Summary of lactose activation

September 9, ff., 1949.

2 l. aerated K12/42 Lac washed and concentrated to 30 ml. Aliquots of 15 ml ea. mixed 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 $CaCl_2$ and 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

	D_i		R.A.$^{20m.}$	
A	089	193		113
B	080	329$^{6min.}$	257 x 10/3 =	860

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

		D_i				
	Benzene	067	3 min		~~3860~~ 3500	= 3500 u/ml A
1	~~Toluene~~					
2	chloroform	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	Prod. u/mg.
A	3280	~~642~~	.642 g.
B	24800	aqueous	.560 g.

u/mg

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

		D_i	D_e	T.	R.A.	u/ml	u/mg.	u/mg prod.
A	.02 ml	040	560	5min.	2080	~~560~~	1040	5.1
B	.01 ml	014	380	5min.	1464	~~380~~	146	44.5

Benzene: 157

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

Lactase activation

September 9, 1949.

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

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

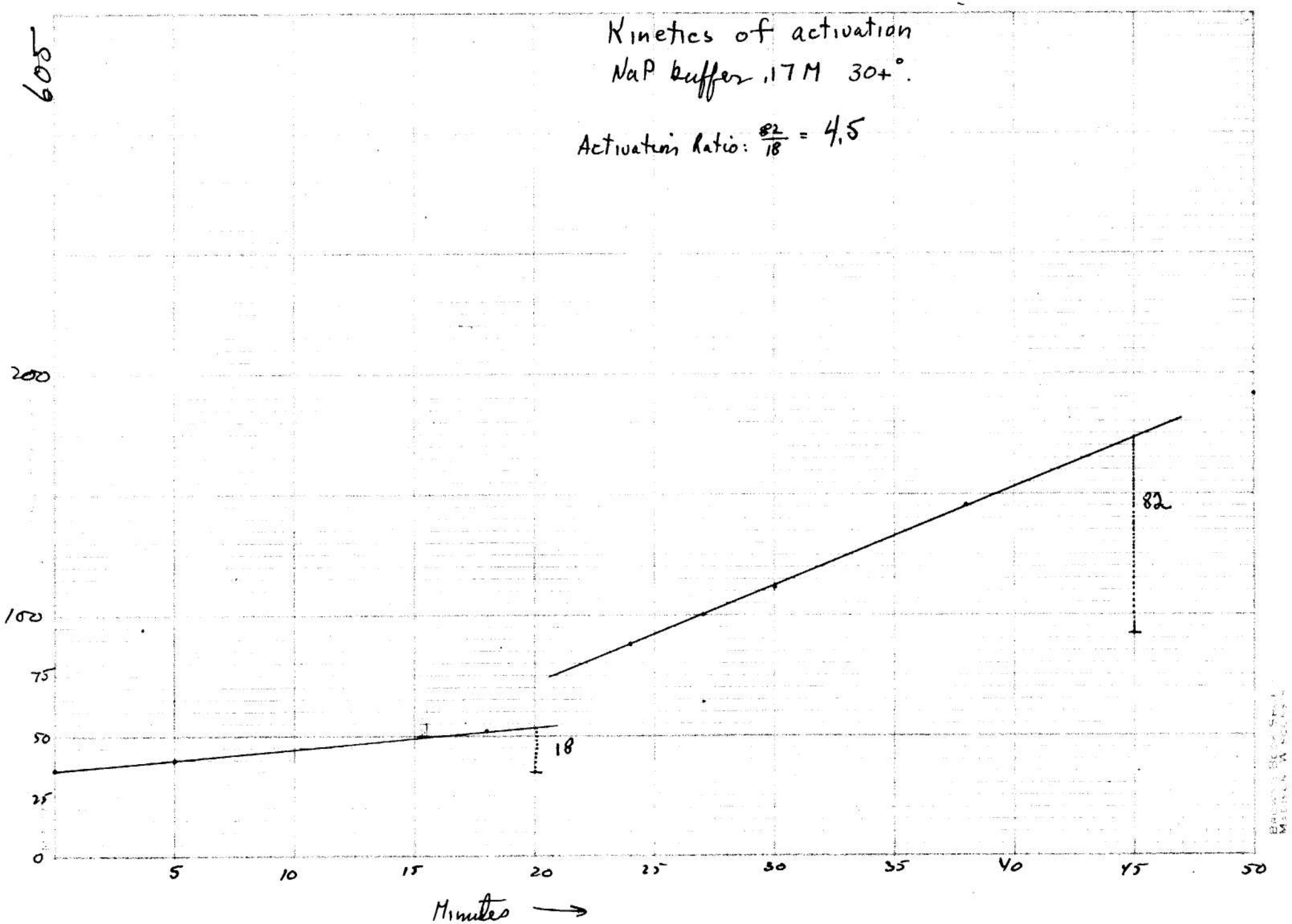
[Dilute $\frac{1}{100}$; $\frac{.5}{10} = \frac{1}{2000}$ for assays.]

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

ONPG Di 034.
Cells Di 200

M/5 buffer NaP. 8.5 ml
cells (add at T0) .5 ml
onpg 1 ml

Time.		D.
2[15]	20 s	036
	60 s	034
	180	035
	240	039
2[20]	5M	040
	~~6M~~	
	7 M	
	8	
	9	
	10	
233	18	052
239	24	087
242 ~~246~~ ~~250~~	27	~~100~~
245	30	112
253	38	146
305	50	191

