Assays on fractionation.

Use ≐ 1 ml. X or Y + 1 ml .5% lactose. Incubate 30 mins. 37°. Then add 4 ml Cu K Sodium. Boil 10 mins. Wash ppt + dissolve in Fe^{+3} and titrate with .02N $KMnO_4$.

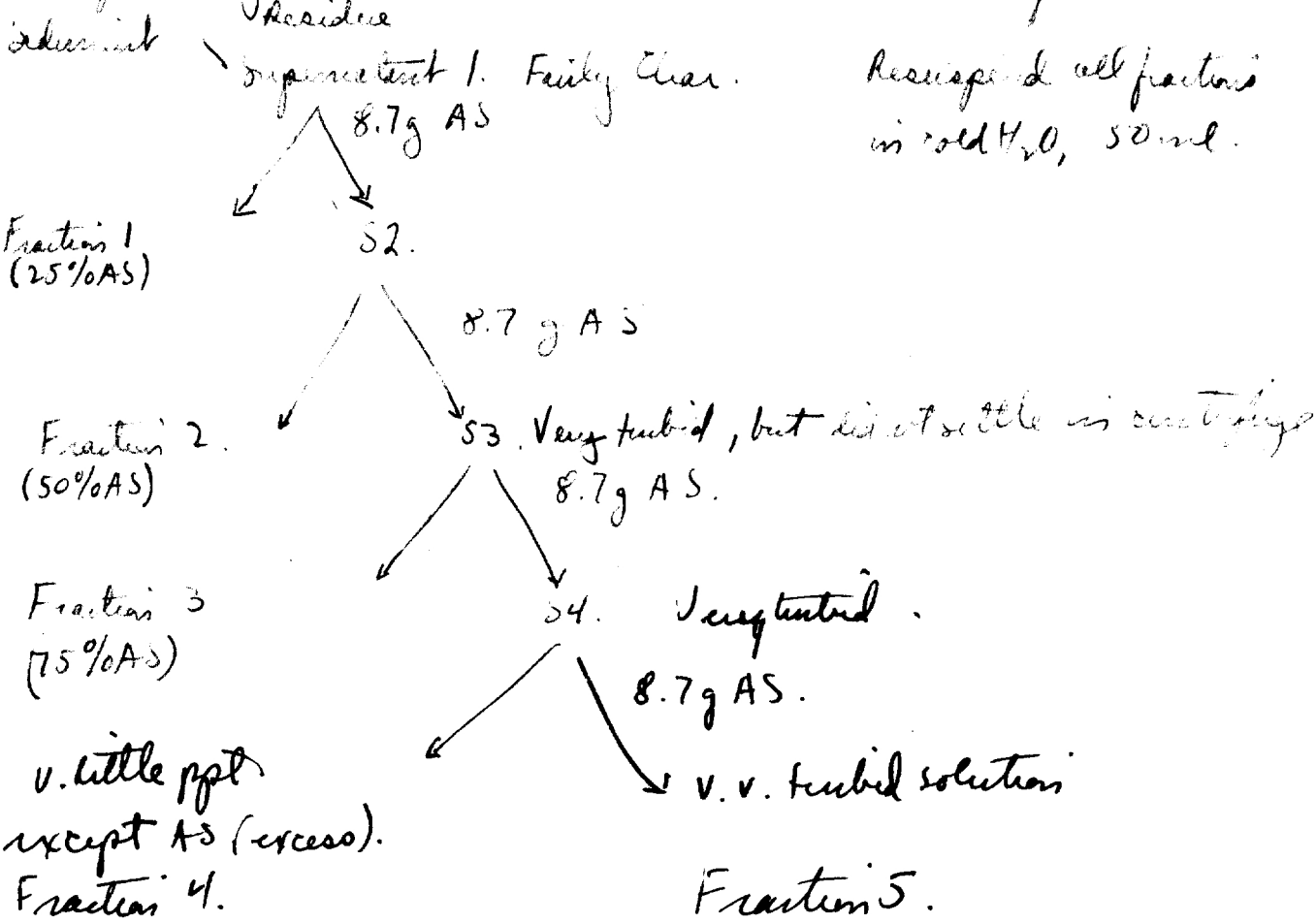
			cc:	
1. X	+++	7.4 +	8	
.5 X	+++	8.19	8	
.1 X	++	4.83	5	Note. ca 3 mg/½ hr.
.01 X	–	.40	.3	
Y. [illegible]	++.	5.84	8	
1.	+++	8.42	8	Acetone
2.	++	7.20	7	(AS)
3. [illegible]	++	3.10	6	(Alcohol)
5. [illegible]	+...	2.67	7	Acetone
6.	–			
7.	–			
8.	–			
9.	–			
10.	–			
Glucose	++	8.98	—	
X + Glucose	+++	8.39	—	Utilization ??
Lactose.	–	0.13	Blanks	

