Zelle 4/4/50 H226 Single cell isolations

3. 3/31/50

		Lac	Mal	EMS Lac
A	8	+	+	+
	10	+	+	+
	19	+	+	+
	23	—	—	o
	24	—	—	o
	25	+	+	+
	26	+	+	+
	27	+	++	+
	28	+	+	+
	31	+	+	+
—	32	+	+	+
	33	+	+	+
	34	+	++	+
	41	+	++	+
	42	+	+	+
	59	+	+	+
	60	+	+	+
	61	+	+	+
	62	+	+	+
	10	+	+	+
	17	+	+	+
	37	+	+	+
B	20	+	+	+
	25	+	+	+
	27	+	++	+
	28	+	+	+
	29	+	+	+
	37	+	+	+
—	38	+	+	+
	39	+	+	+
	40	+	+	+
	53	+	++	+
	54	+	+	+
!	61	—	+	—
	62	+	++	+

A23-24 A25-26

B61 B62 B29

F21 are segregants. F22

Three sibs are indicated —— .

Transfer sibs to D(Lac); also streak out on several sugars.

! Check for partial segregation!

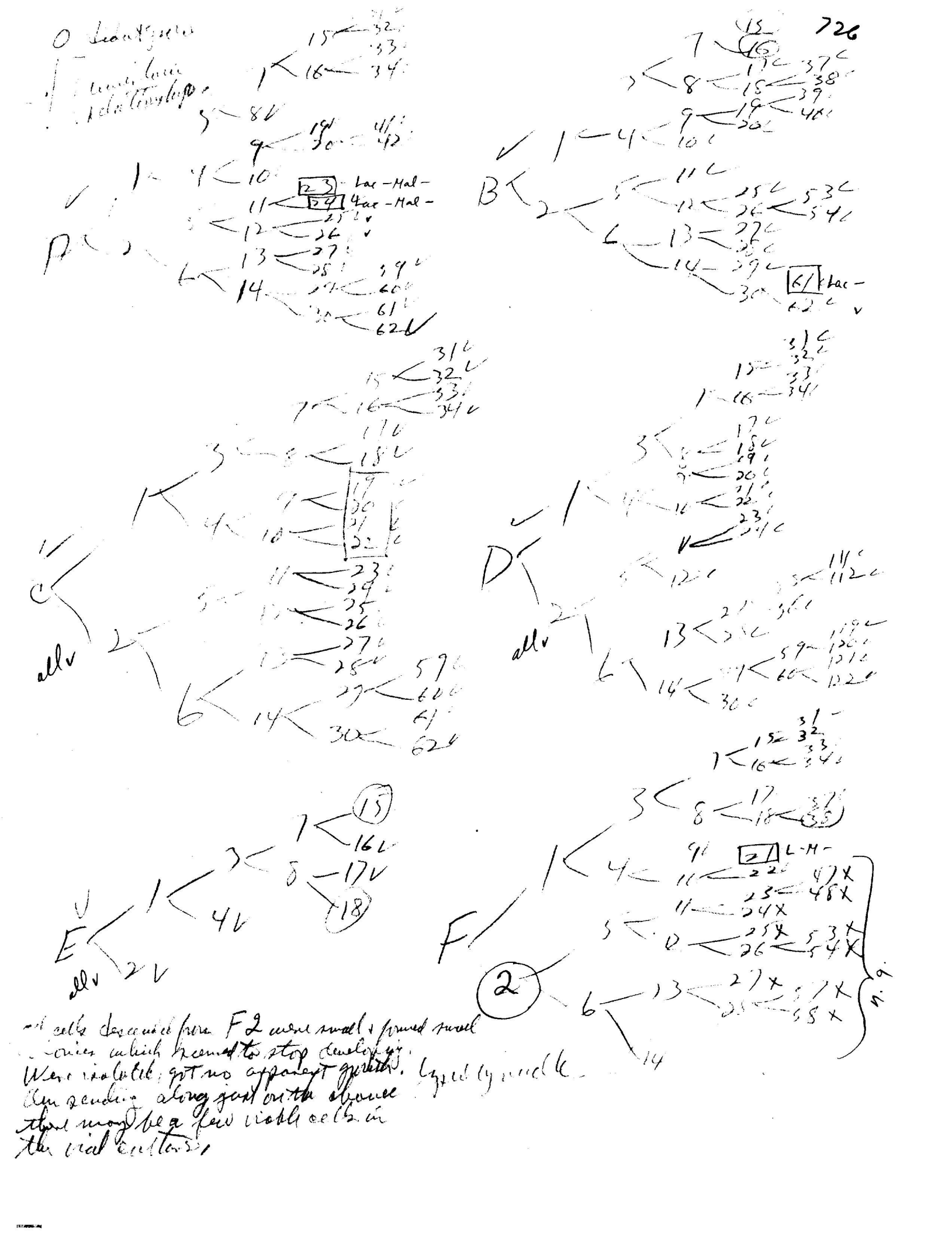
o = n.g

— = lac-gr.

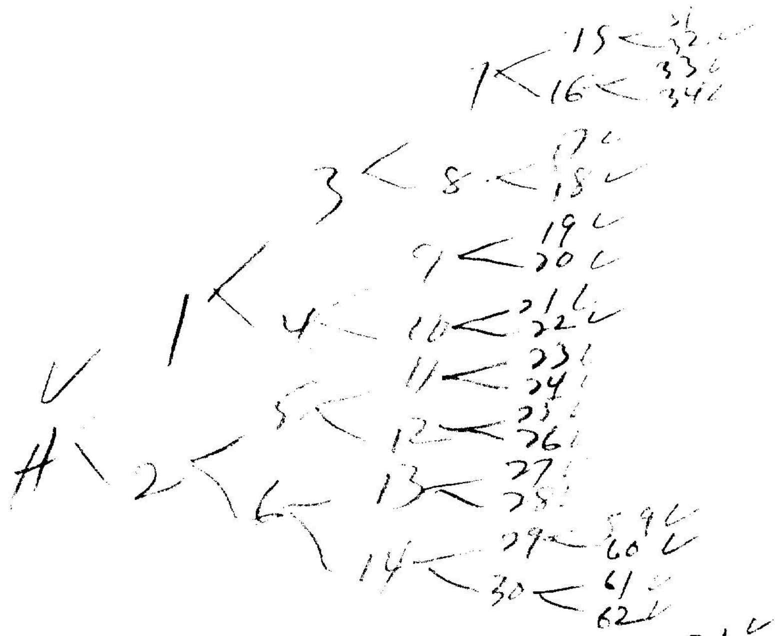
	Lac	Mal	EMS Lac
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28	all x	all x	all x
31			
32			
33			
34			
59			
60			
61			
62			
12			
17			
18			
19			
20			
21			
22			
23			
24			
28			
30	all x	all x	all x
31			
32			
33			
34			
56			
111			
112			
119			
120			
121			
122			
2	+	+	
4	+	+	
16	+	+	
17	+	+	

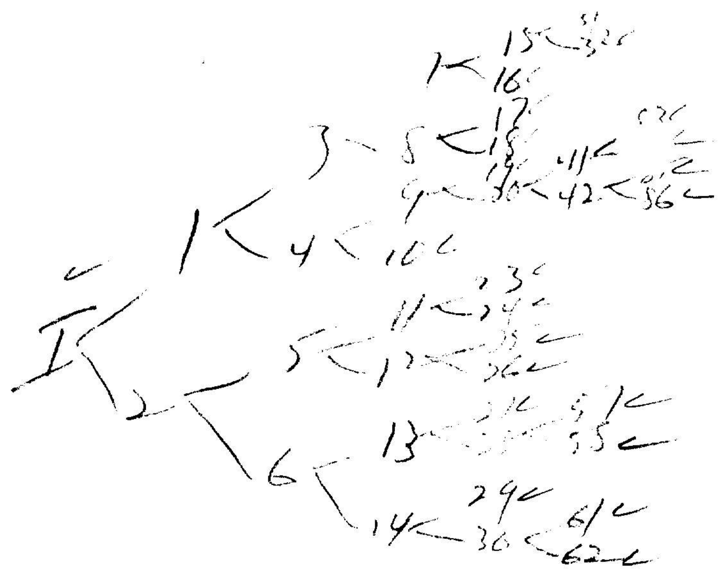
	Vac	Ural	Stac
10			
I 16			
17			
18			
23			
24			
25			
26			
27			
29			
31			
32	all x		
57		all x	
58			all x
61			
62			
83			
84			
85			
86			
19			
12			
16			
17			
18			
K 19			
20			
21			
22			
23	all x		
24		all x	
27			all x
28			
29			
31			
32			
61			
62			

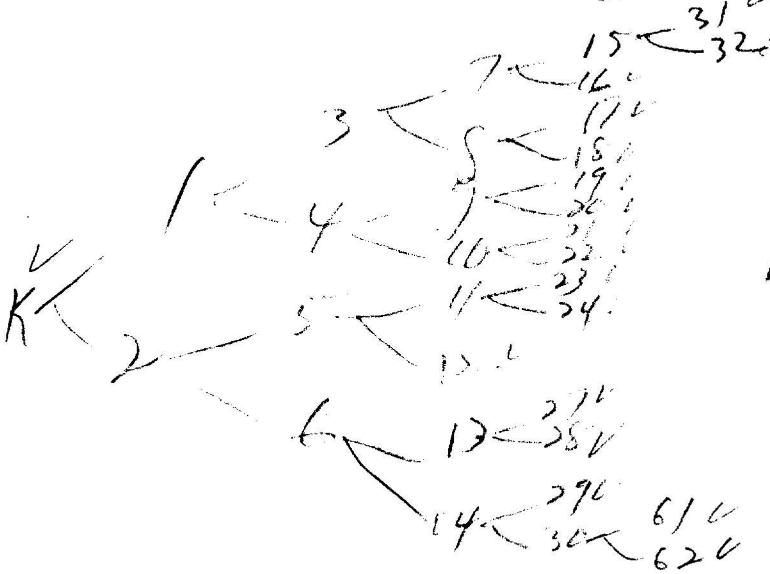
288 ~~vac~~ screw cap vials returned to Zelle.



All cells descended from F2 were small + pinned small colonies which seemed to stop developing. When viable, got no apparent growth. Can pending along just on the chance that may be a few viable cells in the vial [illegible]

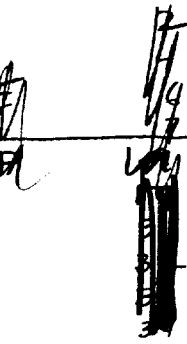






Probably most of these are haploid cells, they grew too well. Initial inoculum was [illegible] in 18 hour Davis minimal culture. (inoculated with an EMS live colony)

I can put the broth in the vials OK. Have [illegible] waiting for bugs to grow. Won't have another chance till next week — traveling again.



	Lac	Mal	EMS Lac	
9	+	+	+	
17	+	+	+	
21	—	+	~~+~~ 0	
22	+	+	+	Check!
24x	0	0	0	
25x	0	0	0	
27x	0	0	0	
31	+++	++	++	
32	+++	++	++	
33	+	++	++	
34	++	++	++	
37	++	++	++	
47x	0	0	0	
48x	0	0	0	
53x	0	0	0	
54x	0	0	0	
57x	0	0	0	
58x	0	0	0	

17
18
19
20
21
22
23
24
25
26
27
28
31
32
33
34
59
60
61
62

all x

all x

all x

Study of Zelle single cell isolations 726b.

A. segregants:		Lac	Mal	Xyl	MTL	Nutr.	
1	A23	−	−	−	−	TLB,	
2	A24	−	−	−	−	TLB,	
3	B61	−	+	+	+	+ MTLB,	Repeat: ✓ no partial segregants.
4	F21	−	+	+	+		

B sibs

1	A25	v	v	v	v	+	no deviations
2	A26	v	v	v	v	+	
3	B24	v	v	v	v	+	
4	B62	v	v	v	v	+	
5	F22	v	v	v	v	+	

C doubtful v.

F 31	v	v+	no deviations.
32	v	v+	
33	v	v+	
34	o	o	
37	v	v+	
B 27	v	v+	
34	v	v+	
41	v	v+	

April 7, 1950.

Take up cultures described on p. 700.

A. P7 Streaked out 698B1, B2 700-2. P8: all are Mal−.

1 698 B1 #2 is Lac+, with sheen for most part.
2 B2
3 700-2 = 698B2! #1, 3 are apparently mixtures of Lac+, Lac −.

Replica apparent pure +.

① 6 colonies streaked out. Each throws off ca 1% Lac − ! No Lac v.

② 4 colonies " ". Mostly +, some v.?, a few % − .

③ " " " . 1% − ; mostly type +.

Test ~~Xyl 2~~, Lac − 's on Xyl, Mtl; Nutrition of parent.

Check nutrition of single + colonies from ①, ③
Also inoculate in O(N2Case) lac for irradiation.

1. 8 Lac − : Mtl+ Xyl+
2. 6 Lac − : all Mtl+; 5 Xyl −; 1 Xyl +.
3. 6 Lac − : Mtl+ Xyl+.

See 731

April 9, 1950.