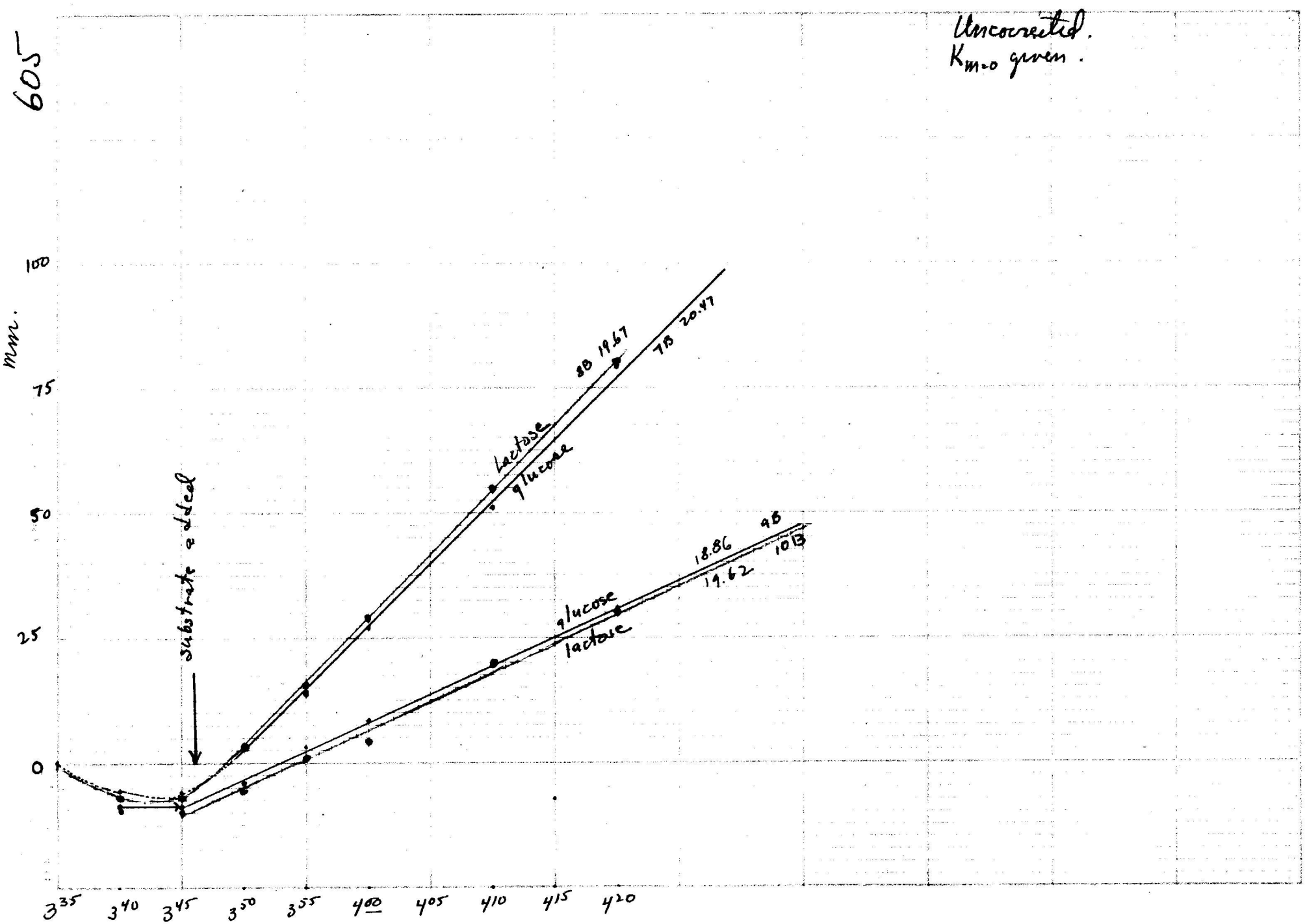

Kinetics of activation
NaP buffer .17 M 30+°.
Activation Ratio: 82/18 = 4.5
600
200
100
75
50
25
0
82
18
5
10
15
20
25
30
35
40
45
50
Minutes →

	Thermo	7-7B	6-8B	9-9B	10-10B
main vessel		← 2cc M/10 NaH_2PO_4 - M/10 Na_2HPO_4 Phosph. →			
	.1cc	← Untreated coli "A" →		treated "B"	M/10 Py buff. 1 hr
side cup	.1cc	10% glucose	lactose	glucose	lactose
3:35	161	104	68	89	73
3:40	148 +3	95 -6	58 -7	78 -8	61 -9
3:45	147 +4	93 -7 -1	57 -1	77 -8	59 -12 -1
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.

Uncorrected.
K_{m-o} given.



Utilization of Isomaltose

September 8, 1949.

D/g D/m M/g M/m M/im 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 mins								
230	(40)	13 -4	09 -1	19 4	18 0	03 -4	42 -6	155 -3
	5	18 0	10 -1	36 20	28 9	03 -5	42 -7	156 -4
	10	25 6	12 0	58 41	43 23	08 -1	47 -3	157 -5
	15	33 14	11 -1	72 55	49 29	04 -5	42 -8	157 -5
	20	36 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