Cu_2O color + ppt roughest.

1. Autolysate active
2. Acetone powder active Alcohol powder active
3. Comes down at ½ saturation. Dry Surf.

Fractionation of W-254 lactase.

Suspend 1g. Acetone Powder 160 in 50 ml. cold H_2O for 24 hours.



Assay: 1 ml .05 ml

1. Acetone Residue
2. Fraction 1 (¼ satn.) sl. opalescent
3. F 2 (½ sat.) clear
4. F3 (¾ sat.) clear
5. F 4 (sat.) clear
6. F5 Residue after AS sat. v. opalescent.

Assay with ½% lactose, ½ hour 37°.

2/20	1.30 – 2.41		1.11
2/1.	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.

d Thees, 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; Insoluble ppt. (Particulate??)

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

a) 9 ml [illegible] + 1 ml [illegible]

b) .9 ml [illegible] 1 ml Ac.

1/2 dilutions:

Brit., ml.	P	S.	
1 ml	0.50	5.17	Enzyme in soluble fractions after AS ppts.
1/2	0.31	3.63	
1/4	—	2.03	
1/8	—		
1/16	—		
1/32	—	.030	Activity is much less than required conditions too close to substrate exhaustion.
1/64	—		
1/128	—		

Blank

When fraction B is ppted. ē AS 50%, three fractions are obtained.

C 1) Supernatant — Cu_2O

C 2) Sedimentable residue after resuspension in H_2O — v. sl. visible Cu_2O

C 3) Non-sedimentable residue. — Cu_2O.

Assay 1/4 ml samples (in 50ml =°) & compare with ~~whole enzyme~~ B. (2.03 ml)

40° may be too low!

Preparation of Lactase : Batch 2.

Grow K-12 in 12 l. N-case 1% Lactose, 30° under strong aeration. After 24h. Harvest in Sharples (Watson).

Fraction 1. 31 g. paste – Add 100 ml H_2O, 5 ml toluene, mix in blender + ~~autolyze~~ autolyze at 37° # 11A 26 –

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

3 Suspend ~~10~~ 5 g. powder in ~~100~~ 50 ml H_2O to extract.

Assay (as in 161 b) .1 ml suspension (20 min, 40°).
3.88 ml .02 N $KMnO_4$.

Extract with cold H_2O 8h. Centrifuge at 40000 rpm 1 hr.

4 Add 17.5 g AS (½ sat.) → small ppt. Redissolve in H_2O. A
supernatant. B.

Test .1 ml samples of each:

162-4A Nonsible Cu_2O
162-4B. " "

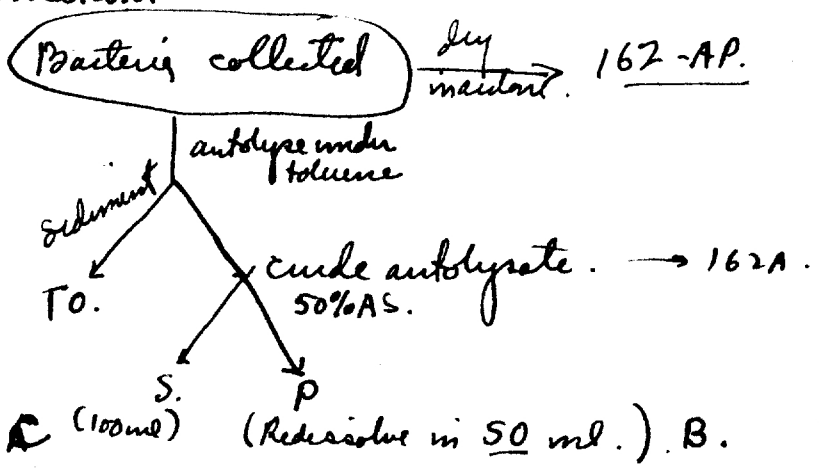
40° may be too high for assay.

