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 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 V_{max}. pH characteristics of activated cells. Rb responses.

September 9, 1949

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

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

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

4:30 – 5:10 in A cells.

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

∴ 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.	Pred. u/mg.
A	3280	~~560~~	.642 g.
B	24800		.560 g.

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

		Di	De	T.	R.A.	u/ml	u/mg.	u/mg pred.
A	.02 ml	040	560	5min. 2080	~~560~~	~~[illegible]~~ 1040	104	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.

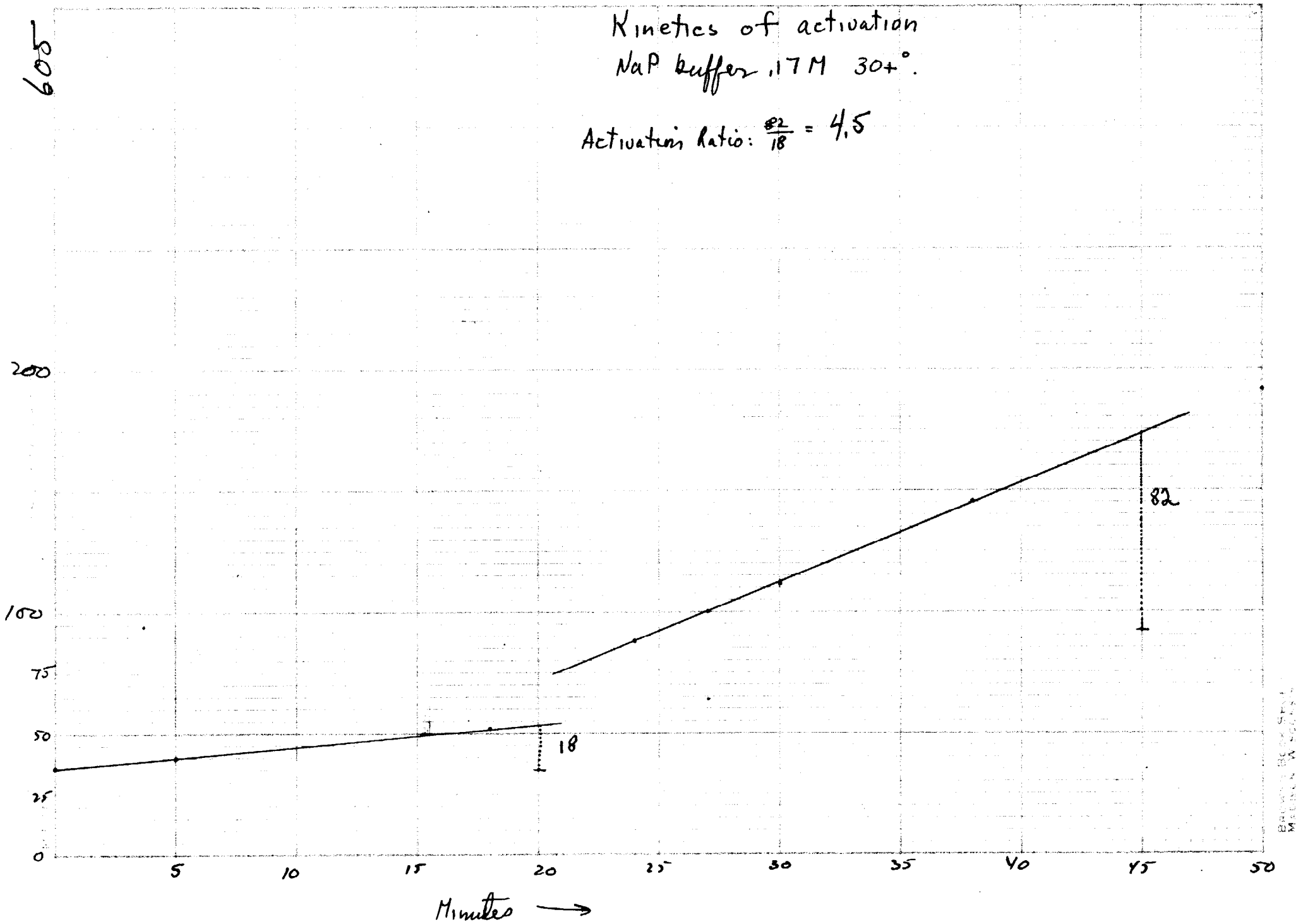
ONPG Di 034.
Cells Di 200

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	~~[illegible]~~ 27	~~[illegible]~~ 100
~~250~~		
245	30	112
253	38	146
305	50	191

