B-phenyl galactoside.

Nov. 10, 1947.

Sample from E E Snell (2 grams).

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

		galactose (A)		lactose (B)		B-φ galactoside. (C)		B-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	B-φ gal	B-φ gal + gal.	
1	++	++	++ ✓	++	
2	++	++	− ✓	++	lac + cells present
3					
4	++	++	− ✓	++	
5	++	++	− ✓	++	
6	++	++	+± ✓	++	
7	++	++	++ ✓	++	
8	++	++	−	++	

Note that none of these cultures originally lac− have grown on B φ galac.

Considerable pigment produced on galactose

Nov 15 1947

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

			A(Galactose .05%)	B (β-ΦGalactoside)	C Galactose +Phenol .02%
TIME::::		Inoculum	SP16	SP16	SP16.
1	58 gal	1a	+++	++	++
2	161 lac	1b	+++	++	++
3	Φβ	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 & 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.

Needs checking & 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+/Bϕ-" is W53. While Y53 is Lac- Bϕ+.

Note: Lac+ Lac-

Bϕ+ Y10 Y53, W-1.

Bϕ- W53 W45, -49.

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

W49 × Y10 Lac- Bϕ- × Lac+ Bϕ+.

Action of all mixtures on lactose.

Trehalose / Maltose Cross adaptation. pulcini.

Dec. 10, 1947.

Prepare 10% suspensions of

a. Y40 Lac+

b. W-1 Lac_1-

c. W-45 Lac_2-

Inc. 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 7.0 added.)

		6P9	9A10	P10	Set up. 3:45 P9
1.	a	–	+++, +++		
2.	b.	–	–		
3.	c.	–	–		
4.	a+b	–	+++, +++		
5.	a+c	–	+++, +++		
6.	b+c.	–	–, –		
a	glycose	+++	+++		
c	glycose	++	+++		

Mixtures of Lac_1- and Lac_2- therefore cannot ferment lactose.

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

Dec. 11.

For 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 on
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 2×10^{-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 (3:20)		21.h.	Lactpse			21.h.-(pH.
1. Azide M/100 X	3:40PM.	6:00PM	9A20.	3:40	6:00PM.	7:00PM	
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							7.37

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

At the initial pH the ratio is ca. 1.6 : 1 There are altogether 10 μM phosphate. At pH 4.50, the ratio is 1:50 → i.e. all but 2% of the base is neutd., and about 6 μM H^+ have been produced (from 30 mg = $\frac{1}{6}$ mM = 167 μM 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.

Dec. 18, 1947.

W-34.
Grow in T(H2O) + .1% trehalose and glucose. No growth (±) on maltose.
Test for activity on glucose and maltose in system like Exp. 65.
Harvest 50ml & conc. to 2ml. 50/2. Set Up. 5P19.

Growth in ↓ Titration →	2h. 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 Hzide 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	±	±	±	—	±	±	—	—
10A 15 (23h.)	+++	+++	+++	±	+++	+++	+	±

Grown ↓ / Tested →	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 variants	+++
l-arabinose	+++	✓	+++
xylose	+++	✓	+++
mannitol	—	occ. var.	++
lactose	—	"	+++
maltose	—	"	+++

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

W108: Test for β-galactosidase activity 94

Jan 26, 1948

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

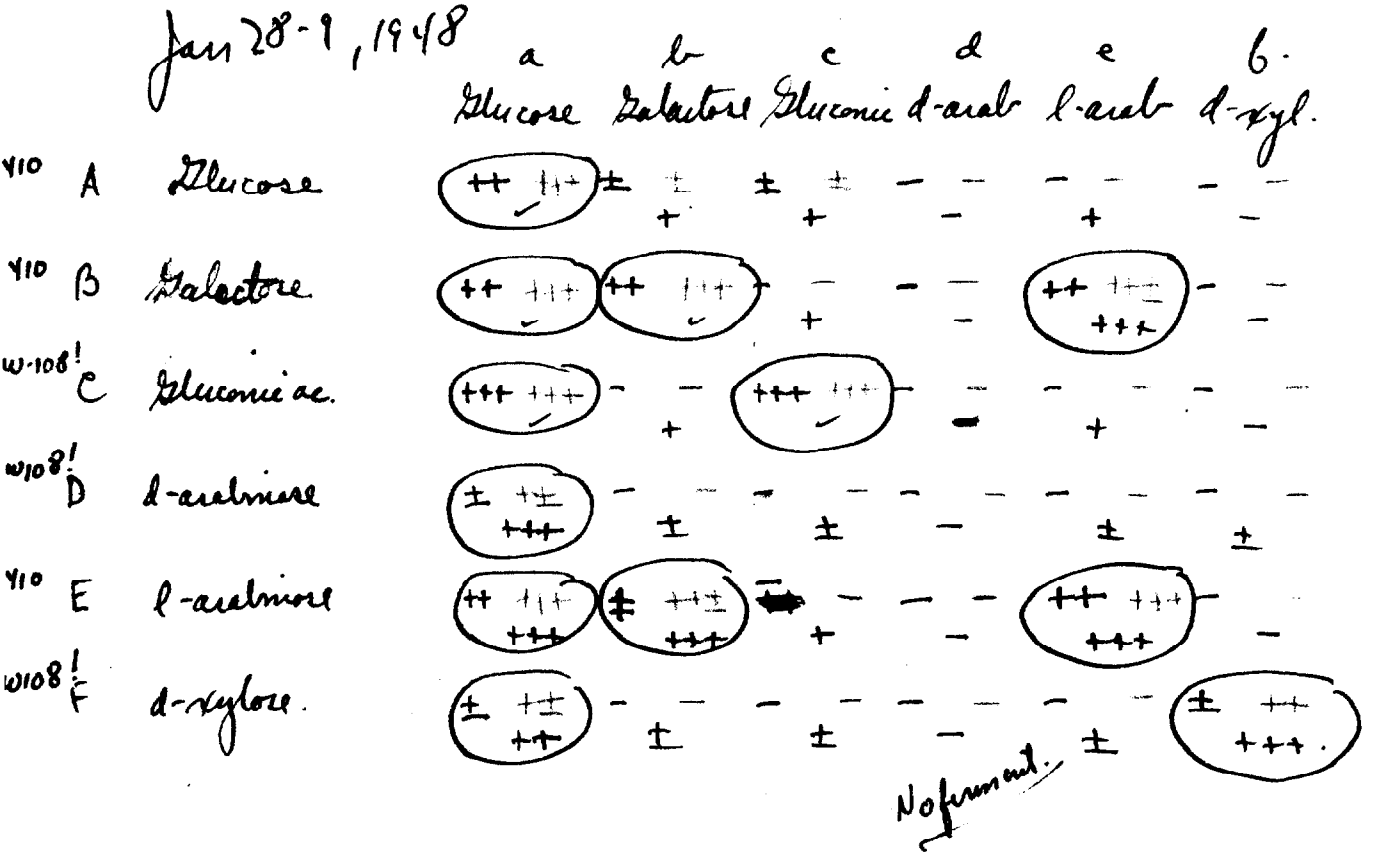
	Test 1.	
1. Blank	−	−
2. Blank medium (BΦgal)	−	−
3. ΦOH .02%	++++	++++
4. 108 a	±	+
5. 108 b	++	+
6. Y10 c	±	+
7. Y10 1	++	+
8. Y10/gluconic only.	−	−

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



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 50ml + 1% sugar. Concentrate to 7ml. Use 1 ml cells, 1ml 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	± ++ +++	± ++ +++	− − ±	± +++		may be too heavily buffered.
5. —	− + +	± − −	− −	− −		
6. W108 * Glucose	− − −	− − −	− −	− −		cells OK
7. * Galactose	± + ++	± +++ ++	− − ±	+ ++		cells OK
8. Gluconic	− 8 ±	− ±	+ +++ +++	+ −	− −	cells OK.
9. * l-Arabinose	± ± +	± ++± +++	− − ±	± +++	− − −	[illegible] galactose by W108 −
10. —	− − −	− − −	− − −	− − −		
11.						
12.						

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

30 m 2 h. 3 h.

* streak out on maltose or glucose

(1). Confirm cross-adaptation of galactose & l-arabinose

~~(2). Glucose is adaptive: Glucozymase is lacking in gluconic 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+ ↓ 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. 1/2

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

of azide (M/200). ~~Adaptation~~ is not completely inhibited by this concentration, 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 phaesphate-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

	gha	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:

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

W-108 C-source utilization. P1 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 solns. ± 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 −

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

Clarify by 5 ml 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	23.55 - 1 drop.	No glucose.
4. W327	23.69 - 23.91.	< maltose control.
5. - Phosphate.	23.91 - < 1 drop.	

Fractionation of Coli Lactase

160

March 20–22, 1948.

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

3/24/48. Test extract on lactose ē Barfoed's method:

1 ml extract, 1 ml 5% lactose + make up to 3 ml. Incubate 3h. at 37°. # cc .01N KMnO4 to equal Cu_2O ppt'd.

XL	>17 cc. (Beyond method)
X	0.23 cc
L	1.18 cc

X+L (added just before Cu ppts). 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.

micelle volume ??

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

3/22. Work in cold.

① 1 ml X + 8 ml acetone. Collect ppt + resuspend in 1 ml

② Do. ~ 95% alcohol.

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

④

⑤ 5 ml Y as ② ↑.
Heavy ppt

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

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

⑧ See 6S. Add .9 g 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 K Sediment. Boil 10 mins. Wash ppt + dissolve in Fe^{+3} and titrate with .02N $KMnO_4$.

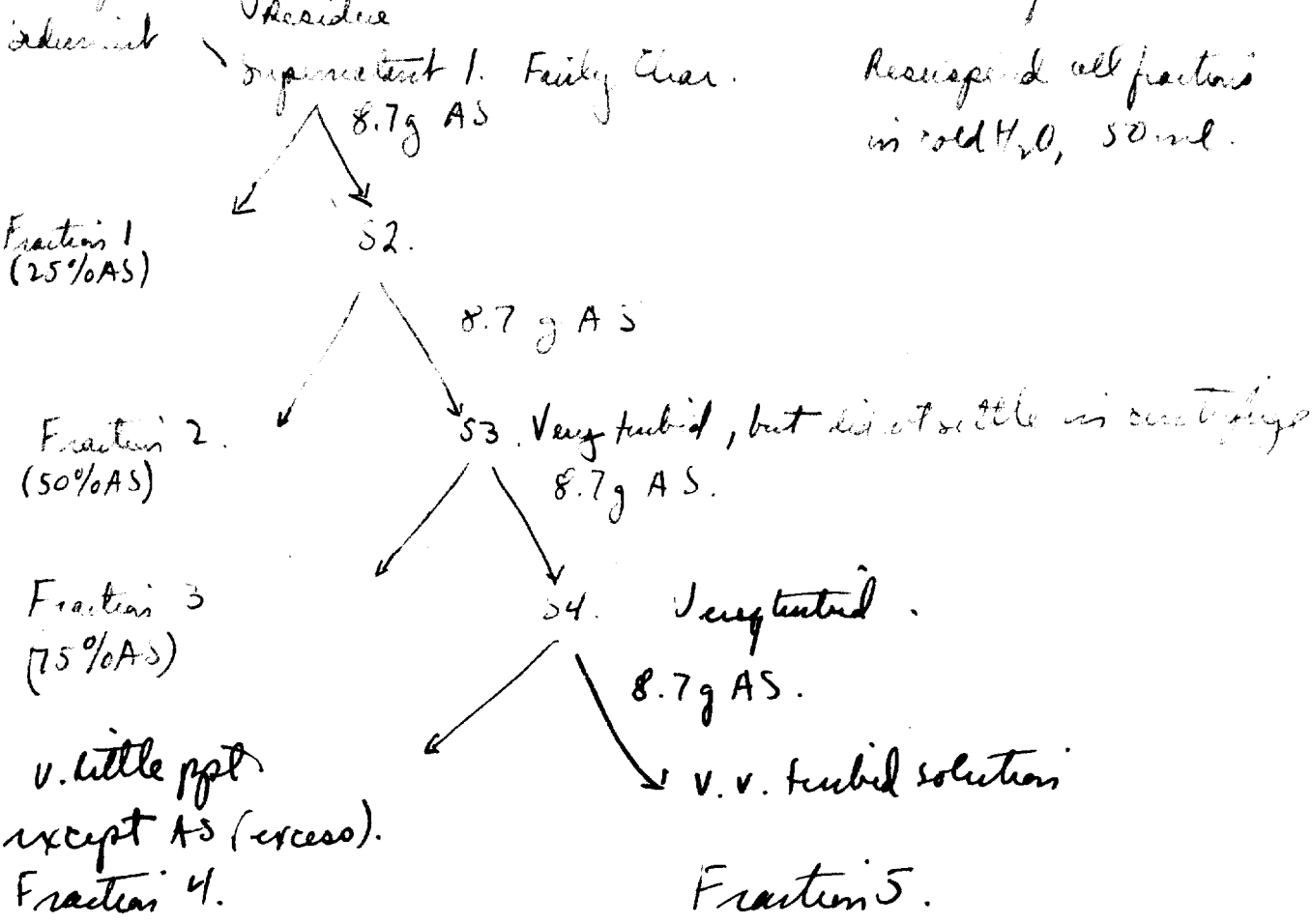
			cc:	
1. X	+++	7.4 +	8	
.5 X	+++	8.19	8	
.1 X	++	4.83	5	Note. ca 3 mg/½ hr.
.01 X	–	.40	.3	
Y.	++.	5.84	8	
1.	+++	8.42	8	Acetone
2.	++	7.20	7	(AS)
3.	++	3.10	6	(Alcohol)
5.	+...	2.67	7	Acetone
6.	–			
7.	–			
8.	–			
9.	–			
10.	–			
Glucose	++	8.98	—	
X + Glucose	+++	8.39	—	Utilization ??
Lactose.	–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

Cu_2O color + ppt roughest.

1. Autolysate active
2. Acetone powder active Alcohol powder active
3. Comes down at ½ saturation. Dry Surf.

Fractionation of W-254 lactase.

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



Assay: 1 ml .05 ml

1. Acetone Residue
2. Fraction 1 (¼ satn.) sl. opalescent
3. F 2 (½ sat.) Clear
4. F3 (¾ sat.) Clear
5. F 4 (sat.) Clear
6. F5 Residue after AS sat. V. opalescent.

Assay with ½% lactose, ½ 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.

d thers, 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; Insoluble ppt. (Particulate??)

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

a) 9 ml H2O + 1 ml [illegible]

b) .9 ml [illegible] + 1 ml [illegible] + 1 ml Ac.

1/2 dilutions:

Brit., 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 required
1/64	—		conditions too close to
1/128	—		substrate exhaustion.

Blank

When fraction B is ppt'd. ē 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 50ml ≈) & compare with ~~whole reaction~~ B. (2.03ml)

40° may be too low!

Preparation of Lactase : Batch 2.

Grow K-12 in 12 l. N2case 1% Lactose 1/2% under strong aeration. After 24 h. Harvest in Sharples (Watson).

Fraction 1. 31 g. paste – Add 100 ml H_2O, 5 ml toluene, mix in blender + ~~au~~ autolyze at 37° ~~#~~ 11A26 –

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°).
3.08 ml .02 N $KMnO_4$.

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

4 Add 17.5 g AS (1/2 sat.) { small ppt. Redissolve in H_2O. A
supernatant. B.

Test .1 ml samples of each:

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

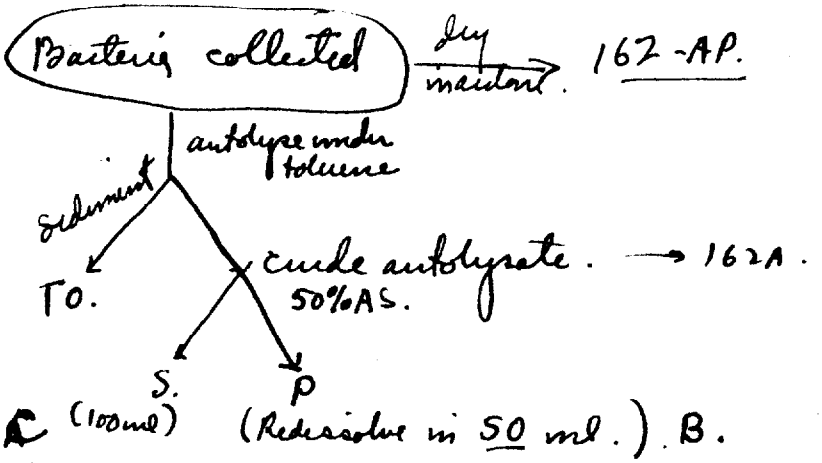
40° may be too high for assay.

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

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

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

No Activity.

Lactose Preparation.

March 29, 1948.

10 liter lots 11-12 in N2 Case + Glucose, (A) N2 Case + Lactose. (B).

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

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

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

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

Collect after 24 h. Store 1P31 in refrigerator.

B. became ~~very clarity~~ opaque on standing in refrigerator overnight On warming this material redissolved. Kelp 10 ml as crude ruddy pate = 163B1; add 14 g. Hm. Sulf. to remainder + cyanide 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 thiosalf. Redissolve ppt in H_2O.
very difficult

Temperature mutants.

March 29, 1948.

85 plates, Y10, 5 secs. Hanovia U.V. ca. EMB Lac
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. Asabove. 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 = A	45 = B.
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. ~~2~~ x 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 ph-saline + 2 ml toluene + autolyze at 37° Sharples and collect supernatant 10 A12. Cool in refrigerator. 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?

Potentiometric measurement of
glucolytic activity

				m.
AO.	0,00	0.01	-0.01	0,00
OB.	1.34	1.34	1.35	1.34
OC.	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 1/1/0
± 1 mg valine ± 1 mg isoleucine ± 1 mg hydroxy aspartic* ± 1 mg aspartic*

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

* overneutralized c 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. Hydroxy aspartic, as 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 Nutrose - PO$_4$ - glucose broth eluted 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 Nutrose .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	[illegible]	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 stock.

From fruit 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− / uncertain or mixed.

Purify 1+ and 1− on maltose.

Temperature mutants.

May 4, 1948.

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

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

Bron. 6P4.
16T. reading 9A5. = 15h.
9A6 = 39h.
9A7 = 63h.

[Note minus 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 deletion, 35° and 36°. This may account for slow development of 382 Maltose, etc.

−106 ∴ At 36°, W-382 is lac + glu −

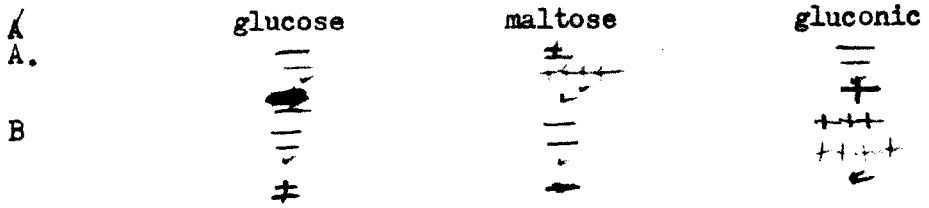
~~9A7 Hohmann~~

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.



To 1 ml. B cells add lcc 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 [illegible] (12 M)
3. 120 [illegible] 1:15 PM
4. 3:30
6 PM.

Glucose "adaptation" 1929.

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

	Glucose	Gna.		Glucose	Gna.
Y10.			W382		
11 AM.	—	—		—	—
1115	—	++		~~+++~~	+++
1130	—	✓		—	✓

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

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

∴ Sorbitol [illegible] may differ [illegible] mannitol.

— 9A7
— [illegible] 17

Galactose - Arabinose X - adaptation.

May 8, 1948.

Harvest K-12 from 16 hour cultures of Y/P sugar broth:

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

10:45 AM. (A7).

	c arabinose	b galactose	c glucose
a	++	− + ++	+++
b	− + ++	+++ ++	+++
c	− − −	− − −	+ ++++

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

See 100 [97]. [Adaptation in presence of azide] Arabinose × galactose + Cohen's letter with Y10.

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	A	B	C	D
glu	1	−	+	±	+	−	−	+	+++
gal	2	−	−	+	+++	−	−	+++	+++
lac	3	−	−	−	+	−	−	−	−

40°

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

		A	B	C	A	B	C
glu	1	+++	+	+++	−	+	+++
gal	2	−	++	+++	−	+++	+++
lac	3	−	−	+++	−	−	±

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

2 P.M. (20-30 min).

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	++45	+++ 30	++15	++30	+15	++30 45 +++15 30
30	+45	++60 45			++45	++60 75	++45	++60 75 ++45 60
60	+75	90 75			+75	90	++75	90 ++75 90
120		160					++160	160
W382 cells. 0	±15	±30 ++75 ++120	-15	-30 ++75 ++120	-	-	+15	++30 45 60 +15 30 120 At 90 still ++
30	-45	-45					+45	++60 ++75 90 +45 60 160 ++ ±45 ++60 ++115 ++120 +45 60
60	-75	-75					+75	++90 120 +75 90 ±75 ++160 +120 +75 90
120							++160	160 -160 ++160

10:00 AM — +++

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

= +++ T

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-387.

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 ~~Y~~ 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- 197

May 15, 1948.

Thor Lac-N2Lac-BCP fermentation tubes amply from 3d. slants of 5P15

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

① N16. ~~NA~~ = 19 hours.

② 7P16 = 25h.

③ 9H17 = 39h.

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+2}. Incubate 10 mins, then boil.

(1) Preliminary tests:

cc cells.	Initial absorption: density		Δ^{420}	Correction:	Final density. λ 420	λ 650	corr. Δ	% hydr.
	λ = 420	λ = 650						
1	.51	.34	.41	.61	.92	.41	.31	ca 50
.1	.065	.049	.08	.071	.145	.054	.074	ca 10
.01	.009	.008	.027	.01β	.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 1 ml cells~~. ~~Vary substrate conc~~. 10 min 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	.066	.0411	.140	.038	.060	.080
.2	.127	.084	.276	.073	.115	.161
~~.3~~						
.4	.250	.164	.520	.112	.225	.295
~~.5~~						
.6	.330	.23	.740	.209	.315	.425
.8	.450	.32	.935	.278	.405	.53
1.0	.540	.370	1.05	.334	.456	.56
after 1 hr	.14		.690	.143		.465
24 hr			.750			.525

ONP. CT.

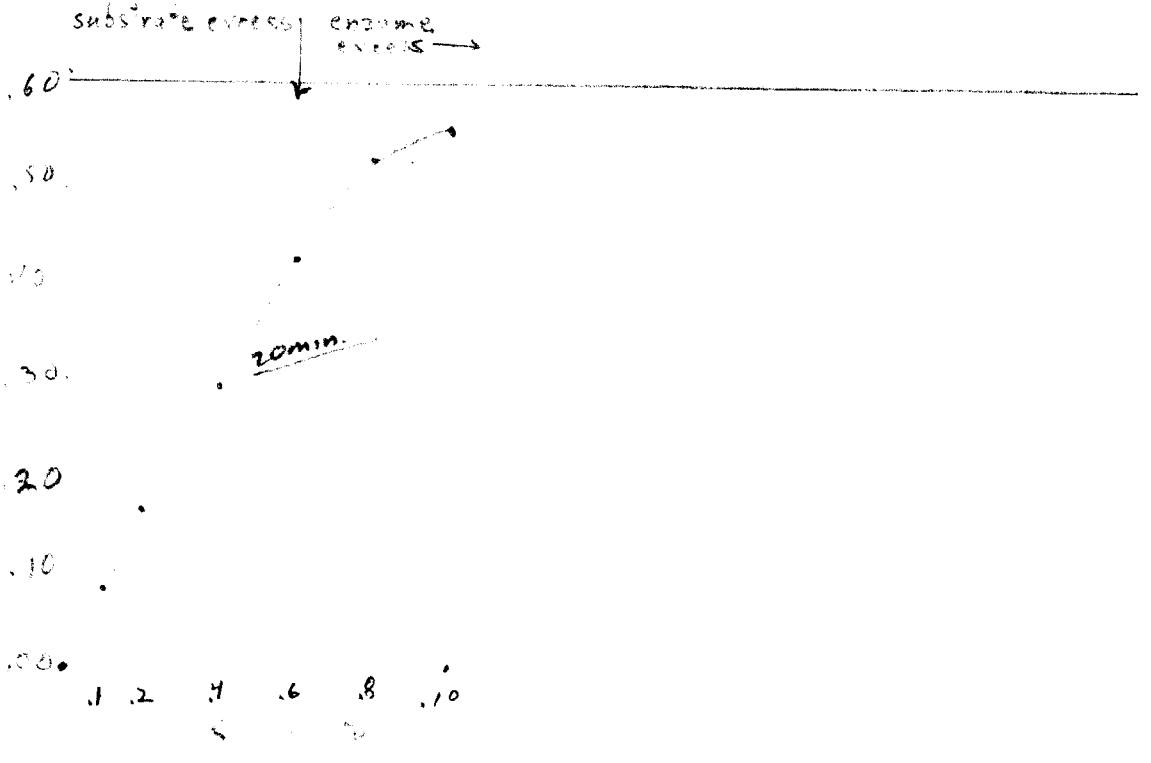
Mx ~~10~~ / 59000 Citrate buffer pH 7.5 M/50. $\lambda = 420$.

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

	$\lambda = 420$	$\lambda = 500$
160	.20	.07
172	.24	.04.

10 mins in NPO system.


substrate excess
enzyme excess
.60
.50
.40
.30
.20
.10
.05
.1
.2
.4
.6
.8
1.0
20min.

Inhibition by maltose.

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

Gall Blade

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
Gall Bladder 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 M/200 ONPG
7.5
20m. 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
W7-11		—	
		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. 100μM 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

Sept 17, 1948

		λ420 vs —	λ650 vs 9—	λ650 vs water	420 H_2O	3 hour exposure of color
L	—			.121	.192	
glucose	1	[illegible]	.015			+
"	2	.100	.017			+
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	.046	[illegible]			−
lac	3	.064	[illegible]			+
"	4	[illegible]	[illegible]			+
lac azide	5	[illegible]	[illegible]			±
lac ATP 5mg	6	[illegible]	.028			−
" " azide	7	[illegible]	[illegible]			−
Bzlm M/1000	8	[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: 2ml cells : 2ml 4% sugar in M/5 buffer + 1ml (supplement if any. Centrifuge once + resuspend in 4ml H_2O Test c ONPG in M/10 citrate buffer as above., 1ml : 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 7 ml cells to 7 ml sugars 4% in M/5 phosphate buffer pH 7.0. Add H_2O as 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	Hydrol. Casein 1%

ONPG as above, but use total volume of 9 ml. (after 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	Y. Extr., Peptone + H.C
3	.499	~~.334~~ .279	1.79	233	are definitely stimulatory to adaptation.
4	.551	~~.279~~ ~~.334~~ .310	1.78	231	
5	.022	.200	.11	14	
6	T=101	.230	0	0	
7	<.005	.200	0	0	
8	.519	.334	1.55	202	

N source: Conditions of adaptation

Sept. 20, 1948

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

		Suppl.	Relative activity
1.	—		03
2.	Lac		35
3.	"	Glucose/mg	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 42 Glu as above.

Indicated supplements ca 1 mg. eq. in 1 ml.

	A	B	C	D	overnight
O	242	224			—
Lac	230	218			-
Megal	246	237			+++
Bugal	319	310			+++
ONPG	240	219			--

A = D₂₀ bact. susp. V = 9 ml
B = D₂₀ + substrate [illegible] 10 ml
C = A [illegible] (x .90)
D = B − C = Δ
E = D/C = relative activity

		A	B	C	D	E		% lac
A12	1	[illegible]	297	202	95	47	—	69
A3	2	246	370	216	104	48	—	71
A4	3	249	333	224	109	49	—	72
A5	4	273	429	246	173	70 ~~64~~	+	103
A6	5	241	380	224	156	70	+	103
alanine	6	239	241	215	76	35	-	52
glutam	7	263	400	237	163	69	+	103
leuc	8	258	356	232	124	53	-	79
φalan	9	250	348	207	141	68	+	102
prol	10	255	371	232	139	60	-	90
lys	11	246	366	222	144	65	+	97
arg	12	238	409	214	195	89	+	133
meth	13	231	383	208	175	84	+	125
+Lac	(14)	251	377	226	151	(67)	—	100
−Lac	15	250	217	207	10	5	—	7.5
H.C.	16	351	870	326	554	176	++	263
H.C.+Tryp.	17	341	860	312	548	178	++	266
T+Leuc	18	263	409	237	172	73	+	109.

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

Amino Acids & Adaptation

Sept. 22, 1948.

5 ml system for adaptations as above. All Lac. K-12.

		A	B	C = A$_{con}$	D (B-C)	E $\frac{B-C}{C}$	% of Lac (1)
1.	—	228	305	205	100	56	100
2.	HC	310	710	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.	" A$_5$	249	illegible	222	257	116	
7.	" A4	291	520	233	287	80	
8.	" A5	241	illegible	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 ea. 10.

.1 ml HC 10% 2.

1 ml ≙ 1% HC 3.

Sept 25-26, 1948.

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

25 g. cell paste recovered. Ca 2½ g. + 10 cc 7.5 PO_4 buffer shaken 2½ h. under toluene. Remove debris & collect supernatant in

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

(B). Ca 1 g. washed ē 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 Y2 lactose. Harvest to 5 cc. 7.5 buffer & autolyze under toluene & shaking 24h & 48h.

(A) 24h. 1 ml withdrawn, debris sedimented & supernatant diluted to 4 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 mins). I.E., calculating 2g/liter, corresponds to 20 mg/ml

See 316

Sept. 27, 1948.

N-12 grown 36 hours in 10 liters S(Lac). 9.4 liters of supernatant were removed leaving 31 grams wet Shaples paste. Make up to ca 45 ml c PO_4 buffer pH 7.5 and grind 75 minutes in Booth-Dienert mill. Combine efflux c 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 calf 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 (acid) hydrolysis!

