++ ✓	− − +	+++ +++ ✓	− − −	− − +	− − −
W108! D	d-arabinose	± ++± +++	− − ±	− − ±	− − −	− − ±	− − ±
Y10 E	l-arabinose	++ +++ +++	± ++± +++	− − +	− − −	++ +++ +++	− − −
W108! F	d-xylose.	± ++± ++	− − ±	− − ±	− − −	− − ±	± ++ +++.

No ferment.

1 hour
2 hours
4 hours.

① Gluconic and galactose are adaptive. Also d-xylose and l-arabinose.

②. D-arabinose is not fermented

③. Galactose and arabinose cross-adapt bilaterally.

④. The resting cell suspensions of W108! utilize glucose !!! (Repeat).

Cells grown overnight and harvested from YP broth 50 ml + 1% sugar. Concentrate to 7 ml. Use 1 ml cells, 1 ml azide buffer + 1% sugar.

→ found to be mostly Glu + revertants.

Cross-adaptation tests.

January 30, 1948.

Grown in: ↓	A Glucose	B Galactose	C Gluconic	D Arabinose	E HDP.
1. Y10 Glucose	+ +++ ±	+	− +	± −	− −
2. Galactose	± ++ +++	± ++ +++	+ +	± +±	− −
3. Gluconic	+ +++	± + +	+++ +++	±	− −
4. l-Arabinose	± ++ +++	± ++ +++	− ±	± +++	−
5. —	− + +	± − −	− −	− −	−
6. * W108 Glucose	− − −	− − −	− −	− −	
7. * Galactose	± + ++	± ++ ++	− ±	+ ++	
8. Gluconic	− 8	− −	+ +++ +++	−	− −
9. * l-Arabinose	± − ±	± ++ +++	− −	+ +++	− −
10. —	− − −	− − −	− −	− −	−
~~11.~~					
~~12.~~					

may be too heavily buffered.

cells OK

cells OK

cell OK.

galactose by W108 −

Design as above. Cells added 11:30 AM. Variable cell yields!
Azide + phosphate.

2 h. 3 h.

* streak out on maltose or glucose

(1). Confirm cross-adaptation of galactose & arabinose
~~(2). Glucose is adaptive. Glucokinase is lacking in gluconi-adapted cells.~~

W108 - C source characterization

T(m) + .05% C source.

	Glucose	MDP	Gluc + MDP
W108	−	+±	+±
Y10.	+++	++	+++

24 hours.

Cross-Adaptation Experiment.

January 31, 1948.

Grow cells of Y10 in 50 ml:

	YP+ Grown on: ↓ Tested on:	glucose a	galactose b	glucose + galactose c	lactose d
A	glucose 1%	+++	− ±±	+++ ✓	− −
B	galactose 1%	+++	+++	+++ ✓	± ++
C	glu + gal 1%	+++	++±	+++ ✓	− −
D.	lactose 1%.	+++	++±	+++ ✓	+++ ✓

Harvest, conc. to 5 ml and
Test in corresponding substrates
in azide buffer.

2 hours. 4/1

Noting that lactose-adapted cells are also galactose-adapted, but galactose-adapted are not lactose adapted. ∴ Galactose is probably an intermediate in lactose utilization.

Adaptation is not completely inhibited by this concentration of azide (M/200). Use (M/100) in future.

Feb. 11, 1948.

Harvest 2 batches (A.B) of W-108 grown in 50 ml. 1% YP-gluconate broth overnight. Test sample for genetic purity.

A. (10 AM) Conc. to 12 ml. Use 1 ml cells per tube, with ½ ml. 10% sugar and phosphate-indicator. (No azide!)

	gna	gna+gl	gl	gal	gal+gl	Bu-gal.	Bugal+glu	Aa
11 AM	+++	+++	++	—	—	—	—	—
12 N	+++	+++	++	—	—	—	—	—

must be in error v. below

Aa: 4 ml. cells + 1 ml. gal. + ½ ml. phosphate-indicator for adaptation, to galactose.

B. 11 AM As above. Conc to 10 ml. 1 ml. cells/tube

	gna	glu	gna	gna-glu	gal	gal	gnagal	glgal	Megal.
11:15	—	—	±	±	—	—	±	—	—
12 N	—	—	+++	+++	—	—	+++	—	—
1:30	—	—	✓	✓	—	—	✓	—	—

① glucose does not inhibit gluconate dissimilation.

C. Cells Aa. wash and test as:

	Gna	Glu	Gal	Xyl	Xyl+Gl	Arab.
1:30PM.						
4:30	—	—	—	—	—	—

W-108 C-source utilization. Pl. 8x

February 13, 1948.

Harvest from 100 ml gluconate broth. Conc. to 7 ml. Use ½ ml/tube contg. ½ ml 10% sugar, 1 ml buffer-indicator soln. ± ½ ml H_2O.
Set up 9:45 AM. Inc. 37°

	Glu	Glu/ml	Dalac	Glu+Gal	Ara	Ara+Glu	Xyl	Xyl+Glu	Gna.
10:20	−	−	−	−	−	−	−	−	+++
11:30	+	+	−	±	−	±	−	+	✓*
12:30	+	+±	±	+±	+	++	−	+	−
2PM	+ *	+±	±	++±	+++	+++	−	++	✓
5PM	+++	+++	+++	+++	+++	+++	++±	+++	+++.
	1:100		1:100		all −		all −		all −

Me Gal	Gal+Me Gal	Glu+Me Gal.
−	−	−
−	−	−
−	−	±
−	±	+±
+±	+++	+++
all −		

streaked out on Glucose plates: —

March 15-16, 1948.

Grow Y-10 & W-254 into YP 1% Lactose, 2x50 ml. each.
Y-10 & W-327 into YP 1% Maltose, do.

Harvest each, and concentrate in 10 ml volumes in sugar .5%, phosphate M/100.

At same time set up no-cells blanks. To 1 ml test samples.

Incubate at 37° 9A-1P 16. Add 4 ml. Barfoed's reagent to clarify. Boil supernatants 10 mins. Cool. Add 1 drop dil. Aerosol OT to wet Cu_2O ppt, and sediment and wash in H_2O. Take up sediment in acid ferric solution and titrate against .0200 N permanganate.

1. Y-10	Lac		0.10
2. Y-10	Mal		0.10
3. 327	Mal		0.30
4. 254	Lac		4.24
5. ---	Glu		9.40
6. ----	Mal	0.98	0.98
7. -----	Lac		0.28

There is therefore an almost equimolar accumulation of monose by 254, but none by 327 on lactose and maltose respectively.

The blanks contain 5 mg. sugar each. Note approximately 10% recovery pf maltose, but negligible recovery of lactose.

Keep remainder of suspensions 1 and 4 for further characterization of the accumulated material.

Take 1ml Exp. suspensions & controls of same carb. comp.

Clarify by 5ml Cu solution, ppt., and boil supernatant 10 min.

Sediment Cu_2O ppt., wash + dissolve in ac. Ferric sulf.

Titrate vs. N/100 $KMnO_4$.

1. Glucose + Phosphate	22.60 - 12.71	
2. Maltose + Phosphate	23.55 - 22.60	
3. Y10 culture	2.3.55 - 1 drop.	No glucose.
4. W327	23.69 - 23.91.	< maltose control.
5. - Phosphate.	23.91 - < 1 drop.	

Fractionation of Coli Lactase

March 20-22, 1948.

X. Ca 20 g. ~~of~~ Sharples paste W-254 ground with Pyrex. Extract overnight in cold with NaCl .9%. Sediment. + dilute to ca. 100 ml

3/2~~1~~/48. Test extract on lactose c̄ Bayford's method:

1 ml extract, 1 ml 5% lactose + make up to 3 ml. Incubate 3h. at 37°. # cc .01N KMnO4 to equal Cu2O ppt'd.

XL	>17 cc. (Bayford method)
X	0.23 cc
L	1.18 cc
X+L (added just before Cu ppt'n).	2.34 cc.

V. High activity thus indicated

Y. Ca 10 g. Autolyze 48h. 37° with toluene. Remove toluene + clarify. Make up to ca. 50 cc. Appreciable yellow color, deeper than X.

incubate overnight 37

Pool Autolysate + Extract. Add 4 vols. Acetone & Collect Sediment. Wash in abs. Acetone. Dry. → 1.6 gms. Acetone Powder.

3/22: Work in cold.

(1). 2ml X + 8ml acetone. Collect ppt + resuspend in 2 ml

(3). Do. ī 95% alcohol.

(2). 5ml X + 1.8g Am Sulf. (AS) Collect ppt. Supernatant ↓
Heavy ppt. and resuspended.

(4).

(5). 5ml Y as (2) ↑.
Heavy ppt

(6). See 2S. Add .9g AS. Collect ppts resuspend. S ↓
Moderate ppt. leaves v. opalescent solution.

(7). See 5S. Do. ↓
leaves clear solution.

(8). See 6S. Add .9g AS (to saturation + drops H_2O) No ppt. But v. opalescent solution.

(9). See 5S. Do. Collect + resuspend ppt.

(10). Supernatant of 9.

Assays on fractionation.

Use ≐ 1 ml. X or Y + 1 ml. .5% lactose. Incubate 30 mins. 37°. Then add 4 ml Cu Ac Sediment. Boil 10 mins. Wash ppt + dissolve in Fe^{+3} and titrate with .02N $KMnO_4$.

			CC:	
1. X	+++	7.4 +	8	
.5 X	+++	8.19	8	
.1 X	++	4.83	5	Note. ca 3 mg/½ hr.
.01 X	—	.40	.3	
Y.	++.	5.84	8	
1.	+++	8.42	8	Acetone
2.	++	7.20	7	(AS)
3.	++	3.10	6	(Alcohol)
5.	+...	2.67	7	Acetone
6.	—			
7.	—			
8.	—			
9.	—			
10.	—			
Glucose	++	8.98	—	
X + Glucose	+++	8.39	—	Utilization ??
Lactose.	—	0.13	Blank	

Cu_2O color + ppt roughest.

1. Autolysate active
2. Acetone powder active Alcohol powder active
3. Comes down at ½ saturation Am Sulf.

Fractionation of W-254 lactase.

Suspend 1g. Acetone Powder 160 in 50 ml. cold H_2O for 24 hours.

Residue

Supernatant 1. Fairly Clear.

Resuspended all fractions in cold H_2O, 50 ml.

8.7g AS

Fraction 1 (25% AS)

S2.

8.7 g AS

Fraction 2. (50% AS)

S3. Very turbid, but did not settle in centrifuge

8.7g AS.

Fraction 3 (75% AS)

S4. Very turbid.

8.7g AS.

v. little ppt except AS (excess). Fraction 4.

v. v. turbid solution

Fraction 5.

Assay: 1 ml .05 ml

1. Acetone Residue
2. Fraction 1 (1/4 satur.) sl. opalescent
3. F 2 (1/2 sat.) Clear
4. F3 (3/4 sat.) Clear
5. F4 (sat.) Clear
6. F5 Residue after AS sat. V. opalescent.

Assay with 1/2% lactose, 1/2 hour 37°.

2/20	1.30 – 2.41		1.11
2/1.	2.41 – 8.71		6.31
1/1	8.71 – 12.5	++	4 +
1/20.	12.59 – 13.40		.81
R	13.40 – 15.70		2.30
R/20.	15.70 – 16.70.		

Residue not uniformly distributed.

Others, 0.

Activity seems to be distributed among the "insoluble residue", the 1/4 AS and the 1/2 AS fractions. Continue to extract the residue + ppt with 1/2 AS. Pool 1/4 + 1/2 AS fractions with these extracted portions.

Pool Extractables from Acetone Powder + Ppt. with 1/2 sat AS. Resuspend in water and centrifuge 30 mins at 4000. Supernatant is very faintly turbid; Considerable ppt. (Particulate ??)

Compare activities: Use 50 ml volumes initially. Assay 20 min. 40°C.

a) 9 ml H/9 + 1 ml S 1/2 dilutions:

b) .9 ml ~~mll~~ 1 ml S 1 ml Ac.

Dil., ml.	P	S.	
1 ml	0.50	5.17	Enzyme in soluble fractions
1/2	0.31	3.63	after AS ppts.
1/4	—	2.03	
1/8	—		
1/16	—		
1/32	—	.030	Activity is much less than original
1/64	—		conditions too close to
1/128	—		substrate exhaustion.

Blank

When fraction B is pptd. c̄ AS 50%, three fractions are obtained.

C 1) Supernatant — Cu_2O

C 2) Sedimentable residue after resuspension in H_2O — v. sl. visible Cu_2O

C 3) Non-sedimentable residue. — Cu_2O.

Assay 1/4 ml samples (in 50 ml ⌀) & compare with ~~whole mixture~~ B. (2.03 ml)

40° may be too low!

Preparation of lactase : Batch 2.

Grow K-12 in 12 l. N-case 1% Lactose 1/20 under strong aeration. After 24h. Harvest in Sharples (Watson).

Fraction 1. 31g. paste – Add 100 ml H_2O, 5 ml toluene, mix in blender + ~~as~~ autolyze at 37° ~~#~~ 11A 26 –

Fraction 2. 42 g paste. Add 100 ml Acetone, shake well, sediment + add fresh acetone. After dehydration, dry in desiccator over paraffin. ⇒ 15.4 g ("nearly dry") acetone powder.

3 Suspend ~~10~~ 5 g. powder in ~~100~~ 50 ml H_2O to extract.

Assay (as in 161 b) .1 ml suspension (20 mins, 40°).
3.~~8~~ ml .02 N $KMnO_4$.

Extract with cold H_2O 8h. Centrifuge at 4000 rpm 1 hr.

4 Add 17.5 g AS (½ sat.) < small ppt. Redissolve in H_2O. A
supernatant. B.

Test .1 ml samples of each:

162-4A No visible Cu_2O
162-4B. " "

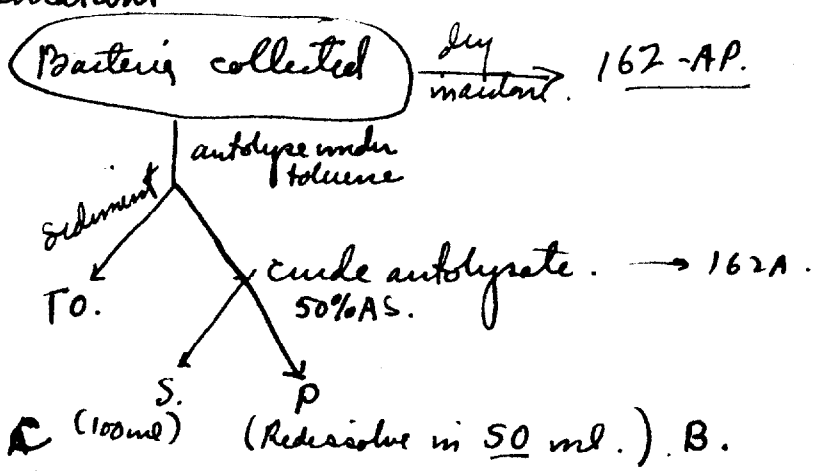
40° may be too high for assay.

No activity!

P28. Clarify 48h. Autolysate (add a few ml $CHCl_3$ to take up toluene and permit sedimentation of solvent) 120 ml autolysate. Almost entirely clear, light. yellow-green solution

Rep 20 ml sample Work with the other 100 ml.

Add 35g. AS. Collect ppt. + redissolve in 50 ml H_2O_2 Fairly clear solution. Pigment is left in supernatant.



Assay .1ml, .01ml samples (on 100 ml basis) 20m. 37°

A
B
C } No visible Cu_2O ppts! [Were cells still adapted?].
[Is glass a factor?].
[Are products being metabolized?].

A29. Repeat using 1ml, .01ml. in M/100 Na Citrate as buffer pH 7.3.
[Previous prepn. autolyzed in citrate].

No Activity.

Lactose Preparations.

March 29, 1948.

10 liter lots 11-12 in N2Case + Glucose (A), N2Case + Lactose (B).

Aerate, 37°. 24h. (Alkateg antifoam). Collect in Sharples.

Bottle A lost. Collect 53g. cell paste from B. [Drop A, B names?]

A]. 10g. put in 100 ml. NaCl-citrate + 1 ml. toluene

B]. 43g. put in 100 ml 5% lactose in citrate buffer. 1½ h. Then wash, autolyze under 1% toluene.

Collect after 24h. Store 1P31 in refrigerator.

B. became ~~very cloudy~~ opaque on standing in refrigerator overnight. On warming this material redissolved. Keep 10 ml as cide ppt. = 163B1; add 14g. Am. Sulf. to remainder + cyanate fractions.

ppt. Redissolved in citrate 163-B2

sup. 163-B3 - from ppt in cold!

Assay 1 x 1 ml enz. + 1 ml 1% lactose, 30 mins. 37°

		Cu_2O
Glucose		+++
Lactose		—
Glucose in citrate		++
A	1.0	±.
	0.1	—
B1	1.0	±.
	0.1	—
B2	1.0	±
	0.1	—
B3	1.0	—
	0.1	—

Probably fermentation in lactose with limited nitrogen served to de-adapt the culture. In future, add fresh lactose to whole medium before centrifuging.

to B2, add 14g thiosulf. Redissolve ppt in H_2O.
v sl. soluble

Temperature mutants.

March 29, 1948.

85 plates, Y10, 5 secs. Hanovia U.V. ca. EMBLac

Incubate at 45° 11A 29 – x ca. 250 ~~plates~~ colonies.

= 20,100 tests.

Recovered W-340

Test at 45°.

Apr. 1, 1948 + 25 plates, x200 = 5000. = 25000 total.

Test W-340 at 36° and 44°.

	36	44
Glucose	+ slow*	–
Galactose	++	++
Gluconic	++	++
Maltose	+ slow	–
Lactose	++	–

* faster at < 36.

At 44° this mutant is similar to W-108, but the lactase activity may be more resistant to 37° than the glucozymase.

April 6, 1948. As above. 100 plates x 300 = 30,100

No detected mutants at 45°

Temperature mutant W-340

W-340 grown on GNA Broth at 37° + 45°, and Lac YP at 37°.

Cells Harvested from 100ml Gna 37 / 6ml H_2O. = 2

37 = A 45 = B.

Cells from YP Lac = 1. (50ml into 2ml H_2O).

Test at 37 + at 45.

Set up 11:35 AM. Apr. 5.

		37 α	45 = β.
11.	1 / Lac	+ ++++	± +++
12.	2A / Gna.	++++	++++
13.	2B / Gna	+++	++++
14.	2A / Lac	–	–
15.	2A / Lac	–	–
16	2B / Lac	–	–
17.	2B / Lac.	–	–

12β was +++ in 5 minutes. 12α in 8-10.

13β " +++ in 8 minutes.

15 MINS.

30 mins.

No further adaptation in next 6 hours.

Lactase production; K-12, lactose synthetic medium

Apr. 9, 1948.

Inoc. ~~2x~~ 50 ml each. K-12 cultures into 10 l. bottles (2) of synthetic medium (v. supra) with 1.5% lactose USP. Aerate at 37° A9-A10. Collect in Sharples.

87 grams damp cell paste.
Suspend in 100 ml M/20 Tris-saline + 2 ml toluene + autolyze at 37° Stir int. and collect supernatant 10 A12. Cool in [illegible]. 150 cc. total.
Save 20 ml. whole clear, yellow autolysate. To remainder (cold), add 45 gms AS. + ppt. During centrifugation, about 2/3 of this material was involved in an accident. The gross glass was removed + the supnt. recovered. The cup + broken glass were washed with 100 ml H_2O, then 35 g. AS added. The ppts collected here were pooled and redissolved in 50 ml. H_2O. (A) Proceed with sedimentation of remaining 1/3, dissolve ppt. in 50 ml H_2O (B).
Assay!

What is ~~green~~ yellow pigment?

Polarimetric measurements of
glycolytic activity

				m.
A0.	0,00	0.01	-0.01	0,00
0 B.	1.34	1.34	1.35	1,34
0C.	1,42	1,44	1,42	1,43
C20	1,38	1,39		1,38
c180	1,47			

No activity!

~~P90~~ P180. 1,46

No activity!

Inhibition of adaptation by amino acid antagonists

April 27, 1948

Each tube is made to 4.5 cc. Cells harvested from YP-glucose or YP-lactose overnight.

Each tube contains

1 ml 5% lactose
1 ml cells
.5 ml conc. BCP indicator + .1 ml Phosphate Buffer M/10
± 1 mg valine ± 1 mg isoleucine ± 1 mg hydroxyaspartic* ± 1 mg aspartic*

	grown L.			grown G.		
1.	−	+++	+++	−		+++
2. IL.	+++		+++	−		+++
3. V.	+++		+++	−		+++
4. V+IL	+++		+++	−		+++.
5.* Asp.	−		± * overneutralized?	−		± *
6.* HOAs.	−		+++	−		−
7.* Asp+HOAs.	+++		+++	−		+++

* overneutralized c̄ NaOH

− 30 m. 3:30.
− 3 h. 6 PM
− 18 h. 9 AM.

By all appearances, valine did not inhibit adaptation, but the experiment is clearly of too long a duration. Hydroxyaspartic, on the other hand seems to have been inhibitory to adaptation even in the presence of excess pantothenate. The clear interpretation of this experiment demands a better control of the adaptation process

* + 5γ pantothenate.

Apr. 29, 1948.

	1:30	2:00	2:30	3:00	3:30
1	—		++±	+++	
2	+++		+++	✓	
3	±		++	+++	
4	—		++±	+++	
5	—		++	+++	
6	—		+±	+++	
7	—		+±	+++	
8	—		+±	+++	
9	—		—	+±	+++
10.	—		+±	+++	

valine inhibits adaptation somewhat and is reversed by isoleucine.

Cells from 400 (in 4 ml.) ml Nzase - PO_4 - glucose broth collected in 10 ml. Each tube contains:

Set up 11:30 A.M.

2 was +++ in <10 mins.

1 ml cells
1 ml 5% lactose
1 ml buffer + indicator BCP.
.1 ml addenda:

1. —
2. (Glucose 5%)
3. + glucose .5%
4. Sod Succ. 1%
5. Nzase .1%
6. TLB.
7. $MgSO_4$ .1%
8. valine } 1 mg/ml .5 ml.
9. isoleucine
10. V + il.

The temperature mutants W-340 and W-382.

May 3, 1948.

Add 1 drop inocula to BCP-fermentation broth, at indicated temperature:

	glucose	lactose	maltose	[redacted]	gluconic
W-340 30°	++ ++	− +±	+ ++	−	+±++
45°	−	−	−	−	~~+++~~
W-382 30°	++±	++	+++	−	+++
37°	−	+	− −	− −	++++
45°	−	−	−	−	++

Broc 5P3.
Fruit Reading 8A4 = 15h. These are both temperature mutants.

W-340 inoculum taken from old slant.

From first test of W-382 on maltose, papillae picked and streaked out.
Mal+ colonies tested on EMB at 37.5°

lactose 19+ 0−

Glucose 13+ 1− 1 uncertain or mixed.

Purify 1+ and 1− on maltose.
mal+ lac− gluc− + al
purify as 30°

Temperature mutants.

May 4, 1948.

Use 1 drop inocula from fresh gna broth cultures & incubate fermentation with BCP tubes as indicated.

	32°				40°			
	glucose	lactose	maltose	galactose	glucose	lactose	maltose	galactose
58-161	+++	+++	++	+++	+++	+++	+±	+++
W-108	–	–	–	++	–	–	–	+++
W-340	+++	++	+++	+++	–	–	–	+++
W-382.	+++	+++	+++	+++	–	–	–	+++

Bro. 6P4.
1st. reading 9A5. = 15h.
9A6 = 39h.
9A7 = 63h.

[Note numerous weakness of 58-161 on maltose]

All readings identical.

do.

TO

Critical temperature: Temperature mutants.

May 5, 1948.

W-340 and W-382 inoculated into BCP broth tubes at indicated temperatures:

30° Plus on glucose, lactose and maltose in 12 hours.
and galactose

32° Ditto. Inocula from gma broth .2 ml

33-34° Ditto.

5P 5. Inoculate W-340, W-382, 58-161, W-108 as above.

	9A6 16h.	glu	lac	mal	gal
340		− ÷	+++	− ÷	+++
382		− ÷	+++	− ÷	+++
108		−	−	−	+++
58-161		+++	+++	+++	+++

Temperature fluctuation between 35° and 36°. This may account for slow development of 382 Maltose, etc.

−1P6 ∴ At 36°, W-382 is lac + glu −

~~9A7 Maltose~~

May 6, 1948.

Harvest cells of W-257 from overnight cultures of YP-broth. 50 ml. / 3 ml suspensions.

A)- maltose 1% B)- gluconate 1%

To 1ml 5% substrate, add 1 ml cells and 1 ml. .01 M Phosphate buffer plus BCP indicator. Incubate at 36°. Set up 11:15 A6.

	glucose	maltose	gluconic
A.	—	±	—
	—	+++++	—
	++	+	+
B	—	—	++++
	—	—	++++
	±	—	+

To 1 ml. B cells add 1cc gluconate and .5 ml 1% triphenyl-tetrazolium hydrochloride.

very deep red by 15 mins. Cytological Study:

1. 15 mins (11.30) 9A7. All tubes were +++
2. 15 mins (12 M)
3. 120 mins 1:15 PM
4. 3:30
6PM. —

Glucose "adaptation" 1929.

Grow Y10, W382 in gna Y2 broth at 34°. Collect cells in 2 ml and test at 34° on glucose and glucanic. Set up 11 AM.

	Glucose	Gna.		Glucose	Gna.
Y10. 11 AM.	–	–	W 382	–	–
1115	–	+++		~~+++~~	+++
1130	–	✓		–	✓

Inoc W-382, W-340 and 58-161 into BCP tubes at 33° + 40° as indicated. 6P6. 1st Rdng 9A7 : 15h.

	33°				40°			
	Mannose	Mannitol	Fructose	Sorbitol	Mannose	Mannitol	Fructose	Sorbitol
340	+++ ✓	±	~~—~~	++ ✓	— ✓	— ✓	— ✓	— ✓
382	+++ ✓	++ ✓	+++ ✓	— ✓	— ✓	— ✓	— ✓	— ✓
58-161	+++ ✓	+++ ✓	++ ✓	+++	+++ ✓	+++ ✓	+++ ✓	± +

[illegible] Sorbitol [illegible] [illegible] [illegible]

Galactose - Arabinose X - adaptation.

May 7, 1948.

Harvest K-12 from 16 hour cultures of YP sugar broth:

a) arabinose b) galactose c) glucose. 50 ml broth, 4 cc suspension

10:45 AM. (A7).

substrate

	a arabinose	b galactose	c glucose
a	+++	− + +++	+++
b	− + ++	+++ +++	+++
c	− − −	− − −	+ (11 AM) ~~+++~~ (11:15)

11:30 1st reading.
12 N 2d reading.

See 100 [Adaptation in presence of azide] Arabinose × galactose with Y10. + Cohen's letter

l-arabinose and d-galactose adapted cells have reciprocally shortened adaptation times. The interconversion is not inhibited by azide.

May 7, 1948.

Prepare 8 ml cell suspensions from 50 ml. YP broth cultures (YZ-sugar)

Cells: A: no sugar, B-glucose C- galactose D- lactose.
Substrates: 1 glucose, 2-galactose 3- lactose.

or at 40°

After harvesting, icubate cells without substrate or buffer at 33-34° for two hours. Then (1:30 P 7) add 1 ml 5% sugar and buffer-BCP

	34° A	B	C	D	40° A	B	C	D
1	−	+	±	+	−	−	+	+++
2	−	−	+	++	−	−	+++	+++
3	−	−	−	+	−	−	−	−

W-340 Exactly as above.
Cells: A-glucose, B-galactose, C-lactose Substrates as above.

	A	B	C	A	B	C
1	+++	+	+++		−	+++
2	−	++	+++		−	+++
3	−	−	+++	−	−	±

Concl Glucozymase is adaptive at 34°, but is produced during galactose adaptation.

①. 2PM. (20-30 mins). 2:30 1 hr. 3:30 2 hr.

[at 34° [illegible]

Summary of ~~#~~ p. 195

Tested for stability at 40°.

W382. + W 340 gave identical results.

Cells grown ↓	Glucose	Galactose	Lactose
Glucose	—	—	—
Galactose	+++	+++	—
Lactose.	+++	+++	—

at 34°

① Glucozymase in glucose adapted cells is unstable at 40° in absence of substrate, but in galactose and lactose adapted cells is stable.

② Glucozymase is adaptive at 34°.

③ Lactase is unstable at 40°.

Suggested:

[Compare enzymes from Y10 and W-382 under otherwise comparable conditions.]

[Does substrate protect stability?]

Stability of adaptive enzymes in absence of substrate at 40°

May 8, 1948.

Grow Y-10 and W-382 in 50 ml. batches YZ-sugar broth at 34°.

A. Glucose (2 flasks each)
B. Lactose (2 each)
C. Gluconic (1 each).

Dispense 1 ml. volumes to tubes with 1 ml indicator buffer (with and without azide) at 40°
At stated times add 1 ml. substrate and record time required to ferment.

Cells: A,B,C. Substrate: a,b Azide +, —

Time substr. added: (minutes).	Aa +	Aa −	Ab +	Ab −	Ba +	Ba −	Bb +	Bb −
Y-10 cells. 0	+15 ++±30	45 ++15	++30		+15 ++±30	30 +15	+15 ++±30	45 +++15 30
30	+45 ++60	45			++45 ++±60	75 ++45 60	+45 ++±60	75 ++45 60
60	+75	90 75			+75	90 ++75	+75	90 ++75 90
120	160	8[illegible]			160	8[illegible]	++160	160
W382 cells. 0	±15 ±30	+±120 +75 -15	+±120 +75 30 -	-	+15 ++30	45 60 +15 30	+15 ++30.	120. At 90 still ++ +15
30	-45	-45			+45	++±60 90 +45 60	±45	160 ++±120 ++75 +60 +45 60
60	-75	-75			+75	++90 +75 90	±75	±160 +120 +75 90
120					++160.	160	-160	++160

[illegible] — [illegible]

t0 = 10:45 AM
15 = 11:00 "
30 = 11:15 "
60 = 11:45 "
120 = 12:45
160 = 1:25
180 = 1:45

[illegible]

Time Required to ferment:

Cells disintegrated at 40° for minutes indicated before addition of substrate.

	Aa+	Aa−	Ab+	Ab−	Ba+	Ba−	Bb+	Bb−
0	45	15			30	30	45	30
30	30	15			45	30	45	30
60	30	15			30	30	30	30
120	40	<40			40	<40	40+	40
0	>120	>120			60	30	45-120	30
30					60	30	45-120	30
60					60	30	45-120	30
120						<40	45-120	45

Y10

W-382.

W-382.

cf. 195.

Needed control on activity of W-382 glucose-glucozymase at 34°!

W-382 glucozymase in glucose adapted cells is very unstable compared to the corresponding Y10 cells or to glucozymase in lactose adapted cells of W 382. Azide does not prevent this instability.

No indication this time of lactase instability.

Check on possible temperature-sensitive Lac-

May 15, 1948.

Incr Lac-NZCase-BCP fermentation tubes amply from st. slants of:

	30°			37.5°			40°		
				5P15					
W-42	−	−	−	−	−	−	−	−	−
W-110	−	−	−	++	+++	+++	++	+++	+++
W-305	±	+	++	±	±	+++	−	±	++
Y-10.	++	+++	+++	++	+++	+++	+++	+++	+++

(1). N16. = 19 hours.
(2) 7P16 = 25 h.
(3) 9A17 = 39 h.

W-42 is not temperature-responsive.

W-110 is − at 30, + above 37.

W-305. is about equally slow at all temperatures compared to Y-10, perhaps slower at 40° than at 37.

Coli Lactase

~~to~~ 50 ml ~~½~~ Lac broth, cells harvested in 10 ml H_2O. Successive 10 fold dilutions in 10 ml M/50 citrate buffer pH 7.5 at 37°, ONPG M/5000. 10 $H_{1\,1\,2}$. Incubate 10 mins, then boil.

(1) ~~Tr~~ Preliminary tests:

cc cells.	Initial absorption: density $\lambda = 420$	$\lambda = 650$	Δ^{420}	Correction:	Final density. λ 420	λ 650	cor. Δ	% hydr.
1	.51	.34	.41	.61	.92	.41	.31	ca 50
.1	.065	.049	.08	.071	↗ .145	.054	.074	ca 10
.01	.009	.008	.027	.010	.036	.010	.025	< 5
.001	.004	.004	.023		.027	—	.023	< 5

$$\text{Correction} = \frac{\lambda_i^{650}}{\lambda_f^{650}} \cdot \lambda_i^{420}$$

(2). ~~Use 2 ml cells~~. ~~Vary substrate conc~~. 10 mins tests 5 boiling. Range .1 – 1.0 seems to be satisfactory. Boiling should be omitted as it causes some 2-3% hydrolysis.

cc cells.	λ^i_{420}	λ^i_{650}	λ^f_{420}	λ^f_{650}	λ^i_{CORR}	Δ
0						
~~.1~~ .1	.066	.041	.140	.038	.060	.080
.2	.127	[illegible]	.276	.073	.115	.161
~~.3~~						
.4	.250	[illegible]	.520	.142	.225	.295
~~.5~~						
.6	[illegible]	[illegible]	.740	.209	.315	.425
.8	.450	[illegible]	.930	.270	.405	.53
1.0	.540	.270	1.05	.339	.486	.56
after 1 hr	.4		.690	[illegible]		[illegible]
2 hr			.750			.525

ONP. CT.