No activity!

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

Rep 20 ml sample Work with the other 100 ml.

Add 35g. AS. Collect ppt. + redissolve in 50 ml H_2O_2 Fairly clear solution. Pigment is left in supernatant.



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

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

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

No Activity.

Lactase Preparation.

March 29, 1948.

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

Aerate, 37°. 24 h. (Allbutex antifoam). Collect in Sharples.

Bottle A lost. Collect 53 g. cell paste from B. [Drop A, B names?]

A]. 10 g. put in 100 ml. NaCl-citrate + 1 ml. toluene

B]. 43 g. put in 100 ml 5% lactose in citrate buffer. 1½ h. Then wash, autolyze under 1% toluene.

Collect after 24 h. Store 1P31 in refrigerator.

B. became ~~very~~ opaque ~~cloudy~~ on standing in refrigerator overnight On warming this material redissolved. Kept 10 ml as crude cell paste = 163B1; add 14 g. Am. sulf. to remainder + cyanide fractions.

ppt. Redissolved in citrate 163-B2

sup. 163-B3 - from ppt in cold!

Assay 1 x 1 ml enz. + 1 ml 1% lactose, 30 mins. 37°

		Cu_2O.
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. In future, add fresh lactose to whole medium before centrifuging.

to B2, add 14g Ammsulf. Redissolve ppt in H_2O.

Temperature mutants.

March 29, 1948.

85 plates, Y10, 5 secs. Hanovia U.V. ca. EMB Lac
Incubate at 45° 11A 29 - x ca. 250 ~~plates~~ colonies.
= 20,100 tests.

Recovered W-340

Test at 45°.

Apr. 1, 1948 + 25 plates, x200 = 5000. = 25000 total.

Test W-340 at 36° and 44°.

	36	44
Glucose	+ slow*	−
Galactose	++	++
Gluconic	++	++
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 glucozymase.

April 6, 1948. Asabone. 100 plates x 300 = 30,100
No detected mutants at 45°

Temperature mutant W-340

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

Cells Harvested from 100ml Gna 37 / 6ml H_2O. = 2

37 = A 45 = B.

Cells from YP Lac = 1. (50ml into 2ml H_2O).

Test at 37 + at 45.

Set up 11:35 AM. Apr. 5.

		37 = α	45 = β.
11.	1 / Lac	+ ++++	± +++
12.	2A / Gna.	++++	++++
13.	2B / Gna	+++	++++
14.	2A / Lac	−	−
15.	2A / Lac	−	−
16	2B / Lac	−	−
17.	2B / Lac.	−	−

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

13β " +++ in 8 minutes.

15 MINS.

30 mins.

No further adaptation in next 6 hours.

Lactase production; K-12, lactose synthetic medium

Apr. 9, 1948.

Inoc. ~~8ex~~ 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 M/10 ph-saline + 2 ml toluene + autolyze at 37° Sat. int and collect supernatant 10A12. Cool in ice water. 150 cc. total.
Save 20 ml. whole autolysate. To remainder (cold), add 45 gms AS. + ppt. During centrifugation, about 2/3 of this material was involved in an accident. The glass was removed + the supnt. recovered. The cup + broken glass were washed with 100 ml H_2O, then 35 g. AS added. The ppts collected here were pooled and redissolved in 50 ml. H_2O. (A) Proceed with. sedimentation of remaining 1/3, dissolve ppt. in 50 ml H_2O (B).
Assay!

clear, yellow.

What is green yellow pigment?

