4/2/49.

Compare carbohydrate utilization by cell suspensions harvested from 20 hour Lac 42 broth, incubated of (A) W710 and (B) W815.

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

		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 3×).

[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.

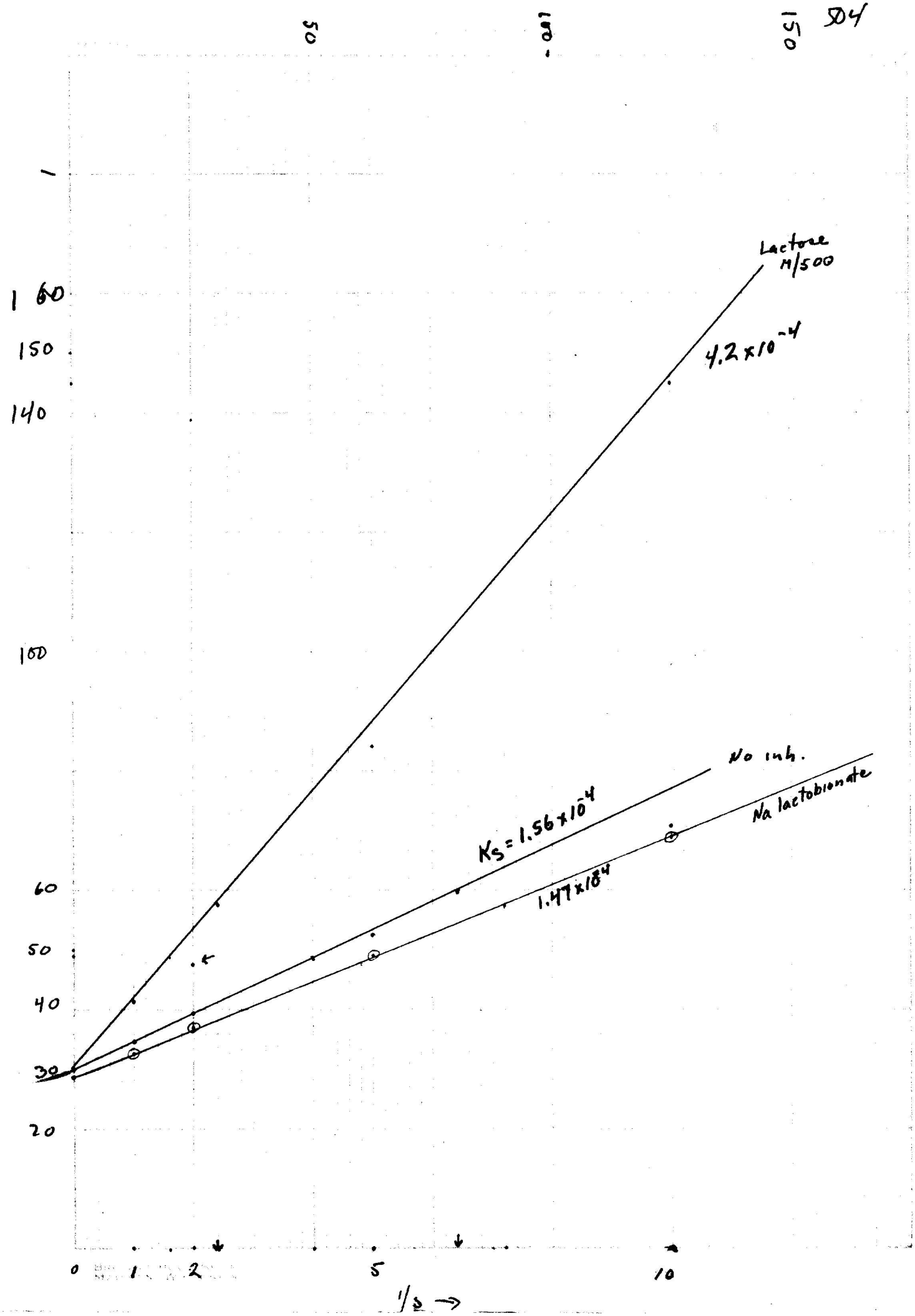
Competitive inhibition of galactosidase

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	32.4
22			013	281	269	37.2
23			005	209	204	49.0
24			003	147	144	69.4

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


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

Glucose.