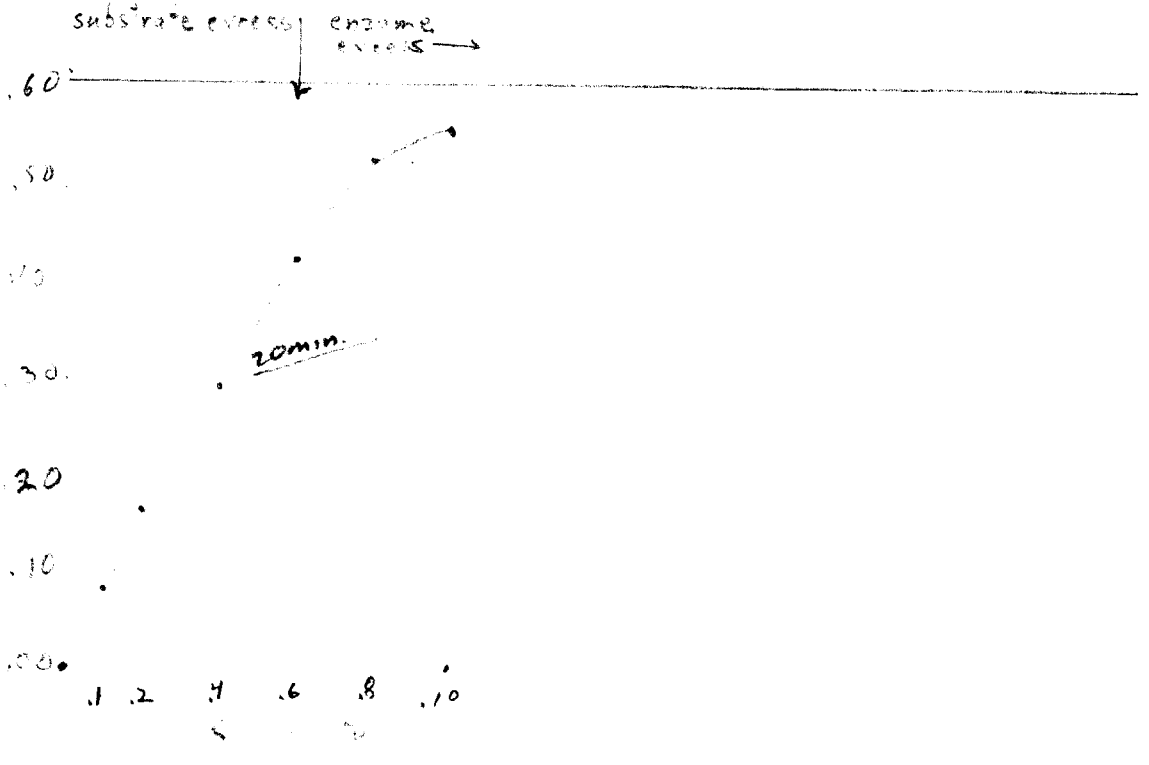
$\frac{M \times \cancel{10}}{50000}$ Citrate buffer pH 7.5 M/50. λ = 420.

uplicates.

	C	D.
A.	1	.070
	1	.065
	2	.140
	2	.132
	4	.270
	4	.272
	6 +	.409
	6	.394
	8	.515
	8	.511
	10	.614
	10	.619

	λ = 420	λ = 500
160	.20	.07
172	.24	.04

10 mins in NPO system.


substrate excess
enzyme excess
.60
.50
.40
.30
.20
.10
.00
.1
.2
.4
.6
.8
.10
20min.

Inhibition by maltose.

[illegible]

2	.032	—
3	[illegible]	.019
4	.062	—
5	.290	.015

Cell blank

1	.249	.161

M/10

Cells .5 ml + 9 ml sugar solutions + 1 ml ONPG All in M/50 buffer.

1. Lac no ONPG
2. Lac ONPG
3. Glu "
4. Mal "
5. — "

20 min readings at 37°.

1 is blank.

	D_{420}	D_{650}
2	.032	0
3	.080	.019
4	.062	0
5	.290	.015
Cell blank 1	.249	.161

Note inhibition by maltose and glucose

Repeat using Sucrose + Maltose.

0	.241	.014
Suc	.239	.010
Mal	.083	.004

Note inhibition by maltose but not by sucrose

Inhibition of galactosidase by carbohydrates.

Sept. 15, 1948

M/50 citrate buffer 7.5 M/200 ONPG
20 m. 37° M/10 (ca.) Sugars

		% inhibition	
K-12	0	0	Control
		− 80	Maltose
		− 83	Galactose
		+ 15	Gluconate
		76	d-Cellobiose
		85	l Arabinose
		55	d Xylose
W211		—	
		87	Maltose
W33		—	
		82	Maltose
Y10		—	
		85	Maltose

PM.	0	.282	—
	glucose	.058	79
	fructose	.133	53
	mannose	.251	11
	raffinose	.000	100
	trehalose	.269	5
	dulcitol	.060	79
	sorbitol	.199	29
	melibiose	.000	100

Qualitative inhibition tests.

Sept. 15, 1948.

Due to paucity of material, the following tests were done in 1.0 ml volumes. 100 μM was dissolved in .9 ml bacterial suspension in buffer as above, then .1 ml M/100 ONPG was added after temp. equilibrium. Color read as + or − :

K-12

	Color	
—	+	
Maltose	−	
Galactosan	+	
Lactitol	−	6±
d-arabinose	+	
Ca Lactobionate	+	
Ca Maltobionate	±	Original color makes this reading doubtful

Adaptation Expt: Preliminary

3502

Sept 17, 1948

		λ_{420}	λ_{650} vs	λ_{650} vs water	420 H_2O	3 hour exposure opt. color
L	—			.121	.192	
glucose	1	[illegible]	.015			+
"	2	.100	.012			+
lactose	3	[illegible]	.014			+
"	4	[illegible]	.019			+
Lac + Azide M/100	5	[illegible]	[illegible]			+
"	6	[illegible]	[illegible]			+
Glu + Az	7	[illegible]	[illegible]			+
"	8	[illegible]	[illegible]			+
water	9	[illegible]	[illegible]			+
G	—			.159	[illegible]	
glu	1	[illegible]	[illegible]			—
"	2	[illegible]	[illegible]			—
lac	3	[illegible]	[illegible]			+
"	4	[illegible]	[illegible]			+
lac azide	5	[illegible]	[illegible]			+
lac ATP 5 mg	6	[illegible]	[illegible]			—
" " + azide	7	[illegible]	[illegible]			—
Br_2 Im M/1000		[illegible]	.013			+
SMT M/2000	9	[illegible]	[illegible]			—
water	10	[illegible]	[illegible]			—

Concentrate cells from Y2 Lac (L) and Y2 Glu (G) 5:1

Adaptation system: 2 ml cells: 2 ml 4% sugar in M/5 buffer + 1 ml (supplement if any. Centrifuge once + resuspend in 4 ml H_2O Test c̄ ONPG in M/10 citrate buffer as above., 1 ml: 9 ONPG + buffer.

SMT, Azide apparently inhibit adaptation, benzimidazole does not at this concentration.

Conditions for gal. adaptation

9/18/48.

Conc 100 ml H-12 from Y2 Glu to 20 ml (5:1)

Add 2 ml cells to 2 ml sugars 4% in M/5 buffer pH 7.0. Add H_2O or suppl. to 5 ml volume 1130 A18. Incubate 5 shaking at 37°.

	Sugar	Suppl.
1.	Lactose	—
2	"	—
3	"	Peptone 1%
4	"	Y. Extr. 1%
5	"	Glucose 1 mg
6	Glucose + Galactose ½ ½	
7	—	—
8	Lactose	Hydol. Casein 1%

ONPG as above, but use total volume of 9 ml. rather than 10, and use 8/9 ONPG previously.

Read tubes a) against water suspensions of same cells, and b) the latter against water, all at 420.

	a (activity)	b (cell dens.)	R.A.	% L.
1	.160	.207	.77	100
3	.499	~~.334~~ .279	1.79	233
4	.551	~~.279~~ ~~.334~~ .310	1.78	231
5	.022	.200	.11	14
6	T=101	.230	0	0
<.005 7	.00[illegible]	.200	0	0
8	.519	.334	1.55	202

Y. Extr., Peptone + H.C are definitely stimulatory to adaptation.

N source: Conditions of adaptation

Sept. 20, 1948

System as above (except 2x for anaerobic expts.)
All tubes contain lactose exc. 1.

		Suppl.	Relative activity
1.	—		03
2.	Lac		35
3.	"	Glucose 1mg	07
4.	"	$(NH_4)_2SO_4$ 1ml 1%	32
5.	"	" , glucose	11
6.	"	asparagine	43
7.	"	TL	75
8.	"	4, anaerobic	28
9.	"	5, anaerobic	21
10.	"	4, Vits. .1ml	33
11.	"	2, Am. Ac.	125
12		M.C.	120

	D^i_{420}	D^t_{420}	$D^i \times \frac{230}{251}$	Δ	$\frac{\Delta}{D^i_{corr}}$
1	.251	.237	230	007	03
1r	.230				
2	229	.282	209	073	35
3	236	.231	215	016	07
4	220	265	201	064	32
5	221	225	202	023	11
6	176	230	161	069	43
7	220	351	201	150	75
8	180	.210	164	046	28
9	215	.238	196	042	21
10	213	.260	195	065	33
11	297	610	271	339	125
12	309	620	282	338	120

Sept. 11, 1948.

Effects of amino acids on adaptation.
K-12 harvested from 1/2 Glu as above.
Irradiated supplements ca 1 mg. eq. in 1 ml.

	A	B	C	D. overnight
O	242	224		–
Lac	230	218		–
Mgal	246	237		+++
Bgal	319	310		+++
CNPG.	240	219		–

A = D_2O lact. susp. V = 9 mC
B = D_2O + substrate check. 10 mC
C = A avg (x .90)
D = B − C = Δ
E = D/C = relative activity

		A	B	C	D	E	% lac ~~lactoflavin~~	
A12	1	224	377	202	95	47	–	69
A3	2	246	370	216	104	48	–	71
A4	3	249	335	224	109	49	–	72
A5	4	273	429	246	173	70 ~~64~~	+	103
A6	5	241	380	224	156	70	+	103
~~A~~ucine	6	239	291	215	76	35	–	52
~~A~~lutam	7	263	400	237	163	69	+	103
~~A~~leuc	8	258	356	232	124	53	–	79
~~A~~alan	9	230	348	207	141	68	+	102
pro	10	255	371	232	139	60	–	90
lys	11	246	366	222	144	65	+	97
arg	12	235	409	214	195	89	+	133
meth	13	231	383	208	175	84	+	125
+lac	14	251	377	226	151	67	–	100
−Lac	15	230	217	207	10	5	–	7.5
H.C.	16	351	870	316	554	176	++	263
H.C.+Tryp.	17	347	860	312	548	178	++	266
T+Leu	18	263	409	237	172	73	+	109.

only arginine and methionine showed significant stimulatory effect for K-12 adaptation.

Sept. 22, 1948.

5 ml system for adaptation in above. All lac. K-12.

		A	B	C = A corr	D (B-C)	E $\frac{B-C}{C}$	% of Lac (1)
1.	—	228	305	205	100	56	100
2.	HC	310	700	279	421	151	
3.	AA. of HC	296	650	266	384	144	
4.	Σ AA	271	520	244	276	118	
5.	AA - A12	229	309	206	103	50	
6.	" A3	249	479	222	257	116	
7.	" A4	259	520	233	287	80	
8.	" A5	241	479	217	212	98	
9.	" A6	250	460	225	235	104	
10.	Arg + Meth.	239	371	215	156	72	130

· 2 ml each AA group in 4-9.

· 5 ml ca. 10.

· 1 ml HC 10% 2.

1 ml ≡ 10% HC 3.

Sept 25-26, 1948.

K-12 grown 24 hours in Synthetic + Lactose 1%, 10 liters.

25g. cell paste recovered. Ca 24g. + 10cc 7.5 PO_4 buffer shaken 24h. under toluene. Remove debris & collect supernatant in

(A). ca 30cc buffer. deep yellow green fluorescence. Ca 1ml/gram bacteria.

(B). Ca 1g. washed c̄ acetone and dried at room temperature. Considerable loss by spattering allows calculation only of final product.

See 316

See 325 for assay.

Sept. 25, 1948.

K-17 grown in 200 ml 1/2 lactose. Harvest to 5 cc. 7.5 buffer & autolyze under toluene & shaking 24h & 48h.

(A) 24h. 1 ml withdrawn, debris sedimented & supernatant diluted to 1/2 ml.

(B). 48h. Remainder (4/5) removed, etc. dilute to 16 ml

each ml corresponds to 10 ml original culture & should have an activity of ca. 10 × bacterial suspensions. (i.e. .05 ml should give ca 100% hydrolysis of 10 ml M/5000 ONPG in 20 minutes). I.E., calculating 2g/liter, corresponds to 20 mg/ml

See 316

Sept. 27, 1948.

N-12 grown 36 hours in 10 litres S(Lac). 9.4 litres of supernatant were removed leaving 31 grams wet Sharples paste. Make up to ca 45 ml c̄ PO_4 buffer pH 7.5 and grind 75 minutes in Booth-Bream mill. Combine efflux c̄ washings. An ~~opaque~~ milky opalescent supernant is obtained, in ca. 100 ml, i.e. .31 grams/ml

(A).

(B). 10 ml sample of culture was taken. Resuspend in eq. H_2O + measure turbidity at D_{420}.

1:20

1:50 dilutions.

1 Unit = A of .100 in D_{420}.
for cell fin prep.

Assays:	A	B	C	D	Act./ml.		
1	008	290		283	14	315A	.2 ml
2	002	205		205	~~100~~ 10	B	.2 ml
3	007	260		254	25	314A	.1 ml
4	001	043		042	40		.01 ml
5	010	020		021	90		.001 ml
6	032	1500		1500	150+	316A	.1 ml
7	002	980		980	980		.01
8	000	290		290	2,900		.001
9	360	1900		1600	(445)	316B(cells)	1 ml
10	079	880.		809	(1100.)		.2 ml
ONPG.	012	012.		0	0.		—

v. high activity!
for non-enzymatic (chem) hydrolysis!

In prep. 316., 1 ml being ≙ culture volume 10 litres/100 ≙ 100 mil cells.

and .001 ml should be equivalent to .1 ml cells, indeed it is, very nearly. Thus a large proportion of the cellular activity is present in extracts. Autolysates are nearly as effective with smaller volumes.

Sept 28, 1948.

K-12 grown on 100 ml T(O) glucose (A) & do. + H.C. (½ ml/100) (B) shaken 16 hours. Adjust densities:

(A) 1:10 dilution D_{650} 259

(B) HC. 319

ratio of 1.23 : 1.

Re-suspend ~~Dilute HC~~ (O) culture in ~~50~~ 20 ml H_2O; the HC culture in 24.6 ~~61.5~~ ml H_2O to adjust initial densities.

The adaptation system consists of 1 ml cells + 3 ml T(O) + lactose + H [illegible] supplement. Adapt 3 hours, in duplicate. Resuspend in ½ ml [illegible] 8 ml buffer for A., Add 1 ml ONPG 20 minutes for B.

		A	B	C	D	E
	1. Lac. buffer pH 7.0 M/50.	.201	745			
		.196	641			
	2. T/O Lac	.248	669			
	12	.260	720			
	3. T(O) Lac + .1 ml H.C.	.256	710			
	13	.260	731			
(B	4. Lac buffer	.177	169			
	14	.184	175			
	5. T(O) Lac	.187	171			
	15	.188	170			
	6. T(O) Lac + .1 ml HC	.189	153			
	16	.172	168			

	A.	B.
A. .25/9	.232	.219
B. .25/9	.239	.200

Negligible activity of unadapted culture and of B series.

Sept 28, 1948.

(NZ) W478 x W583 on Lac B_1. 20 colonies streaked on Lac EMB: All ++.

Fractionation of galase 316.

Sept. 28-9, 1948.

Original extract (316) consisted of 2900 u/ml in 100 cc or 2.9×10^5 units all together. To fractionate remove 50 ml and dilute c̄ 50 ml H_2O. (1.5×10^5 units; ~~1.5×10^3~~ 1500/ml). "316" is fraction 0. Add $(NH_4)_2SO_4$ in 4 aliquots of 17.5 g. each in ice bath to give ¼ sat'd fractions. Take up sediments in 10 ml M/50 $PO_4^{\equiv}$ except for the final fractions.

	Prop. ~~Conc.~~ ~~Activity~~	Act.	Prop. Act.	Assay .01	.001
0	1.00.		1.00.	615	.089
1 (¼ sat).	5.00			129	019
2 (½ sat)	5.00			390	055
3 (¾ sat)	5.00			194	028
4 (sat.)	10.00			101	015
5 Supernat.	1.00.			060	015.
					.140

Assay at the equivalent of .01 and .001 ml of ~~the original~~ fraction 0. 1 ml M/500 ONPG in M/50 PO_4 buffer.

Enzyme activity is probably not quite linear. Fractions have higher total activity than the original "soup".

Pool fractions ~~1~~, 2 + 3 (40 ml) and add 24 gms $(NH_4)_2SO_4$ (¾ sat). Take up ppt in M/50 citrate buffer, [illegible] 319A

P30. To remaining 50 ml (1.5×10^5 units) add 250 ml collacetone, let stand, and filter off 330 mg. dry powder. 319B. This should have an activity of about 500 u/mg. Take up 10 mg in 10 ml Phosphate buffer.

Effect of phosphate on lactase

Sept. 29

Lactase preparation 319A is suspended in M/50 citrate buffer. pH 7.5 (Ethylene diamine - citric acid) = (EDC buffer), and should have a potency ca. [100/20] × (.58 + .23) ×10³ u/ml. = 4000 u/ml.

Assay

.001 ml in citrate and in phosphate buffer M/50. pH 7.5.

Triplicate series.

Phosphate seemed to be melted. After 7 mins, use 7 ml EDC + 1 ml Phosph. + 1 ml enzyme + 1 ml ONPG.

1	~~EDC~~ PO_4	.371
2	"	369
3	"	390
11	~~PO_4~~ EDC	012.
12		013
13		012.

ONPM/5000 m

21	EDC	640
22	"	640
31	PO_4	750
32	"	745

41. (7 mins later).
EDC + PO_4. 0

may be due to inhibition by citrate.

Sept 30, 1948.

K-12 in A) T(O) B) T(Pro) .5 ml 1 mg/ml. C) T(AA) .5 ml 1% HCl
shake overnight. Resuspend in 5 ml H_2O. Turbidity at
1:100 dilution:

	Dens
A	119
B	119
C	52
	050

Dilute A and B to 11.9 ml to equalize c.

Adaptation system: 5 ml. 3 hours 37°. 10:30 A – 1:30 P

1 ml cells
3 ml substrate.

① Phosph M, 50 7.5 + 2% lac
② T. (2%) Lac.
③ ② + ~~supplement~~ proline 1 mg% .2 ml
④ ② + ~~HCl~~ AA. ~~1%~~ 1 ml

		A.	B.
A	1	176	220
	2	259	331
	3	162	218
	4	160	291
B	1	169	215
	2	167	206
	3	186	226
	4	174	272
C	1	150	281
	2	190	310
	3	226	589
	4.	249	778

T(O) cells did not adapt!! T(AA) cells were stimulated by T(O). & further by amino acids.

	Urine A	B	C = .9A	D = B-C	E = D/C	% Lac	Suppl.	
1	25.7	3.68	231	137	59	120	Σ AA	
2	248	3.27	223	106	48	98	AA-A12 + arg	
3	241	3.35	217	118	54	110	" lys	
4	242	3.29	218	111	51	104	" meth	
5	241	3.00	217	83	38	77	" cyst	INHIBITORY!
6	259	4.02	233	169	73	149	AA - arg.	
7	242	3.66	218	152	70	143	- lys	
8	241	3.67	212	145	65	132	- meth	
9	249	3.72	224	148	66	135	- cyst	
10	250	4.27	225	202	54	110	AA-A4 + dal	
11	260	4.10	234	176	75	153	+ tyr	
12	[illegible]	4.51	243	208	85	174	+ thyp	
13	269	4.89	242	247	102	208	AA - dal	dal inhib? thyp stimulatory.
14	211	4.52	244	208	85	173	- tyr	
15	269	4.41	242	199	82	167	- thyp	
16	230	[illegible]	207	102	49	—	0	
17	319	7.75	287	508	177	362	M.C.	
~~18~~								

Activation of Lactase.

Sept. 30, 1948.

A. Phosphate vs. EDC citrate. System is, as usual, 10 ml and M/2000 in ONPG. .001 ml of Lactase 319A used for test.

1. 1 ml M/5 Phosphate	222
2. 1 ml M/5 citrate	021
3. 1 ml each.	022

All contain 1 ml Phosphate Buffer

B.

Add	
1 —	189
2 1 ml EDC	012
3. 1 ml Na citrate M/5.	190.

The inhibition is clearly due to the ethylene diamine component of the EDC buffer!

Oct. 1. Test .002 ml of 319A in the following buffers, each at M/50 pH 7.5

	D420.
1. Phosphate	310
2. Glycerophosphate	488
3. " + Phosph.	477
4. Barbital	513
5. " + "	494

Deficiency in phosphate was visibly apparent. A NaCl effect?

Phosphate is not required for the reaction.

ONPM/5000 in: M/50

1. Phosphate	694
2. Barbital	645.
3. Glycerophosphate	725

Activation of lactase + other assays 321a.

To test influence of NaCl add 1ml of M/5 NaCl, KCl, and Na_2SO_4 respectively to a phosphate buffer system as above. 319A .02ml Phosphate M/50+.

1. — 2.75
2. NaCl 3.95
3. KCl 2.59
4. Na_2SO_4 5.14.

M/50. Repeat

1. ~~NaCl~~ 3.17
2. NaCl 5.12
3. Na_2SO_4 5.92
4. KCl 2.98
5. LiCl 2.18 Inhibitory.
6. NH_4Cl 2.30
7. $(NH_4)_2SO_4$ 2.52
8. $MgSO_4$ 2.57

NaCl concentration series:

1. — 3.16 .10
2. .1 4.05 M/50x
3. .5 3.88
4. 1.0 ↓
5. 5.0

Sept 30, 1948.

17g. wetpaste K-12 harvested from 20 ~~gallons~~ litres (low yield!
S(Lac)

Add ca 50cc cold acetone to dehydrate, filter, and desiccate the residue. Assay sample of cells for activity.

	A.	B
D420.	[illegible]	621

Also, other assays:

325

314B.	1mg	1150	ca. 35 u/mg.
	.1mg	379	
	.01mg	046	
319B.	1mg	1070	ca. 190 u/mg.
	.1mg	960	
	.01mg	193	

→ 5.2 grams dry powder obtained: Lactase 325A.

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 ~~1/50 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 hydolyzed casein/10%.

10 ml

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

Lactase: Phosphate assay.

Oct. 4, 1948.

~~2~~ 2 ml 219A + 2 ml 10% TCA. Remove sediment. Assay in indicated aliquots against 10^{-4} - 10^{-3} Phosph. sulfate standards. Extreme 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.

		D_{660}
H/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. Impure activity + response to Na. Expires at 1:1000

	D_{470}.	Enzyme.	Na_2SO_4
1	095	C	0
2	140	C	M/50
3	171		
4	219		M/1000
5	277		M/100
6	290		M/50
7	178		M/10,000.

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

Oct 4, 1948.

Septums contain .001 + .005 ml 319 A and 1, 5 ml M/200 ONPG
= 1 ml K_2HPO_4 buffer + 1 ml N/50 Na_2SO_4 vol 10 ml.

37°.

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

Approximate T	Times T.	A	B	C	D.
	0	004	001	009	007
	1:20			069	154
	3:30	048			
	4:00		083		
	4:30			225	
	5:10				320
	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			
	14 —		191		
	14 30			640	
	15 —				750
	15 30	172			
	16 —		213		
	16 30			710	
	17 —				815
	17 30	195			
	18 —		238		
	18 30			780	
	19 —				870
	19 30	212			

Mins+Secs.	A	B	C	D
+11 20		258		
30			825	
4				920
11 30	236			
12		280		
			860	
13 ~~30~~ ~~25~~				955
	258			
14		300		
			905	
15				995
	277.			
16		320		
			940	
17				1005
	298			
18		341		
			955	
19	~~316~~			1045
	316			
30		363		
			980	
31				1050
	334			
32		381		
			1000	1060
33				1060
	351			
34.		400		
			1000	
35				—
	370			
36		420		
			1030	
37				1080
	389			
38		440		
			1045	
39				—
	404			—
40		459		
41				1095
	421			
42		473		
			1050	
43	~~438~~			—
	438	~~490~~		
44		490		
			—	—
45				

	A	B	C	D
~~45~~				1100
~~46~~	451			
		509		
			1050	
53. ~~47~~				1095
~~48~~				
~~49~~ 30				
~~53~~		560		
			1050	
~~54~~.				1100
~~55~~ 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			
~~79~~		740.		
~~83~~.	700			
~~d~~.	1145	1250	1145	1250.
1:10	209	1:11 690	213	810.

evaporation may have intervened overnight.

Oct 5, 1948.

49 g. Stiff Shapler 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.

A6 Remove debris. Left a 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	

Inhibition by serum.

319 A.	5.	—	780	
.002 ml.	6.	+ 476F41:10	599	
	7.	+ 471F51:10	710	
—cmy.	8.	+ 476F41:10	023	
—cmy.	9.	+ 471F51:10.	[illegible]	

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

Assaying "C"

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

throw out!

Lactase activation and inhibition
Mini assays

(1 ml Suppl.) In Na P buffer.

				Dy 20.
	1. 329B	-2		45.3
	2. " B	-3		075
329A	3. 329 C		—	082
	4.		NaCl M/50	081
10^{-3}	5.		KCl M/50	092
	6.		LiCl M/50	078
	~~7. 8. 9.~~		~~Et(NH$_2$)$_2$~~	
	In K PO_4			
.001	7. 319 A		—	150
	8		Et DiNH$_2$ M	017
	9		M/10	022
	10		M/50	049
	11.		M/100	082
Na P.	1 [redacted]			
	B [redacted]			

Repeat Assays of C!

		Buffer	Inhibit			
329C	1	Na	.001			116
	2.	Na	"			111
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	1 ml	006 !
	9	"	$CuSO_4$	M/10	.1 ml	- 119
	10	"	$HgCl_2$	M/10	.1 ml	- 412
	11		IAcONa	M/20	1 ml	266
	12.		"	M/20	.5 ml	286
	13.		"	"	.1 ml	335.

Tried D_i = 129
" D_i = 420

v. sl. inhibition.

may be related from release of Tris

Mechanism of fluoride inhibition
+ Km.

Oct. 5, 1948.

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

		Buffer.	D_{420}
1.	–	NaP	290
2.	NaF M/100	NaP	019
3.	$Na_4P_2O_7$ 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.	α 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 made to be shown. Conc. of ONPG from 2×10^{-5} to 2×10^{-4} mols/l. be explored.

∴ fluoride inhibits only in presence of phosphate. M/500 needed for substantial inhibition. (Mg effect?)

Lactase. mechanism of fluoride inhibition

Requirement for Mg^{++}? Km.

Oct 7, 1948.

319A .001ml in M/500 NaP buffer. Using Kontag's phosphate ONPG M/2000

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

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

	ONPG.	5m	10m	15m	20m.	0	30	$K_m(\times 10^{-5})$
	11	1						
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	221	015 323	5
	15	10	↑ 094 90 slope	141	203	262	017 381	—

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.

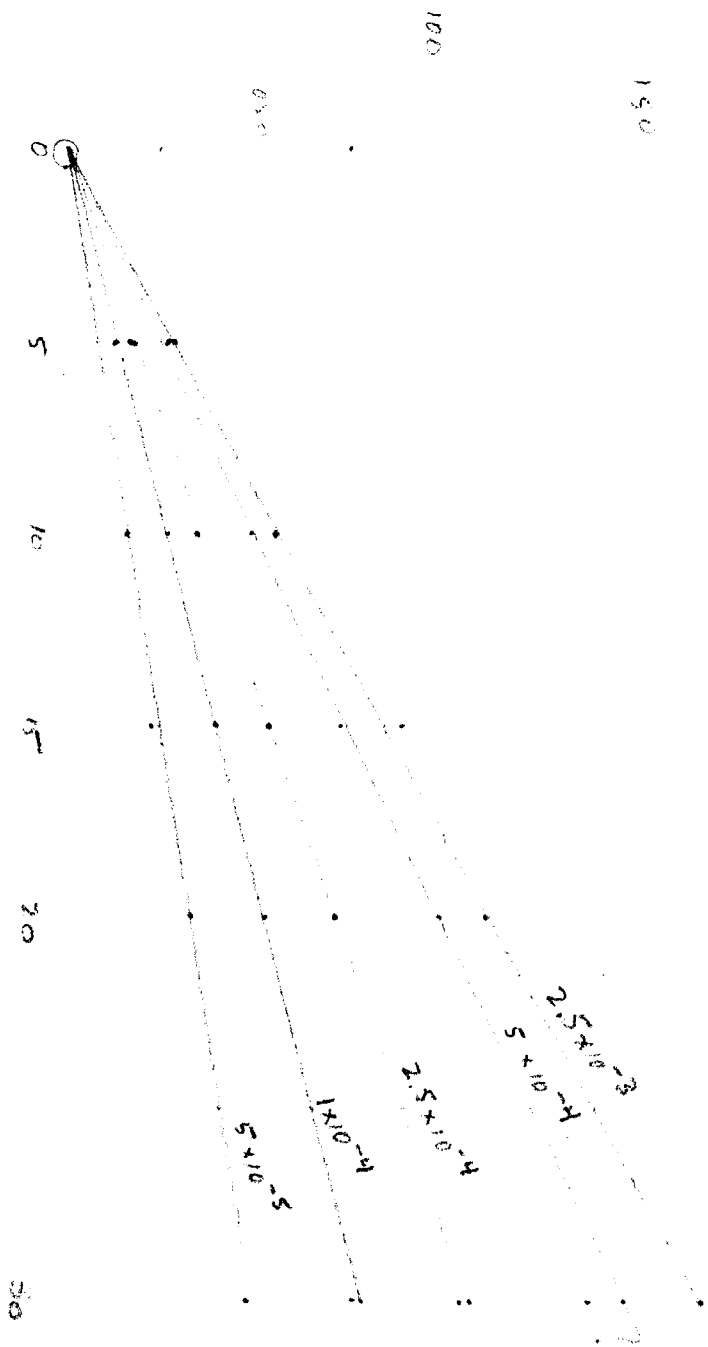
Consider 20 min. data

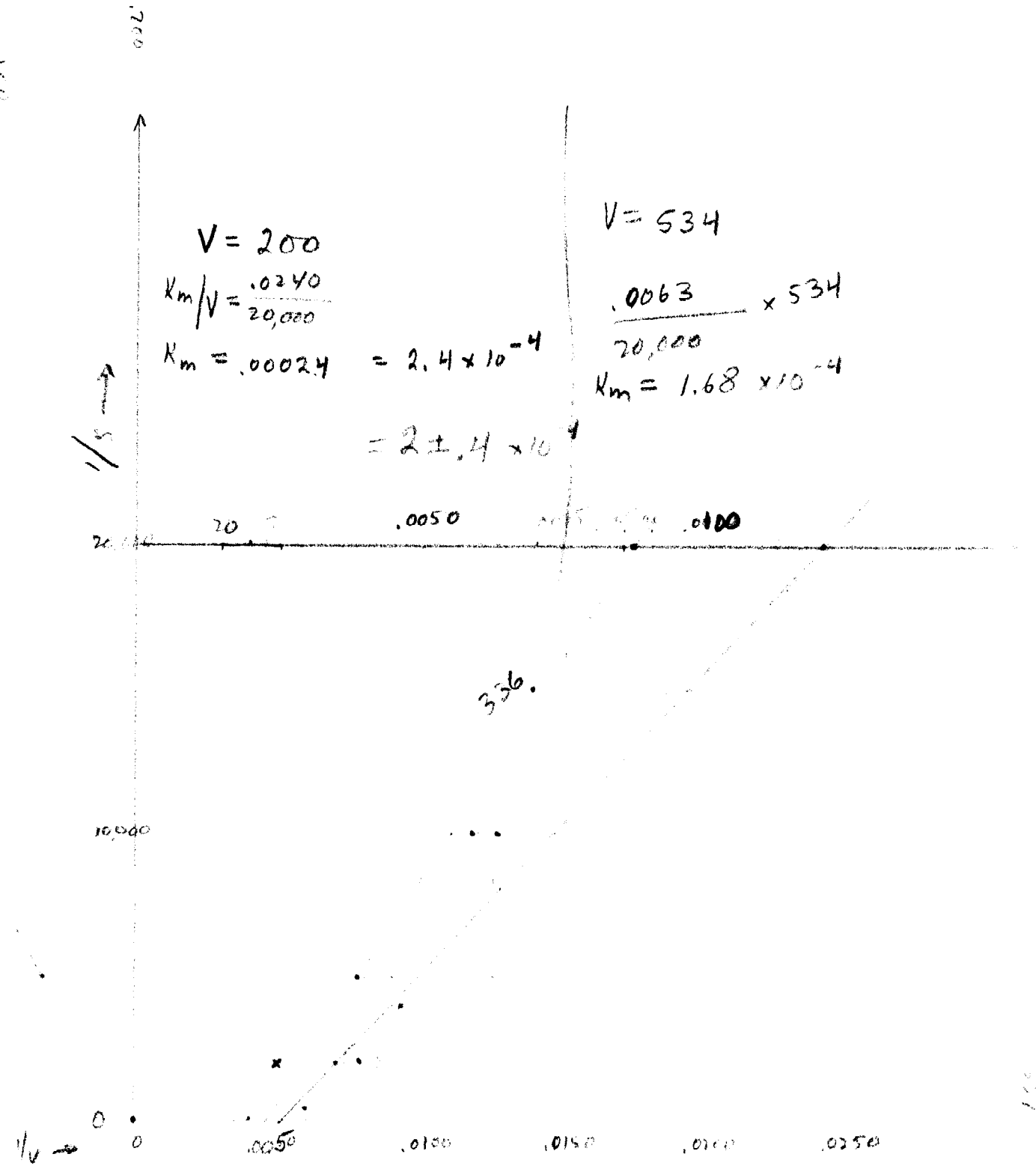
	V	1/V	1/S
1			
2	121	.0083	13,300
3	17—	.00105	10,000
4	212	.0047	4,000
5	245	.0041	2,000

$\bar{V}$ = 315 ±32

Careful extrapolation gives

K_m =


0
5
10
15
20
25
2.5×10⁻³
5×10⁻⁴
2.5×10⁻⁴
1×10⁻⁴
5×10⁻⁵


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
10,000
0
1/V →
0
.0050
.0100
.0150
.0200
.0250

In 3 determinations, Km was 1.4

1.5

1.18 $\times 10^{-4}$.