Grow 727-1 and 727-3 in D-Y2-plus. aer. est. 10^{10}/ml.
Dilute each 10^{-4} for irradiation. 20 secs 30cm.
Dilute to 10^{-6}. controls .05
UV .1 of this dilution/plate.

		"+"	"v"	s	−
1. Control.		117	13	1	31
		107	10	0	25
1 UV=x		227	40		46

Negligible killing. Repeat expt. with higher doses and better populations

---

Mal+ segregants of 727: 1, 3

~~Cut~~ Pick papillae from EMB Mal and purify. No frank Mal v.
Pick to Lac EMB; streak out on EMB Mal.

MA = 7271
MB = 727-3

A: 10 picked and purified Mal+ from EMB Mal brushed on EMB lac
3 and 6 were lac− # 10 a, b lac− c, d lac+.

B. All 7(+) lac+. Streaks out on Mal EMB for hemi zygosity tests.

A: all lac ++, − Mal pure ++. ∴ 727-1 and 727-3

B: all lac ++, − Mal pure ++. are pure Mal −

Hemizygosity tests: ~~H237~~ H237 728

4/9/50.

see 720. From W67 x W950. Lac v Xyl v Mal− Gal− MH? SH?
check from slant. Incr. D(Lac) 10 ml for heavy suspensions.

H 237 may not be heterozygous diploid: it does not give typical Lac v.

One Mal+ 3 Gal+ each pure +.
but lac appearance peculiar. Recheck H237: appears to be Lac +.

Irradiated 17226 on EMS lac

April 9, 1950.

20 secs. 50 cm Irradiate 17226 1/3 at 10^{-4} in H_2O.
Dilute ~~to~~ to ~~10^{-5}~~ 10^{-6}; plate .1 ml

A Control

B UV.

EMS Lac

	V	—
A	32	15
	55	22
~~B~~	28	15
B	2	12
	6	7
	6	16
	2	15

Not a highly pure suspension.

EMS lac ——— A - too many lac -

G. J.

Fermentation of neolactose

April 8, 1950

A	58-161	/100ml	Y2 1% lac
B	"	3 x 25 ml	" agar
C	W1301	/100 ml	"
D	"	" agar	" .

	Flask	Cells	substrate
1	2A	B	—
2	9B	B	glucose 10mg.
3	3A	B	lactose "
4	4B	B	neolac "
5	6A	D	—
6	10A	D	glucose 10mg
7	11A	D	lactose "
8	14A	D	neolac "
T	1		

This experiment was designed to determine whether lactose-adapted cells could utilize neolactose.

Since 58-161, fully lactose-adapted, ferments neolactose 1/4 as rapidly as it does lactose, the non-fermentability may be due to a block of adaptation comparable to Lac: Bugal or Lac. —: However, the falling off of the fermentation may speak for inhibition by allolactose, or a relatively high K_s for neolactose.

	T	1	2	3	4	5	6	7	8
12[20]	137+39	22	17+	−2	25+	50	30	20+	7
12[25]	~~89~~	23	17+	−1	25	50	26-28 (−30')	21	8
Typ 12[30]	140	27	95	34	30+	58+	85 (−85) cl	54	11
12[35]	140	26	103	90	34	55	145	106	16
12[40]	141	27	269 (+211) 295 to 84	186 217 to 69 148	51	58	244 +130 283 to 68	203 242 to 75 [167]	41
12[45]	143	30	155	124 − 114	77	60	136	129	77
12[50]	145	33	251 +488 273-96	213 239-48 329	105	62	230 +338 261-53	214 245-64	117
12[55]	142	33	165	98	131	63	110	106	154
1[28]	143	34	249 +645 252-99	172 200-150	159	64	210 251-79	208	204
1[0]	144	34	181	214 379	208	66	+510 127	304? 306-71	261 265-112 153

	T	1	2	3	4	5	6	7	8
1 15	148	35	250	274	229	64	258	147	141
			272-110 +883	282-88	247-122 (125)		269-82		
1 25	150	40	184	170	154	66	164	228	181
		+931	232-114	219-126 681				263-118	
1 35	149	40	150	162	198	70	249	172	234
							269-73		247-104 296
1 45	150	43	205	219	230	70	118	231	140
1 55	154	46	246	262	261 282	75	161	275	180
			+ 254-110	267-119 829	273-116			285-113	
2 05	154	43	129	131	134	71	199	136	215
2 15	152	45	147	151	163	74	204	163	241
2 25	150	47	159	161	187	76	217	168	270
									278 -115
2 35	150	43	162	163	204	77	230	165	130
2 45	154	48	172	166	221	77	235	165	148

4/14/50. Cells had been kept in refrigerator

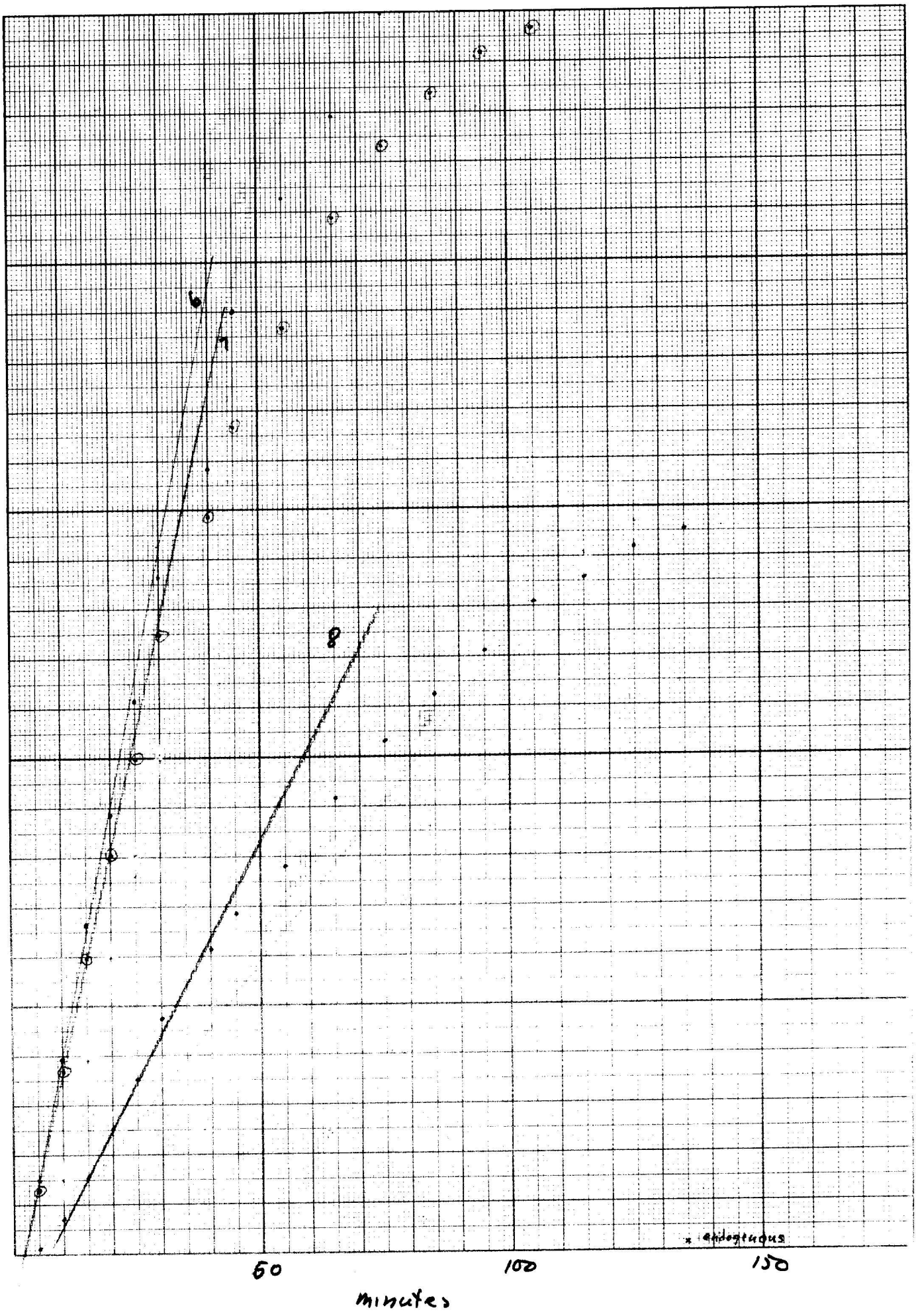
Estimate optical density at 4200 Å. dilute in distilled water

A	10^{-2}	287
B	10^{-2}	281

min.	T	Th	1	2	3	4	5	6	7	8
0	1230	0	0							
5	1235	0	-1	68	56	4	-1	60	52	5
10	40	-1	-1	173	151	20	-1	~~158~~	148	29
15	45	-3	0	268	235	44	1	263	239	63
20	50	-5	1	362	322	70	1	355	~~355~~ 322	101
25	55	-2	4	456	401	99	5	446	~~398~~	141
30	100	-3	4	539	474	126	5	545	499	190
40	10	-4	3	623	565	174	6	633	594	246
45	15	-8	0	688	621	191	0	760	668	275
55	25	-10	3	782	724	239	0	851	747	313
65	35	-9	4	867	810	284	6	937	837	367
75	45	-12	4	919	864	313	2	999	893	413
85	55	-14	5	958	905	342	1	1040	935	451
95	2 05	-14	2	985	922	372	1	1078	968	486
105	15	-12	6	1005	944	~~364~~ 343	6	1085	997	514
115	25	-12	8	1117	954	427	8	1098		543
125	35	-10	6	1022	958	446	11	1113		568
135	2 45	-14	7	1028	957	459	7	1114		582
Flask Const.			1.84	1.64	1.69	1.81	1.58	1.60	1.70	1.58
Initial linear rates:		mm	~~1224~~	1224	1080	262		1230	1080	
per hour.								~~1854~~	~~1670~~	390
		μl/h.		2007	1825	474		1968	1836	616
		N/L	=	.24						.335

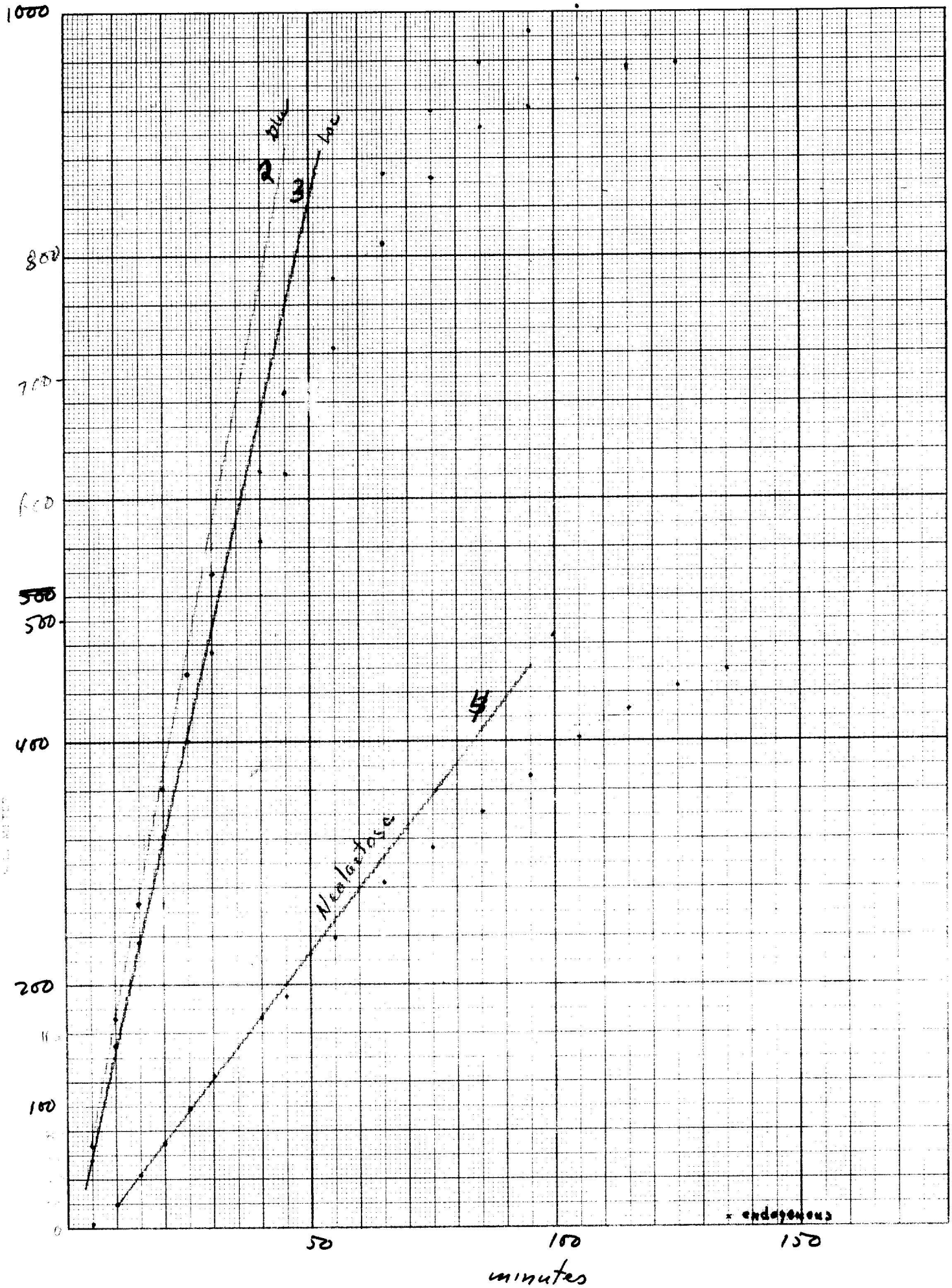
W1301/Lac

230



58-161/Lac

730



Nicotine response

730A

April 10 (9?) 1950.

