

Assays on fractionation.

Use ≥ 1 ml. X or Y + 1 ml. 5% lactose. Incubate 30 mins. 37°. Then add 4 ml Cu⁺⁺ Sediment. Boil 10 mins. Wash ppt + dissolve in Fe⁺³ and titrate with .02N KMnO₄. CC: 8

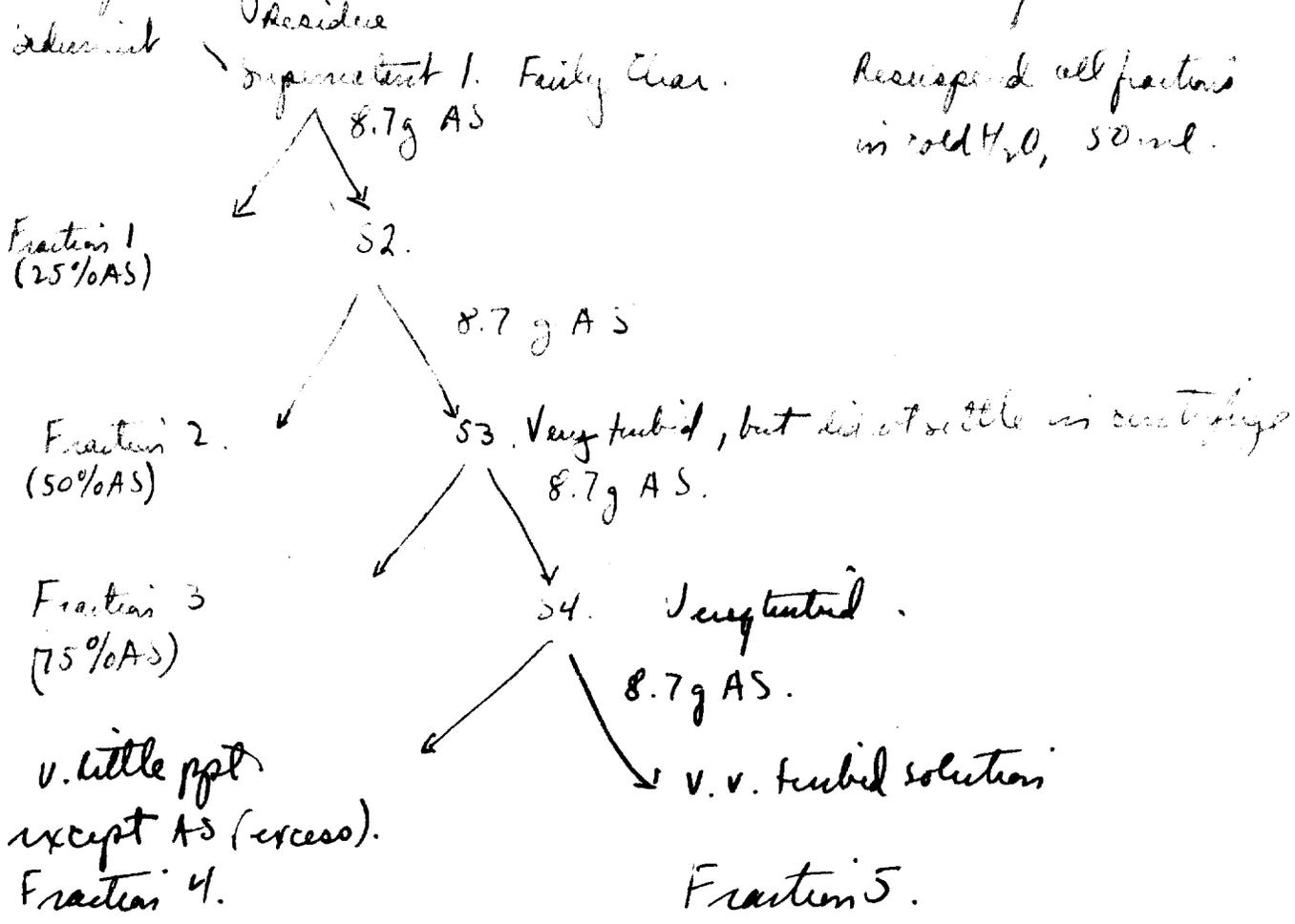
1. X	+++	7.44	8	
5 X	+++	8.19	8	
.1 X	++	4.83	5	[Note. ca 3 mg/1/2 hr.
.01 X	-	.40	.3	
Y. 1st 1/2	++	5.84	8	
1.	+++	8.42	8	Acetone
2.	++	7.20	7	(AS)
3. 1st 1/2	++	3.10	6	(Alcohol)
5. 1st 1/2	+...	2.67	7	Acetone
6.	-			
7.	-			
8.	-			
9.	-			
10.	-			
Glucose FR	++	8.78	-	
X + Glucose	+++	8.39	-	Utilization??
Lactose.	-	0.13		Blank