$\frac{4.1}{3} = 1.4 \times 10^{-4}$

Km o-nitrophenyl galactoside
K-12 Lactose.

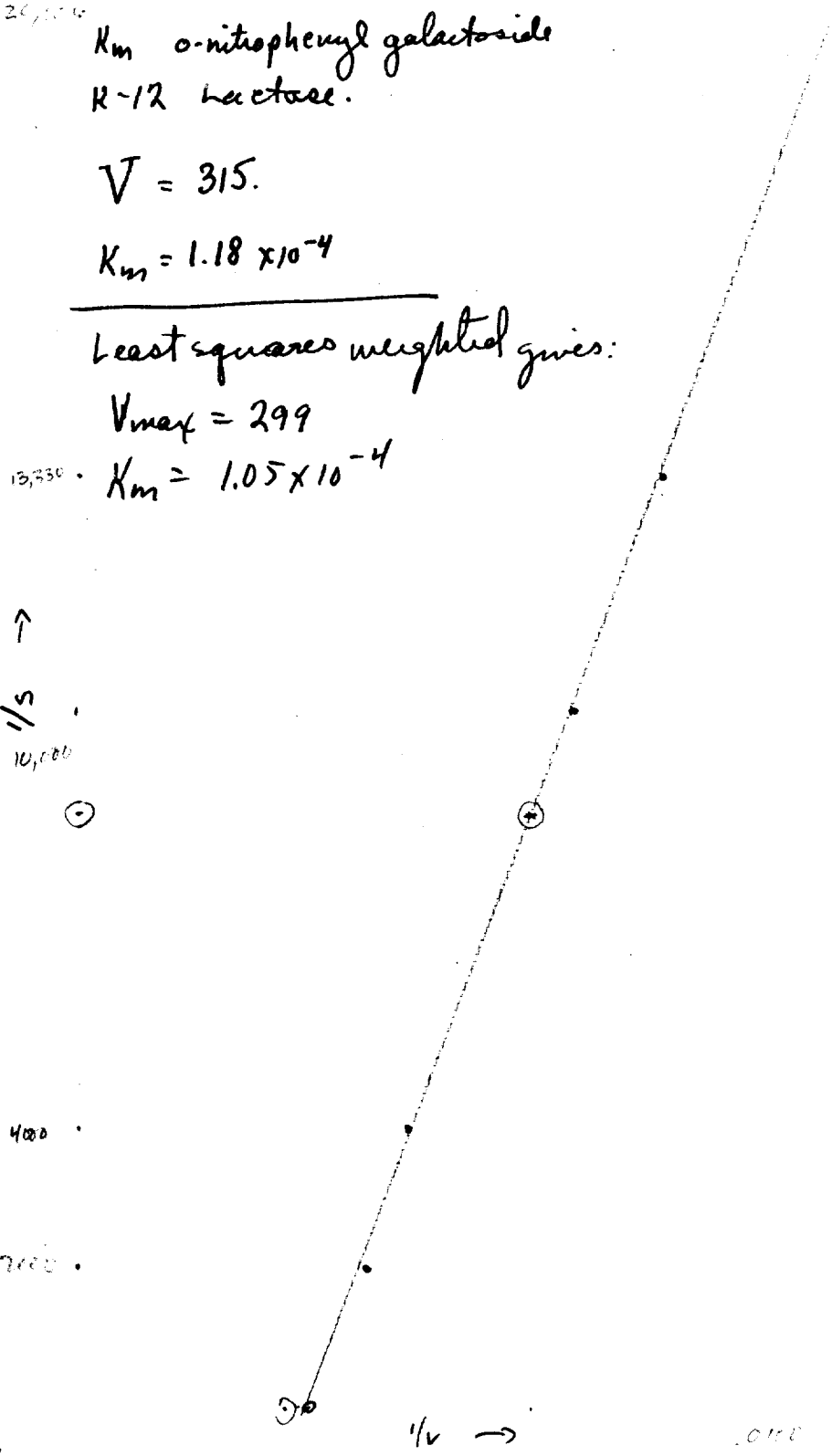
$V = 315.$

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

Least squares weighted gives:

$V_{max} = 299$

$K_m = 1.05 \times 10^{-4}$



Analysis of 3/29 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	2.02	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		28.87	62.44	119.66		~~1252.9~~ 1252.89	222.02

$$r = \frac{222.02}{62.44} = 3.56 \qquad 2r = 7.11$$

$$r^2 = 12.64$$

$$a = \frac{28.87}{62.44} = .462$$

$$b = \frac{119.66 - \overset{102.77}{3.56(28.87)}}{1252.89 - \underset{1578.56}{7.11(222.02)} + \underset{789.24}{12.64(62.44)}}$$

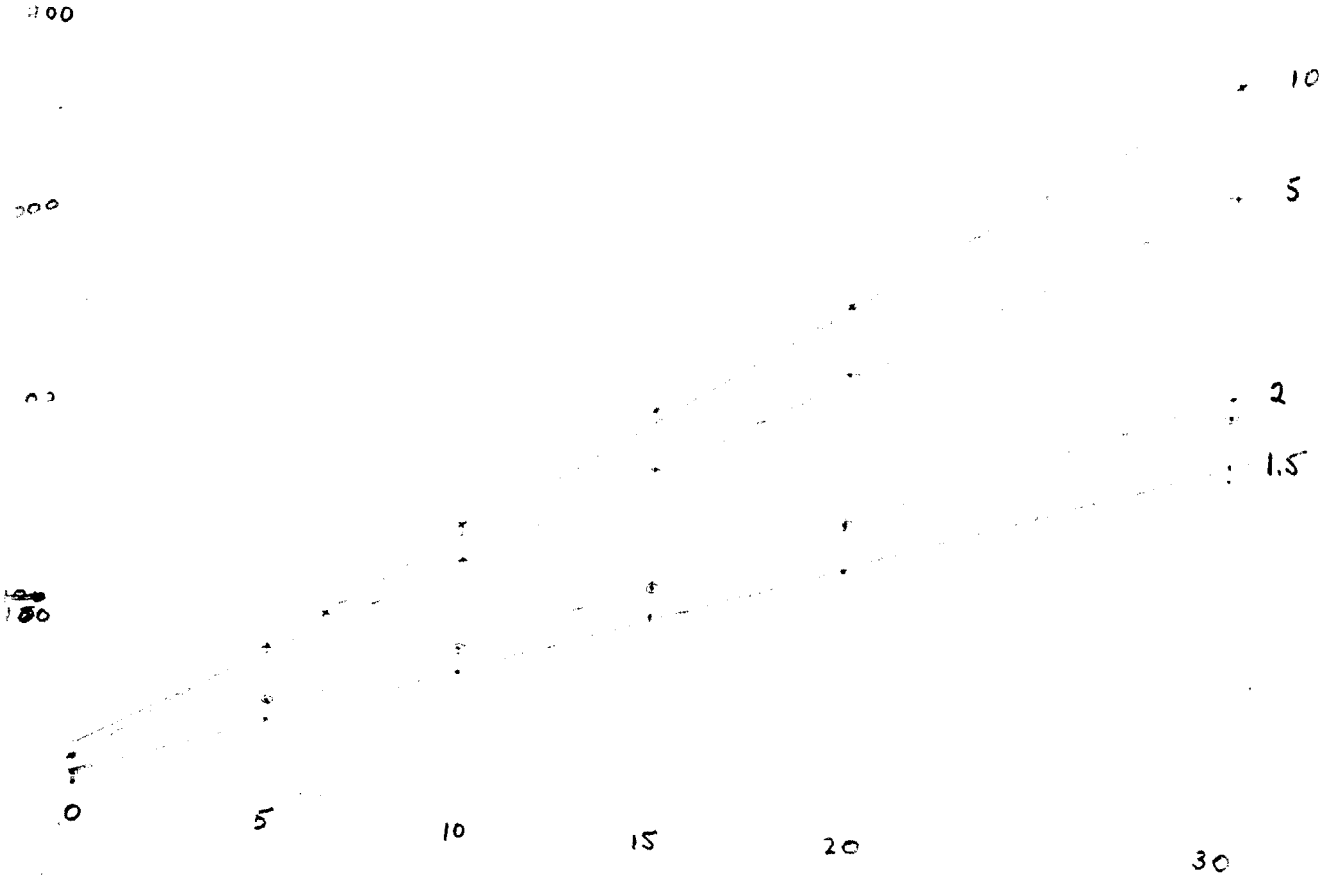
$$= \frac{16.89}{480.46}$$

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

$$\frac{1}{V_{max}} = a - br = .462 - .128$$

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

$$= .105$$


0
5
10
15
20
30
10
5
2
1.5

Kinetics ; $Mg^{++} + F^-$

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

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

ONPG x M/20,000.

		to	5M	10M	15M	20M	30M
0	50	051	080	10[illegible]	[illegible]	162	219
1	10	011	[illegible]	[illegible]	[illegible]	110	149
2	5	[illegible]	027	[illegible]	[illegible]	[illegible]	114
3	2	000	017	027	[illegible]	053	
4	1	−003	01[illegible]	014	[illegible]	031	046

(2). in M/100 NaP buffer.

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

Corrected values of (1).

	to	5	10	15	20	30	$V_{corr.}$	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	.02040	20000

Km is estimated at $\underline{\underline{2.4 \times 10^{-4}}}$

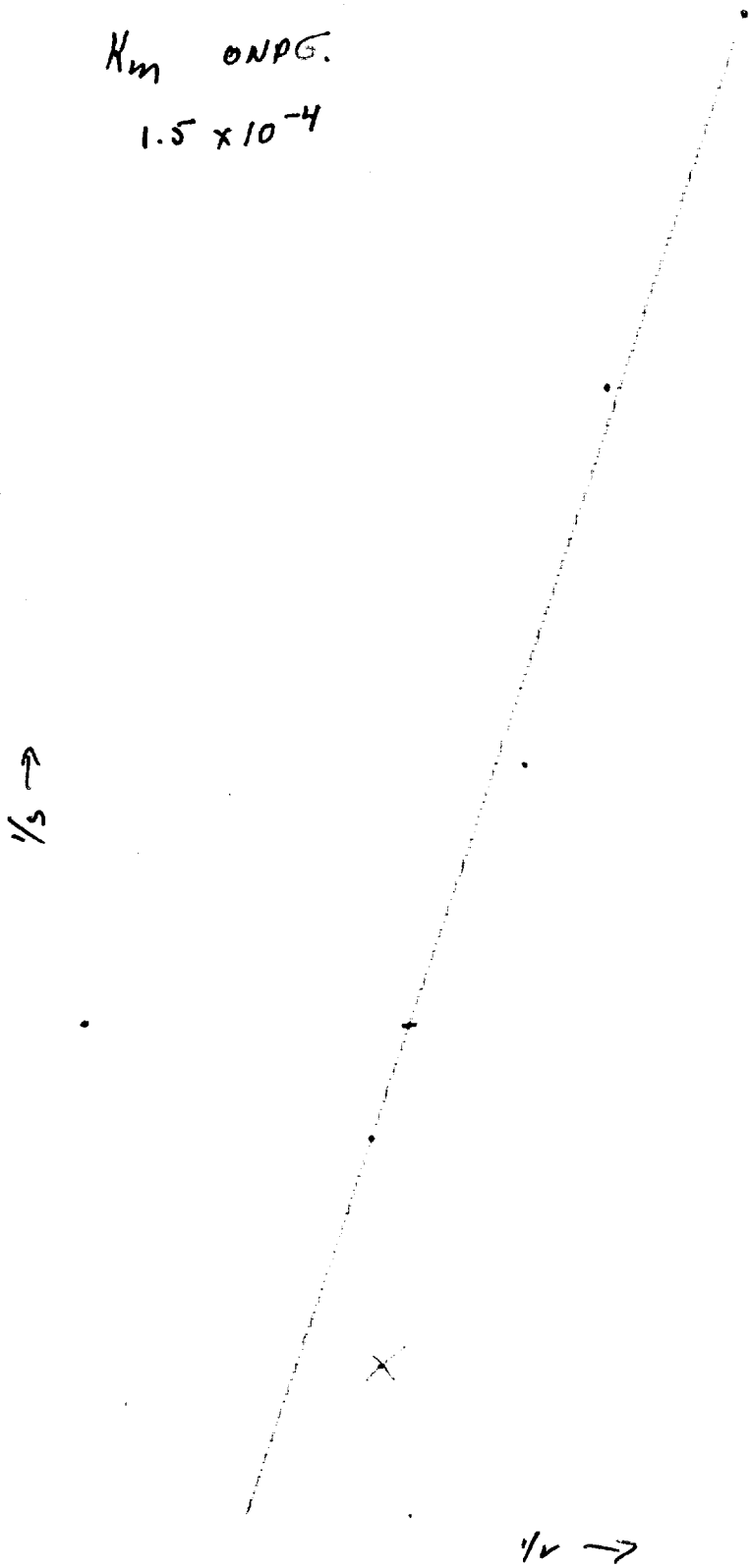
should be 1/[illegible] 69.
too high.

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 319A.

	ml M/20000	D_0^{V20}	D_{10}^{V20}			1/s	1/v
1.	1.00	000	115	115		20000	.0051
2.	1.33	002	146	144		15000	0069
3.	2.00	007	180	173		10,000	0058
4.	4.00	009	272	263		5000	0038
5.	10.00.	026	281	255		2000	0039

Note discrepancy
inactivity = 3/34.

② In M/100 NaP buffer. + M/50 salts.

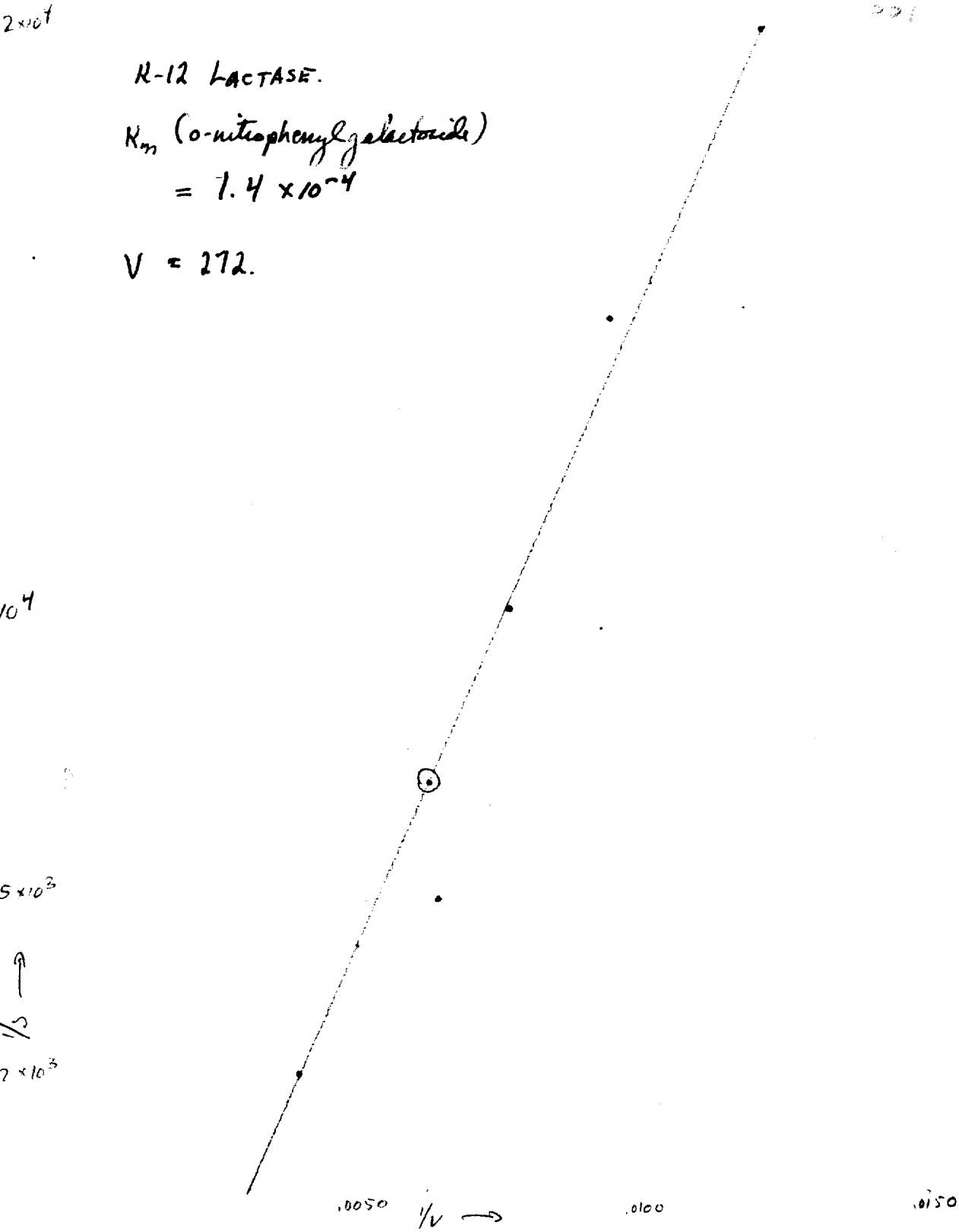
11.	—	340
12.	NaCl	351
13.	KCl	316
14.	LiCl	305
15.	RbCl	087
16.	CaCl	302

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.

10/12/48.

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

N2Case 1%
Yeastr. .5%
Lactose 2%
Tween 80 0.1%
Na Acetate 0.1%
} LB medium.

heavy growth noted!

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

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

84	.0119	252	1	11	1.0	006	090
107	.0093	328		12	1.33	0	107
132	.0076	472		13	2.0	005	137
157	.0061	830		14	4.0	003	160
252	.0040	1200		15.	10.0	009	261

272. 1/V = .0031

d (decomposition)	3. M/100 Na P buffer.	Salts M/50.
1200	1 —	250
1200	2. NaCl	258
172	3. RbCl	101
575	4. Na + RbCl.	163

V =

te the enzyme?

Does Rb^+ irreversibly inactivate

L. bulgaricus lactose.

tare 79. wt. 128

49 g. wet Sharples paste collected
grown 24h. in 12 litres LB-lactose broth
5 aeration.

A) 4g. in M/100 NaP buffer for autolysis. Ca 10 ml (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-Gum 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 enzyme tests in 9.0 ml. Add .5 ml M Na_2CO_3 to develop color and stop reaction.

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

Lactase; Coli & L. bulgaricus.

October 15, 1948.

		D_i^{420}	D_{30m}^{420}	D_i^{30m}	Δ.
1	Coli 319A. .001 ml —	002	295		293.
2	+ Ethylene diamine·HCl M/10	010	029		020.
3	+ Ethanolamine·H_2SO_4 M/10	040	130		094.
4	+ Ethylene Glycol M/10.	−001	378!		379.
21	+ PbCl M/50	0	050		050.
22	+ KCl M/50	−001	284		285.
23	+ PbCl + KCl. M/50 ea.	0	126		126.
5	L. bulgaricus. Cell suspension.	220	321		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	152	1250		—
9	" .1 ml	022	361		341
10	" .01 ml	010	030		024	$= \frac{1}{4500} \times 20g$
11	" .001 ml	0	022		022	?
12	" 10^{-4} ml	−002	021		023	} probably ONPG!

All tests in M/100 Na P. pH 7.5 & M/20,000 ONPG. 37°. 20m. λ = 420.
This may not be the opt. pH for bulgaricus.

Note: Intense stimulation by glycol!, Reversal of Pb inhibition & K.
Relatively low activity of cells (L.B.) may aect. for poverty of extract.

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 RbCl
11	EtOH	400 – [illegible]	
12	PrOH	469	
13	iPrOH	395	
14	$Pr<(OH)_2$	390	
15	BuOH	450	
16	Dioxan	300	
17	MeOH	441	
18	$Et<^{Cl}_{OH}$	151	
19.	$Pr<(OH)_3$	449	

.05 ml 338C KP buffer M/[redacted]50. Salt 0 M/50. [illegible] + 200 [illegible]

21	-	pH 1.5	257	(or C)
22	NaCl M/50.	"	390	Na required
23	"	8.0	074	
24.	"	1.0	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 and 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.	

? [illegible] PrOH + enzyme + ONP [illegible] to [illegible]
? [illegible] of products of PrOH.

L. bulgaricus lactase

October 18, 1948.

33°C .01 ml/tube. 9 ml. pH 7.5 Stock H_3PO_4 & Na_2CO_3 in various buffers, M/100. Add ~~M~~ Na_2PO_4 buffer additional M/~~50~~ when called for. NaCl M/50 in all tubes.

	buffer.		+ 1 mL H_2SO_4, 4/1
1.	NaP	110	120
2.	NaBarb	~~175~~ 116	160
3.	" + NaP	170	188
4.	Et $SNH_2 \cdot Cl$	020	
5	" + NaP	025	
6.	Na Glycine P	080	070
7.	" + NaP.	109.	110
8.	NaP, $MgSO_4$.	175	

A) [illegible] B) [illegible] .05 mL [illegible]

Mg, PO_4 are stimulating.

Lactase - ONPG competition
K_m.

October ~~10~~ 26 1948. — 10/28/48.
NaP M/50 pH 7.5. 3MA 10^{-3} cc. 70m. 37°

		ONPG	Lac.	D_{t_0}	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]~~ 010		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	+ .1ml antiserum.		290	360	070
	9.	~~[illegible]~~						
	10.	~~[illegible]~~						

~~no [illegible] 10^{-3} [illegible]~~

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	–	+
Gal	–	+
Suc	–	–
Xyl	–	–

Results 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	− (+ papill.)	−	+; −	−	− *	
15	89		−	++ ; (−)	++	++	
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 +	−	− (two +)	−		
23	97	− *	−	V	−	− (⊙±)	
24	98	V	−	+	++	++	
25	99	V bulging + sectors	−	+	++	++	

* − colonies and some v. slow +

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

"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~~

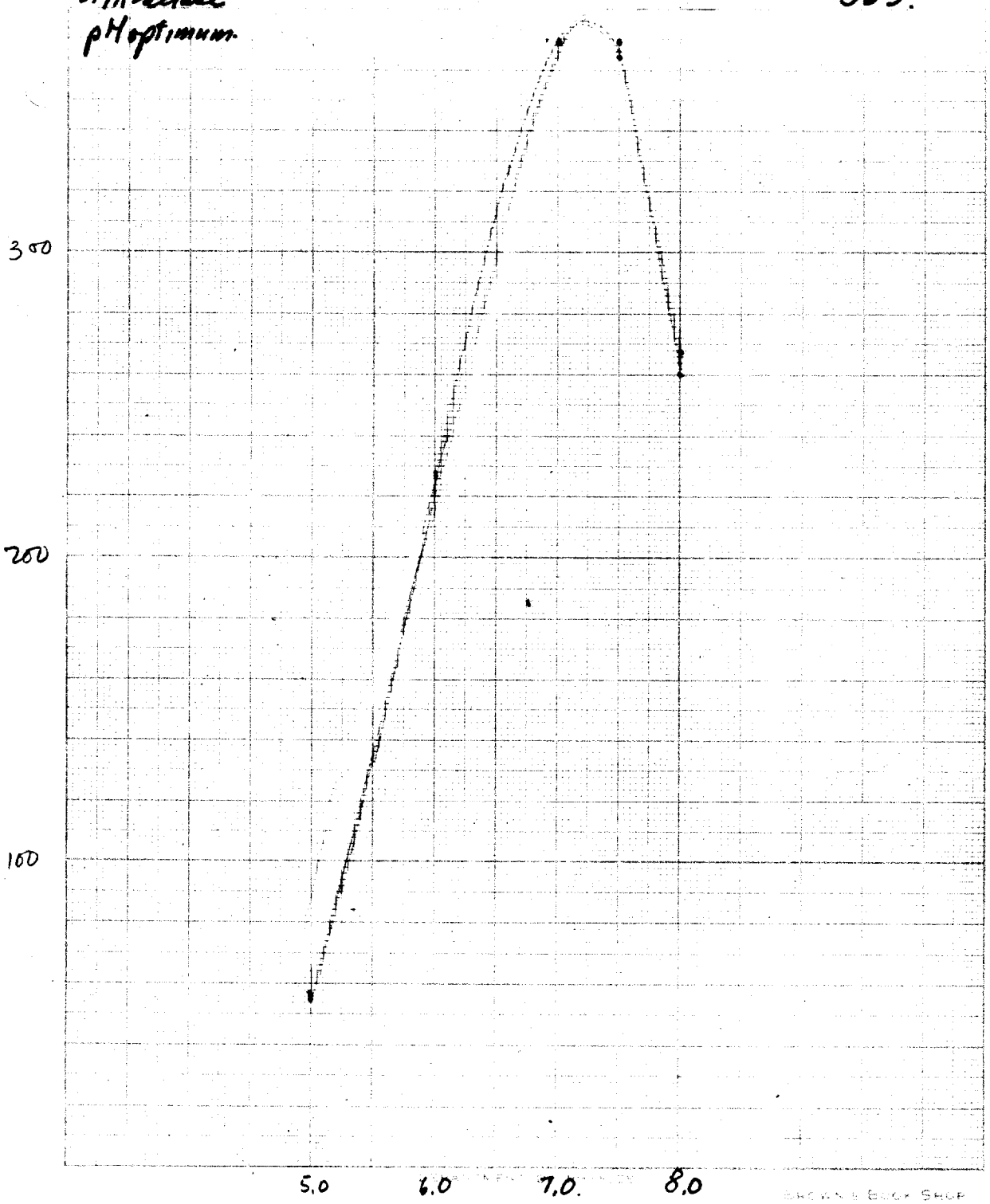
Na_2SO_4 M/50.

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 1/2 glucose shake 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 trypt.
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	~~170~~ 171
10. A		199	161
11. T+5MT	gelatinous		
12. T+5MT	pH > 8-9		
13. C+A	D_i = 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.
exp pH = 7.3

12/8/48.

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

Exp. ~~27~~ A. 5 gms. meal were shaken 24 hr. c. 10 ml. H_2O. The clear was reduced and supernatant diluted to a 10 ml. gel. (A).

Kinetics at pH 4.0 Na acetate buffer 1/1/100 (after Veibel who showed optimum at 3.4). He finds Km for ortho 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 KCl + stim by Sodium. In M/100 Acetate buffer. salts M/50 each. Ferricyanide = M/1 Na_2CO_3 1 ml.

	alt	Dyzo.
1.	No enzyme	ambr
2.	—	167 ✓
3.	Na	248 ✓ (av. determin.)
4.	Rb	196
5.	Na+Rb	212.

may be a chloride effect

1	—	220 ✓	Note appr stim by Na
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.

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

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

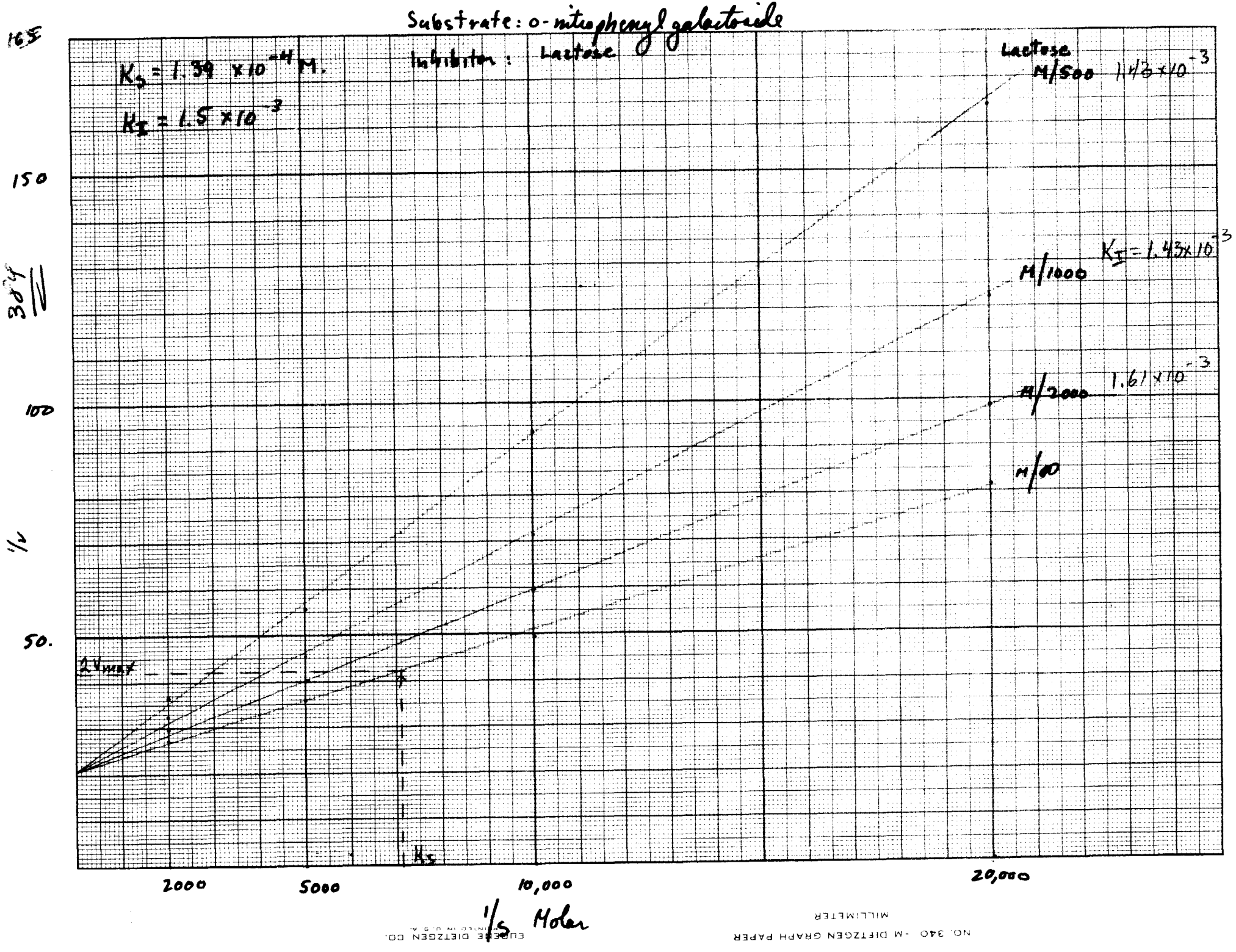
∴ Barley lactase is constitutive

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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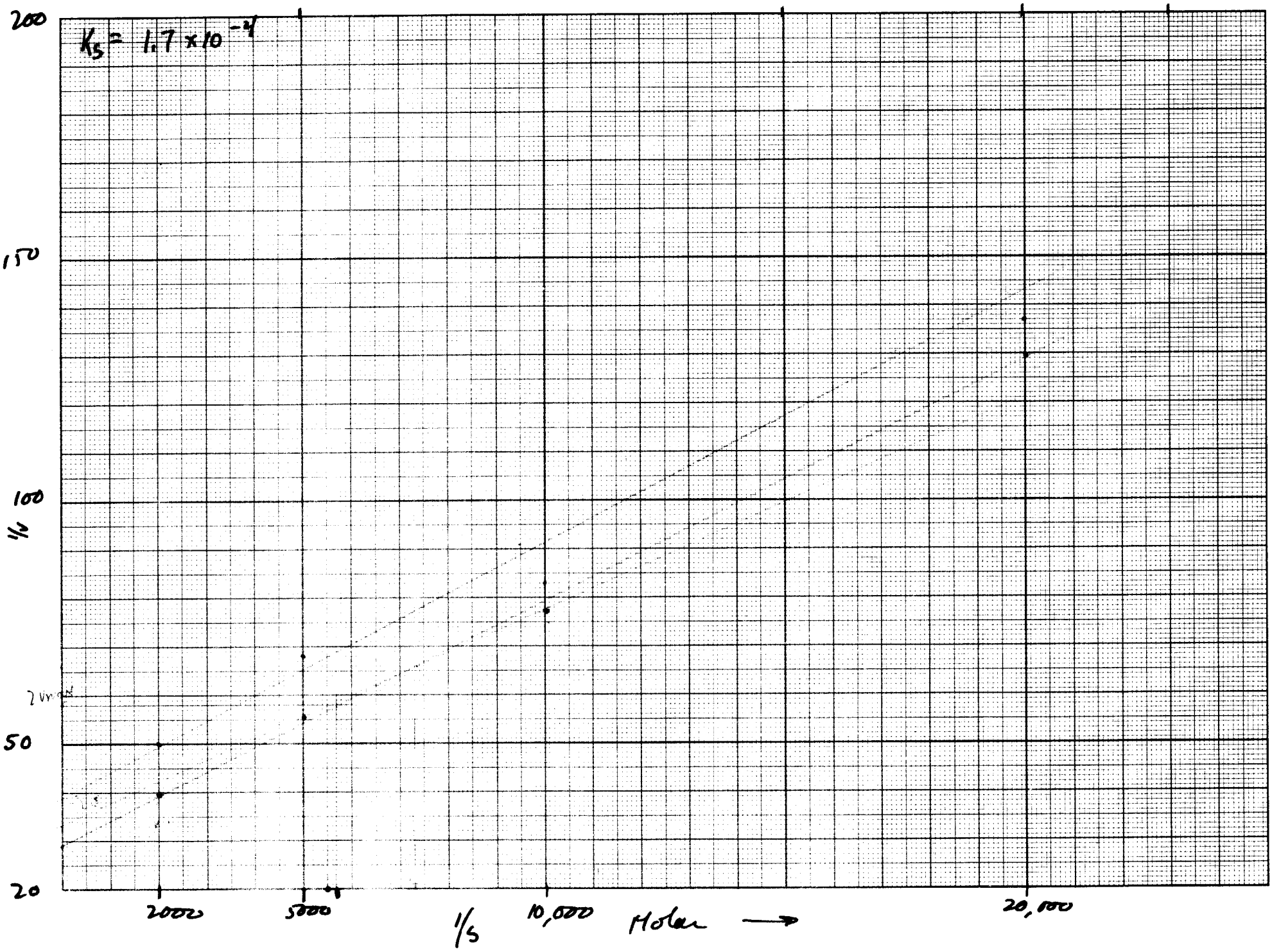

385.