	Cells	Substr	Flask
1	3	—	12A
2	3	Glu	13A
3	3	Lac	5 B
4	3	Nicot	2B
5	6	—	10B
6	6	Glu	6B
7	6	Lac	7B
8	6	Nicot	8B

3 = 58-161 / Nicotine N2 agar

6 W1301 " " "

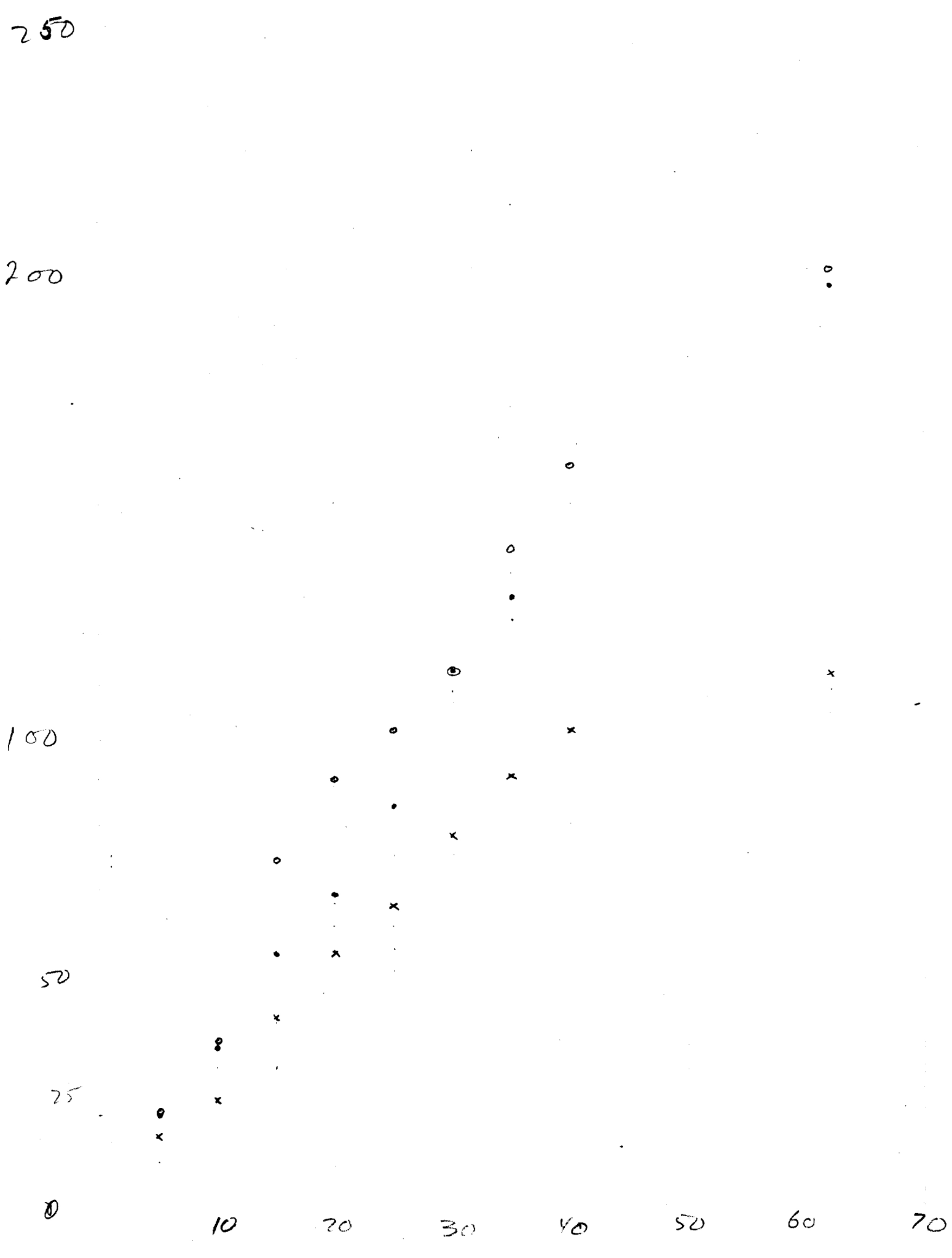
barometer very low!

	T	1	2	3	4	5	6	7	8
310	149	68	45	61	75	9	39	60	68
320	147-50	64	45	61	75	9	39	62	72

TIP

320	149	64	47-	58	73	4	34	57	62
335	150	68	68	65	79	12	58	74	70
340	151	68	83	65	78	11	72	82	70
345	156	74	108	71	83	17	95	105	76
350	156	73	121	70	83	16	115	118	81
355	159	75	143	74	86	17	136	131	81
~400?	150	75	170	76	90	22	157	145	86
~405	164	81	192	80	91	22	189	164	94
~410	165	82	(14) 191-112	81	92	24	218	175	97
" 415 (4322)	156	72	181 182-131 (15)	73	81	14	250 256-67	187 193-95	88
" 445"	158	77	182	79	87	17	119	119	99

98
57
41


250
200
100
50
25
0
10
20
30
40
50
60
70

Calculations

	T	Th	—	Gluc 1	Lac 2	Molac 3	—	Gluc 6	Lac 7	Molac 8
330	0	0		64	47	38	73	4	34	57	62
335	5	1	+3	20	6	5		7	21	16	7
340	10	2	+2	34	5	3		5	36	23	6
345	15	7	-1	54	6	3		6	54	41	7
350	20	7	-2	67	5	3		5	74	54	12
355	25	10	-3	86	6	3		3	92	64	9
400	30	9	-2	114	9	6		9	114	79	7
405	35	15	-2	130	7	3		3	140	92	17
410	40	16	-2	+32	7	3		4	158	102	19
432	62	17	-13	196	-2	-3		7	199	113	9
445	75	7	+6	265 (83)	14	7		6	257	153	30
				•					o	x	

data later

See Protocol for repetition 730B

Autosomes "stable diploid" 731
dec 727

April 10, 1950.

Cross 727-1 and -3 as follows., on EMS lac

A	1-	x	Y10	Streaks of 727-1 and -3 streaked out. 99%+.
B			W677	
C	3		Y10	
D			W677.	

A and C gave good yields, almost all lac+. B, D gave ~~poor yield~~ moderate yields, lac-:lac+ ca 3:1

Picks + and streak out on EMB lac.

A: 100+. No distinctive lac v but some colonies have lighter, possibly mottled ~~ed~~ centers. Mark these for further purification. Repick all to EMB Mal, MH, Xyl.

B

C. 7~~8~~+ As A.

D 2 + retest: hold in incub.

A. Spot-tests of purified lac+ [1-6 excl. 3. all Xyl+ Mth+
20, 21, 22, 40, 53, 54, 55, 56, 93 Mal –
others Mal+.

C. All Mth ++. All Xyl+ #35, 87 Mal – 87 Mal-+?
others Mal+
+Hold Mal plates in refrigerator.

Treg 727-3 x
WS88

A. 6 restreaked on lac EMB as possible from lac V.
Each of these threw off considerable lac - and has appearance suggesting a rather stable lac V.

C. Ditto. C3 destinctly variegated. } Each is pure Mal +.

B. 12 EMS lac + picked and streaked on EMB lac Colonies resemble those of A + D. #1, 2 are like C3

D. like B.

Kept on EMS lac
Pick single "4" colonies and restreak on EMB....

B:	lac	Mal	Xyl	Mtl
1	++ -	—	+	+
2	++ -	—	+	+
3	++ -	—	+	+
4	++ -	—	—	—
5	V	—	—	—
6	++ —	—	+	+
7	++ V	—	—	—
8	++ —	—	+	+
9	++	—	+	+
10	++	—	+	+
11	++ -	—	+	+
12				

D:

	Lac	Mal	Xyl	Mtl
1	++ −	−	++	++
2	++ −	−	−	−
3	++	−	++	0
4	++ −	−	++	++
5	+ −	−	−	−
6	++	−	+	+
7	++	−	+	+
8	++	−	−	−
9	+ −	−	+	−
10	++	−	++	+
11	++	−	++	+
12	+ −	−	−	−
13	+ −	−	−	−
14	++ −	−	++	++
15	++	−	++	++
16	++ −	−	++	++
17	+ −	++ −	++	++
18	++	++ −	++	++
19	✓	− −	−	−
20	++ −	++ −	++	++
21	++ −	++ −	[illegible] −	−
22	+ −	−	++	−
23	++	−	++	++
24	++	−	++	++
25	++ −	−	[illegible] −	−
26	++ −	−	++	+
27	++ −	−	++	+
28	++ −	−	++	+
29	++ −	−	++ ++	+
30	+ −	−	−	−
31	+ −	−	++	+
32	++	−	++	+
33	+ −	−	−	−
34	++ −	−	++	++
35	+ −	−	++ ←	[illegible] −

Constitutive galactosidase of W1301

April 18 ca., 1950.

A 58-161 Glu
B "
C W1301 Glu
D "

2 plates each O(×2) ± glucose 0.1%
growth in 10 ml H_2O. .1 ml in 10 ml
Residue in spotplate for dying.

A	383	403 (20m)	5.8	Add benzene to each tube and refrigerate
B	209	236 "	3.1	
C	388	++++	180	
D	262	++++	144	
—	001	014		

C and D are too active to assay at this dilution. Use .01 ml/10

1u = Δ=100 in 20m.

				Units/ml
C'	048	150 (10m)		180
D'	027	113 "		144

E, F are suspensions from 4/15. Delete benzene treated suspensions

1:100, use .2/10 E = 58-161 F = 1301 /no sugar

	Di	Dom Pg (10m.)	
E	003	017	?
F	004	222	>2000

ca 150 before activation. See Manometric Protocol 4/12.

4/15 called. See manometric protocol 4/12

∴ The "constitutive" lactase differences persist through "activation".

4/20. Qualitative test (spotplate) for galactosidase in W1301 grown in D (BMTLB,) — various sugars.

Glu	+++
Lac	+++
Mal	+++++
Gal	+++
Ara	− ?
Mtl	++++
Stl	++++
K. Pneu.	+ −?

April 18, 1950.

W1301

A. DN2 — 6 plates Use ca 1/5 in 20 ml

B. DN2/Glu 7 plates. Use ca 2/5 in 20 ml

Hold 24 hours. Run manometry 4/19. See Protocols.

In this experiment, the cells (B) were tested on a variety of substrates glucose, galactose, maltose, lactose, but not arabinose, were rapidly utilized by B. Later that PM, with same suspensions, xylose, sorbitol, mannitol and gluconate were tested, and were not utilized:

+ A	— B
glucose	arabinose
galactose	xylose
lactose	mannitol
maltose	sorbitol
	gluconate

This was taken to mean that W1301 is preadapted to sugars A, because previous work had indicated that K-12 was not preadapted under these conditions. But see 4/20.

Cells (A) also utilized lactose and maltose, but rather more slowly in relation to glucose.

Cell density of suspensions used. Dilute: .1/10 ml

	D_i	D_{onpg}^{10}	Δ	Δ/D_i
A	128	370	236	1.84
B	160	560	397	2.48
Δ_{onpg}		019		

W1301 : Synthetic medium. 734

April 18, 1950.

~~Synthetic~~ Grow 20h. aer. 37°. D (Lac or Glu) + BM 1/10

			Di	Dorpg	$\Delta^{corr.}$	Di 1:10	Δ/Di	R.A.
1	58-161	Glu	016	033	1	208	—	
2	"	Lac	034	190	142	321	~~4.3~~ 4.43	
3	W1301	Glu	020	620	585	228	25.6	
4	"	Lac	036	434	385	332	11.6	
	—		~~0~~	017				
5	58-161	Glu	208	200 (20m.)	—			—

Theo W1301 produces a "constitutive" lactase on very simple medium.

Selection against W1301 is anticipated in non-lactose medium. Transfer successively in D(BMTiB.) glucose, lactose, and maltose. Streak out on EMBLac. 1st transfer from W1301/Glu above. 0th: pure lac+

0th: colonies from EMBLac picked to DN2 Glu. Spot test for constitutive lactase:
12 colonies: all onpg+

1: 12 from Mal to DN2 Glu
4 Lac
4 Glu
} all onpg+ 58-161 4 colonies onpg−.

2: EMBLac − all lac+.

3: " " . Test from DN2 Glu to onpg. 4 each: onpg ++

Galactosidase in W1301: Synthetic medium with various carbon sources.

[redacted] 73/9.

April 20, 1950.