Cu₂O color + ppt roughest.

1. Autolysate active
2. Acetone powder active Alcohol powder active
3. Comes down at 1/2 saturation. Am Sulf.

Fractionation of W-254 lactase.

Suspend 1g. Acetone Powder 160 in 50 ml. cold H₂O. for 24 hours.



Assay: 1 ml .05 ml

1. Acetone Residue
2. Fraction 1 (1/4 sat.) sl. opalescent
3. F 2 (1/2 sat.) clear
4. F 3 (3/4 sat.) clear
5. F 4 (sat.) clear
6. F 5 Residue after AS sat. v. opalescent.

Assay with 1/2% lactose, 1/2 hour 37°.

2/20	1.30 - 2.41	1.11
2/11	2.41 - 8.71	6.31
1/1	8.71 - 12.5	++ 4 +
1/20	12.59 - 13.40	.81
R	13.40 - 15.70	2.30
R/20	15.70 - 16.70	

Residue not uniformly distributed.

Others, 0.

Activity seems to be distributed among the "insoluble residue", the 1/4 AS and the 1/2 AS fractions. Continue to extract the residue + ppt with 1/2 AS. Pool 1/4 + 1/2 AS fractions with these extracted portions.

Pool extractables from Acetone powder + ppt. with 1/2 sat AS. Resuspend in water and centrifuge 30 mins at 4000. Supernatant is very faintly turbid; considerable ppt. (Particulate??)

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

- a) 9 ml 1/4 + 1 ml 1/2
- b) .9 ml 1/4 + 1 ml 1/2

1/2 dilutions: Assay 20 min. 40°C.

Ampl., ml.	P ^A	S.
1/2	0.50	5.17
1/4	0.31	3.63
1/8	—	2.03
1/16	—	
1/32	—	.030
1/64	—	
1/128	—	

Activity AS 6.31 + 2.30 = 8.61. B
 Enzyme in soluble fraction after AS pptn.

Activity is much less than original conditions too close to substrate exhaustion.

Danks

When fraction B is pptd. \bar{c} AS 50%, three fractions are obtained.

- C 1) Supernatant - $C_{4,0}$
 C 2) Sedimentable residue after resuspension in H_2O v. sl. visible $C_{4,0}$
 C 3) Non-sedimentable residue. - $C_{4,0}$.

Assay $1/4$ ml samples (in 50 ml \bar{c}) & compare with ~~whole culture~~
 B. (2.03 ml)

40° may be too low!

Preparation of lactase : Batch 2.

162 -

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

Fraction 1. 31g. paste - Add 100ml H₂O, 5ml glucose, mix in
blender + ~~autolyse~~ autolyse at 37° # 11A 26 -

Fraction 2. 42g paste. Add 100ml acetone, shake well,
sediment + add fresh acetone. After dehydration, dry in
desiccator over paraffin. → 15.4 g ("nearly dry") acetone powder.

Suspend ⁵ 10g. powder in ⁵⁰ 100 ml H₂O to extract.

Assay (as in 161 b) .1 ml suspension (20 min, 40°).
3.8 ml 102N KMnO₄.

Extract with cold H₂O 8h. Centrifuge at 4000 rpm 1hr.