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		
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	70.4
32	5	"		
33	10	"	74	135.1
34	20	"	40	250

KP 7.5 M/100

1/V extrapolation

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

Non-competitive inhibition

K_2HPO_4 buffer!

No inhibition

1/10 glucose

Na buffer

1/v →

K_s

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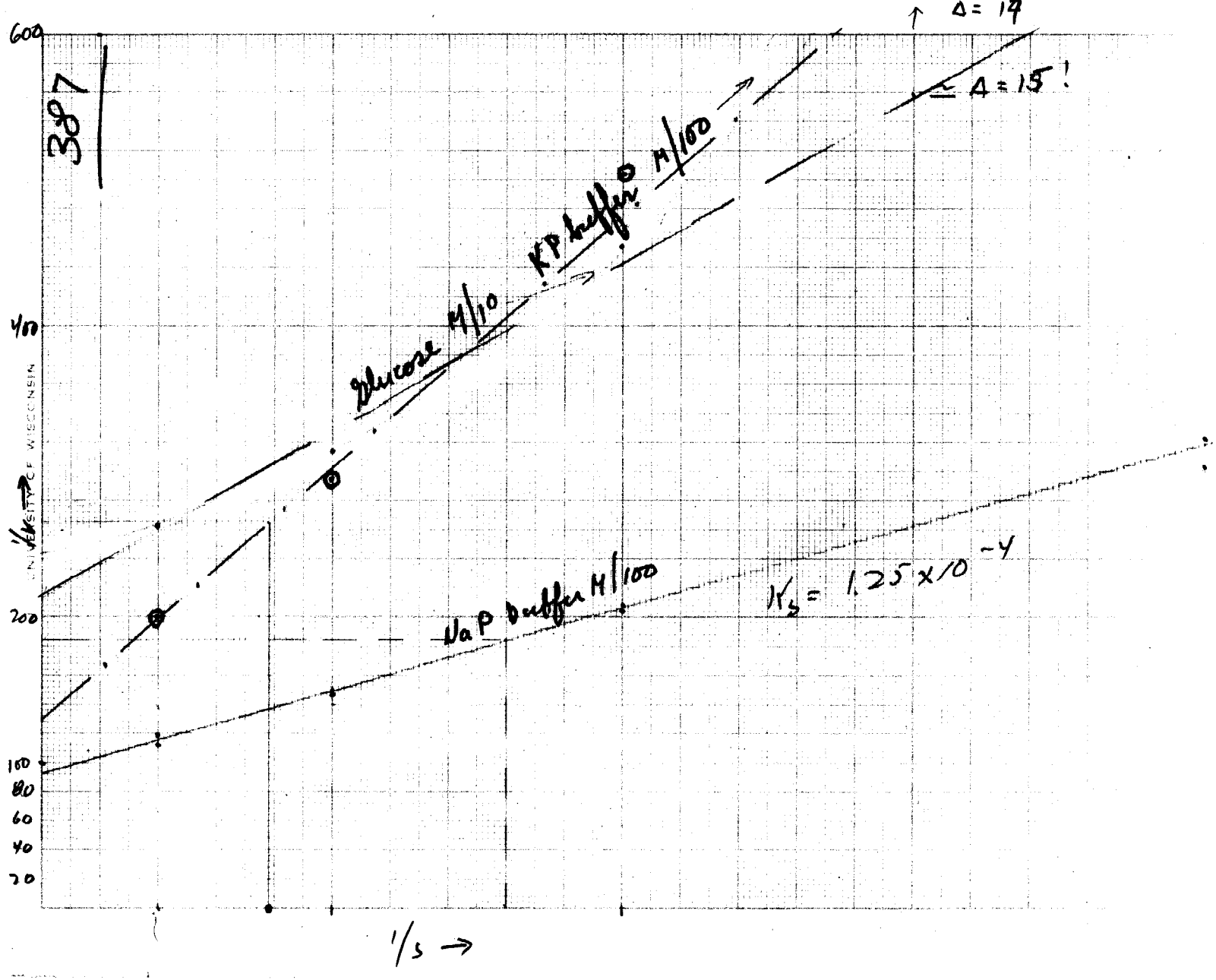

387
600
400
200
100
80
60
40
20
1/V →
1/s →
Δ = 14
Δ = 15 !
Glucose M/10
KP buffer M/100
NaP buffer M/100
1/Vs = 1.25 x 10 -4

December 13, 1948.

	ONPG. M/-1000.	Suppl.	Buffer.	1/V	2	F	Δ = V
			NaP ~~M/100~~ M/100				
1.	2			112	10	99	89
2.	5	"K_s 1.25×10⁻⁴"	"V_{max} = 109"	147	2	70	68
3.	10			208	—	48	48
4.	20			303	-3	30	33
		Glucose M/10.	NaP M/~~1~~00				
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

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 ~~the~~ 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. [illegible] 500
2. 319B. 10γ 18
3. 319C 2×10^{-3} ml 70.
   ca 500 u/ml

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

		pH.	
(B) lactose	11	4	84
	12	5	[illegible]
	13	6	[illegible]
	14.	7	
(A). glucose	21	4	97
	22	5	[illegible]
	23	6	[illegible]
	24.	7	[illegible]

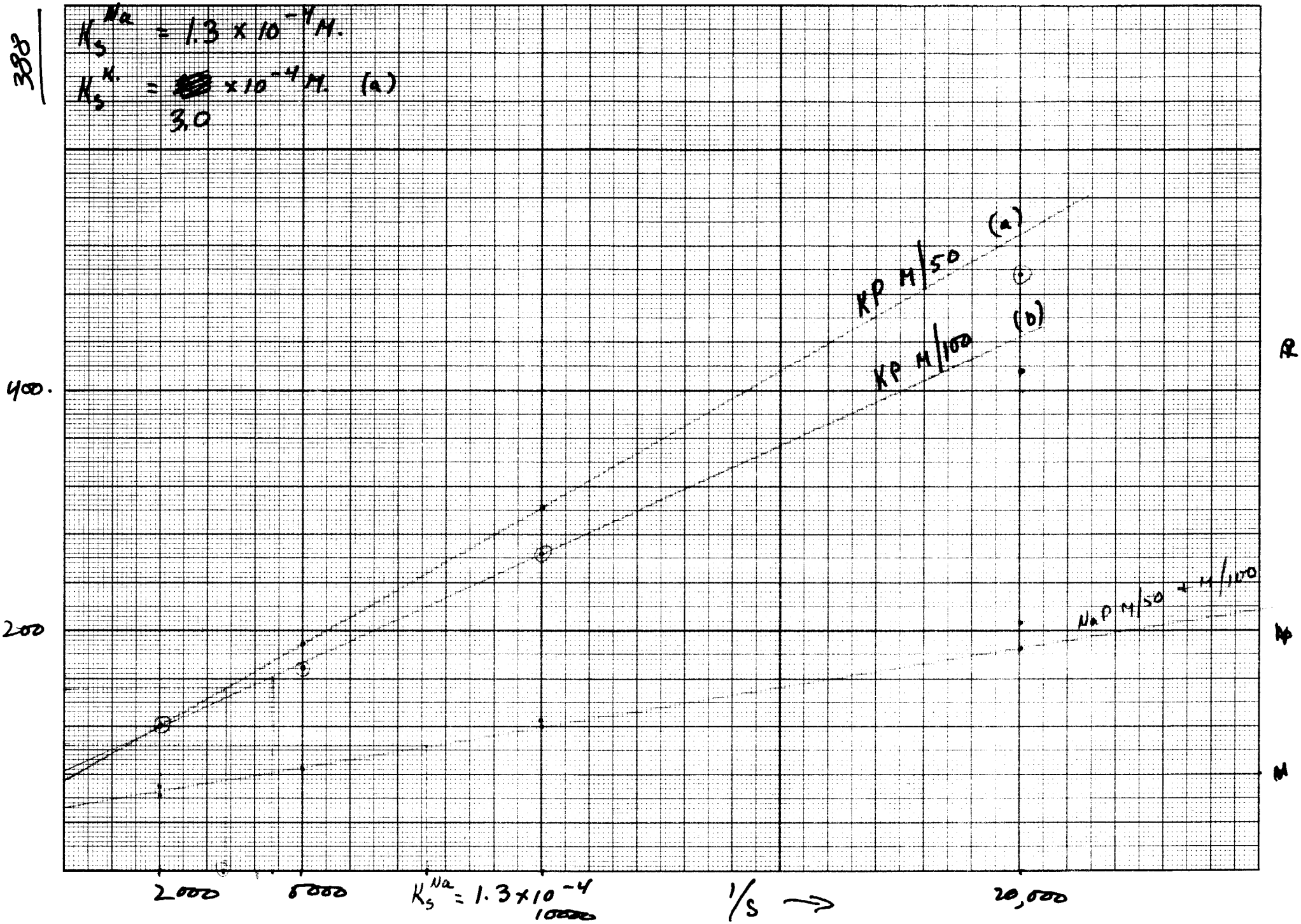
Nearly [illegible] not [illegible] – there [illegible] dilute [illegible] suspension.

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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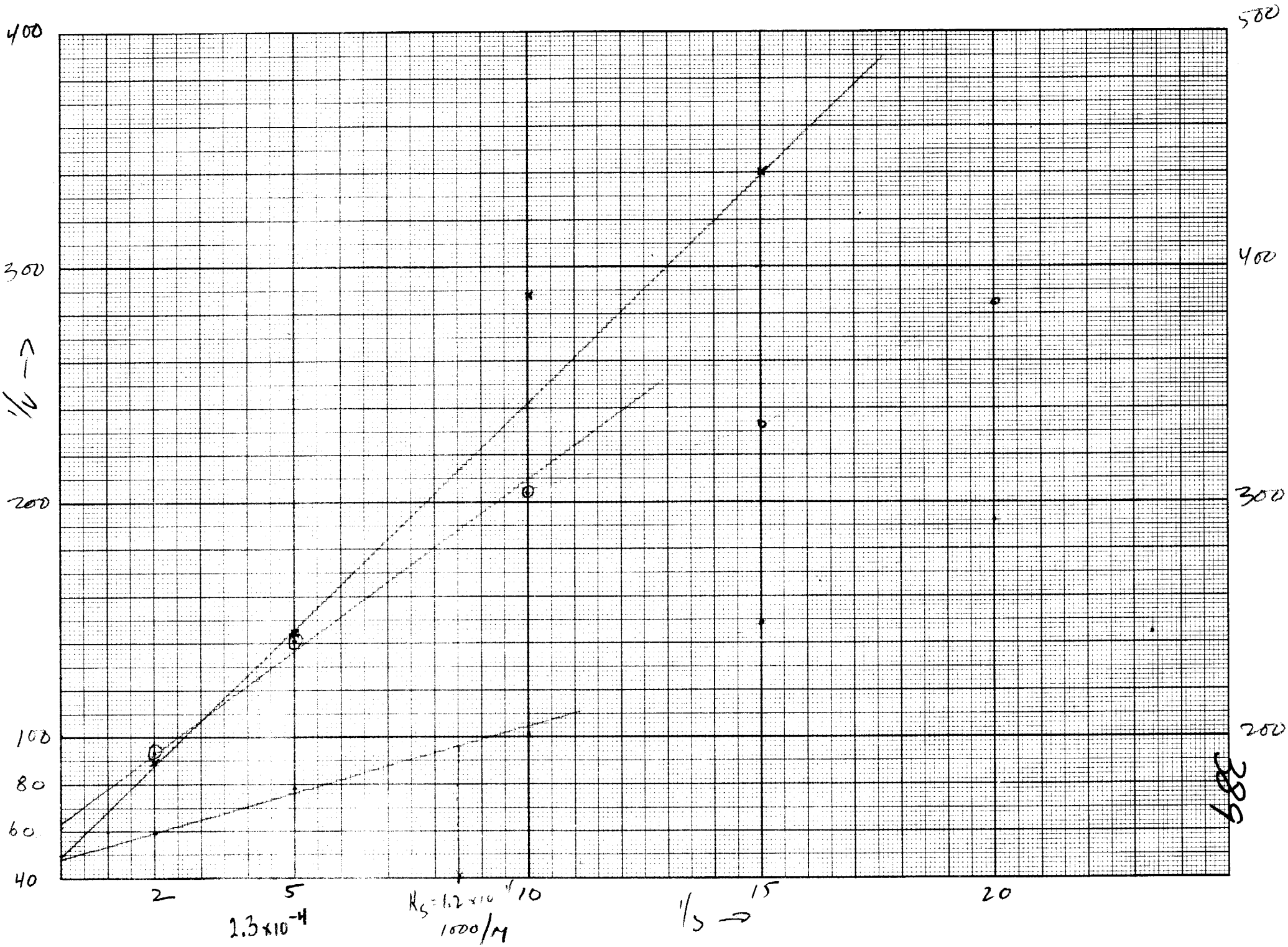


Influence of metal ions on K_s (ONPG).

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

12/21/48.

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

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

Study [illegible] in Na [illegible]

Tubes 1+2. 10^{-3} mG [illegible] buffer, incubated 90 [illegible] 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.

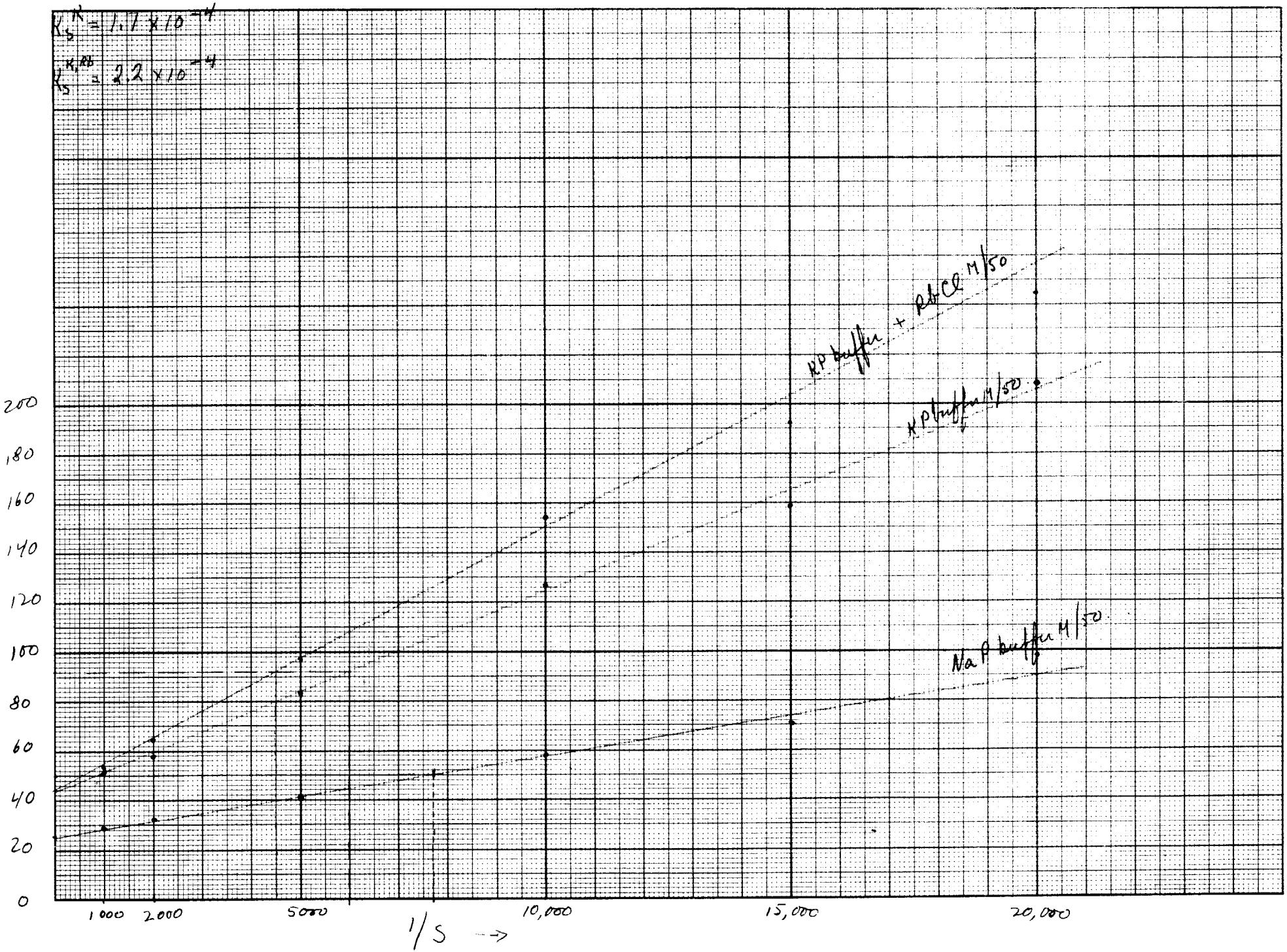
Series 0 - NaP. M/50 pH 7.5

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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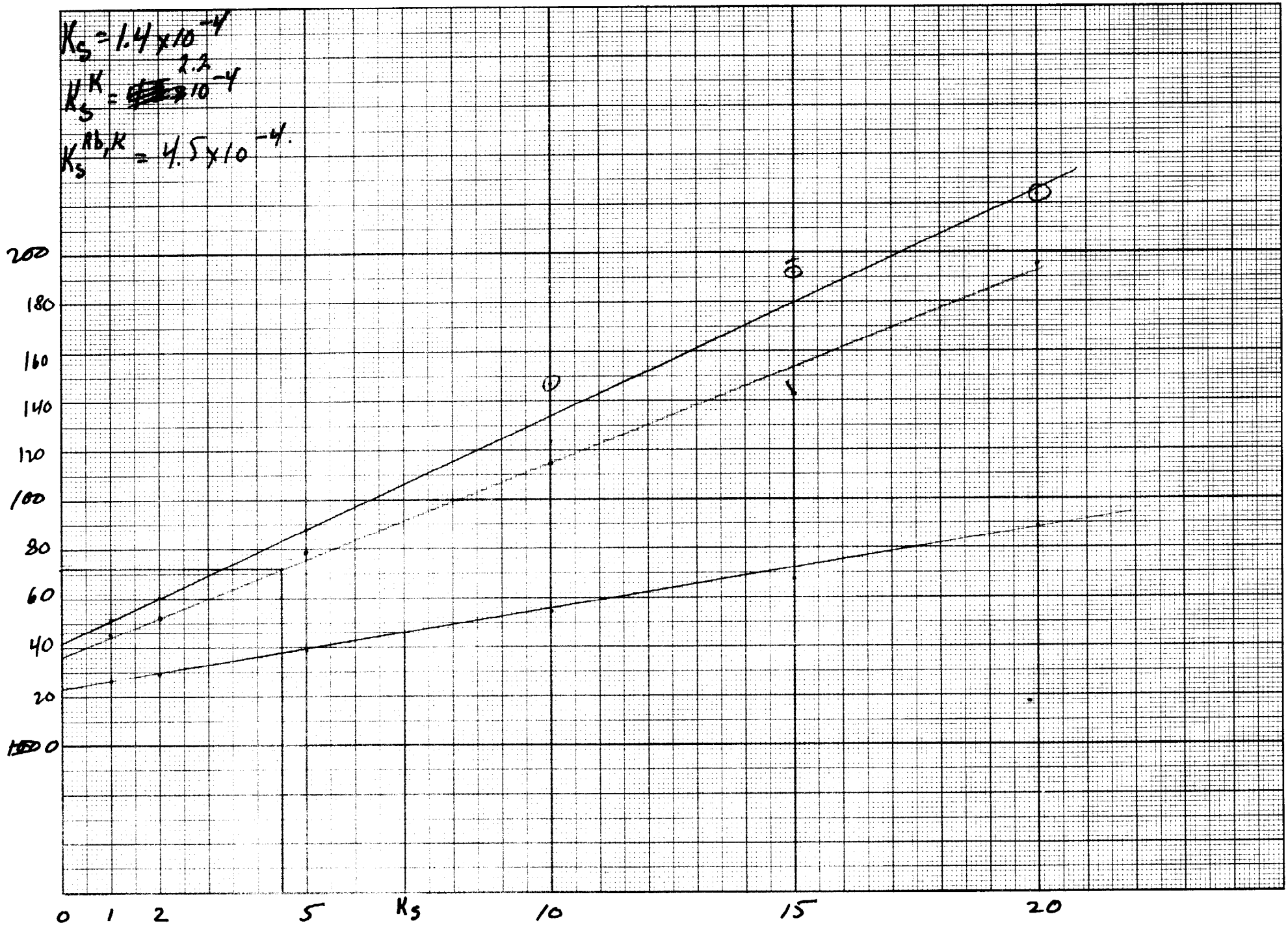

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Dec. 28, 1948.

3194 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

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

In this expts., substrate + buffer are made up; enzyme is pipetted diluted before dumping it in at To. Cf. 339 in which absorption curves show marked effects (enzyme salts, substrate added first.)

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 100mg cells in 10ml M/50 NaP. Shake 2 hrs. Remove solid + assay 1 ml in 10ml. (C).
≐ 50 mg/ml wet cells

D). Assay against cell suspension in M/50 NaP 7.5 (–12.0 mins.)

	Di		Lc	Δ
A. .001 ml	.003		241	243
B. .01 ml	.06	71	77	77
C. .01 ml	~~.082~~ .02	82	59	59
D. ~~.02 ml~~ 0.2 ml	.082		113	31

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

~~Ref~~ 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

Suppressor lactase

Jan. 9, 1948

Grow batches of W661 & 662 in S(lac). Harvest and dry over P_2

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

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

Jan. 10, 1949.

Lactase adaptation in W-112 (Lac$_1$)

Grow W-112 in Y2 1/2% 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 P buffer + BCP
1 ml 5% sugar. 2 hour reads

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

Check by streaking out cells used.

Lac$_1$ — 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 x 50 ml Y2/sugal. Harvest, wash + dry over P_2O_5. Yield 33 mg. dry cells. 1/13. very active on ONPG.

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

18h. Bugal actively fermented; heavy growth. Much space; no ferm. in Y2 Lac

Harvest + test:

a) spot plate ONPG: B: +++ L: —

b). E .1ml 4/50 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 e

a) Add ONPG to enzyme-buffer. NaPM/100. 7.5 (RbClM/50) ONPG M/200.
b) "enzyme ONPG".

		10^{-3} mol.		
a)	1	319A	—	510
	2	"	Rb	470
	3	395	—	630
	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 / 2000 ~~ml~~

—	289
old RbCl	268
new RbCl.	200

Pb 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.	PbCl$_2$ old	Na	409		07				
3.	CoCl	Na	393		10				
4.	PbCl new	Na	316		28				
5.	—	K	239		—(45	)			
6.	PbCl$_2$ old	K	220		08				
7.	CoCl	K	182		24				
8.	PbCl new	K	100		58				

Rb inhibition

January 14, 1949.

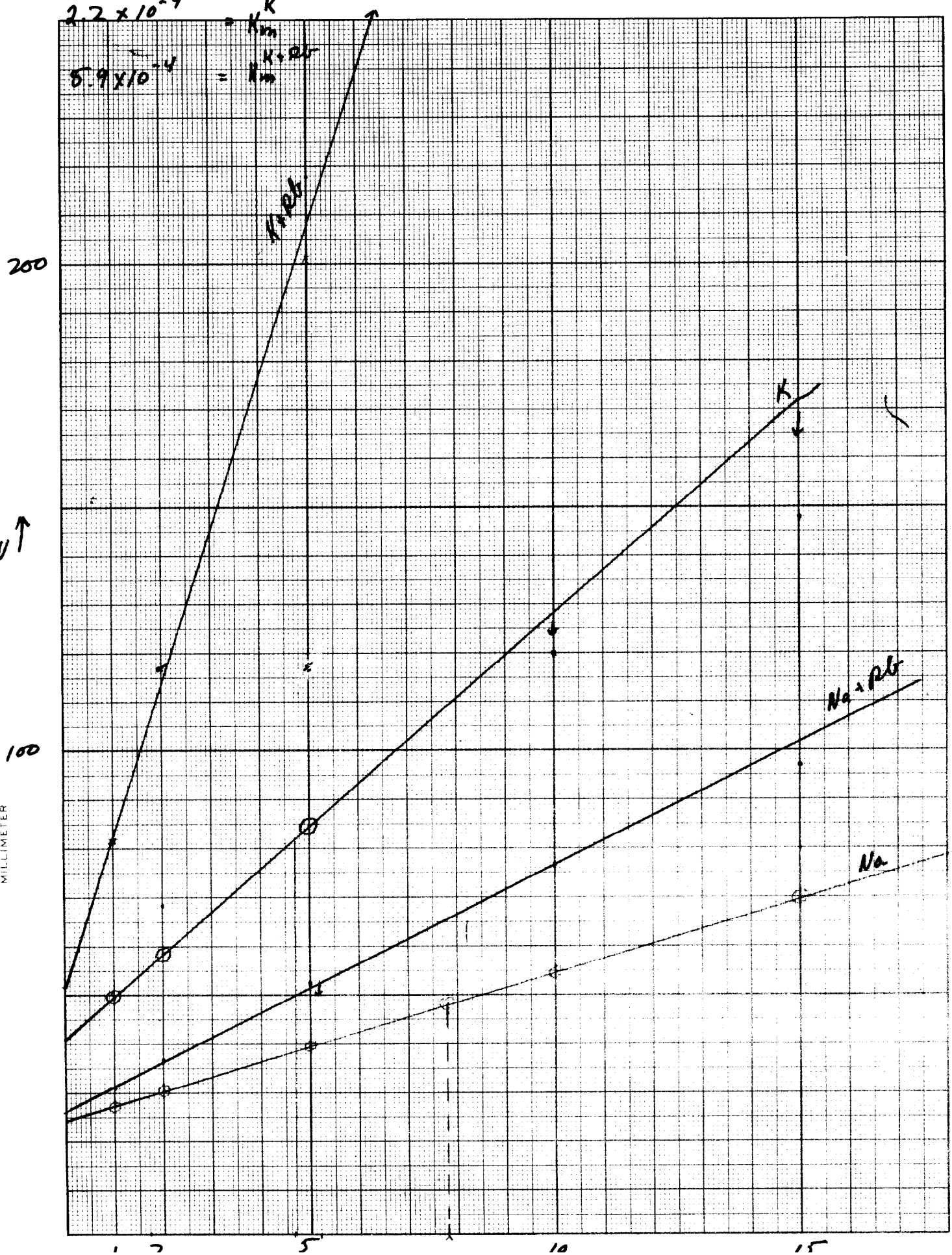
	1/s	1/v.	Δ			Di	1/v corrected (+ 1/3).
NaP							
15m	1	27.2	368		388	20	
	2	30.4	329		340	11	
	5	39.4	254		255	1	
	10	~~31.1~~ 54.9	182		184	2	
	15	69.0	145		142	−3	
NaP+RbCl							
15m	1	31.1	322		339	17	
	2	36.9	271		280	9	
	5	52.1	192		198	6	
	10	76.9	130	[131]	~~(104)~~	1	
	15	97.1	103		103	0	
KP							
20m	1	37.3	268		286	18	49.7
	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							
20m	1	61.3	163		181	18	81.7
	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

$1.28 \times 10^{-4} = K_m^{Na}$ $1.92 = K_m^{Na+Rb}$ 411

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

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



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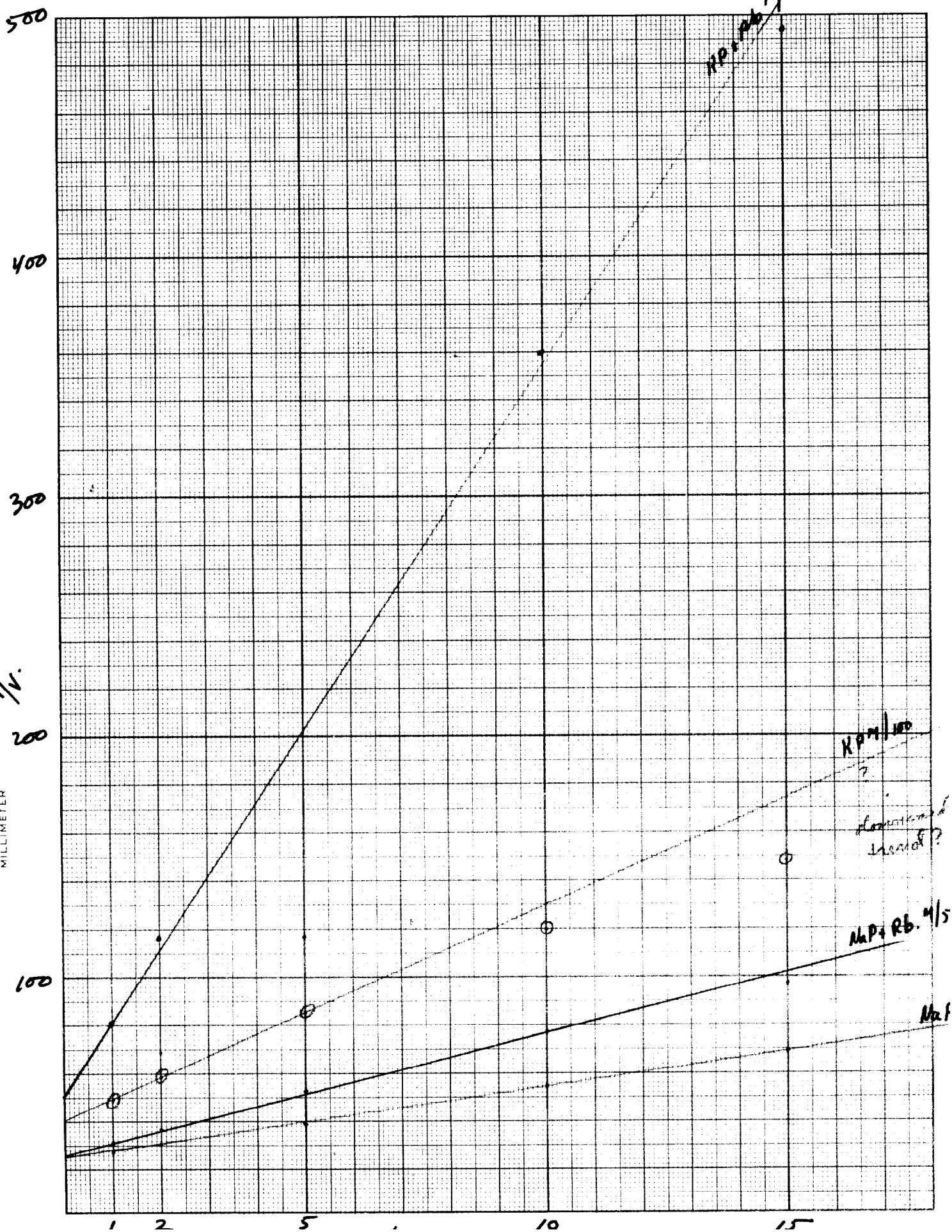

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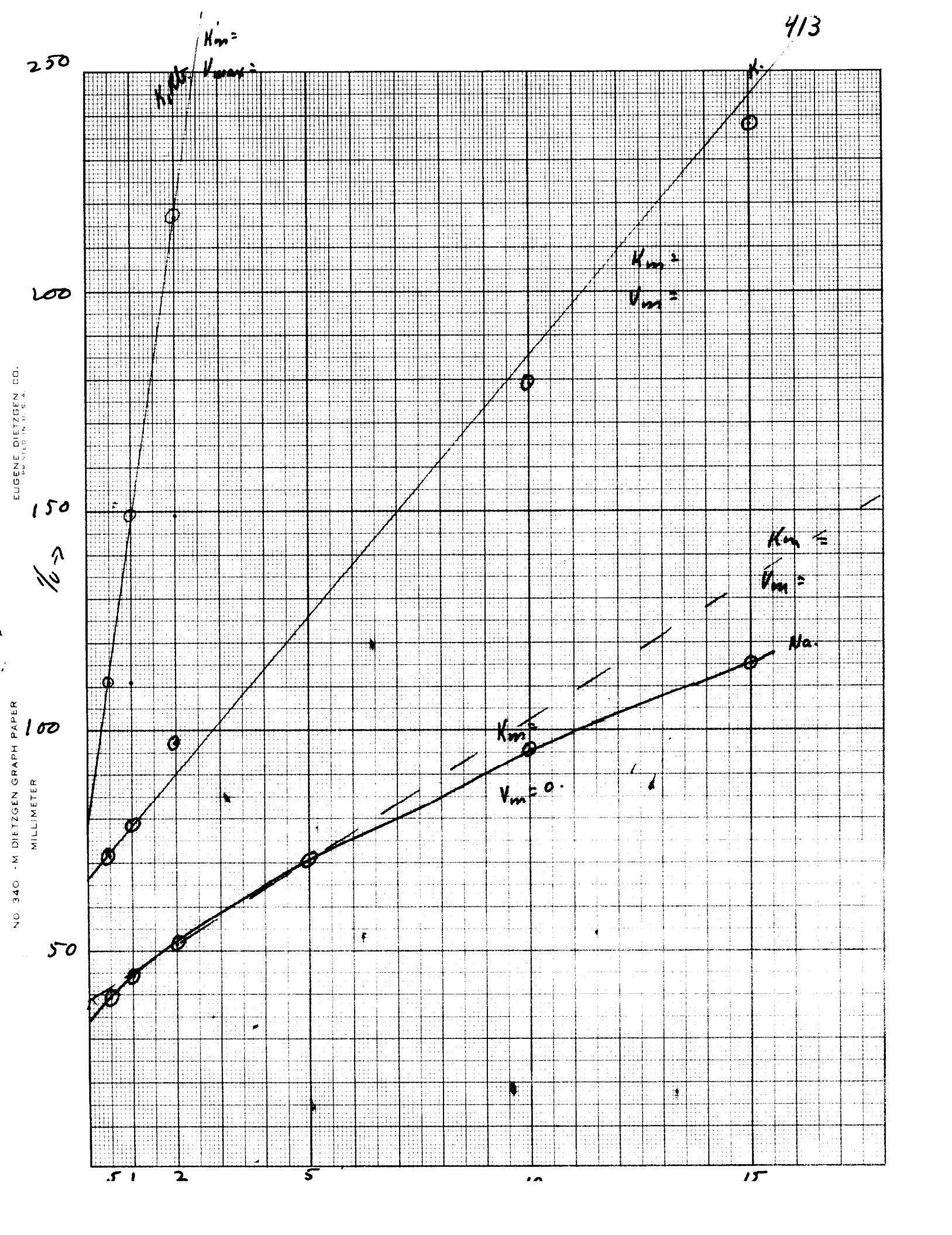
413
250
200
150
100
50
1/v →
K
Km =
Vmax =
Km =
Vm =
Km =
Vm =
Na.
Km =
Vm = 0.
5
15
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V_{max} / K_m'

411 beta
413.