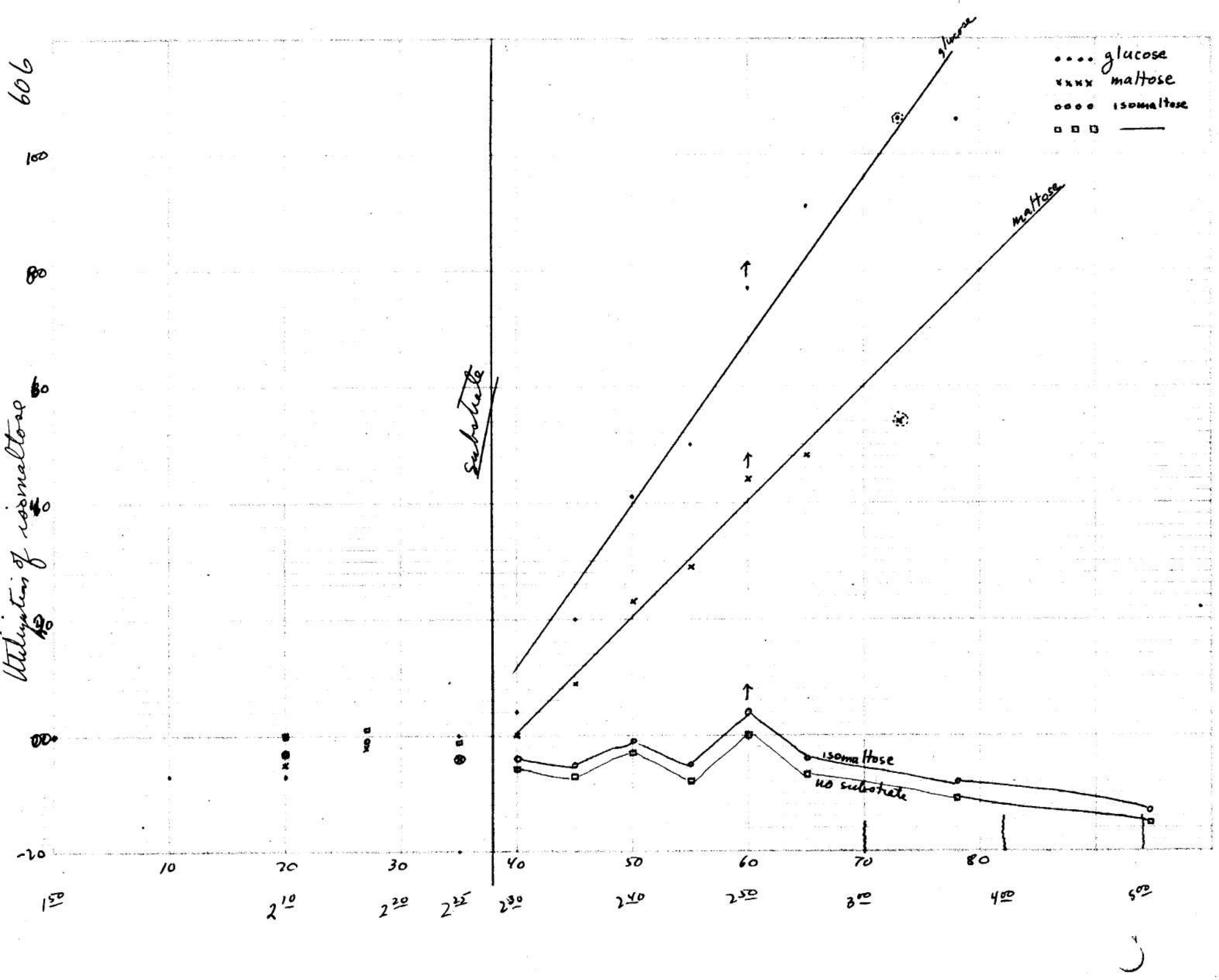
4A M, g

8A M, m

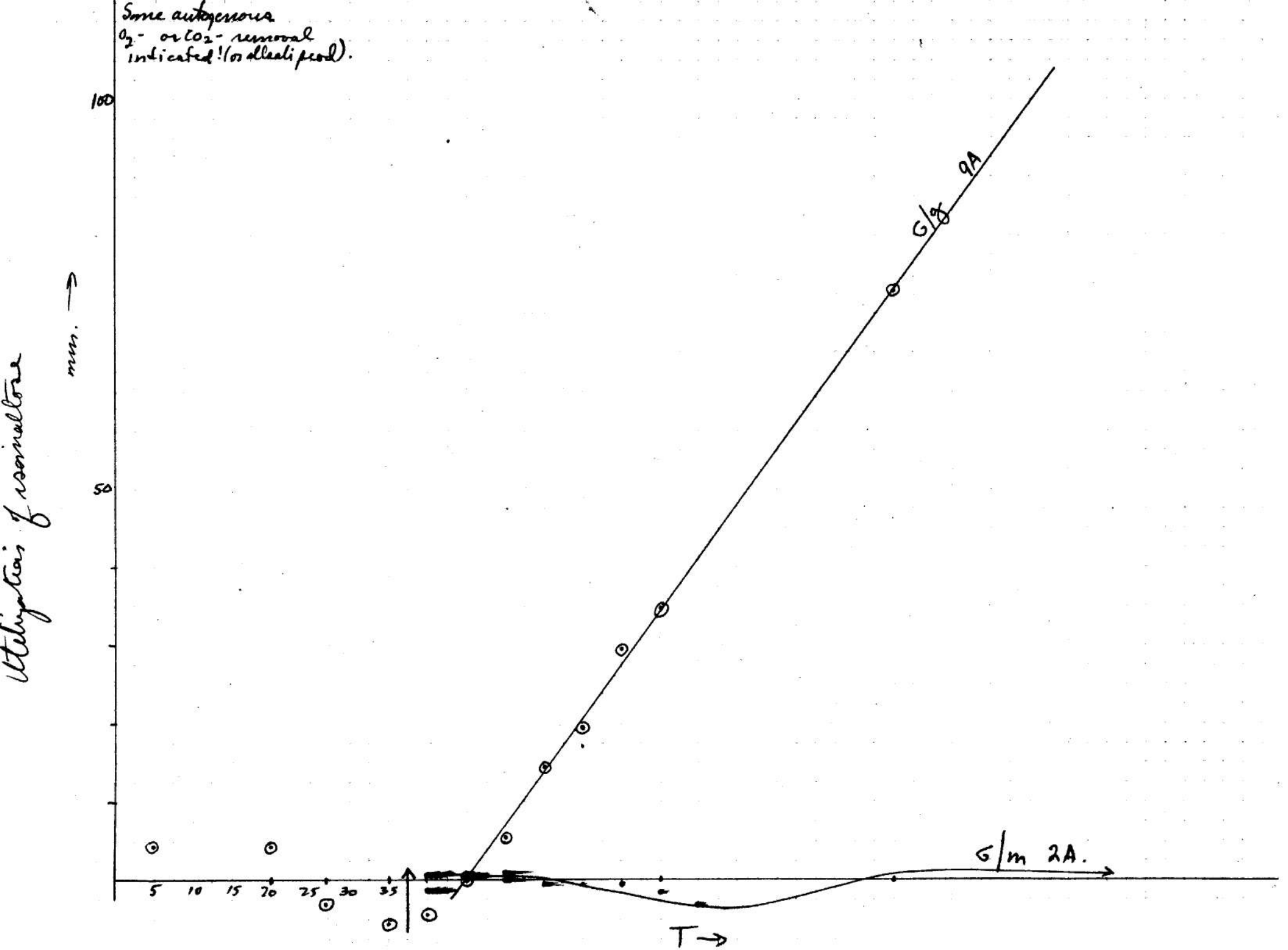
10A M, isomaltase

7A M —

Isomaltose not utilized by maltose-adapted K-12!


606
Utilization of isomaltose
100
80
60
40
20
0
-20
Substrate
glucose
maltose
isomaltose
no substrate
10
20
30
40
50
60
70
80
1:50
2:10
2:20
2:25
2:30
2:40
2:50
3:00
4:00
5:00

Some autogeneous
O_2- or CO_2- removal
indicated! (or alkali prod).



Cross adaptations.