W1301 grown on [redacted] D BATLB, ... overnight ĩ aeration

	Sugar	.1/10 Di	Donpg	1/10 D	Δ	Δ/Di 10 R.A.	Galactosidase spot test
1	Glu		269	157	240	15.3	+++
2	Mal		930	146	900	62	+++++
3	Lac		372	149	340	23	+++
4	Arab		119	156	90	5.7	±
5	Mtl	10	452	146	420	29	++++
6	Stl		875	153	840	55	++++
7	Gna	5	128	160	100	7.9	+
8	Galac		320	168	290	17	+++
0	—		021				

fermentable
Incl Na2CO3
at 10m.

Repeat expt. with fresh cells. 0.1/10

		Di	10 Donpg	1:10 Di	Δ cor	Δ/Di 10
234 -2	1 Glu	0.8	202	114	197	17
	2 Mal		538	223	533	24
	3 Lac		147	180	142	7.9
	4 Arg		109	208	104	5.0
	5 Xyl		179	182	174	9.6
	6 Succ. 2 ml		373	120	376	15

as above.

Verification: Preadaptation of W1301 to maltose, galactose

735

April 20, 1950.

Flask	T	Cells	Sidearm (10mg.)	uncorr. Δ 40m.
9B	1	A	—	1
2B	2	A	Glu	300
3B	3	A	Gal	167
5A	4	A	Mal	202
10A	5	B	—	1
6B	6	B	Glu	107
4A	~~8~~	B	Gal	94
8A	8	B	Mal	80

A = 58-161 2 plates DN2 Glu } 10 ml.
B = 1301 1 plate " . }

2 ml per flask. M/20 $NaHCO_3$ CO_2.

Time → / Flask ↓

	200 ±	210	225	240	317	uncorr. Δ 40M.
T	155	152	155	152	154	-3
1	13	11	15	13		1
2	18	72	203	>310		
3	43	91	186	217		
4	47	85	162	246		
5	52	52	52,	50	54	
6	54	76	115	158	265	
7	61	78	111	152	244	
8	59	77	104	136	205	

Galactozidase: use .1 ml / 10 minutes

	Dil	210 min. Dompg	Δ	R.A.
A	128	370		
B	160	560		
	Δompg	019.		
A	319	329	023	~~.072~~ .072
B	132	273	137	~~1.04~~ 1.04

Preadaptive Galactosidase tests on E.Z.L. "suppressor" cultures

April 19, 1950.

Grow from EMB colony to Permassay 10ml. Washings:

See EZL code

	Di	Dongg	
A	010	−	
B	002	−	
C	009	−	
D	007	++	W716 B
E	2DIE 019	±	W716 C
G	017	++++	Read diffuse ?? between Lac and Mal
L	019	++++	
M	016	++++	

see 734-1

Quantitative readings interrupted by rain.

Repeat "D" from DN2/Glucose = W716B. in ca 10ml

4/20/

	Di	Dongg 20
D	083	1070
—	0	019

W1301 x W945

April 20, 1950.

"C" W1301 x W945 on EMS Lac
+, slow, – colonies seen.
Purify.

28 Lac+ — 2 possible npg – — no! Both are npg+
Replate on DN2 glu

12 Lac slow — 1 possible npg – — But very slow
Replate and streak out EMB Lac

Store purified cultures on NSA slants

Restreak purified Lac – prototrophs and hold on EMB Lac.
5/6. Pick separated – and Lac+ revertants (corresponding) to T(0), for test for constitutive lactase

"W1312" is not a prototroph! Repeat cross! (W1301 x W1177
W1301 x W1178)

---

ca 50 Lac – allowed to revert and Lac+R tested from D(0) glu for constitutive lactase. None were Const+.

Conclusion: Const locus is closely linked to Lac, or Const+ is epistatic to Lac–.

TRY: Lac,– on EMB Neolactose

Preadaptation of 58-161; W1301

737

A- 58161
B=1301 April 21, 1952.

	Cells		Flask.	0	↓ 340	350	400	410	435
					Time →				
1	A	—	B	26	25	23+	28	27	25+
2	A	Glu	A	75+	75+	101	156+	168	257
3	A	Gal	"	70	70	93	127	157	212
4	A	Mal	"	47	47+	64	88	106+	163
5	B	—	"	31	25+	26	29+	27	28
6	B	Glu	"	20	20	58	99	136	242
7	B	Gal	"	23	19	49	84	114+	201
8	B	Mal	"	56	54+	77	101	122	187
Thermobarometer.				136+	136	140	140+	139+	140
				0	↓				
9	A	Lac	9B	34	TIP 35	40	38	41	40
10	B	Lac	11A	61	67	97	118	150	179
Thbm					140	142	137	141	139
					440 ↑	452	500 (-1)	510	520

∴ 58-161 is preadapted to galactose, maltose, glucose but not to lactose. W1301 is preadapted to lactose.

K12?
Y10?

A, B gram on DN2 Agar

Cf. K-12, 58-161, 1301 for preadaptation

April 22, 1950.

Flask	Cells	Substr	12 50	100	↓ 105	110	120	130
10 A	K-12	Glu	08	09	29	83	174+	297
2B		Gal	50	51+	68	113	179+	202
3B		Mal	31	29	34	50	88+	147
4B		Lac	58	59	60+	60	63	67
5B	161	Glu	41+	42	58	114	209	>300
6B		Mal	54	53+	61+	82	140	224
13A		Lac	51+	52	58+	60	64+	64+
8B	1301	Glu	67	68	76	128+	216	310
9A		Lac	28	29	30+	83	166+	246+
	Thermobar		134	135	136	138	143	137+

2 plates DN2 Glu / 10 ml 2 ml each.

∴ K-12 grown on D(N2), Glucose is preadapted to galactose and maltose, but not to lactose. This speaks for a medium influence previous work with cells gave no such preadaptation. Compare the media used!!

Bacterial densities: .1/~~ml~~ 9 4200Å.

		D.	Galactosidase spot
	K-12	329	±
A)	58-161	460	−
B)	W1301	400	+++

Assay A and B for galactosidase. Then add benzene and store overnight.

Separate aliquots. To A'; B' add ~~toluene~~. octyl alc.

Preadaptation of K-12.

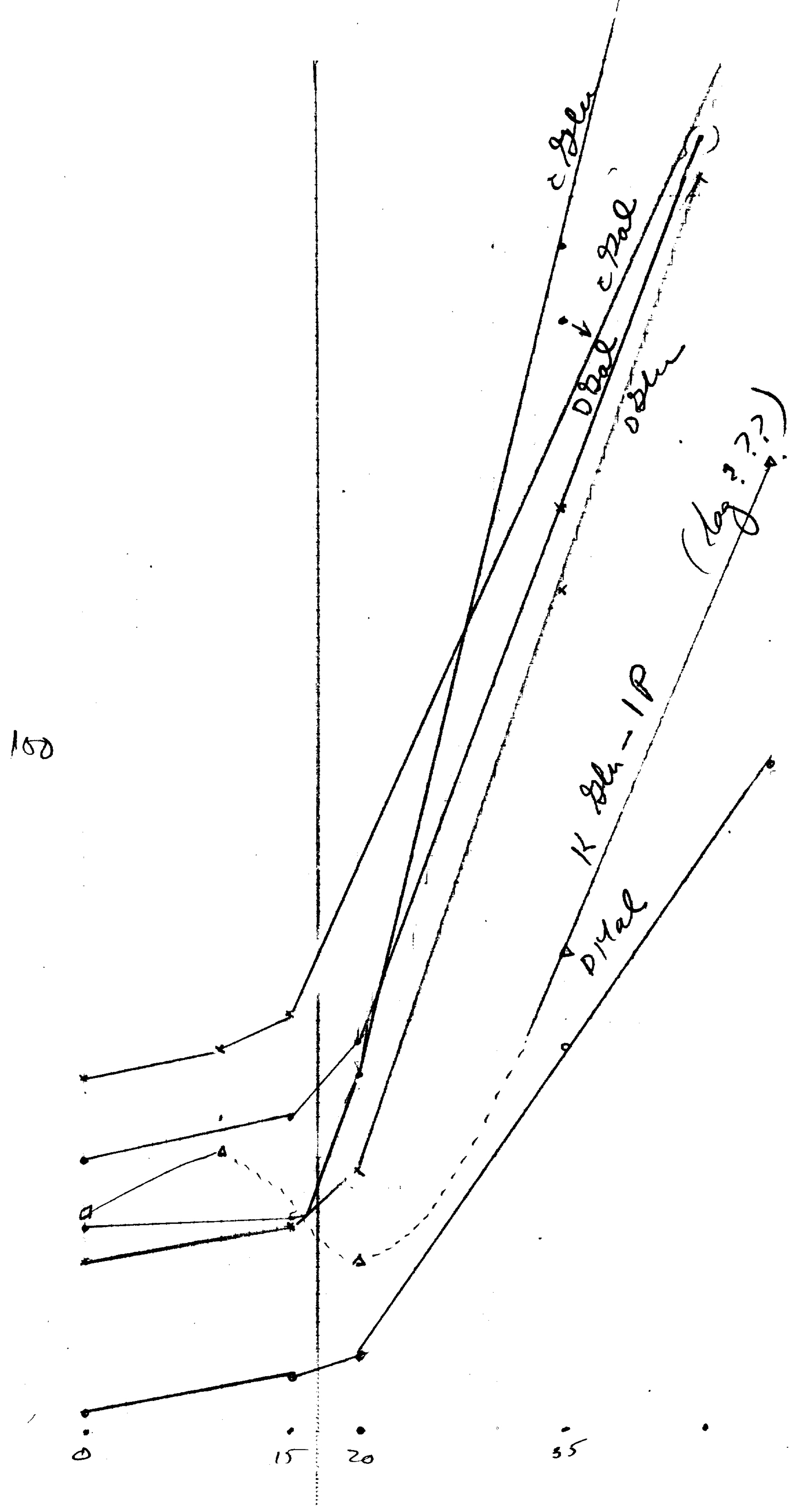
April 25, 1950.

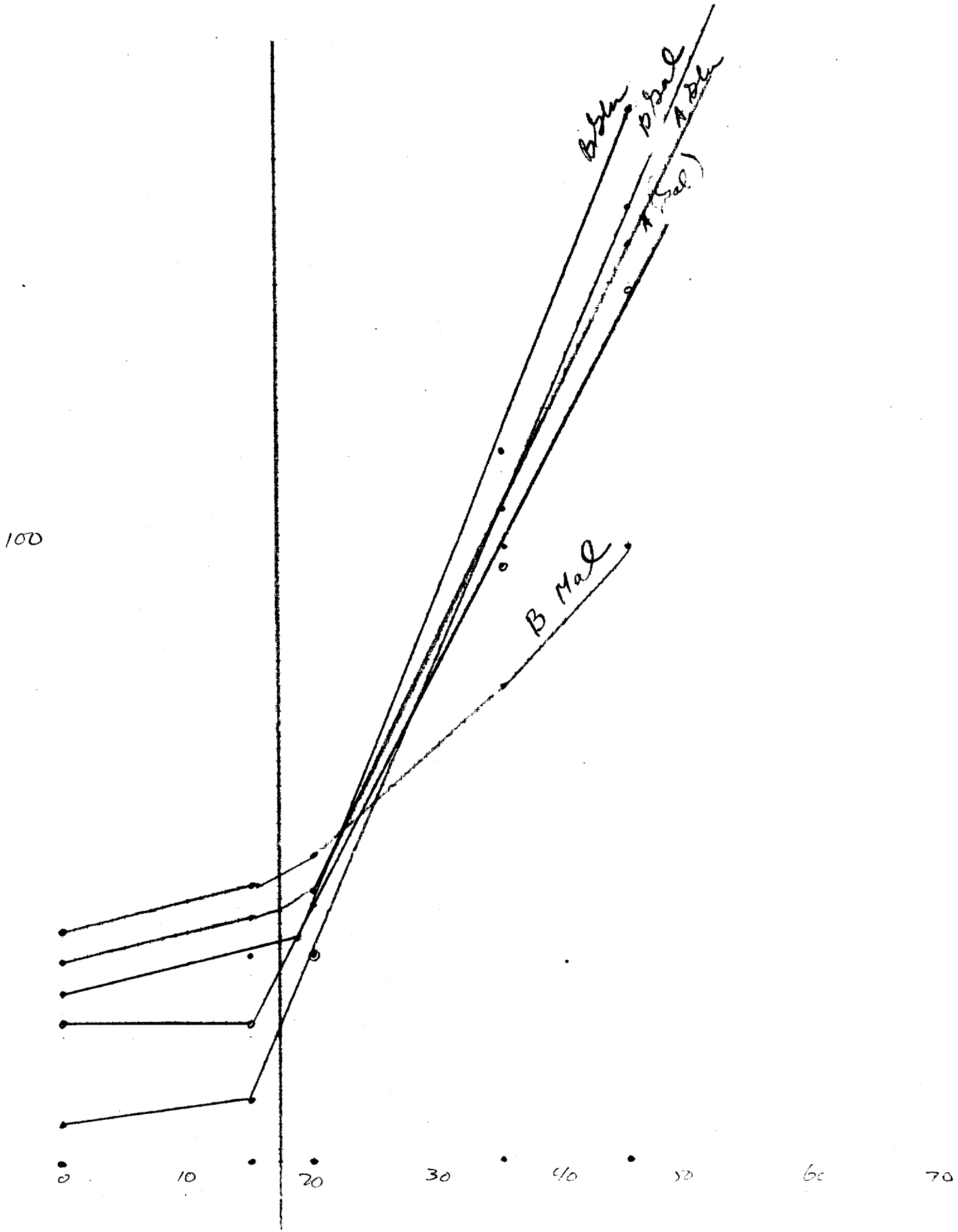
A	Perm.		100 ml
B	"	Agar	100 ml (3 plates)
C	D N2 Glu .1%		"
D	"	Agar	"

Flask	Cells	Side 1	Side 2	0 4^{30}	15 4^{45}	(1) ↓	20 4^{50}	35 5^{05}	45 5^{15}	
B 1	A	Glu		06	10		33'	104	152'	
A 2	A	Gal	Mal	22	22		33	95'	139	
6A 3	B	Glu		27	33		41	113'	167	
7A 4	B	Gal	Mal	32'	39		43	98'	146	
A 5	B	Mal		37'	44		49	76'	98	
3A 6	C	Glu		30	31		52	173	252	
4A 7	C	Gal	Mal	40	46		57'	162	188	?
A 8	D	Glu		25	30		38'	123	188	
B 9	D	Gal		52	56		61	135	183	
A 10	D	Mal		03	8		11'	56'	97	
A. 11	D	~~T~~ Glu-1-P 5mg (.25ml)		32	41'		25	70'	141	
3B/12	TB			135'	137'		137' fluctuating	140	140	low pressure day!