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

Competitive Inhibition

4/4/49.

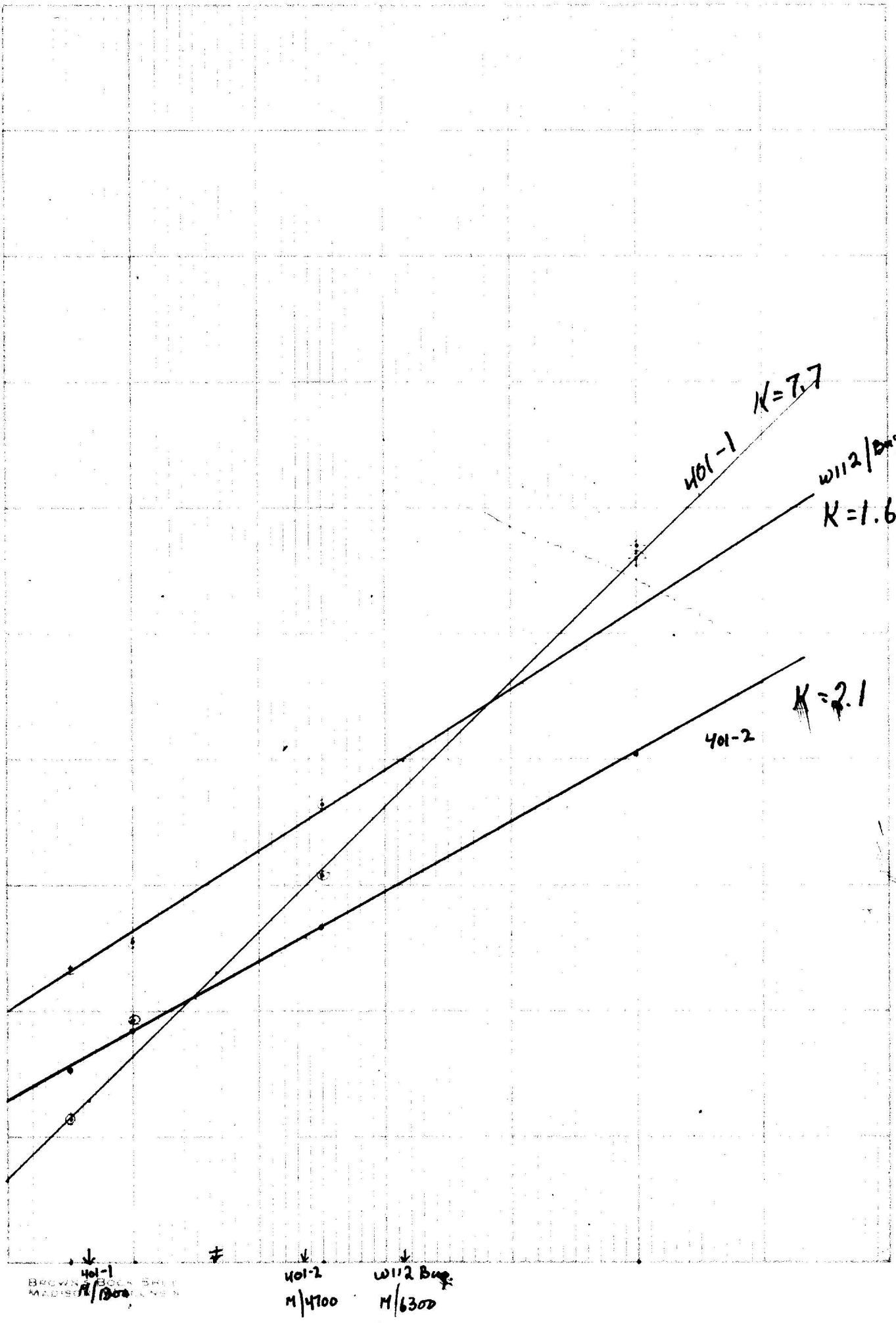
	[399 1:200]		NaP 7.5 M/50 + Na_2SO_4 M/50 · 1/vol			
1		—	023	387	368	27.2
2			010	339	336	30.2
3			003	259	256	39.1
4			—	180	180	55.6
	Lactitol ~~lactobionate~~					
11		M/400	021	360	343	29.1
12		"	012	301	298	34.4
13		"	001	210	209	47.8
14		"	002	141	139	71.9
21		Bregal	027	278	256	39.1
22			017	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