September 8, 1949

		11A 1	16A 2	5A 3	8A 4	13A 5	12A 6	8A 7	T
		T/Tr	T/gl	T/mal	T/ar	D/gal	D/ar	D/—	
11 50	0	17	9	18	19	43	18	8	151
11 55	5	13 -4	5 -4	14 -4	16 -3	40	17	9	151
→									
12 01	11	20 3	23 14	21 3	17 -2	46	9	4	151
12 05	15	33 16	56 47	25 7	21 +2	66	15	6	151
12 10	20	43 26	86 77	22 4	19 0	82	13	7	151
12 15	25	54 37	118 109	19 1	18 -1	100	13	7	151
12 20	30	73 56	160 151	25 7	26 +7	121	22	14	151
12 25	35	93 76	202 193	32 14	35 16	131	27	17	151
		X	X			X			
12 48				38 20	41 27		29	16	151
1 12				51 32	72 52		36	14	152 +1
133				51 31	70 56		36	14	153 +2
2 20				70 43	152 128		46	19	160 +9
3 17				92 56	281		55	23	169 -18
				×					

stopped stock

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

Test on maltose, glucose, trehalose, and arabinose

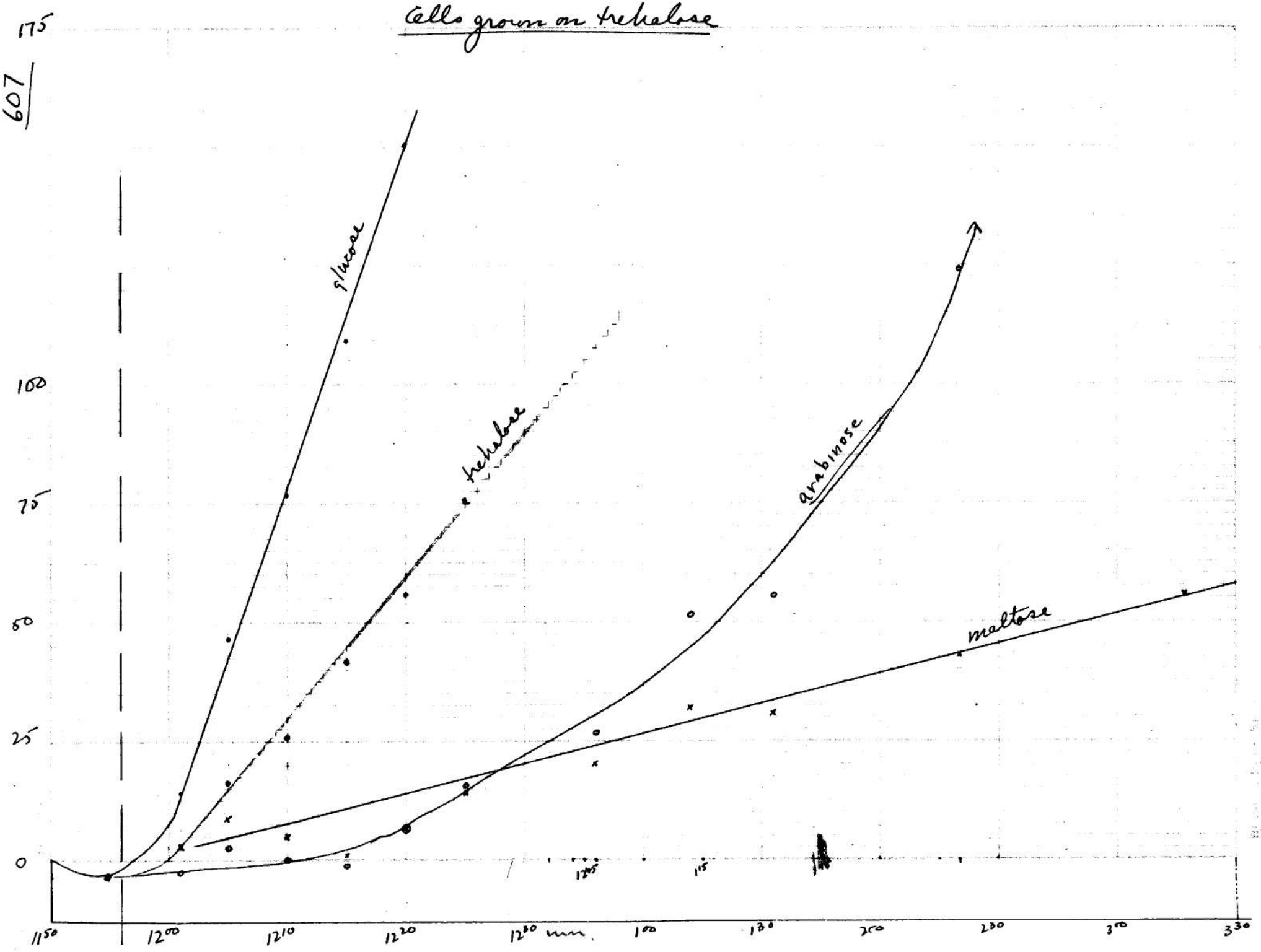
Cells 5×, 2 ml in $NaHCO_3$ M/20 NaP M/1000 set CO_2 32°.

.1 ml 10% sugar at →

trehalose // maltose. Need autoferment. control.

Note rapid adaptation to arabinose (30 minutes)

Cells grown on trehalose



Trehalose in maltose-adapted 607'

		glucose 4	maltose 8	trehalose 10	Thermo
2ml cells in $NaHCO_3$ + CO_2	9 10	27	08	10	148
M/20	9 15	23	06	09	146
K12 / Tip Sub. maltose 32°		*			
	9 20	20	09	16	149
	9 [illegible]	38 +18	29 +20	21 +5	149 0
	9 30	54 +15	43 +13	22 0	150 +1
Bubble later autom.	9 [illegible]	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	