In prep. 316., 1 ml being = culture medium 10 liters/100 = 100 ml. cells.

and .001 ml should be equivalent to .1 ml cells, which 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(0) glucose (A) & do. + H.C. (B) (½ ml/100) shaken 16 hours. Adjust densities:

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

(B) HC. 319

ratio of 1.23 : 1.

Re-suspend ~~dilute~~ HC ~~culture~~ (0) culture ~~in~~ 20 ml H_2O; the HC culture in 24.6 ml H_2O to adjust initial densities.

The adaptation system consists of 1 ml cells + 3 ml T(0) lactose + H supplement. Adapt 3 hours, in duplicate. Resuspend in ½ ml dup 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(0) Lac	.248	669			
12	.260	720			
3. T(0) Lac + .1 ml H.C.	.256	710			
13	.260	731			
(B) 4. Lac buffer	.177	169			
14	.184	175			
5. T(0) Lac	.187	171			
15	.188	170			
6. T(0) 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 galaxe 316.

Sept. 28-9, 1948.

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

	Prop. fract.	Act.	Prop. Act.	Assay .01	.001
0	1.00.		1.00.	615	089
1 (¼sat).	5.00			129	019
2 (½sat)	5.00			390	055
3 (¾sat)	5.00			194	023
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. 1ml M/500 ONPG in M/50 PO_4 buffer.

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

Pool fractions ~~1~~, 2 + 3 (40ml) and add 2½ vols AS (¾sat). Take up ppt in M/50 citrate buffer. ~~pH 6.8~~ 319A

P30. To remaining 50ml (1.5×10^5 units) add 250 ml cold acetone, 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 10ml 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 needed. After 7 mins, are

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.

ONP M/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.

Nutrition - Adaptation

Sept 30, 1948.

K-12 in A) T/O) ,5ml 1mg/ml. 5ml 1% HCl =
shake overnight. 1:100 dilution: Resuspend in 5 ml H_2O. Turbidity at

	Dens.
A	119
B	119
C	252
	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

A. B. 1 ml cells, 3 ml substrate.

① Phosph M/50 7.5 + 2% lac
② T. (2%) Lac.
③ ② + ~~supplement~~ proline 1mg% .2 ml
④ ② + ~~HCl~~ AA. ~~H2O~~ 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	24.8	3.27	223	106	48	98	AA − Arg + arg	
3	241	3.35	217	128	54	110	" lys	
4	242	3.29	218	141	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	217	145	65	132	− meth	
9	249	3.92	224	148	66	135	− cyst	
10	250	4.27	225	202	54	110	AA − AA + Øal	
11	260	4.10	234	176	75	153	+ tyr	
12	270	4.51	243	208	85	174	+ thyp	
13	269	4.89	242	247	102	208	AA − Øal	Øal inhib? thyp stimulatory.
14	211	4.52	244	208	85	173	− tyr	
15	261	4.41	242	199	82	167	− thyp	
16	230	2.72	207	102	49	—	0	
17	319	7.15	287	508	177	362	M.C.	
~~18~~								

Sept. 30, 1918.

A. Phosphate vs. citrate. EDC 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.

1 Add —	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

	D 420.
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 32½.

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	395
3.	KCl	2.59
4.	Na_2SO_4	5.14.

M/50. Repeat

1.	~~[illegible]~~	317
2.	NaCl	512
3.	Na_2SO_4	592
4.	KCl	298
5.	LiCl	218
6.	NH_4Cl	230
7.	$(NH_4)_2SO_4$	252
8.	$MgSO_4$	257

inhibitory.

NaCl concentration series:

1.	—	316	.10
M/50+ 2.	.1	405	
3.	.5	388	
4.	1.0	↓	
5.	5.0		

Lactase.

Sept 30, 1918.

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

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

Dyno.	A.	B
		621

Also, other assays:

325

314B.	1 mg	1150	ca. 35 u/mg.
	.1 mg	379	
	.01 mg	046	
319B.	1 mg	1070	ca. 190 u/mg.
	.1 mg	960	
	.01 mg	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 ~ shaking, to 10 ml ~~1/50 Phosphate Buffer (PB) 7.5~~ T(0)-Sugar.

Adaptation system ~~[redacted]~~ 5 ml, containing 1 ml T(0)~ 5% Lactose + varying amounts of cells. A (no supplement). B. .1 ml hydrolyzed casein/100 ml.

		Cells.	4(-)	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 (2.9) ml	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 dilute suspensions

b. pronounced stimulation of " by hydrolyzed, although cells were grown in T(0). This medium, therefore, offers no advantage.

Index: Phosphate assay. 327

Oct. 4, 1918.

2 ml 219A + 2 ml 10% TCA. Remove sediment. Assay in indicated aliquots against 10^{-4} - 10^{-3} P'orgh sulfuric standards. Extreme of original 219A. ~~Also~~ assay 1 ml of ~~1:500 dilution~~ of 219A in M/50 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 paddle
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 dialyzed 4 hours against distilled water. Final volume, 13 ml. = 219C. Impure activity + response to Na. Expresses at 1:1000

	D_{420}.	Enzyme.	Na_2SO_4
1	095	C	0
2	140	C	N/50
3	171		
4	219		N/1000
5	277		N/100
6	290		N/50
7	178		N/10,000.

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

Lactose kinetics.

Oct 4, 1948.

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

37°.

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

Stopwatch T	Time T.	A	B	C	D.
	0	004 wk 001		009 wk 007	
	1:20			069 200 154	
	3:30	048			
	4:00		083		
	4:30			225	
	5:00				320
	5:30	069			
	6:00		102		
	6:30			326	
	7:00				411
	7:30	089			
	8:00		178		
	: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	~~16~~		
	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		
13			860	
	258			955
14		300		
			905	
15				995
	277.			
16		320		
			940	
17				1005
	298			
18		341		
			955	
29	~~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
	451			
~~46~~		509		
			1050	
53. ~~47~~				1095
~~48~~				

↓

~~49~~ ~~30~~				
~~53~~		560		
			1050	
~~54.~~				1100
~~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			
~~N.~~	1145	1250	1145	1250.
1:10	209	1:11 690	213	810.

evaporation may have intensified overnight.

Oct 5, 1948.

49 g. Stiff Shapley 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.

AB Remove debris. Left a opalescent yellow green solution, 17 ml.

Assays. (in M/50 Na. Phosphate). ONPG M/2000. 20 m. 37°

D_i 420.

329. 1. B .01
2 .001
3. C .01
4. .001

Inhibition by serum.

319 A. 5. —
.002 ml. 6. + 476F41:10
7. + 471F51:10
—enz. 8. + 476F41:10
—enz. 9. + 471F51:10.

730
(341
430
540

780
599
710
023

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

Discarding "C"

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

throw out!

Lactase activation and inhibition
Micro assays

(1 ml Suppl.) In Na P buffer.

				D_{420}
	1. 329B $^{-2}$			45.3
	2. " B $^{-3}$			075
329A [illegible]	3. 329C	—		082
[illegible]	4.	NaCl M/50		081
10^{-3}	5.	KCl M/50		092
	6.	LiCl M/50		078
	~~7~~	~~$Et(NH_2)_2$~~		

In KPO_4

.001	7. 319A	—		150
	8	Et $DiNH_2$	M	017
	9		M/10	022
	10		M/50	049
	11		M/100	082

Na P. [illegible]

Repeat Assays of C!

		Buffer	Amt		
329C	1	Na	.001		116
	2.	Na	"		114
319A.	3	K	—		167
.001	4	K	EDA M/100		128
	5	K	" " + Na_2SO_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.

Turbid D_i = 129
" D_i = 420

} v. sl. inhibition

may be protected from inhibition by [illegible]

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.	Duro
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 $\times$ M/2000	in NaP.	at time	
10	0.1	091	258	(8.5×10^{-5}).
11	0.5	210	920	K_m may be estimated in the neighborhood of $5 \times 10^{-5} - 10^{-4}$
12	1.0	254	1110	Linearity needs to be shown. Conc. of ONPG from 2×10^{-5} to 2×10^{-4} must be explored.

∴ fluoride inhibits only in presence of phosphate. M/100 needed for substantial inhibition. (Mg effect?)

Lactase. Mechanism of fluoride inhibition

Requirement for Mg^{++}? Km.

Oct 7, 1948.

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

	Suppl.		
1.	—	019!	Con?
2.	NaF M/100	013	
3.	" M/500	180	
4.	" $MgSO_4$ M/500	132	Note aggravation.
5.	" M/1000 $MnCl_2$ M/200		
6.	" M/500 " "		
7.	— " "		
8.	— $MgSO_4$ M/200.	251	No marked stimulation!

.001 ml in M/50 NaP buffer. Vary amounts of M/2000 ONPG added.

Measure q. 5 min.

	ONPG.	5m	10m	15m	20m.	0	30	K_m ($\times 10^{-5}$)
	11	1						
ng	12	1.5	038	065	094	123	178	8.3
	13	2	049	079	111	149	210	7
	14	5	077	124	173	201	323	5
	15	10		141		262	381	—

↑ 094 90 slope

These data show a substantially linear decomposition of the galactoside in the interval studied, but taking V_{15} as V_{max}, we 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.

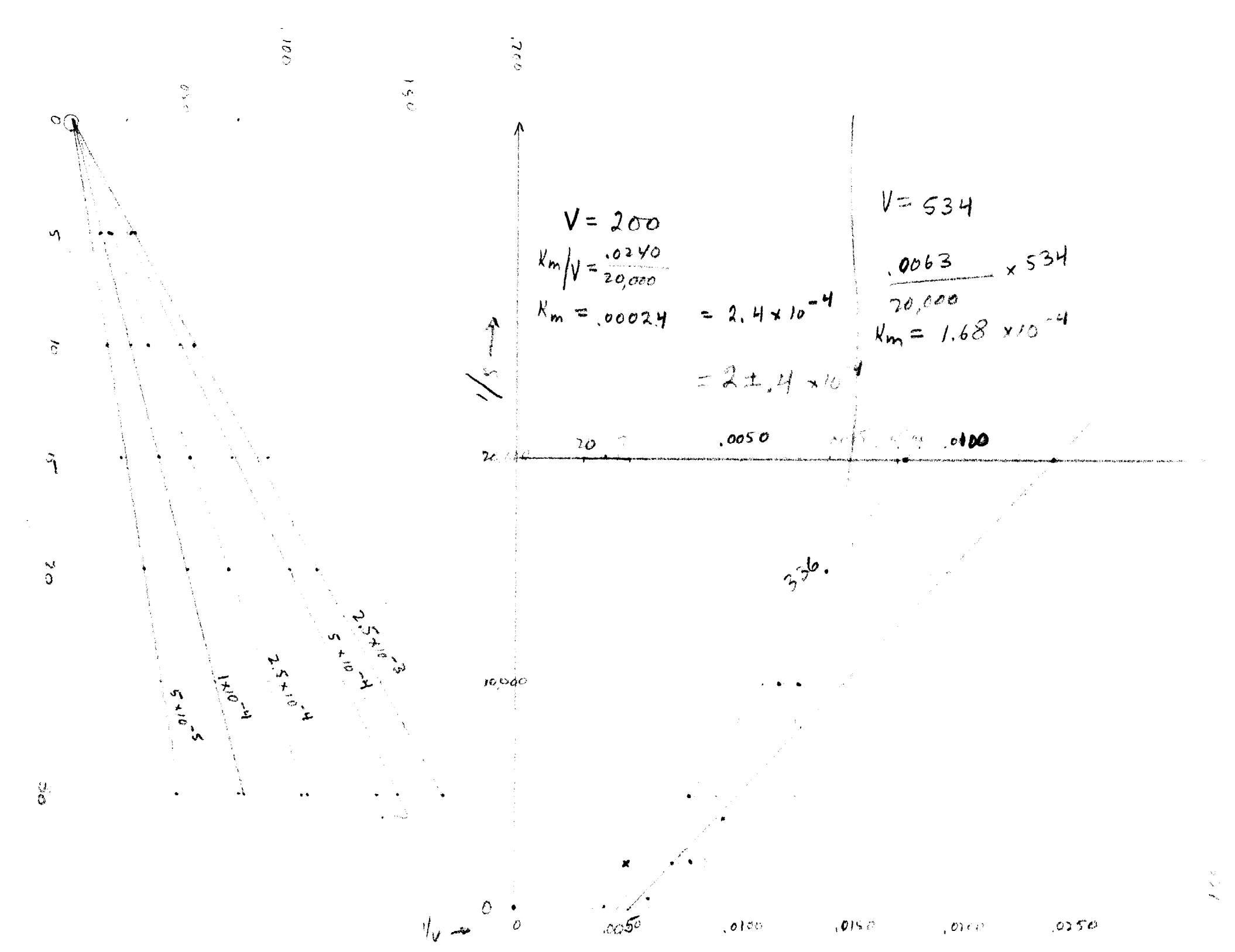
Use first 20 min. data

	V	1/V	1/S
①			
②	121	0083	13,300
③	141	00705	10,000
④	212	0047	4,000
⑤	215	0046	2,000

$\bar{V}$ = 315 ± 32

Careful extrapolation gives

K_m =


V = 200
$K_m/V = \frac{.0240}{20,000}$
$K_m = .00024 = 2.4 \times 10^{-4}$
$= 2 \pm .4 \times 10^{-4}$
V = 534
$\frac{.0063}{20,000} \times 534$
$K_m = 1.68 \times 10^{-4}$
1/S
1/V
.0050
.0100
.0150
.0200
.0250
20
10,000
5 × 10⁻⁵
1 × 10⁻⁴
2.5 × 10⁻⁴
5 × 10⁻⁴
2.5 × 10⁻³

In 3 determinations, Km was 1.4
1.5
1.18 $\times 10^{-4}$.

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

K_m o-nitrophenyl galactoside
K-12 Lactose.

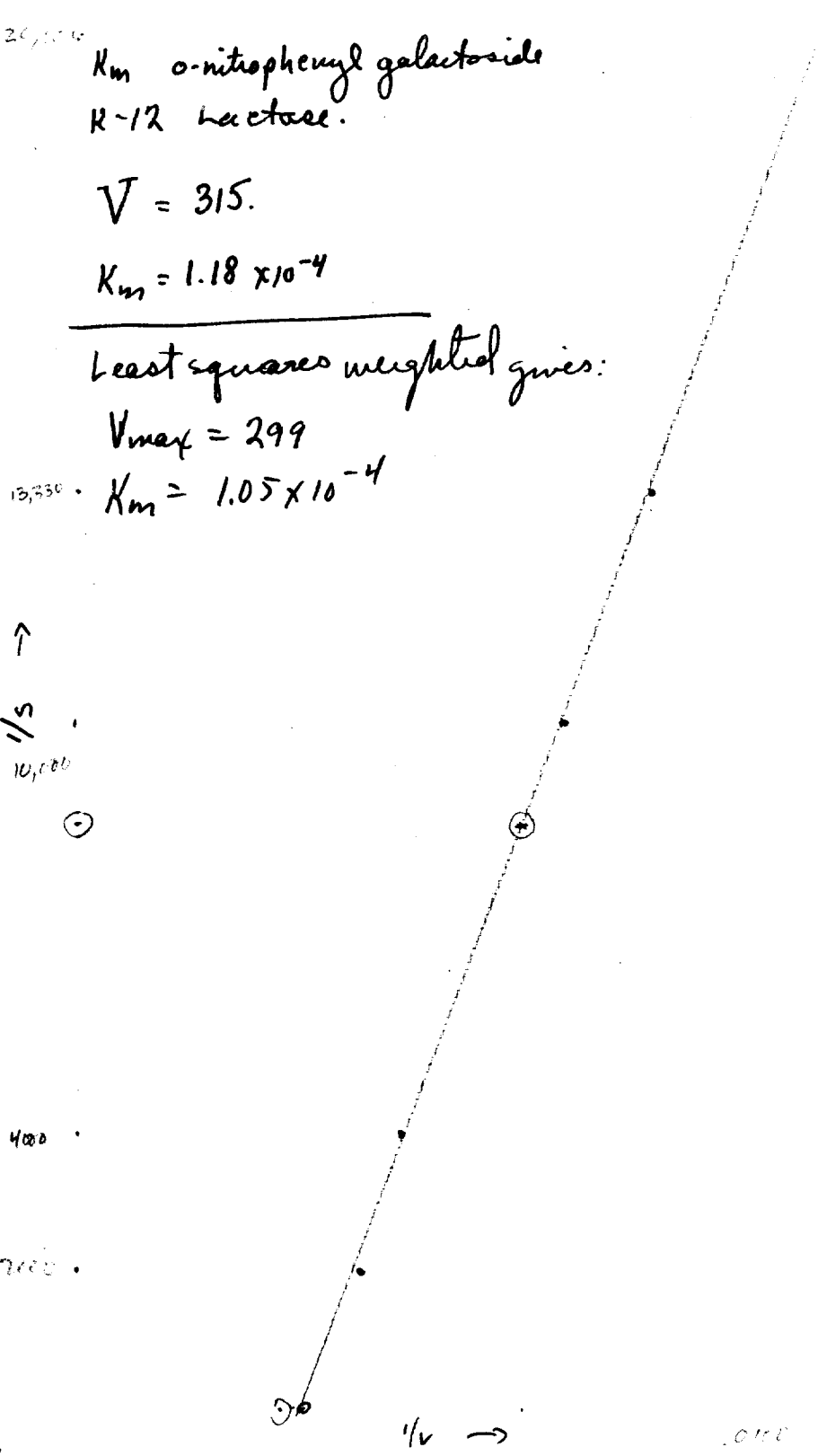
$V = 315.$

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

Least squares weighted gives:

$V_{max} = 299$

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



Analysis of 3rd 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	2.12	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		28.81	62.44	119.66		1252.89	222.02

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

$r^2 = 12.64$

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

$$b = \frac{119.66 - 3.56(28.87)}{1252.89 - 7.11(222.02) + 12.64(62.44)}$$

(102.77; 1578.56; 789.24)

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

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

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

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

$= .105$

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 c̄ substrate dilution. D420.

ONPG x M/20,000.

		t_0	5 M	10 M	15 M	20 M	30 M
0	50	051	080	10[illegible]	[illegible]	162	219
1	10	011	[illegible]7	060	0[illegible]	110	149
2	5	009	027	[illegible]	063	[illegible]	114
3	2	000	017	021	01[illegible]	053	
4	1	−003	010	014	[illegible]	031	046

(2). in M/100 NaP buffer.

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

Corrected values of (1).

	t_0	5	10	15	20	30	v calc.	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	.02400	20000

Km is estimated at 2.4x10⁻⁴

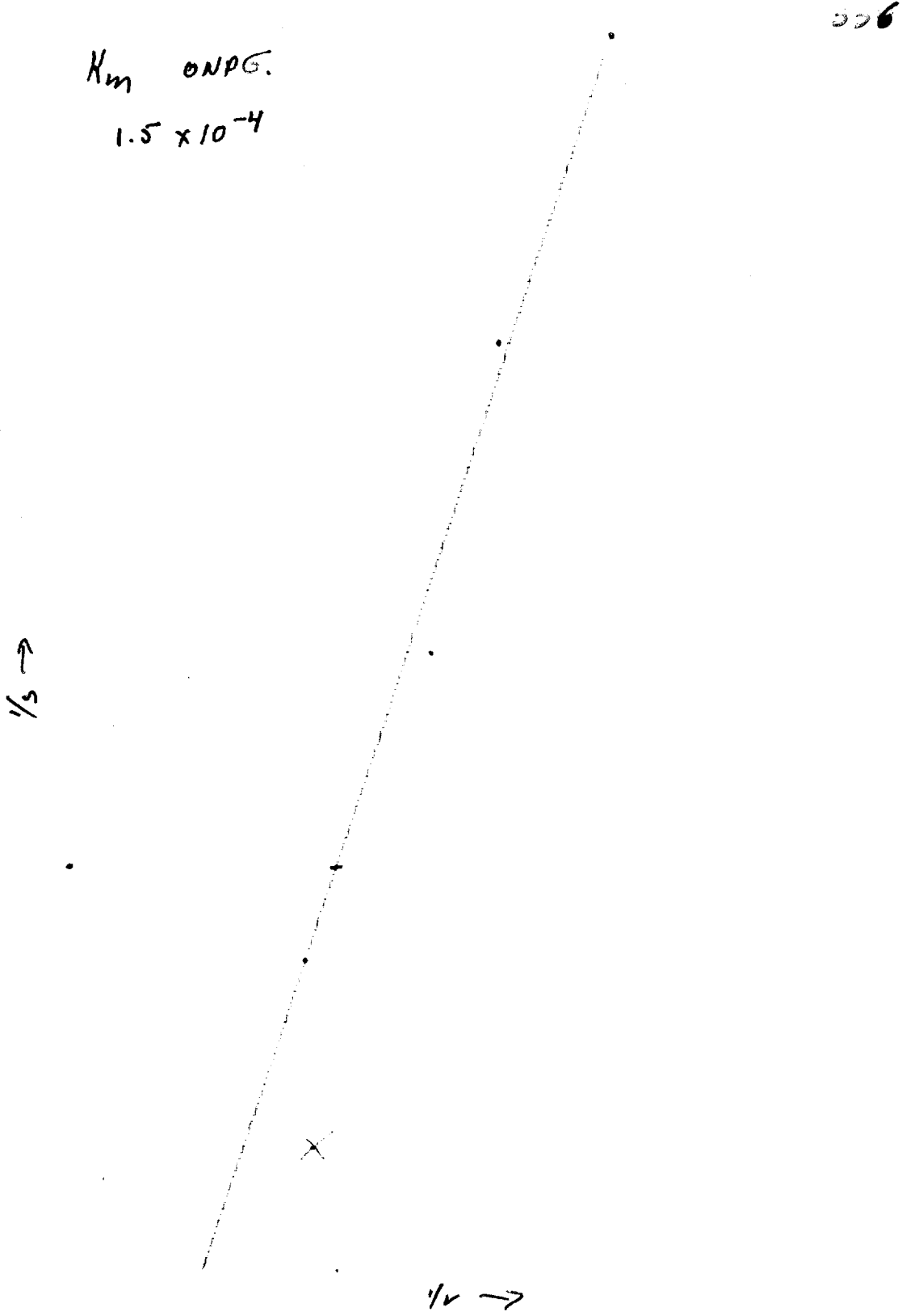
should be / [illegible] too high [illegible] 69.

V at 200/30 m.

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 3/9A.

	ml M/20000	$D_0^{V/20}$	$D_{10}^{V/20}$			1/s	1/v
1.	1.00	000	115	115		20000	.0051
2.	1.33	002	116	114		15000	.0067
3.	2.00	001	180	179		10,000	.0038
4.	4.00	001	272	271		5000	.0035
5.	10.00.	026	281	255		2000	.0039

Visible discrepancy / inactivity = 3/34.

② In M/100 NaP buffer. + M/50 salts.

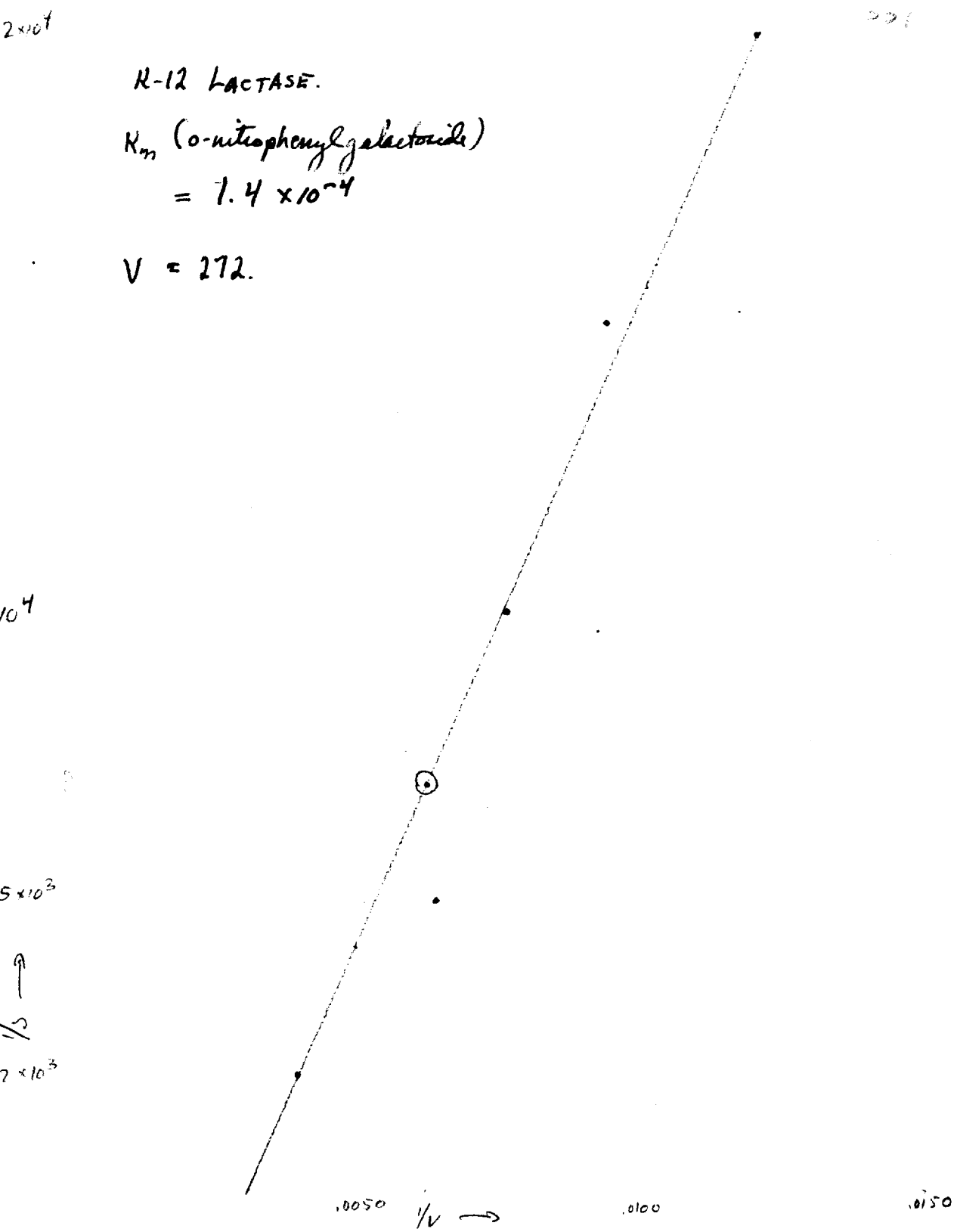
11.	—	3[illegible]
12.	NaCl	351
13.	KCl	316
14.	LiCl	305
15.	RbCl	087
16.	CaCl	302

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

N Z case	1%
Yeast extr.	.5%
Lactose	2%
Tween 80	0.1%
Na Acetate	0.1%

} LB medium. heavy growth noted!

Wash and concentrate 1:5. Use 1:10 ε ONPG M/2000 pH 7.5

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

84	.0119	252	1
107	.0093	328	
132	.0076	472	
157	.0041	830	
252	.0040	1200	

11	1.0	006	090
12	1.33	0	107
13	2.0	005	137
14	4.0	003	160
15.	10.0	009	261

272. 1/V = 0031

d (decomposition)	
1200	
1200	
172	
575	

③. M/100 Na P buffer. Salts M/50.

1 —	250
2. NaCl	258
3. Rb Cl	101
4. Na + Rb Cl.	163

to the enzyme?

Does Rb+ irreversibly inactivate

L. bulgaricus lactose.

tare 79. wt. 128 — 49 g. wet Staples paste collected grown 24h. in 12 litres LB-lactose broth 5 aeration.

A) 4g. in M/100 NaP buffer for autolysis. Ca 10ml (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-Gem Mill for extraction → 45 ml

L bulgaricus lactase pH opt. 039

10/16/48.

338C .05 ml. of buffers, 1/5 M/50. ONPG 4/2000.
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 ~~1/7~~ ~~[illegible]~~ to develop color and stop reaction.
1 M Na_2CO_3

NaCl needed! Repeat above + addition of 5 ml 1/5 NaCl.

Lactase; Coli & L. bulgaricus.

October 15, 1948.

			D_i^{420}	$D_{30m.}^{420}$	D_i^{zero}	Δ.	
1	Coli 319A. .001 ml	—	002	295		293.	
2	+ Ethylene diamine · HCl M/10		010	029		020.	
3	+ Ethanolamine · HSO$_4$ M/10		040	130		094.	
4	+ Ethylene Glycol M/10.		−001	378		379.	
21	+ RbCl M/50		0	050		050.	
22	+ KCl M/50		−001	284		285.	
23	+ RbCl + 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	1210		—	
9	"	.1 ml	022	361		341	
10	"	.01 ml	010	030		024	$= \frac{1}{4500} \times 20g$
11	"	.001 ml	0	022		022	probably ONPG!
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. $\lambda = 420$.
This may not be the opt. pH for bulgaricus.

Note: Intense stimulation by glycol!, Reversal of Rb inhibition ± K.
Relatively low activity of cells & extract may acct. for poverty of extract.

October 18, 1948.

.001 ml 319A. NaP buffer M/100. Alcohols... M/10. ONPG M/[illegible]

1.	—	7	341 – 410	
2.	RbCl	7	089	
3.	Ethyleneglycol	7	423	
4.	" + RbCl	7	190	No marked displacement of Rb.
11	EtOH	7	400 – 4[illegible]	
12	PrOH	7	469	
13	iPrOH		395	
14	$Pr<(OH)_2$		390	
15	BuOH		450	
16	Dioxan		300	
17	MeOH		441	
18	$Et<^{Cl}_{OH}$		151	
19.	$Pr\ll(OH)_3$		449	

.05 ml 338C KP buffer 11/[redacted]50. Salts M/50. [illegible] +200 Na_2CO_3 [illegible]

21	–	pH 7.5	7	257	
22	NaCl M/50.	"		390	Na (or Cl) required
23	"	8.0		079	
24.	"	7.0		590 ←	pH optimum between
25.	"	6.0		410.	6 and 7.
26	338A 1 ml in NaP M/50. 7.5.		7	032.	Inactive.