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

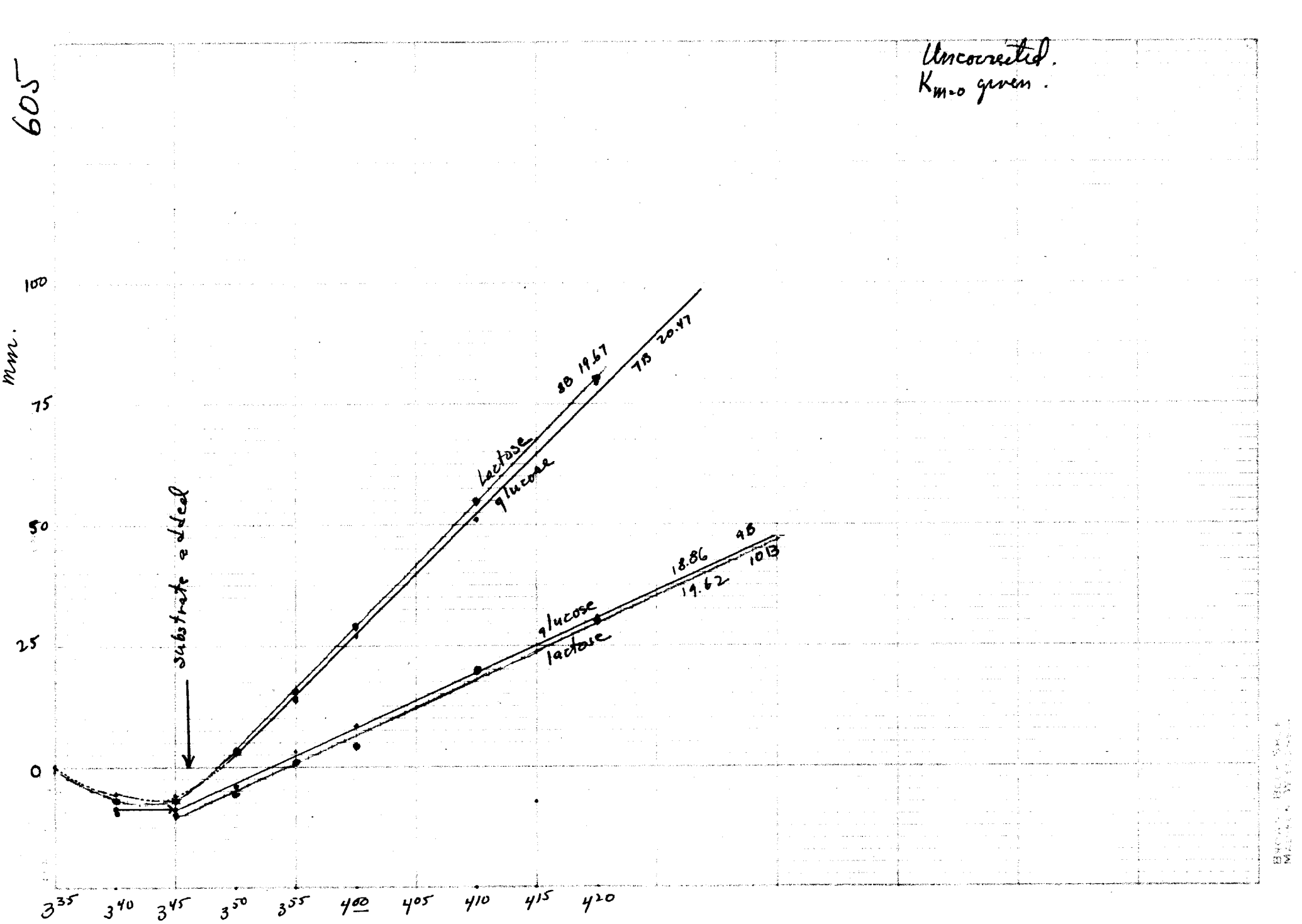
Activation Ratio: $\frac{82}{18} = 4.5$



	Thermo	7-7B	6-8B	9-9B	10-10B
main vessel		← 2cc M/20 $NaHCO_3$ - M/100 Phosph →			
	.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	39	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 -10 -1
3:50	150 +1	106 3 +10	70 +3 +10	84 -4 +4	66 -6 +4
3:55	149 +2	119 17 +14	84 16 +15	90 +1 +7	72 +1 +7
4:00	147 +4	127 27 +10	93 29 +11	93 +8 +5	73 4 +3
4:10	149 +2	153 51 +24	121 55 +26	107 20 +12	91 20 +15
4:20	153 +2	185 79 +28	157 80 +26	122 31 +11	105 30 +10

Manometric tests on "activated" cells.

ca. 50% inactivation of buffer treated cells.


605
Uncorrected.
$K_{m=0}$ given.
mm.
100
75
50
25
0
substrate added
Lactose
Glucose
80 19.67
75 20.47
Glucose
lactose
18.86
19.62
96
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/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 minutes								
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