Add 17.5g AS (1/2 sat.) small ppt. Residue in H₂O. A
supernatant. B.

Test .1ml samples of each:

162-4A
162-4B.

No visible C₄H₂O
" " " "

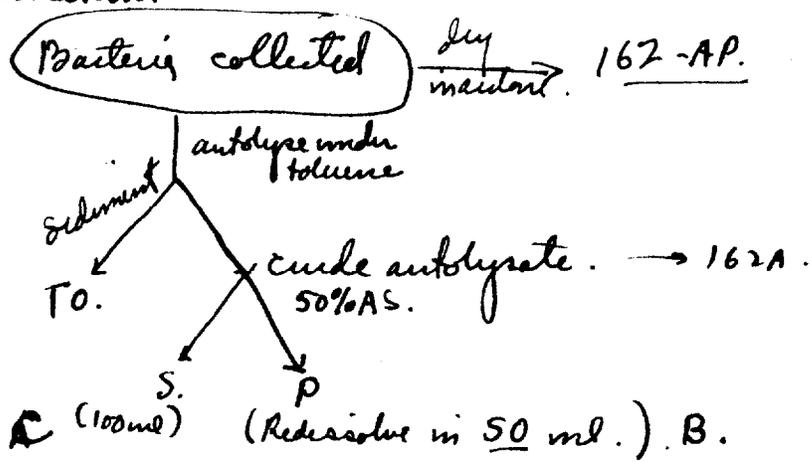
40° may be
too high for assay.

No activity!

P28. Clarify 48h. Autolyse (add a few ml $CHCl_3$ to take up volume and permit sedimentation of solvent) 120 ml autolyse. Almost entirely clear, light yellow-green solution.

Keep 20 ml sample. Work with the other 100 ml.

Add 35g AS. Collect ppt. + residue in 50 ml H_2O_2 . ^{Fairly clear solution.} Pigment is left in supernatant.



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

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

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

No Activity.

Lactose Preparations.

March 29, 1948.

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

Aerate, 37°. 24h. (Allotery autiform). Collect in trays.

Bottle A lost. Collect 53g. cell paste from B. [Drop A, B versus?] AI. 10g. put in 100 ml. NaCl-citrate + 1 ml. toluene

BI. 43g. put in 100ml 5% lactose in citrate buffer. 1 1/2 h. Then wash, autolyze under 1% toluene.

Collect after 24h. Store 1P31 in refrigerator.

B. became ^{opaque} ~~very cloudy~~ on standing in refrigerator overnight. On warming this material redissolved. Keep 10ml as crude saddy rate = 163B1; add 14g. Hm. Sulf. to remainder + separate fractions.

ppt. Redissolved ^{in citrate} 163-B2

sup. 163-B3 - from ppt in cold!

Assay \bar{c} x 1 ml eny. + 1 ml 1% lactose, 30 mins. 37°

	CH ₂ O.
Glucose	+++
Lactose	-
Glucose in citrate	++
A 1.0	±±
0.1	-
B1 1.0	±.
0.1	-
B2 1.0	±
0.1	-
B3 1.0	-
0.1	-

Probably fermentation in lactose with limited nitrogen served to de-adapt the culture. As future, add fresh lactose to whole medium before centrifuging.

to B2, add 14g H_2SO_4 . Residue ppt in H_2O .
v. small ppt

Temperature mutants.

March 29, 1948.

85 plates, 410, 5 sec. Harvick U.V. ca. EMBLac
 incubate at 45° 11A 29 - x ca. 250 ~~plates~~ colonies.
 = 20,000 tests.

Recovered W-340

Test at 45°.

Apr. 1, 1948 + 25 plates, x 200 = 5000.

= 25000 total.

Test W-340 at 36° and 44°.

	36° *	44°
Glucose	+ slow*	-
Saccharose	++	++
Glucosic	++	++
Maltose	+ slow	-
Lactose	++	-

* faster at < 36.

At 44° this mutant is similar to W-108, but the lactase activity may be more resistant to 37° than the glucosylase.