Note stimulatory effects of primary alcohols, especially n-propyl and n-butyl alcohol, and inhibition by chloroethanol.

A 18. Cf. ONP $\bar{c}$ and $\bar{s}$ nPrOH. ONP ca. M/25000. NaP buffer etc.

1. + PrOH.	168
2. –	165.

PrOH at dilution of M/10 does not influence absorption of ONP.

? With PrOH + enzyme + ONP galactoside lead to color [illegible]?
? Absorption spectrum of products c PrOH.

L. bulgaricus lactase_

October 18, 1948.

338C .01 ml / tube. 9 ml. pH 7.5 Stop Rx c̄ Na_2CO_3.
In various buffers, M/100. Add ~~[illegible]~~ Na_2PO_4 buffer additional M/[illegible] where called for.
NaCl M/50 in all tubes.

Buffer.		+ 1 ml $MgSO_4$ 4/1
1. NaP	110	120
2. NaBarb	~~175~~ 116	160
3. " + NaP	170	188
4. Et_3NH_2·Cl	020	
5 " + NaP	025	
6. NaGlycinoP	080	070
7. " + NaP.	109.	110
8. NaP + $MgSO_4$.	175	

A) No activity B) Repeat with .05 ml enzyme [illegible]

Mg, PO_4 are stimulatory.

Lactose - ONPG competition.
Km.

October ~~1~~ 26 1948. — 10/28/48.
NaP M/50 pH 7.5. 319A 10^{-3} cc. 20 m. 37°....

		ONPG	Lac.	D_{t0}	Di	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	
(1 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 + .1 ml antiserum.	~~[illegible]~~ 290		360	070	

~~9. ... KP ... M/200~~
~~10. ... NaOH M/10.~~

~~[illegible] 10 [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	−	+
Sal	−	+
Suc	−	−
Xyl	−	−

Retests 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 (bulkiness + 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) ~ E coli K12, O.D. λ420, =1.00; B) do. +M/5000 ONP + c) ONP only in acetate buffer .04M, pH 4.0. Compare readings (as O.D.).

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

Lactase pH optimum

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

acetate buffer?

no enzyme

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

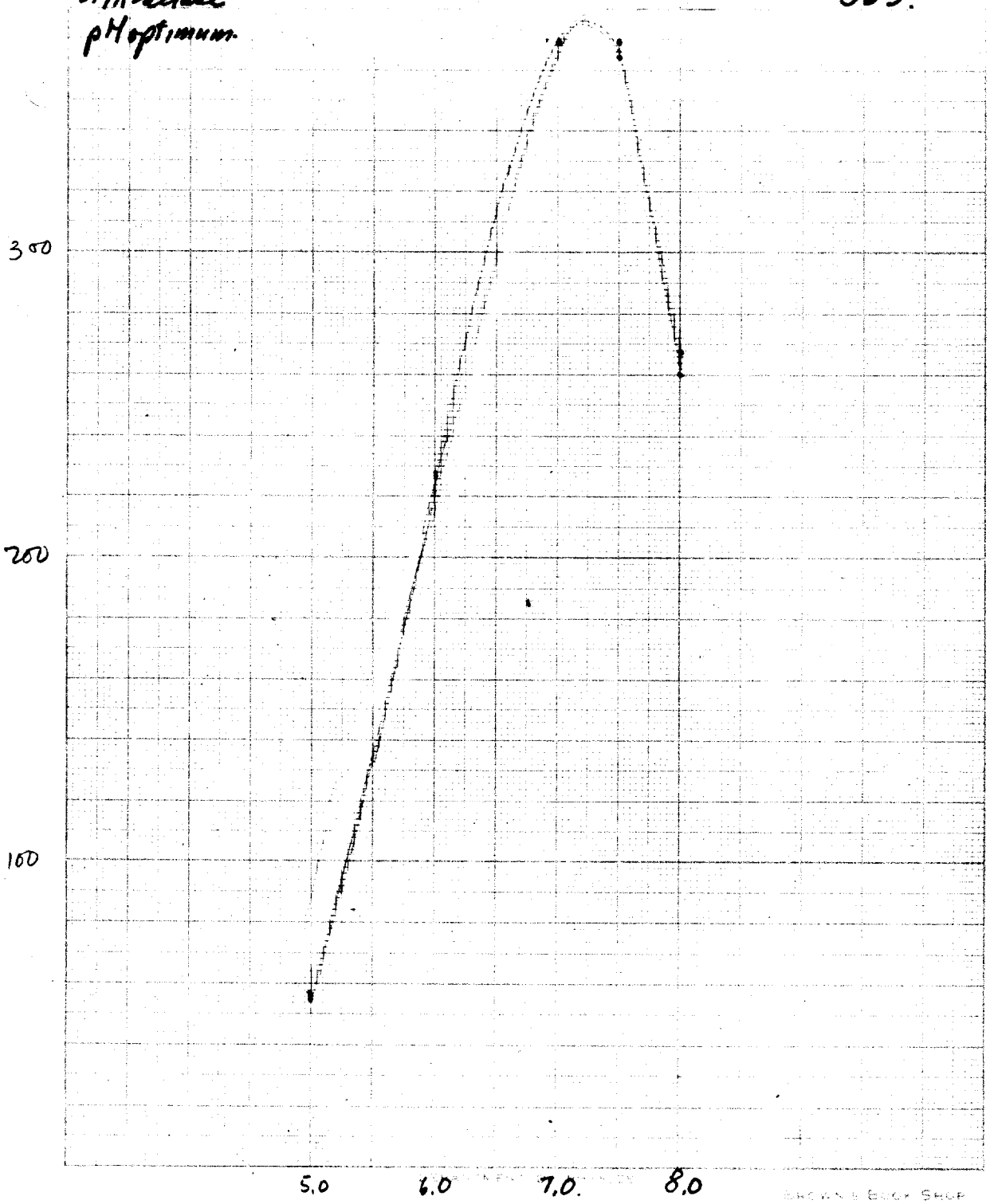
Na_2SO_4 ~~M~~/50.

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

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

Repeat, using phosphate buffer only!

3/19 Lactase
pH optimum.



pH optimum - colitectase

11/18/48.

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

Duplicate tubes. Add Na_2CO_3 M/10 at conclusion:

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

AA antagonists and adaptation.

11/27/48.

Harvest K-12 from 200 ml 1/2 glucose shaken overnight and resuspend in 40 ml 1/5 Na 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~~	
~~4. 5MT 500γ~~			
~~5. T~~			
~~6. T~~		060	172
7. C		140	179
8. C		~~140~~ 171	~~177~~ 171
9. A		190	~~170~~ 171
10. A		199	161
11. T+5MT	gelatinous		
12. T+5MT	pH > 8-9		
13. C+A	Di = 178 381	A_{cn} =	
14. C+A		159	170
15. A+5MT			
16. C+T.			

No inhibition by canavanine

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

12/8/48.

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

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

Assay preparation A; 20 min determinations.

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

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

	salt	D420.
1.	No enzyme	[illegible]
2.	—	167 ✓
3.	Na	248 ✓ (av. [illegible] determin.)
4.	Rb	196
5.	Na+Rb	212.

may be a chloride effect

1	—	220 ✓
2	NaCl	250
3	Na_2SO_4	270

Note appz stimulating by Na_2SO_4

Lactose : competition with ~~galactose~~ ONPG on ~~bi~~ lactase
alfalfa

12/9/48.

Run ONPG conc. series ē various lactose concentrations.

10 ml .05 M .001 ml 381A add 1 ml Na_2CO_3 to terminate. in M/100 Na_2CO_3.
20 m.

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

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

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

Barley Seedling lactase

12/8/48.

Seedlings from Dr. Nancy Kent.

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

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

∴ Barley lactase is constitutive

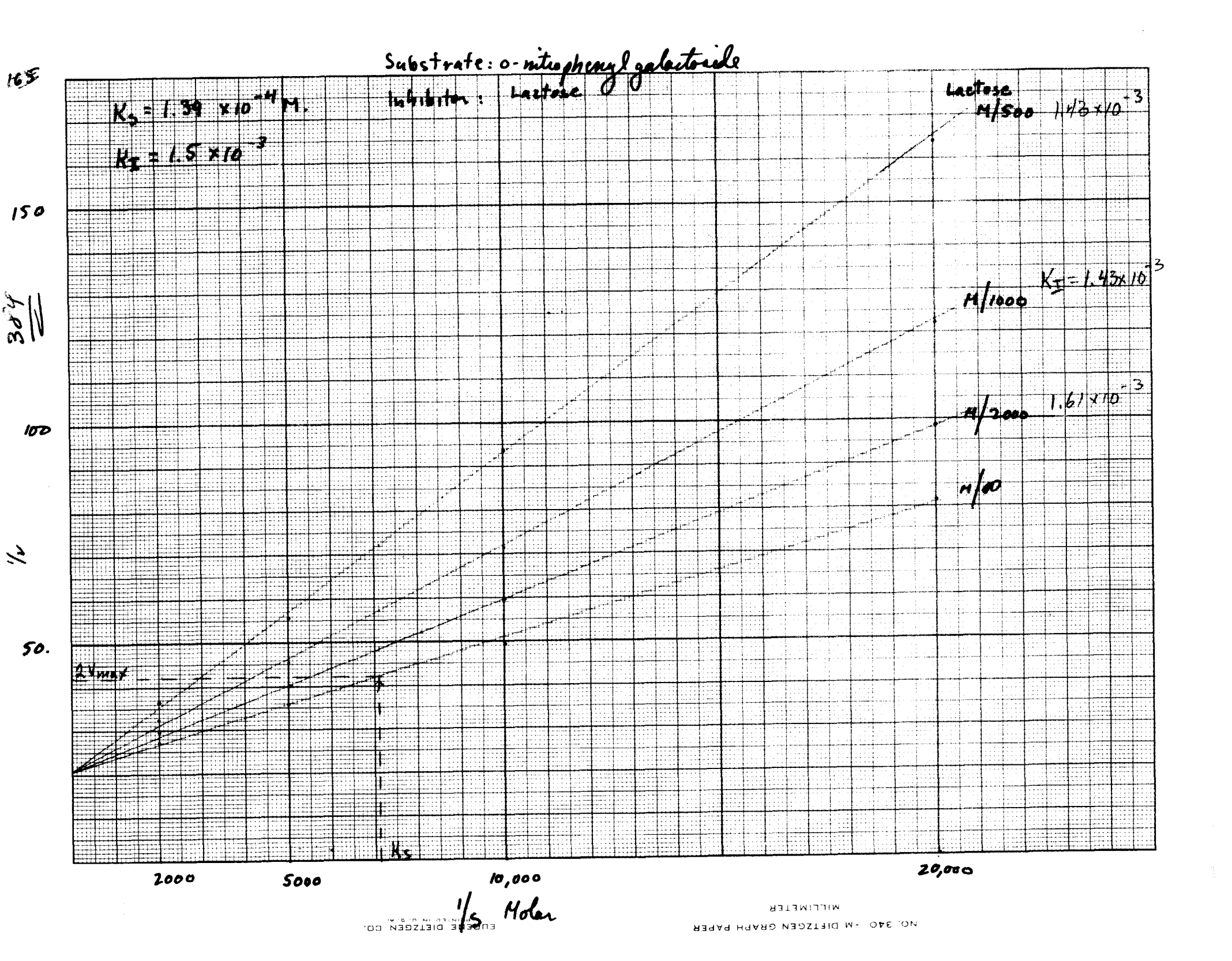
12/10/48. Qualitative tests on malt extract show no lactase activity.

Competitive inhibition of coli lactase

December 10, 1948.

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

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


Substrate: o-nitrophenyl galactoside
Inhibitor: Lactose
$K_S = 1.39 \times 10^{-4}$ M.
$K_I = 1.5 \times 10^{-3}$
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.
1/v
ΔV_{max}
K_S
2000
5000
10,000
20,000
1/s Molar

Kinetics of inhibition of coli lactase with glucose

Dec. 11, 1948.

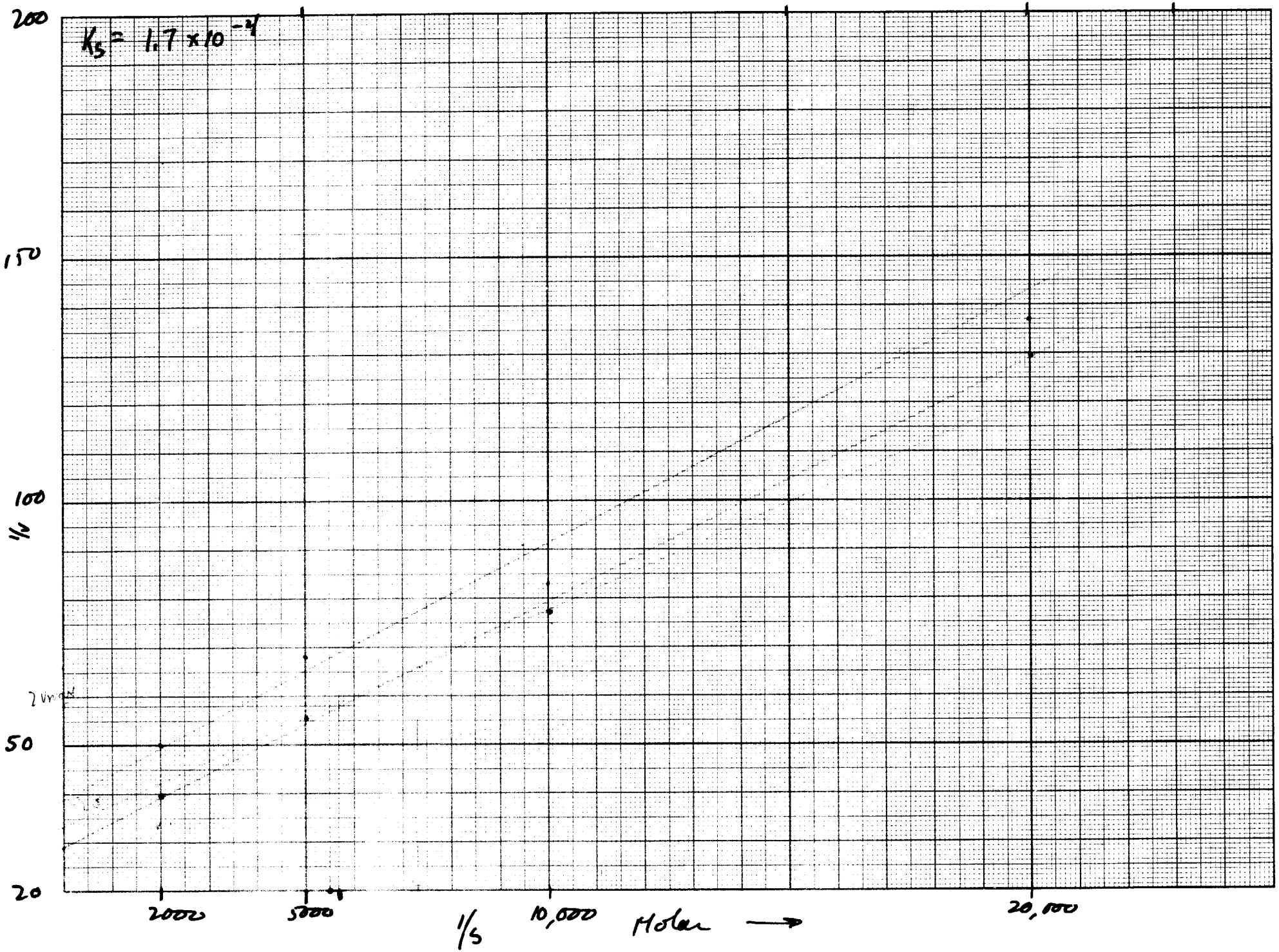
Set up parallel to 384. But use .001 ml enzyme; 20 mins.

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

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

K_s estimate here is 1.7×10^{-4}.

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


$K_5 = 1.7 \times 10^{-4}$
200
150
100
50
20
1/s
2000
5000
10,000
20,000
1/s
Molar

Glucose inhibition of lactase.

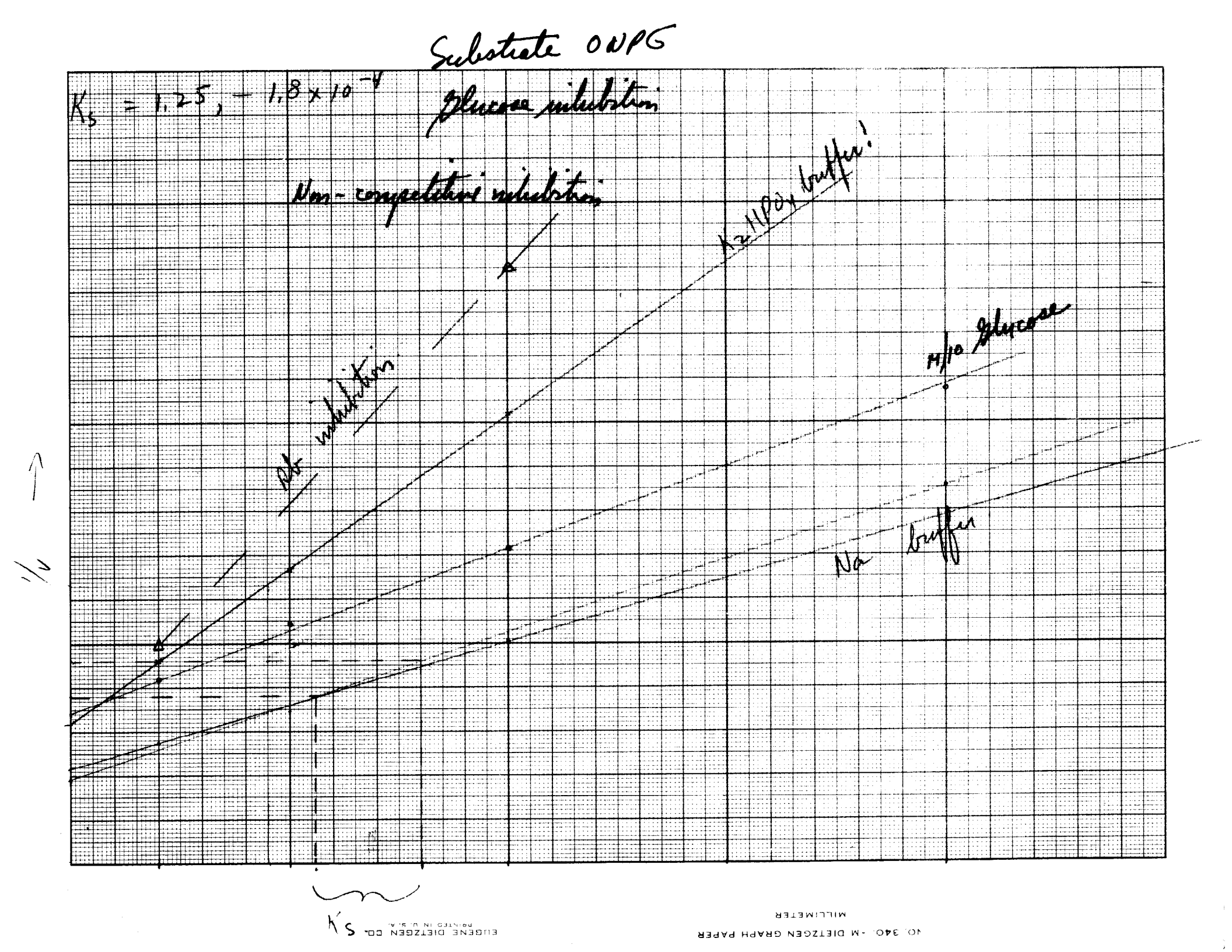
12/11/48.

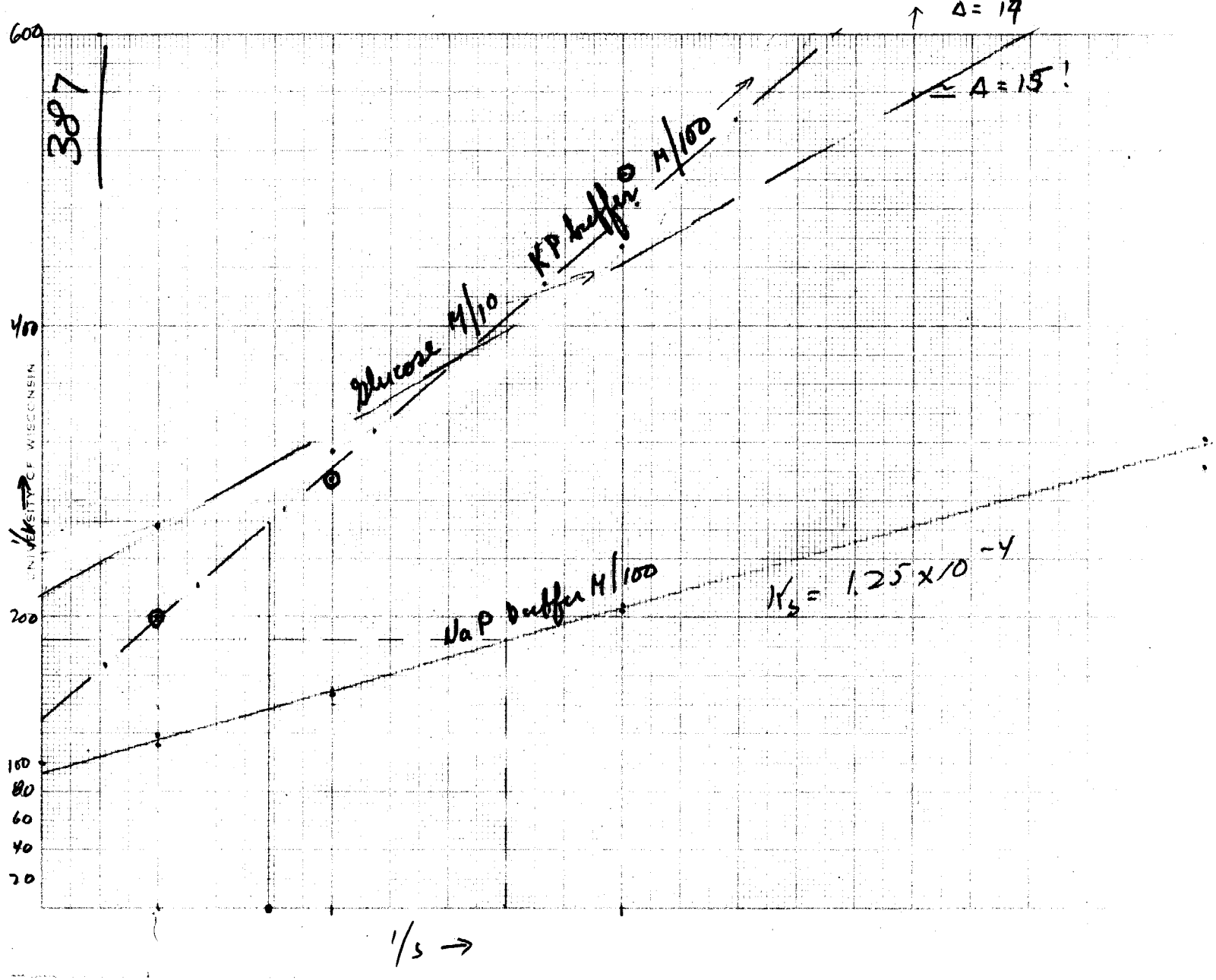
Ls 385. .002ml 10 Mins. Val 7.5 M/50.

Compare 0 and M/10 Glucose at various concentrations.

	ONPG	Glu		1/V	
1	2	—	365	27.4	
2	5	—	290	34.5	✓
3	10	—	197	50.8	
4	20	—	117	85.5	
11	2	M/10	239	41.8	✓
12	5	"	184	54.3	
13	10	"	140	71.4	
14	20	"	93	107.5	
		RbCl			KP 7.5 M/100
21	2	—	218	45.9	
22	5	—	150	66.7	✓
23	10	—	98	102.1	
24	20	—	57	175.4	
31	2	M/50		142 × 1.4	1.4 conversion
32	5	"			
33	10	"		74	135.1
34.	20	"		40	25

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
1/10 glucose
No buffer
1/v
K_s


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

December 13, 1948.

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

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

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

Coli lactase: Summary assays

Yeast

ONPG M/2000. NaP M/50. 15 min[illegible]

1.	319A.	2×10^{-3} ml. [illegible]	500
2.	319B.	10 γ	1·8
3.	319C	2×10^{-3} ml ca 500/ml	70.

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

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

} [illegible] c these dilute suspensions.

Cell density indicated by light absorption.

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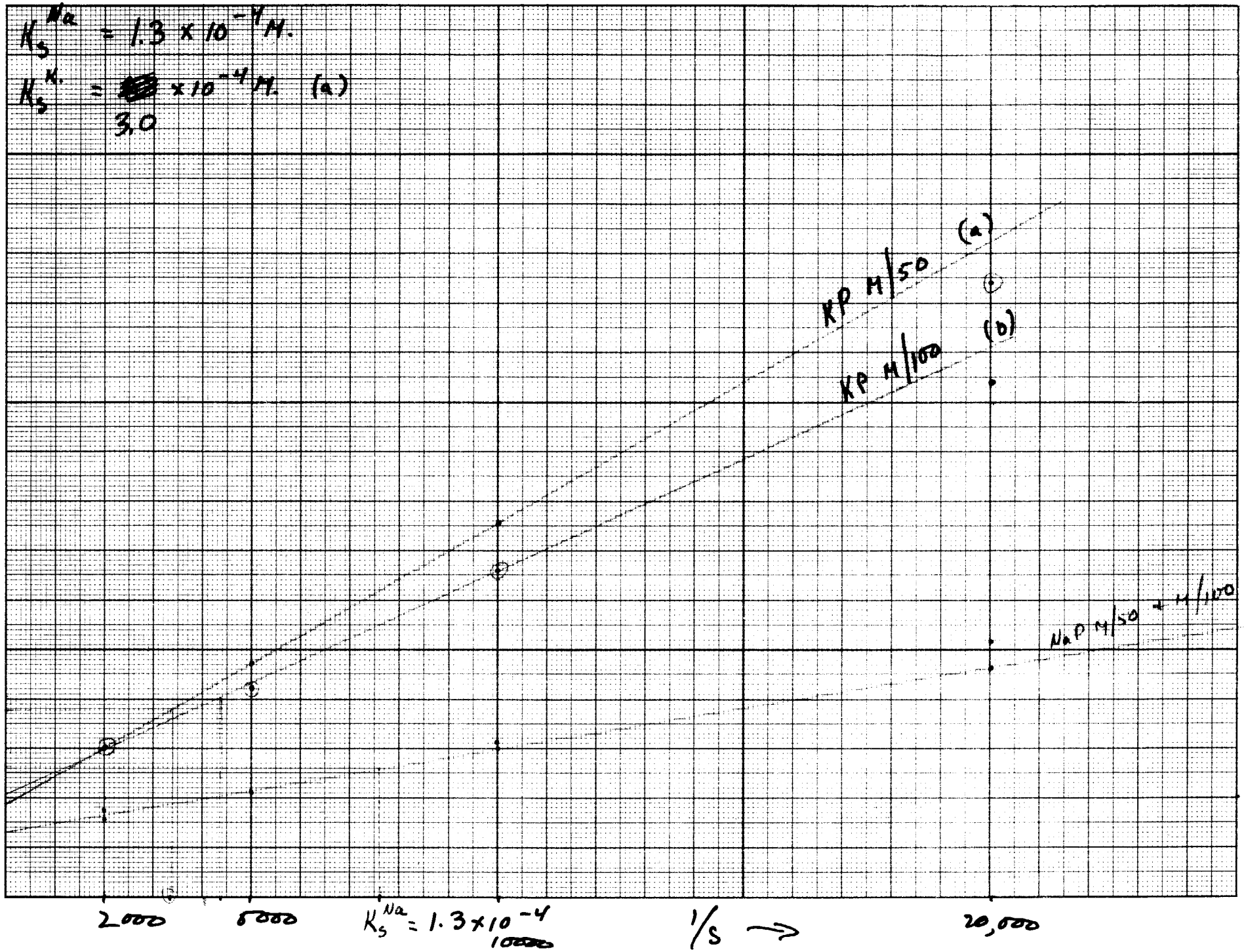
$K_s^{Na} = 1.3 \times 10^{-4} M.$

$K_s^{K.} = 3.0 \times 10^{-4} M.$ (a)



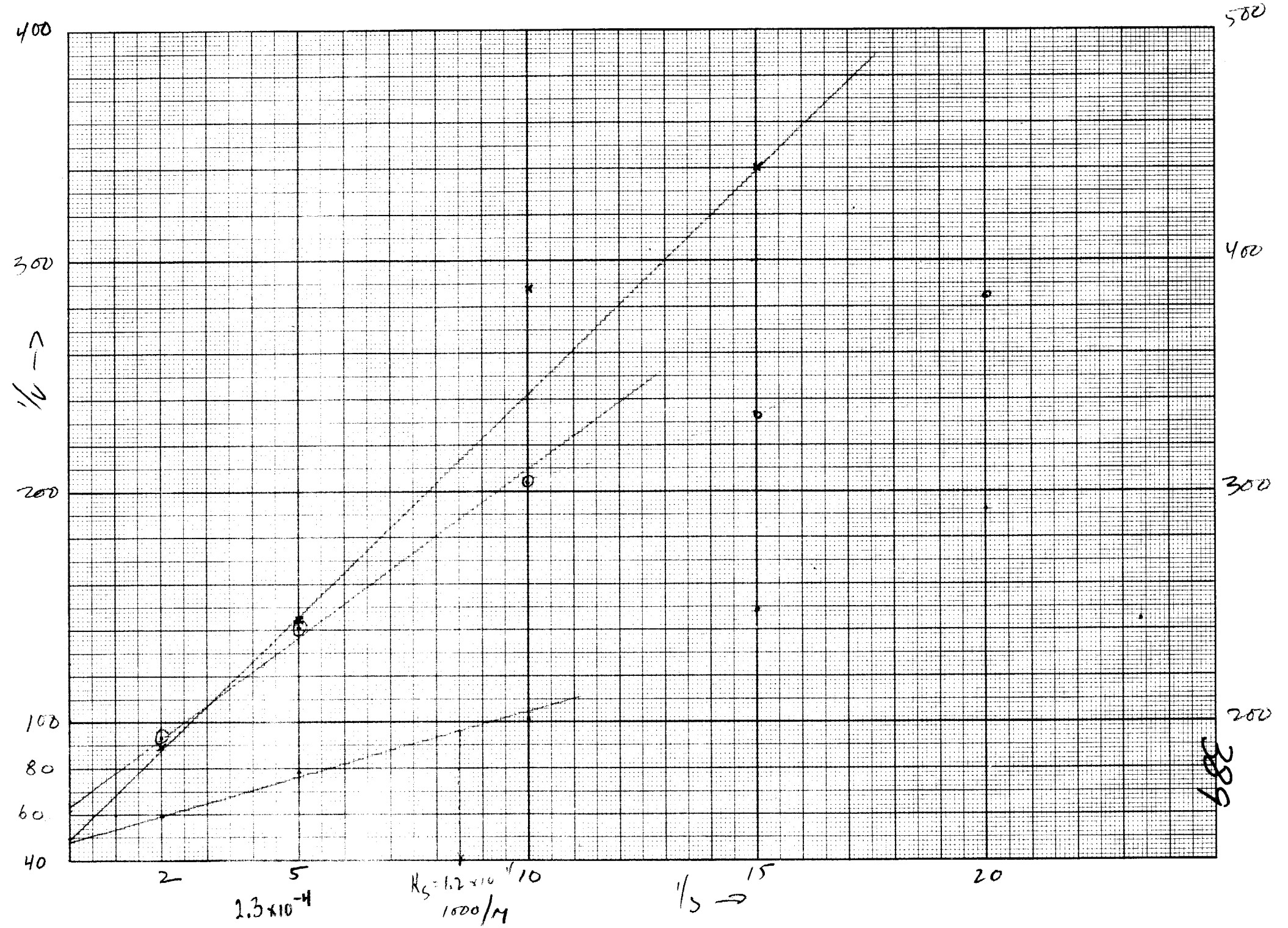
K_s / salt concentration

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

	ONPG 1022/M		1/v	I	+	Δ	
1.	2	NaPM/50	62.9	13	172	159	
2	5		84.7	4	122	118	
3	10		120.	0	83	83	
4	20		185	-3	51	54	
11	2	NaP M/100	64.9	18	172	154	
12	5		83.3	9	129	120	
13	10		125.	7	81	80	
14	20		208	3	51	48	
21	2	KP M/50	120	10	93	83	
22	5		185	10	64	54	
23	10		303	6	39	33	
24	20		417	0	24	24	Calculation of K_s
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 pipette in 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



Influence of metal ions on K_s (ONPG).