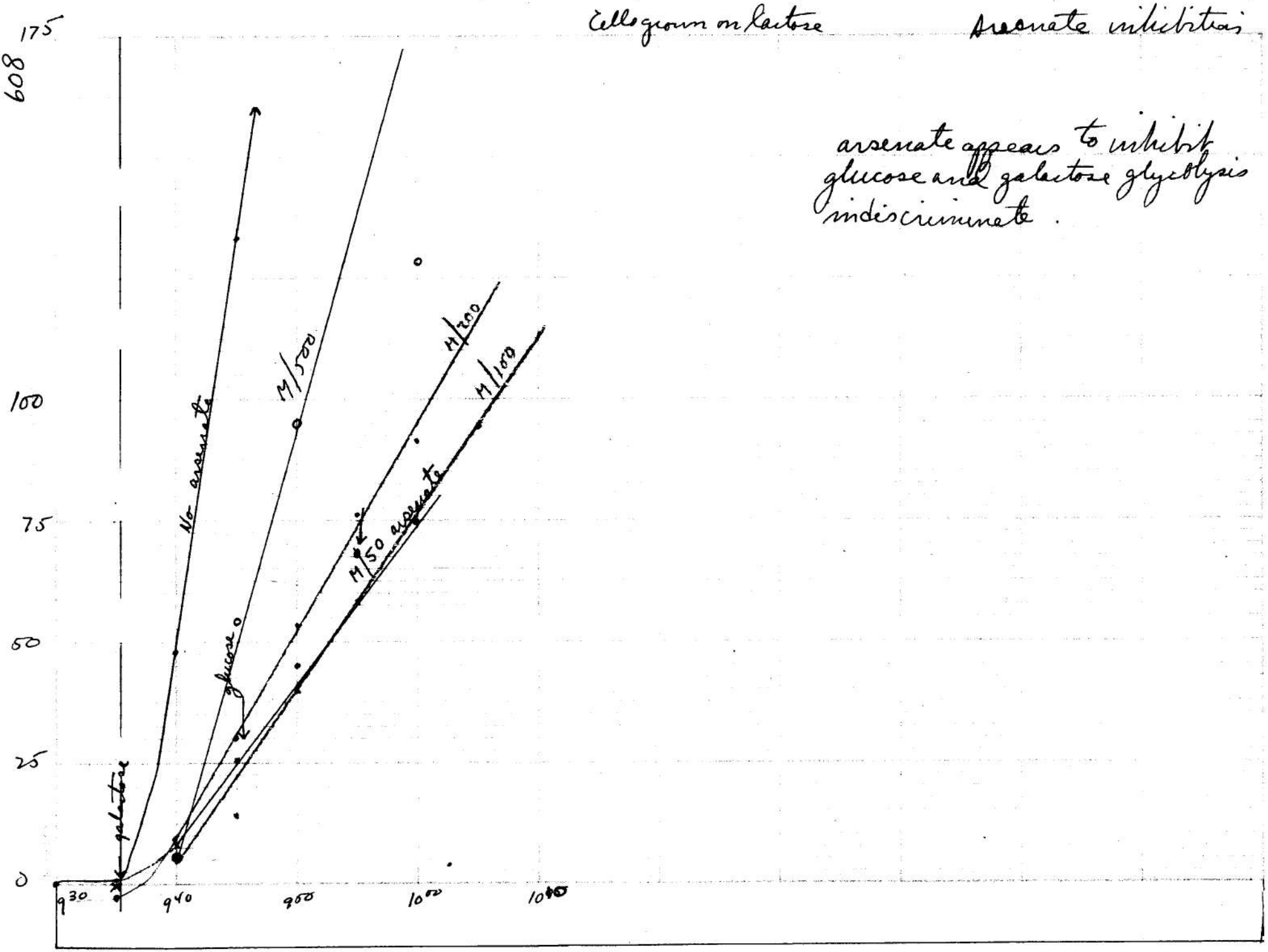
Arsenate inhibition of galactose fermentation.

September 9, 1949

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

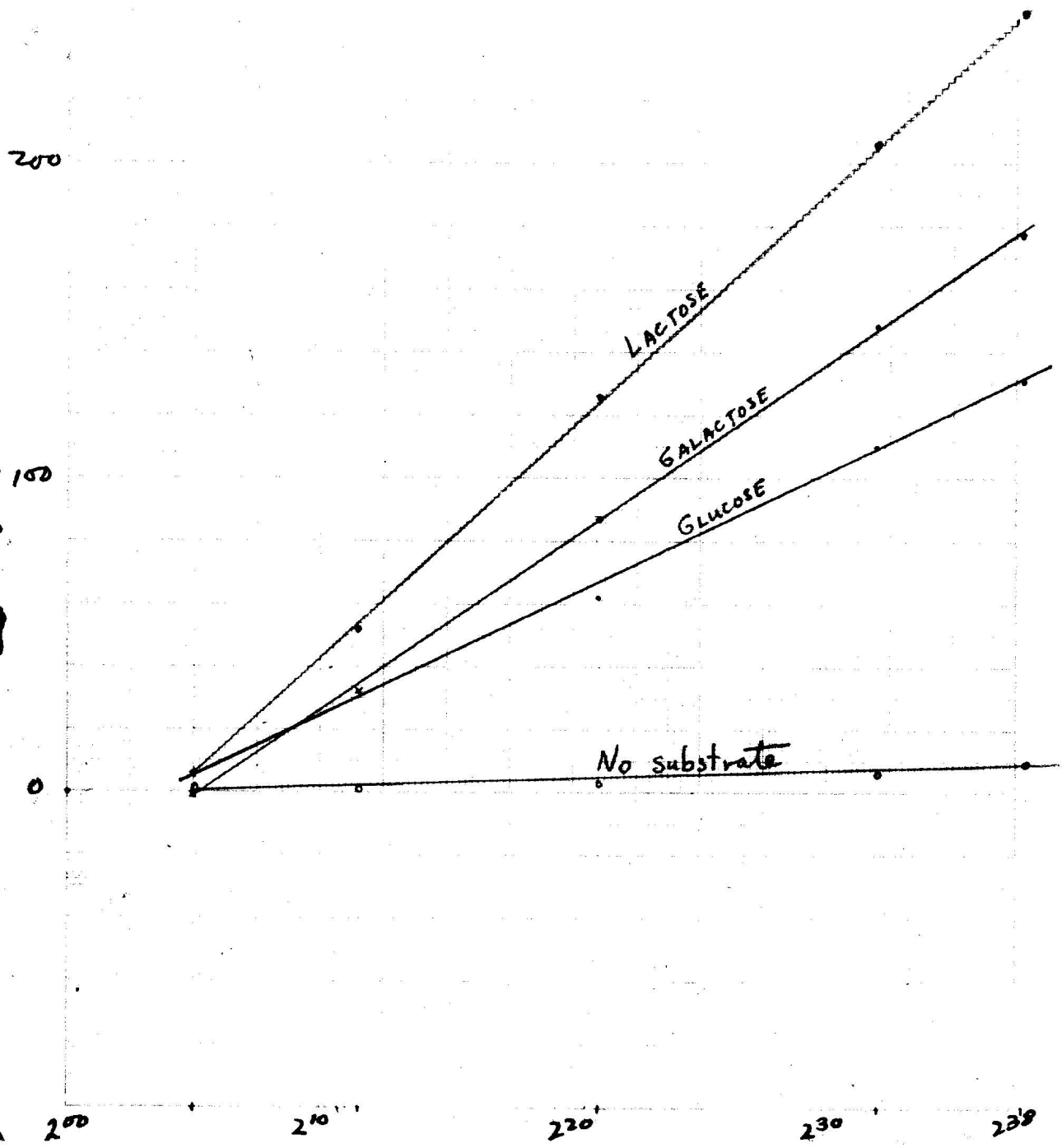
$KAsH_2PO_4$	—	M/50	M/100	M/200	M/500		T
	1 9A	2 7A	3 4A	4 2A	5 10A	6	T
930	47	18	16	30	03		151
935	47 +1	17 0	~~12~~ 16	27 -2	02 0		150 +1
→ galactose 940	95 47	27 8	22 5	39 8	10 6		152 -1
945	181 133	45 26	41 14	61 30	58 54		152 -1
	X → glucose						
~~950~~		58 40	61 45	83 53	98 95		151 0
955		75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

Cells grown on lactose Arsenate inhibition



arsenate appears to inhibit glucose and galactose glycolysis indiscriminate.