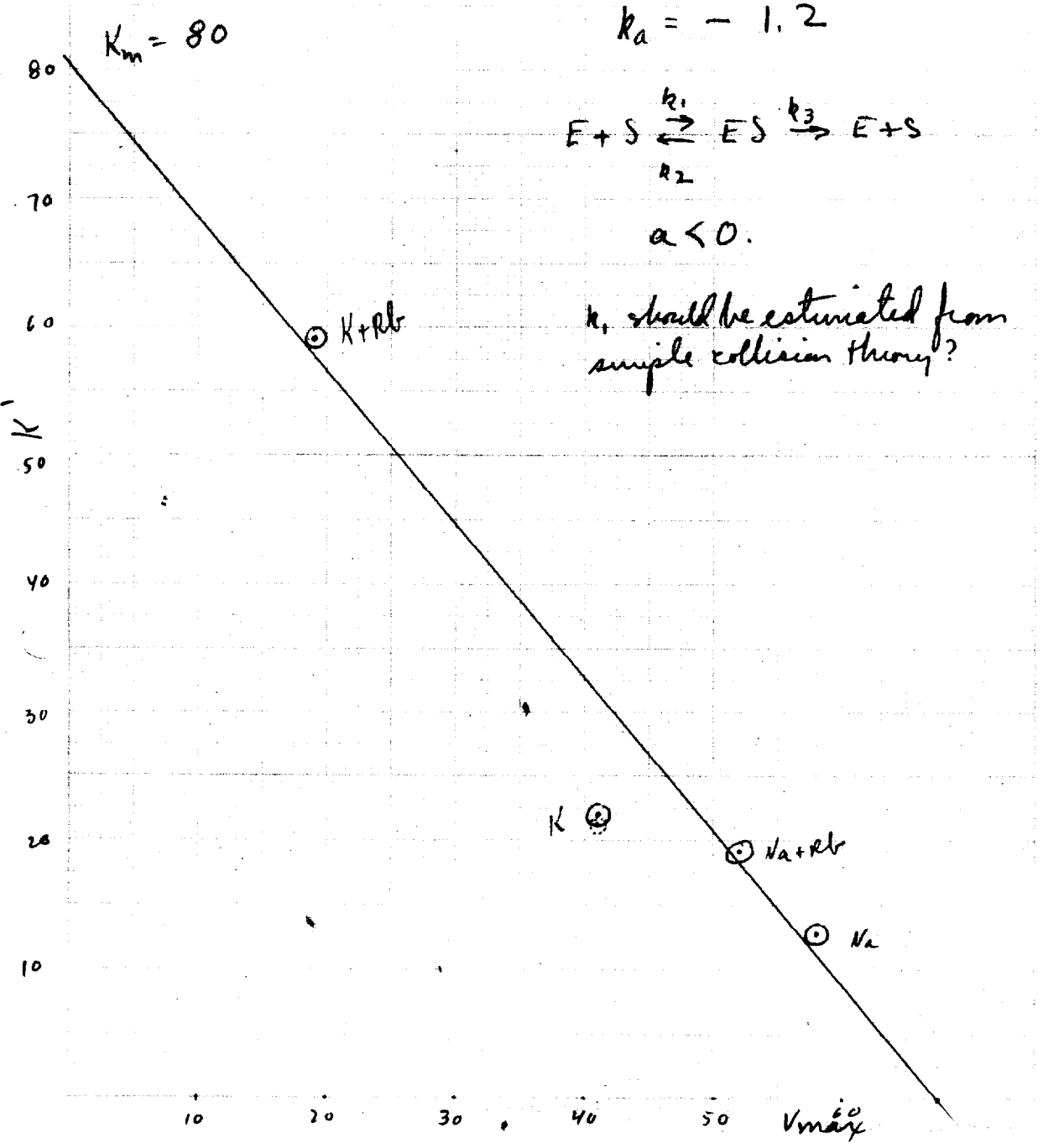
$$K_s' = K_m + k_a V_{max}$$

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

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

$a < 0.$

k_1 should be estimated from simple collision theory?



January 16, 1919.

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.

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

Note. 5 ÷

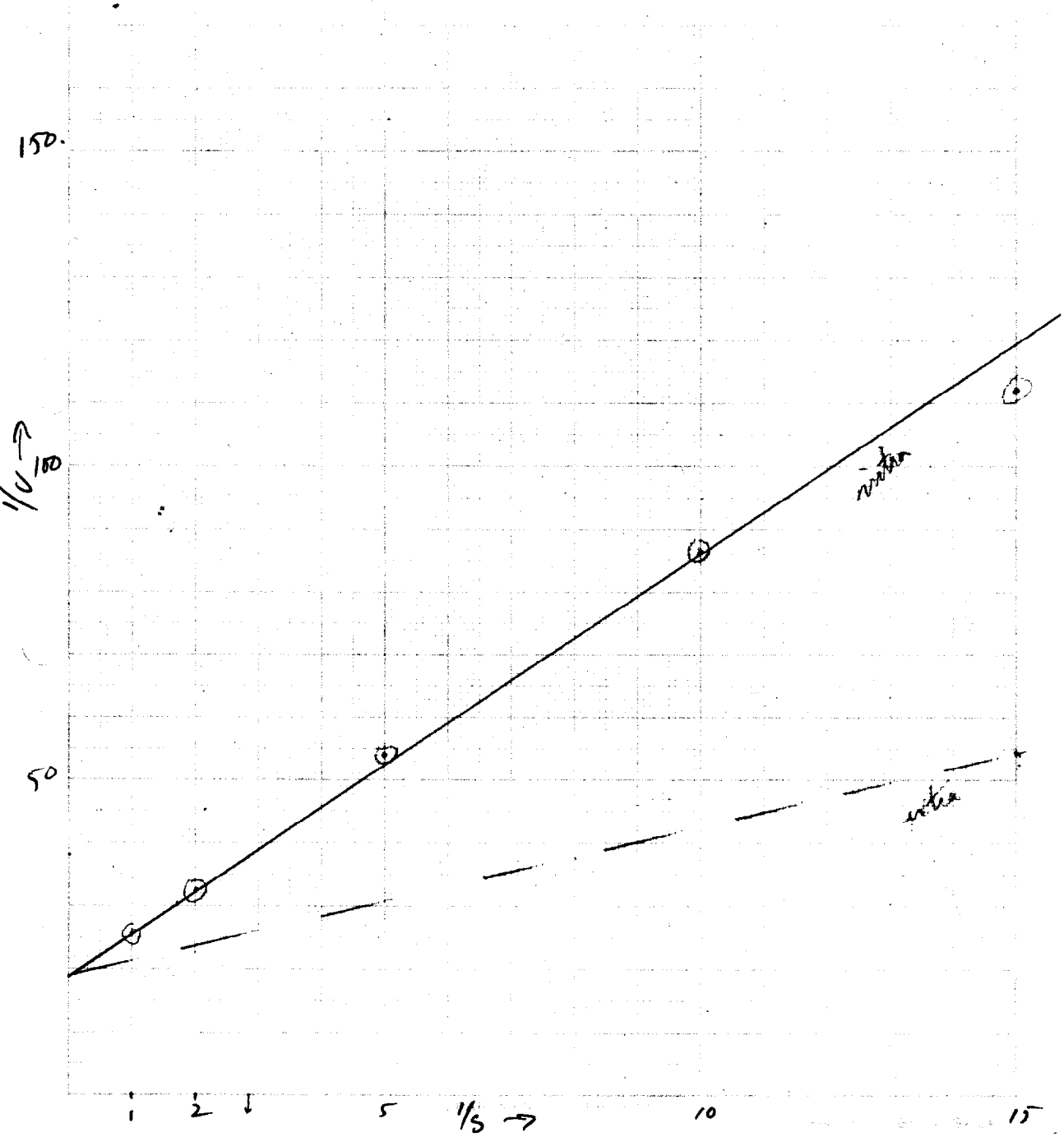
Note M/100 OAP ÷

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

$V_{max} = 527$

Kinetics of intracellular galactosidase.

Na P buffer pH 7.5 M/100.



Intracellular galactosidase

Jan. 17-18, 1949.

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

Preliminary assay: 10 mins in NaP M/100 7.5

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

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

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

a) pH optimum. Use M/100 KP buffer M/50 NaCl. ONPG M/5000 unless stated

	pH	Δ		Δ
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

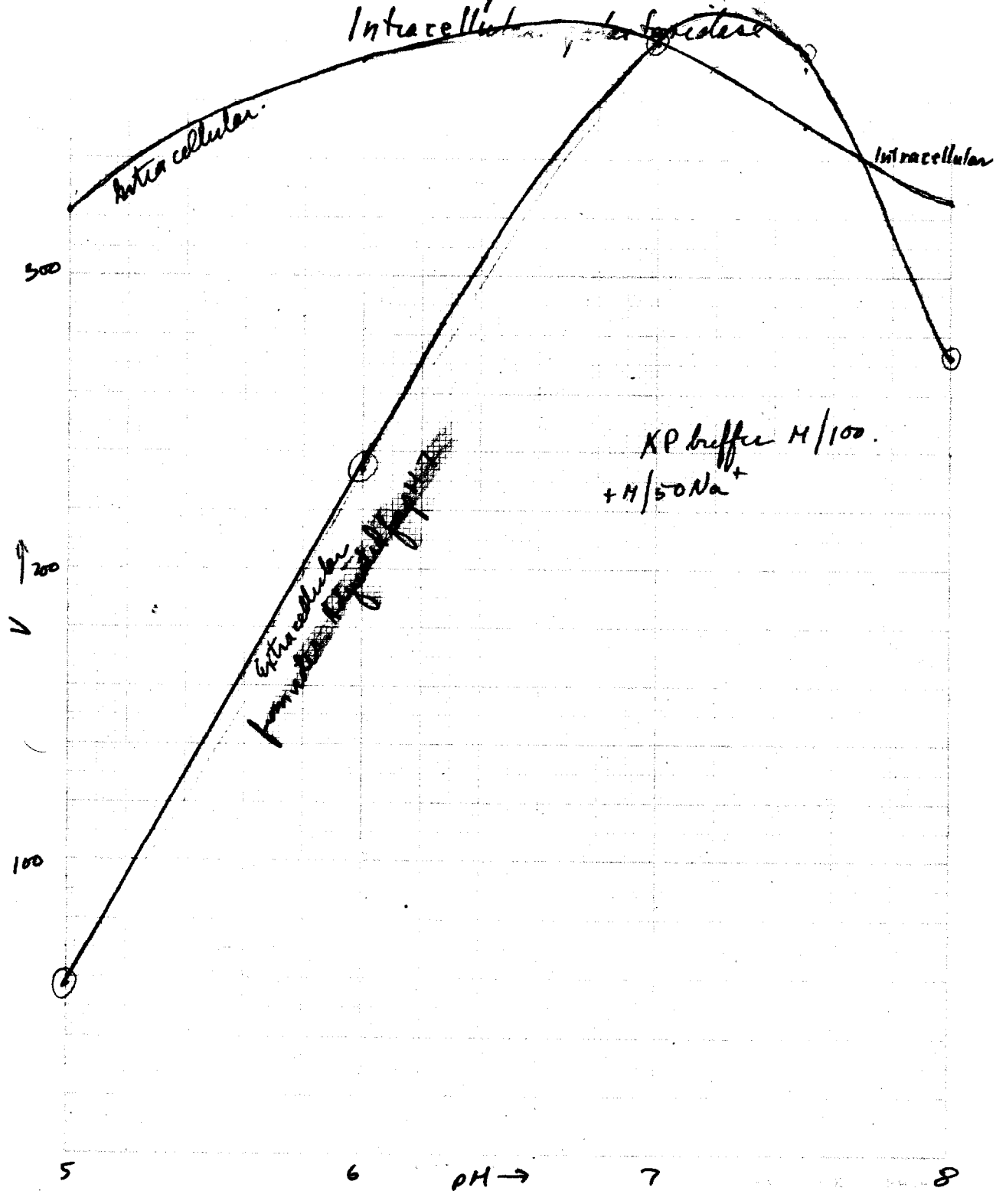
c) Kinetics. Na buffer M/100. 7.5 V_{max} = 527, K_m = 4.5×10^{-4}

	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
KP buffer M/100.
+M/50 Na+
500
200
100
V
5
6
pH →
7
8

Adaptation of ML; K-12 on galactose

		Δ_1	Δ_2	[12:45 AM] Corrected:	i	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-	gal	60	424	116	129	176		540	52	49
	lac	228	—	111	123	339		970	206	—
	glu	002.	30	117	130	119		147	~~<2~~ <2	3.4
blank	—									

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

Galactose therefore has ca. 14x activity

for ML Lac/Gal = 16
K-12 Lac/Gal = 4

Mutant adaptations to galactose.

1/22/49.

Harvest cells from 10 ml 1/2 - 1% sugar broth and resuspend in Butyl galactoside 1/2%. Tubes ± BCP indicator. Also check counts on EMB Lac plates.

	Lac	✓	Gal	✓	~~Gal~~ Bug.	✓	~~Bugal.~~ Glu	✓
K-12	114 680 514		150 298 120		147 1000 590		BCP ++ 120 ~~1000~~ 131 -010	
W108	~~8~~102 518	+	~~8~~05 150	+	~~8~~126 1100	+	85 112	✓
W45	~~8~~110 122	✓	140 146	✓	83 120	✓	- 140 150	✓
W112	~~8~~ 106 160 (30)	✓	117 196 49	✓	210 870 310	✓	++ 123 134	✓
W255	~~8~~ 127 1050 800+	✓	~~8~~ 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. Given 108/Glu had 1% +

Adaptation to related substrates

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

	D_i	5:20 PM	D_i cor.	Δ	Δ/D_i^{cor} = R.A.	7:15 PM	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	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 %	

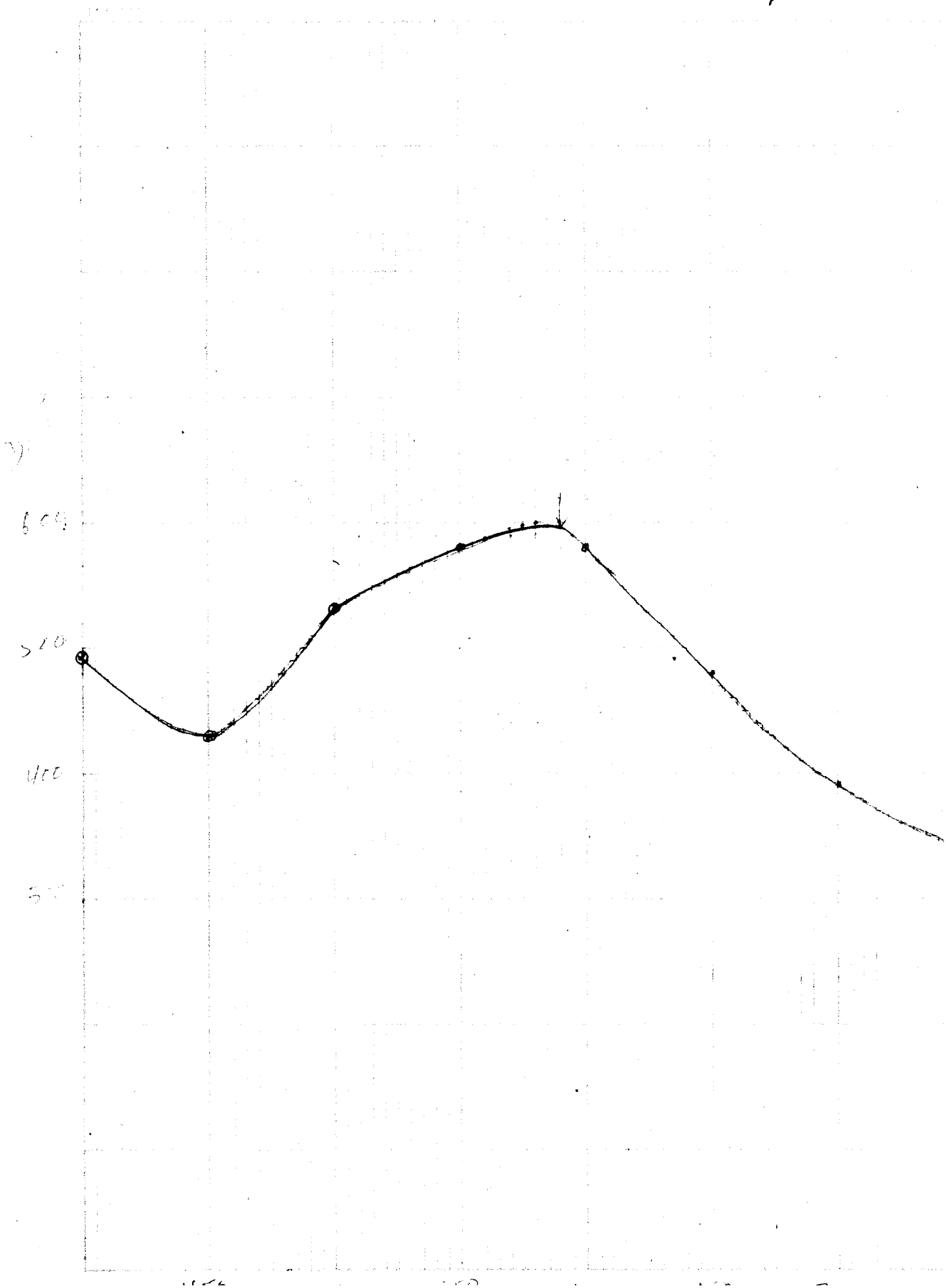
Absorption spectrum of E. coli + formazan. (tetrazolium)

#23.

1	2
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% tetraphenyl tetrazolium, and study absorption spectrum. Peak at $\lambda = 5800$ Å but not very sharp.


600
500
400

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	+	−	∓	−	−
30	+++	±	+	±	±
45	+++	±	+	±	±
60	++++	±	+	±	±

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

Galactosidase in W815.

3/1/49.

Harvest cells from 42 Lac and 42 Glu. dilute, etc. &
test E M/2000 ONPG KP 7.5 M/50.

	D_{λ}^{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 in 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.	4h 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 dry as does that of K-12.

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

4/2/49.

Compare carbohydrate utilization by cell suspensions harvested from 20 hour Lac Y2 broth, unshaken of (A) W710 and (B) W815.

Add 10 mg sugar to 1 ml cell suspension and 1 ml (μM) buffer BCP.

		A 10m	15m	B 5m	10m	15m	20 m.	25	60
1	[illegible]	[illegible]		−	−	±	−	±	++±
2	[illegible]	+++		−	−	±	±	++±	+++
3	lactose	+++		±	++	+++	+++	+++	+++
4	butyl galactoside	+++		±	++	+++	+++	+++	+++

∴ butyl galactoside is fermented much more quickly than galactose (ca 3x).

[Is glucose accumulated from lactose? cf. W255 and W815 grown on lactose. Also W1089L3+. J.

Query? does galactose permeate the cell? Use inhibition of galactosidase.

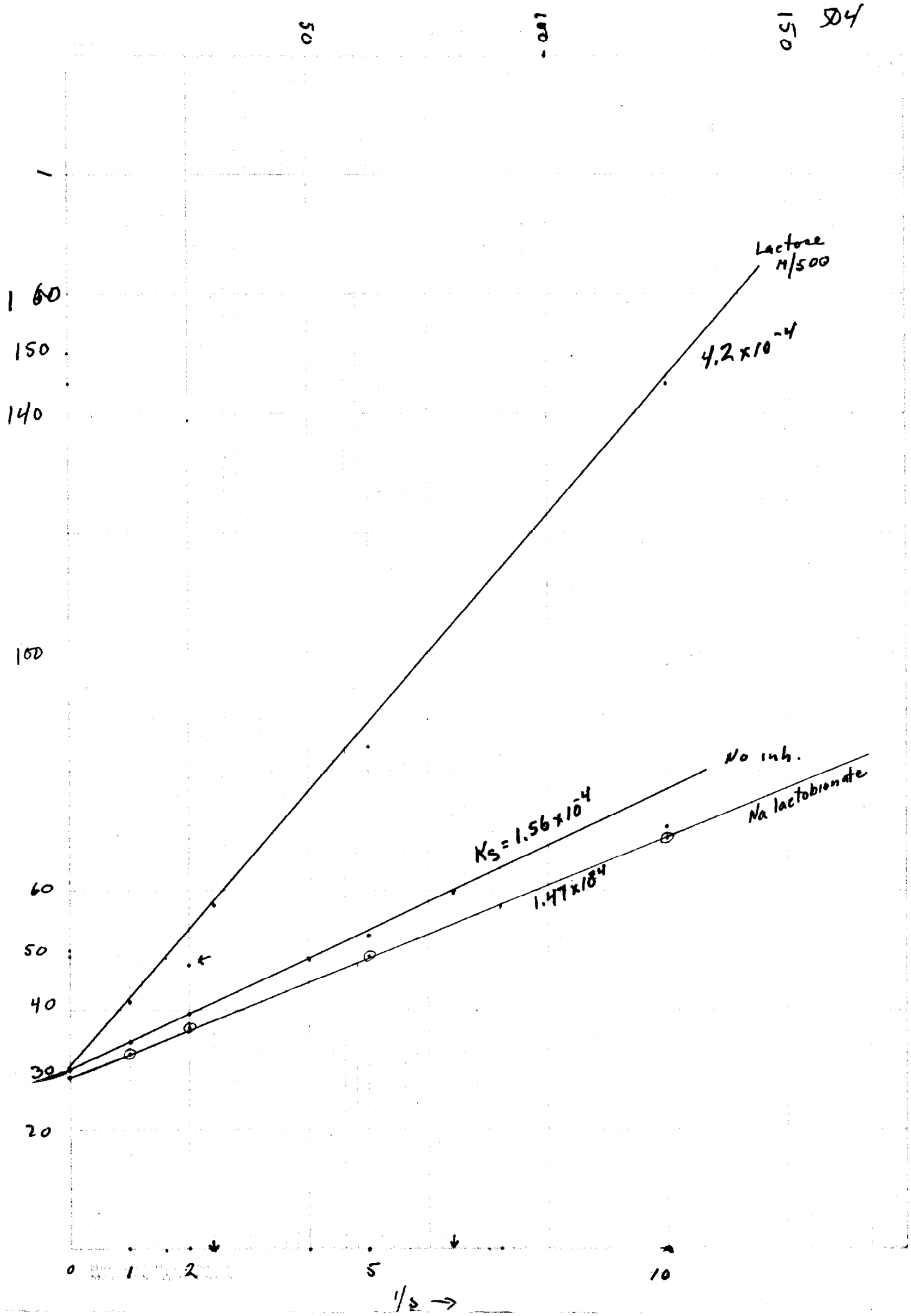
Competitive inhibition of galactosidase

4/3/49.

Extract 399 dry cells. Dilute 1% aqueous extract 1:200 and use 1 ml aliquots.
Na P buffer pH 7.5 M/50.

	ONPG M/.		Di	Df	Dcor	1/v
1	100		020	307	289	34.6
2	200		010	263	254	39.4
3	500		002	193	191	52.4
4	1000		-003	129	132	75.7
11	100	Lac M/500.	020	261	243	41.1
12	200		013	221	~~210~~ 209	47.8
13	500		003	122	119	84.0
14	1000		-001	68	69	145
21		Lba M/500	021	338	319	32.4
22			013	281	269	37.2
23			005	209	204	49.0
24			003	147	144	69.4

Lba = Calactobionate; Ca replaced by Na & oxalate, barium and Na_2SO_4.
Make substrate etc. to 9 ml. Add 1 ml enzyme dilution at t_0. 36°.


Lactose M/500
4.2×10⁻⁴
No inh.
Na lactobionate
Ks = 1.56×10⁻⁴
1.47×10⁻⁴
160
150
140
100
60
50
40
30
20
0
1
2
5
10
1/s →

		Di		Df
1.	—	177 ~~141~~ ~~132~~	550	
2.	Azide	178	520	
3.	Lac	180	510	
4.	Azide + Lac	190	510	

Glucose.

			Di cor.	
1.	—	178	160+010 = 170	165.

Competitive Inhibition

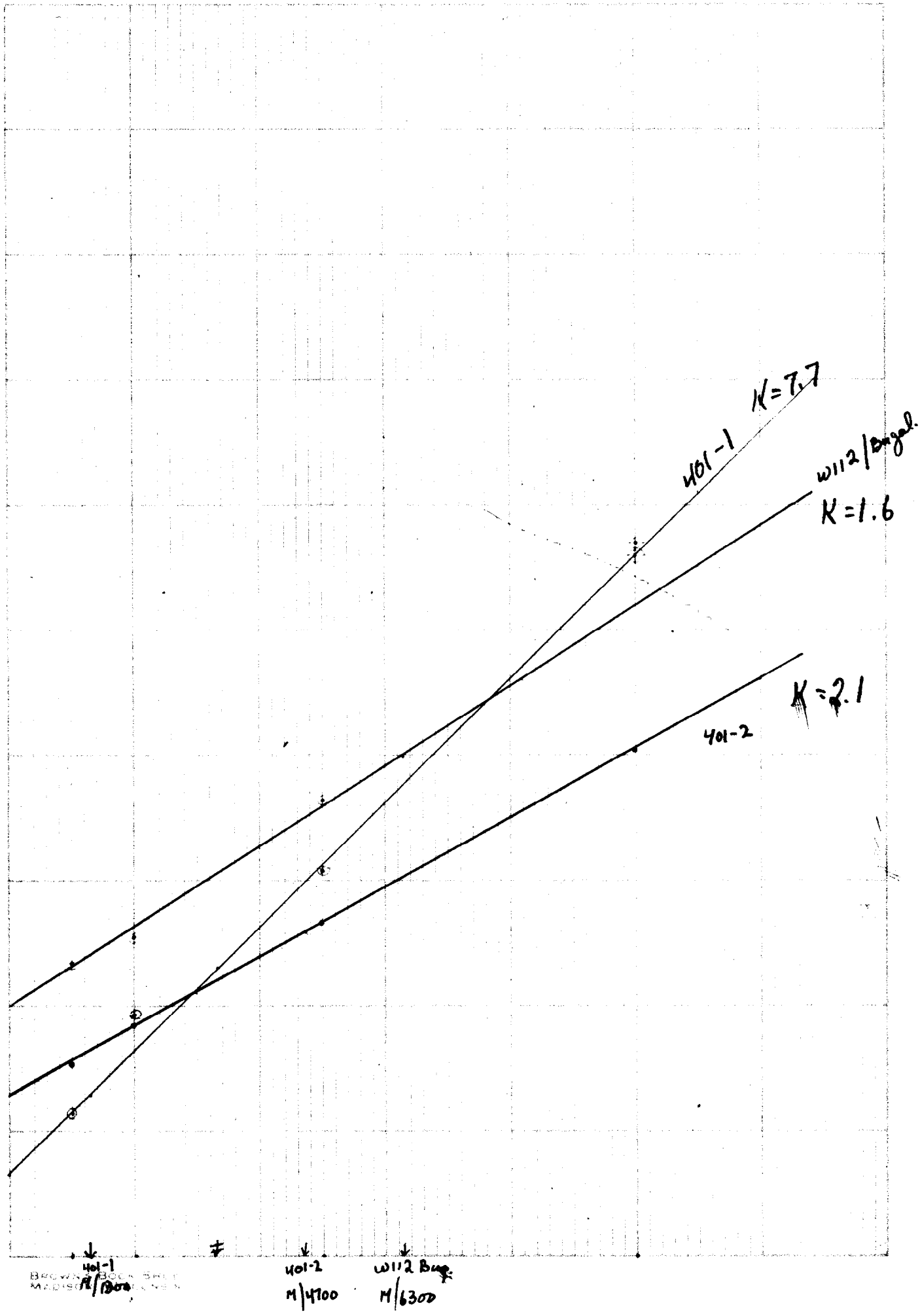
4/4/49.

	[399 1:200]		NaP 7.5 M/50 + Na_2SO_4 M/50 · 1/vol			
1		—	023	387	368	27.2
A 2			010	339	336	30.2
3			003	259	256	39.1
4			—	180	180	55.6
	Lactitol ~~lactobionate~~					
11		M/400	021	360	343	29.1
12		"	012	301	298	34.4
13		"	001	210	209	47.8
14		"	002	141	139	71.9
21		Bugal	027	278	256	39.1
22			017	203	189	~~34.8~~ 52.9
23		M/500	009	109	102	98.0
24			005	62	58	172
31		Megal	024	379	359	27.8
32			017	330	316	31.6
33		M/500.	007	241	238	42.0
34			004	173	170	58.8

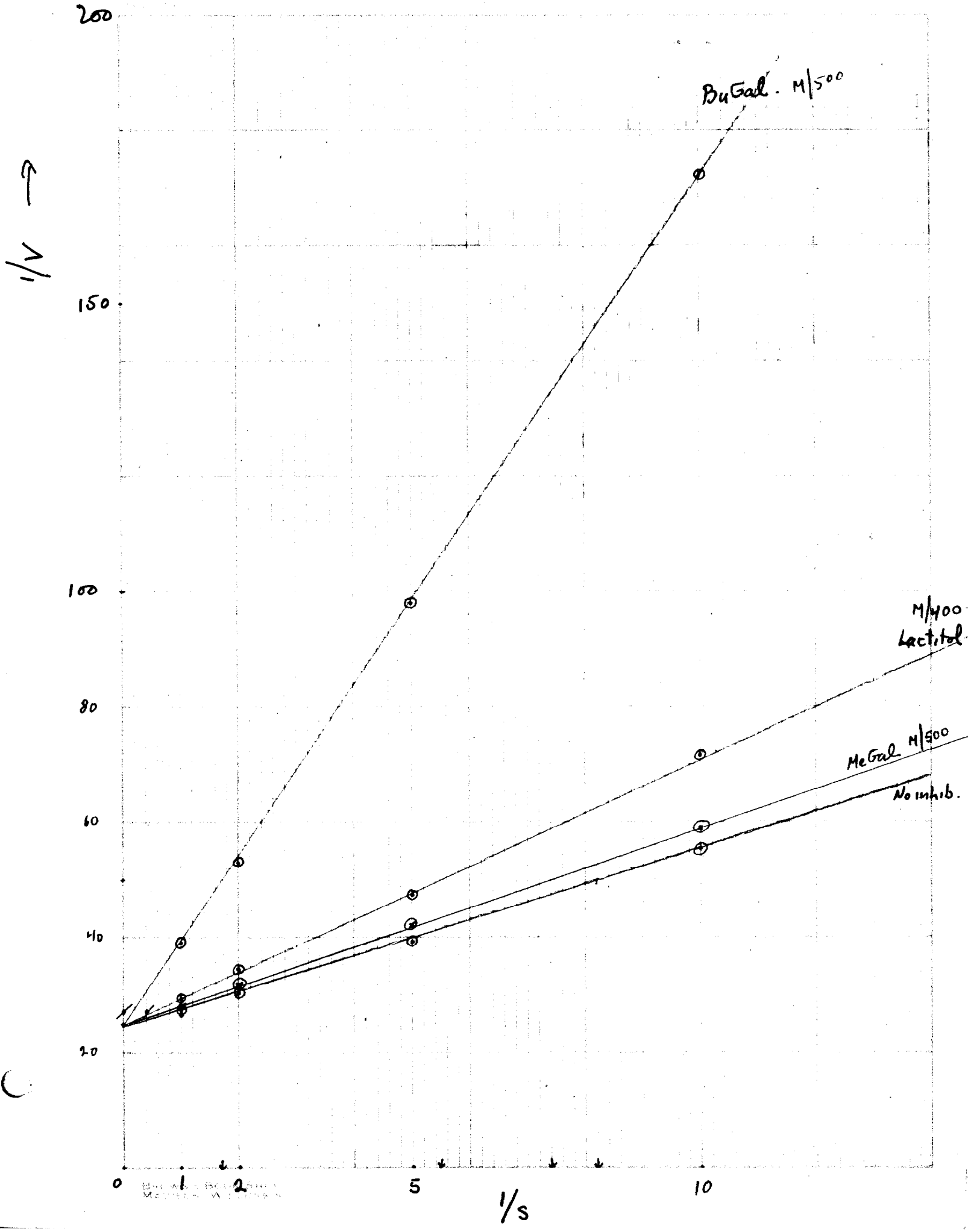
correction is increase from 9 to 11 ml. Subtract 9/11 of Di from Df.