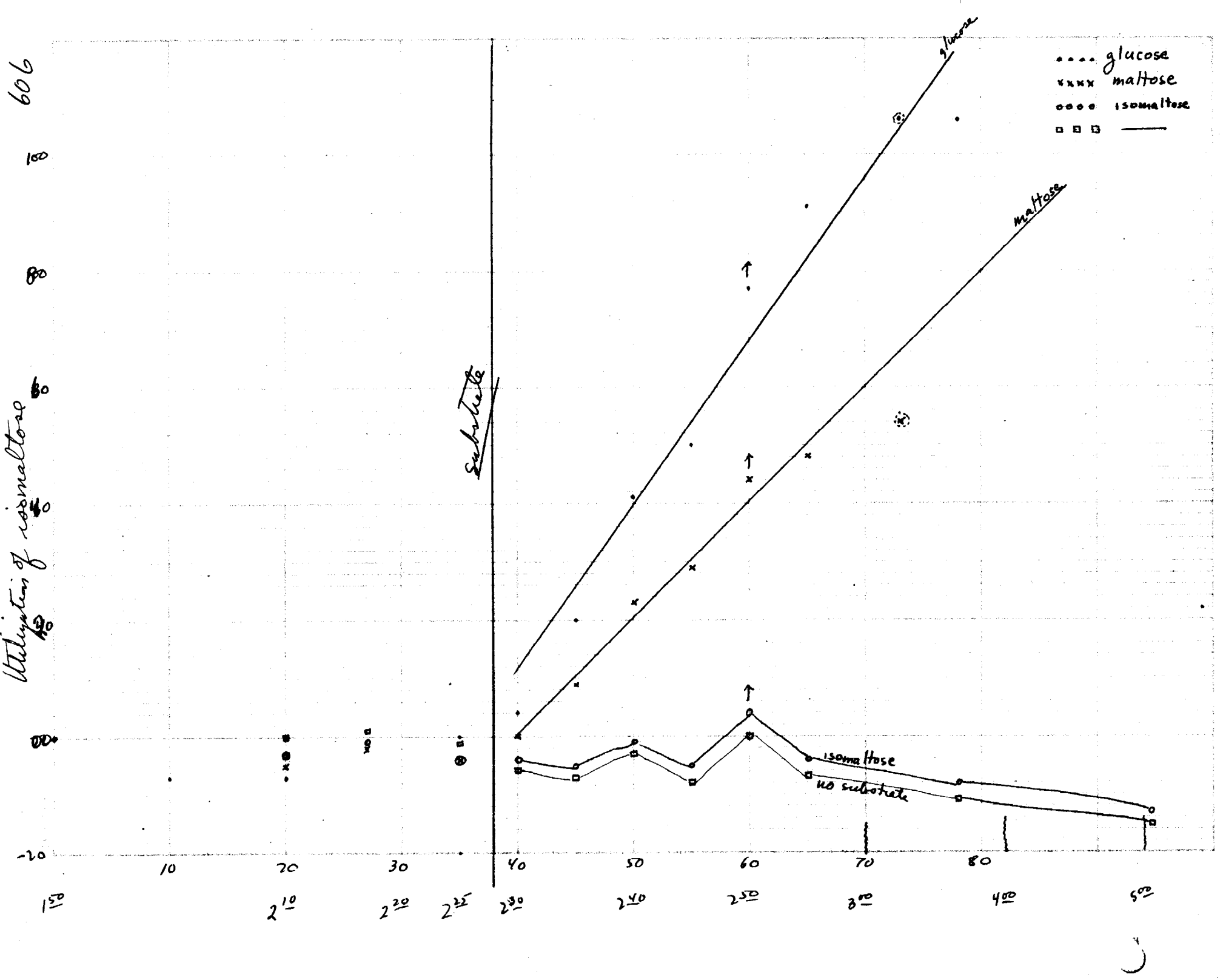
4A M, g

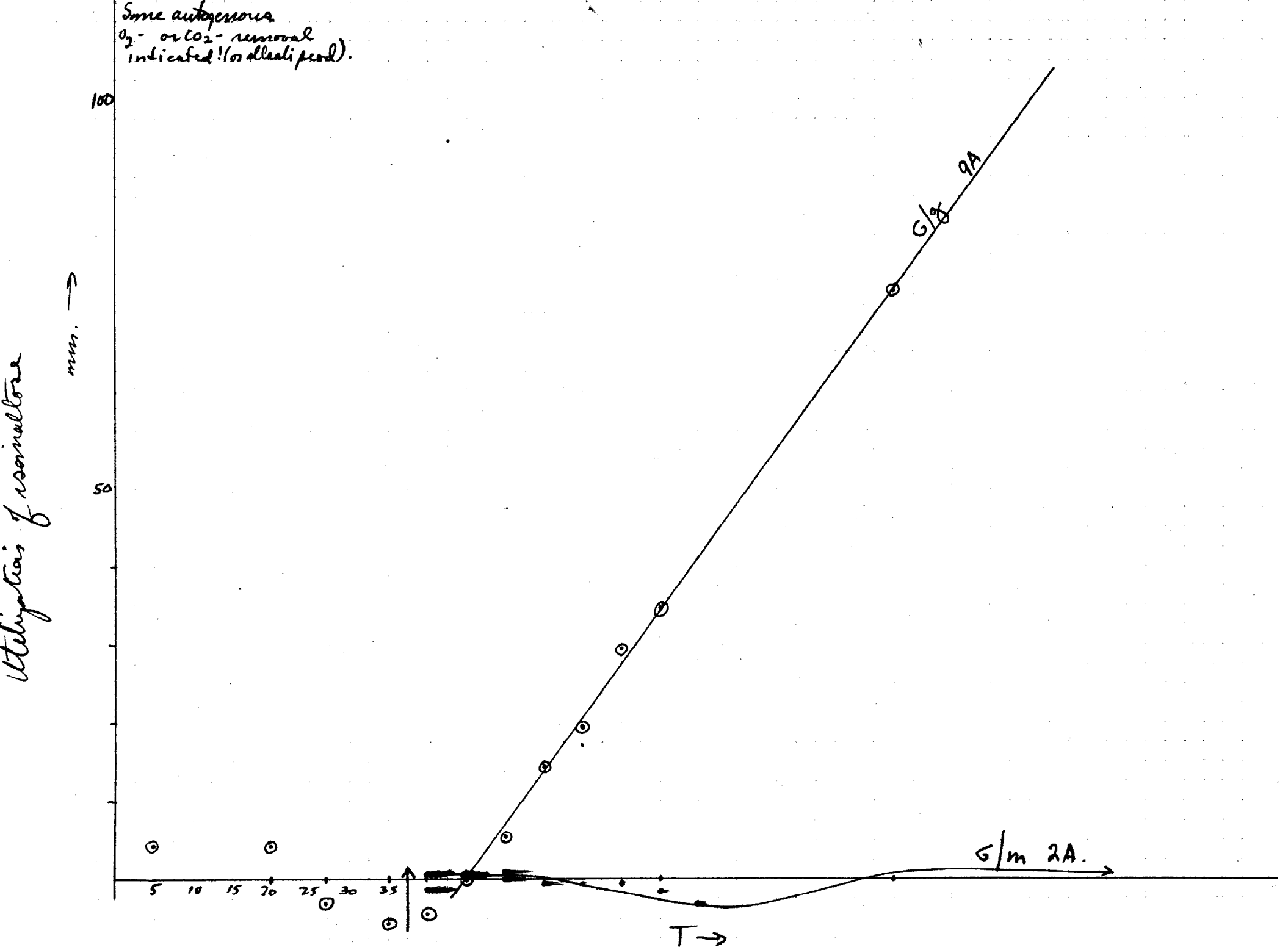
8A M, m

10A M, isomaltase

7A M —

Isomaltose not utilized by maltose-adapted K-12!


606
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
Substrate
glucose
maltose
isomaltose
no substrate
glucose
maltose
isomaltose


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

Cross adaptations.

September 8, 1949

		1A 1	6A 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	
→										
1201	11	20 3	23 14	21 3	17 -2	46	9	4	151	stoppers stuck
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	
		×	×			×				
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 ½ Trehalose ½% (T) or Galactose 1% (D).