A

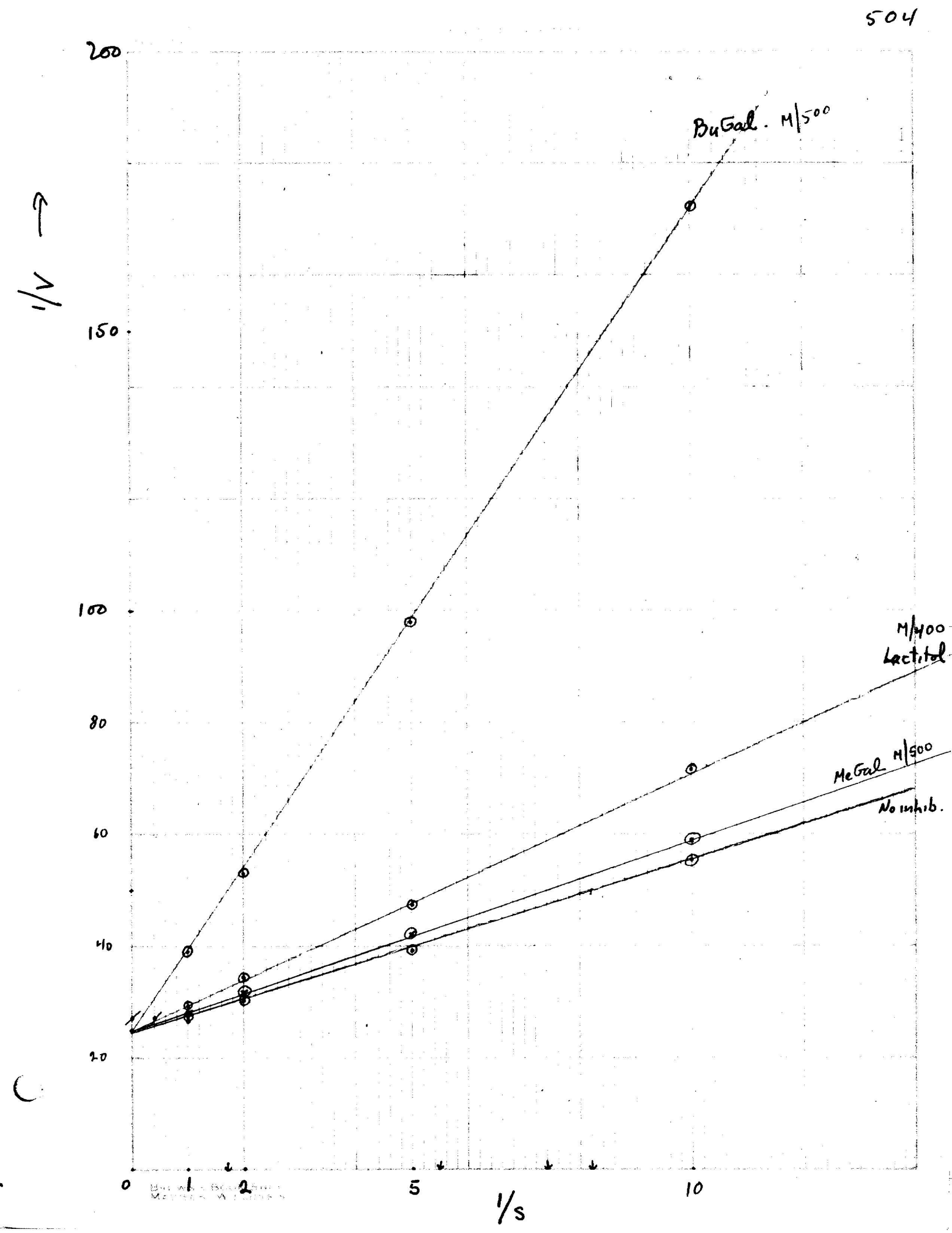
correction is increase from 9 to 11 ml. Subtract 9/11 of Di from Df.