Apparent K_m : $\times 10^{-4}$

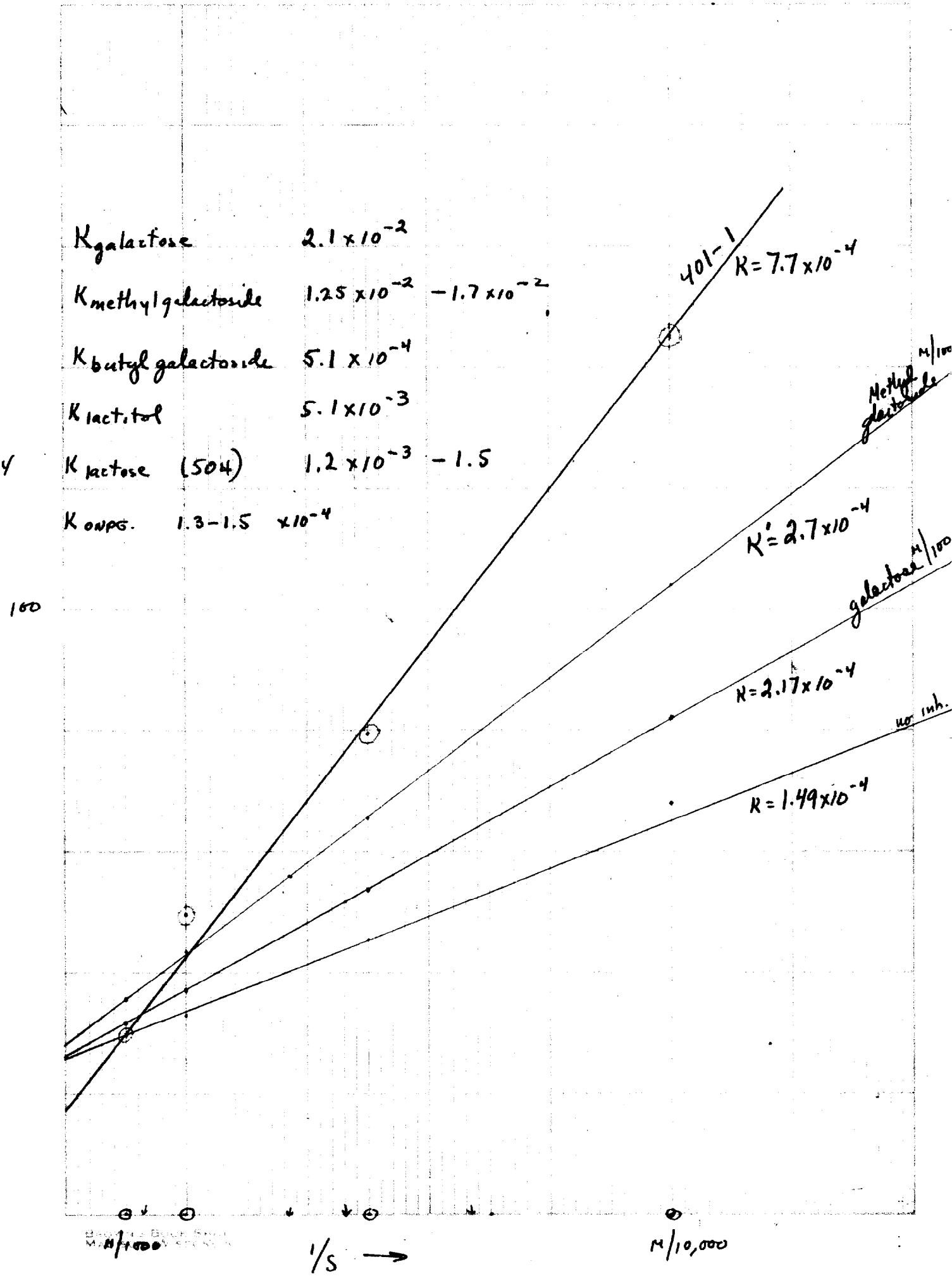
Blank:	1.22
Megalactoside	1.35
Bugalactoside	5.9
Lactitol	1.82

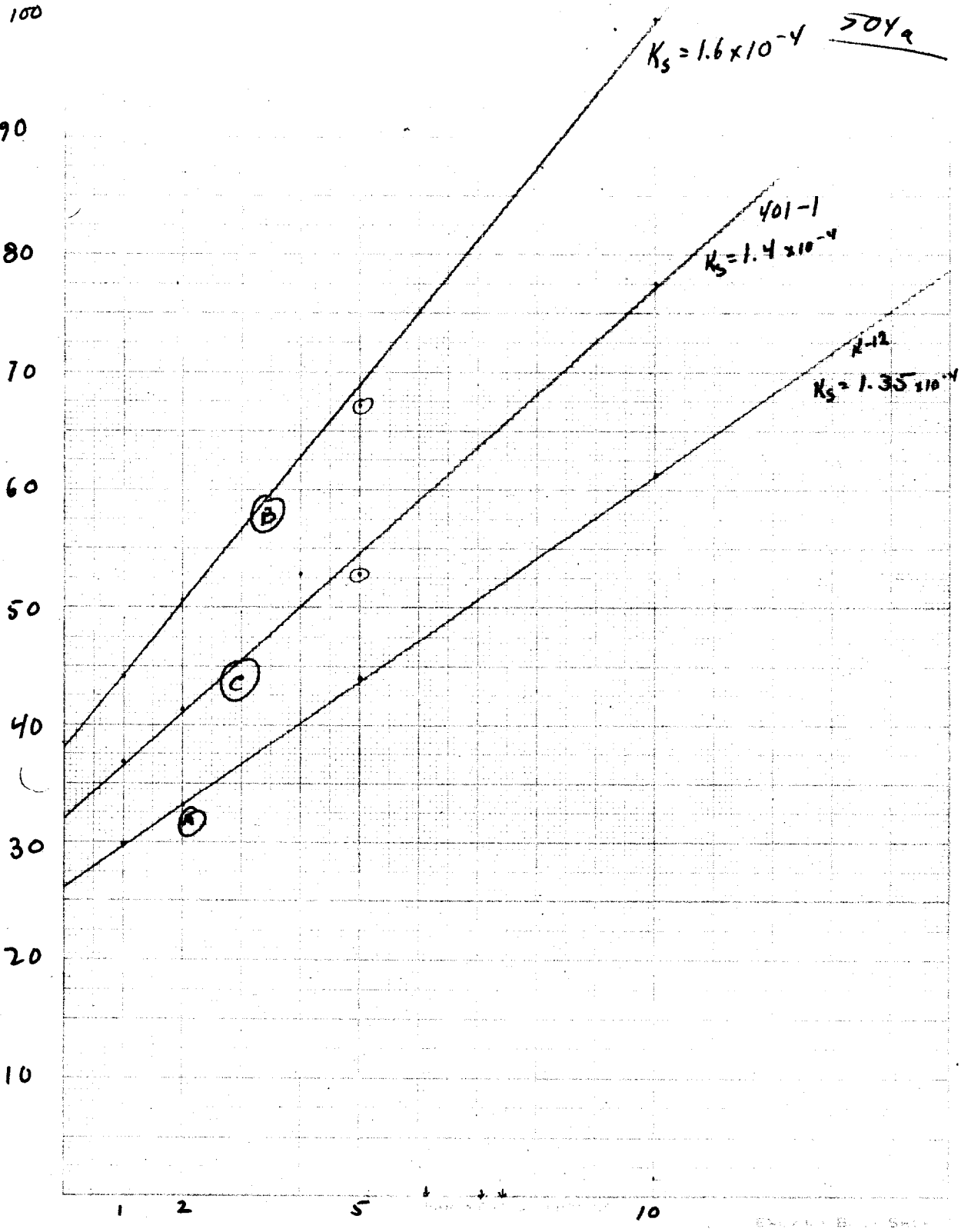

K=7.7
401-1
w112/Bugal.
K=1.6
K=2.1
401-2
401-1
M/3000
401-2
M/4700
w112 Bug.
M/6300

						$V_{cor.}$	$1/v$	$1/v_{adj}$			
	1			019	349	334	29.9				
	2		Blank	009	311	304	32.9				
	3			003	221	219	45.7				
	4			002	149	147	68.0				
							35.7				
	1		Megal	025	300	280	~~43.5~~				
	2			012	240	230	43.5				
+1	3		M/100	009	159	152	65.4				
	4			004	99	96	104				
	1		Galactose	021	330	313	31.9				
	2			010	280	272	36.8				
+5	3		M/100	003	188	186	53.8				
	4			1	121	122	82.0				
1:50											
W112	1		~~[illegible]~~	028	239	216	46.3				
Bergal.	2			013	208	197	50.8				
	3			004	140	137	73.0	$K_m = 7.7 \times 10^{-4}$			
+6	4			002	090	088	114				
									T		
401-1	1		Blank	0023	450 ✓	432	23.1	29.9			
	2			016	273 ✓	260	38.5	49.8			
.2/100	3			005	166	162	61.7	79.8			
	4			004	92	89	112	145.			
401-2	1		Blank	019	339	324	30.9				
	2			014	280	269	37.2				
.4/100	3			003	188	186	53.8				
	4			005	128 ✓	124	80.6				
	841			207	361						
	51			202	451						
	52			232	880						
	470			160	220						


200
150
100
80
60
40
20
1/V
0
1
2
5
10
1/S
BuGal. M/500
M/400
Lactitol
MeGal. M/500
No inhib.

$K_{galactose}$	2.1×10^{-2}	
$K_{methylgalactoside}$	1.25×10^{-2}	-1.7×10^{-2}
$K_{butylgalactoside}$	5.1×10^{-4}	
$K_{lactitol}$	5.1×10^{-3}	
See 384 $K_{lactose}$ (504)	1.2×10^{-3}	-1.5
$K_{ONPG.}$	$1.3-1.5 \times 10^{-4}$	




100
90
80
70
60
50
40
30
20
10
1
2
5
10
$K_s = 1.6 \times 10^{-4}$
>0Y a
401-1
$K_s = 1.4 \times 10^{-4}$
K-12
$K_s = 1.35 \times 10^{-4}$
A
B
C

Kinetics of suppressor lactases 504a

4/7/49

1/s ONPG NaP 7.5 M/100.

		100/M	Di	Df	Vcor.	1/V	Ks	Vmax
A	1	1	017	358	344	29.9		
399	2	2	009	309	302	33.1	1.35	
1K-12	3	5	003	230	227	44.0		
10mins.	4	10	0	163	163	61.3		
B	1	1	023	~~240~~ 245	226	44.2		
(Y01-1)	2	2	013	209	198	50.5	1.6	
	3	5	006	154	149	67.1		
	4	10	—	100	100	100.—		
C	1	1	022	~~240~~ 290	272	36.8		
(Y01)	2	2	011	251	242	41.3	1.4	
	3	5	003	192	189	52.9		
	4	10	006	134	129	77.5		
D	1	1	019	760	744			excessive
W112	2	2	013	680	669			enzyme
Bugal.	3	5	003	500	500			
prep.	4	10	006	331.	325			
10mins.								

These determinations show no unusual deviations! and are in consistent with 504

F

galase from Megal. grown cells

4/5/49.

Grow K-12 overnight in 200 ml 42 Megalac. ½%
Harvest P5 and dry over P_2O_5.

Yield: 85 mg dry cells.

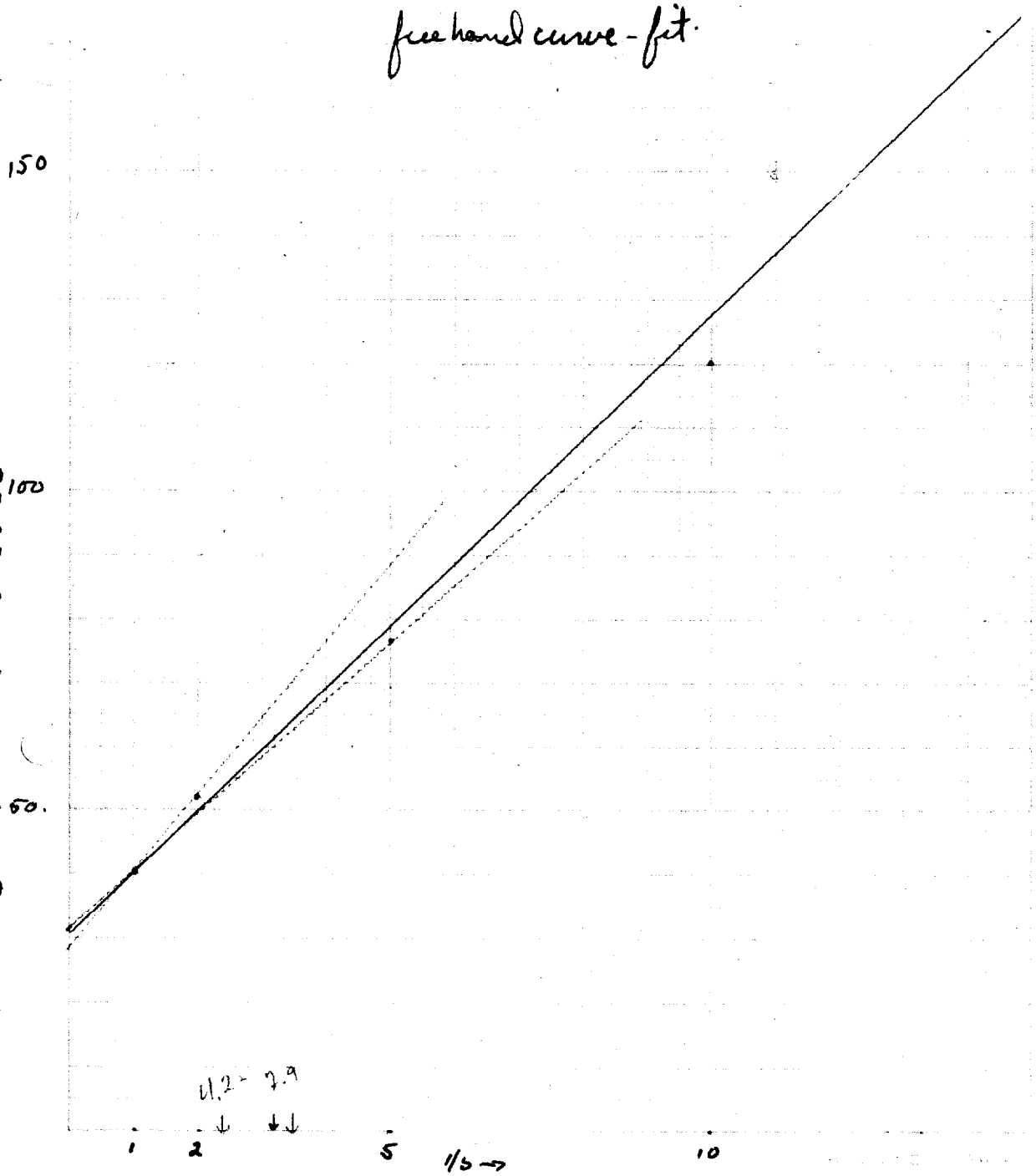
Triturate and extract 40 mg / 10 ml H_2O for extract 506A.
Extract potency ca. 600 u/ml.

4/5/49.

Grow K-12 in 2x 50ml 1/2 Mal 1% Harvest and dry over P_2O_5. Yield: 29 mg.

Intracellular lactase.

free hand curve-fit.



Kinetics of cellular lactase

4/6/49.

K-12 harvested from Y2 bac. 5X. then .2ml in 10 NaP buffer M/100 pH 7.5

ONPG	Rdg.	Est. Con.	V + O2.	V	1/V
M/1000	346 -	370-358	338	249	40.2
2000	283 -	299-291	281	192	52.1
5000	220 -	222 221	219	130	76.9
10,000	167 -	178 173	172	83	120.5
12000 (stirred vigorously).	260		240		
∞		0	89		

V_{max} = 322.

K_m = 3.2×10^{-4}

Stirring does not stimulate enzyme action!

Km is here at least twice that of isolated enzyme.

Kinetics of enzyme from lactose and fungal grown cells.

Temperature coefficients at enzyme saturation

4/7/49.

1+2 at 37° 3+4 at 32°. ONPG M/1000 NaP M/100

1,3 K-12/Lac cells, Di controlled.
2,4 K-12 (399) extract.

	Di 4:41 PM	D20		Vcor	D31
1	22		461	342	[illegible]
2	25		307	287	454
3	23		262	142	319
4	20		159	143	~~319~~ 234
	cells		101		

Q_{15} extract = ~~302~~/287/143 = 2.01 Q_{10}: 1.6

Q_{15} cells = 342/142 = 2.41 Q_{10} = 1.8

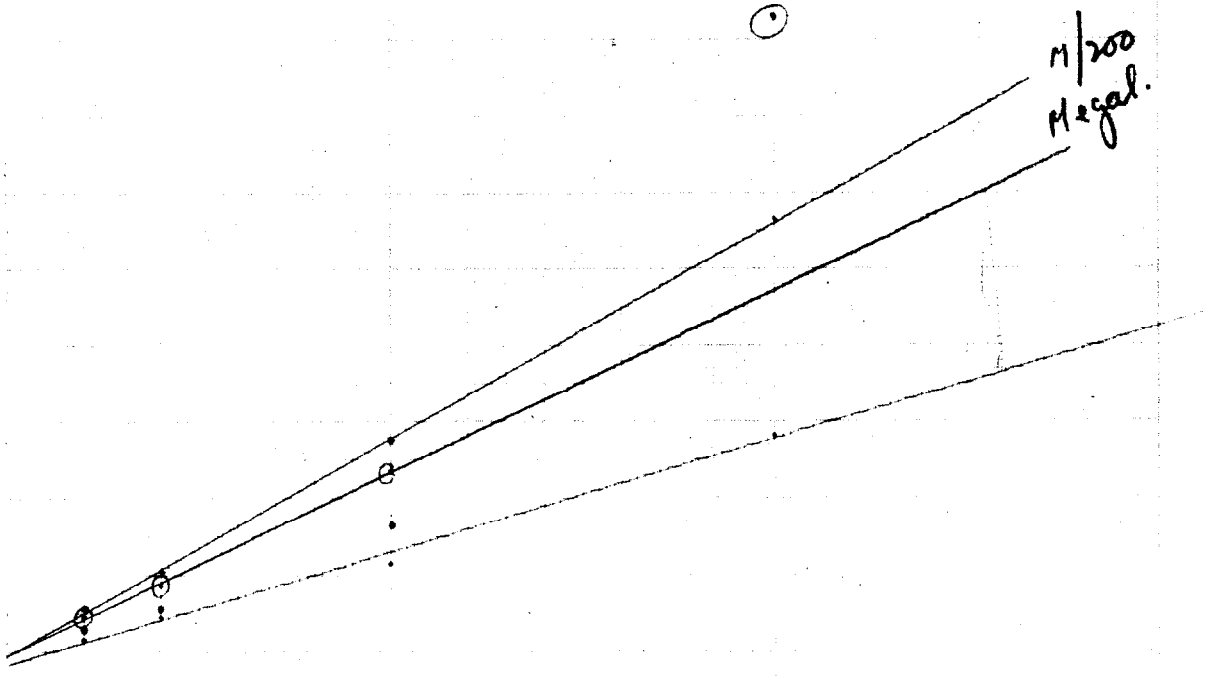
or calc. $Q_{10} = (Q_{15})^{2/3}$

Note: Q_{10} cells is higher than Q_{10} extract at this high substrate concentration.

2:25 K-12 + W-349 grown on lactose tested on lactose; lactitol.

K:12: +++ on Lac ++ on Lol in 5 mins. Glu +++ + Lol in 10 mins.
W349. — —

Add glucose pair at 2:30 2:55: W349 - - - .


M/200
Megal.

4/7/49.

NaP M/100 7.5 20 min 37°

			Suppl.				1/v
399 1:200	1	1		020	478	462	21.6
	2A	2		018	421	406	24.6
	3	5		—	319	319	31.3
	4	10		-5	203	208	48.1
	1	1	Megal	019	421	406	24.6
	2B	2		009.	353	346	28.9
	3	5	M/200	-001	229	230	43.5
	4	10		003	200	97	103.1 ??
506 1:150	1	1		020	451	435	23.0
	2C	2	~~Meg~~	011	400	391	25.6
	3	5		007	281	275	36.5
	4	10		-004	204	208	48.1
							25.8
	1	1	Megal	020	404	388	~~20.5~~
	2D	2		010	337	329	~~26.4~~ 30.4
	3	5	M/200.	008	217	210	47.6
	4	10		-003	128	131	~~18.1~~ 76.3

Data n.g. expt. made repetition.

Test for induction period in cellular utilization of ONPG.

514

4/8/49.

Harvest K-12 from Y2 tac. Conc. 5x. Use .2ml/10.
Make up in Na P buffer & ONPG M/2000. Run expt in cuvette.
Temperature: 22.5 initially. 24° at 16m. at 20m. Add substrate at t_0.

	Time	D		Time	D
	–	134		30	263
	t_0 calc.	131		60	270
	~~t_0~~	~~[illegible]~~		90	271
	20	121 (mixing).		120	282
	30	137			
	40	141			
	60	146	12		
	70	148	1min		
	100	149	13		293
	120	159			
	140	162	14		307
	160	167			
	180	170	15		319
	200	177			
	220	180	16		330
	240	184			
	270	190	17		341
	300	197	18		357
6m.	330	202	19		369
	360	209	20		380
	90	212			24°
	120	219			
	150	225			
	180	231			
	210	239			
	240	245			
	270	252			
	300	259			

Correction: −13.4 for dilution of cells. + 10 for substrate. ∴ −3.4 +134 gives initial. = 131.

April 10, 1949

W349 is listed as lactase + B-14-. Inoculate 2 × 500 ml
½ lactose and aerate 24 hours. Harvest + dry over P_2O_5.
Yield: 672 mg.

Linear dilution response
galactosidase

4/14/49

399A, ca. 1:100 1ml/tube NaP M/50 7.5 ONPG M/2000.

	Di	Df	Vcor
.1	003	41	25
.2	004	72	56
.3	0	96	80
.4	0	127	111
.5	003	158	142
.6	003	189	173
.7	002	212	196
.8	010	267	242
.9	001	318	302
1.0	001	354	338
0	+ Substrate	016	

non linear

Subtract (~~[illegible]~~ 016 from all vc.
~~and Di from~~ #8. − .025 on this one.
for Vcor

Galactosidase in Lac. adapted cells.

4/29/49

Grow K-12 shaken overnight in M/100 Lac 5 ml. Harvest and compare with Lac M/100 adapted cells, etc.

		2:10 PM Di	2:30
1	Lac	119	800
2	Lac	106	126
3	Glu (Y2)	157	172
4	NSB	162	170

These tubes were made up from Stodola's purified lactobionate. Either the pure prep. is inactive or M/100 is too dilute.

App. increases of 2-4 probably artifacts; no visible color. ~~[illegible] and yellow after hours~~
Lac does not adapt to ONPG. after 1 hour, progressive color in controls, ca 50. Probably adaptation.

Effect of azide on pH sensitivity.
Compare activity of Lac adapted cells above in KPM/50 buffer pH 5.0 and 7.0 all tubes receive M/50 NaN_3 and M/2000 ONPG.

pH	Azide	Di	Df.	R.A.
7.0	−	54	340	
7.0	+	60	361	
5.0	−	53	94	
5.0	+	51	145	SIC!

Azide stimulates cells!

Should use KN_3 to eliminate Na effect

April 17, 1949.

Prepare M/10 Na Lba. from Link's crude material with Sod. Carbonate equiv. to pH 9.5

1. Make up YZ- M/40 Lba. Grow K-12 and Y-53 in 5 ml. ea. overnight with shaking. From these suspensions, inoculate 180 ml aerated flask for dry-cell prep'n.

Test galactosidase activity of washed suspensions. ONPG M/2000; NaP M/50 7.5 20 mins. 37 C.

	D_i	D_f	R.A.
K-12	229	880	300
Y-53	229	222	008

This prep. of lactobionate certainly elicits a very active galactosidase, but not from Lac_1-/

The cells harvested fermented glucose, lactose very very slowly.

2. Inhibitions. Make up tubes with .01 ml 399A lactase, M/1000 ONPG, NaP as above.

To 3,4 add M/100 Lba.

	D_i	D_f		$1/v_o$	$1/v_i$
1	002	251			
2	0	252	250	40	
3	010	169			
4	014	164	155	64.5	

Taking K_{onpg} as 1.3×10^{-4}, K_{lba} can be calculated:

$$K_I = I\left[\frac{1/v_o\left(1 - \frac{S}{K_s+S}\right)}{1/v_i - 1/v_o}\right]$$

$$= \frac{M}{100}\left[\frac{40}{24.5}\left(1 - \frac{1/10^{-4}}{2.3\times10^{-4}}\right)\right]$$

$$= M/100\left[\frac{40}{24.5}\left(1 - \frac{1}{2.3}\right)\right]$$

$$= \frac{(40)(.57)}{24.5} \times 10^{-2} = .93\times10^{-2}$$

$$\frac{K_I}{I} = k_i = \frac{v_i\, k_s}{(v_o - v_i)(1+k_s)}$$

$$= \frac{155}{95}\,\frac{1.3}{2.3}$$

$$= .83$$

$$= 8.3\times10^{-3}$$

(crude lactobionate)

3. Prepare dried cells from 180 ml aerated Y2-Lba. Yield 160 mg. Well aerated culture was very dense

Lactobionate.

4/17/49.
399A 15/100 M/1000 ONPG
Repeat c̄ purified lactobionate from F. Stodola. NaPM/50 pH 7.5

Lba.	Di	Df.
—	003	400
M/200	004	367
M/100.	010	359

ONPG added. Correction .010

$$\frac{K_I}{I} = \frac{V_i\, k_s}{(V_0 - V_i)(1 + k_s)} = \frac{359 \times 1.3}{(41)(23)} = \frac{367}{23} \cdot \frac{1.3}{2.3} \cdot M/200$$

$$= 4.9 \times 10^{-2} \qquad 4.5 \times 10^{-2}$$

use m̄ $= 4.7 \times 10^{-2}$

Concentration effects on adaptation

4/20/49.

Lactose 3.6% stock. = M/10 Make up Y2: 2 each.

	Di	Df	Δ	R.A.
1. M/50	041	432	400	1000
2. M/100	044	570	530	>1000
3. M/500	056	395	350	650
4. M/1000	053	477	430	900
5 M/10,000	045	120	80	170
6 M/100,000	048	77	35	75

Harvest K-12 grown overnight in Y2 + each of above conc. (10 ml aliquots). Conc. ca 5x; use 1 ml/10 ml tube in assaying for galactosidase.

Retest adaptation to galactose (1%) and Lba purified (M/40 in Y2)

Gal	087	139	60	75
Lba	063	97	40	55

The cut off of adaptive response appears to be much lower than for combination of the enzyme!

The response to lactobionate is undoubtedly due to lactose impurity. If M/40 lactobionate is used, an impurity of 1% will give M/4000, in the range of effective response! = Check if Lba potentiates adaptation!

Enzyme dilution

	Di	De	V_{cor}		
1	−3	048	37		
2	0	083	69		
3	0	124	110		
4	0	159	145		
5	0	198	184		
6	4	253	235		
7	0	274	260		
8	2	321	305		
9	4	337	319		
10	1	406	391		
0	−2	+12	−14		
NaPM/50	7.5	M/1000	ONBG	379	10^{-2} – 10^{-3}

Quantitative adaptation data

4/23/49

			Di	Df	
1	K12	Y2Lac	090	349	
2	"	" Glu	090	087	
3	K12	Bug M/500	120	790	(7 min. reading!)
4	"	M/1000	99	529	
5	W112	Y2Lac	132	170	
6	"	Glu	80	073	
7	"	Lac M/500	80	095	
8		" M/1000	93	106	
9		Bug M/500	113	310	
10		" M/1000	120	228	

10 min. readings
Note tremendous activity
of Bugal adapted cells of K-12!

Comparison of Lac,- and Bugal.

4/24/49.

Grow W112 overnight in Y2 Lac M/500; Bugal M/500 and Glu M/500

A = K-12 B = Y70 C = W112 (8 min. 10)

1 = Lac M/50 2 = M/500 3 = Bug M/500

		D_i cells	D_i cor		Δ	Δ/D_i	R.A. 20 min.	
K-12	A 1	70	73	281	208	297	600	(410) 514
	2	110	109	223	114	104	200	
	3	81	83	470	387	478	950	(800) 590
Y70	B 1	117	115	140	025	021	042	
	2	111	110	120	010	009	018	
	3	113	112	178	064	057	113	
W112	C 1	90	91	127	036	040	080	(23) 30
	2	113	112	127	015	013	027	
	3	88	89	239	150	171	341	(700) 310

These cells are shaken, and therefore pneumatically aerobic!
Compare earlier data which show a wider discrepancy.

[Cf ~~See~~ 421. — in last column I.
EML 194. (Y10 for K-12)

Much greater differentials.

Compare Y10 (K) and W112 (Lac-)

April 25.

Without shaking: 20 min tests

		Di Y20		Acor	R.A.
Y10	1 Lac M/50	048	152	96	200
	2 M/500	078	174	91	116
	3 Bug "	033	113	52	098
	NSB	063	070	0	000
	Y2 Glu	047	056	01	002
W112	1	072	086	08	011
	2	109	119	08	007
	3	97	143	43	044
	Blank + orgeg		013		

Shaken: 20 min.

Y10	1	108	460	350	324
	2	119	570	452	380
	3	097	441	341	331
	4	130	150	020	015
	5	080	086	005	001
W112	1	096	119	020	021
	2	~~096~~ 103	120	014	014
	3	122	262	139	114

These data can be used:

	M/500 Lac	M/500 Bug	M/50 Glu
Y10 Lac,+	380	331	001
W112 Lac,−	014	114	—

Adaptivity of galactosidase

5/6/49.

Y10 after 3 transfers in NSB, grown overnight shaken in

	15 min.	Di	Df.	Con Δ.	Δ	R.A. 15m.	20m.
Lac Y2		100	441	351-22	341	329	439
Pneumococcus (Ely)		111	128	6	17	005.4	007.2
NSB.		109	127	6	18	005.5	007.3
0				22			

Increase upon adaptation is 61 x

i.e., unadapted cells have activity ca. 1.6% of adapted!

This may be incipient adaptation.

Kinetics of adaptation

5/25/49

Harvest Y10 from 6 hr. heavily noc: Y2 Blue shaken.
Suspend 2 ml in 2 ml 1% oxalate, 2 ml H_2O, 1 ml M/5 buffer.
Take 4 ml samples into M/100 oxalate M/50 buffer then ONTO M/100

		Di	Df	Acr.	R.A.
7PM	T=0	104	97		
7 45	45 m.	101	100		
9 30	150 m.	086	097		
9 50	170 m.	079	090		

No adaptation found

5/26/49

Y10. 2 ml cells 1 ml 1% Lac ½ ml buffer ½ ml H_2O or M.C. (A) (B)

T_0 = 2:35 PM. Assay in azide phosphate

cells very clumpy! apparent in both media

	T	O_i	D_f
A. (O)	T_0	121	133
	3 PM	130	168
	3:35	117	144
	5 PM	109	132
	7 PM	106	134
B (MC)	T_0	128	133
	3 PM	130	148
	3:35	120	129
	5 PM	118	147
	7 PM	118	133

Minute adaptation

Adaptation rate.

7/5/49.

Harvest K-12 from standing culture in Y2 Bkr. Conc. ca ~~20~~ 10 X. in H_2O. Ad. Syst. contains ~~H_2O~~ 1ml NaP M/5 7.5, 1ml 2% lactose, 1ml cells and 1ml supp.

Take .3ml samples to 9ml ONPG test system.

A). No supplement — ONPG concentration 021.

B). Peptone 1ml 2%. — 4PM Start.

T.		A Di	A Df	A Δ(corr)	A R.A.	B Di	B Df	B Δ	B R.A.
15m.	4^{15}	061	071	−005	——	064	087	008	012
	5^{00}	056	077	+ 005	009	067	098	038	057
	7^{00}	048	098	+ 034	071	083	310	214	261
	8^{00}	~~051~~				099	780	670	680

Deadaptation.

Harvest K12 freshly grown on Y2 Lac. 8^{40} PM

.5ml sample (from c). 8 mins.

071 152 067 236 (60 MIN)

A) 1ml cells 1ml buffer 1ml glucose 1ml H_2O

B) do. 1ml M/100 Azide

c) do. 1ml lactose 1ml H_2O.

		Di	D_f^{10}	R.A. (60)
10PM	A	062	267	
	B	062	300	
	C	062	260.	

(80 mins)

Inappreciable deadaptation!

c should be corrected for inhibition by 0.1% lactose.

Deadaptation

July 6, 1949

Harvest K12 from 50 ml Y2 Lac overnight. Conc. ca 10x.
System (4 ml)

1 ml cells (a) 1 ml buffer (b) 1 ml 2% sugar (c) 1 ml peptone water (d)

A. a b — Buffer only $10^{-4.5}$ M.
B. a b — d peptone
C. a b glucose — glucose (final conc. 2.2×10^{-3} M)
D. a b glucose d peptone + glucose.

Assay in M/100 azide M/50 Na buffer. .2 ml samples (d = .05)

	Di	Df.	Δcor	R.A.
A	050	143		
B	050	181		
C	049	100		
D	048	118.		

Does glucose compete for entrance into cell?

1 45 PM

A	038	552		
B	049	226		
C	046	380		
D	080	234.		

Note augmented activity of cells incubated in buffer.

Sediment this tube and examine supernatant.
.5 ml supernatant. ca 120 Most activity is still in cells!

Storage Effects on galactosidase

7/14/49.

32 hour cells from 42 hr 9:35 to 2:30

Assay is azide.

A. 1 ml cells 1 ml buffer B. water. Incubate

C " " D. " Refrigerate

	Initial Di	Initial Df.	Final Di	Final Df. 10 min.
A	059	472	059	730
B	061	242	061	109
C	056	930	056	590
D	060	241	060	160

A.

a). Note irregular excess of buffer treated cells over water treated. Buffer was M/10 Na. pH 7.5

Is activation related to Na^+?, λ? Assay in K buffer.

7/14/49.

PM. Harvest 10 hr. cells from Y2 Lac.

Dilute equal volume ē a) water b) NaP M/5 pH 7.5 c) KP do.
d) NaCl M/5 e) Sucrose M/5

.1 ml samples assayed.
β^{30} -10^{25}

Di H_2O 10 min.	084.	274	
a	075	158.	
b	042	>750 [5 mins].	✓
c	040	>750 [5 mins].	✓
d	066	410	
e	071	375	

[phosphate buffers, which also permit lysis, are most effective in augmenting activity.
pH effect? concentration? Measure pH's.]

verify lysis by uv absorption of supernatant.

Suspensions A and B contain ca. 1.5 and 2.2 mg/ml respectively. [For ≐ .1 mg, use $\frac{1}{15}$ ml for A and $\frac{1}{22}$ ml for B.]

Assay .05 ml each.

	D_i	D_f	Δ_{cor}
A	018	18.4	155
B	030	430 (5 mins).	390 × 4
Blank	001	014.	

−013 for substrate + 10% for dilution.

B) .11 mg had activity of $\frac{20}{5} \times 4 = 16u$. ≐ 150u/mg = full activity of the cells dried.

A) .075 mg had 1.5 u. ≐ 20u/mg. ≐ full activity, not augmented.

Differences between treated and untreated cells persist on drying.

?? Can inactive, cell-free or dried preparations be activated?

Sediment A and B. Resuspend sediment in 5 ml H_2O (= 1) and keep supernatant (= 2). B2 is much more opalescent than A1.