Dec. 14, 1948.

	ONPG 1000/M.		1/V		NaP M/50.	I	F 40 mins!	A	A/2 Final
20 mm	1.	2	59.5			11	179	168	
	2.	5	78.1			0	~~128~~	128	
	3.	10	101			1	100	99	
41 mm	4.	15	149			0	134	134	67
	5.	20	192			-3	101	101	52
					KP M/50.				
20 mm	11.	2	93.5			10	117	107	
	12.	5	141.			0	71	71	
	13.	10	204.			1	50	49	
41 mm	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 mm	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 mm
-4,5 40 mm

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

Glucose also causes an alteration of slope!

These data NVG enzyme prep low assay!

Used 388: 319A diluted 1:2.5

E. coli lactase.

12/17/48.

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

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

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

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

12/21/48.

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

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

Study heat inactivation in Na buffer.

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

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

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

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

December 14, 1948.

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

Succ 0 — NaP. M/50 pH 7.5

10 — KP " "

20 — KP " " + RbCl M/50.

	ONPG	1/V		Di.	D_f	Δ	
0	M/1000	29.1		20	363	343	
1	2000	32.5	$V_{max} = \frac{1}{25} = 400.$	12	320	308	$K_s = 1.3 \times 10^{-4}$
2	5000	41.5		0	241	241	
3	10000	58.1		-4	168	172	V_{max} =
4	15000	70.4		-3	139	142	
5	20000	98.0		-2	100	102	
10	1000	51.0		23	219	196	
11	2	58.5		11	182	171	
12	5	83.3	$V_{max} = \frac{1}{43} = 232.$	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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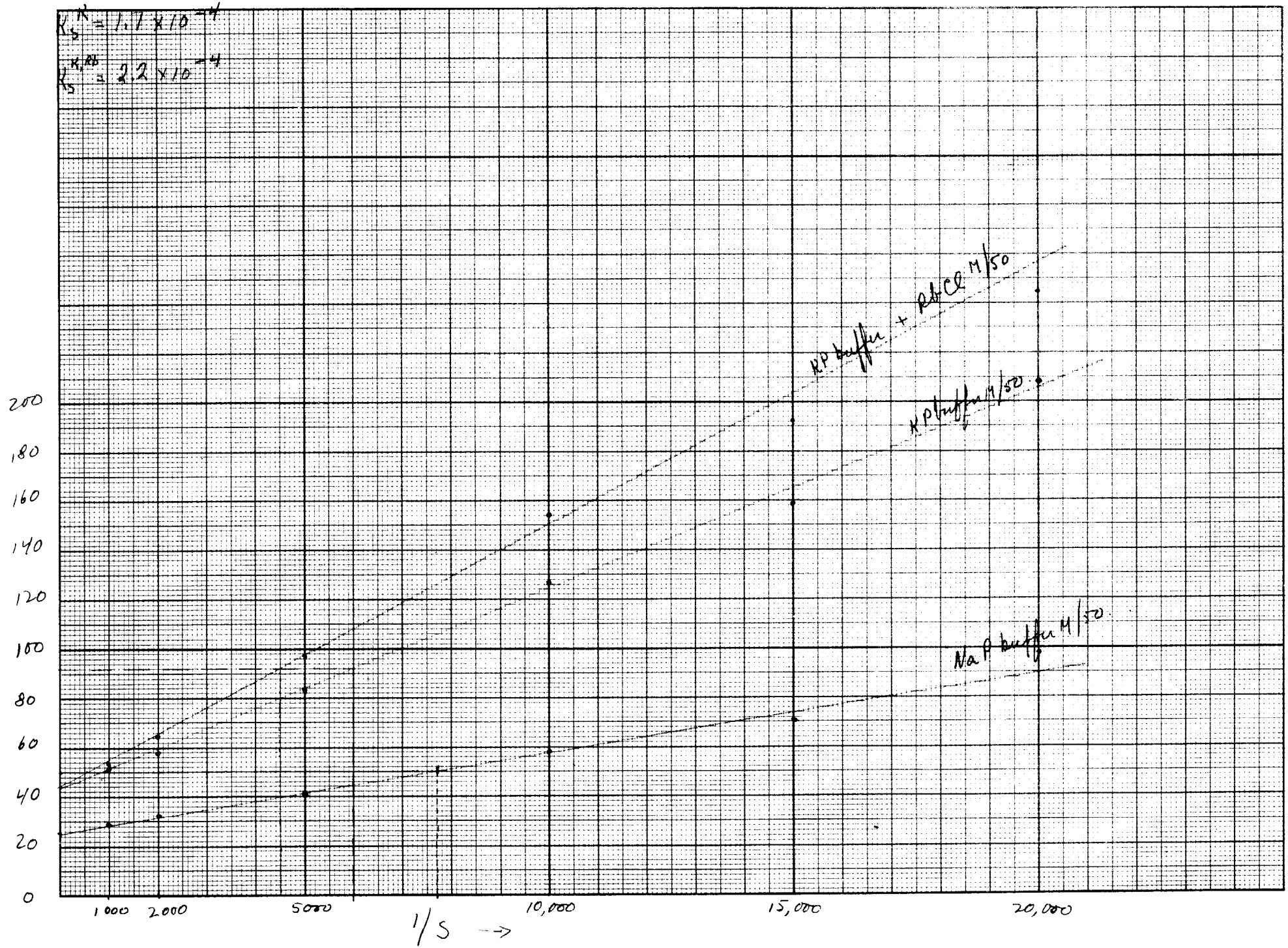
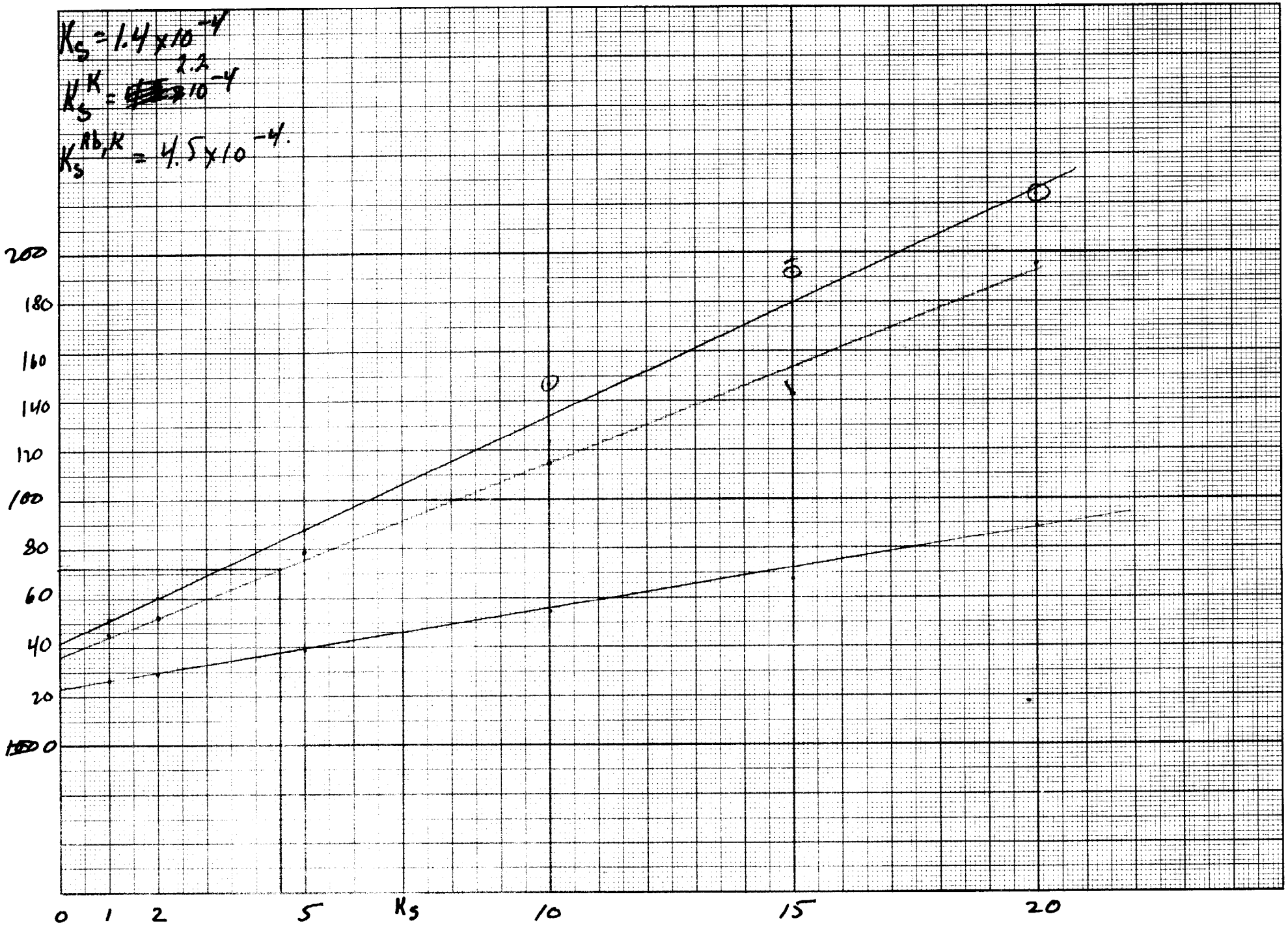
$K_S^{K} = 1.7 \times 10^{-4}$

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





Dec. 28, 1948.

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

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

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

In this expts., substrate + buffer are mixed up; enzyme is pipetted & diluted before dumping it in at To. Cf. 339 in which pipetted enzyme mixed effects (enzyme salts, substrate added 2nd.)

K-12 Lactose.

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 150 mg dry cells in 10 ml M/50 NaP. Shake 2 hrs. Remove solids + centrifuge to ppt in 1 hr. (C).
≏ 50 mg/ml wet cells

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

	Di		Δ
A. .001 ml	.003	241	243
B. .01 ml	.006	71 ; 77	77
C. .01 ml	~~.002~~ .002	59	59
D. ~~0.2 ml~~ 0.2 ml	.082	113	31

B .01 ppt ∴ Wet cells is only about ⅕ as efficient as
C .01 ml extract dry cells.

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

A-1 Lactates.

1/3/49.

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

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

Ion-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.
~~5~~.							
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 maybe 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 dur
400	.69	.93	1.38
420	.68	.91	1.43
450	.67+	.87	1.23
500	.65	.83	0.74
550	.65	.79	0.35
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_2O_5

#1 = W661 44g. wet paste → 10g.

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

Jan. 10, 1949.

Lactose adaptation in W-112 (Lac_i)

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

A glucose
B butyl galactoside
C lactose.

Wash + resuspend in 4 ml H_2O.

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

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

Checks by streaking out cells used.

Lac_i — produces lactase with butyl galactoside but not with lactose. Cf. Escherichia expts. showing same result with nitrophenyl galactoside.

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

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

18h. Bugal actively fermented; heavy growth. Whole space; w ferm in ½% Lac

Harvest + test:

a) spot plate ONPG: B: +++ L: —

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

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

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

Rb inhibition of lactase

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

b) "enzyme ONPG".

			10^{-3} mol.	
a)	1	319A	—	510
	2	"	Rb	470
	3	395	—	680
	4	"	Rb	630
	5	399	—	310
	6	"	Rb	309
b)	7	319		660
	8	"	Rb	650.

∴ no appreciable inhibition!

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

—	289
old RbCl	268
new RbCl	200

Rb inhibition of K-12 lactase.

1/15/49.

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

Rb inhibition

January 14, 1949.

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

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

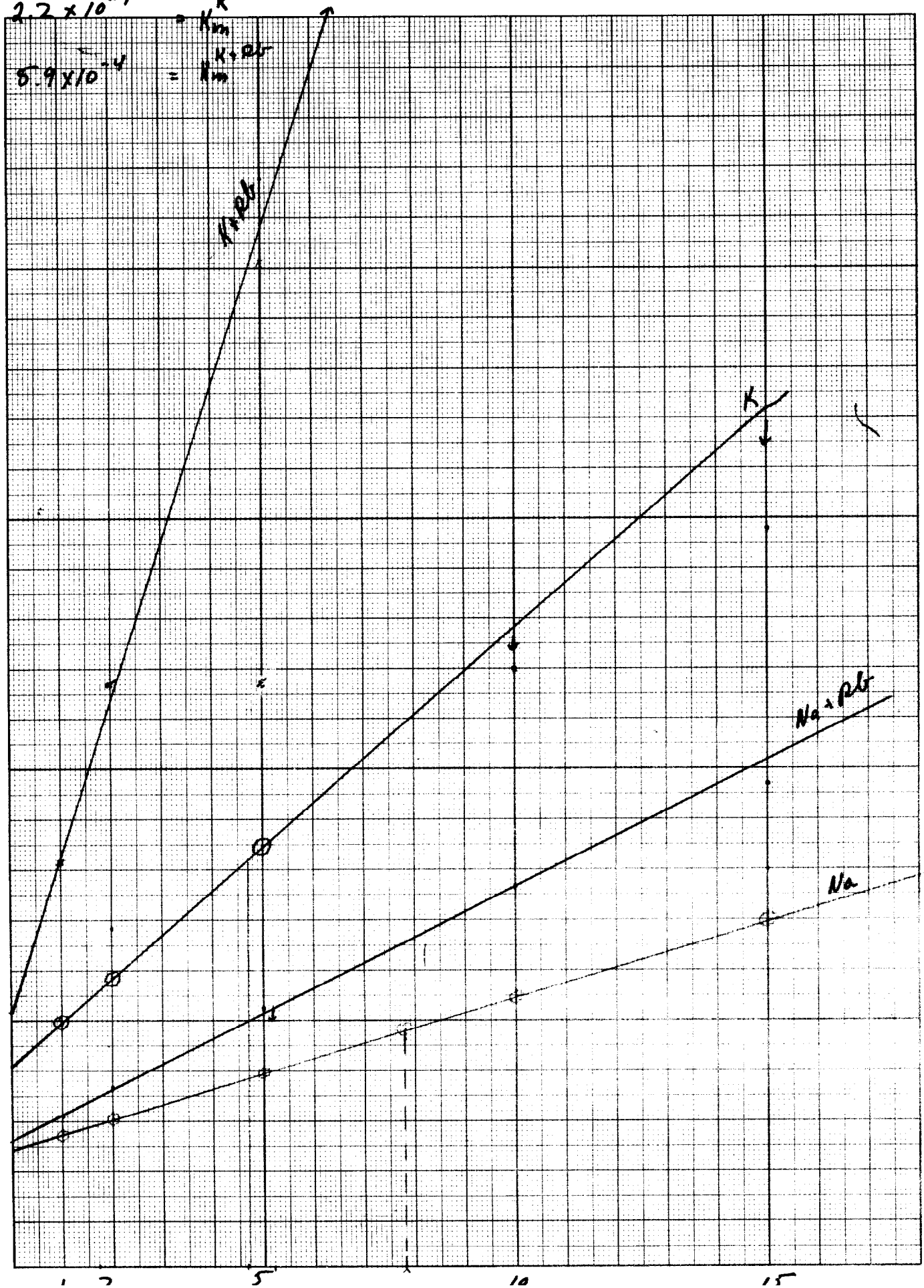
$1.28 \times 10^{-4} = K_m^{Na}$

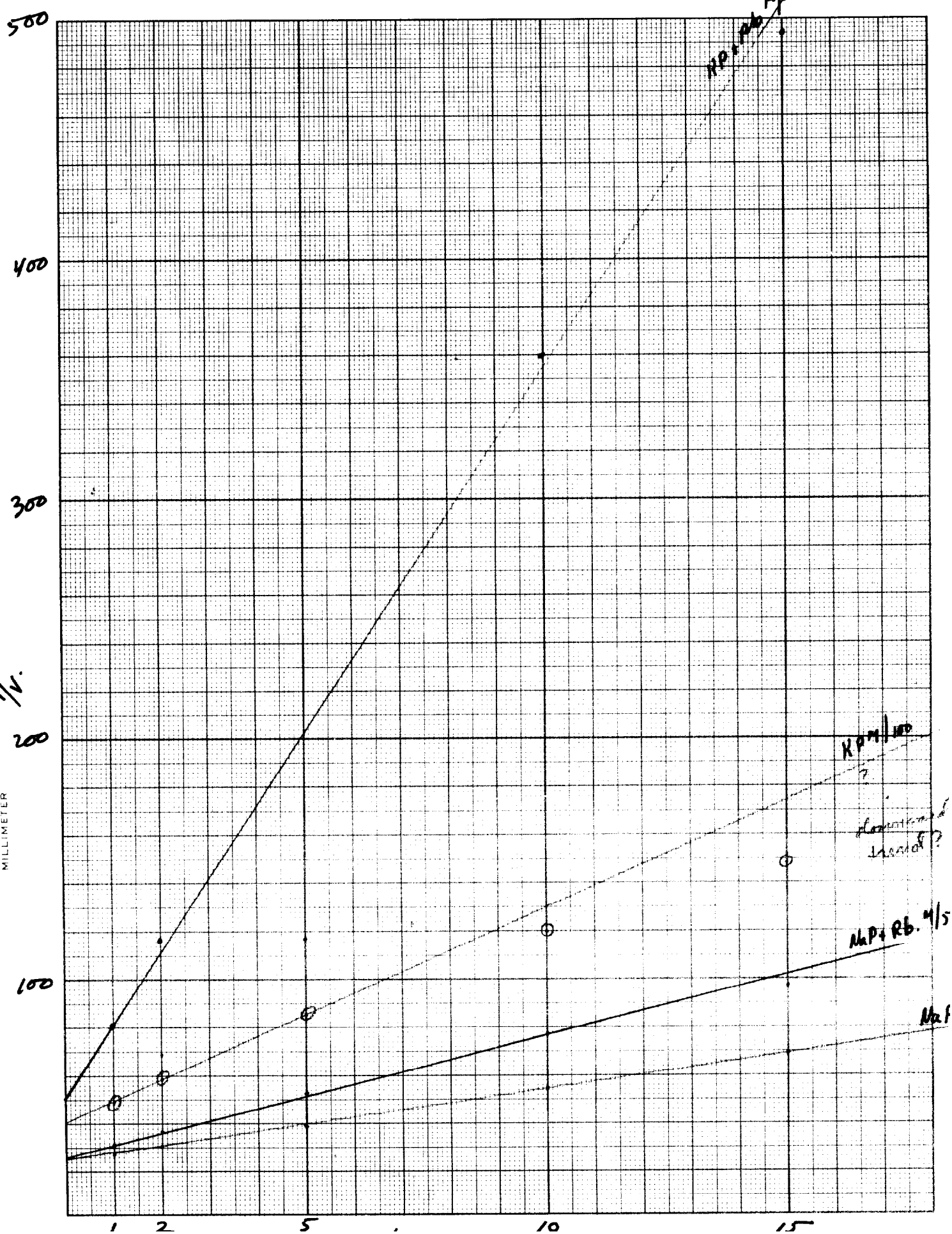
$2.2 \times 10^{-4} = K_m^{K}$

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

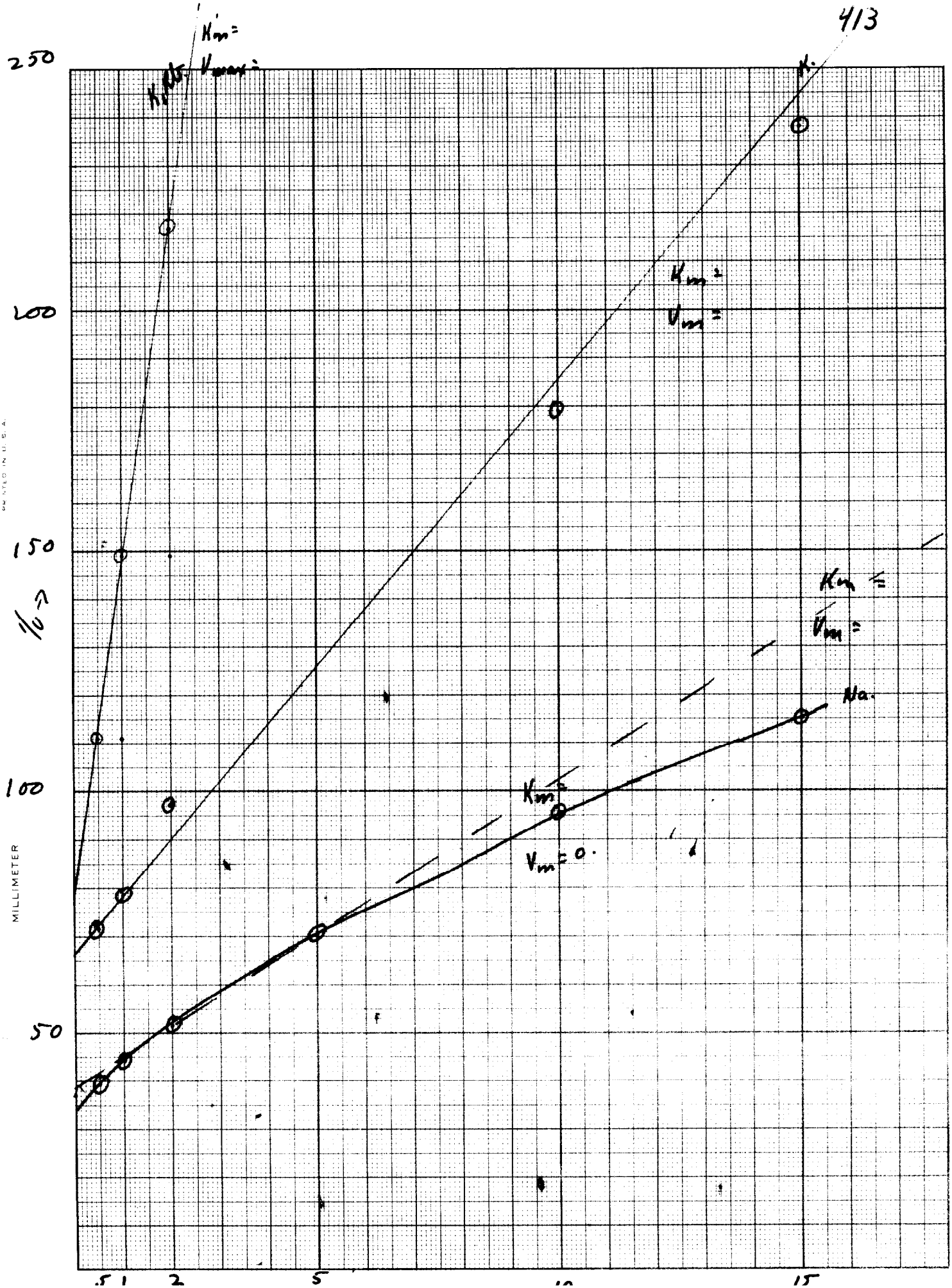
$1.92 = K_m^{Na+Rb}$

411





1/V.



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

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

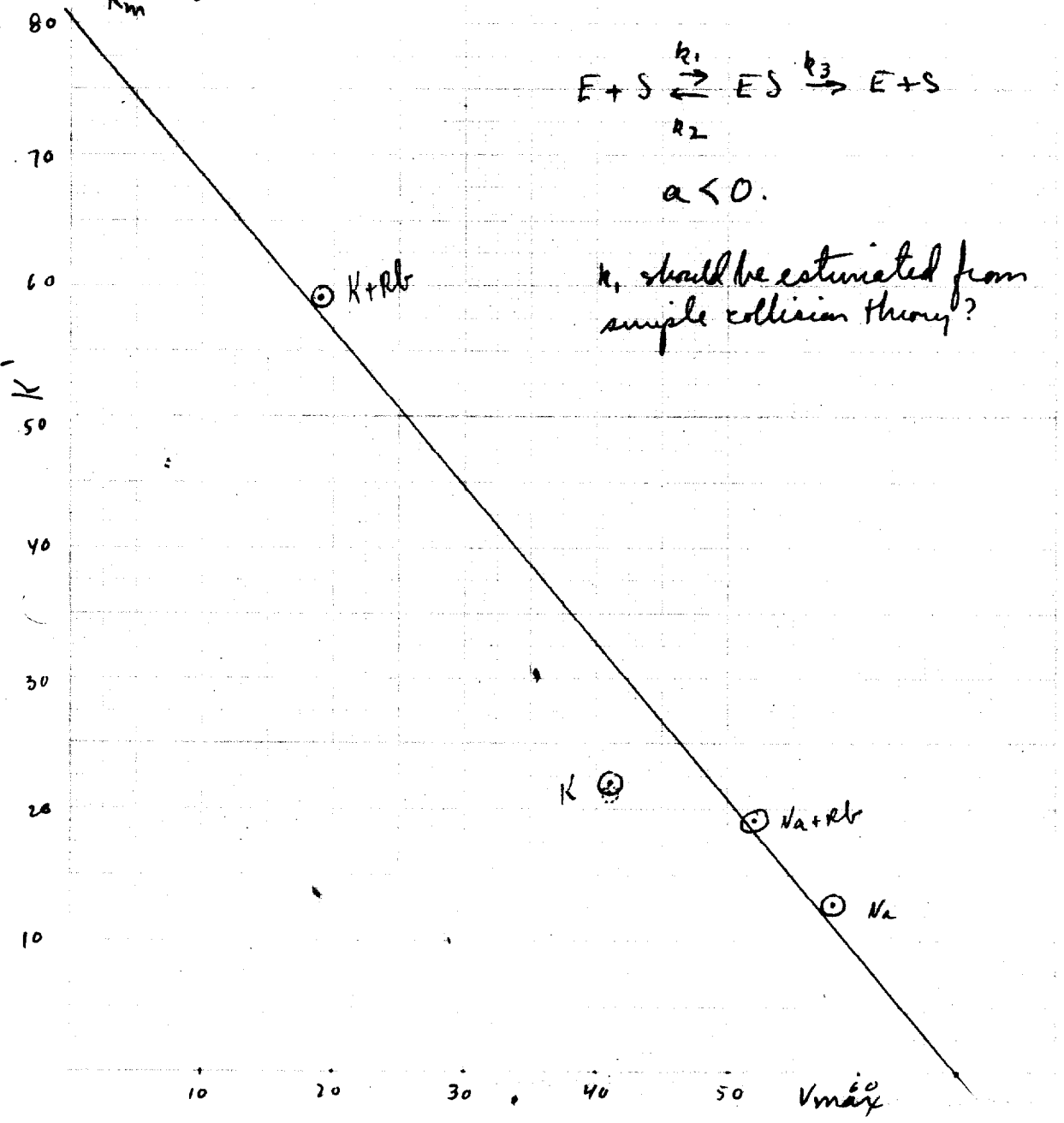
k_a = " -1.2 "

$K_m = 80$

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

$a < 0$.

k_1 should be estimated from simple collision theory?



January 16, 1949.

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

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

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

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

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

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

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

The enzyme dilutions + other preps stood at room temperature ~~at rest~~ for several hours. This may acc't for the exc. variations seen.