T.


200
100
0
LACTOSE
GALACTOSE
GLUCOSE
No substrate
2^00
2^10
2^20
2^30
2^39

W25 1a Lactose utilization 609a.

9/13/49

See

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

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 accl. for the poor lactose activity seen here.

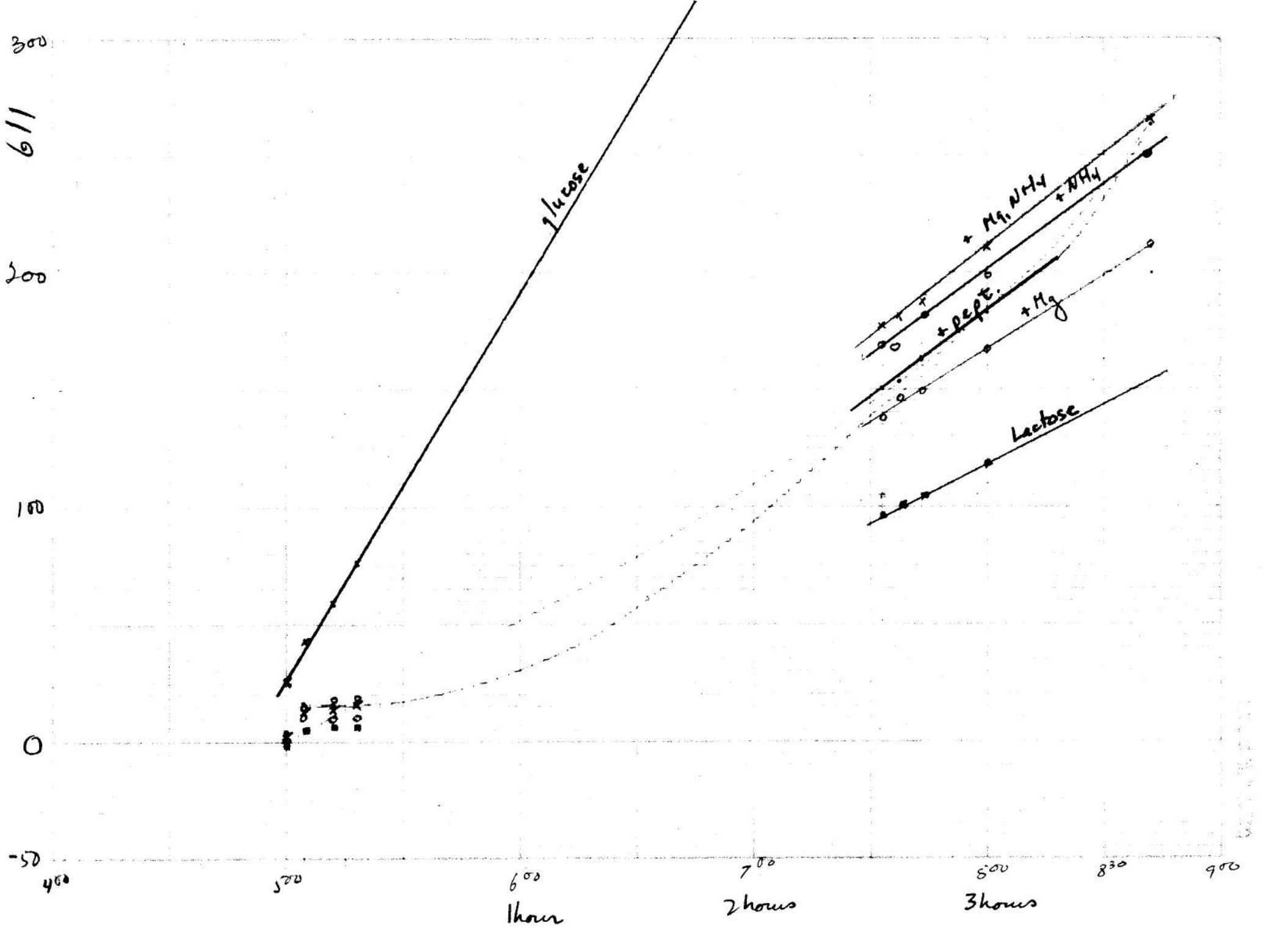
	glucose 1	5B	galactose (glucose) 2	2B	lactose 3	6B	/ purg gal 4	4B	T
1240	-2		18		-3		16		147
1245	-1		23		04		27	(153)	147
1246	05		28		08		27		153
1250	10		32		12		31		157
1255	04		28		08		25		151
100	04		30		10		20		148
110	14		40		19		31		153
116	22		48		25		32		150
121	28		52		27		29	(151)	154
131	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
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	26 0	37 4	22 0	40 3	57 -1	40 25	33 -5	69	161
505	30 17	36 16	19 10	36 12	50 +5	45 43	20 +8	61	161
510	33 16	42 18	23 10	41 13	55 6	65 59	24 +4	64	161
518	33 16	43 19	23 10	43 15	55 6	82 76	24 +4	65	161
733	174 150	200 169	158 138	212 177	152 96	199 [illegible]	36 -3	76	161
737½	179 153	201 168	163 146	217 180	158 100	213	33 -5	76	161

	I R	II L
1.	Lactose	Glu
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/100.

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

		D_i	$D^{onpg}_{10MN.}$	Δ'	
540 L4	water	007	438	384	
L2	glucose	002	217	166	
L3	lactose	0	165	116	!
M4	water	001	072	022	
M2	glucose	021	058	008	
M3	lactose	0	071	022	
M4	Mgso4 + lactose	0	074	025	
—		-007	042 = 49	0	

Cell density L 19.9
M 13.3

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

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

K12 / mal and / lac
showing decrement of activity
when incubated with lactose or glucose!

Consistency of benzyne 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 benzyne / tube.

5 PM add 9 ml H,O to 1-5. (#2 mediat.).

Assay. 2 ml samples.

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

Too crude to be used in present stage of development.

Effect of N-supply on lactase deadaptation.

September 24, 1949.