April 6, 1948. As above. 100 plates x 300 = 30,000

No detected mutants at 45°

Temperature mutant W-340

166.

W-340 grown on GNA broth at 37° + 45°, and Lac YP at 37°.

Cells Harvested from 100ml Gna 37 / 6ml H₂O. = 2
37 = A 45 = B.

Cells from YP Lac = 1. (50ml into 2ml H₂O).

Test at 37 + at 45.

Set up 11:35 AM. Apr. 5.

37 = A

45 = B.

		37 = A	45 = B.
11.	1 / Lac	+ +++	I ++
12.	2A / Gna.	++++	++++
13.	2B / Gna	+++	++++
14.	2A / Lac	-	-
15.	2A / Lac	-	-
16.	2B / Lac	-	-
17.	2B / Lac.	-	-

12 β was ++ in 5 minutes. 12 α in 8-10.

13 β " ++ in 8 minutes.

15 MINS.

30 mins.

No further adaptations in next 6 hours.

Lactase production; K-12, lactose synthetic medium

Apr. 9, 1948.

Inoc. ~~2~~ x 50 ml each. K-12 cultures into 10 l. bottles (2) of synthetic medium (v. supra) with 1.5% lactose USP. aerate at 37° A9-A10. Collect in Sharples.

87 grams damp cell paste.

Suspend in 100 ml 1/10 wt% saline + 2 ml toluene +
autolyse at 37° Sediment and collect supernatant

10A12. Cool in Sharples. 150 cc. total.

Save 20 ml. whole ^{clear, yellow.} autolyse. To remainder (cold),
add 45 gms AS. + ppt. During centrifugation, about 2/3
of this material was involved in an accident. The gross glass was
removed + the supernat. recovered. The cup + broken glass were
washed with 100 ml H₂O, then 35 g. AS added. The ppt. collected
here were pooled and redissolved in 50 ml. H₂O. (A) Proceed with
sedimentation of remaining 1/3, dissolve ppt. in 50 ml H₂O (B).

Assay!

What is ~~green~~ yellow pigment?

Parametric measurement
of lactate activity

172a

				m.
A0.	0.00	0.01	-0.01	0.00
OB.	1.24	1.34	1.35	1.34
OC.	1.42	1.44	1.42	1.43
C20	1.38	1.39		1.38
C180	1.47			

No activity!

~~P90~~ P180. 1.46

No activity!

Inhibition of adaptation by amino acid antagonists 174

April 27, 1948

Each tube is made to 4.5 cc. Cells harvested from YP-glucose or YP-lactose overnight.

Each tube contains

1 ml 50% lactose

1 ml cells

5 ml contg. BCP indicator & 1 ml Phosphate Buffer 17/10

± 1 mg valine ± 1 mg isoleucine ± 1 mg hydroxy aspartic* ± 1 mg aspartic*
 grams L. grams G.

1.	-	+++	✓	+++	-	✓	+++
2.	IL.	+++	✓	+++	-	✓	+++
3.	V.	+++	✓	+++	-	✓	+++
4.	V+IL	+++	✓	+++	-	✓	+++
5.*	Asp.	-	✓	±	-	✓	±*
6.*	HOAs.	-	✓	+++	-	✓	-
7.*	Asp+HOAs.	+++	✓	+++	-	✓	+++

*overneutralized?

* overneutralized with NaOH

- 30 m. 3:30.

- 5 h. 6 PM

- 18 h. 9 AM.

By all appearances, valine did not inhibit adaptation, but the experiment is clearly of too long a duration. Hydroxy aspartic, on the other hand seems to have been inhibitory to adaptation even in the presence of excess pantothenate. The clear interpretation of this experiment demands a better control of the adaptation process.

* + 5% pantothenate.

HA of ...

Apr. 29, 1948.

	1:30	2:00	2:30	3:00	3:30
1	—		+±	+++	
2	+++		+++	✓	
3	±		++	+++	
4	—		+±	+++	
5	—		++	+++	
6	—		+±	+++	
7	—		+±	+++	
8	—		+±	+++	
9	—		—	+±	+++
10.	—		+±	+++	

valine inhibits adaptation somewhat and is reversed by isoleucine.