Save samples; also mix A1, A2 etc. 1:1 = NaP M/5.

Incubate $3^{30} \rightarrow 5^{30}$

Test .1 ml samples A1, A2 and A1P, A2P.

	Pi	
A1	040	155 10
A2		N 3 20
B1	068	530 5
B2		470 6
A1P	016	140 10
A2P		099 20
B1P	030	300 5
B2P		260 6

Y10 and Y70 grown on lactose. Incubate 1:1 with water, buffer M/10.

a) 4:30 — 7:00 Assay.

b) K-12 grown on lactose. Incubate 1:1 with water, buffer, etc.

K-12 / glucose [KG]. water, M/10 buffer.

1:1:1 lactose, 2%, water, M/100 buffer, M/10 buf.

	Di.		
KG O	062		
KG P	041		
KG-L-O	032	neg.	10m.
L-P	030		
L-P	027		
KL-O	139	371	6M
KL-P	111	520	6M
KL-P	078	>1150	5M
Y10-O	095	119	10M.
Y10-P	072	960	7M.
Y70-O	113	negl.	
Y70-P	076	152	9M.

Alcohols on galactosidase

August 8, 1949.

Lactase K-12 extract 2%. Activity ca 1200 u/ml.

.001 ml in M/50 NaP 7.5 M/2000 onpg. Stop Rx ε Na_2CO_3

Alc	Conc	20m. Rdg.
—		119
Mannitol	M/10	132
Sorbitol	M/10	133
PrOH	M/100	119
"	M/10	134 ← Optimal concentration.
"	M/1	113
"	2M	029
"	5M	006

Recheck Mannitol and PrOH concentration. Also, of 3 4/1 which showed larger alcohol effects.

8/9/49 .002 ml, as above

1	—	
2	—	
3.	PrOH	M/10
4.	Et<OH OH	M/10
5.	Mannitol	M/10
6	~~BuOH~~	~~M/10~~

Summary of lactose activation

September 9, ff., 1949.

2 l. aerated K12/42 Lac washed and concentrated to 30 ml. Aliquots of 15 ml ea. mixed c̄ A) 15 ml H_2O ; B) 15 ml NaP 1/5. and incubated 1 hour at 30°. After removal of 1 ml, 29 ml samples were dried over $CaCl_2$ over P_2O_5, and subsequently found to yield .642 and .560 g. respectively after washing, or 22.1 and 19.3 mg/ml respectively.

Assays of A and B before and after drying were (U./mg.)

	wet	dried
A	5.1	104
B	44.5	146

After ~~the~~ benzene treatment, an activity of 157 U./mg was recovered.

2. Can dried cells be further activated? Relate these activities to Vmax. pH characteristics of activated cells. Rb responses.

September 9, 1949

Assay aliquots of A and B. ~~1/10~~ 1/10; .1/10 = .01 ml

	D_i	~~[illegible]~~	20 m.	R.A. 20 m.	
A	089	193			113
B	080	329 6 min.		257 × 10/3 =	860

1 ml A = $\frac{.642 \text{ mg}}{29 \text{ ml}}$ = 22.1 mg, assuming complete recovery.

		D_i				
	Benzene		3 min			
1	~~Toluene~~	067	530 × 20/3	~~3860~~ 3500	= 3500 u/ml A	
2	Chloroform	048	430 20/3	2500.	≐ 157 u/mg.	

4:30 – 5:10 m A cells.

∴ autolysis strongly activates galactosidase.

.01 ml samples of A, B suspensions have activities of 113; 860 u respectively, ≐ (113, 860) u/ml. Total samples should be 29 x... or

	Total.	grams dry	Pud. u/mg.
A	3280	~~[illegible]~~	.642 g.
B	24800	aqueous	.560 g.

u/mg

Use .01 to .02 ml samples of 1% aqueous suspensions of dried cells for comparison.

		D_i	D_t	T.	R.A.	u/ml	u/mg.	u/mg pud.
A	.02 ml	040	560	5 min.	2080 ~~560~~	~~[illegible]~~ 1040	1040	5.1
B	.01 ml	014	380	5 min.	1464 ~~380~~	1460	146	44.5

Benzene: 157

This drying has resulted in optimal activation of E. coli lactase.

Lactase activation

September 9, 1949.

Harvest and water wash K-12 from 2 l. aerated 37° Y2 Lac 1½%.

Suspend in 35 ml. Remove 5 ml, and separate 15 ml portions of remainder: A) + 15 ml H_2O B) + 15 ml NaP M/5 pH 7.5. Incubate in stoppered flask at 30° 1^{30} to 2^{30}, for subsequent dry cell preparation. At 2^{30} Remove 1 ml aliquots, and sediment + dry remainder

[Dilute $\frac{1}{100}$; $\frac{.5}{10} = \frac{1}{2000}$ for assays.]

A) Assay in dil (M/50) and conc. (M/10) buffer. Do latter in colorimeter. Use cells & ONPG as blank.

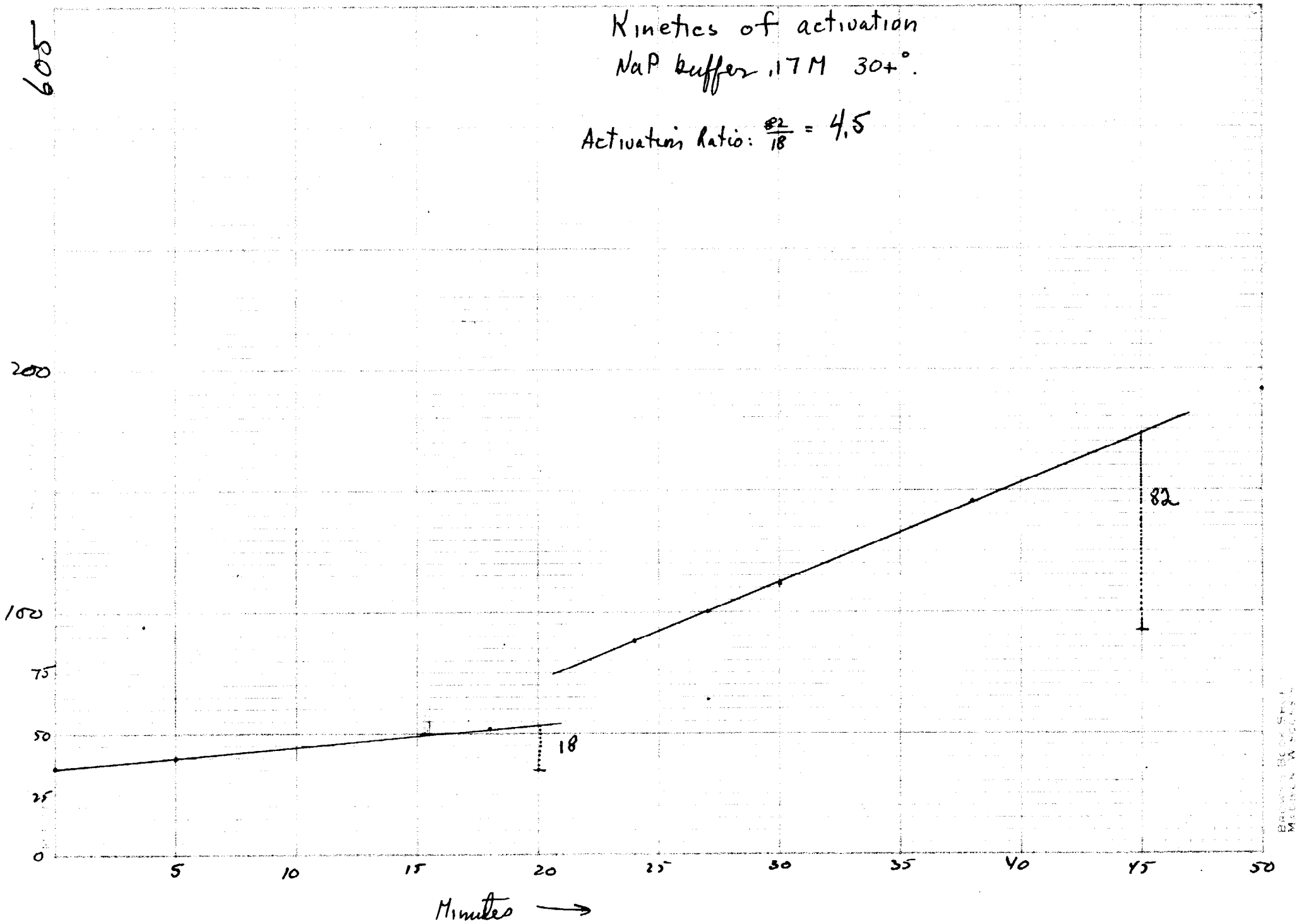
ONPG Di 034.
Cells Di 200

M/5 buffer NaP.	8.5 ml
cells (add at To)	.5 ml
onpg	1 ml

Time.		D.
2^{15}	20 s	036
	60 s	034
	180	035
	240	039
2^{20}	5 M	040
	~~6 M~~	
	7 M	
	8	
	9	
	10	
233	18	052
239	24	087
242 ~~[illegible]~~	27	~~[illegible]~~ 100
~~250~~		
245	30	112
253	38	146
305	50	191

Kinetics of activation
NaP buffer .17 M 30+°.

Activation Ratio: $\frac{82}{18} = 4.5$



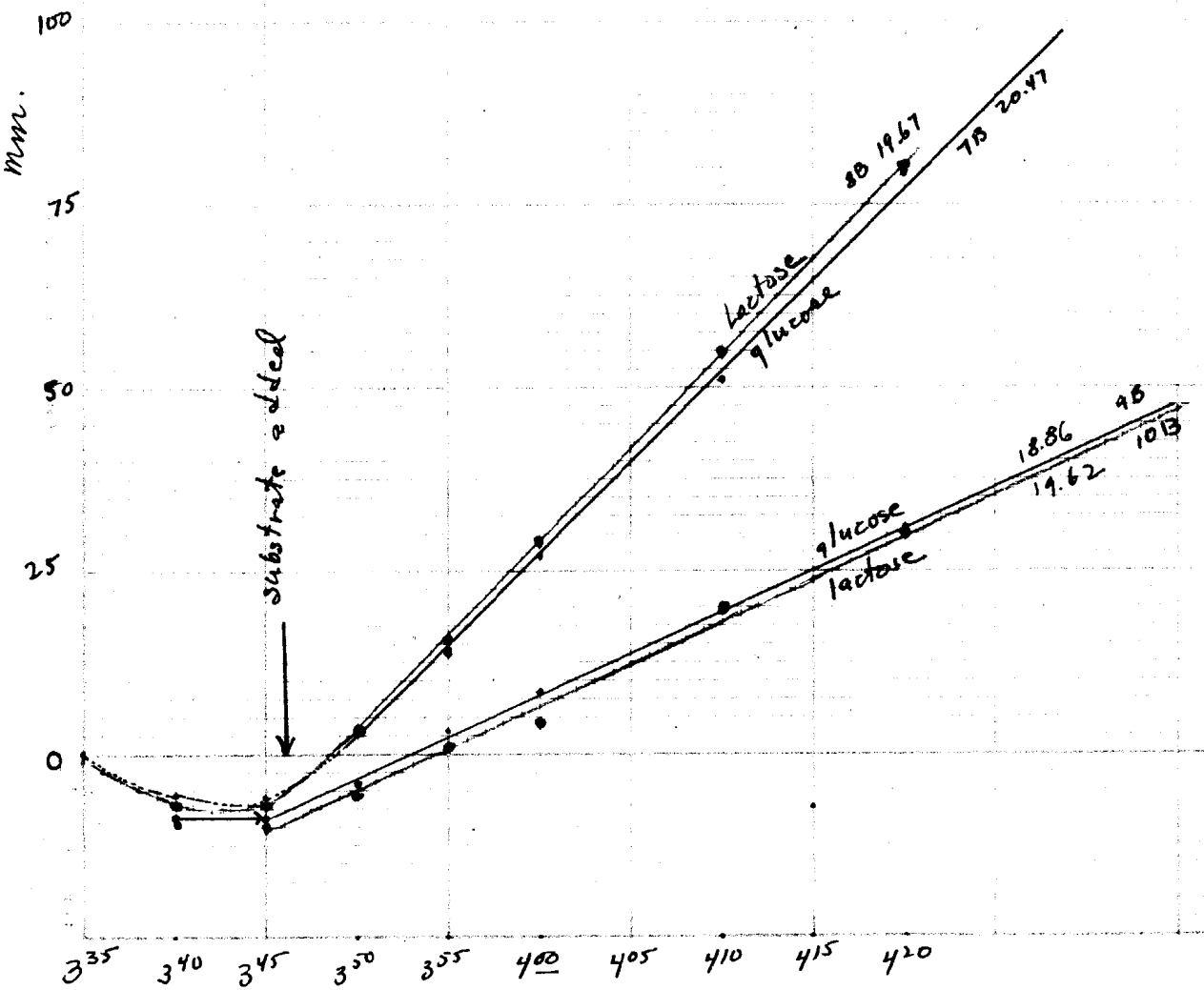
	Thermo	7-7B	6-8B	9-9B	10-10B
main vessel		← 2cc M/10 NaHCO3 - M/100 Phosph. →			
	.1cc	← untreated coli "A" →		treated c "B"	M/10 PO4 buffer 1 hr
side cup	.1cc 10%	glucose	lactose	glucose	lactose
3:35	151	104	68	89	23
3:40	148 +3	95 -6	58 -7	78 -8	61 -9
3:45	147 +4	93 -1 -1	57 -1 -	77 -8	59 -12 1
3:50	150 +1	106 +10 (3)	70 +10 (3)	84 +4 (-4)	66 +4 (10)
3:55	149 +2	119 +14 (17)	84 +15 (16)	90 +7 (+1)	72 +7 (+1)
4:00	147 +4	127 +10 (27)	93 +11 (29)	93 +5 (+8)	73 +3 (4)
4:10	149 +2	153 +24 (51)	121 +26 (55)	107 +12 (20)	91 +15 (20)
4:20	153 -2	185 +28 (79)	157 +26 (80)	122 +11 (31)	105 +10 (30)

Manometric tests on "activated" cells.

ca. 50% inactivation of buffer treated cells.

605

Uncorrected.
$K_{m=0}$ given.



Utilization of Isomaltose

September 8, 1949.

		9A	2 A	4 A	8 A	10 A	7 A	T
		D/g	D/m	4/g	4/m	4/m	4/–	
150	0	14 0	07 0	12 0	15 0	04 0	45 0	152 0
210	5	18 4	07 0	05 -7	10 -5	01 -3	45 0	152 0
217	10	14 -3	15	13 -2	16 -2	06 -1	49 1	155 -3
225	35	11 -6	12	09 0	14 -4	03 -4	47 -1	155 -3
3 mins								
230	40	13 -4	09 -1	19 4	18 0	03 -4	42 -6	155 -3
	5	18 0	10 -1	36 20	28 9	03 -5	42 -7	156 -4
	10	25 6	12 0	58 41	43 23	08 -1	47 -3	157 -5
	15	33 14	11 -1	72 55	49 29	04 -5	42 -8	157 -5
	20	38 19	09 -1	92 77	62 44	09 +2	48 0	155 -3
	25	51 29	14 -1	111 91	71 48	08 -4	46 -7	160 -8
308	30	56 34	12 -3	126 106	77 54	04 -8	42 -11	160 -8
338	60	101	14	218	133	06 -9	43 -13	163 -11
		X		X	X			
503			09			03 -13	42 -15	164 -12
			X			X	X	

K12 Cell growth in glucose or maltose (D, M)

2 ml cells .1 ml substrate 10%. = Temp. g, m.

$NaHCO_3$ M/20

CO_2

NaP M/1000

9A D, g

2A D, m

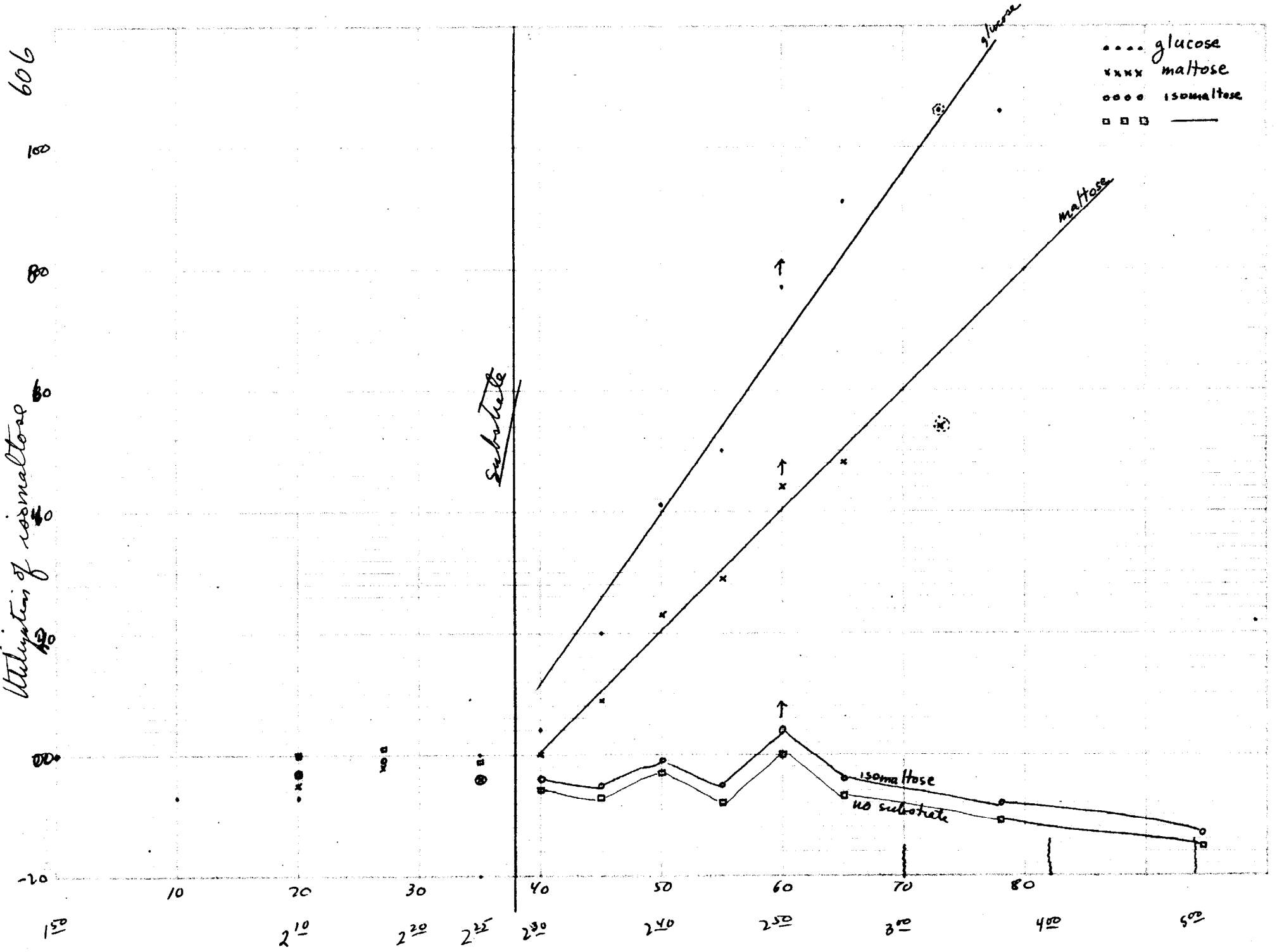
4A M, g

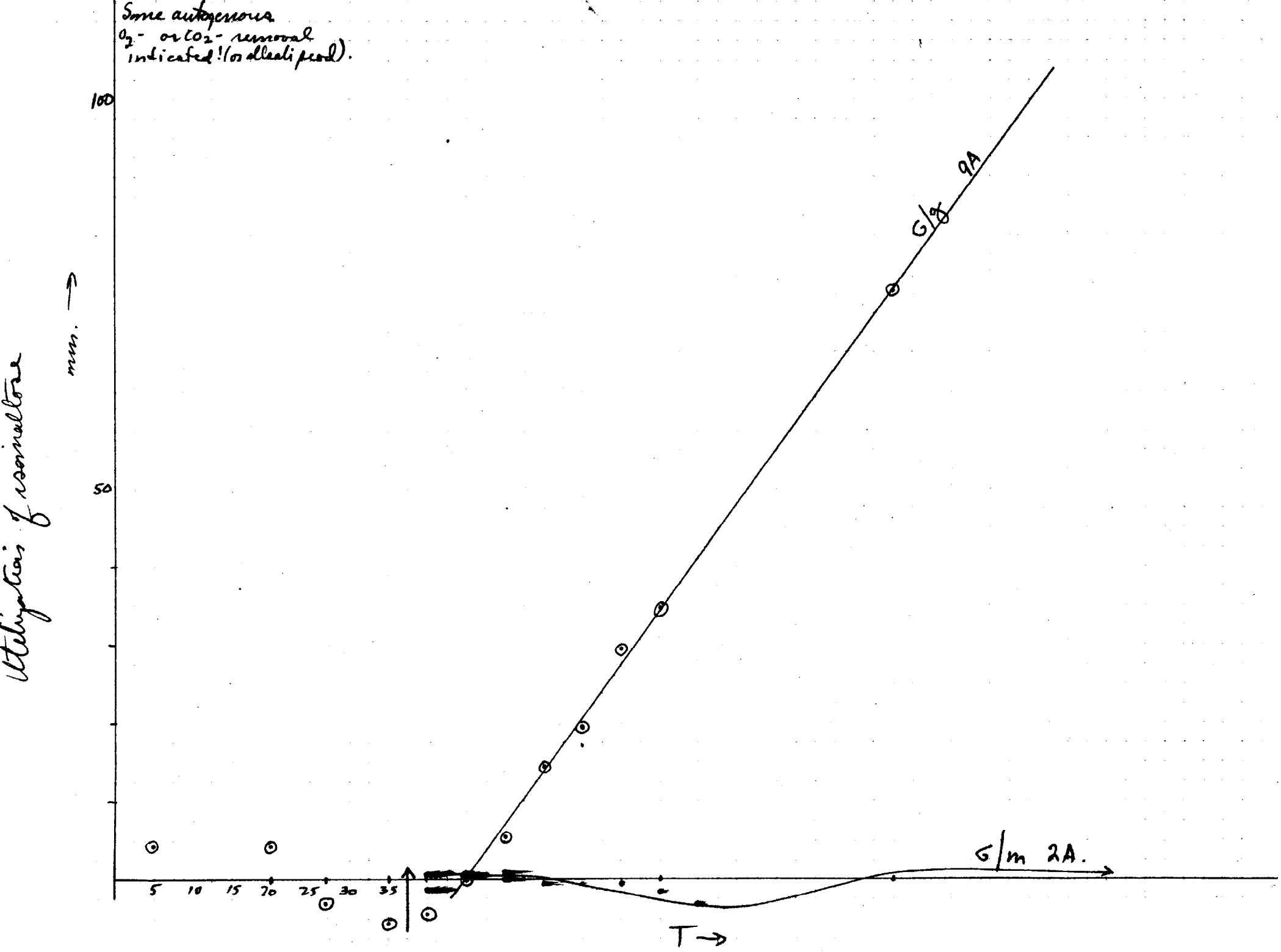
8A M, m

10A M, isomaltase

7A M –

Isomaltose not utilized by maltose-adapted K-12!


glucose
maltose
isomaltose
glucose
maltose
isomaltose
no substrate
Substrate
Utilization of isomaltose
100
80
60
40
20
0
-20
10
20
30
40
50
60
70
80
1:50
2:10
2:20
2:25
2:30
2:40
2:50
3:00
4:00
5:00


Some autogenous
O_2- or CO_2- removal
indicated! (or alkali prod).
100
50
mm. →
Utilization of resonator
G/g 9A
G/m 2A.
5
10
15
20
25
30
35
T →

Cross adaptations.

September 8, 1949

		11A 1	16A 2	5A 3	8A 4	13A 5	12A 6	8A 7	T
		T/Tr	T/gl	T/mal	T/ar	D/gal	D/ar	D/—	
11 50	0	17	9	18	19	43	18	8	151
11 55	5	13 -4	5 -4	14 -4	16 -3	40	17	9	151
→									
12 01	11	20 3	23 14	21 3	17 -2	46	9	4	151
12 05	15	33 16	56 47	25 7	21 +2	66	15	4	151
12 10	20	43 26	86 77	22 4	19 0	82	13	7	151
12 15	25	54 37	118 109	19 1	18 -1	100	13	7	151
12 20	30	73 56	160 151	25 7	26 +7	121	22	14	151
12 25	35	93 76	202 193	32 14	35 16	131	27	17	151
		X	X			X			
12 48				38 20	41 27		29	16	151
1 12				51 32	72 52		36	14	152 +1
1 33				51 31	70 56		36	14	153 +2
2 20				70 43	152 128		46	19	160 +9
3 17				92 56	281		55	23	169 -18
				×					

stopped stock

K12 grown overn. in YP Trehalose ½% (T) or Galactose 1% (D).

Test on maltose, glucose, trehalose, and arabinose

Cells 5 ×, 2 ml in $NaHCO_3$ M/20 NaP M/1000 sat CO_2 32°.

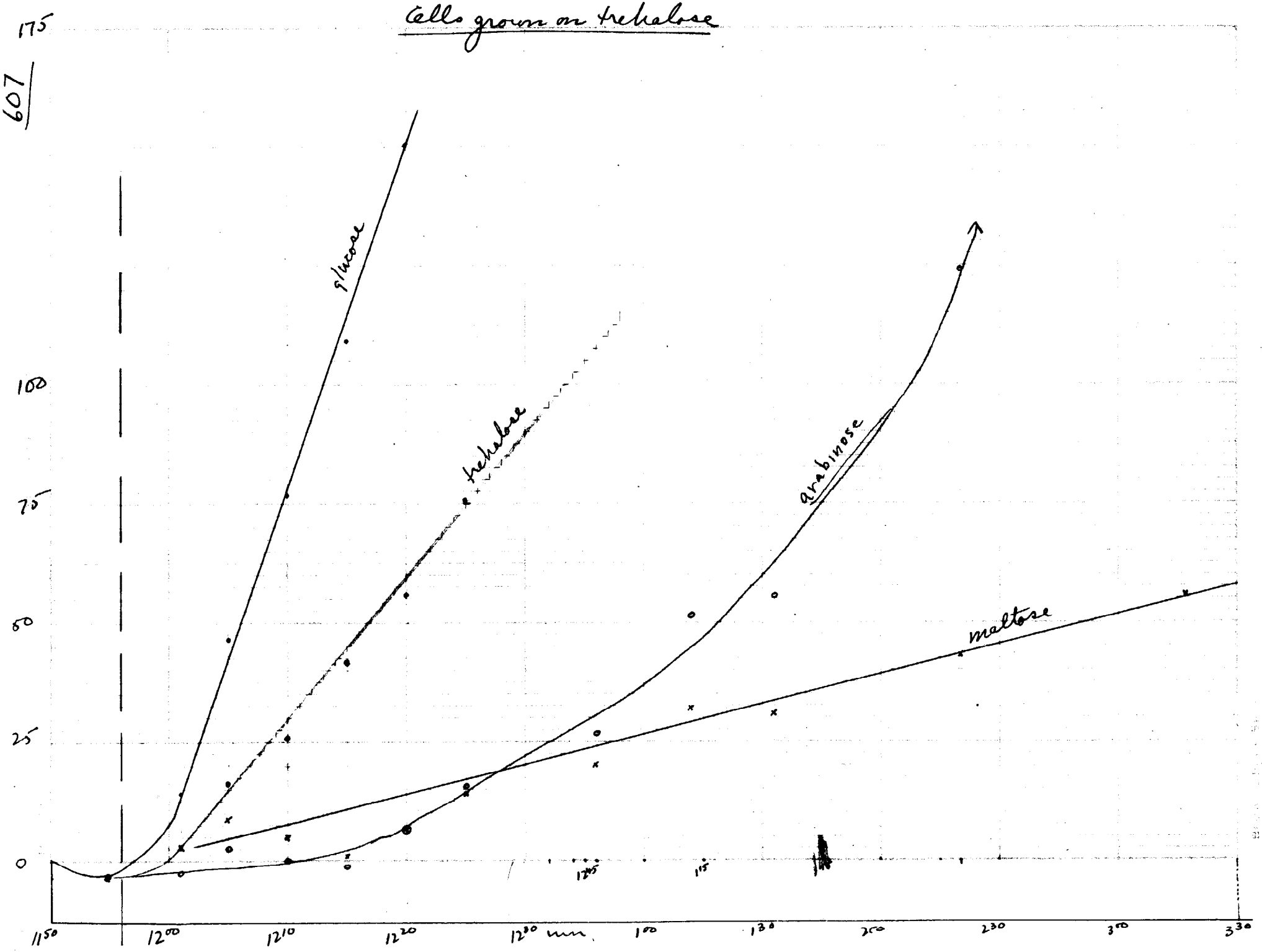
.1 ml 10% sugar at →

trehalose // maltose. Need autoferment. control.

Note rapid adaptation to arabinose (30 minutes)

Cells grown on trehalose

607



Trehalase in maltose-adapted 607'

		glucose 4	maltose 8	trehalose 10	Thermo
Inol cells in $NaHCO_3$ + CO_2	9 10	27	08	10	148
M/20	9 15	23	06	09	146
K12 / Tip Sub.		*			
maltose	9 20	20	09	16	149
32°	9 26	38 +18	29 +20	21 +5	149 0
	9 30	54 +15	43 +13	22 0	150 -1
*Bubble later autom.	9 36	79 +26	71 +29	21 0	149 +1
	9 40	97 +16	91 +18	23 0	151 -2
	9 45	118 +23	116 +27	25 +3	150 +1
	9 50	137 +19	140 +26	27 +2	150 -1
	10 05			32 +3	152 -2
	11 02			60 +26	154 -2
	11 07			61 +1	154
	24m.	99 98	109	04	
	hour			26	

Arsenate inhibition of galactose fermentation.

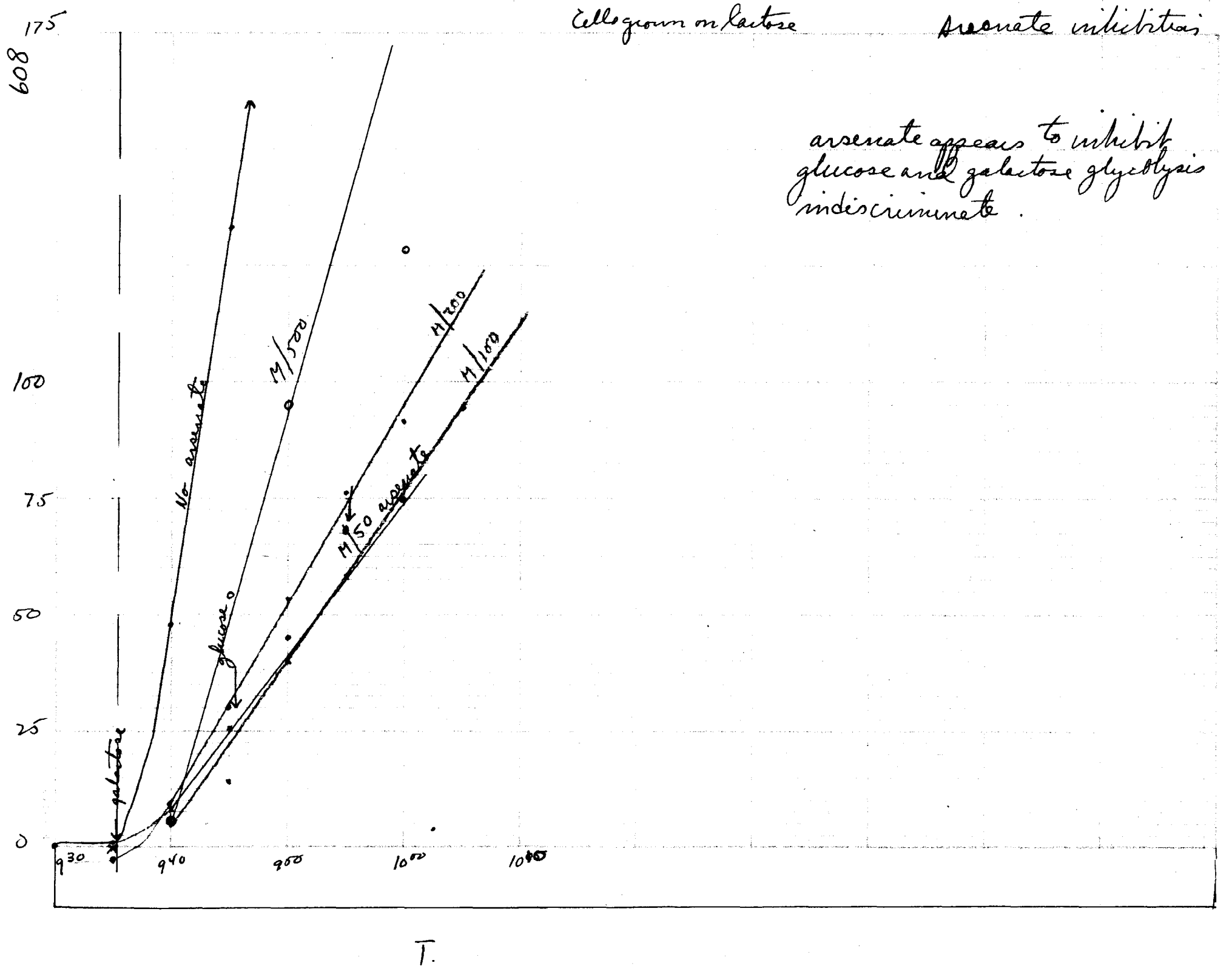
September 9, 1949

K12/Lac. 10mg gal in one sidearm; 10mg glu in 2d.
2ml diluted cells from exp., in $NaHCO_3$ M/20 - NaP M/1000 / CO_2 32°.