Note. 5 ÷ .

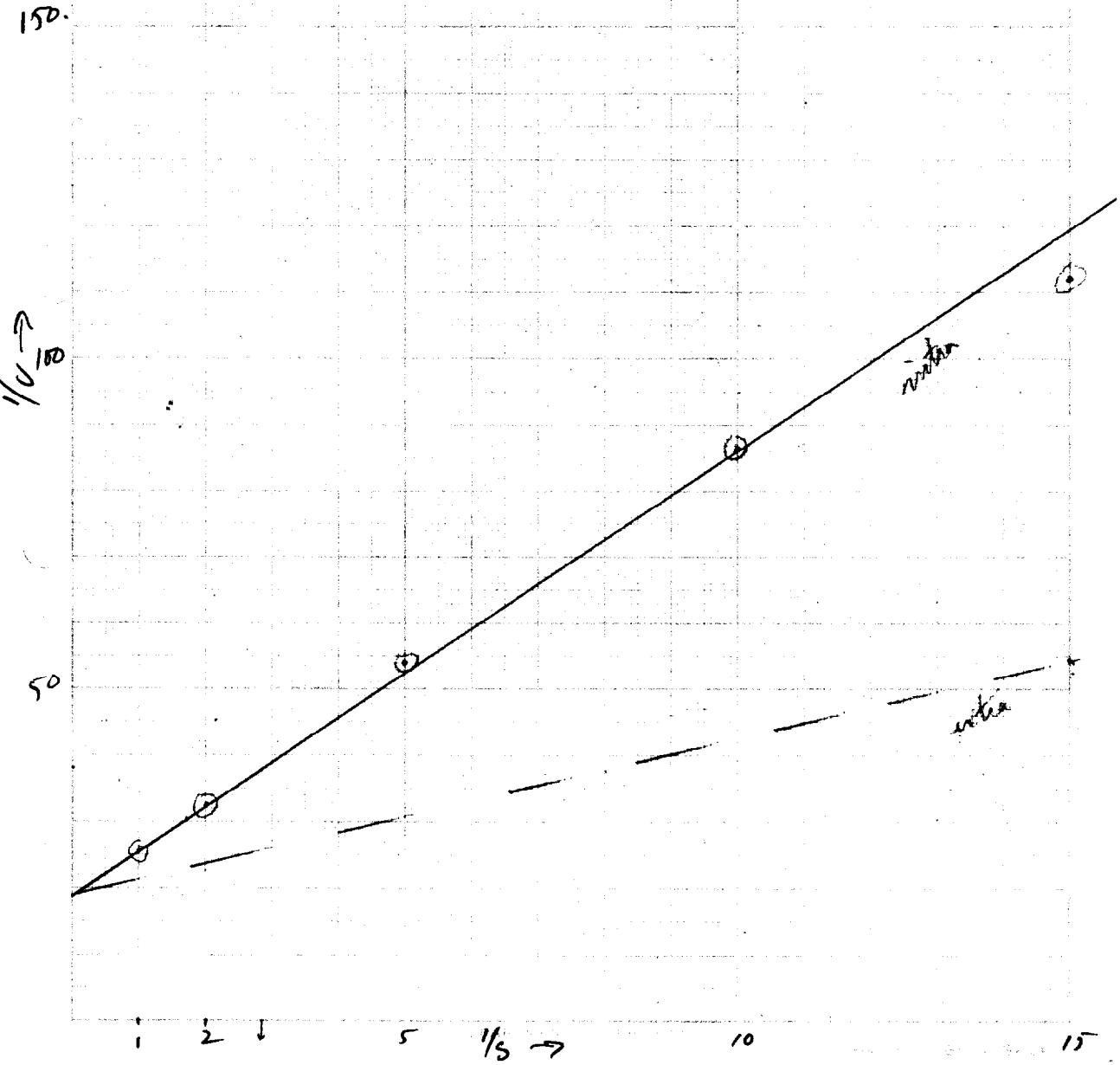
Note M/... [illegible] ÷ .

$K_m = 4.5 \times 10^{-4}$ 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 100ml Y2 Lactose broth. Resuspend in ca 20 ml.

Preliminary assay: 10 min. in NaP M/100 7.5

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

Ca 40γ/ml. Relative activity 20m. 4

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

a) pH optimum. Use M/100 Kphos buffer M/50 NaCl. 0.006 M/5000 unless otherwise stated

	pH	Δ		Δ hr.
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

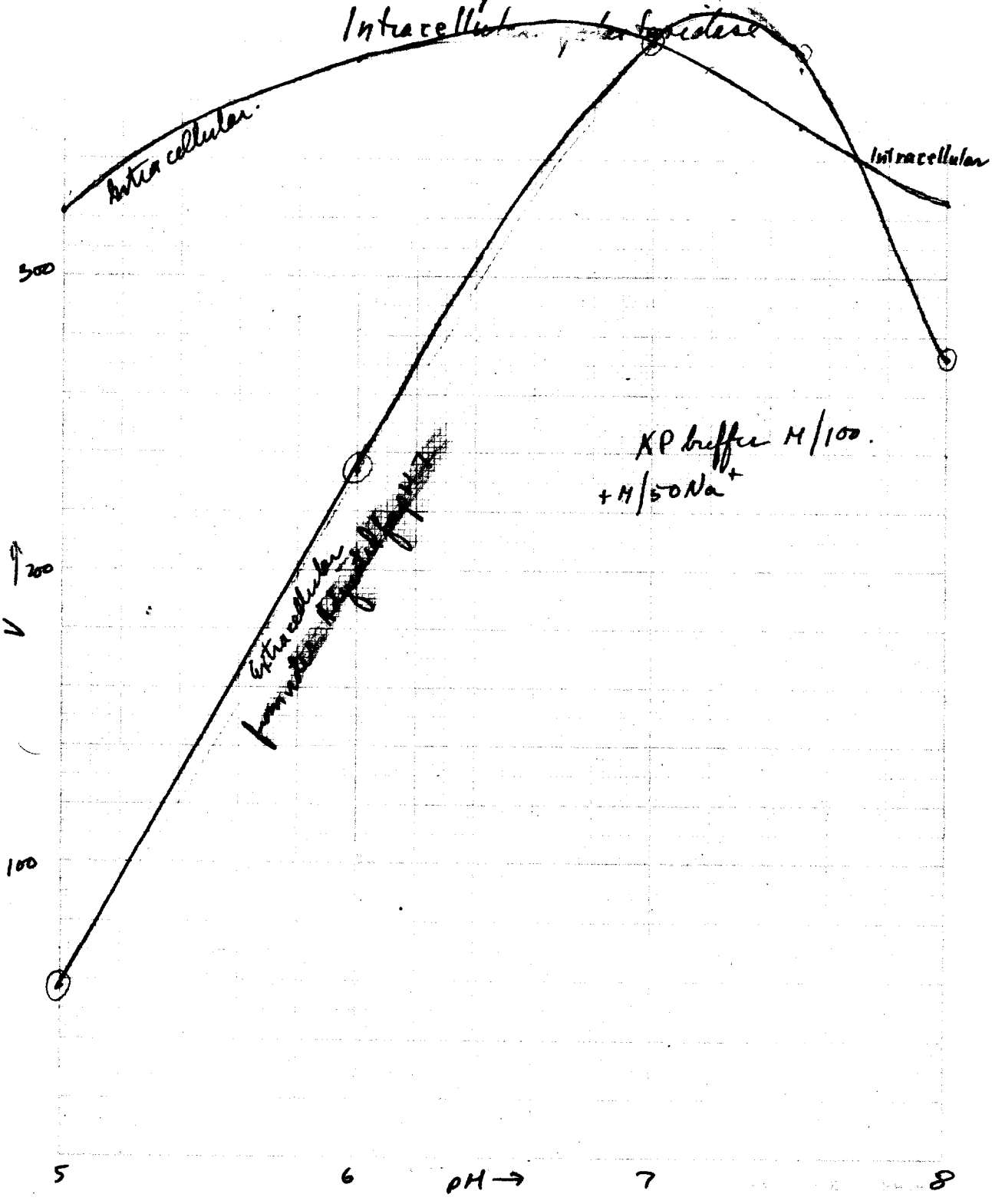
K_m = 4.5×10^{-4}

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

Adaptation of ML; K-12 on galactose

		Δ_1	Δ_2	[12:45 AM] Corrected:		20 m.		315 PM	20 m. R.A.	37. R.A.
Mhm.	gal	20	301	180	~~200~~	200	≡	481	11	22
	lac	308	—	180	200	488		1100+	171	
	glu	—	126	95.5	105	84 ?		121	<0	18
K-12	gal	60	424	~~116~~ ≡	129	176		540	52	49
	lac	~~128~~	—	111	123	339		970	206	—
	glu	002.	30	117	130	119		147	~~<1~~ <2	3.4

blanks —

Glucose cells may have grown and/or 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 Y2 - 1% sugar broth and resuspend in Butyl Galactoside ½%. Tubes ± BCP indicator. Also check counts on EMB Lac & Bugal plates.

	Lac		Gal		Bug.		BCP Bugal.	Glu
K-12	114 680 514		150 298 120		147 1000 590		++ 120 -10	131
W108	102 518	+	105 150	+	126 1100	+	85	112 ✓
W45	110 122	✓	140 146	✓	83 120	✓	- 140	150 ✓
W112	106 160 (30)	✓	117 196 49	✓	210 870 310	✓	++ 123 -	134 ✓
W255	127 1050 800+	=	89 386 305	=	93 930 1000	=	- 86 -6	104 =

Substrate: 33

ONPG readings: For K-12 with Lac as 100%
initial in —
final in —
R.A. —
Bugal. 115%
Galactose 22%

1:30P-

✓ 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. Cmen 108/Glu had 1%+

Adaptation to related substrates

Harvest K-12 from 1% sugar Y2 broths 10 ml quants 5:20 PM

	Di	Di cor.	Δ	Δ/Di cor. = R.A.	Df 7:15 PM	Δ'	R.A.
✓ Glucose	141	135	139	−004	147	008	
✓ Galactose	187	250	180	+ 70	810	630	—
✓ Lactose	153	470	150	320	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 %	

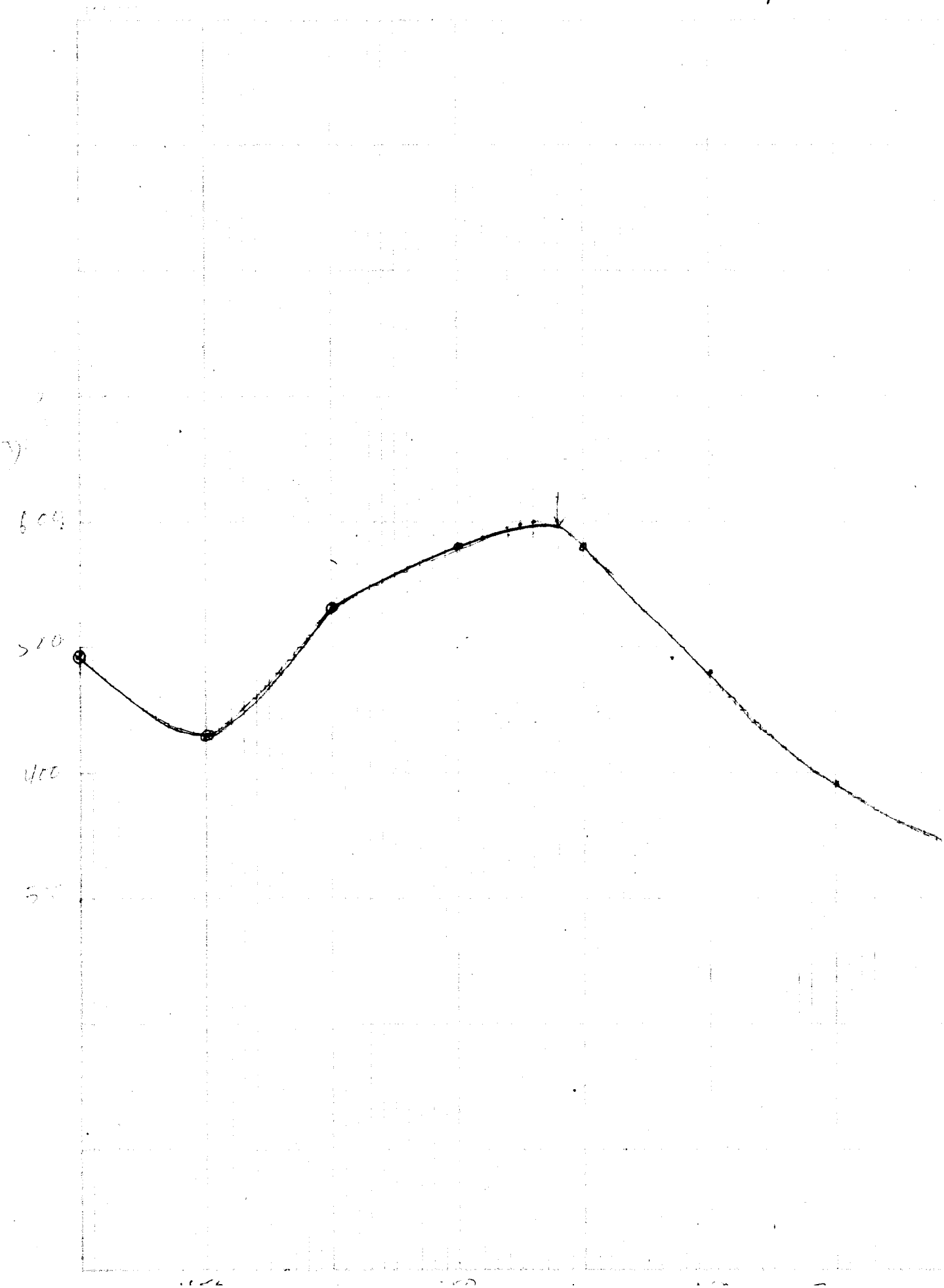
Absorption spectrum of E. coli + formazan. (tetrazolium)

423.

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

Jan 25, 1949.

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



Sugar utilization by W815.

Feb. 28, 1949.

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

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

Time (m).	L/Lac	L/Glu	L/Gal	G/Lac	G/Glu
15	+	−	∓	−	−
20	+++	±	+	±	±
~~25~~ 45	+++	±	+	±	±
60	++++	±	+	±	±

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

Galactosidase in W815.

446b.

3/1/49.

Harvest cells from 42 Lac and 42 Glu. dilute, etc. + tet ≡ M/2000 ONPG KP 7.5 M/50.

	D_i^{420}	corr.	D_t	R.A.
Glu	300	270	280	< [redacted]
Lac.	436	—	>> 1000.	> 300

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

Lactase of W815 cf. K-12.

3/2/49.

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

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

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

10:45

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

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

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

Carbohydrate utilization by W815.

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 1ml cell suspension and 1ml buffer BCP. (1M)

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

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

[Is glucose accumulated from lactose? Cf. W255 and W815 grown on lactose. Also W1089 L_3 + J.

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

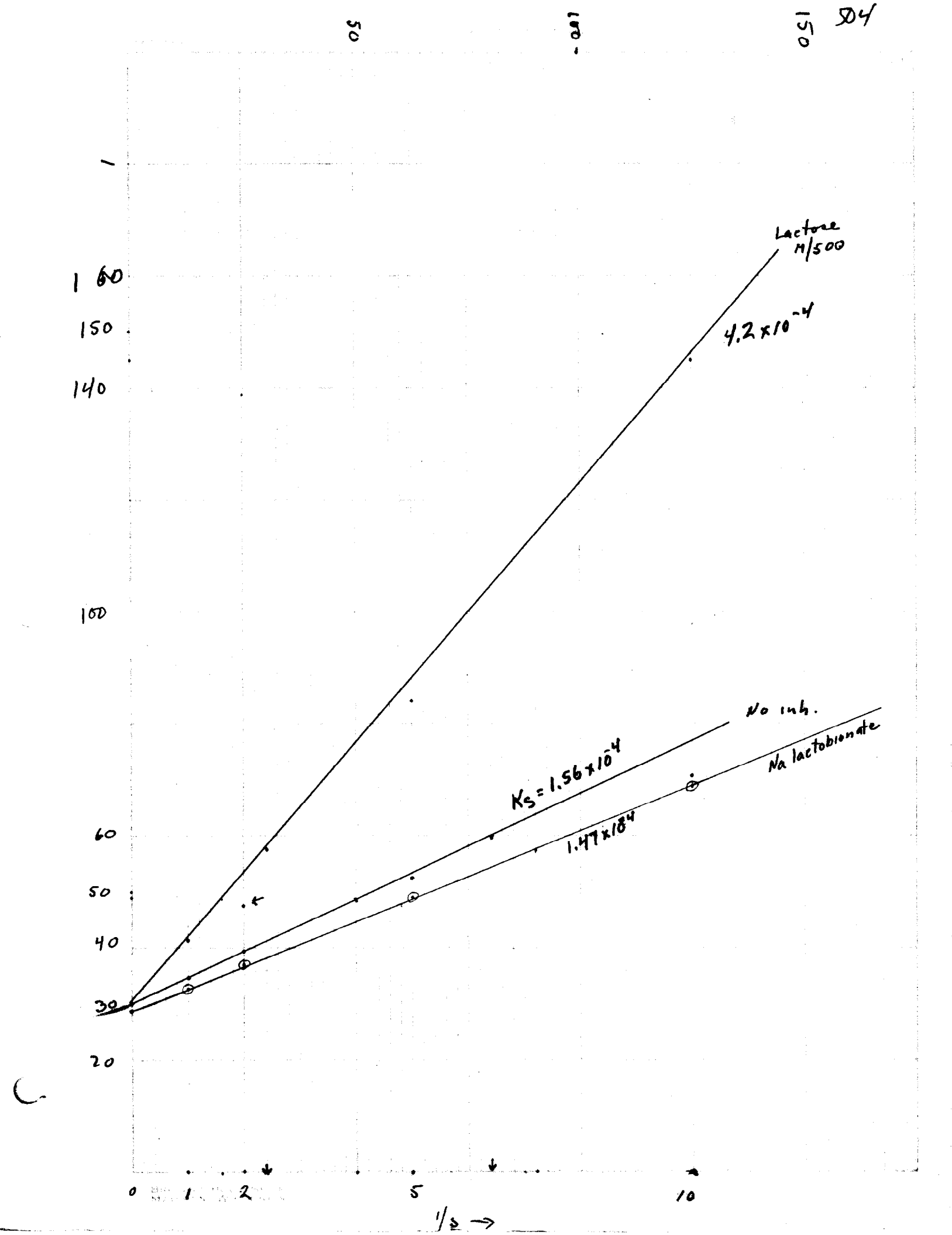
Competitive inhibition of galactosidase

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	D_f	D_{cor}	1/v
1	100		020	307 ✓	289	34.6
2	200		010	263	254	39.4
3	600		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	32.1
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 ext. to 9 ml. Add 1 ml enzyme dilution at t_0. 36°.


Lactose M/500
4.2 x 10⁻⁴
No inh.
Na lactobionate
Ks = 1.56 x 10⁻⁴
1.47 x 10⁻⁴
1/s →

	Di		Df
1. —	177 ~~181 182~~	550	
2. Azide	178	520	
3. Lac	180	540	
4. Azide + Lac	190	570	

Glycerin.

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

Competitive Inhibition

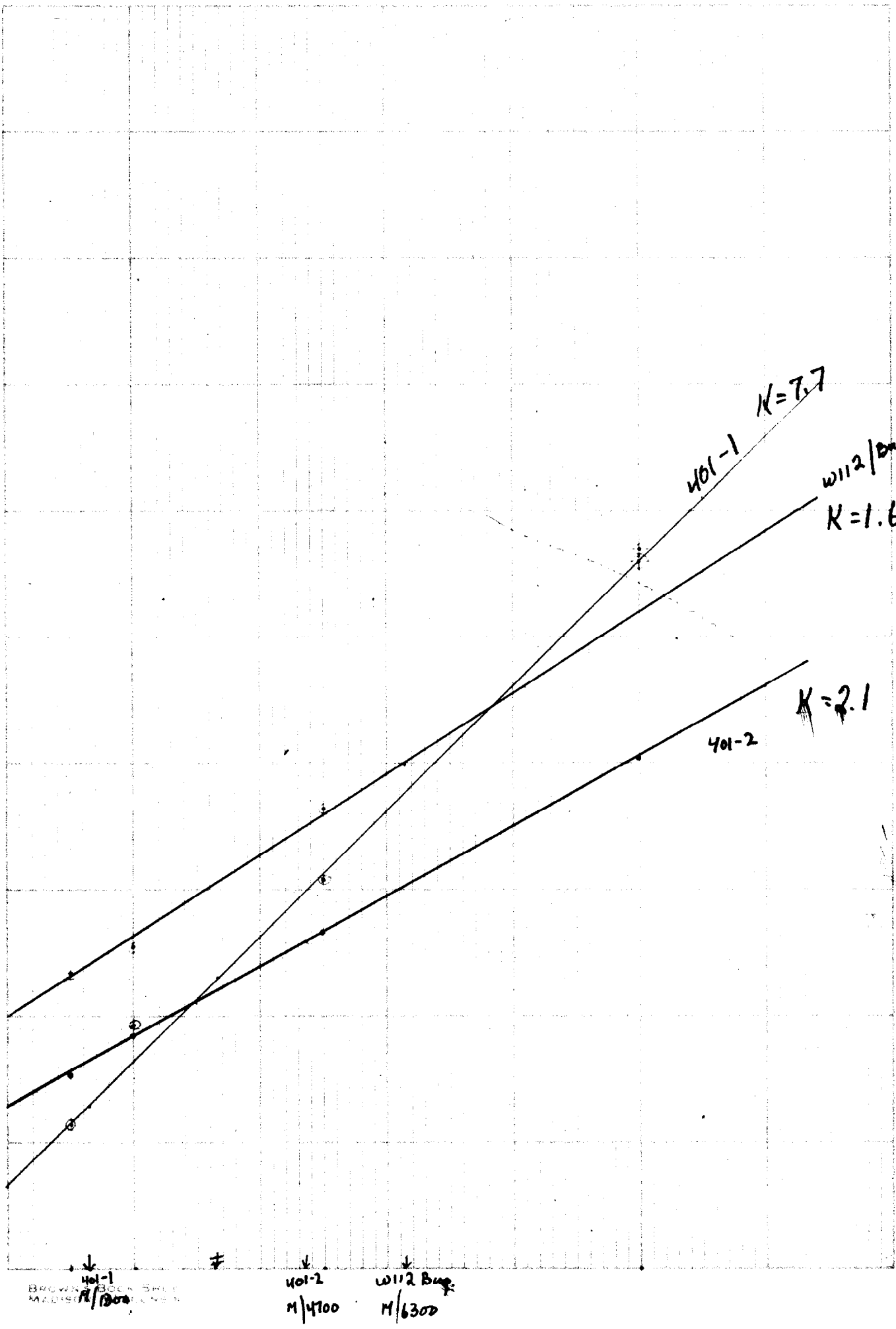
4/4/49.

	[399 1:200]		NaP 7.5 M/50 + Na_2SO_4 M/50 · 1/v.v.			
1		—	023	387	368	27.2
A 2			010	339	330	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		Bregal	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

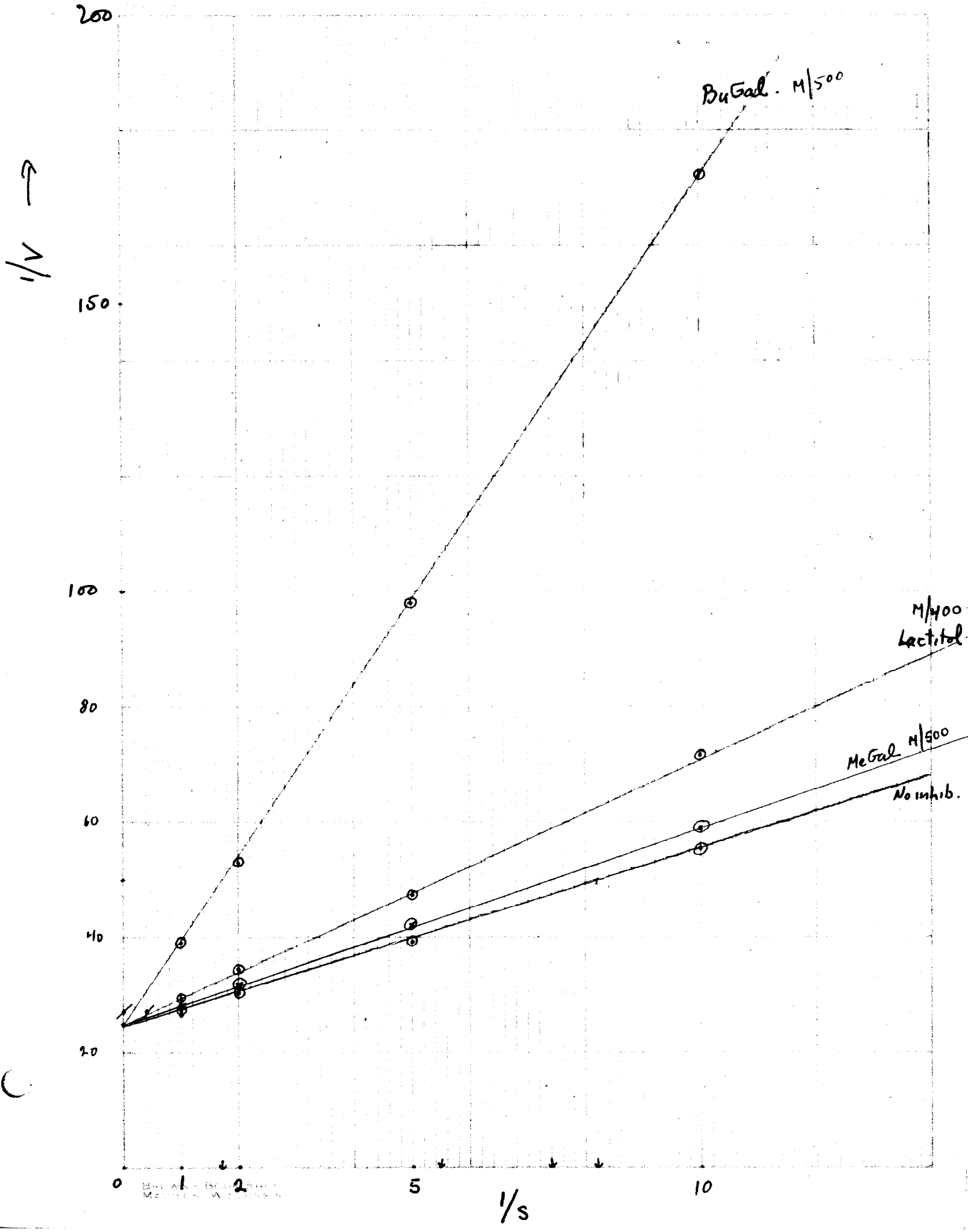
correction is increase from 9 to 11 ml. Subtract 9/11 of Di from Df.