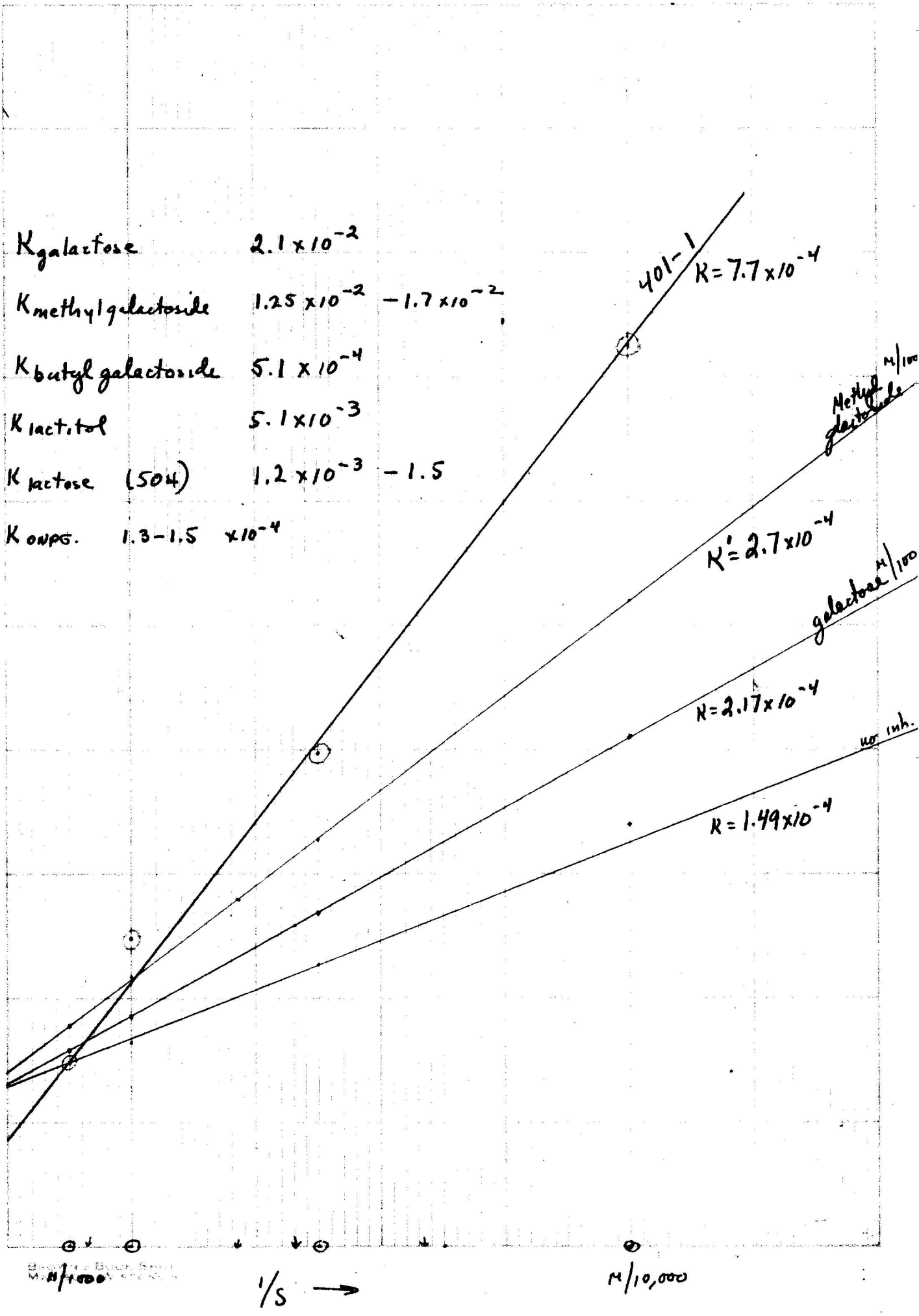
Apparent K_m : $\times 10^{-4}$