Cells from 400 (in 4 fl.) ml $NH_4Cl - PO_4 -$ glucose broth collected
in 10 ml. Each tube contains:

1 ml cells
1 ml 5% lactose
1 ml buffer + indicator BCP.

.1 ml addenda:

1. —
2. (Glucose 5%)
3. + glucose .5%
4. + food succ. 1%
5. NH_4Cl 1%
6. TLB,
7. $MgSO_4$.1%
8. valine } 1 mg/ml
9. isoleucine } .5 ml.
10. V + il. }

Set up 11:30 A.M.
2 was +++ in < 10 mins.

The temperature mutants
W-340 and W-382.

May 3, 1948.

Add 1 drop inocula to BCP-fermentation broth, at indicated temperature:

W-340	glucose	lactose	maltose	sucrose	gluconic
30°	++ +	- ±	+ ++	-	+ ± +
45°	-	- ✓	-	- ✓	+++ ✓
W-382					
30°	++ ±	++	+++ ±	-	+++
37°	- ✓	± ✓	- -	- -	+++
45°	- ✓	- ✓	- ✓	-	✓ ++

Proc 5P3.

Fruit Reading 8A4 = 15h. These are both temperature mutants.

Serial 12-14-48

W-340 inoculum taken from old stock.

From fruit test of W-382 on maltose, papillae piled and streaked out.
Mal+ colonies tested on EMBA at 37.5°

Lactose 19+ 0-

Glucose 13+ 1- 1 uncertain or mixed.

Purify 1+ and 1- on maltose.

mal+ test glucose + at 37°

purify as 30°

Temperature mutants.

May 4, 1948.

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

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

Gen. 6 P4.
1st. reading 9A5 = 15h.

9A6 = 39h.

9A7 = 63h.

All readings identical.

do.

TO

[Note ^{reads} weakness of 58-161 on maltose]

May 5, 1948.

W-340 and W-382 inoculated into BCP broth tubes at indicated temperatures:

30° Plus on glucose, lactose and maltose in 12 hours.
and galactose

32° Ditto. Inocula from gaa brath .2 ml

33-34° Ditto.

5P 5. Inoculate W-340, W-382, 58-161, W-108 as above.

	9A6 16h.	glu	lac	mal	gal
340		- $\frac{+}{-}$	+++	- $\frac{+}{-}$	+++
382		- $\frac{+}{-}$	+++	- $\frac{+}{-}$	+++
108		- $\frac{+}{-}$	- $\frac{+}{-}$	- $\frac{+}{-}$	+++
58-161	9A6	+++	+++	+++	+++

*Temperature fluctuates
between 35 and 36. This
may account for slow
development of 382 Maltose,
etc.*

- 1P6 .∴ At 36°, W-382 is lac + glu -

~~9A7~~ ~~W-382~~

May 6, 1948.

Harvest cells of W-257 from overnight cultures of YP-broth. 50 ml. / 3 ml suspensions.

A)- maltose 1% B)- gluconate 1%

To 1ml 5% substrate, add 1 ml cells and 1 ml. .01 M Phosphate buffer plus BCP indicator. Incubate at 36°. Set up 11:15 A6.

	glucose	maltose	gluconic
A.	— — — —	± +++ — —	— — + —
B	— — — — ±	— — — — —	+++ +++ — —

To 1 ml. B cells add 1cc gluconate and .5 ml 1% triphenyl-tetrazolium hydrochloride.

very deep red by 15 mins.

Cytological Study:

- 1. 15 mins (11.30)
- 2. 45 mins (12.11)
- 3. 120 mins 1:15 PM
- 4. 3:30
6 PM. —

9A7. All tubes were +++

Glucose "adaptation"

1929.

Grow Y10, W382 in gna 42 broth. ^{at 34°} Collect cells in 2ml and test at 34° on glucose and glucanin. Set up 11 AM.