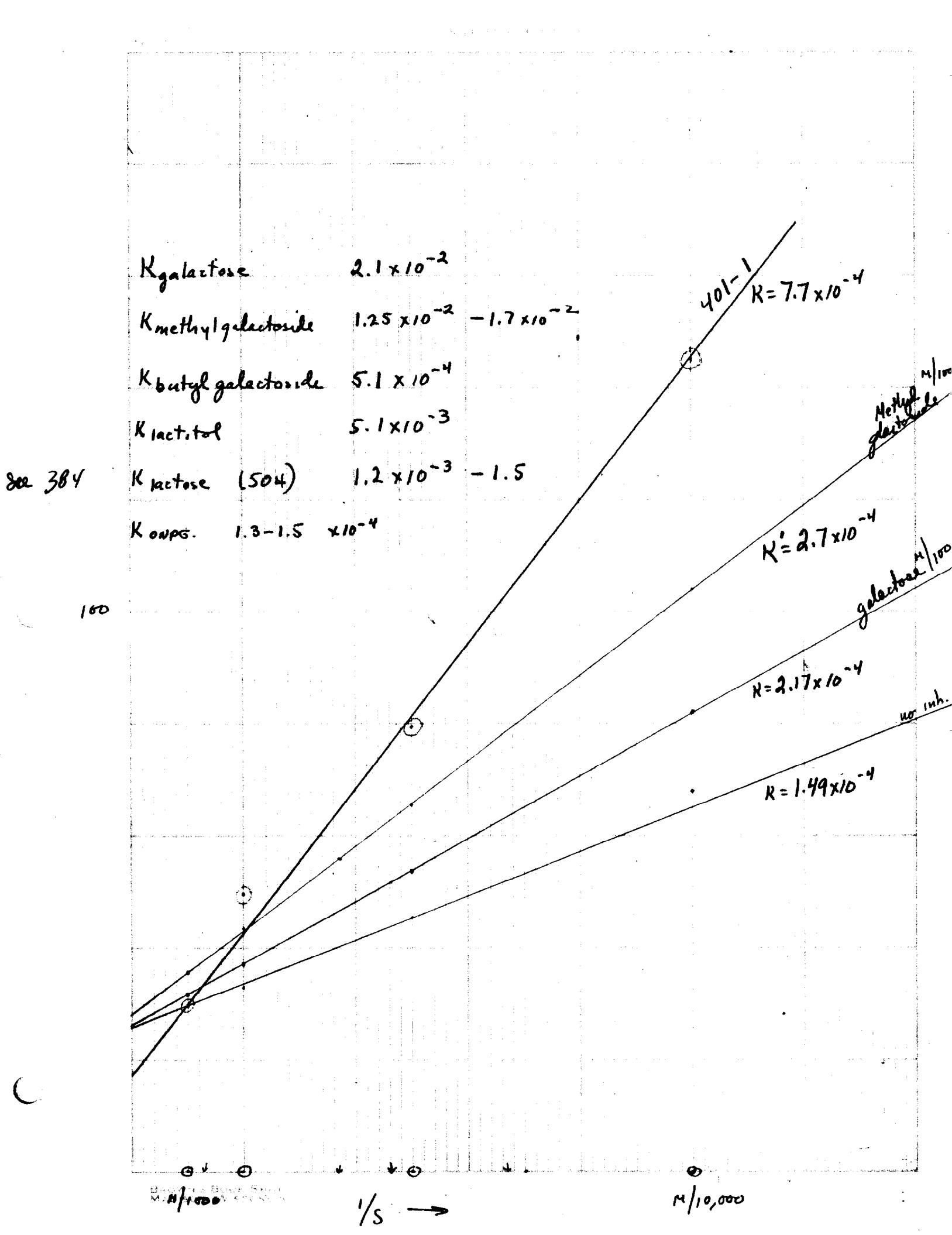
Apparent K_m : $\times 10^{-4}$

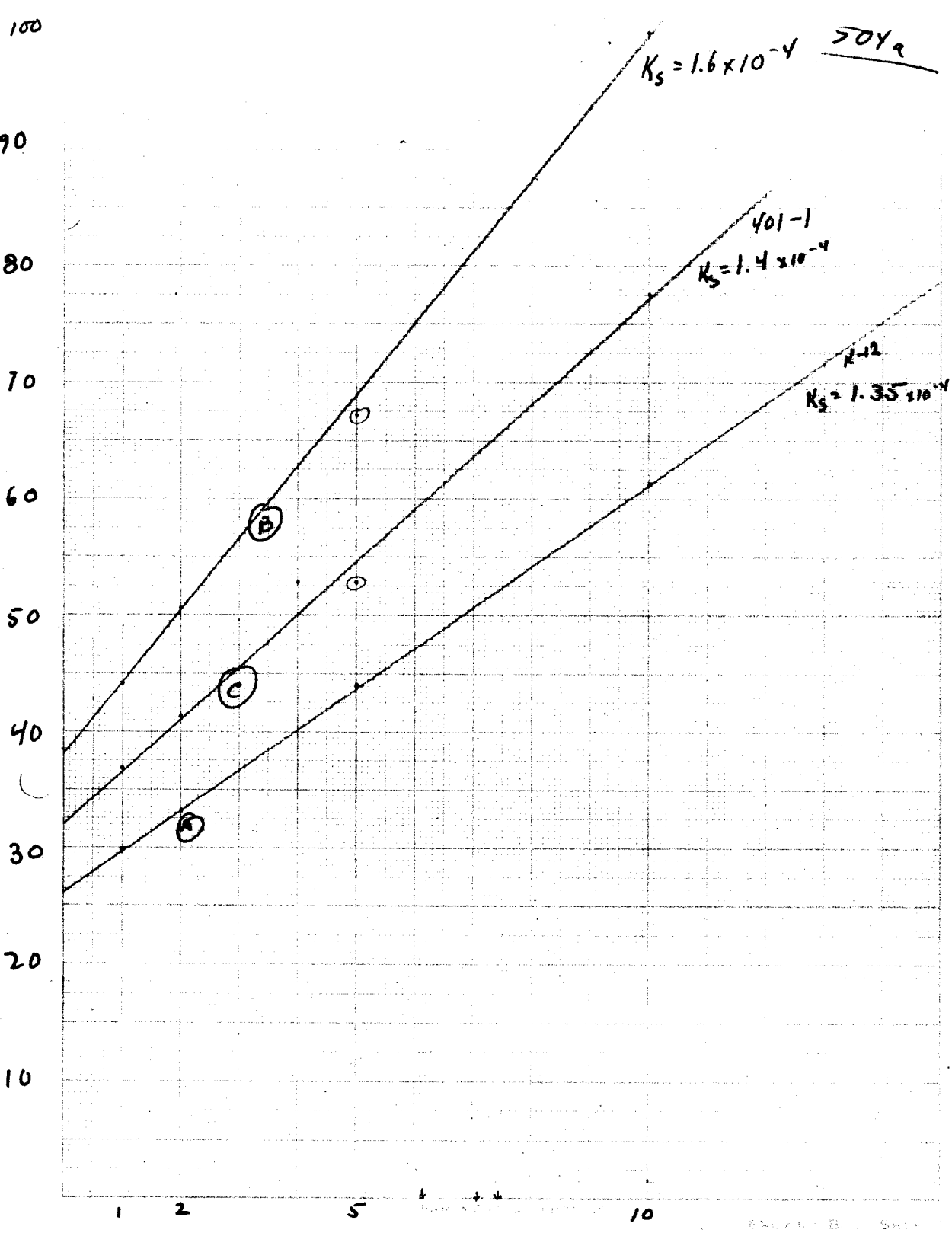
Blank:	1.22
Megalactoside	1.35
Bugalactoside	5.9
Lactitol	1.82


401-1
K=7.7
w112/Bergel.
K=1.6
K=2.1
401-2
401-1
M/8000
401-2
M/4700
w112 Bergel
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	Galastone	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]	0028	239	216	46.3				
Bengal.	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				
	8Y1		207	361						
	S1		202	451						
	S2		232	880						
	Y70		160	220						


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


$K_{galactose}$ 2.1×10^{-2}
$K_{methylgalactoside}$ $1.25 \times 10^{-2} - 1.7 \times 10^{-2}$
$K_{butylgalactoside}$ 5.1×10^{-4}
$K_{lactitol}$ 5.1×10^{-3}
$K_{lactose}$ (504) $1.2 \times 10^{-3} - 1.5$
K_{ONPG} $1.3-1.5 \times 10^{-4}$
See 384
160
401-1
$K = 7.7 \times 10^{-4}$
M/100
Methyl galactoside
$K' = 2.7 \times 10^{-4}$
galactose M/100
$K = 2.17 \times 10^{-4}$
no inh.
$K = 1.49 \times 10^{-4}$
M/1000
1/S →
M/10,000


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
$K_s = 1.4 \times 10^{-4}$
401-12
$K_s = 1.35 \times 10^{-4}$
A
B
C

Kinetics of suppressor lactases 504a

4/7/49

		$1/S$ ONPG 100/M	NaP 7.5 M/100. Di	Df		$V_{corr.}$	$1/V$			K_s	V_{max}
A	1	1	017	358		344	29.9				
399	2	2	009	309		302	33.1			1.35	
(K-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				
(401-1)	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				
(401)	2	2	011	251		242	41.3			1.4	
2	3	5	003	192		189	52.9				
	4	10	006	134		129	77.5				
D	1	1	019	760		744					
W112	2	2	013	680		669				excessive	
Buged. prep.	3	5	003	500		500				enzyme	
10mins.	4	10	006	331.		325					

E

F

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

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.

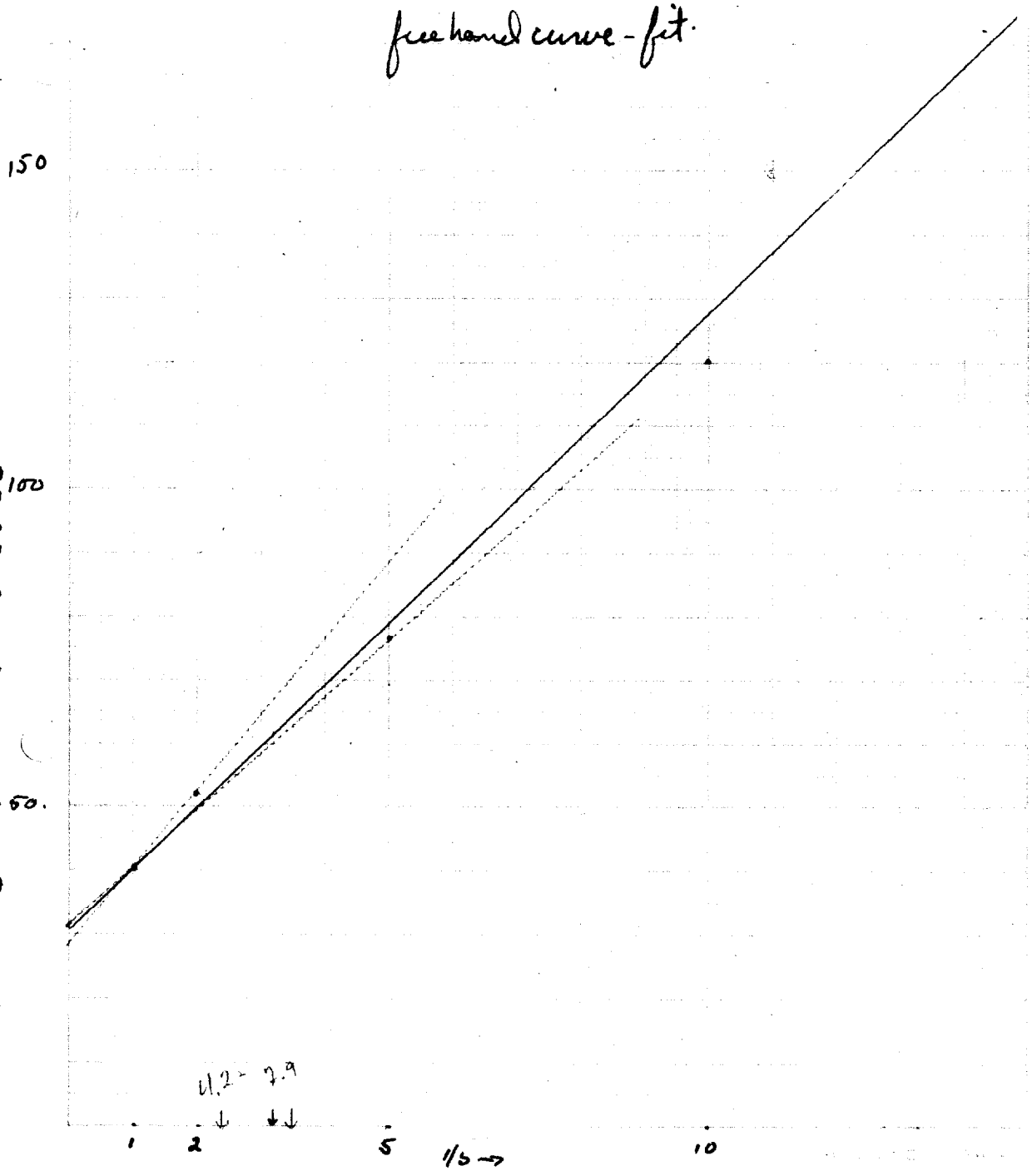
K-12: amylomaltase

4/5/49.

Grow K-12 in 2x 50ml Y2 Mal 1% Harvest and dry over P_2O_5. Yield: 29 mg.

Intracellular lactase.

freehand curve-fit.



Kinetics of cellular lactase

4/6/49.

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

ONPG	Rdg.	Est. Con.	V + Or.	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		

$V_{max} = 322.$

$K_m = 3.2 \times 10^{-4}$

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 NaPM/100

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

	D_i 4:41 PM	D_{20}		V_{corr}	D_{31}
1	22		461	342	
2	25		307	287	
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.

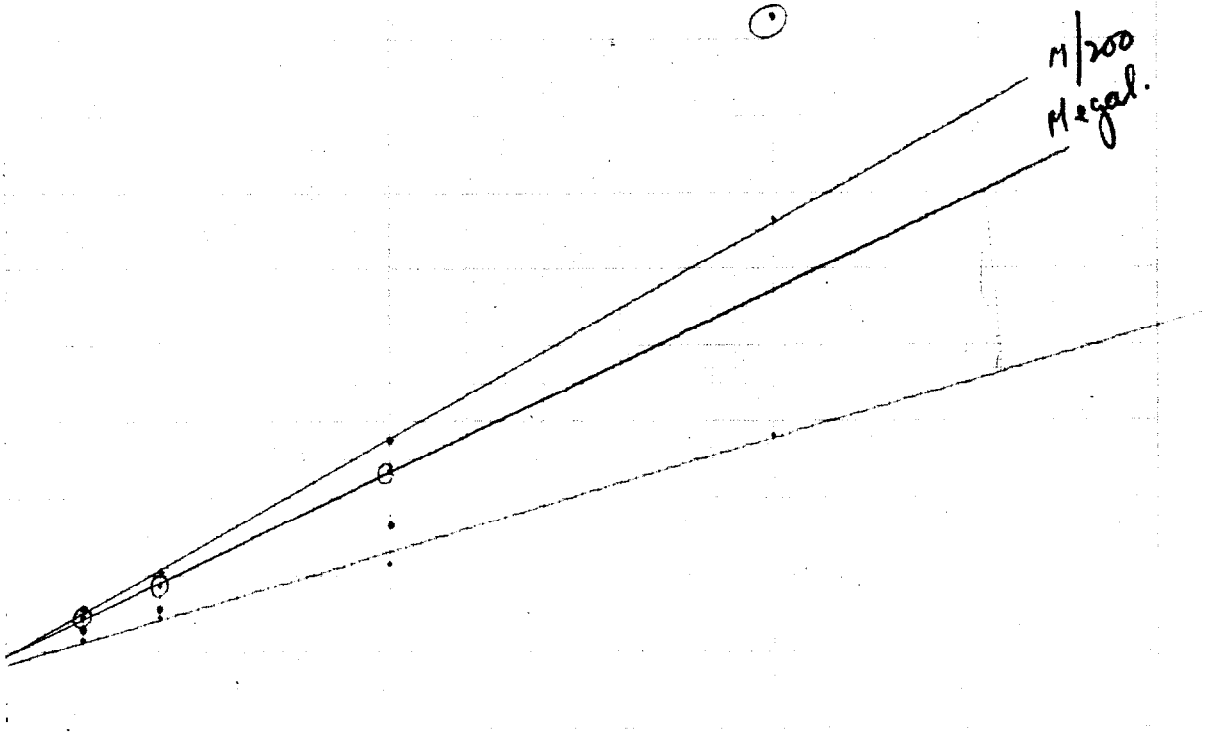
2:25 K-12 + ω-349 grown on lactose tested on lactose; lactitol.

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

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

~~E+~~

510



4/7/49.

NaP M/100 7.5 20 mins 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						
	2B	2	Megal	019	421	406	24.6	
	3	5	M/200	009	353	346	28.9	??
	4	10		-001	229	230	43.5	
				003	200	97	103.1	
506 1:150	1	1						
	2C	2	~~Meg~~	020	451	435	23.0	
	3	5		011	400	391	25.6	
	4	10		007	281	275	36.5	
				-004	204	208	48.1	
							25.8	
	1	1						
	2D	2	Megal	020	404	~~388~~	~~20.5~~	
	3	5		010	337	~~329~~	~~30.4~~ 30.4	
	4	10	M/200.	008	217	210	47.6	
				-003	128	131	~~76.1~~	
							76.3	

Data n.g. — expt. made repetition.

Test for induction period in cellular utilization of ONPG.

4/8/49.

Harvest K-12 from Y2 tac. Conc. 5x. Use .2 ml/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		D	
	–	134		30	263
	t_0 calc.	131			
	~~t_0~~	~~131~~		60	270
	20	121 (mixing).			
	30	137		90	271
	40	141			
	60	146	12	120	282
	70	148	1 hour		
	100	149			
	120	159	13		293
	140	162			
	160	167	14		307
	180	170			
	200	177	15		319
	220	180			
	240	184	16		330
	270	190	17		341
	300	197	18		357
6 m.					
	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 Y2 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	non linear
.9	001	318	302	non linear
1.0	001	354	338	non linear
0	+ Substrate	016		

Subtract 016 from all Df. and Di from Df for Vcor

#8. − .025 on this one.

Galactokinase in Hba-adapted cells.

4/29/49

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

		2:10 PM Di	2:00
1	Lac	119	800
2	Hba	106	126
3	Gal (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. ~~Hba tube~~ Hba does not adapt to ONPG. after hours, progressive color in controls, ca 50. Probably adaptation.

Effect of azide on pH sensitivity.

Compare activity of Lac adapted cells above in KPM/5 buffer pH 5.0 and 7.0 all tubes receive M/50 Na_2SO_4 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		$1/V_0$	$1/V_i$
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:

$$K_I = \frac{I}{\left[\frac{1/V_0\left(1-\frac{S}{K_S+S}\right)}{1/V_i - 1/V_0}\right]} \qquad \frac{K_I}{I} = k_i = \frac{V_i k_s}{(V_0-V_i)(1+k_s)}$$

$$= \frac{M}{100}\left[\frac{40}{24.5}\left(1-\frac{1/10^{-4}}{2.3\times10^{-4}}\right)\right] \qquad = \frac{155}{95}\,\frac{1.3}{2.3}$$

$$= M/100\left[\frac{40}{24.5}\left(1-\frac{1}{2.3}\right)\right] \qquad = .83$$

$$= \frac{(40)(.57)}{24.5}\times10^{-2} = .93\times10^{-2} \qquad = 8.3\times10^{-3}$$

(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 ē purified lactobionate from F. Stodola. NaPM/50 pH 7.0

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

ONG added. Concn. .010

$$\frac{K_I}{I} = \frac{V_i\, k_s}{(V_0 - V_i)(1 + k_s)} = \frac{359 \times 1.3}{(41)(2B)} = \frac{367}{23} \cdot \frac{1.3}{2.3} \cdot M/200$$

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

use in $= 4.7 \times 10^{-2}$

Concentration effects on adaptation

4/20/49.

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

		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 shakers). Conc. ca 5×; use .1 ml/10 ml tube in assaying for galactokinase.

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 dilutions

	Di	De	V_{con}			
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}

4/23/49

			Di	Df	
1	K12	V2Lac	090	349	
2	"	" Glu	090	087	
3	K12	Bug M/500	120	790	(7 min. reading!)
4	"	M/1000	99	529	
5	W112	V2Lac	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 min.)

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

		D_i cells	D_i con		Δ	Δ/D_i	R.A. 20 min.	
K-12	A 1	70	73	281	208	297	600	(470) 514
	2	110	109	223	114	104	200	(800)
	3	81	83	470	387	478	950	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	(760) 310

These cells are shaken, and thus presumably aerobic!
Compare earlier data which showed a wider discrepancy.

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

Much greater differentials.

Compare Y10 (K) and W112 (Lac-)

April 25.

Without shelving:				20 min. kata		
			D_i^{Y10}		Δ_{cor}	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 Blu	047	056	01	002
W112	1		072	086	08	011
	2		109	119	08	007
	3		97	143	43	044
		Blank + oxpg		013		
Shaleen:				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		~~076~~ 103	120	014	014
	3		122	262	139	114

These data can be used:

	M/500 Lac	M/500 Bug	M/50 Blu
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	160	441	351-22	341	329	439
Pneumococcus (Felix)	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.

5/25/49

Harvest Y10 from 6 hr. heavily inoc: Y2 Glu shaken.
Suspend 2 ml c 2 ml 1% lac, 2 ml H_2O, 2 ml M/5 buffer.
Take .4 ml samples into M/100 azide M/50 buffer then ONPG M/200

		Di	Df	Acor.	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

Adaptation kinetics

5/26/49

Y10. 2 ml cells | 1 ml 1% Lac | ½ ml buffer | ½ ml H₂O or M.C.

(H) (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

Terminate adaptation

Adaptation rate.

7/5/49.

Harvest K-12 from standing culture in Y2 Bleu. Conc. ca ~~20~~ 10 X. in H_2O. Ad. Syst. contains ~~H_2SO_4~~ 1 ml NaP M/5 7.5, 1 ml 2% lactose, 1 ml cells and 1 ml supp.

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

A). No supplement — ONPG conc. in 021.

B). Peptone 1 ml 2%. — 4PM start.

T.		A Di	A D_f	A Δ(corr)	A R.A.	B Di	B D_f	B Δ	B R.A.
15m.	4[15]	061	071	−005	——	064	087	008	012
	5[00]	056	077	+ 005	009	067	098	~~059~~ 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

5 ml sample (from c). 8 mins.

071 152 067 236 (60 min)

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

B) do. 1 ml M/100 Arside

c) do. 1 ml lactose 1 ml H_2O.

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

10PM

(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	$10^{.45}$ 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 = .059)

	Di	Df.	Δ cor	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 lac 9:35 to 2:30

Assay is azide. Incubate Refrigerate

A. 1 ml cells 3/4 ml buffer B. water.

C " " D. " "

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

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. Hancock 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.

$E_{\frac{30}{}} - 10^{25}$

$D_{i\ 10\ min.}$	H_2O	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_c	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 = NaPM/5.

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

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

	Pi	
A1	040	155 10
A2		M3 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) $\frac{420}{}$ — $\frac{700}{}$ 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.

	D:		
KG O	062	} negl. 10m.	
KG P	041		
KG-L-O	032		
L-P	030		
L-P	027		
KL-O	139	37'	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 a/ml.

.001 ml in M/50 NaP 7.5 M/2000 ONPG. Stop $N_{a_2}CO_3$

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, 134 which showed larger alcohol effects.

8/4/49 .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/Y2 Lac washed and concentrated to 30 ml.
Aliquots of 15 ml ea. mixed ē 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: 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 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 = .01 ml

	Di		20m.	R.A. 20m.	
A	089	193			113
B	080	329 6min.		257 × 10/3 = 860	

1 ml A = 642 mg / 29 ml = 22.1 mg, assuming complete recovery.

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

4:30 – 5:10 in 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	~~560~~		.642 g.
B	24800	aqueous		.560 g.

u/mg

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

		Di	De	T.	R.A.	u/ml	u/mg.	u/mg pud.
A	.02 ml	040	560	5min. 2080	~~560~~	1040	1040	5.1
B	.01 ml	014	380	5min. 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° 1/2 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

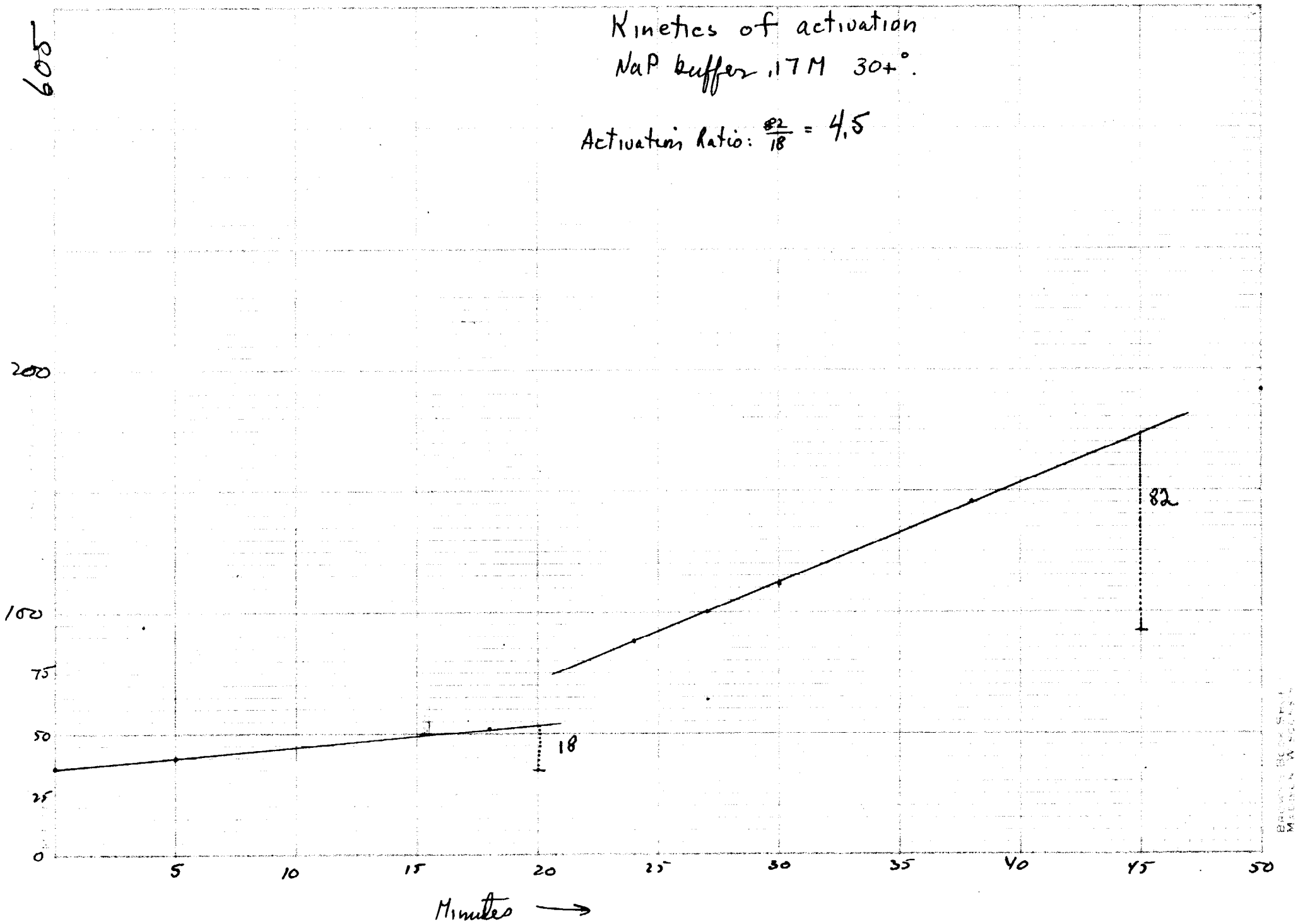
[Dilute $\frac{1}{100}$; $\frac{.5}{10} = \frac{1}{2000}$ for assays.]

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 T_0)	.5 ml
onpg	1 ml

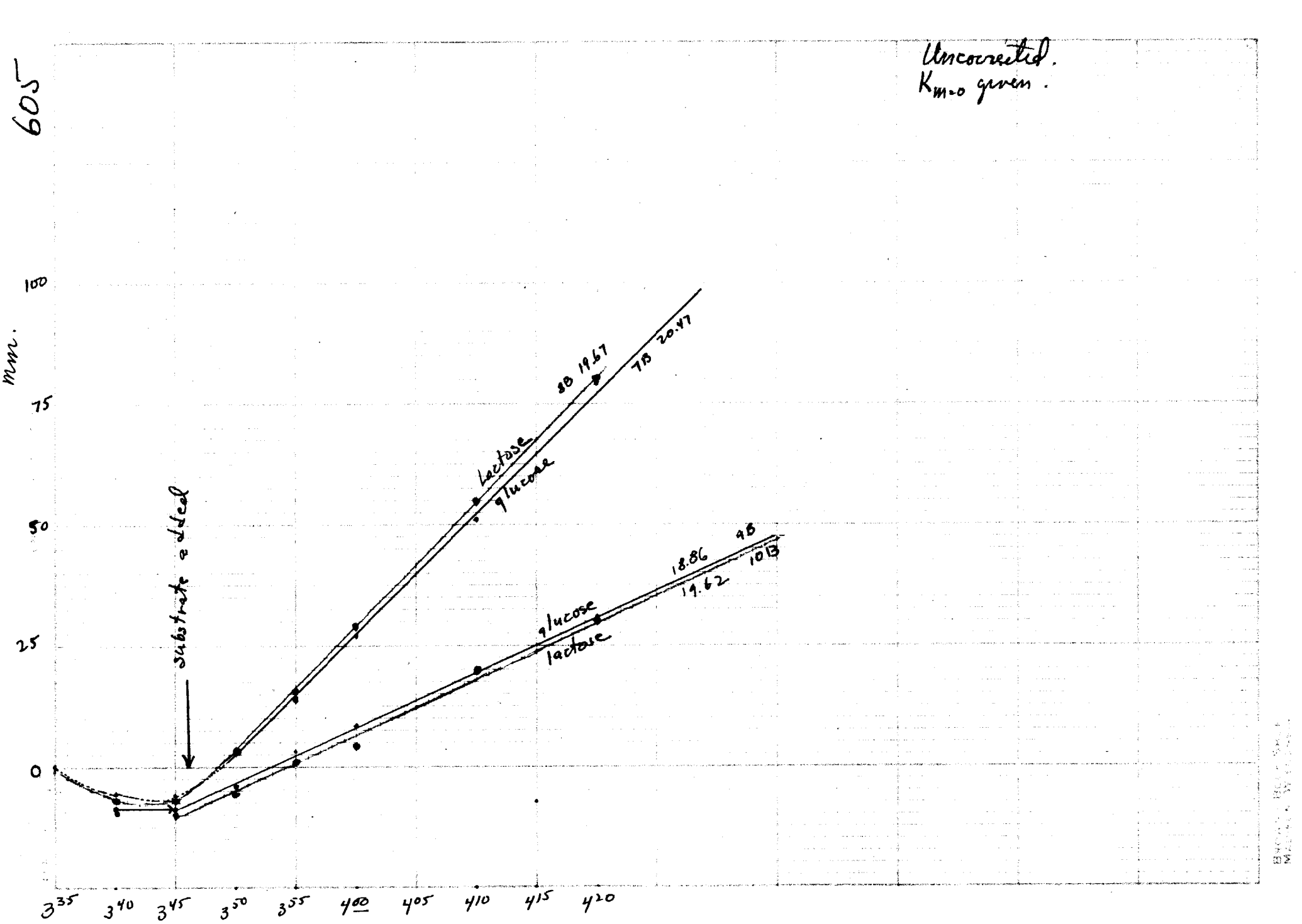
Time.		D.
215	20 s	036
	60 s	034
	180	035
	240	039
220	5M	040
	~~6 M~~	
	7 M	
	8	
	9	
	10	
233	18	052
239	24	087
242	~~27~~	~~100~~
~~250~~		
245	30	112
253	38	146
305	50	191


Kinetics of activation
NaP buffer .17 M 30+°.
Activation Ratio: 82/18 = 4.5
600
200
100
75
50
25
0
82
18
5
10
15
20
25
30
35
40
45
50
Minutes →

	Thermo	7-7B	6-8B	9-9B	10-10B
main vessel { 2cc	← 2cc M/10 $NaHCO_3$ - M/1000 Phosph →				
main vessel { .1cc		← untreated coli "A" →		treated c̄ M/10 PO_4 buffer 1 hr "B"	
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 -7 -1	57 -7 -	77 -8	59 -12
3:50	150 +1	106 +10 (3)	70 +10 (+3)	84 +4 (-4)	66 +4 (-6)
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-o} given.
mm.
100
75
50
25
0
substrate added
Lactose
Glucose
80 19.67
75 20.47
Glucose
lactose
18.86
19.62
98
103
3 35
3 40
3 45
3 50
3 55
4 00
4 05
4 10
4 15
4 20

Utilization of Isomaltose

September 8, 1949.