Test on maltose, glucose, trehalose, and arabinose

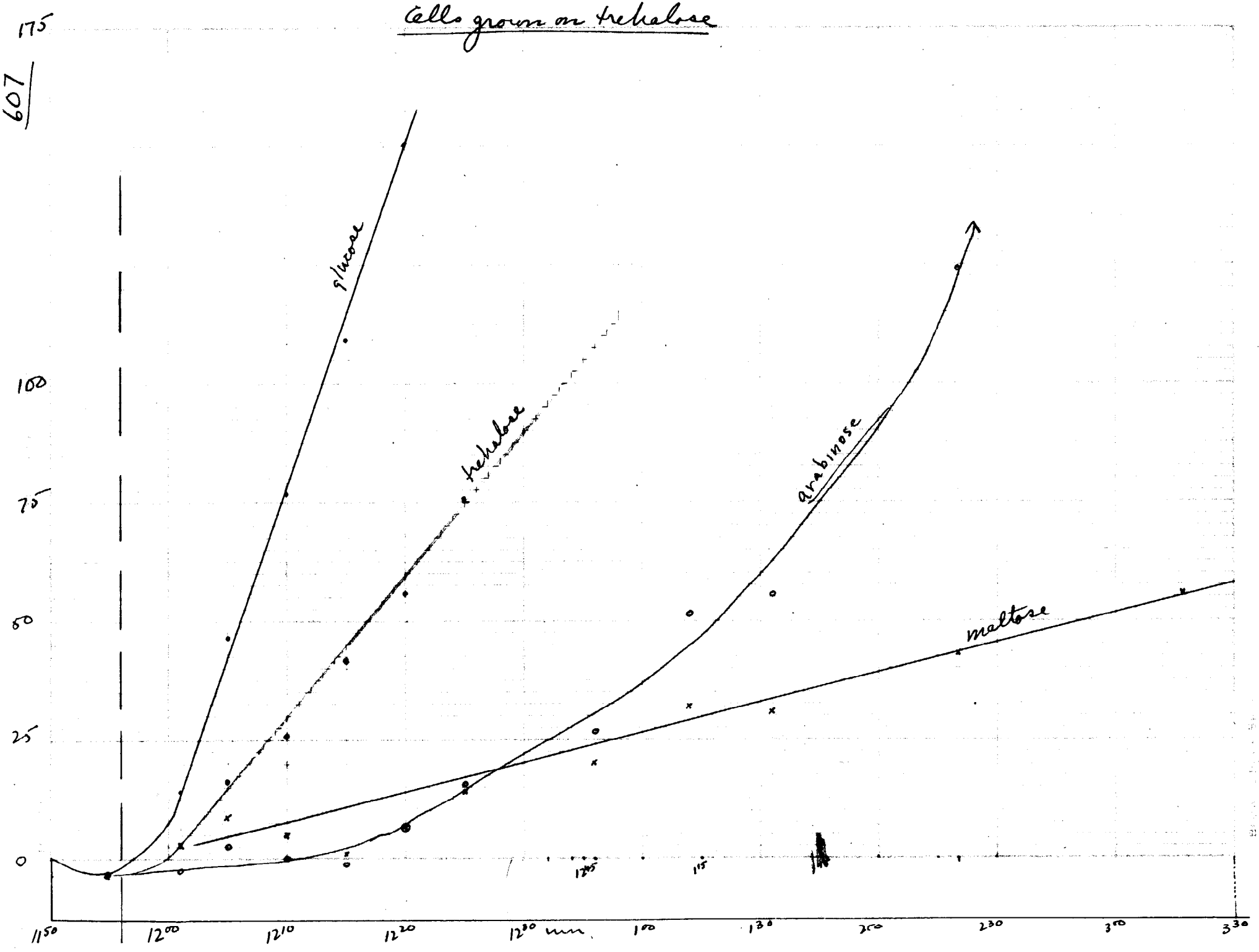
Cells 5×, 2 ml in $NaHCO_3$ M/20 NaP M/1000 set CO_2 32°.

.1 ml 10% sugar at →

trehalose // maltose. Need autoferment. control.

Note rapid adaptation to arabinose (30 minutes)

Cells grown on trehalose



Trehalase in maltose-adapted 607'

		glucose 4	maltose 8	trehalase 10	Thermo
ml cells in $NaHCO_3$ + CO_2	9^{10}	27	08	10	148
M/20	9^{15}	23	06	09	146
K12 / Tip Sub.		*			
maltose	9^{20}	20	09	16	149
32°	9^{26}	38 + 18	29 + 20	21 + 5	149 0
	9^{30}	54 + 15	43 + 13	22 0	150 − 1
Bubble later	9^{36}	79 + 26	71 + 29	21 0	149 + 1
sodium.	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.