Y10. #	Glucose	Gna. W382	Glucose	Gna.
11 AM.	-	-	-	-
1115	-	+++	+++	+++
1130	-	✓	-	✓

Temperature mutants - other hexoses.

193

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

	33°				40°			
	Mannose	Mannitol	Fructose	Sorbitol	Mannose	Mannitol	Fructose	Sorbitol
340	+++ ✓	±	---	+++ ✓	- ✓	- ✓	- ✓	- ✓
382	+++ ✓	± ✓	+++ ✓	- ✓	- ✓	- ✓	- ✓	- ✓
58-161	+++ ✓	+++ ✓	++ ✓	+++	+++ ✓	+++ ✓	+++ ✓	± +

∴ Sorbitol may be a fermentable carbon source for these mutants.

= 9A7
= 23017

May 7, 1948.

Harvest K-12 from 16 hour cultures of YP sugar broth:

a) arabinose b) galactose c) glucose. 50 ml broth, 4% suspension
10:45 AM (A7).

	substrate		
	arabinose	galactose	glucose
a	+++ ✓	- + +++	+++ ✓
b	- + ++	+++ +++	+++ ✓
c	---	---	+ 11 AM +++ 12:15

cells

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

See 100⁹⁷. [Adaptation in presence of azide] Arabinose x galactose + Cohen's letter with Y10.

l-arabinose and d-galactose adapted cells have reciprocally shortened adaptation times. The interconversion is not inhibited by azide.

May 7, 1948.

Prepare 8 ml cell suspensions from 50 ml. YP broth cultures (YZ-sugar)

Cells: A: no sugar, B-glucose C- galactose D- lactose.

Substrates: 1 glucose, 2-galactose 3- lactose.

or at 40°

~~After~~ After harvesting, incubate cells without substrate or buffer at 33-34° for two hours. Then (1:30 P 7) add 1 ml 5% sugar and buffer-BCP

		34°				40°			
		A	B ^{glu}	C ^{gal}	D ^{lac}	A	B	C	D
glu	1	-	+++	±	+++	-	-	+	+++
gal	2	-	-	+++	+++	-	-	+++	+++
lac	3	-	-	-	+++	-	-	-	-

W-340 Exactly as above.

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

		34°			40°		
		A ^{glu}	B ^{gal}	C ^{lac}	A ^{glu}	B ^{gal}	C ^{lac}
glu	1	+++	+++	+++	-	+	+++
gal	2	-	+++	+++	-	+++	+++
lac	3	-	-	+++	-	-	±

Concl Glucosylase is adaptive at 34°, but is produced during galactose adaptation.

① 2PM. (20-30min). 2:00-1 hr. 3:00-2 hr.

[at 34° hold for 1 hr before glucose added]

Tested for stability at 40°.

W382. + W340

gave identical results.

Cells grown on ↓	Glucose	Galactose	lactose
Glucose	—	—	—
Galactose	+++	+++	—
lactose.	+++	+++	—

at 34°

- ① Glucosylase in glucose adapted cells is unstable at 40° in absence of substrate, but in galactose and lactose adapted cells is stable.
- ② Glucosylase is adaptive at 34°.
- ③ lactase is unstable at 40°.

Suggested.

[Compare enzymes from Y10 and W-382 under otherwise comparable conditions. I.]

[Does substrate protect stability? I.]

Stability of adaptive enzymes in absence of substrate at 40°

May 8, 1948.

Grow Y-10 and W-382 in 50 ml. batches YZ-sugar broth at 34°.

- A. Glucose (2 flasks each)
- B. Lactose (2 each)
- C. Gluconic (1 each).

Dispense 1 ml. volumes to tubes with 1 ml indicator buffer (with and without azide) at 40°
At stated times add 1 ml. substrate and record time required to ferment.