D/g D/m M/g M/m M/m M/-

		9A	2A	4A	8A	10A	7A	T
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 min.							
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	08 -1	92 77	62 44	09 +2	48 0	155 -3
	25	51 29	14 -1	111 91	71 48	08 -4	46 -1	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

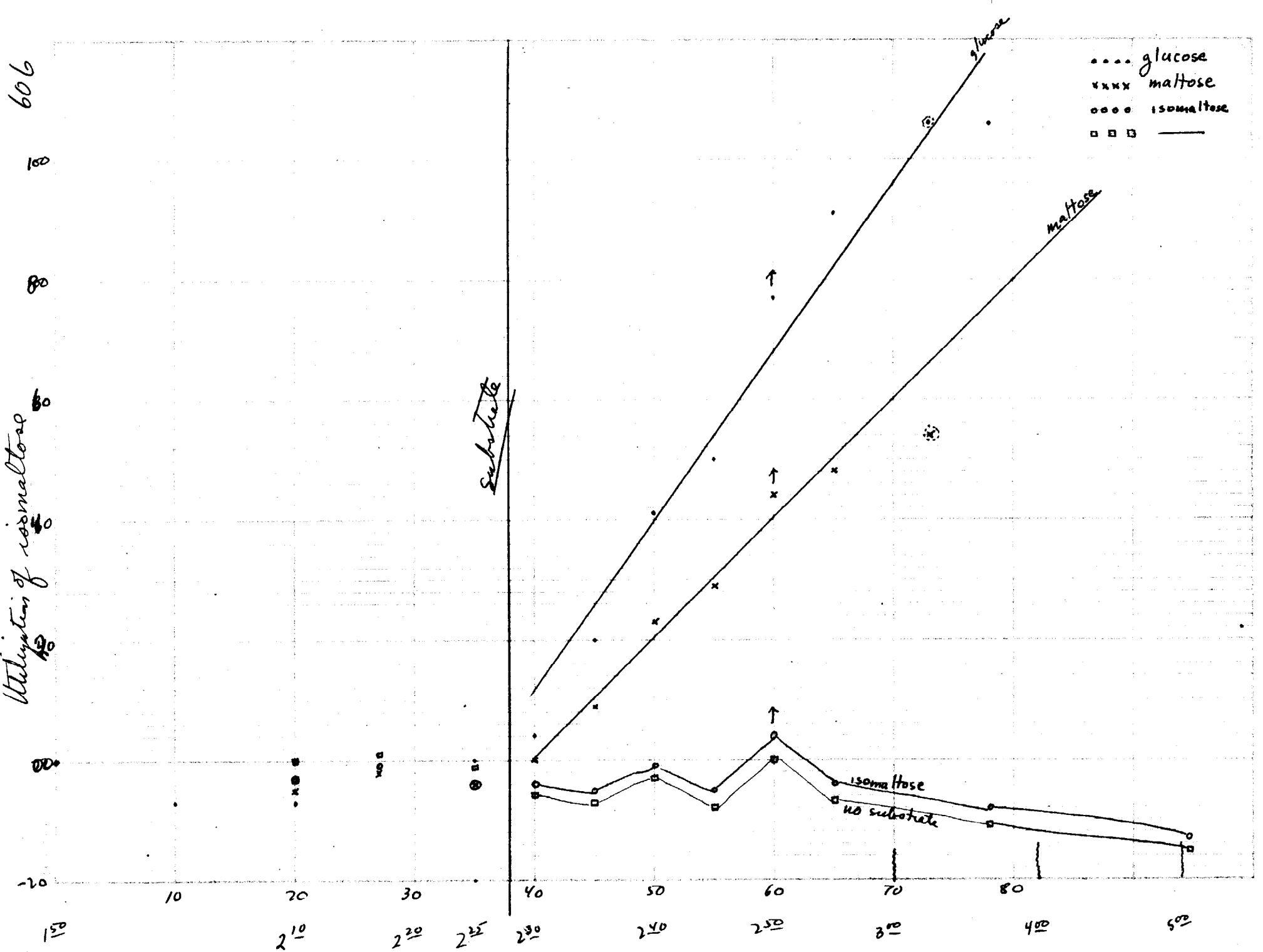
CO$_2$

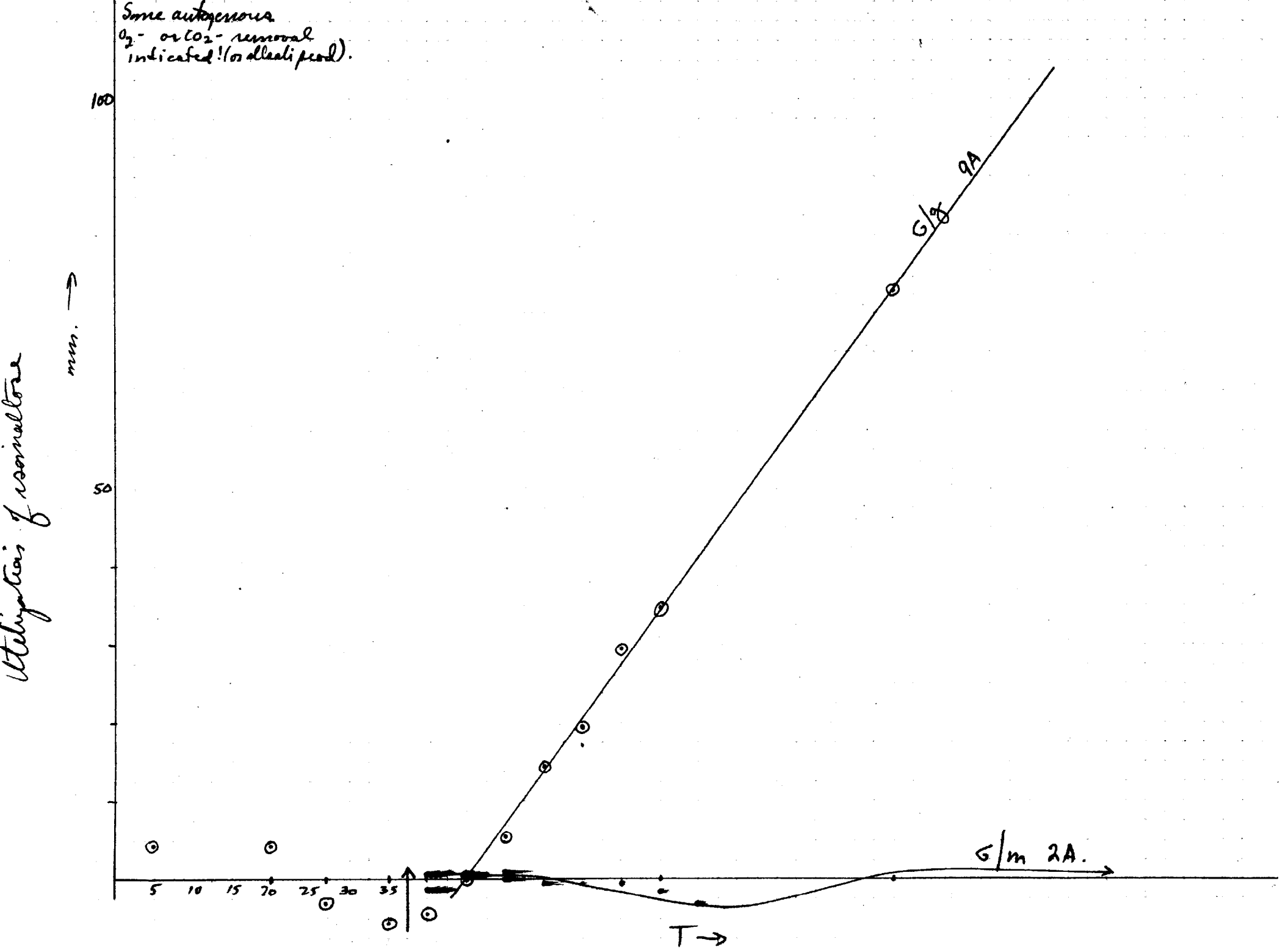
NaP M/1000

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!

606


glucose
maltose
isomaltose
Substrate
Utilization of isomaltose
100
80
60
40
20
000
-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
glucose
maltose
isomaltose
no substrate


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

Cross adaptations.

September 8, 1949

		11A 1 T/Tr	16A 2 T/gl	15A 3 T/mal	8A 4 T/ar	13A 5 D/gal	12A 6 D/ar	8A 7 D/—	T	
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	
→										
1201	11	20 3	23 14	21 3	17 -2	46	9	4	151	staggers to stick
1205	15	33 16	56 47	25 7	21 +2	66	15	6	151	
1210	20	43 26	86 77	22 4	19 0	82	13	7	151	
1215	25	54 37	118 109	19 1	18 -1	100	13	7	151	
1220	30	73 56	160 151	25 7	26 +7	121	22	14	151	
1225	35	93 76	202 193	32 14	35 16	131	27	17	151	
		X	X			X				
1248				38 20	41 27		29	16	151	
112				51 32	72 52		36	14	152 +1	
133				51 31	70 56		36	14	153 +2	
230				70 43	152 128		46	19	160 +9	
317				92 56	281		55	23	169 -18	
				×						

K12 grown overn. in 1/2 Trehalose 1/2% (T) or Galactose 1% (D).

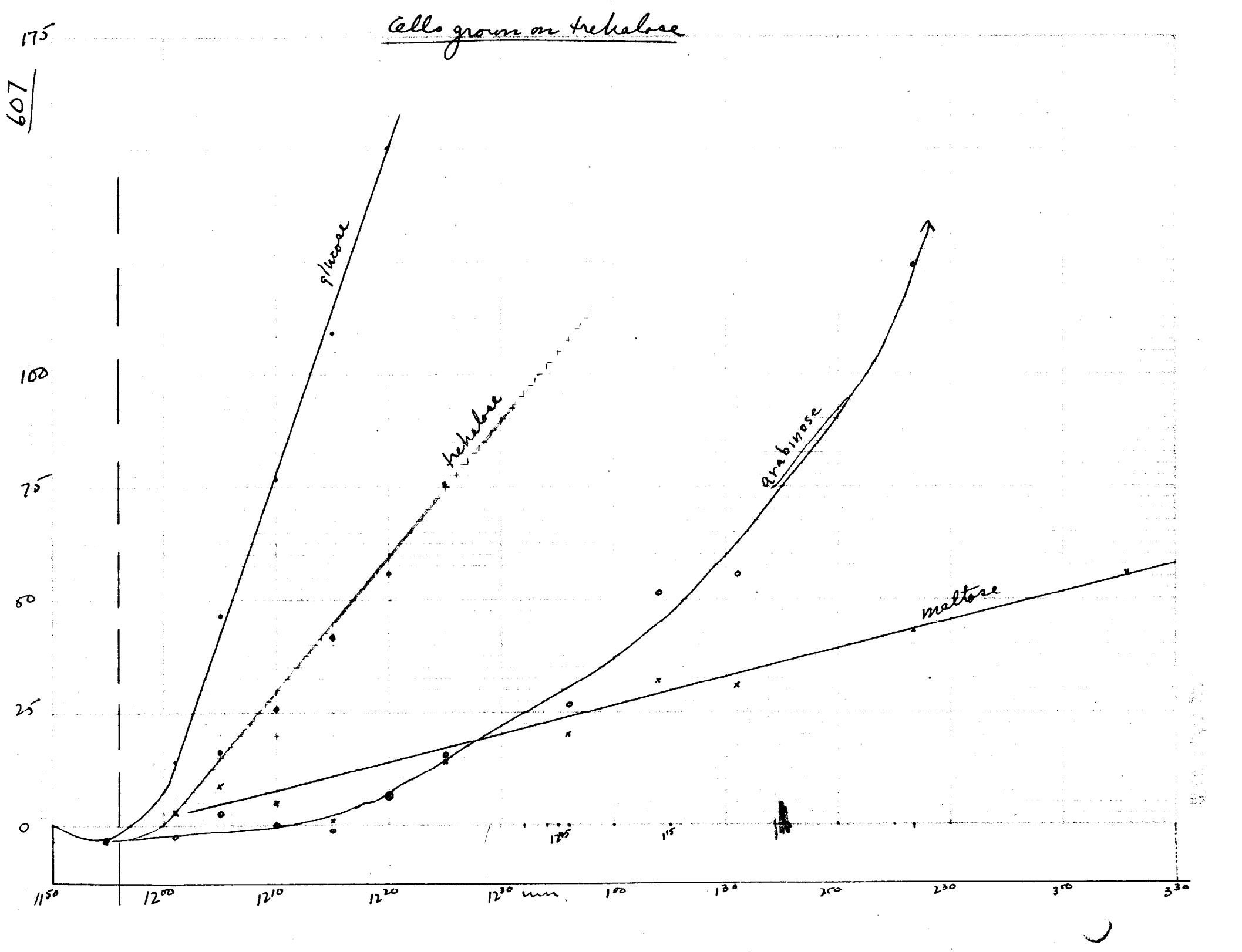
Test on maltose, glucose, trehalose, and arabinose

Cells 5×, 2 ml in $NaHCO_3$ M/20 NaP M/1000 set 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
175
100
75
60
25
0
glucose
trehalose
arabinose
maltose
11⁵⁰
12⁰⁰
12¹⁰
12²⁰
12³⁰ min.
1⁰⁰
1³⁰
2⁰⁰
2³⁰
3⁰⁰
3³⁰

Trehalase in maltose-adapted 607'

		glucose 4	maltose 8	trehalose 10	Thermo
Inoculo in $NaHCO_3$ + CO_2	9 10	27	08	10	148
M/20	9 15	23	06	09	146
K12/ maltose 32°	Tip Subs.	*			
	9 20	20	09	16	149
	9 26	38 +18	29 +20	21 +5	149 0
	9 30	54 +15	43 +13	22 0	150 +1
Bubble later obsm.	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.

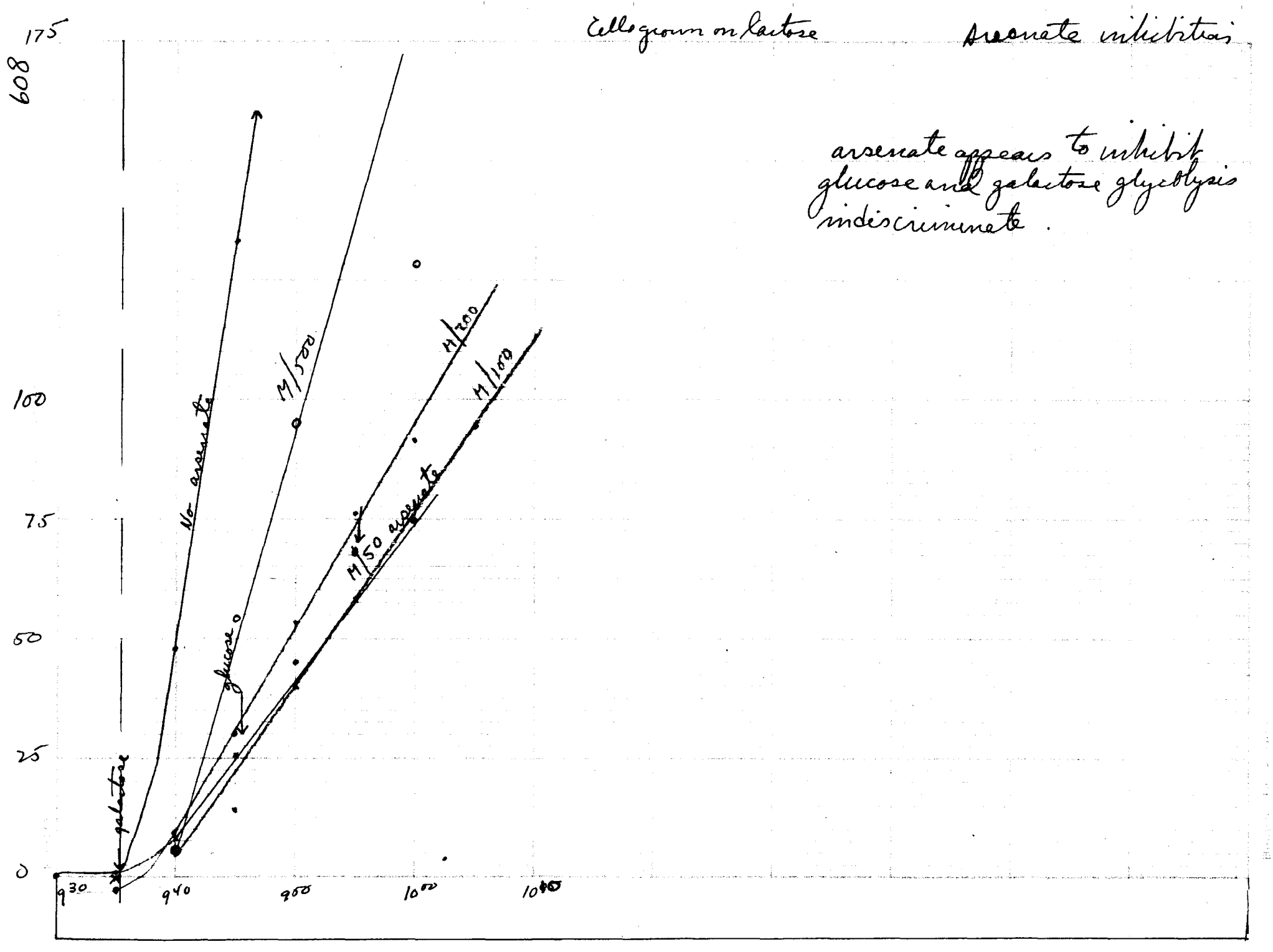
September 9, 1949

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	6	
930	47		18		16		30		03			151
935	47	+1	17	0	12 ~~bulk~~ 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

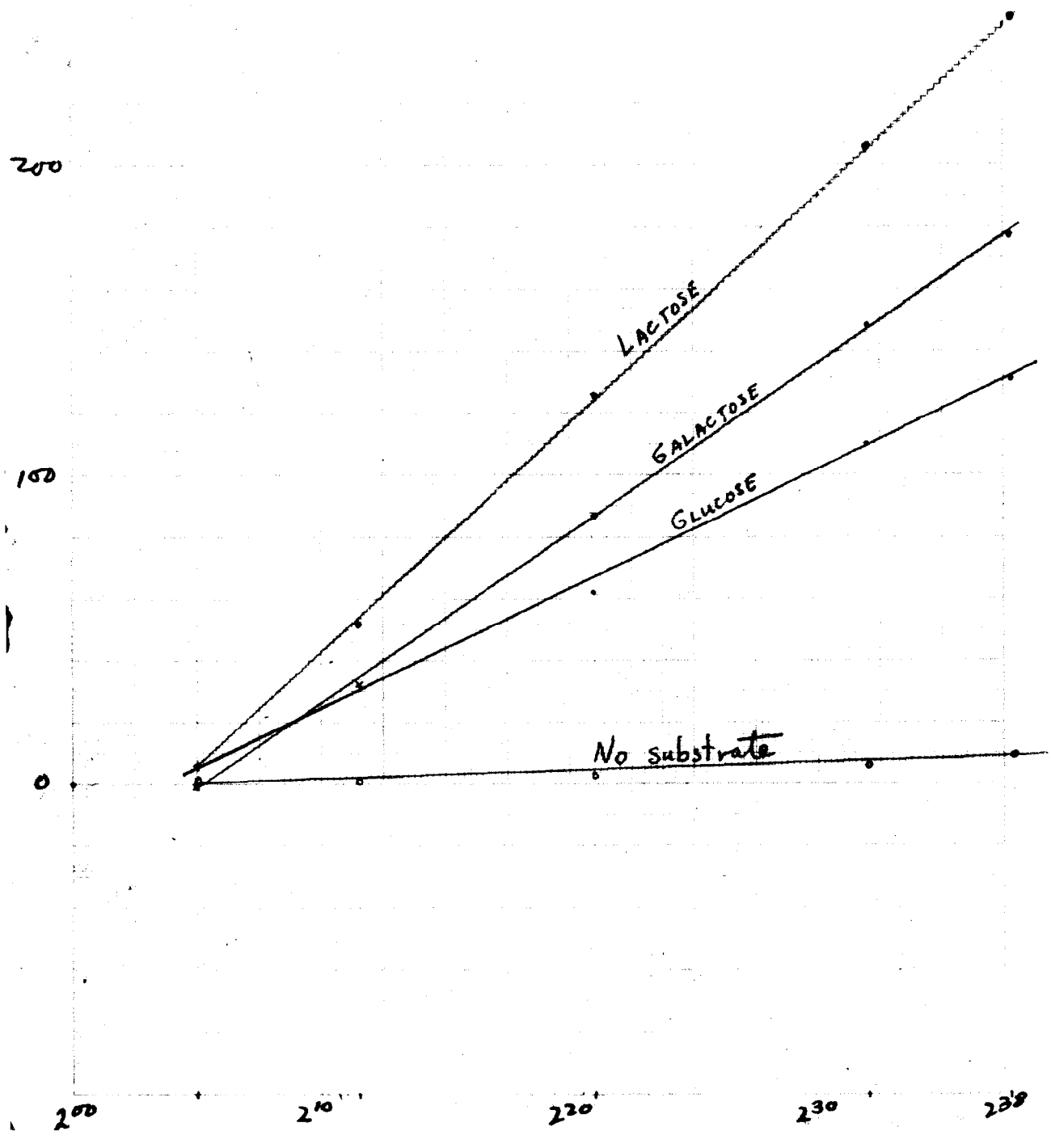
Cells grown on lactose

Arsenate inhibition



arsenate appears to inhibit glucose and galactose glycolysis indiscriminately.

T.


200
100
0
LACTOSE
GALACTOSE
GLUCOSE
No substrate
2^00
2^10
2^20
2^30
2^38

W251a Lactose utilization 609a.

9/13/49

See

	glucose		galactose		lactose		/		T
	1	1B	2	8B	3	3B	4	5B	
200	06		04		01		09		108
→ 2 05	20	5	12	-1	16	6	19	1	117 -9
2									
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

Equilibrate ca 1½ hrs!

Streak out 1B: ca 30% Glu+!

Streak out culture 1

W251a Lactose utilization

9/11/69

1.5 mC cells 1 ml 10% sugars $NaHCO_3$ 4/20 NaP 4/1000

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

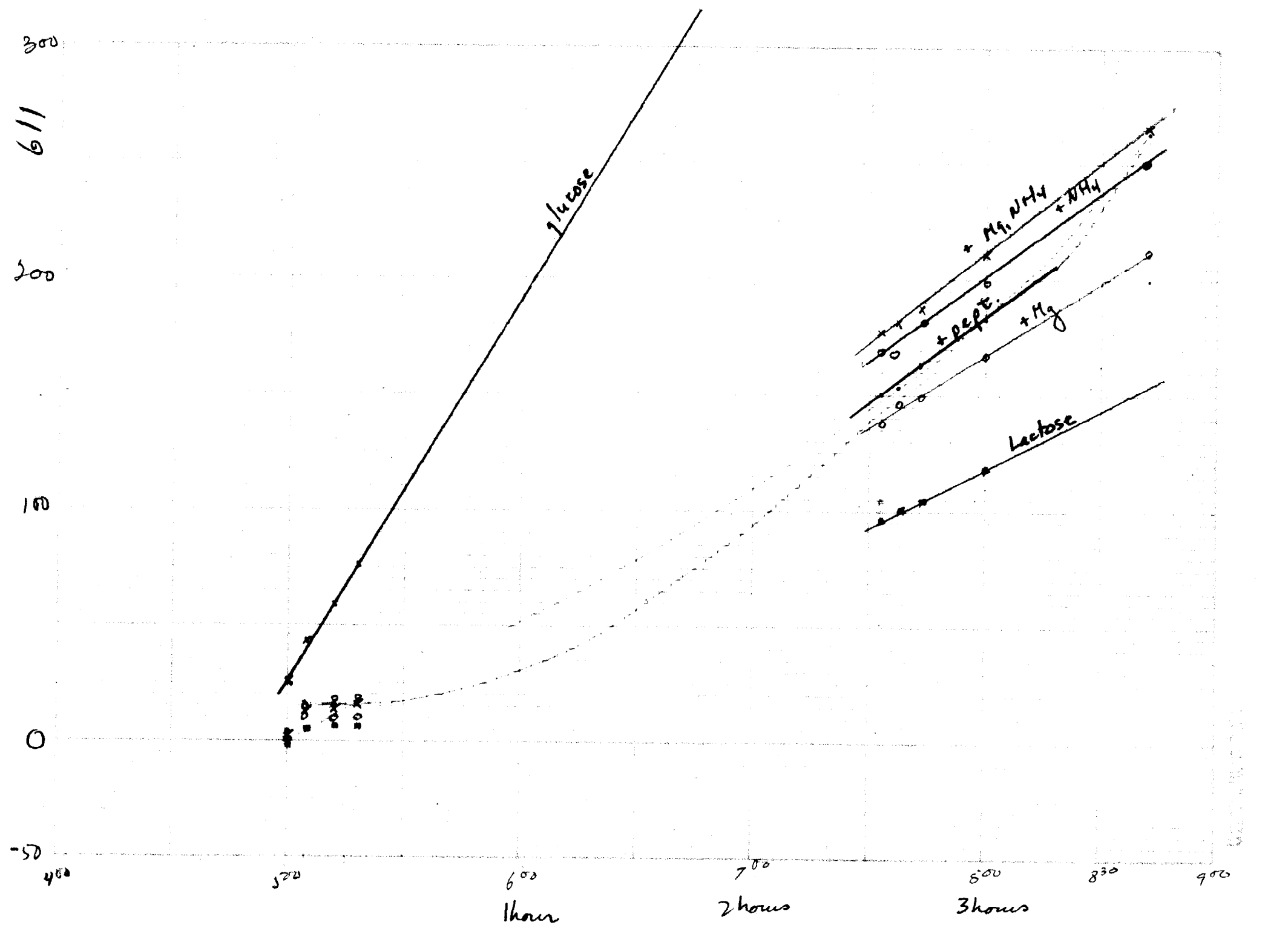
	1 5B	2 2B	3 6B	4 4B	T
	glucose	galactose (glycerol)	lactose	per g/gal	
1240	-2	18	-3	16	147
1245	-1	23	04	27 (153)	147
1246	05	28	08	27	153
1250	10	32	12	31	157
1255	04	28	08	25	151
100	04	30	10	20	148
110	14	40	19	31	153
115	22	48	25	32	150
121	28	52	27	29 (151)	154
131	41	69	42	35	156
150	62	84	51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

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

		Volume	$k_{0ml\,32°}$	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
Klett
Glucose
+ Mg, NH4
+ NH4
+ pept.
+ Mg
Lactose
4:00
5:00
6:00
7:00
8:00
8:30
9:00
1 hour
2 hours
3 hours

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	~~8~~ 21	~~3~~ 28	17	32	53	10	28	45	161
→	LAC	LAC	LAC	LAC	LAC	GLU	—	PEP	
450									
500	260	374	220	403	57 -1	40 25	33 -5	69	161
505	3017	3616	19 10	36 12	50 +5	45 43	20 +8	61	161
510	3316	4218	23 10	41 13	55 6	65 59	24 +4	64	161
515	3316	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 [illegible]	30 -3	76	161
737½	179 153	201 168	163 146	217 180	158 100	213	33 -5	76	161

	I A	II L
1.	Lactose	Glu
2.	Lactose	Glu
3.	Lactose	Glu
4.	Lactose	Glu
5	Lactose	Glu
6.	~~Lactose~~	~~Glu~~ Glu
7	—	Glu
8	—	Peptone

Peptone .5%
$(NH_4)_2SO_4$ 1%
$MgSO_4$ M/100
$(NH_4)_2SO_4$ + $MgSO_4$ M/10.

K12 grown on maltose, aerated.

743	183 163	208 181	165 149	218 187	156 104	225	27 +1	70	161			
800	~~168~~ 209 183	~~220~~ 231 198	188 166	247 210	176 118		33 -5	81	(173)	165	-4	
841	297 263	291 250	241 211	310 265	×		41 -13	87		168	-7	

ONPG ✓
after toluene ++

September 23, 1949

5[40]

		D_i	$D^{0mpg}_{10MN.}$	Δ'	
M	L1 water	007	438	384	
M	L2 glucose	002	217	166	
M3	L3 lactose	0	165	116	!
M4	M4 water	001	072	022	
	M2 glucose	001	058	008	
	M3 lactose	0	071	022	
	M4 Mgt lactose	0	074	025	
	—	007	042 = 49	0	

Cell density L 19.9
M 13.3

Cells incubated from 3[30] PM
on indicated supplements:
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 blanks

	D_i	D^{0mpg}_{10M}
L1		438
L2		125
L3		54
M1		28
M2		10
M3		32
M4		28

mg. dry wt.

K12 / mal and / lac

showing decrement of activity
when incubated with lactose or glucose!

Consistency of benzene activation of galactosidase

September 24, 1949.

2 PM

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

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

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

Too erratic to be used in present stage of development.

Effect of N-supply on lactase deadaptation.

September 24, 1949.

12 hour cells aerated not washed

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

	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 .1 ml	022	[illegible]	
6	$(NH_4)_2SO_4$ M/10 .1 ml	023	264	
7	Lac .2 %	022	364	
~~8~~	—	— 003	011	014
~~9~~				

CO_3 1 ml
M/1 Na 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 →										
2:05 →	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	56
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 —

	T	1	2	3	4	5	6	7	8	9
5:30	163	127	108	99	135	182	219	191	209	155
6:45	159	127	127	110	137					55

1 ml cells W251a/Lac aer. in $NaHCO_3$ M/20 .05 - .10 microliters.