These data show a clearly constitutive glycolysis of maltose and galactose from E. coli K-12 harvested from Permassay! They also show glycolysis of glucose-1-phosphate (barring simple phosphatase!)

(See Leloir et al on metabolism of E. coli)


c Glu
c Gal
D Gal
D Glu
(log ???)
K Glu - 1P
D Mal
100
0
15
20
35


B Mal
100
0
10
20
30
40
50
60
70

Irradiation of #226

April 25, 1950.

Dilute #226 stock (from D Lac 4/24) 2×10^{-6}.
Retain aliquot; UV aliquot 20 sec. at 50 cm. Plate .05 ml (−) (uv)
on EMB Lac EMS Lac EMB Mal

A. Unirradiated 10^{-7}
B Irradiated 2×10^{-7}.

A	EMB Lac	V	−		Mal EMB	"+"	Vars	−
	1	45	29			9	17	1
	2	22	18			8	17	3
	3	61	37					
	4	51	32					
	5	16	10					

B	EMB Lac (2x)	V	−			
	1	11	27	3	8	1
	2	6	36	7	3	3
	3	2	12	12	18	4
	4	13	27	2	3	1
	6	40	23	10	3	0

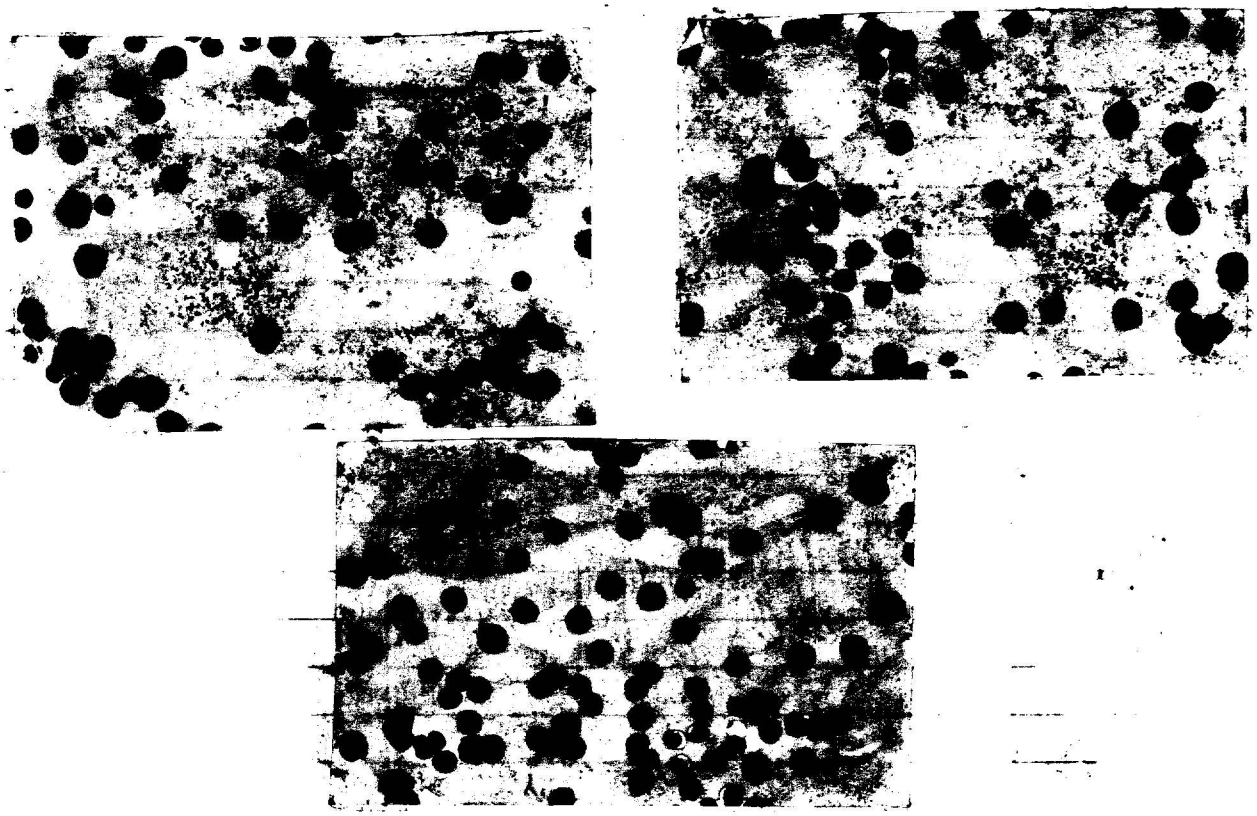
EMS Lac:

A 12+ 0−; 33+ 2− 0 Sectored.

B
0+ 1−
3+ 1−
5+ 3−
2+ 1−
4+ 2−
1+ 0−
1+ 1−
0+ 1− 1 sectored.

16+ 10− 1 sec.

Repeat for larger numbers.



A

B

Irradiation of H226

April 30, 1950.

Dilute to ~~10^{-3}~~ 10^{-4} for irradiation. A. Control. Plate at 10^{-7}

B. 20 secs UV. Various dilutions.
("7" = 2×10^{-7})

Plate on EMB Lac; Mal EMS Lac; Mal.

A. EMB Lac

v	–
116	8
128	18
115	8
121	19
480	53* / 533

EMB Mal. 2 plates.
+ and v not distinguishable with certainty.
No – seen.

* 4, circled, later scored as Lac v

B EMB Lac

B7 24h.

24	58
47	63
71	121

all v are delayed.

Mal: see above. All are + or v.

B7 42h.

34	53
~~48~~ 52	~~36~~ 45
86	98

Jerad. H226

May 3, 1950.

A. EMS Lac	+	−	Mal	+	o
	128	0		129	0
	130	0		155	0
	94	0			
	88	0			

B EMS Lac	+	−	sect.
"B7"	33	25	10
	32	20	5
	30	18	6
	46	19	10
	141	82	31

Note "induced" lac - "mutation".

B6: too dense to count well. However, on each of two EMS Mal no - or see were seen! Streak out Lac see on EMS Lac

---

H226 from A; EMS Lac : + were streaked out as EMS Lac, etc. Each of 8 colonies was

Mal++ Xgl+ Mtl+ Lac+ ! This is, then, a partial segregant! Pick to slant as 739-1.

Reisolate type H226 from slants.

Kinetics and stability of cellular lactase

April 25th. 1950

Harvest X-12 from aerated Y2 Lac (1%?) after ca 18 hours. Wash 2x and concentrate from 100 to ca 10 ml.
Remove ca 2-3 ml and toluene with benzene ~~at room temp~~ for autolysis overnight. (B). Assay remainder with ~~orig~~ M/2000 in Na P buffer M/50 pH 7.5 [Stored overnight in refrigerator]
On basis of preliminary assay, dilute A 1/50 and B 1/500 to give convenient ranges of activity in 20 minutes.
Assay system: 5 ml H_2O 3 ml M/15 Na P buffer 7.5 1 ml cells. 1 ml substrate (or H_2O). Add 1 ml Na_2CO_3 M/1 to stop reaction. Use dum scale of spectrophotometer. Time with stop watch, with 30 sec. interval between additions. Run in 38° "Precision" water bath with motor stirrer. Preliminary turbidity gives variance of tubes. Use volumetric pipettes. Remove from bath to room temperature when Na_2CO_3 is added.
Check, time sequence with A.

(1)

	Time	Di		$D_{Na_2CO_3}$ ①	+1 hr	$A_{corr.}$	$A_{\frac{corr}{Di}}$
1	5	100		134	133	080	080
2	"	095		220	220	166	161
3	"	098		353	357	299	298
4	"	097		458	462	404	403
5	"	099	(085) (081)	045	038		
6	"	−008	(+004) ∞	001	013		

no cells / no cells

These data are clearly non-linear !! (presumably due to "activation" ~~of~~ during assay !)

Corrections
① = −009 −045 = −054
② = 021 038 = −059

(2) ~~Incubate cells in buffer prior to assay (12 45 to 4 20 PM)~~

April 26, 1950.

A Y2 –
B Y2 0.1% glucose } agar 1 plate each. A, C to 7 ml
C Y2 1% glucose B to 10 ml

Use 2 ml. per vessel. + .1 ml 10% Substrate

Flask	Cell	Sub	5'	10'	15'	TIP ↓ 25'	30'	35'	45'	55'	A20M + Substrate
B	1 A	Gal	51	51	55	64	78	89	117	155	91
A	2 A	Glu	37	34	37	51	68	78	109	150	99
"	4 B	Glu	63	57	61	76	97	114	161	218	132
"	5 B	Gal	52	44	47	57	75	91	134	175	118
"	6 B	Mal	45	33	38	48	54	58	76	101	53
"	7 C	Glu	54	45	48	71	95	115	166	209	138
"	8 C	Gal	39	27	32	53	73	92	136	131	78
"	9 C	Mal	47	36	39	47	46	43	50	39	—
"	10 B	Glu 1mg	7	− 3	− 3	7	30	47	106	143	136
"	11 B	Glu .5mg	23	16	13	20	40	54	114	107	87 ≡
	TB		169	171	171	176	180	180	189	189	13

Readings by P. Phandi.

Note: Utilization of maltose by B but not C. Galactose utilized by each of them! (at a good rate). Experiment needs repetition!! Galactose is preadaptive! (purity of galactose ??)

Utilization of galactose; maltose
K-12 from Y2

April 28, 1950.

A. Y2 — 0.1% glucose agar — 2 plates
B Y2 glucose 1% — 2 plates
} to 2 ml per vessel
ml. .1 ml 10% substr.
Both Galactose and Maltose Recrystallized.

Flask		Cells	Substr	Time → 0:00	10	20	↓ 25	30	35	45	100	A_{24}
1A	1	A	—	05	07	07	9	6	8	12'	15	3
2B	2	A	Glu	32'	35	35	65	121	191	310	—	24
3B	3	A	Gal	44	49	47	48	48	48	56	66	8
4B	4	A	Mal	45'	52	50	58	66	81	116'	308	58
5B	5	B	—	37'	46'	42'	44	44	44	50	52	6
6B	6	B	Glu	16	27	22'	55	121	185	296'	—	24
9B	7	B	Gal	46	58	54	53'	57'	55	61	69	8
8B	8	B	Mal	54	65	62	65'	71'	69	73	79	8
		Th Bac.		146'	156	151	151	154	152	155	157	4

Thus K12 from .1% glucose ferments maltose ca 1/5 - 1/4 the rate of glucose; from 1% glucose, at only a negligible rate! Galactose is not used significantly by either, previous discrepancy presumably due to impure galactose.

W1301 - Preadaptation to galactose

May 1, 1950

-W1301 and 58-161, each harvested from 3 plates DN2 Glu. 1 Wash and resuspend in ~~25~~ 30 ml. 1/20 $NaHCO_3$. Flasks 2 ml cells, .1 ml 10% substrate in sidearm.