Cells: A,B,C. Substrate: a,b Azide +, -

Time subst. added: (minutes)

	Aa+	Aa-	Ab+	Ab-	Ba+	Ba-	Bb+	Bb-
0	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅
30	t ₄₅ t ₆₀ t ₄₅							
60	t ₇₅ 90 75							
120	100 < 110	100 < 110	100 < 110	100 < 110	100 < 110	100 < 110	100 < 110	100 < 110

Y-10 cells.

W382 cells.

	Aa+	Aa-	Ab+	Ab-	Ba+	Ba-	Bb+	Bb-
0	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅	t ₁₅ t ₃₀ t ₄₅ t ₃₀ t ₁₅
30	-45 -45	-45 -45	-45 -45	-45 -45	-45 -45	-45 -45	-45 -45	-45 -45
60	-75 -75	-75 -75	-75 -75	-75 -75	-75 -75	-75 -75	-75 -75	-75 -75
120								

Y-10 cells 9:20 AM - 11:45

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

= + + + T substrate

Time Required to ferment:

196.

Cells disseminated at 40° for minutes indicated before addition of substrate.

	Aa +	Aa -	Ab +	Ab -	Ba +	Ba -	Bb +	Bb -
0	45	15			30	30	45	30
30	30	15			45	30	45	30
60	30	15			30	30	30	30
120	40	<40			40	<40	40+	40
0	>120	>120			60	30	(45-120)	30
30					60	30	45-120	30
60					60	30	45-120	30
120						<40	45-120	45

W-387.



W-382.

cf. 195.

Needed control on activity of W-382 glucose-glucosylase at 34°!

W-382 glucosylase in glucose adapted cells is very unstable compared to the corresponding ~~W~~ 410 cells or to glucosylase in lactose adapted cells of W-382. Aside does not prevent this instability.

No indication this time of lactase instability.

Checks on possible temperature-sensitive Lac- 197

May 15, 1948.

Proc Lac-N222cc. BCP fermentation tubes empty from st. slants of:

	30°			5P15 37.5°			40°		
W-42	-	-	-	-	-	-	-	-	-
W-110	-	-	-	++	+++	+++	++	+++	+++
W-305	±	+	++	±	±	+++	-	±	++
Y-10.	++	+++	+++	++	++	+++	+++	+++	+++

① N16. ~~11h~~ = 19 hours.

② 7P16 = 25h.

③ 9A17 = 39h.

W-42 is not temperature-responsive.

W-110 is - at 30, + above 37.

W-305. is about equally slow at all temperatures compared to Y-10, perhaps slower at 40° than at 37.

Coli lactase

to 50ml 1/2 Lac broth, cells harvested in 10ml H₂O. successive 10 fold dilutions in 10 ml 1/50 citrate buffer pH 7.5 at 37°, ONPG 14/5000. 10 min. incubate 10 min, then boil.

① Preliminary tests:

cc cells.	initial absorption: density				Final density.		corr. Δ	%lysis.
	λ=420	λ=650	Δ ₄₂₀	Correction:	λ 420	λ 650		
1	.51	.34	.41	.61	.92	.41	.31	ca 50
.1	.065	.049	.08	.071	.145	.054	.074	ca 10
.01	.009	.008	.027	.018	.036	.010	.025	< 5
.001	.004	.004	.023		.027	—	.023	< 5

$$\text{Correction} = \frac{\lambda_{650}^{420}}{\lambda_{650}^{650}} \cdot \lambda_i$$

② Use ~~1 ml cells~~. Vary substrate ~~concn~~. 10 min tests & boiling. Range .1 - 1.0 seems to be satisfactory. Boiling should be omitted as it causes some 2-3% hydrolysis.

cc cells.	λ ₄₂₀	λ ₆₅₀	λ ₄₂₀	λ ₆₅₀	λ _{CORR}	Δ
1	.066	.041	.140	.038	.060	.080
.2	.127	.087	.276	.073	.115	.161
.3						
.4	.250	.181	.520	.142	.225	.295
.5						
.6	.370	.280	.740	.209	.315	.425
.8	.450	.360	.930	.270	.405	.53
1.0	.540	.470	1.05	.339	.486	.56

after 1 hr

.4

.690

.143

.265

hr

.750

.525

ONP. CT.