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. Y2 — 50ml. Conc. to 5ml (10X)
Leave water suspensions on table top 10A — 730P 30.

1 ml aliquots incubated ± benzene 7:30 – 9:00 PM (90 mins.)
Test samples per standard ONR (M/3000 mpg; 20 mins; 37°; Na P M/50 7.5

Untreated samples: (.1ml/10)

	Di	Dampg (12 min.)	R.A./ml	R.A./ml/Di,00/10³
K/Lac	250	800	94	38
K/Mal	307	475	19	6.2
K/Glu	118	119	0.2±	.0.2

TREATED

(.01 ml)	K/Lac	017	540 (7 min.)	1.5 x 10³	.58	100
(.01 ml)	K/Mal	027	380	.36 x 10³	.12	21
(.1 ml)	K/Glu	070	269	.02 x 10³	.02	3

Activation of ca $\frac{1500}{94}$ = 16 x fairly concentrated 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.

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

1 ml aliquots to small tubes and incubate ½ gm in reagent.
Assay standard mpg.

Intact Cells.

			Δint.	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 aeration	4	/Lac	.1	054	218	293	100
" "	5	/Mal	.2	129	193	51	17

Benzene treated 2hrs?						RA/ml	RA/ml/Di	
	1	Lac	.01	007	310	292	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 amts = .01 ml (excl. 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 7.5.

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

Negligible diff. even if carried over

Kinetics of thymol activation
Balance in W842. (test).

Oct. 2, 1949.

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

Add a crystal (10-15 mg) of thymol at 4[15] P.M. .005 ml samples
C = Phenol. 1 ml start at 4[30]

T.	Mins.		Dongs.
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]~~ 8[00]	~~190~~ 215	A	> 1000
		B	650
	(185)	C	231

Note: slow process.
Needs >> 1 hr.
Some evaporation possible.

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

		Amt	Di	Dongs	RA
Cells.	K/L	.02	052	149	~~8~~ 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.

1: .5 ml susp.

~~2: .5 ml " + 4.5 ml H₂O~~

3: .5 ml " + 4.5 ml "

3: .5 ml (1/10) + 4.5 ml "

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

all in duplicate

A thymol

B ~~hexanol~~ octanol

C. benzene

1/2 hour tests

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

Neglect D: (.007 ± .03). 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

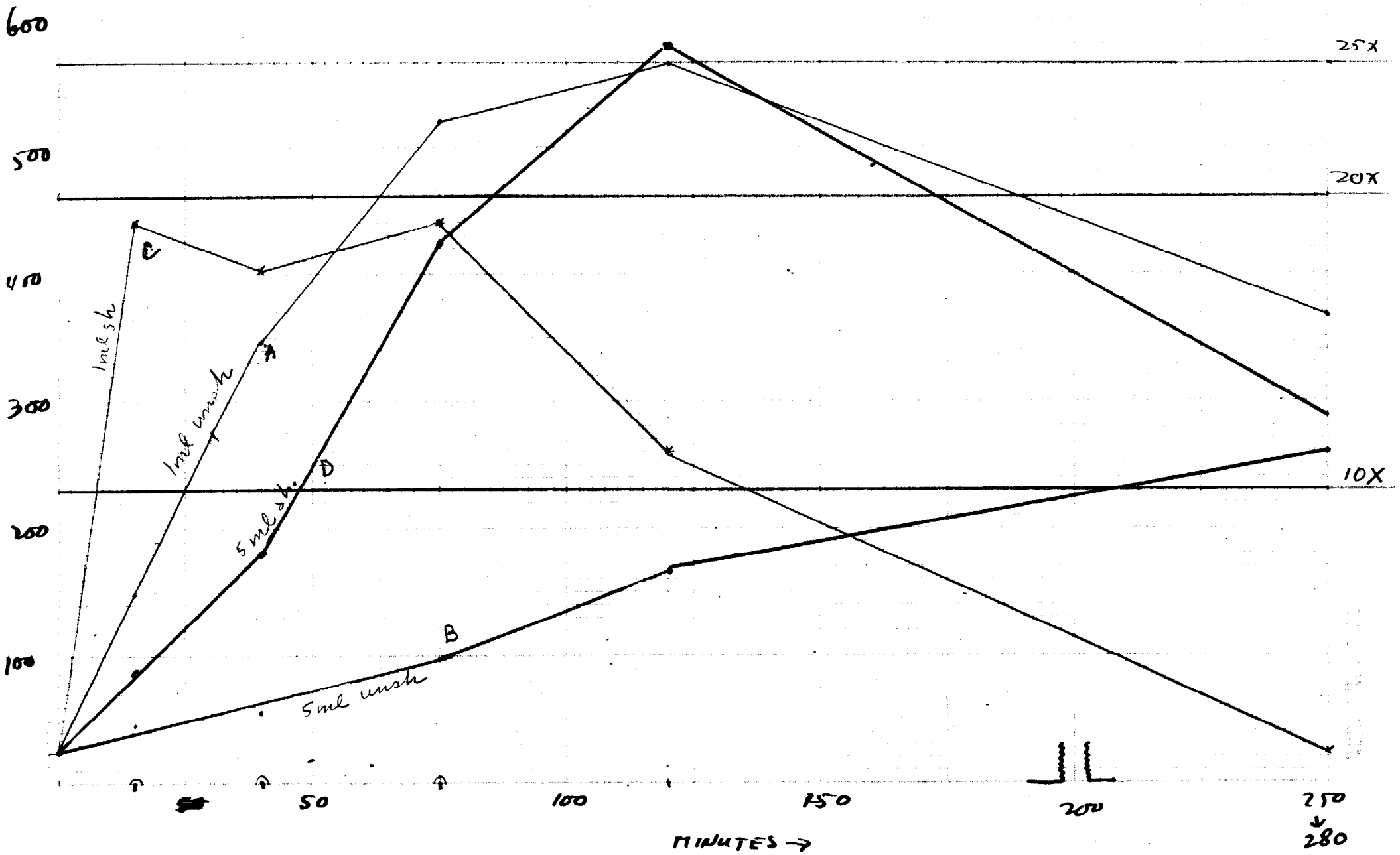
Time may have been insufficient for complete activation! Thymol seems to act most rapidly. I Try Phenol, other ϕ-OH's

Reassay 1, 3 4P2.

A: ~~4~~ 1773.

624

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: $3\frac{PM}{20}$.

A) 1ml unsh. B) 5ml unsh. C) 1ml sh. D) 5ml sh.

Remove .1 ml samples from time to time; dilute in water 10ml and assay. Terminate c Na_2CO_3, exc. intact cells incid ant.

3^{35} T	MINS 15	Di	D^{20}_{onpg}	
Start .1ml	0	089	318	23
10/ml		003		
1 A	15		149	
1 B			46	
1 C			~~460~~ ← (440)	
1 D			87	
4^{00}	40			
½ A			173x2 = 346	
1 B			53	
½ C			201 x 2 = 402 (!)	10% < above!
1 D			180	
½ A	75		260 x 2 520	
B			98	
½ C			220 x 2 440	
D			423	
1 A	120		281 x 2 562	
1 B			83 166	
1 C			120 240	
1 D			289 x 2 578	
A			369	
B			260	
C			025	
D			289	

(left margin: ml 1/100 dil; 4^{35}; 5^{20}; 8^{00})

2 hours optimum for unshaken cultures.

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

	2½h.	18h.	
thymol	479	178	Repeat
phenol	016		
benzene	466	685	overnight.
octanol	369	222	

Gal. base 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; Gler. Ser. [Benzene from 12N+I. ca 8 hours.

						RA	u/mg
Intact:	K/L .1	130	520		Δ corr 392	3.02	14
	K/M .1	129	151		24		0.9
	K/G .2	204	182		— 0		0
	—	— 007	± 004	Counts = +11			
Benzene	K/L .005	— 004	410		403	×20	~~62~~ 62	297
	K/M .01	+ 004	074		59	×10	4.6	22
	K/G .1	074	060		—		—	

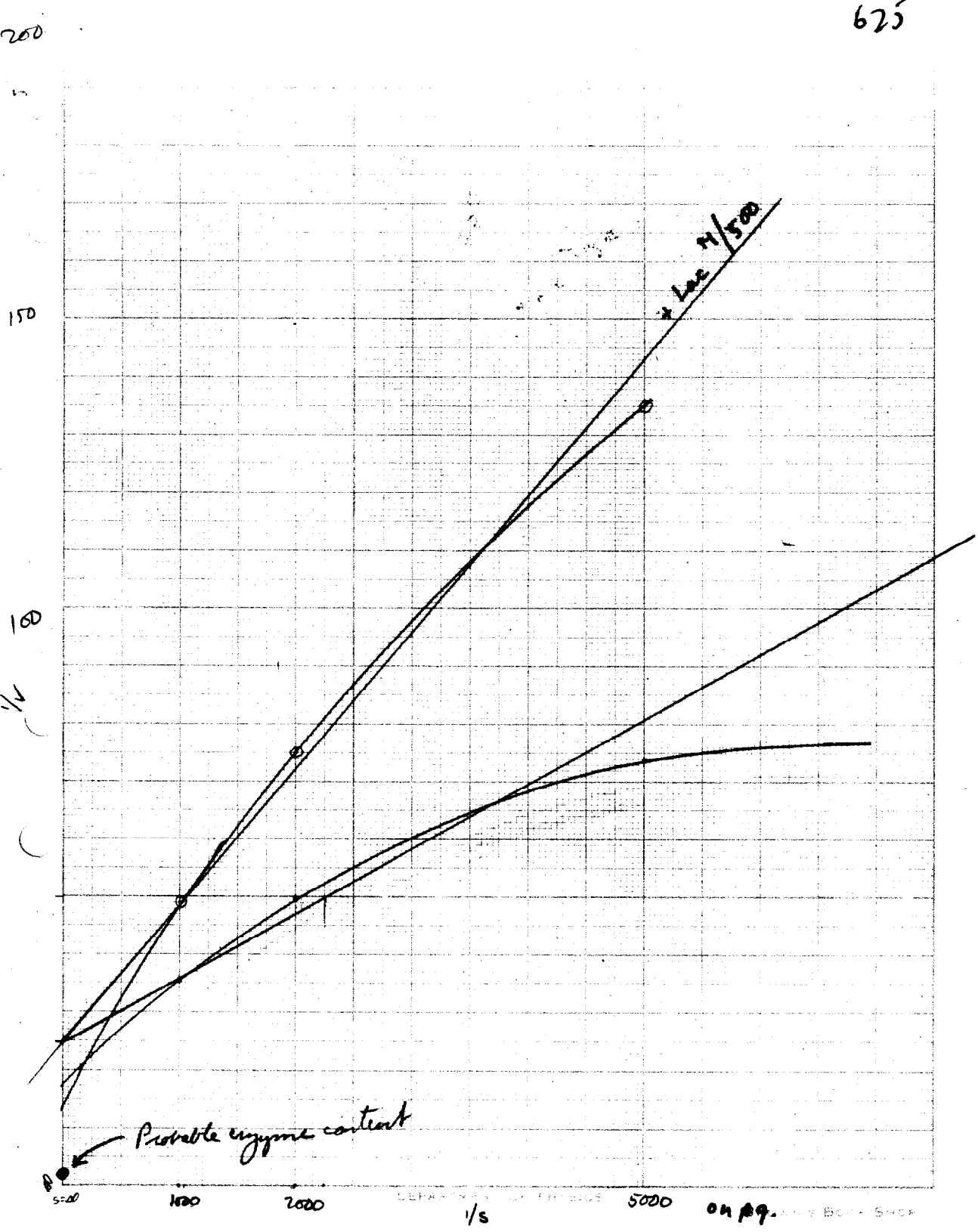
		I	Di	Dongg
Instant	L	.05	087	246
	M	.1	157	189
	G	.1	130	124
	—	.1	214	250
B2 4h.	L	.005	0	530
	M	.02	018	194
	G	.1	069	074 (5:35 PM)
	—	.02	032	267

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

	Di	Dong9		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 6MMS.	196	380		100	~~408~~ 300
Mal 11	104	174 20M	69	38		10	1.8
— 11	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 Lac Y2

Konpg and Klac.

M/100 Na P

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

Graph: scale: Vmax = 1/25 = 400

Konpg = M/2000 = 5×10^{-4} M ✓ pure onpg.

$K_{Lac} = [Lac]$ = 2×10^{-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 binding needs to be investigated.

Lactase in non-adapted cells.

Oct 13 - 1949.

Harvest from T(m) ½ % sugar. K12. 24 hours.
Held 24h. after in H_2O; benzene

		Di		
Intact.				
	Lac .1	119	no (or v. weak!) activity!	
	Mal .1	078		
	Glu .1	112		
Benzene	Lac .005	002		
	Mal.1	075		
	Glu.1	111		
Fresh ~~intact~~	Lac .1	122	107 139	v. low act. [illegible]

Washed cell adaptation

Oct. 15, 1949.

K12 harvested from an. Y2 Glu. 5x. Conc + Wash

System: 1 ml cells 1 ml M/10 NaP. 5 ml M/100 lac or glu 10 AM – 1 PM. 3 hours.
1 ml supplement (YP broth) H_2O qs. 10 ml. Inc. in tubes 37°.

	Cells	Sugar M/200	Supp.	Di	D$_{20}$ anpg	R.A. (in. culture)	R.A. (conc. cells)
1	L	L	–	134	511		
2	L	G	–	138	671		
3	S	~~G~~ L	–	134	162		
4	S	~~L~~ G	–	~~148~~ 150	151		
5	"	–	YP	132	142		
6	"	S	YP	180	190		
7	" aer!	L	YP	183	540		
8	"	L+G M/500	YP	189	530		
9	"	L ~~aer~~	YP	275	458		
10 0-G	G	(L)	–	128	128		
11 0-L	L	(L)	–	141	330		

correction

8	S	L	YP	189	530
9	S	L+G aer.	YP	275	458

Take samples of 0-G; 0-L; 3, 7 under benzene.

YP = yeast peptone broth. Use 1:10

Assay 8 PM.

		Di	Danpg
3	1.0 ml	106	120
7	.1 ml	008	184
10	1.0 ml	080	106
11	.1 ml	002	160
3	1 ml intact (10 hrs.)	117	130 13-14

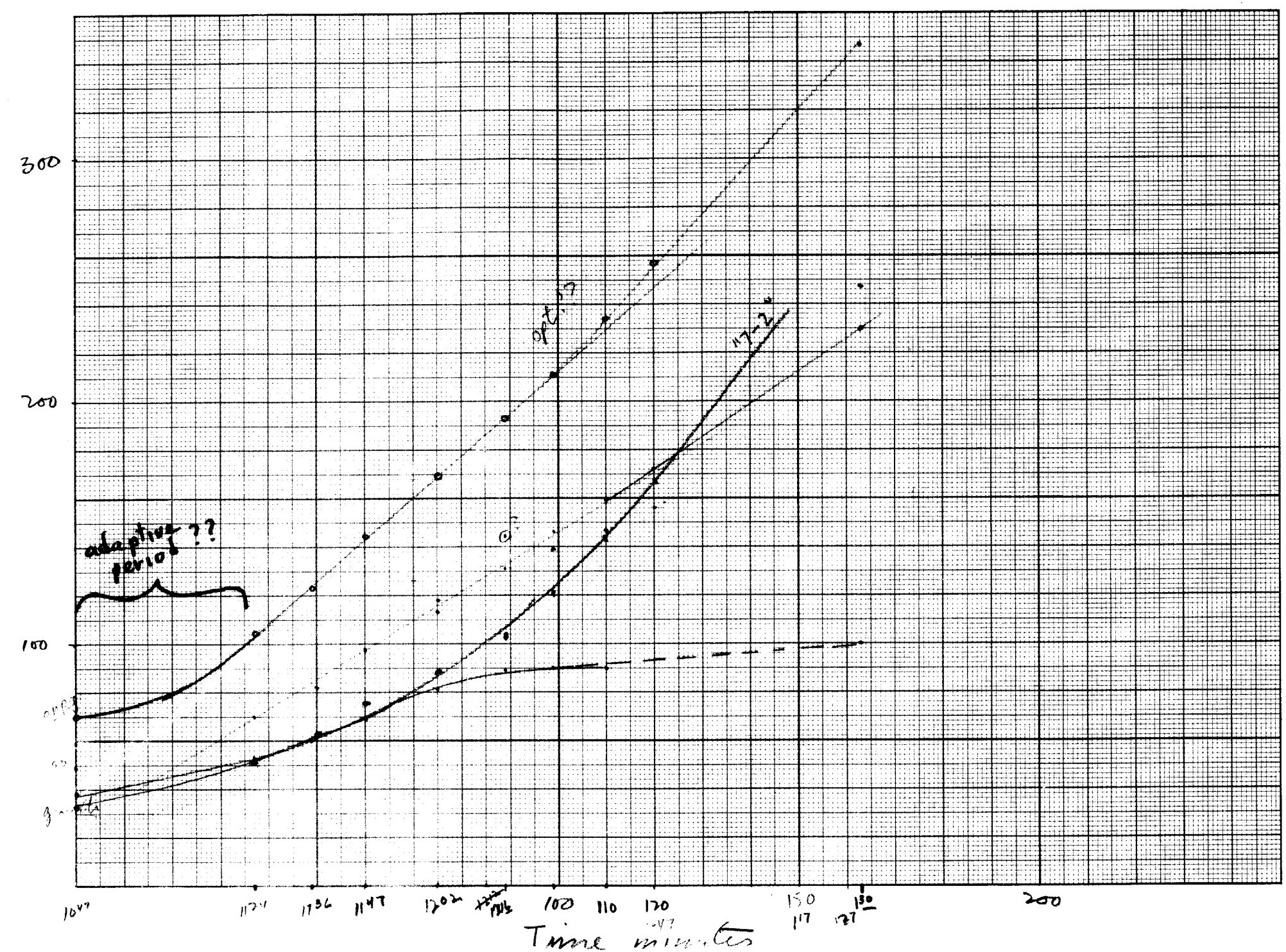
of 1:10 tubes

Adaptation to onpg

		0	37	49						
	Di	10^{47}	11^{24}	11^{36}	11^{47}	12^{02}	12^{12}	12^{16}	12^{26}	12^{37}
1	019	040	040	042		038				
2	031	038	053	060	069	081	083	089	90	090
3	162	180	183	183	187	189				
4	006	032	031	030		032				
5	002	018	019	018		019				
6	005	009	012	012		013				
7	042	070	104	123	144	169	180	193	211	234
8	034	049	070	082	097	118	123	144	146	159
9	032	042	068	078	090	113	110	120	130	136
10	036	050	072	083	100	113	119	131	139	147
		10^{40}								
						AZIDE M/100				
7-2		32	51	63	75	18^{52} 88		104	121	144

These all were left in water bath & pipettes etc. back is onpg for 20-30 mins for temp equilibration. All should be added back.

	Cells	Supp.	ONPG M/				Δ
					12^{47}	1^{30}	$10^{49}-1^{30}$
1	0	YP	1000			040	0
2	1	YP	0		090	100	62
3	1 (10x)	YP	0		0	200	20
4	1	-	1000			034	02
5	1	-	[illegible] 2000			019	01
6	1	-	4000			011	02
7	1	YP	1000		257	347	277
8	1	YP	2000		172	230	181
9	1	YP	4000		146	183	141
10	1	YP	2000		156		
				7-2	167	247	



October 17, 1949

Expt. B Use 2x cells. Add cells at T/O)

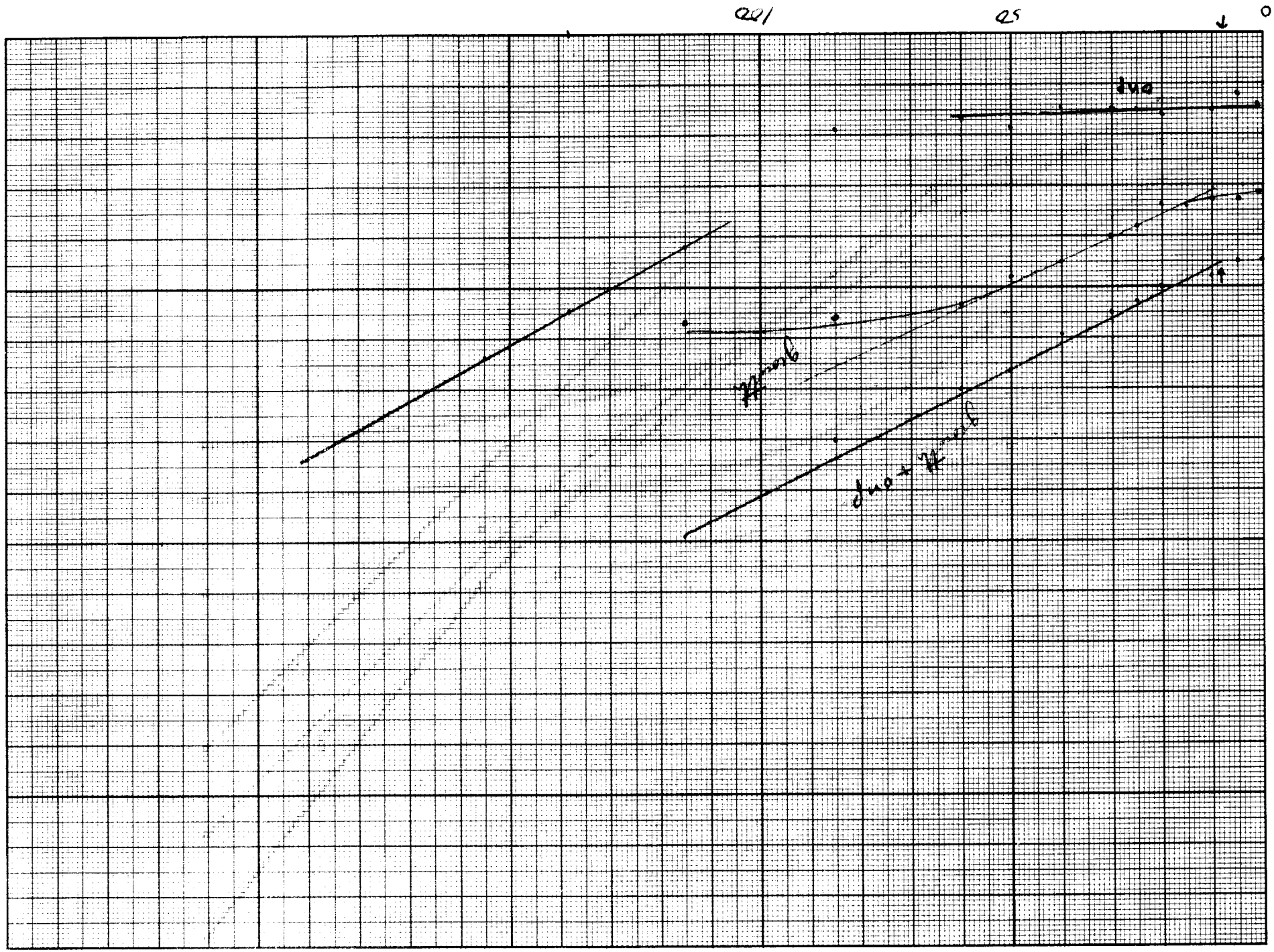
2.15 Cells added

	D_0	2^{16} 1 M	2^{20} (6)	2^{25} (10)	2^{35} (20)	2^{40} (25)	2^{45} (30)	2^{55} (40)	3^{05} (50)	3^{15} (60)
1	050	091	090	096	100	106	110	119	133	140
2	049	090	090	094	101	106	109	121	133	145
3	047	093	093	097	100	107	110	121	132	140
4	051	094	095	096	092	092	096	097	097	100
5	017	063	066	066	068	076	080	090	096	107
1		0	-1	5	9	15	19	28	42	49
5		0	3	3	5	13	17	27	33	44
1-5		28	24	30	32	30	30	29	37	33

Correction: $-.1 \times 50$ leave $45 + (63-17)\ 46 = 91$

		3^{30} (75)	4^{10} (115)	4^{33} (135)	4^{50} (155)
1	~~330~~	150	198	229	253
2		150	191	231	260
3		157	190		250
4		102	121		131
5		112	114	120	126
1-5		38	84	109	127

100 only 2 pts?



Adaptation kinetics: cell density effect.

October 18, 1949.

10 ml colorimeter tube. 1 ml Y2. 1-3 ml cells (1:100, 5× from Y2 tube, K-12)

		M/100 Na P. Di	M/1000 onpg.	a) 1 onpg	b) 1 onpg			
		9^{45}	10^{05}	10^{20}	10^{35}	10^{50}	12^{05}	12^{20}	1^{15}
growth + onp.	1a	080	083	090	99	110	180	200	260
	2a	090	098	110	124	139	215	225	288
	3a	103	112	129	146	157	240	257	327
growth	1	050	053	063	070	080	109	115	120
	2	060	069	080	095	106	128	128	131
	3	078	090	106	121	126	146	149	149
A onp		0	20	35	50	65	140	155	210
	1	30	30	27	29	30	71	85	140
	2	30	29	36	29	33	87	97	157
	3	25	22	23	25	31	94	108	178

Bacteria = 010. Subtract 040 from 1-3

October 18, 1949.

Inoculate 3ml of 632 suspension
into a) + mpg (1-2)

b - mpg (3-4

1
2
3
4

Galactosidase in Basutt

November 21, 1949.

Cells.			Di	Dump
+	1 ml	1:100	034	134
−	1 ml	1:100	030	177

Thymol treated

+	1 ml	1:500	−004	027
−	1 ml	1:100	−008	156

041

K-12 X

		9 ml / Di	11 ml / 20 M. (6 mM Strep) Donpg	
Intact cells.				R.A.
.05	Lac	120	370	ca 370 (Low!)
.1	Mal	120	100	
.1	Glu	147	118	
.1	Suc	176	158	
B_2. Hd. (6h.)				
.005	Lac	011	013	
.01	Mal	012	013	
.1	Glu	183	80	
.05	Suc	080	067	~VIS.
—	onpg	009	003	

Gal'ase in Garrett

A + 1 Lac
B + 1 Gluc
C − 1 Lac
D − 1 Gluc

Conc. 50/10.

1 ml. samples under benzene 11AM − 8PM. for x series.

cells.

		Di	Donpg
A	.02	020	379
B	.1	089	072
C	.05	060	481
D	.1	090	076

Extr.

A	.005	−010	447
B	.1	050	064
C	.05	059	690
D	.1	049	047

11/23 Garr + 1/42 Mal. as above. Harvest 10^30 AM.
Assay 11^30 AM Also take aliquots for "activation"

			35+ mins	R.A
E	.1	199	340	ca 50

~~Assay~~ 4PM .1 ml samples:

	Di	Df	Δincr
A	207	303	96
B	114	460	346
C	123	520	397
D	122	530	408

Note activation of gal'ase c̄ treatment other than incubating cells in water!!

Compare stability of K-12; Barrett +.

Nov. 24/1949.

Harvest K-12; Barr + from ½ Mal 50 : 10.

Assay 1 ml samples immediately. Also store 1 ml samples at 37° 12 15 16

	Di	Dongy.
K	198	269
B.	~~159~~ 181	309
O	- 003	047

Reassay 7th PM

1	K (Refr.)	204	260
2	K (inc.)	190	277
3	K (thymol)	159	228
4	G (Refr)	201	297
5	B (inc)	154	520
6	B (thym.	150	670
	O	003	

Note greater fragility in water of Barrett.

12/8/49

Harvest K-12 from 30 hr. unshaken cultures in Davis minimal medium (new).

Conc. 50:10.

Intact cells 0.2 ml per tube ea

	D_i	D_{onpg}		Δ_{cor}	R.A.	R.A.'
Lac	044	5210		473	1070	
Mal	059	064		003	—	
Glu	053	057		001	—	

Extr. (Bz ttment)

							"Activation"
Lac	.01	-003	103		098	1960/	4.1
Mal	.2	053	057		001		
Glu	.4	108	097		−008	—	
0		-004	004	008			

$\Delta_{cor} = -(D_i)(0.9) + 008 + D_{onpg}$

Galactosidase: cells from synthetic medium 648

12/9/49

Harvest K-12 from 48 hr. Shaken succ } Davis minimal
24 hr. " sugar }

Conc 50:10

Intact cells: 0.1 ml / tube ; Extr. as indicated (Benzyme 5 hours)

			RA	u/mg
Lac	141	498		12.0
Mal	101	100		0
Glu	111	111		0
Suc	084	091		0.4

Lac	.005	-001	439	20x	297	
Mal	.2	127	140		0.3	0.3
Glu	.2	142	155		0.3	0.3
Suc	.2	086	144		1.7	

Conditions of adaptation

12/12/41

Prepare tubes of D(o) + maltose + supplements as indicated

1. —	—	—	all +++
2. Peptone .1%	+	A12	
3. Peptone .5%	++	-Cy	
✓ 4. Y. Extr. .1%	++	-M	Supraadaptation ?
✓ 5. Ac. hyd. casein .1%	±	-Hy	
✓ 6. " " .5%	+±	-Ar	
~~7. NAA~~		7 Cy	
~~8. CAA~~		8 Ar	
✓ 9. Vits	—	9 M	
✓ 10. RNA + YNA.	—	10 Hy	
✓ 11. AA. ≐ .1% Casein	+	11 $S_2O_3^=$	
✓ 12. A12	+++		
— 13 A3	—		
✓ 14 A4	+		
✓ 15 A5	—		
✓ 16 A6	+		
17 B12	—		
(after aut.) 18 Liver Extr. sterile filtered.			

Response to A12 was outstanding !

1 ml samples of culture under AcOH 2-4 hours.
Add 4 ml M/10 NaPbuffer; ½ ml M/200 orpg.
Read qualitatively after 20 m.