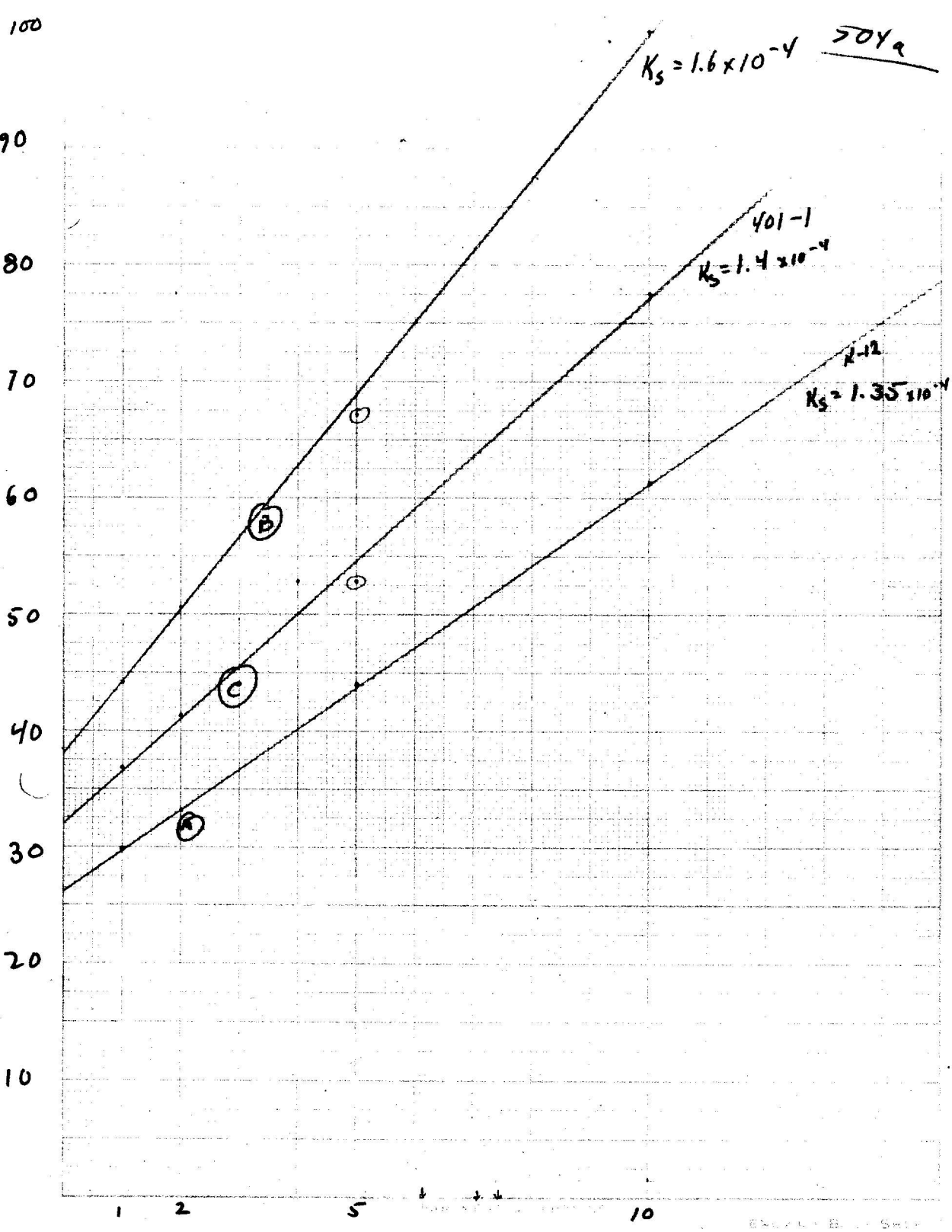
Blank:	1.22
Megalactoside	1.35
Bugalactoside	5.9
Lactitol	1.82