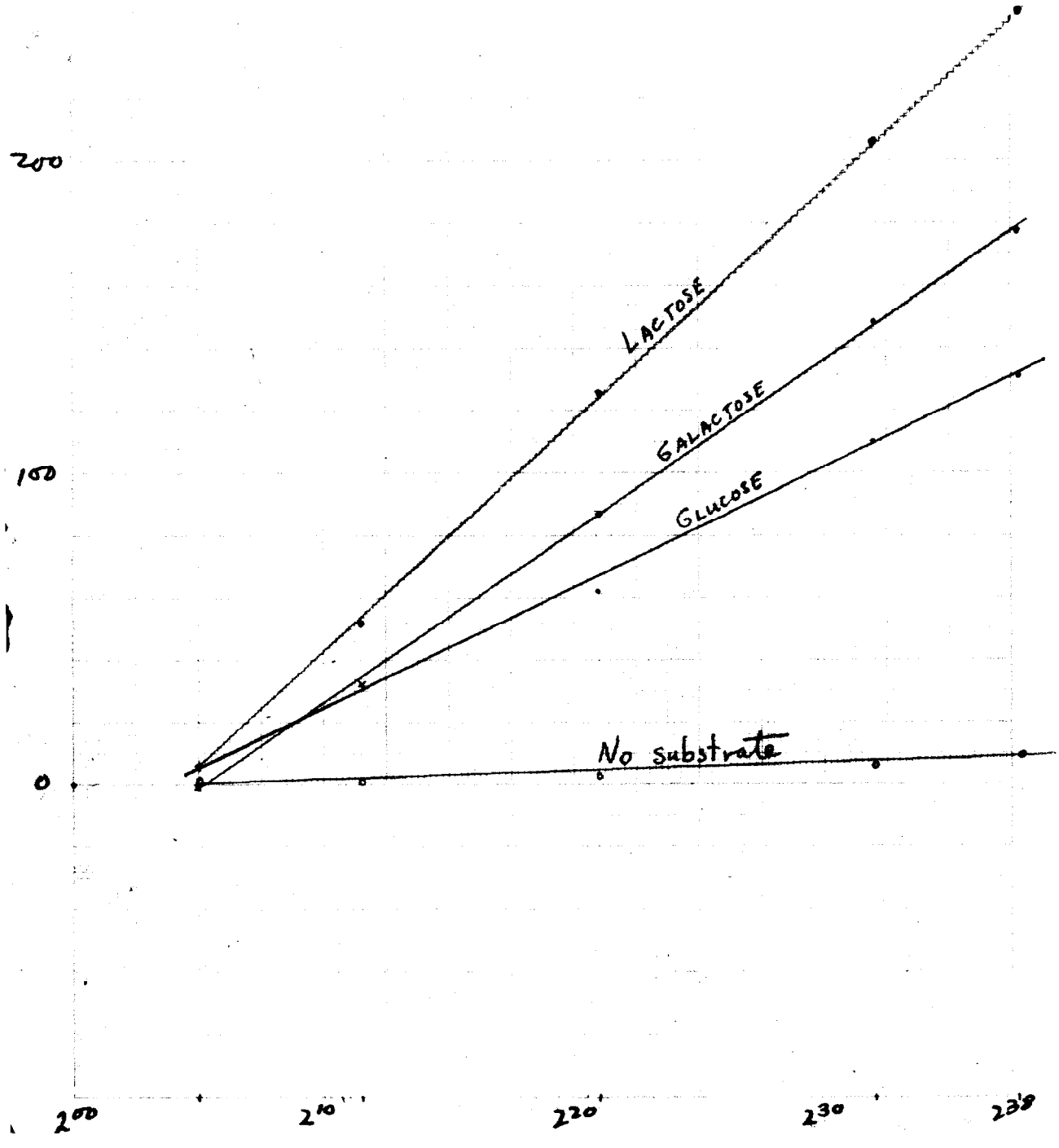
$KAsH_2PO_4$	—		M/50		M/100		M/200		M/500			T
	1	9A	2	7A	3	4A	4	2A	5	10A		T
930	47		18		16		30		03			151
935	47	+1	17	0	12	16	27	-2	02	0		150 +1
→ galactose												
940	95	47	27	8	22	5	39	8	10	6		152 -1
945	181	133	45	26	41	14	61	30	58	54		152 -1
	X		→ glucose									
950			58	40	61	45	83	53	98	95		151 0
955			75	58	79	67	105	76	130	128		150 +1
			→ glu									
1000			89		88	74	119	91	149	148		149 +2
1005			111		112	96	X		X			149 +2

Cells grown on lactose

Arsenate inhibition



arsenate appears to inhibit glucose and galactose glycolysis indiscriminate.


200
100
0
LACTOSE
GALACTOSE
GLUCOSE
No substrate
200
210
220
230
238

W251a Lactose utilization 609a.

9/13/49

See

Equilibrate ca 1½ hrs!

	glucose		galactose		lactose		/		
	1	1B	2	8B	3	3B	4	5B	T
200	06		04		01		09		108
2 05	20	5	12	-1	16	6	19	1	117 -9
2 11 30	43	32	41	32	58	52	15	1	113 -5
2 21	80	62	101	87	137	126	22	3	118 -10
2 32	125	110	161	148	216	206	24	6	117 -9
2 38	143	131	187	177	249	242	24	9	114 -6

Streak out 1B: ca 30% Glu+!

Streak out culture 1

9/11/49

1.5 mC cells 1 ml 10% sugars $NaHCO_3$ 7/20 NaP 7/1000

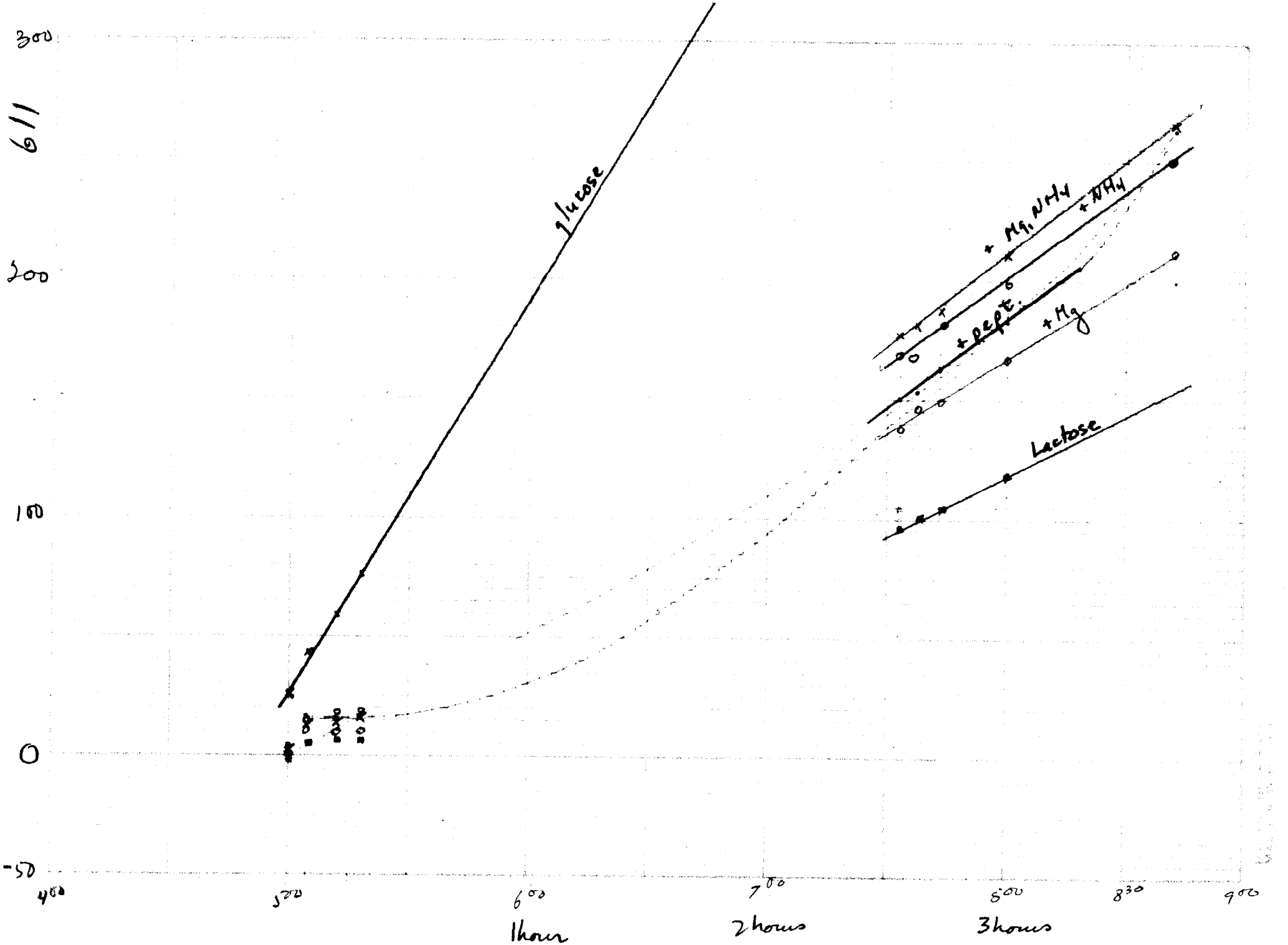
Cells were grown in 50 ml x2 lac overnight c̄ aeration. However, the medium evaporated to ca 15 ml. This may acct. for the poor lactose activity seen here.

glucose galactose (glycerol) lactose / methyl gal

	1 5B	2 2B	3 6B	4 4B	T
1240	-2	18	-3	16	147
1245	-1	23	04	27 (153)	147
1246	05	28	08	27	153
1250	10	32	12	31	157
1255	04	28	08	25	151
100	04	30	10	20	148
110	14	40	19	31	153
115	22	48	25	32	150
121	28	52	27	29 (151)	154
131	41	69	42	35	156
150	62	84	51	27	152
205	89	114	68	29	152

$\alpha^{CO_2}_{32°}$ = ca .63 Subtract

		Volume	$k_{0\,ml\,32°}$	1ml	2ml					
	1									
	2	21.12	1.88							
	3	19.51	1.74							
	4	20.19	1.80							
	5	19.97	1.78							
	6	18.20	1.62							
A	7	18.43	1.64							
	8	18.99	1.69							
	9	19.02	1.69							
	10	18.44	1.64							
	11	19.60	1.75							
	12	18.86	1.68							
	13	19.61	1.74							
	14	18.26	1.63							
	1	19.81	1.76							
1.82	2	19.88	1.77							
	3	20.45	1.82							
	4	20.85	1.86							
	5	19.85	1.77							
	6	18.95	1.69							
	7	20.47	1.82							
	8	19.67	1.75							
	9	18.86	1.68							
	10	19.62	1.75							
	T	19.11	1.70							
				subtract .0891	subtract .178					


300
200
100
0
-50
400
500
600
700
800
830
900
1hour
2hours
3hours
glucose
+ Mg, NH4
+ NH4
+ pept.
+ Mg
Lactose

K12 / maltose
Lactose adaptation

September 14, 1949

	Lac + pep.	Lac + NH_4	Lac + Mg	Lac + Mg + NH_4	Lac	Glucose	/	Peptone	
	1 10A	2 11A	3 5A	4 7A	5 2A	6 13A	7 12A	8 4A	T
	16	16	16	16	(25)	(15)	(15)	(15)	(16)
445	21	28	17	32	53	10	28	45	161
→	LAC	LAC	LAC	LAC	LAC	GLU	—	PEP	
450									
500	26 0	37 4	22 0	40 3	57 -1	40 25	33 -5	69	161
505	30 17	36 16	19 10	36 12	50 +5	45 43	20 +8	61	161
510	33 16	42 18	23 10	41 13	55 6	65 59	24 +4	64	161
518	33 16	43 19	23 10	43 15	55 6	82 76	24 +4	65	161
733	174 150	200 169	158 138	212 177	152 96	199 [0=]	36 -3	76	161
737½	179 153	201 168	163 146	217 180	158 100	213	33 -5	76	161

	I R	II L	
1.	Lactose	Glu	Peptone .5%
2.	Lactose	Glu	$(NH_4)_2SO_4$ 1%
3.	Lactose	Glu	$MgSO_4$ M/100
4.	Lactose	Glu	$(NH_4)_2SO_4$ + $MgSO_4$ M/100.
5	Lactose	Glu	
6.	~~Lactose~~	~~Glu~~ Glu	
7	—	Glu	
8	—	Peptone	

K12 grown on maltose, aerated.

Conditions

743	183 163	208 181	165 149	218 187	156 104	225	27 +1	70		161		
800	~~168~~ 209 (183)	~~210~~ 231 (198)	188 166	247 210	176 118	x	33 -5	81	(173)	165	-4	
841	297 263	291 250	241 211	310 265	×		41 -13	87		168	-7	

ONPG ✓ ✓
ethylbenzene ++ ±?

September 23, 1949

5^40		D_i	$D^{ONPG}_{10MIN.}$	Δ'
~~M1~~	~~L1~~ water	007	438	384
~~M2~~	~~L2~~ glucose	002	217	166
~~M3~~	~~L3~~ lactose	0	165	116 !
~~M4~~	~~L4~~ water	001	072	022
	M2 glucose	001	058	008
	M3 lactose	0	071	022
	M4 Mgr + lactose	0	074	025
—		007	042 = 49	0

Cell density L 19.9
M 13.3

Cells incubated from 3^30 PM in indicated supplement:
1 ml cells
.2 ml 1% sugar
.1 ml KP buffer pH 7.0 M/5.
(4) + .1 ml $MgSO_4$ M/5.

8^00 vs ONPG blank

	D_i	D^{ONPG}_{10M}
L1		438
L2		125
L3		54
M1		28
M2		10
M3		32
M4		28

mg/ml

K12 / mal and / lac

showing decrement of activity when incubated with lactose or glucose!

Consistency of benzene activation of galactosidase

September 24, 1949.

2PM

A) 5 tubes each receive 1 ml K12/bac. #6 the same, dil. 1:10.
Add 1 ml benzene/tube.

5PM add 9 ml H2O to 1-5. (#2 mix ab.).
Assay. 2 ml samples.

	Di	D
1	010	400 sic!
2		157
3		173
4		163
5		172
6		205.

Too crude to be used in present stage of development.

Effect of N-supply on lactase deadaptation.

September 24, 1949.

12 hour cells aerated, not washed

Harvest K-12 from Y2 Mal and Y2 Lac.
Add NaP 7.5 to M/50. 1ml cells + 1ml supplement
incubate from 12 50 to 3 50 PM = 3 hours. 37°
Add. 1ml benzene to activate. cell density (~~after~~ before 1:1)
onpg M/2000 in M/100 NaP 7.5 37°. 6.7 mms.

	Suppl.	Di	10 m. Donpg	Δ'
1	Y2	029	387	
2	Y2 Lac	027	590	
3	Lac 1%	028	217	
4	H_2O	024	236	?
5	Lac .2% + $(NH_4)_2SO_4$ M/10 .1ml	022	[illegible]	
6	$(NH_4)_2SO_4$ M/10 .1ml	023	264	
7	Lac .2 %	022	36[illegible]	
[illegible]	—	003	011	014
[illegible]				

M/1 Na_2CO_3 1ml added

W251a/ Lac

	T	1 5A	2 2A	3 4A	4 13A	5 3A	6 12A	7 6B	8 10A	9 9A
1 50	155-53	30	22	32	44	39	33	57	46	45
1 55	154-48	32	24	34	43	35	29	51	41	38
2 00	157-53	32	24	34	45	38	32	56	45	43
201 →										
205 →	158-54	30	24	33	44	36	33	57	45	43
2 10	157-48	28	24	32	42	35	34	57	45	40
2 15	158-53	28	22	31	41	34	36	59	49	42
2 20	150-58	27	21	32	44	38	39	66	60	49
2 25	150-59	30	26	34	46	44	53	74	67	51
2 30	161-60	35	30	39	53	51	63	82	73	52
2 35	161-61	48	31	40	53	51	63	80	75	52
2 40	154-58	44	39	48	64	62	76	88	81	51
2 45	157-60	51	46	53	67	66	80	90	85	51
2 50	162-58	57	48	54	70	68	84	93	89	51
2 55	162-56	60	50	55	71	71	89	96	92	49
3 00	161-57	62	51	57	73	75	93	100	97	50
3 10	162-55	62	52	57	71	80	101	104	103	47
3 20	158-55	70	53	58	79	84	106	107	108	46
3 30	160-52	75	57	62	83	90	116	113	116	44
4 00	155-60	90	71	73	100	119	150	141	148	55
4 30	155-58	102	76	75	104	128	164	150	161	50
	1 LAC	1 mg.								
	2 GLU+GAL	.5 ea.								
	3 GLU	.5								
	4 GAL	.5								
	5 LAC	10								
	6 GLU+GAL	5 ea								
	7 GLU	5								
	8 GAL	5								
	9 —									
530	163	127	108	99	135	182	219	191	209	155
	1 ml cells	W251a/Lac	Aer.	in	$NaHCO_3$	M/20		.05 - .10	in cultures.	
645	159	127	127	110	137					55

** streaked out on EMB glucose: essentially pure Glu -! (>99%-) But note overall slow fermentation. Culture may have gone too acid.

Gal'ase activity in unadapted cells.

Sept. 30, 1949.

Harvest K-12 from 12 hour aer. 42 — 50 ml. Conc. to 5 ml (10 x)
Leave water suspension on table top 10A — 730 P 30.

1 ml aliquots incubated c̄ benzene 7^30 – 9^00 PM (90 mins.)
Test samples per standard ONPG (M/2000 mpg; 20 mins; 37°; Na_2P 7.5 M/50

Untreated samples: (.1 ml / 10)	Di	Dampg	R.A./ml	R.A./ml / Di,100 / 10^3
K / Lac	250	800 (12 min.)	94	38 / 100
K / Mal	307	475	19	6.2 / 17
K / Glu	118	119	0.2±	.0.2 / 1±

TREATED					
(.01 ml)	K / Lac	017	540 (7 min.)	1.5×10^3	.58 / 100
(.01 ml)	K / Mal	027	380	$.36 \times 10^3$	.12 / 21
(.1 ml)	K / Glu	070	269	$.02 \times 10^3$	.02 / 3

Activation of ca $\frac{1500}{94} = 16 \times$ fairly consistent here, but 1½ h. may not provide maximal activation with benzene.

Lactase is present in glucose and especially in maltose-adapted cells.

Gal'ase activation in K12
Octyl alcohol, thymol, benzene

Oct. 1, 1949.

Harvest K-12 12 hr. acc. 42/— 50 ml. Wash 2x and conc. 10x.

1 ml aliquots to small tubes and incubate i gmin reagent.
Assay standard onpg.

Intact Cells.

			Ainit.	Di	O^{20} onpg	R.A./[redacted]/Di=100		
A.	1	/Lac	.025	043	140	210		(100)
	2	/Mal	.1	140	167	21		10
	3	/Glu	.2	141	130			
no activation	4	/Lac	.1	054	218	293		100
" "	5	/Mal	.2	129	193	51		17
Benzene treated 2hrs?	1	Lac	.01	007	310	RA/ml 292	RA/ml/Di 171	~~(100)~~ (100)
	2	Mal	.01	007	046	29	21	~~(10)~~ (12)
	3	Glu	.1	040	062	1.5		~~(1)~~ (2)
	4	an. Lac	.01	0	169	158	292	(100)
	5	an. Mal	.01	0	072	61	43	(17)
Octyl alc.	1	Lac	.01	007	418 (11 min.)	750		

Note superiority of octyl alcohol activation.

P1. .5 ml aliquots. At time add 4.5 ml H_2O for $\frac{1}{10}$. Take 1 ml assts = .01 ml (exc. 3)
2 hour treatments (430 - 630).

		Di	Donpg	R.A./Di	
Octyl Alc (.1 ml)	1	012	268 (5)	573	(100)
	2	003	061	34	6
	3	043	052	2	<1
	4	001	367	657	(100)
	5	-002	110	85	13
Benzene	1	012	230 (5)	484	(100)
	2	005	061	32	7
	3	042	056	5	1
	4	-001	230	418	(100)
	5	-001	073	49	12
Thymol 1 crystal	1	009	419 (5)	932	

Octyl alc. > Benzene
Thymol >> Octyl alcohol.

Test OcOH, Thymol for partition of NO_2 OOH at pH .75.

		O^{onp} M/50,000
		100
octyl alc	1:20	88
thymol		99.

Negligible diff. even if carried over

Kinetics of thymol activation
Gal'ase in W842. (test).

Oct. 2, 1949.

K/Lac of 10/1/49. A) 1ml B) 5ml in 10ml cent. tube
unshaken, 37°.

Add a crystal (10-20mg) of thymol at 4 15 PM. .005 ml samples

C = Phenol. 1ml start at 4 30

T.	Mins.		Dongq.
4 20	5	A	No visible color
		B	
4 40	25	A	251
		B	103
5 00	45	A	444
		B	126
	(30 MIN)	C	132
~~7 40~~	~~190~~		
8 00	215		
		A	> 1000
		B	650
	(185)	C	231

Note: slow process.
Needs >> 1 hr.

Some evaporation possible.

P2. Hawaii as usual W842/Mal; W842/Lac K-12/Lac.

		Bmt	Di	Dongq	RA
Cells.	K/L	.02	052	149	178
	W/L	.05	172	150	—
	W/M	.05	130	109	—
Thymol	K/L	.005	004	670	655
	W/L	.005	019	018	0 !
	W/M	.005			

Activation = $\frac{655}{178} \times \frac{5}{2}$ = 9.2 fold

No activity!

Consistency of Gal'ase activation by thymol, octyl alcohol.

PM

1: .5 ml susp.

~~2: .5 ml " + 4.5 ml H_2O~~

3: .5 ml " 4.5 " "

4: .5 ml ($\frac{1}{10}$) + 4.5 ml "

all in duplicate

A thymol

B octanol

C. enzyme

$\boxed{1/2 \text{ hour tests}}$

8:30 Make up to 10 ml (exc. 3)

Test .1 ml samples 1, 2; .5 ml of 3 (≡)

Neglect D: (.007 ± .003). Add Na_2CO_3 to terminate Rx.

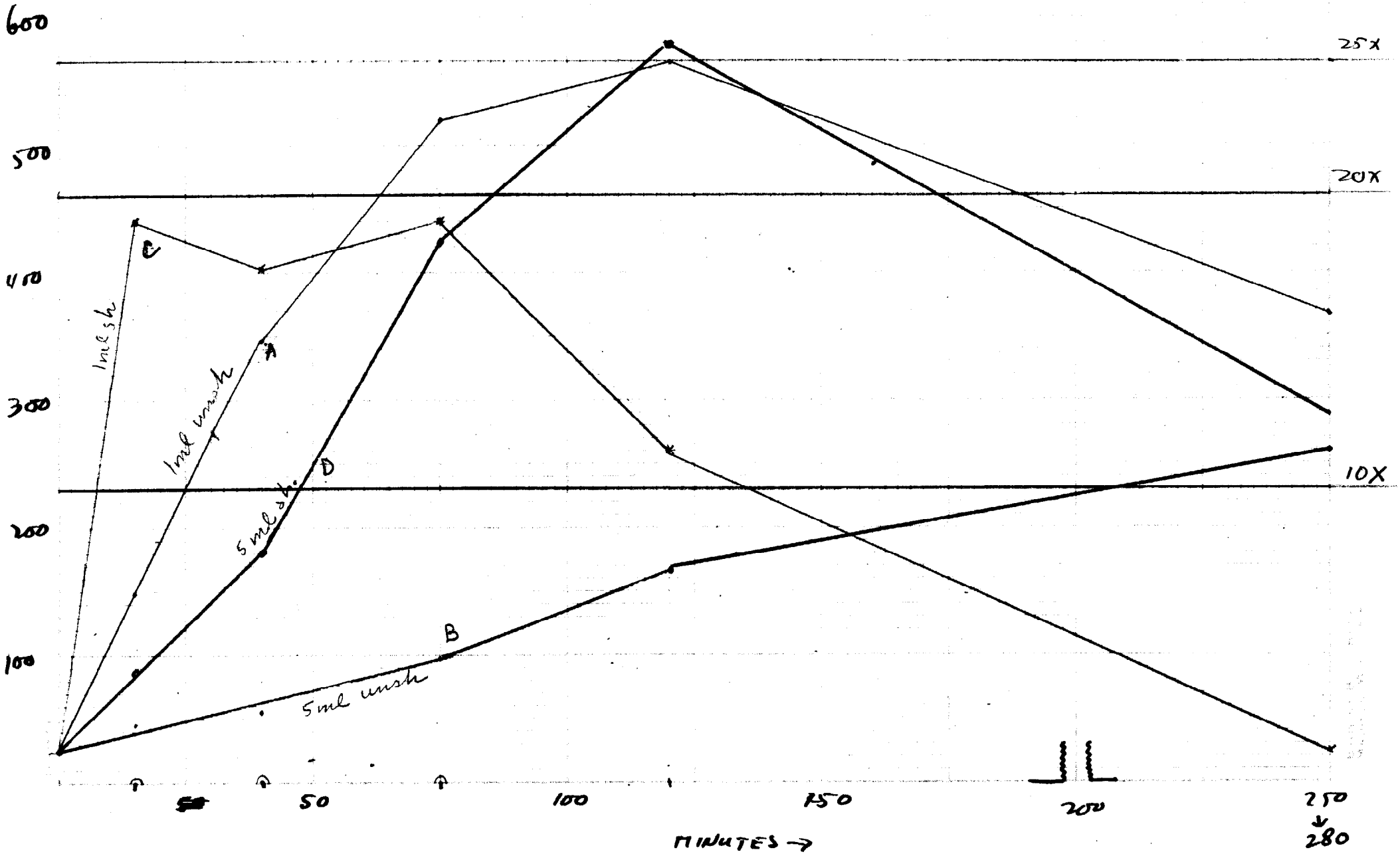
	A(Thy)	B(Oc)	C(B_2)
1	318	131	200
1	359	118	171
2	062	054	054
2	060	057	062
3	082	069	067
3	093	053	064

Time may have been insufficient for complete activation! Thymol seems to act most rapidly. [Try Phenol, other ϕ-OH]

Reassay 1, 3 4P2.

A: ~~4~~ 1>>3.

Kinetics of gel'ase activation by thymol



Kinetics of Gal'ase activation. Effect of shaking

10/3/49.

Harvest aer. K/Lac conc 50/20. H_2O. Add thymol: 3^{PM}_{20}.

A) 1 ml unsh. B) 5 ml unsh. c) 1 ml sh d) 5 ml sh.

Remove .1 ml samples from time to time; dilute in water 10 ml and assay. Terminate = Na_2CO_3, exc. intact cells mid ante P

		MINS	Di	D^{20}_{onpg}	
3^{36} T		15			
Intact .1 ml		0	089	318	
.01 ml			003		23
1	A	15		149	
1	B			46	
1	C			~~460~~	(440)
1	D			87	
4^{00}		40			
1/2	A			173x2	= 346
1	B			53	
1/2	C			201 x 2	= 402 (!) 10% < above!
1	D			180	
1/2	A	75		260 x2	520
	B			98	
1/2	C			220 x2	440
	D			423	
1	A			281 x2	562
1	B	120		83	166
1	C			120	240
1	D			289 x2	578
	A			369	
	B			260	
	C			025	
	D			289	

mul 1/100 dil

4^{36}

5^{20}

8^{00}

2 hours optimum for unshaken cultures.

2½h. 1 ml. treated 5^{45} – 8^{15} test + compare:

	2½h.	18h.
thymol	479	178
phenol	016	
amyne	466	685
octanol	369	222

Repeat overnight.

Gal'ase of adapted + unadapted cells; Lac, -

674a

October 5, 1949:

a) W112 harvested from Y2 Lac; Y2 Mal; K-12/Lac. as above.

		Di	Donpg	
(1) Intact cells.	.01/ml			
	K/L	131	710	
	W/L	98	—	
	W/M	124	—	
(2) Benzene 24 hours.	K/L .01	006	590	(12.5 min; Na_2CO_3)
	W/L .1	~~073~~ 068	261	
	W/M .1	~~068~~ 073	092	

b) K12 from Y2 Lac; Mal; Gly. Ser. [Benzene from 12N + I. ca 8 hours.

					Δ cor		RA	u/mg
Intact:	K/L .1	130	520		392		3.02	14
	K/M .1	129	151		24			0.9
	K/G .2	204	182		— 0			0
	—	— 007	± 004	Correction = +11				
Benzene	K/L .005	— 004	410		403	x20	~~3~~ 62	297
	K/M .01	+ 004	074		59	x10	4.6	22
	K/G .1	074	060		—		—	

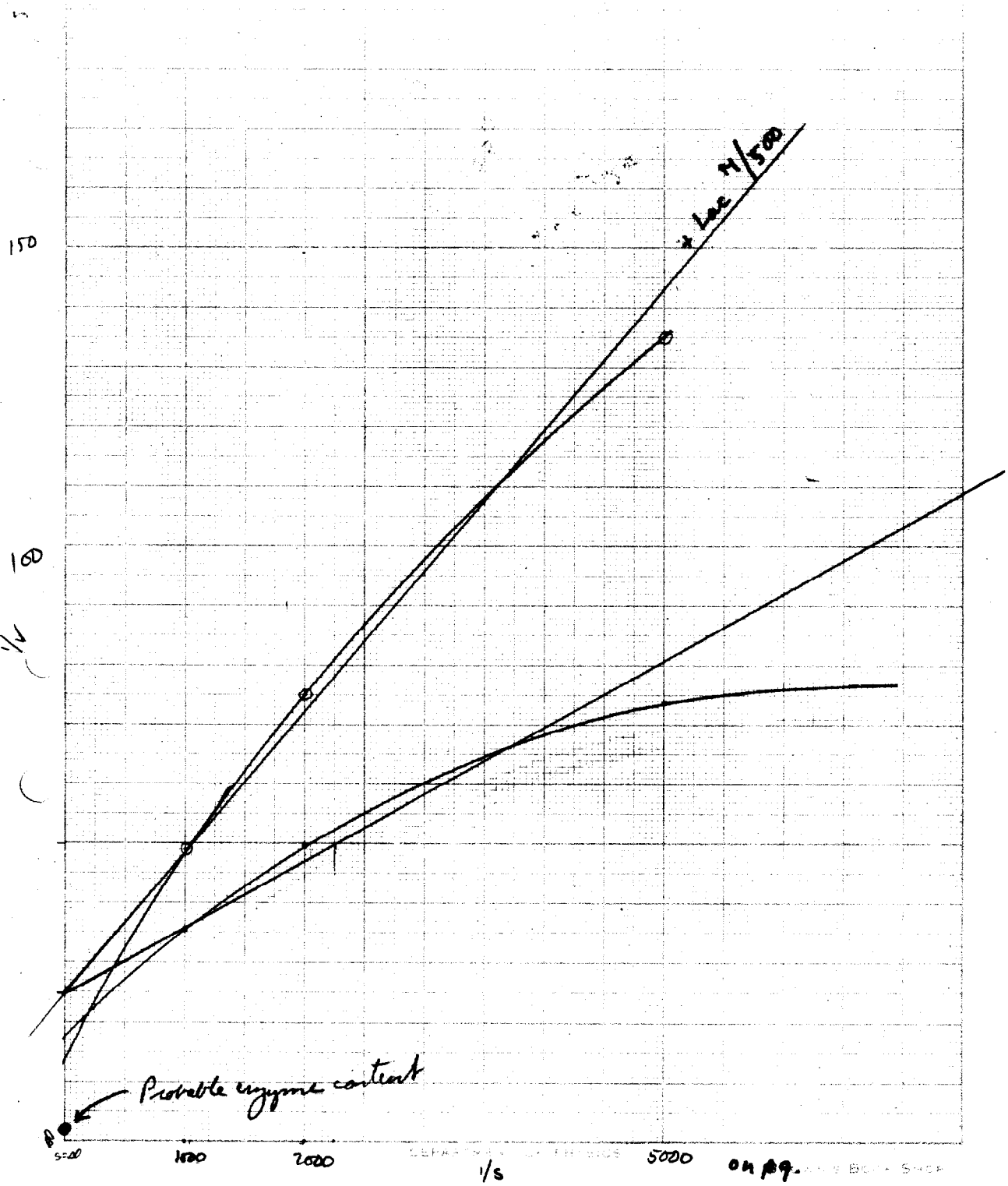
		I	Di	Donpg
Instant	L	.05	087	246
	M	.1	157	189
	G	.1	130	124
	—	.1	214	250
B2 4h.	L	.005	0	530
	M	.02	018	194
	G	.1	069	074 (5:35 PM)
	—	.02	032	267

K12 harvested from yeast - peptone (YP) / sugars. 50ml/10ml.

	Di	Donpq		A	R.A.	u/mg
Lac	173	408		231	134	6.4
Mal	181	177	182 40m.	8 40	2	0.1
—	122	125	131	10	4	0.2
		maybe inaccurate				

			A'	R.A./Di	R.A.	R.A./Lac	u/mg
Lac 005	005	212 6MINS.	196	380		100	~~408~~ 300
Mal "	104	174 20M	69	38		10	1.8
— "	080	141 "	58	47		12	2.3

Activation $\frac{20 \times 196 \times 3\frac{1}{3}}{231}$. 57x !!


200
150
100
1/v
+ Lac M/500
Probable enzyme content
s=∞
1000
2000
5000
1/s

Kinetics of Gal'ase in intact cells.

Oct. 7, 1949.

K-12 harvested from LacY2

Konpg and Klac.

M/100 NaP

	M onpg	lac	Di	Na_2CO_3 Donpg		1/V
cells	100			352	282	35.5
	200			274	202	49.5
	500			183	136	73.5
cells	100	M/500		274	204	49.0
	200	500		185	133	75
	500	500		121	74	135
cells		—	089	050	047	
no cells.	100			020		
	200			002		
	500.			-003		

Graph calc: $V_{max} = 1/25 = \underline{\underline{400}}$

$K_{onpg} = M/2000 = 5 \times 10^{-4}$ M ✓ prev. meas.

$K_{Lac} = [Lac] = 2 \times 10^{-3}$

Note: In extracts + cells, of: (K_s): ($\times 10^{-4}$)

	cell	ex
onpg	5	1.3
lac	20	14

i.e., transport blocks to lac << onpg. But still note that the 1/s : 1/v plots do not extrapolate to the full Vmax for extracts! Possibility of bending inds to be restricted.