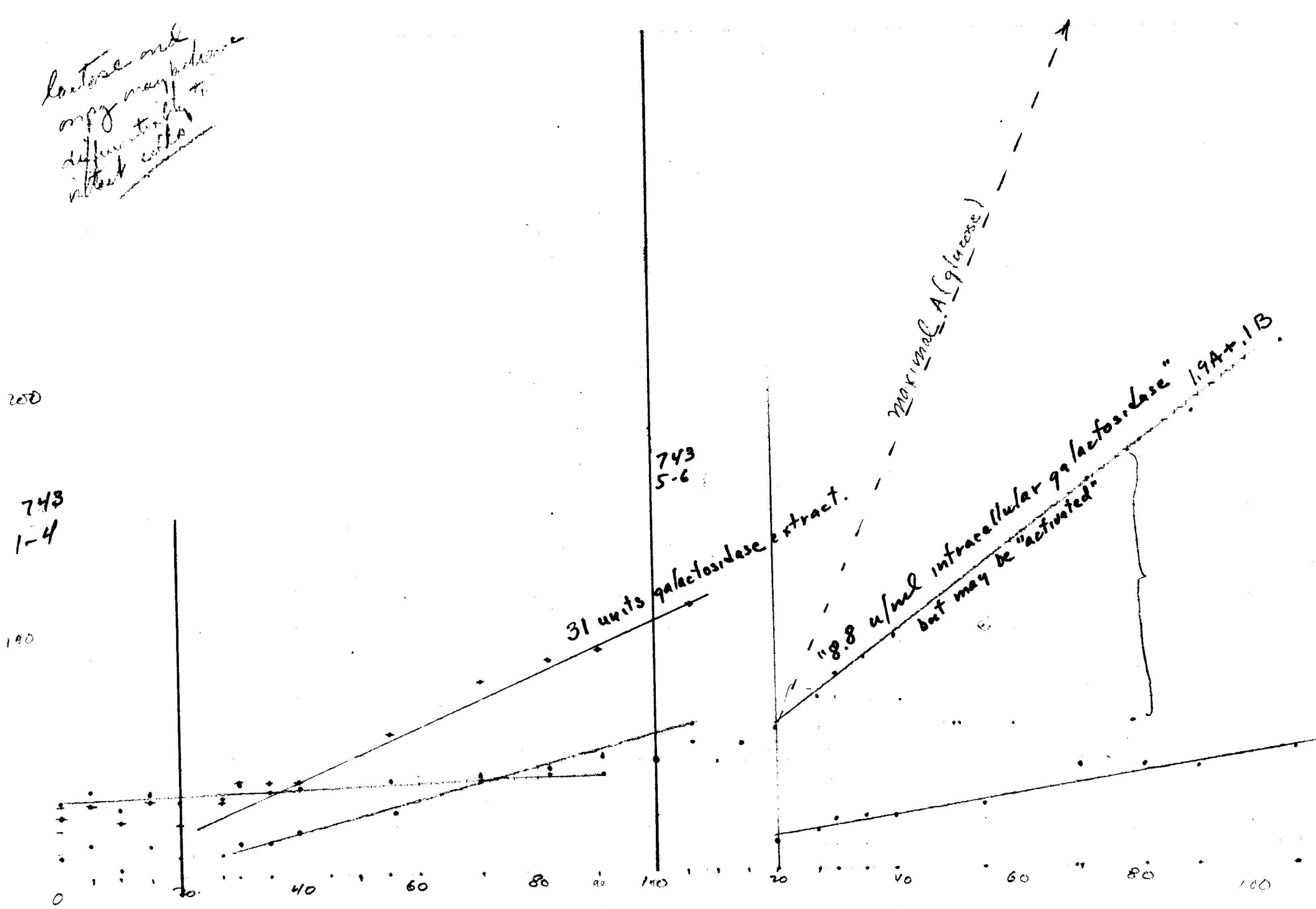
			0	5	10	15	25	30	35	45
1A	—	161	55	57'	59'	57'	61	64	62'	61
2A	Glu 2 mM	161	48'	50	61	93	153	183	209	262
3A	Gal	161	13	10	10	17	20	20	23	21'
4A	Lac	161	51	52'	51'	55'	59	58'	61	60
6A	~~Gal~~	1301	60'	65	63	70	71	71'	72	70
5A	~~Glu~~	1301	62	64	77'	119	181'	206	236'	290
7A	Gal	1301	76	74	70'	84	82'	82	84'	85
8A	Lac	1301	24	27	28	69	127'	150	177	224'
	Th Bar		133	135	132	138	139	136	140	139

W1301 is preadapted to lactose but not to galactose (galactose should be accumulating! Uses lactose as fast as glucose.

1/10	Di	Donpg		Δ
A	252	260 (10 min)		—
B	249	473 (6 min)	ca 220	∴ B shows an activity of $\frac{4 \times 2.2}{1} = 88$ u/ml.

(Test by B, exposed to conditions of Warburg vessel.)

Judging from efficiency of B in mixture with A, B contains approximately 7x the amount of lactase needed to keep pace with glycolysis, assuming equal potency within and outside the cell.


lactose and
may be
743
1-4
743
5-6
200
100
0
40
60
80
100
20
40
60
80
100
31 units galactosidase extract.
"8.8 u/ml intracellular galactosidase"
but may be "activated"
1.9A+.1B.

M243 segregation pattern

743
b

	Mal	Gal	MH	Xyl	Mal	Gal	MH	Xyl	Mal	Gal	MH	Xyl	Mal	Gal	MH	Xyl
(1)	+	−	+	+	−	−	−	−	−	−	−	−	+	−	+	+
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	±	−	−	+	−	+	+	−	−	−	−	−	−	−	−
	−	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−
	−	−	−	−	+	−	+	+	+	−	+	+	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	+	−	−	+	−	+	+	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	+	−	−	+	+	+	+	−	−	−	−				
	−	−	−	−	−	−	−	−	+	−	−	+				
(2)	−	−	−	−	−	−	−	−	+	−	+	+	±	−	−	−
	−	−	−	−	−	−	−	+	+	−	+	+				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	+	−	−				
	−	−	−	−	−	−	−	−	+	−	+	+				
	−	−	−	−	−	−	−	−	−	−	−	−				
	+	−	+	+	±	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	+	−	−				
(3)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	+	−	+	+	−	−	−	−	+	+	+	+
	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−				
	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
(4)	−	−	−	−	−	±	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+
	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−
	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−
	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	−	−	−	−	−	−	−	−	+	+	±	+	+	−	+	+
	−	−	−	−	−	−	−	−	−	−	−	−				

Gal − Mal − MH Xyl − −
+ + + +
= predominant

All− 108
All+ 6
Gal− +++ 15
Gal+ − − − 5
Xyl/+++
7:0 2

Segregation of H243

May 1, ... 1950.

Picks 8 colonies of H243 from EMS Lac and inoculate into ~~1~~ Dilution Permissay. After 48 hours, plate out on various media:

(1)

7	EMBLac	142 total.	4 clear Lac⁻. Anumber (±5) of others are faded lac⁻ reminiscent of Gal-Lac⁻.
	EMSLac	3+ 0- (some minute: hold)	
	EMS Gal	7+ 1-	
	EMS Mal	2+	
6	S Lac	12+ well defined. several small- hold.	
	S Gal	11+ "	
	S Mal	18+ 6-	
5	S L	++ numerous poorly defined -	
	S G	ca 20 -	
	S M	30 -	

---

(2).

7	BL	196 total	1 Lac⁻.
	SL 0		
	SM 0		
	SG 2+	? - hold.	
6	SL 7+	Many small -?	
	SG 12+	" "	
	SM 1+		
	BL 3+	Many -.	
5			
	SM	181+ 31-	2 sec.
	SG	26-	2 sec?

---

(3)

7	BL	84 tot.	2 L.
	SL	2 + tiny +	
	SM	2+ 1-	
	SG	1+ 1-	
6	BL	13 +	Many small
	SG	25+ 4-	" "
	SM	23+ 1-	
5	SL	Many large+.	
	SM	194+	75- ✓
	SG	184+	62-

Segregation of H243

May 3, 1980.

(4) 7 BL [redacted] SV (1 faint)
SL 4+
SG
SM

6 SL 11+ many small
SG 18+ 1- many small.
SM 11+ 7-

5 SL [redacted]
SG 12-
SM 147+ 33-

Partial segregant. Picks EMS: Gal- and [redacted] Mal- to EMB; [redacted] Lac for partial segregants.

Repicks Lac+ and streak out on EMS Lac, EMB Lac, Mal, Gal for verification and resolation

(4) Gal- : 6 tests 2 Lac+
Mal- : 22 tests 2 Lac+

(5) Gal- : 33 tests 9 Lac+
Mal- 37 tests 8 Lac+

Segregants: Picks Lac- at random and test on four sugars:
(1) Mal Gal Xyl Mtl

Partial segregants

May 6, 1950.

Restricale possible Gal- or Mal- Lac±, from EMB Lac+.

(3)

EMS→	Lac	Mal	Gal	EMS Lac
1	+?	+−	v(+,−)	
	v	v	v	
	v	v	v	
	v	−	−	
5	+(v?)	+−		
6	v	v	v	
7	v	v	v	
8	v	v	v	

	Lac	Mal	Gal	Stac
9	+(?-?)	−+	−,+	
10	v	v	++	
11	v	v	v	
12	v	v	v	
13	v	v	v	
14	v	v	+v	
15	v	v	v	
16	v	v	+v	
17	v	v	v	

#4, 5, 9 → 1, 2, 3. should be looked at again. How can the − appearance be accounted for?

Each appears to be:
Lac+ pure
Gal−
Mal−
B_1−
} probably many Lac+ crossovers.

(4)

1	v	v	v
2	v	v	v
3	v	v	v
4	v	v	v

M242 Reversions (Mal+ − purified and selected):

1-8: pure Mal+ app. Lac− (i.e., segregated) but hold.

Repeats Gal and Mal− from EMS (#1, 3, 4) (ca 3 each)

	Gal−	Mal−
Gal EMS	all −	7+ 7−
Mal EMS	12− 1+	13− 1(−+)
Lac EMS	all −	

May 1, 19[illegible]

6 plates [illegible] 1 × 10 to 10 ml. 9 ml dried over P_2O_5.
1 ml = dilute to 10. [illegible] = .6 plate / 10 ml [illegible] 10 [illegible]
2 parts [illegible] incubated under benzene [illegible]
[illegible] [illegible]ous.)

Dry [illegible] mg.
Extract [illegible] 5 [illegible] Retain aliquot of suspension of dry cells (2 ml)
Also [illegible] 4.5 ml [illegible] centrifuge [illegible] supernant.
Make preliminary assay [illegible] metric [illegible]
Extract has activity [illegible] $\frac{2}{5m}$ × [illegible]

Use [illegible]

		Juent	Di	
Intact cells.		1 ml		ca 15
Dry cell susp.		.01 ml = .003		
Benzene cell susp.		.02 ml	6 m	

dried [illegible]

Note very low intact [illegible] may have de[illegible]
744E Add benzene to aliquots [illegible] assays.

Prelimi[illegible] assays.

		Di	D'orig		
1 744 A		.192	.500	20m.	ca 10 × 3 = 30u/ml
2 744 B		.022	.358	5m.	ca 50 × 4 × 3 u/ml = 6[illegible]
3 744E	[illegible]	.039	.470	5m.	[illegible]

① mg equivalence of bacterial density unit. Let 1 BDU = quantity of bacteria giving gst. tens of .10 in 10 ml.

Then 276 mg dry cells were obtained from 9 ml of a suspension which had a density of $10 \times \frac{1}{.1} \times 1.39$ per ml $= 9 \times 139$ BDU.

$\therefore$ 1 BDU $= \frac{276 \text{ mg}}{9 \times 139} =$ 217 γ/mg.

Discard this expt.

Manometric efficiency

May 6, 1950.

Cells harvested from D V2 1% Glu (stored 24h.) 200 ml agar.

Suspend in ca 40 ml $NaHCO_3$ M/20.

Dispense 2 ml (D) per flask. + added cells.

Flask	Vessel.	Sidearm (10 mg.)	TIME → 0	10	35	↓40	45	50	55
B 1	D	—	33	34	39	37	43'	43	41
B 2	D	glucose [17]	−02	−07	[18] 06'	[15] 20	103'	167	219
B 3	D	lactose	42'	42	49'	50	52'	51'	48
B 4	D + .1ml A	lactose	48'	46'	54'	60	64	66	67
B 5	D + .1ml D	lactose	24	23'	30'	34	37'	37'	37
B 6	.1ml A	lactose	23	22'	25	24'	25	25	25
8B 7	.1ml D	lactose	34	32'	35	38'	38	35	32'
9B 9	Th Bac		44	43	46'	49'	48	45'	43

1	65
2 [21 / 71]	38
3	78
4	47
5	74
6	41
7	26
TB	32 / 44

Inadequate lactase!

May 5, 1950.

200 ml DN2 Lac 1% K-12 harvested. Wash; suspend sediment in 10 ml. (D). Dry 9 ml aliquot; Dilute 1 ml to 10. (A).
Treat ca 2 ml of A with Benzene (B). 40M —

Preliminary assays (see 744.)

A: 30 u/ml. Dilute 1:15
B: 50 × 4 × 3 units/ml, ca. = 600 u/ml. Dilute 1:200

D: 430 mg dry cells (two stage drying).
∴ Calculate 1 BDU = $\frac{430 \text{ mg}}{9 \times 192}$ = .248 mg (Cf. 744 .217

Assume about .23 mg.

D) Prepare a 2% suspension of dry cells. Remove aliquot for use, and dilute 1:10 (2 mg/ml) (D) (2× in anti.)