401-1
K=7.7
W112 Bedrock
K=1.6
K=2.1
401-2
401-1
M/8000
401-2
M/4700
W112 Bedrock
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	[redacted]	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}
$K_{methylgalactoside}$ 1.25×10^{-2} -1.7×10^{-2}
$K_{butylgalactoside}$ 5.1×10^{-4}
$K_{lactitol}$ 5.1×10^{-3}
See 384 $K_{lactose}$ (504) 1.2×10^{-3} -1.5
K_{ONPG} $1.3-1.5 \times 10^{-4}$
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
$K_s = 1.6 \times 10^{-4}$
SOY 9
401-1
$K_s = 1.4 \times 10^{-4}$
K-12
$K_s = 1.35 \times 10^{-4}$
A
B
C

Kinetics of suppressor lactases 504a

4/7/49

		1/s ONPG	NaP 7.5 M/100.					
		100/M	D_i	D_f	$V_{corr.}$	1/V	K_s	V_{max}
A	1	1	017	358	344	29.9		
	2	2	009	309	302	33.1	1.35	
	3	5	003	230	227	44.0		
399	Y	10	0	163	163	61.3		
(K-12								
10 mins.	1	1	023	~~240~~ 245	226	44.2		
	2	2	013	209	198	50.5		
B	3	5	006	154	149	67.1	1.6	
	Y	10	—	100	100	100. —		
(Y01-1)								
	1	1	022	~~240~~ 290	272	36.8		
	2	2	011	251	242	41.3		
	3	5	003	192	189	52.9	1.4	
C	4	10	006	134	129	77.5		
(Y01)								
2	1	1	019	760	744			
	2	2	013	680	669			
D	3	5	003	500	500			excessive
W12	Y	10	006	331.	325			enzyme
Bugal. prep.								
10 mins.								
	1							

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

F

galase from Megal. grown cells

4/5/49.

Grow K-12 overnight in 200 ml 1/2 Megalac. 1/2 %
Harvest P5 and dry over P_2O_5.

Yield: 85 mg dry cells.