12 hour cells aerated not washed

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
150	155-53	30	22	32	44	39	33	57	46	45
155	154-48	32	24	34	43	35	29	51	41	38
200	157-53	32	24	34	45	38	32	56	45	43
201 →										
205 →	158-54	30	24	33	44	36	33	57	45	43
210	157-48	28	24	32	42	35	34	57	45	40
215	156-53	28	22	31	41	34	36	59	49	42
220	150-58	27	21	30	44	38	39	66	60	49
225	150-59	30	26	34	46	44	53	74	67	51
230	161-60	35	30	39	53	51	63	82	73	52
235	161-61	48	31	40	53	51	63	80	75	52
240	154-58	44	39	48	64	62	76	88	81	51
245	157-60	51	46	53	67	66	80	90	85	51
250	162-58	57	48	54	70	68	84	93	89	56
255	162-56	60	50	55	71	71	89	96	92	49
300	161-57	62	51	57	73	75	93	100	97	50
310	162-55	62	52	57	71	80	101	104	103	47
320	158-55	70	53	58	79	84	106	107	108	46
330	160-52	75	57	62	83	90	116	113	116	44
400	155-60	90	71	73	100	119	150	141	148	55
430	155-58	102	76	75	104	128	164	150	161	50

1 LAC 1 mg.
2 GLU+GAL .5 ea.
3 GLU .5
4 GAL .5
5 LAC 10
6 GLU+GAL 5 ea
7 GLU 5
8 GAL 5
9 —

530	163	127	108	99	135	182	219	191	209	155

1 ml cells W251a/lac aer. in NaHCO3 4/20 .05 - .10 microaerns.

645	159	127	127	110	137					55

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

Gal'ase activity in unadapted cells.

Sept. 30, 1949.

Harvest K-12 from 12 hour aer. Y2 — 50ml. Conc. to 5ml (10X)
Leave water suspensions on table top 10A — 730P 30.

1 ml aliquots incubated ē benzene 7:30 – 9:00 PM (90 mins.)
Test samples per standard ONR (M/2000 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.)	1.5×10^3	.58	100
(.01 ml)	K/Mal	027	380	$.36 \times 10^3$	.12	21
(.1 ml)	K/Glu	070	269	$.02 \times 10^3$	.02	3

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

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

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

Oct. 1, 1949.

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

1 ml aliquots to small tubes and incubate ½ gum 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 activation	4	/Lac	.1	054	218	293	100
" "	5	/Mal	.2	129	193	51	17

						RA/ml	RA/ml/Di	
Benzene treated 2hrs?	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. 2 hour treatments (430 – 630). At time add 4.5 ml H_2O for 1/10. Take .1 ml amts = .01 ml (except 3)

		Di	D_{onPg}	R.A./Di	
Octyl Alc (.1 ml)	1	012	268 (5)	573	(100)
	2	603	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	605	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 OcOH at pH 7.5.

		$D^{onP.}_{M/50,000}$	
		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/ PM. .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~~ 210		
		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.

Cells.		Amt	Di	Dongs	RA
	K/L	.02	052	149	~~0~~ 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

Not activity!

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

CM

1: .5 ml susp. all in duplicate

~~2: .5 ml + 4.5 ml H₂O~~

2: .5 ml " 4.5 " A thymol

3: .5 ml (1/10) + 4.5 ml " B ~~to~~ octanol

C. enzyme

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

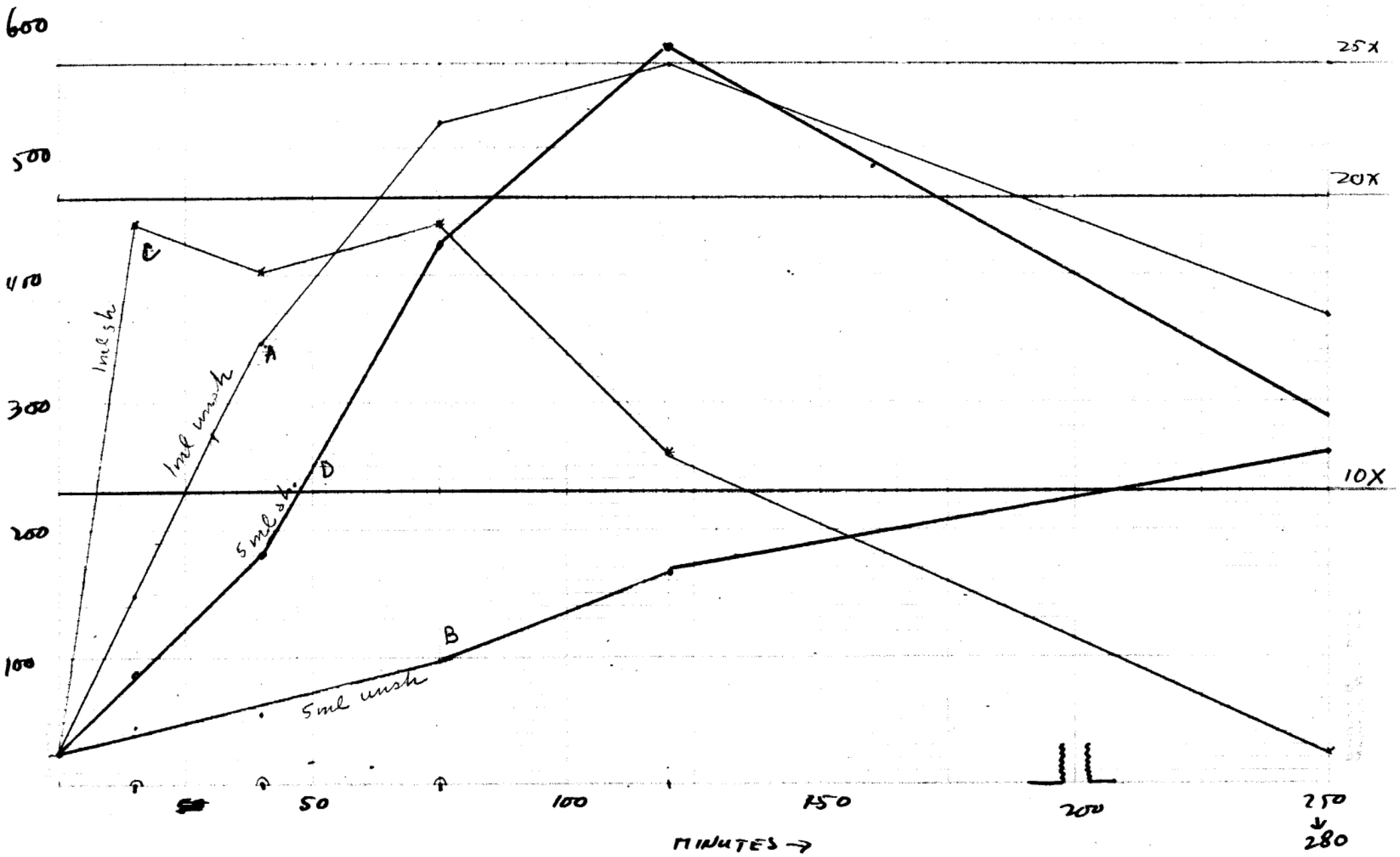
	A (Thy)	B (Oc)	C (B_2)
1	318	131	200
1	359	118	171
2	062	054	054
2	060	057	062
3	082	069	067
3	093	053	064

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: ~~1~~ 1 2 3.