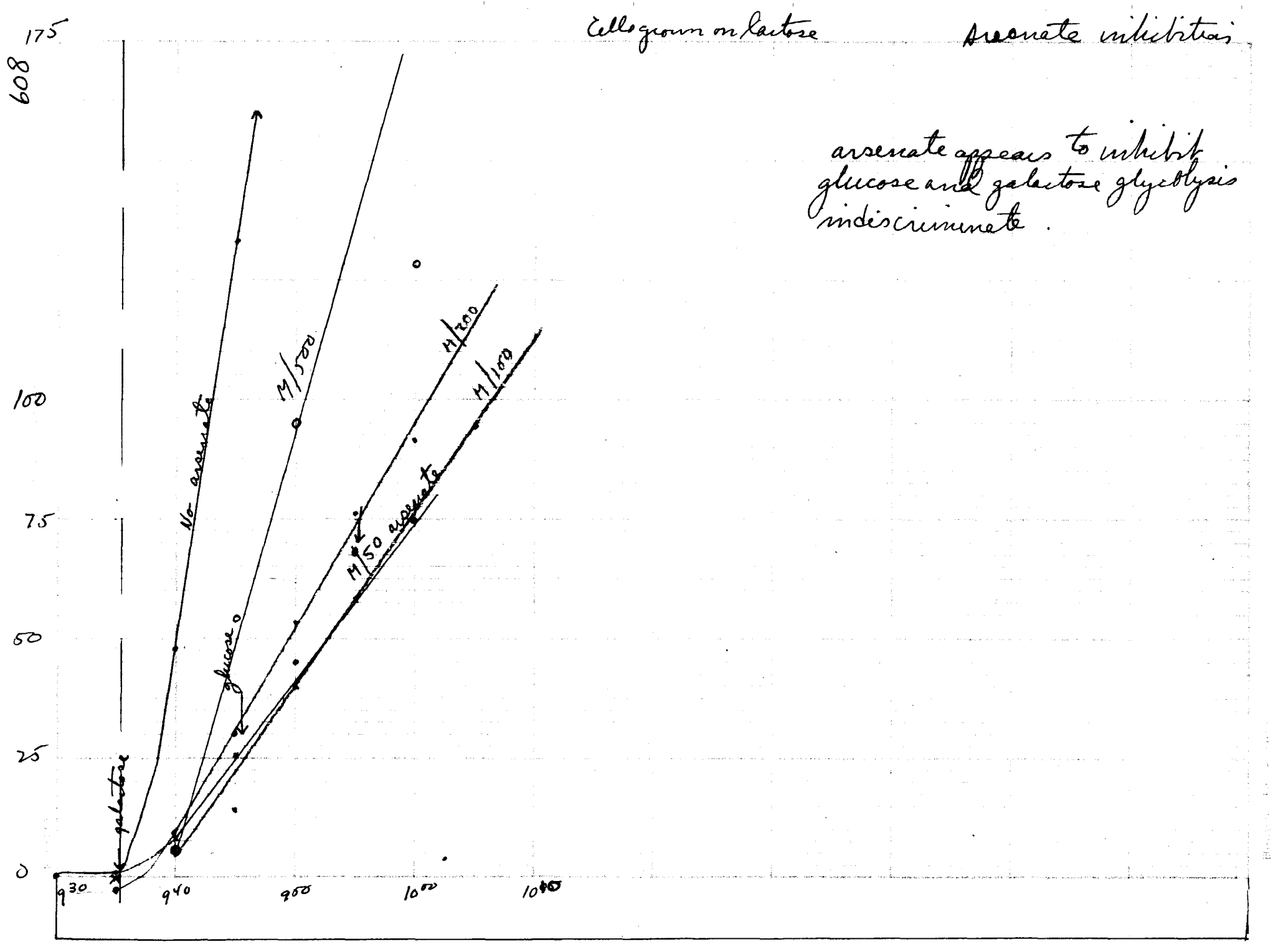
September 9, 1949

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 ~~bril~~ 16		27	-2	02	0		150 +1
→ galedrose 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

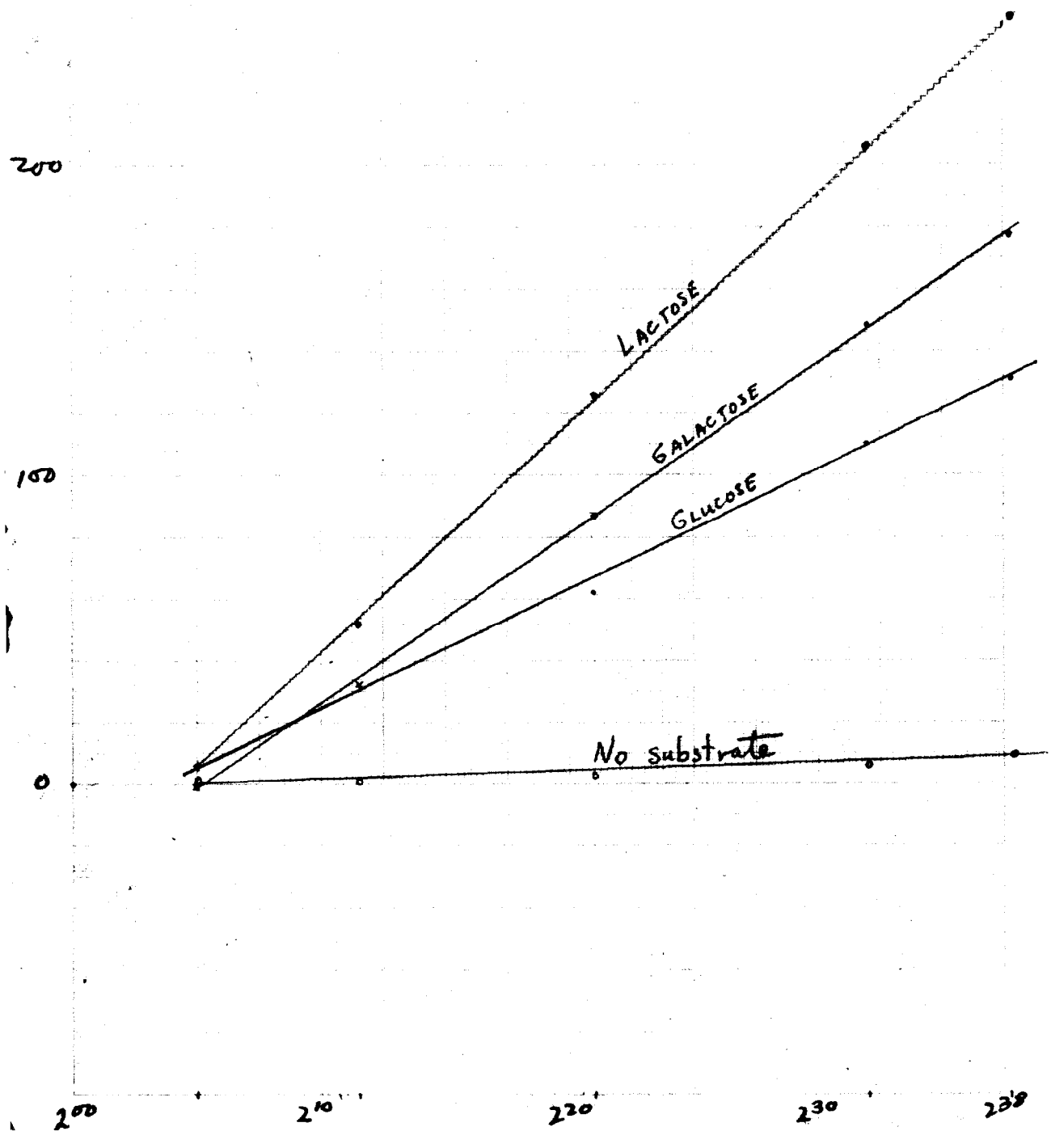
Cells grown on lactose

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!