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

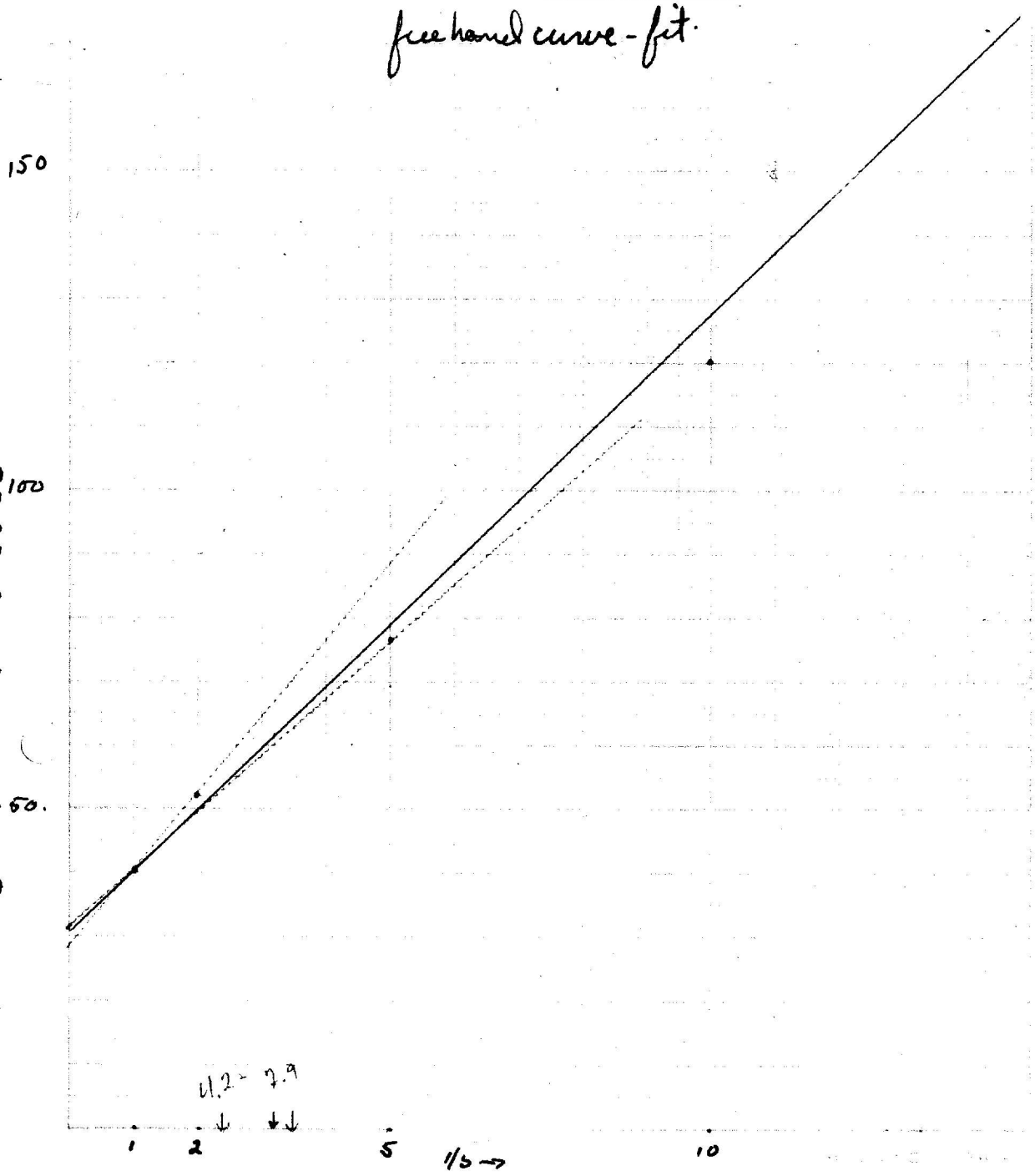
K-12: amylomaltase

4/5/49.

Grow K-12 in 2x 50ml ½ Mal 1% Harvest and dry over P_2O_5. Yield: 29 mg.

Intracellular lactase.

freehand curve-fit.



Kinetics of cellular lactase

4/6/44.

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

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

V_{max} = 322.

K_m = 3.2×10^{-4}

Stirring does not stimulate enzyme action!

K_m 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_{con}	D_{31}
1	22		461-114	342	[illegible]
2	25		307	287	454
3	23		262	142	319
4	20		159	143	~~310~~ 231
	cells		101		