X_1 Extract remainder 3 hours and spin sediment. (Re-extract = X_2)
Yellow, viscid, opalescent supernatant

Cells

Kinetics of ~~[illegible]~~ un-treated cells. Dilute ~~[illegible]~~ 1/15

	Final conc		Corr A	Corr B	Σ Corr	Δ = V (392)	1/V	1/S
1	M/500	422	79	042	121	301	33.2	500
2	M/1000	339	79	021	100	239	41.8	1000
3	M/2000	260	79	010	89	171	58.5	2000
4	M/5000	177	79	004	83	94	106	5000
5	M/10,000	140	79	002	81	59	169	10000
6	0	079				↓ (Would be zero on linearity)		
7	M/1000 No cells	021						

Terminate with Na_2CO_3. Read within a few minutes.

~~10~~ 20 minutes 37° Cells fresh (a few hours in H_2O)

NaP M/50. 10 ml volume + 1 ml Na_2CO_3.

Volumetric Quant Technique

K_s (cells) = 6.3×10^{-4}

V_{max} ≐ 1/25.5 ≐ 392 (3.92 units / ml suspension.)

The cell suspension ~~control~~ was $1/15 \times 1/10 \times 1/9 \times 430$ mg/ml $= \frac{430}{1350} = 318$ γ/ml

(Calculated density unitage : $\frac{2}{3} \times 192 = 128$
≐ 318 γ
in perfect agreement, as demanded!)

Lower O.D. due to alkali).

Therefore V_{max} was 3.92/.318 = 12.3 u/mg.

Kinetics: Benzene treated cells.

May 5, 1[illegible].

B) Dilute ~~1:100~~ 1:200 to place in convenient range for assay.

M/—	Dompg	Corrn	Δ	1/Δ	D'	Corrn	Δ'	1/Δ'
500	482	049	433	23.1	490	039	451	22.2
1000	460	028	432	23.1	461	022	439	22.8
2000	403	017	386	25.9	400	013	387	25.8
5000	293	011	282	35.5	300	008	292	34.2
10000	200	009	191	52.3	197	006	191	52.3
	007				004			
					002			
					033			

H_2O M/500 no with

See 744 for correction factors.

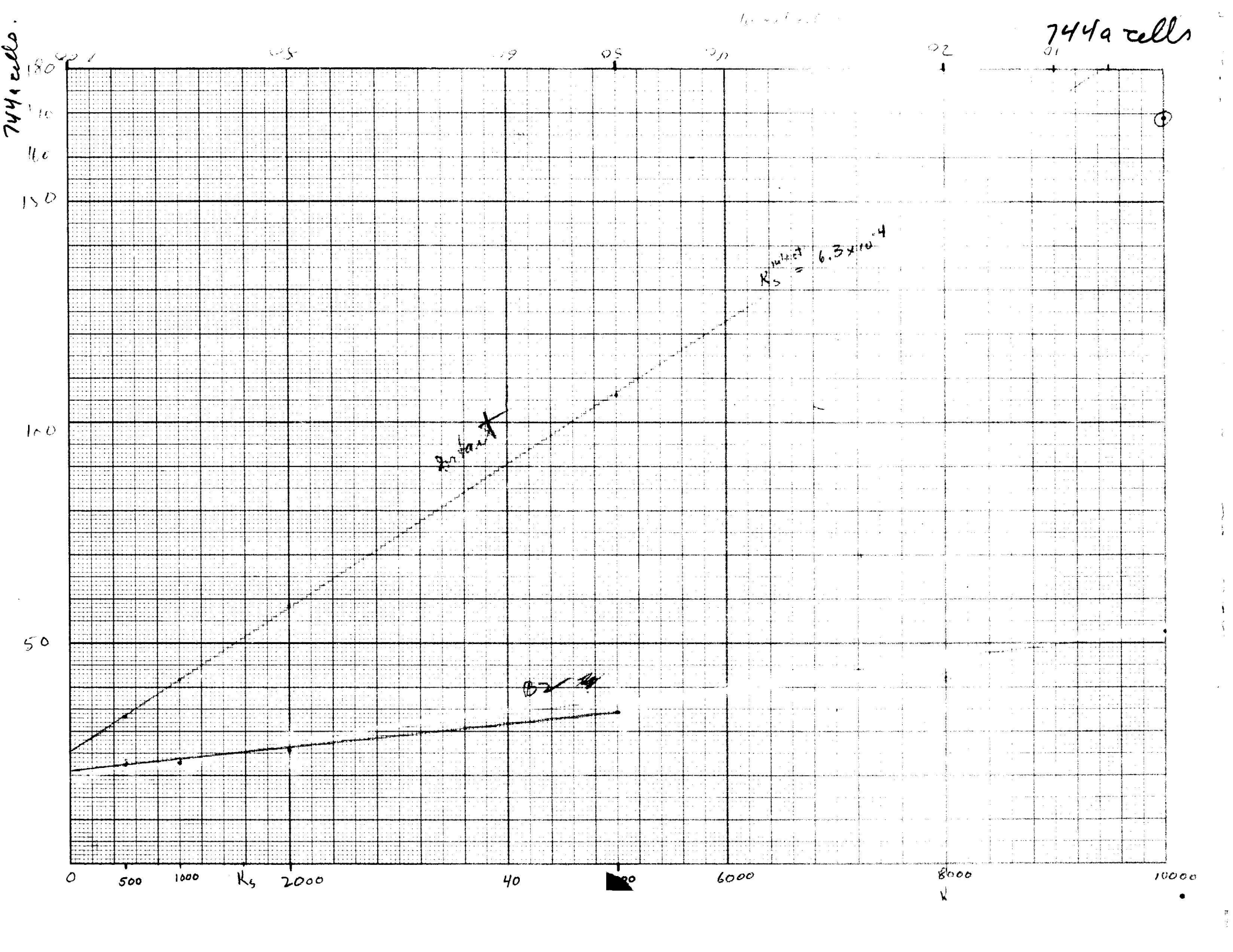
Repeat readings using a single tube D'

$$K_s = 1.25 \times 10^{-4}$$

Note difference in V_{max} : Cells are $\frac{200}{15}$ conc

$$V_{max} = \sqrt{21.0} = 476$$

$$\text{Absolute activity} = \frac{476}{392} \times \frac{\cancel{200}\ 40}{\cancel{15}\ 3} \times 12.3 = 199$$


744a cells.
744a cells
180
150
100
50
0
500
1000
K_s
2000
40
6000
8000
10000
$K_s = 6.3 \times 10^{-4}$

Manometric assay of lactase

May 1, 1950.

Dry cells of K-12 harvested from ca 100 ml Y2 Lac, P_2O_5, room temp. Yield: ca 35 mg. Tetanate and shake in 3.5 ml H_2O. Sediment and retain supernatant.

a) Preliminary assay in mmg of extract (rather weak! – no pancreatic salt treatment?) → 77u/mol

b) optical density of 742-A cells (for assay!)

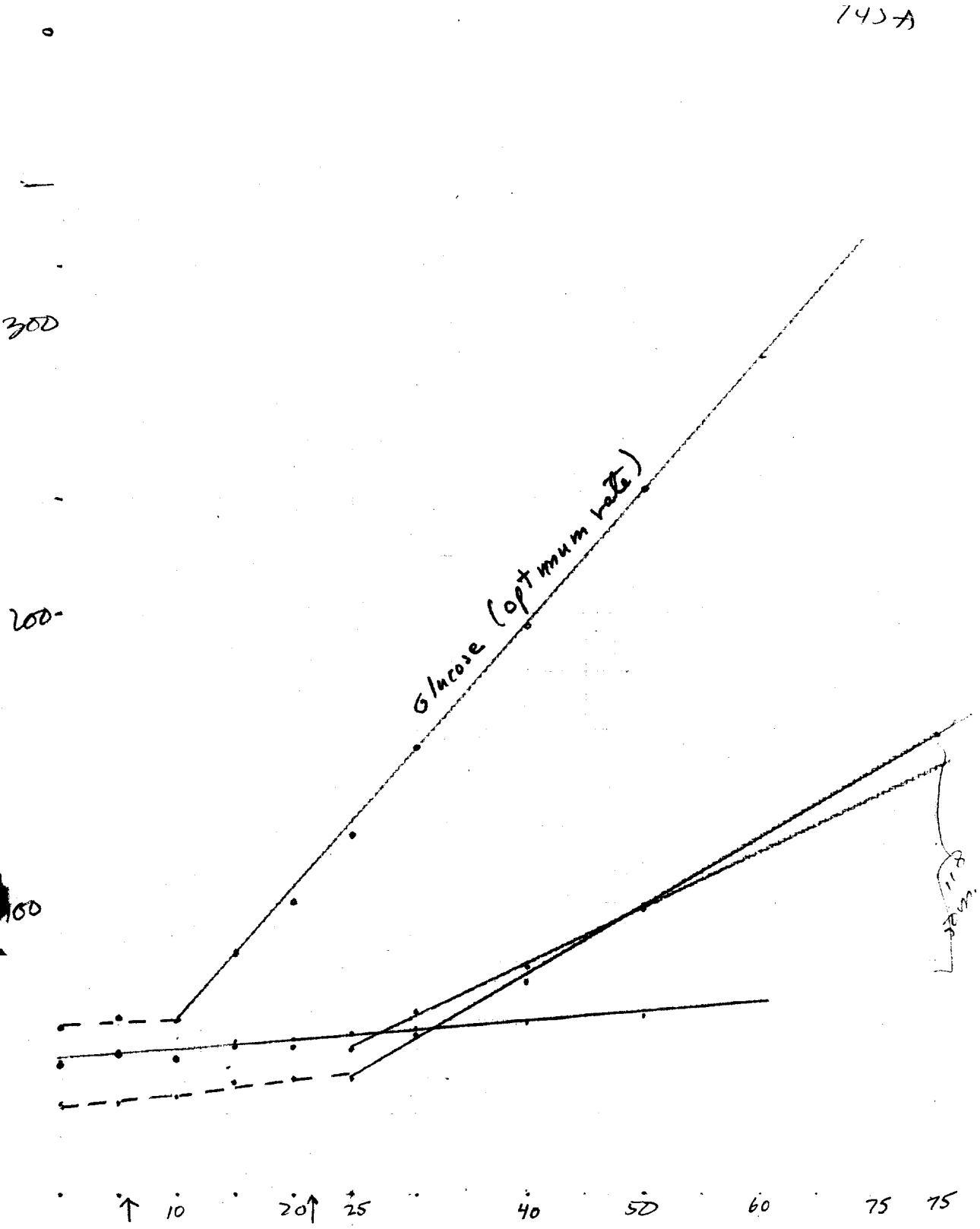
See 742

Use 2.0 ml 742A cells. Time →

	Side 1 / Side 2	0	5	① 10	15	20	27	30
1	lactose 10mg —	31	36	28	35	32'	33'	38
2	" Extr .2ml	8'	14	3'	13	8'	09'	14
3	" " .4ml	25	30'	23	32	22	31'	39
4	glucose Extr .4ml	28'	35	29'	38	32	34'	40
5	1.9ml A 0.1ml B - lactose	46	54	46'	53'	59'	72	82
6	1.9ml .1ml B lactose	8'	18	7	16	12'	16'	21
TB.		157	162	156	162'	[illegible]	160	163'

	35	40	55	71	82	91	106
1	35	37	39	41	41	41	47'
2	14'	18	26	39	43'	48	61
3	39'	39'	58	80	88'	93	112
4	37	37'	36'	42'	42	37'	40
5	88'	97	101'	161	177	189	217
6	22'	22	26	42	42'	41	47
TB	164	162	161	169'	168	163	167

growth?


300
200
100
Glucose (optimum rate)
10
20
25
40
50
60
75
75

Manometric assay of lactase

May 4, 1950.

Use same cells (742 A!) for assay of glucose liberated.

	Cells 2 ml	Substrate 2 ml		0	5	10	15	20	25
C 1	A	Lac	—	46	49'	47	53	54	55'
A 2	A	Glu	—	58	61	60	83'	101	124
A 3	A	Lac	744 with 1 ml	45	48	45	51	51	50'
A 4	A	Lac	" 2 ml	31	32	34'	39'	40	40

31	40	50
57'	59	61
154	196	243
63	78'	98
55	73	99

Glucose A = 228 / 50 min

Lact

Lactase A = 118 / 50 min.

(opt.) Should steady state be reached at a suboptimum level?

Manometric Efficiency

May 8, 1950.

Harvest K-12 from DN2 .1% Glu(G) 3 plates and Lac(L) 2 plates.

~~Resuspend in 5 ml $NaHCO_3$ M/50 buffer.~~

~~↓ cell density computed (from opt. density)~~

Resuspend in 20; 15 ml respectively.

Optical density: (.1/10) (9 ml)

Use 1 BDU ≙ .23 mg as conversion factor

	O.D.	mg/ml	Do/pg
G	360	8.3	
L	291	6.7	[illegible]

Repeat .03 ml/10 L 091 122 !

very low activity!

effect of $NaHCO_3$?

or no adaptation?

Effect of Bicarbonate buffer.

Manometric assay.

May 9, 1950.

Cells harvested from 2 plates each D/2 Glu; Lac (.1%) and resuspended in 10 ml H_2O. From "L", aliquot A diluted 1:1 with H_2O; B with M/10 $NaHCO_3$. Let stand (under CO_2) at room temperature from ca 3PM – 8PM. Assay in M/50 Na P 7.5 ...

Assay (.1/10) 20

		Di	Donor
Putative mutant	A	207	258
	B	191	271
	—	-009	012

2 ml B (Lactose) cells.