Struck out 1B: ca 30% Glu+!

Struck out culture 1

W251a Lactose utilization 689

9/11/49

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

Cells were grown in 50 ml Y2 lac overnight c̄ aeration. However, the medium evaporated to ca 15 ml. This may acct. for the poor lactose activity seen here.

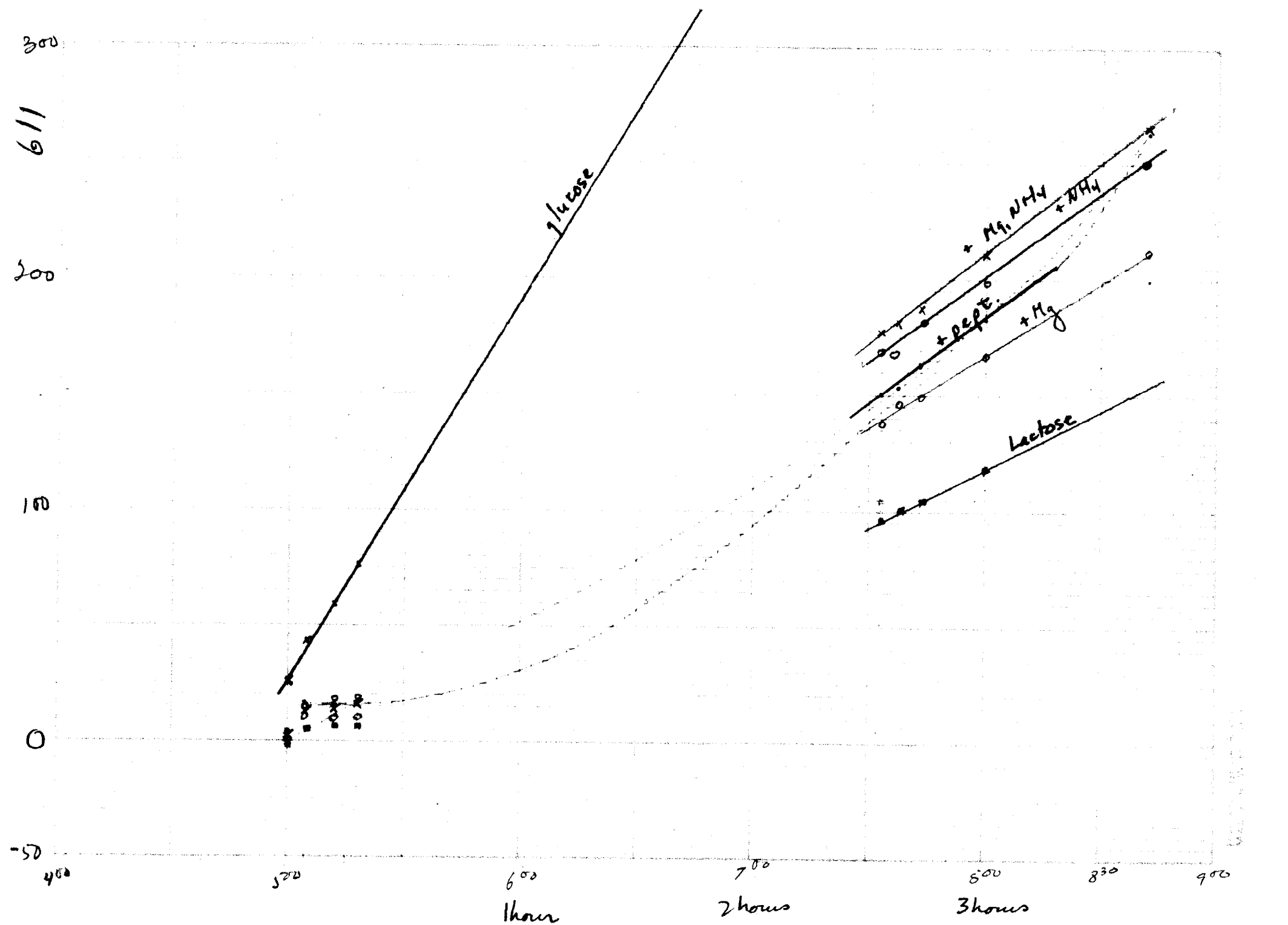
	glucose	galactose (glucose)	lactose	bioglyal	
	1 5B	2 2B	3 6B	4 4B	T
1240	-2	18	-3	16	147
1245	-1	23	04	27 (153)	147
1246	05	28	08	27	153
1250	10	32	12	31	157
1255	04	28	08	25	151
100	04	30	10	20	148
110	14	40	19	31	153
115	22	48	25	32	150
121	28	52	27	29 (151)	154
131	41	69	42	35	156
150	62	84	51	27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

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

		Volume	$k_{0 ml\ 32°}$	1 ml	2 ml
	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
611
Glucose
+ Mg, NH4
+ NH4
+ pept.
+ Mg
Lactose
4[00]
5[00]
6[00]
7[00]
8[00]
8[30]
9[00]
1hour
2hours
3hours

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 L II

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

K12 grown on maltose, aerated.

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	$D^{onpg}_{10MN.}$	Δ'	
M	L1 water	007	438	384	
M	L2 glucose	002	217	166	
M	L3 lactose	0	165	116	!
M	M1 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^{ONPG}_{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

6/16

September 24, 1949.

2PM

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

5PM add 9 ml H_2O to 1-5. (#2 mix act.).
Assay. 2 ml samples.