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

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

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

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

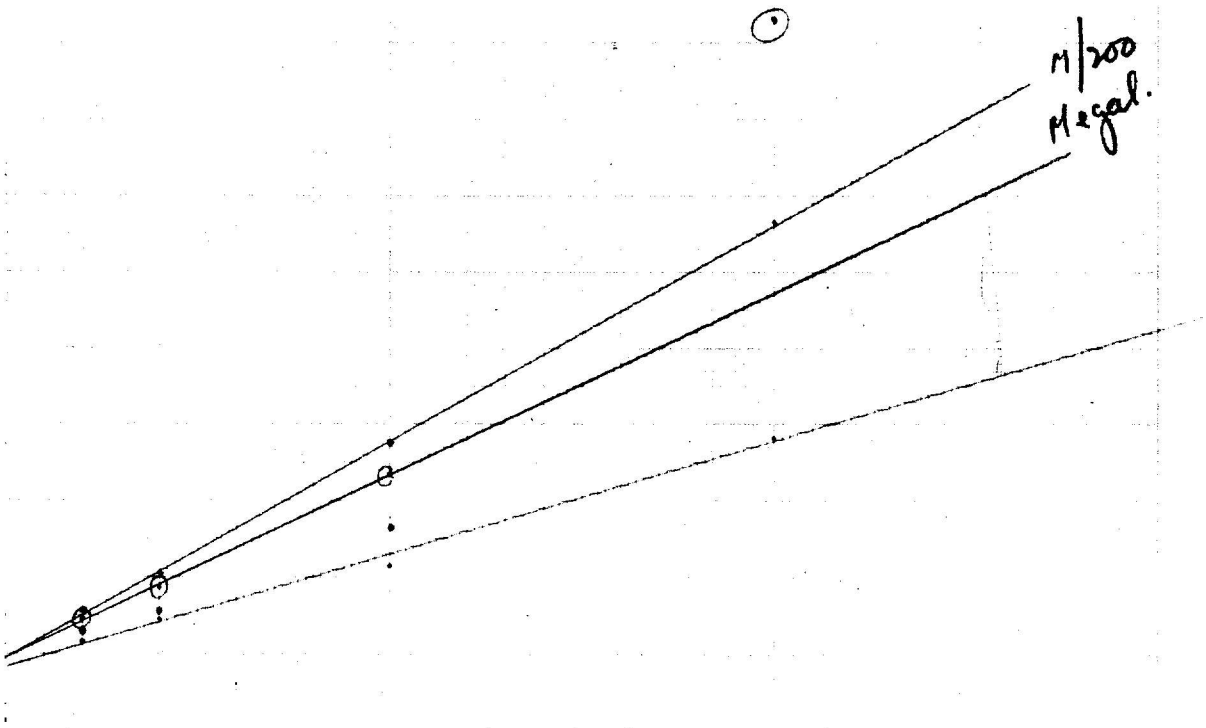
2:25 K-12 + W-349 grown on lactose tested on lactose; lactitol.

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

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

~~510~~

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

Data n.g. ~~expt.~~ needs 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		Time	D
	–	134		30	263
	t_0 calc.	131		60	270
	~~t_0~~	~~131~~		90	271
	20	121 (mixing).		120	282
	30	137	12	In air	
	40	141	13		293
	60	146	14		307
	70	148	15		319
	100	149	16		330
	120	159	17		341
	140	162	18		357
	160	167	19		369
	180	170	20		380
	200	177			24°
	220	180			
	240	184			
	270	190			
	300	197			
6m.	330	202			
	360	209			
	90	212			
	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		

Substrate (~~Df~~) 016 from all exc. #8. − .025 on this one.
~~and Di from~~ for Vcor

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 and yellow~~ Hba does not adapt to ONPG. after 4 hours, progressive color in controls, ca 50. Probably adaptation.

Effect of azide on pH sensitivity.

Compare activity of Lac adapted cells above in KPM/50 buffer pH 5.0 and 7.0 all tubes receive M/50 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:

$$K_I = I \left[\frac{1/v_0 \left(1 - \frac{S}{K_s + S}\right)}{1/v_i - 1/v_0} \right] \qquad \frac{K_I}{I} = k_i = \frac{v_i k_s}{(v_0 - v_i)(1 + k_s)}$$

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

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

$$= \frac{(40)(.57)}{24.5} \times 10^{-2} = .93 \times 10^{-2} \qquad = 8.3 \times 10^{-3}$$

(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 ⊂ purified lactobionate from F. Stodola. NaPM/50 + H2O

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

ONPG added. Caution .010

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

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

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

810

Concentration effects on adaptation

4/20/49.

Lactose 3.6% stock. Make up ½: 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 ½ + 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 ½)

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!