~~Mx 10~~
59000

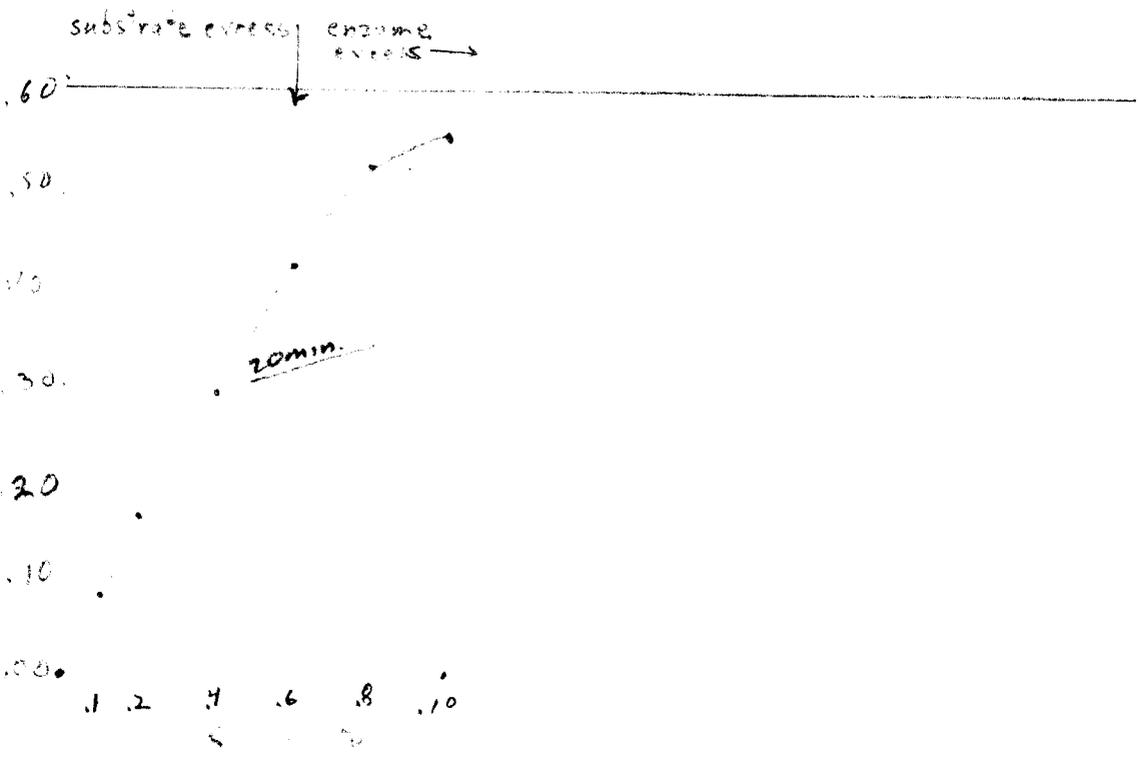
Citrate buffer pH 7.5 M/50.
uplicates.

$\lambda = 420.$

C	D.
1	.070
1	.065
2	.140
2	.132
4	.270
4	.272
6 +	.409
6	.394
8	.515
8	.511
10	.614
10	.619

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

10 mins in NPE system.



12/10

Inhibition by maltose.

Plate 1

1	.032	0
2	.032	0
3	.080	.019
4	.062	0
5	.290	.015

Blank

1	.249	.161
---	------	------

M/10

Cells .5ml + 9ml sugar solutions + 1ml ONPG All in 4/50 buffer.

1. Lac no ONPG
2. Lac ONPG
3. Glu "
4. Mal "
5. -- "

20 min readings at 37°.

1 is blank.

Note inhibition by maltose and glucose

	D410	D650
2	.032	0
3	.080	.019
4	.062	0
5	.290	.015
blank 1	.249	.161

Repeat using Sucrose + Maltose.

0	.241	.014
Suc	.239	.010
Mal	.083	.004

Note inhibition by maltose but not by sucrose