	Di	8 m D 0 and 9
1	010	400 sic!
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	156-53	28	22	31	41	34	36	59	49	42
2 20	150-58	27	21	32	44	38	39	66	60	49
2 25	150-59	30	26	34	46	44	53	74	67	51
2 30	161-60	35	30	39	53	51	63	82	73	52
2 35	161-61	48	31	40	53	51	63	80	75	52
2 40	154-58	44	39	48	64	62	76	88	81	51
2 45	157-60	51	46	53	67	66	80	90	85	51
2 50	162-58	57	48	54	70	68	84	93	89	51
2 55	162-56	60	50	55	71	71	89	96	92	49
3 00	161-57	62	51	57	73	75	93	100	97	50
3 10	162-55	62	52	57	71	80	101	104	103	47
3 20	158-55	70	53	58	79	84	106	107	108	46
3 30	160-52	75	57	62	83	90	116	113	116	44
4 00	155-60	90	71	73	100	119	150	141	148	55
4 30	155-58	102	76	75	104	128	164	150	161	50
	1 LAC	1 mg.								
	2 GLU+GAL	.5 ea.								
	3 GLU	.5								
	4 GAL	.5								
	5 LAC	10								
	6 GLU+GAL	5 ea								
	7 GLU	5								
	8 GAL	5								
	9 —									
5 30	163	127	108	99	135	182	219	191	209	155
	1 ml cells W251a/Lac aer. in $NaHCO_3$ 7/20 .05 - .10 in side arms.									
6 45	159	127	127	110	137					55

~~OK~~ 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 — 7:30 P 30.

1 ml aliquots incubated ē 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.

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

1 ml aliquots to small tubes and incubate & given reagent.
Assay standard on pg.

Intact Cells.

			Aint.	Di	D^{20} on pg	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 2 hrs?						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 (exc. 3)
2 hour tr ments (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_2OOH at pH .75.

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

Negligible diff. even if carried over

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

Oct. 2, 1949.

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

Add a crystal (10-20 mg) of thymol at 4^{15} PM. .005 ml samples c = phenol. 1 ml start at 4^{30}

T.	Mins.		Donpg.
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	Donpg	RA
Cello.	K/L	.02	052	149	178
	W/L	.05	172	150	–
	W/M	.05	130	109	–
Thymol	K/L	.005	004	670	655
	W/L	.005	019	018	0 !
	W/M	.005			

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

No activity!

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

PM

1: .5 ml susp.

~~2: .5 ml " + 1.5 ml H_2O~~

3: .5 ml " 4.5 " "

3: .5 ml ($\frac{1}{10}$) + 4.5 ml "

all in duplicate

A thymol

B ~~to~~ octanol

C. benzene

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

8^{30} Make up to 10 ml (exc. 3)

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

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

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

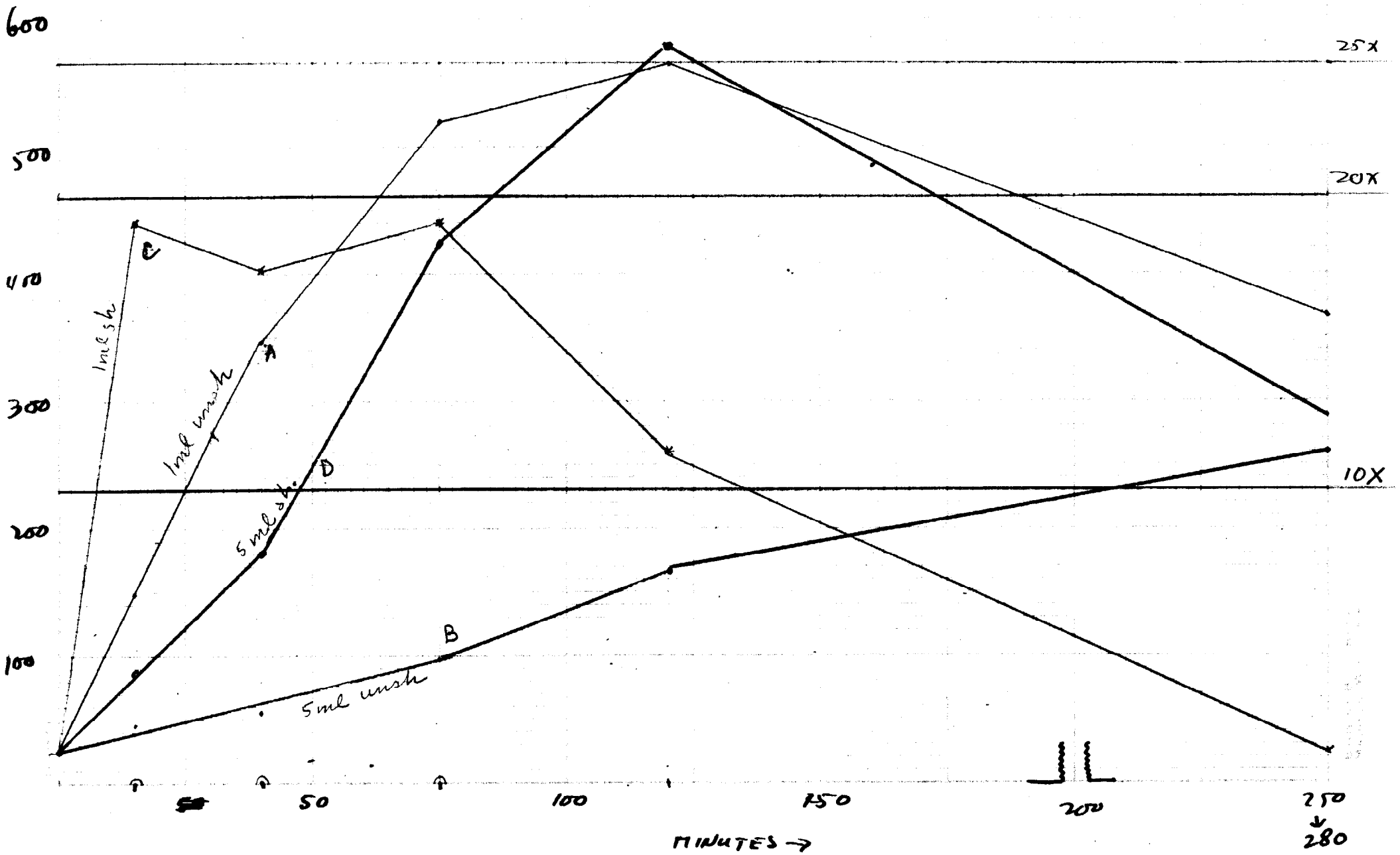
Time may have been insufficient for complete activation! Thymol seems to act most rapidly. [Try Phenol, other ϕ-OH]

Reassay 1, 3 4P2.