Kinetics of gel'ase activation by thymol



Kinetics of Gal'ase activation. Effect of shaking

10/3/49.

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

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

Remove .1 ml samples from time to time; dilute in water 10 ml and assay. Terminate F Na, CO_3, exc. intact cells mid amts.

		MINS	Di	D^{20}_{onpg}
3^{36} T		15		
Intact	.1 ml	0	089	318
	.01 ml		003	23
1	A	15		149
1	B			46
1	C			~~460~~ ← (440)
1	D			87
4^{00}		40		
½	A			173 x 2 = 346
1	B			53
½	C			201 x 2 = 402 (!)
1	D			180
½	A	75		260 x 2 520
	B			98
½	C			220 x 2 440
	D			423
1	A			281 x 2 562
1	B	120		99 166
1	C			120 240
1	D			289 x 2 578
	A			369
	B			260
	C			025
	D			289

mc 1/100 dil

4^{36}

5^{20}

8^{00}

10% < above!

2 hours optimum for unshaken cultures.

2½h. 1 ml. titrated 5^{15} - 8^{15} test + compare:

	2½h.	18h.
Thymol	479	178
phenol	016	—
enzyme	466	685
octanol	369	222

Repeat overnight.

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.				
	K/L .01/ml	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; Glyc. Ser. [Benzene from 12N ± I. ca 8 hours.

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

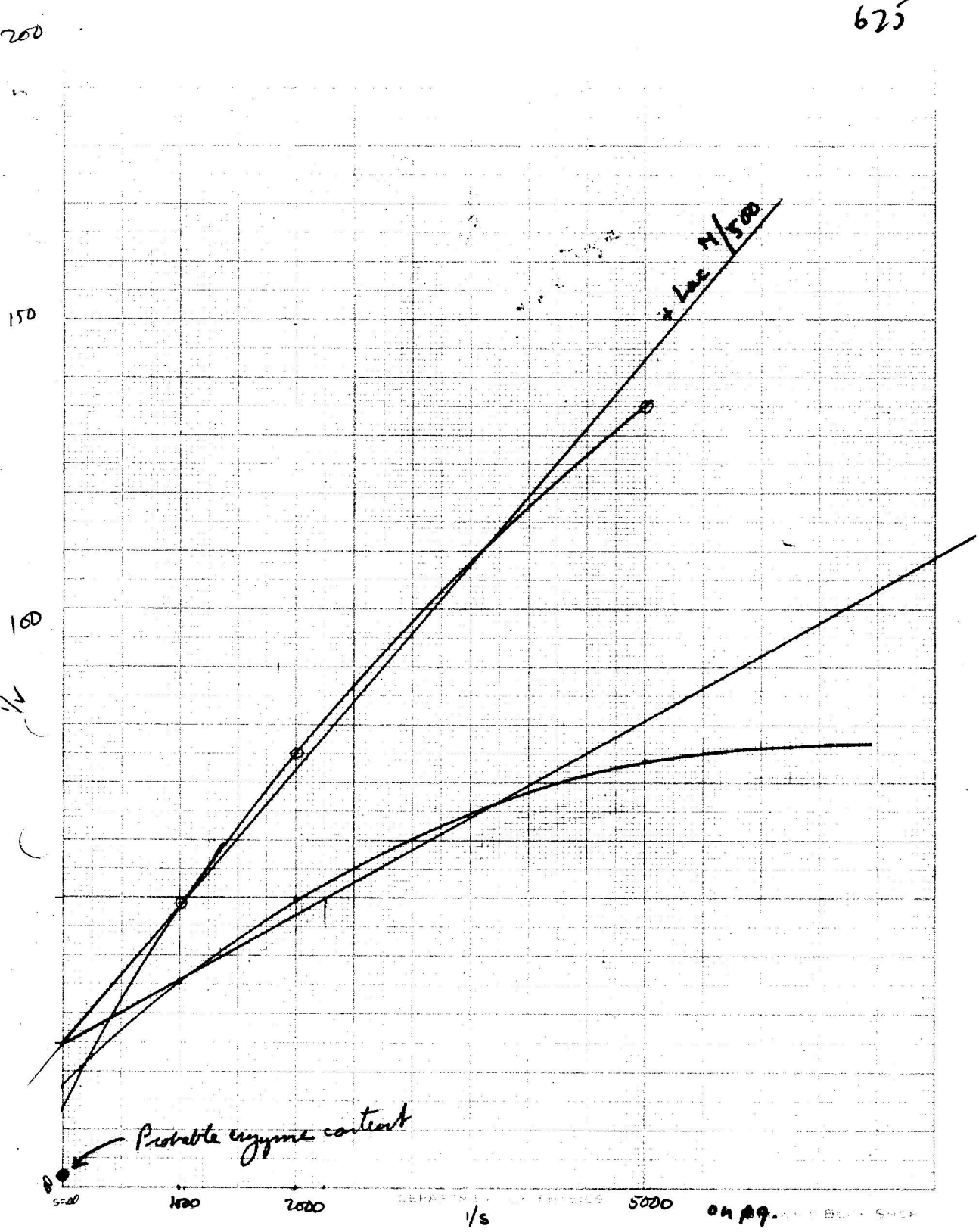
		I	Di	Dongg
Instant	L	.05	087	246
	M	.1	157	189
	G	.1	130	124
	—	.1	214	250
	L	.005	0	530
	M	.02	018	194
B2	G	.1	069	074 (5:35 PM)
4h.	—	.02	032	267

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

	Di	Dong		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 "	104	174 20M	69	38		10	1.8
— "	080	141 "	58	47		12	2,3

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


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

Kinetics of Gal'ase in intact cells.

Oct. 7, 1949.

K-12 harvested from Lac Y2

Konpg and Klac.

M/100 Na P

	M onpg	lac	Di	$D_{onpg}^{Na_2CO_3}$		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: cale: $V_{max} = 1/25 = \underline{\underline{400}}$

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

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

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

	cell	ex
onpg	5	1.3
lac	20	14

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