Sidearm
B { B 1 Lac
B 2 Glu
B [redacted]

B 4 A cells - Glu
A { A 5 " Lac
6 6 A 2ml B 1ml Lac

A 7 A —

A 8 TB

	T→0	5	10	15	20	30
1	22	22	22	24'	28	39
2	25'	24	30	42'	59'	91
4	45'	47	52'	59	74	94'
5	25	28	28	25'	30'	30
6	65	69	68	64	72	71
7	54	59'	59	56'	63	60'
TB	121	127'	126	124	131	127

	40
1	41
2	114'
4	107'
5	25
6	66
7	54
TB	122

No bicarbonate effect, but these cells have very low lactase activity!

Irradiation of M226.

May 9, 1950.

A - Control 10^{-7} M226 resolated from single colonies.

B - UV 20 sec. Dilutions indicated. Irradiate the 10^{-4} dilution. ϕ 30 P/M.

"7" = 2×10^{-7} ~~$6 = 10^{-6}$~~ ~~$6 = 2 \times 10^{-6}$~~

EMB Mal

	+	−	V	
A.	19	1	131	Some "7" may be V.
	10	3	122	
B	64	20	142	Not accurately countable.
	87	28	146	Other plates not counted

EMB Lac

	V	−	
A	188	17	
	212	10	
	400	27	
	~~200~~	14	
B	127	108	Many colonies ⊙
	134	80	
	80	128	
	341	316	
	170	162	

Pick Mal − colonies and test for Lac V. See 747A.

Pick whole lac − colonies and streak out for Mal+/− content.

~~Tests~~

32

Mal+	Mal −	Mal+, −
14	13	5

+	−	+ and −
18	9	5

B2 did not grow on synthetic (EMS)

A and B1-3 appear to be Mal- Lac^{v}.

See 747D

H226 : UV.

747A.

a) Picks apparently pure ~~Lac~~ Mal− colonies to EMB Lac, for "partial segregants". (1, 2, 4)

A] 4 Mal "−" : 3 Mal− Lac− #3: Mal−, +?; Lac −+? Restreak (747 ~~A~~) has −v in both Lac and ~~Mal~~ REISOLATE

B] 38 Mal "−". 13 of these have apparent Mal+ in brush, usually not so clearly evident Lac+.

← 3 possible Mal− Lacv. Restreak: 747AB-[1-3]. May be Mal− Lac+ appearance for some colonies

Remaining 22 are apparently pure Lac− Mal−.

b) SECTORED COLONIES

Pick from Mal sectored colonies (EMB), the − sectors possible, and the + sectors. Brush on EMB Mal; Lac:

Mal		Lac		Mal		Lac		Mal		Lac		Mal		Lac	
−	+	−	+	+	*	+	−	+	−		+	−	+	−	*	+	−
−	*	+	−	−	+		+	−	+	+	*	+	−	−	+		+
−	+	−	+	+	*	+	−	−	−		+	−	−	−	*	+	−
−	*	+	−	−	−		+	−	+	−	*	+	−	+		+	+
−	*	+	−	−	−	*	+	−	−		+	−	+	−	*	+	−
−	*	+	−	−	+	*	+	−	+	+	*	+	−	+	*	+	−
−	*	+	−	−	+		+	+	−	−	*	+	−	−	*	+	−
−	*	+	−	−	+		+	−	+	+	*	+	−	−	*	+	−
−	*	+	−	−	−	*	+	−	−	−		+	−	+	*	+	−
−	*	+	−	−	+	*	+	−	−	−		+	−	+	*	+	−
					+	*	+	−	−	−	*	+	−	−	*	+	−
					+	*	+	−	−	+		+	−	+		+	+
					+	*	+	−	−	+		+	−	+	−	o	+	+
					+	*	+	−	−	−	*	+	−	−			
−	o	+	+	−	−	*	+	−	−								
+		+	−	+	+		+	−	+								
+		+	−	+	−		+	−	+								
−	*	+	−	−	+		+	−	+								
−	*	+	−	−	+		+	−	+								
+	*	+	−	−	−	*	+	−	−								
−		+	−	+	+	*	+	−	−								
+	*	+	−	−	−	*	+	−	−								
+		+	−	+	+	*	+	−	−								
							+	+	+								

− denotes absence of visible + .

These data are of limited value owing to the difficulty of identifying a minute fraction of Lacv in a Lac− brush (picked finally to plate). * types would appear to represent Mal+/Mal− mixed colonies & Lacv.

o might be Mal− Lacv

Restreak as 747c 1-2

#1 is Lac− Mal−, +, prototroph

#2 is Lacv Malv

Pick Mal− brushes from c) and check on Xyl EMB

40 *
2 o

19 Xyl+ 17 Xyl− ! (High crossover frequency?)

EMS Platings

747B

May 12, 1950

6 clones.

		+	−	sectoral
EMS Lac		74	0	0
		101	0	0
		140	1	0
EMS Mal		49	0	0
		155	0	0
		141	2	0
		134	1	0
B	EMS Lac	104	15	12
		82	9	16
		101	13	9
		98	23	20
		120	15	11
	Mal	102	4	11
		89	6	10
		65	5	14
		65	5	3
		102	4	12

5/22. Pick − and sectoral −, + from Mal EMS to Lac EMB, Lac S, and Mal B.

Lac	Mal	Lac	Mal
−+	− ++ +	*+	− − +
−	−	*+	+ − +
−* +	−	−	−
−	− + +	−	−
−* +	−	−* +	− − +
−	−	* +	−
	−+ −	· −	−
−	−	*+ +	− +
−* +	−	*+	−
* +	+ +	*+	−
−	−	−	−
		*+	+ − +
		*+	+ + −
		−	+ − +
		−	+ − +

Streak * from EMS Lac to EMS Mal. 6 "−" and 7 "+" sectors.

Analysis of these plates was interrupted (by weekend vacation trip and lack of assistance)

Experiment to be repeated on more appropriate scale. Test for:

a) auxotrophic Lac; Mal v

b) Partial segregants in mitect and sectored Mal− (EMS) colonies.

c) Carefully examine "induced haploids" for trace residual diploid (cf. 747A-[B]).

UV-induced partial segregants

747D

May 16, 1950.

1-3 = 747B-B1-3 4 = 747B-A1

a) from EMS (excludes 747D2)

b) from EMB Lac.

		EMB Lac	EMB Mal	EMS Lac	Mal Xyl MR
a.	1	V	—	+	
	2				
	3	V, —	+, —, v?	+	not part. veg.
	4	V	—	+	

		EMB Lac	EMB Mal	EMS Lac		Mal	Xyl	MR
b	1	V	—	+			V	V
	2	V, —	V	no growth	(Methionine less not part. veg.)	—	V	+
	3	V	V	+				
	4	V, —	—	+			—	—

5/18 c Repicks 3 single Lac V colonies from 2a, b. Restreak on EMB Lac; Mal and determine nutrition: Methionine less.

Repicks single Lac V colonies to Mal EMB to obtain mixtures, in hemizygosity test.

SUMMARY:

#1.	Lac V	Mal- Xyl V MR V	Prototroph
#4	Lac V	Mal- Xyl- MR-	Prototroph (spontaneous)
H244 #2	Lac v	Mal- Xyl v MR +	Methionine less.

PROJECT:

1. Hemizygosity of Mal- in 1, 2, and 4
2. Identification of triploids in outcrosses of #2.

5/24

747E. Auxotrophic segregants of H244 (Xyl-) to Y10.

M+ Lac- Mal- Xyl- Mtl+ × Y10

1.

H244 Reversion hemizygosity test

#2. Segregation: Streak out Lac v. on EMB Lac

a) Test 5 Lac- on Xyl EMB and BATLB, Each was M- Xyl+

a' Test 5 Xyl- Lac- Each was M+ =747E1

5/21/50. b) Test addnl. Lac- on Xyl EMB: 34 Xyl+ 15-.

Check Xyl- on BATLB.

H244M+

c) Bush on Mal EMB. for reversions (hemizygosity tests): ~~33~~ 23 Bushes.

5/21 3B Papillae picked from these and streaked on EMB Mal, Lac

~~#1,4: Bush on EMB Mal for reversions.~~

5/22 When predominantly + or v on Mal and Lac, repick single ~~Mal+~~ Lac v to Mal ~~Lac~~ for verification as possible Lac v reversions.

5/23 27 groups sampled. Mostly Mal-. 4 groups had ~~Mal+ Lac~~ Mal+: 1/4[b]; 1/4[c]; 3/6[a]; 1/3[d]

Restreak each of these on Lac, Mal EMB, and restreak single Lac v colonies:

	Lac	Mal
1	- v	+ v -
2	v	+ v -
3	v	+ v -
4	v	- v

Pick - to EMB Xyl. M+ may be v

Mal-		Mal+	
4X-	1X±	9X+	CIS
5X-	1X+	7X+	CIS
2X-	~~2X+~~	1X+	CIS
3X-	5X+	2X+	TRANS??

W1301 crosses.

May 8, 1950.

A × 1272
B × 1178 } EMS lac
C × 340

Picks 100+ from B and streak out on EMS Lac for Lac v. Yields very low on A. C considerable.

c) 73 Lac+ picked to EMS Mal. All +.

c) v. good yield. Lac+ and Lac slow (incubator at 36+° - threshold for Lac_3^t).

20+ and 20 sl picked to water susp.

and spotted on EMS lac, B Lac, ~~Mal~~ Glu

20 Lac+ : ~~Mal~~ Glu

20 Lac sl : ~~Mal~~ Glu -. Are any of these const Lac+? (at 30°?)

~~Pick to DNZ Glu for further test.~~ Picks from EMB Glu for spot plate tests with onpg.

Glu -:

onpg +	1, 3, 4, 6, 8, 10, 11, 13, 14, 15, 16, 17, 18, ~~20~~	70%
onpg -	2, 5, 7, 9 12, ~~19~~ 11, 12.	30%

The onpg + glu - cultures must represent the genotype Const + Lac_3^t. Purify to verify temperature behavior. Streak out the two types. Pick from EMS Lac. on EMB Lac.

~~Inoculate NZB with #~~ 11, 12, 1301 and 58-161. ~~Incubate at~~ 30 and 40°.
of 16 Glu + cultures, 10 +, 6 - on onpg.

---

see 749:

Test various suppressor stocks for constitutive lactase: (escape from Glanto)

W252: very strong +++ on onpg.

are lac 3 constitutive?

May 11, 1950.

B 100 lac+ picked and streaked on EMB Lac.
Likely; 2 improbable lac v. Picks four colonies and restreaks
88 further lac+ picked and streaked: 1 definite Lac v.
Of previous set, # 1 is Lac v, others probably not. Keep as
748B1 and B2. Brush on DN2 tube for spot test.
onpg spot tests: Both B1 and B2 are constitutive.

C: Segregation of Col among ~~Lac+~~ Lac+ (from EMS spots to DNB onpg spot plate tests

1-12 5+ 7- (5, 6, 9, 10, 12) These data not very informative.

#11 (npg+) is lac+ at 44° Both are Glu —.
#12 is Lac — " "

May 11, 1950.

Test various suppressor strains for constitutive lactase:

a) scrape growth from (old) slants for npg spot plate:

	W	npg	Constitution			
1	251a	±	W108	SLac I	+ Lac_3 −	(ferments Lac + Sal) L+ M+ D −
2	252	+++	W108	SLac	"	L+ M− D −
3	327	−	W108		"	M+ L− D−
4	329	−	W108		"	M+ L+ D −
5	349	±	58-161	LOL +		
6	716D	+	Y70	Lac_1− Su+		
7	716E	+	"	"		
8	1301	++	58-161	Lac+ Const+		

b) Inoculate into Penassay and incubate 11AM — . Assay. After 36 hours, spot-plate test with one drop of grown culture

251	−
252	+++
328	−
329	±? Repeat:
349	−
396	−
397	−

Lactase economy

May 13, 1950.

Harvest K12 from 200 ml aerated Y2 .1% lac, wash, into 10 ml H_2O

Galactosidase assay: .02 ml /10. Rel. Act. ca 1.3

Original suspension has ca 800 u/ml lactase.

Di	20M. Donpg
132	309

K12 / Glucose from 5/11/50. used for assay

For manometric assay, dilute this suspension 1/1+2, to a final becarb. buffer of M/20. Cell densities (.1/10) Dilute G 1/1+1 likewise

L see [illegible] should have ca 250 units of activity [illegible]. [illegible] flask contents at [illegible]

L	180
G	200

	Flask	Side 1	Side 2	4:25	4:32	4:35	4:40	4:45	5:00
A	1 G	glucose		39'	~~40~~ 39↓	42	48	49'	
B	2 G	lactose		31	30	32'	33'	33	
	3 S	lactose	.2 ml L	43	42	43,	47	46	
	4 S	lactose	.1 ml 744 extr.	55	55	58'	58,	58	
	5 L	glucose		43	43,	53'	108'	159	
	6 L	lactose		46	45'	48	83,	117	
A	7 S	—		63	64'.	66',	62'	65	
B	8 L	—		42	44'	45'	40	45	
B	9 —	—		133	135'	133'	129	133	

6 ugm glucose

N.G.