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

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 ē Na_2CO_3, exc. intact cells mid amts.

		MINS		
3^{35} T		15	Di	D^{20} onpg
Intact	.1ml	0	089	318
	.01ml		003	23
1	A	15		149
1	B			46
1	C			~~460~~ (440)
1	D			87
4^{00}		40		
1/2	A			173x2 = 346
1	B			53
1/2	C			201 x 2 = 402 (!) 10% < above!
1	D			180
1/2	A	75		260 x2 520
	B			98
1/2	C			220 x2 440
	D			423
1	A			281 x2 562
1	B	120		83 166
1	C			120 240
1	D			289 x2 578
	A			369
	B			260
	C			025
	D			289

ml 1/100 dil

4^{36}

5^{20}

8^{00}

2 hours optimum for unshaken cultures.

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

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

Retest overnight.

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

624a

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±]. ca 8 hours.

				Δ cor		RA	n/mg
Intact:	K/L .1	130	520	392		3.02	14
	K/M .1	129	151	24			0.9
	K/G .2	204	182	— 0			0
	—	— 007	± 004	Correction = + 11			
Benzene	K/L .005	— 004	410	403	×20	~~3~~ 62	297
	K/M .01	+ 004	074	59	×10	4.6	22
	K/G .1	074	060	—		—	

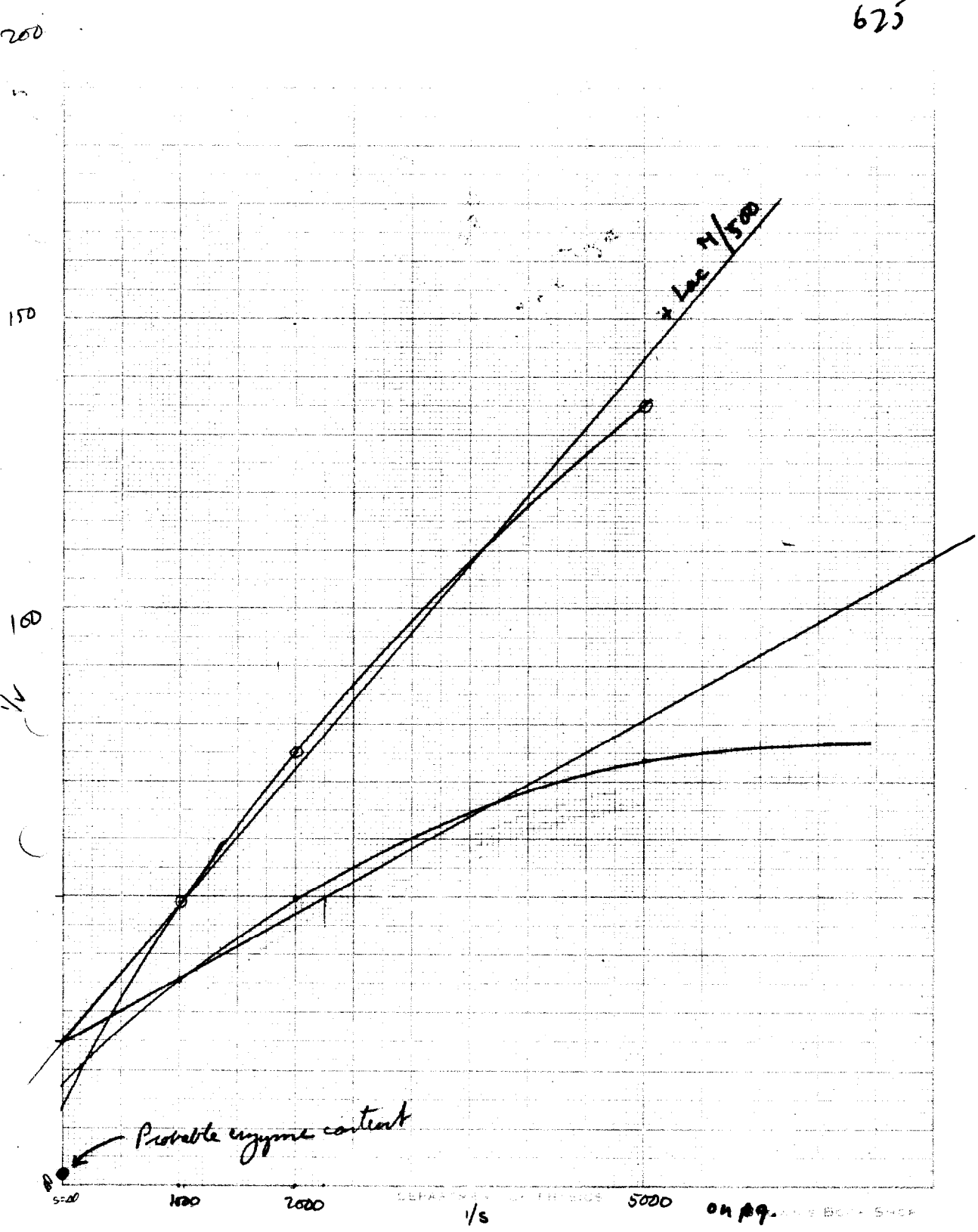
		I	Di	Dongg
Instant	L	.05	087	246
	M	.1	157	199
	G	.1	130	124
	—	.1	214	250
B2 4h.	L	.005	0	530
	M	.02	018	194
	G	.1	069	074 (5:35 PM)
	—	.02	032	267

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

	Di	Dong		A	R.A.	u/mg
Lac	173	408		231	134	6.4
Map	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
Map 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/ onpg	lac	Di	$D_{onpg}^{Na_2CO_3}$		1/V	M/100 Na P
cells	100			352 ✓	282	35.5	
	200			274	202	49.5	
	500			183	136	73.5	
cells	100	M/500		274	204	49.0	
	200	500		185	133	75	
	500	500		121	74	135	
cells		—	089	050	047		
no cells.	100			020			
	200			002			
	500.			-003			

Graph scale: Vmax = 1/25 = 400

K_{onpg} = 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 bending curves to be considered.