Pneumatic [illegible]
glandular activity

				m.
AO.	0,00	0.01	-0.01	0,00
OB.	1.34	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

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 5% lactose
1 ml cells
.5 ml conc. BCP indicator + .1 ml Phosphate Buffer 7.1/10
± 1 mg valine ± 1 mg isoleucine ± 1 mg hydroxy aspartic* ± 1 mg aspartic*

	grams L.		grams G.	
1.	−	+++	−	+++
2. I.L.	+++	+++	−	+++
3. V.	+++	+++	−	+++
4. V+IL	+++	+++	−	+++.
5.* Asp.	−	± *overneutralized?	−	±*
6.* HOAs.	−	++	−	−
7.* Asp+HOAs.	+++	+++	−	+++

* overneutralized c NaOH

− 30 m. 3:30.
− 3 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

* + 5γ pantothenate.

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 ml.) ml N-Z case - PO_4 - glucose broth eluted in 10 ml. Each tube contains: Set up 11:30 A.M.

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

2 was +++ in <10 mins.

1. —
2. (Glucose 5%)
3. + glucose .5%
4 Sod Succ. 1%
5 N-Z case .1%
6 TLB,
7 $MgSO_4$ .1%
8 valine } 1 mg/ml .5 ml.
9 isoleucine }
10 V + il.

The temperature mutants W-340 and W-382.

May 3, 1948.

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

		glucose	lactose	maltose	[illegible]	gluconic
W-340	30°	++ ++	− +±	+ ++	−	++±
	45°	−	−	−	−	[illegible]
W-382	30°	++±	++	+++	−	+++
	37°	−	+	− −	− −	++++
	45°	−	−	−	−	++

Broc 5P3.
Fruit Reading 8A4 = 15h. These are both temperature mutants.

W-340 inoculum taken from old stock.

From first test of W-382 on maltose, papillae picked and streaked out.
Mal+ colonies tested on EMB at 37.5°

Lactose	19+	0−
Glucose	13+	1− / uncertain or mixed.

Purify 1+ and 1− on maltose.

May 4, 1948.

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

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

Incr. 6 P4.
1st. reading 9A5. = 154.
9A6 = 396. All readings identical.
9A7 = 636. do. TO

[Note variation weakness of 58-161 on maltose]

Critical temperature: Temperature mutants.

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		— ±	+++	— ±	+++
382		— ±	+++	— ±	+++
108		—	—	—	+++
58-161		+++	+++	+++	+++

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

-196 ∴ At 36°, W-382 is lac + Glu —

~~9A7 Maltose~~

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 dry 15 mins. Cytological Study:

1. 15 mins (11.30) 9A7. All tubes were +++
2. [illegible] (12 M)
3. 120 1:15 PM
4. 3:30
6 PM. —

Glucose "adaptation" 1929.

Grow Y10, W382 in gma Y2 broth at 34°. Collect cells in 2ml and test at 34° on glucose and glucanic. Set up 11 AM.

	Glucose	Gma.		Glucose	Gma.
Y10. 11 AM.	—	—	W382	—	—
1115	—	+++		+++	+++
1130	—	✓		—	✓

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

	33°				40°			
	Mannose	Mannitol	Fructose	Sorbitol	Mannose	Mannitol	Fructose	Sorbitol
340	+++ ✓	± ~~[illegible]~~	++ ✓	− [illegible]	− ✓	− ✓	− ✓	− ✓
382	+++ ✓	+± ✓	+++ ✓	− ✓	− ✓	− ✓	− ✓	− ✓
58-161	+++ ✓	+++ ✓	++ ✓	+++ +	+++ ✓	+++ ✓	+++ ✓	± +

∴ Sorbitol [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] [illegible] may affect [illegible] mannitol.

— 9A7
— [illegible]17

Galactose - Arabinose X - adaptation.

May 8, 1948.

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

a) arabinose b) galactose c) glucose. 50 ml broth / 4 cc suspension

10:45 AM. (AT).

	substrate c arabinose	substrate b galactose	substrate c glucose
a	++	− + ++	+++
b	− + ++	+++ ++	+++
c	− − −	− − −	+ ~~+++~~

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

See 97, 100. [Adaptation in presence of azide] Arabinose × galactose with Y10. + Cohen's letter

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 harvesting, icubate 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° A	B	C	D	40° 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.

		A	B	C	A	B	C
substrate glu	1	+++	+	++	−	+	+++
gal	2	−	±	++	−	+++	+++
lac	3	−	−	++	−	−	± later +

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

Ø. 2PM. (20-30 minis). 2:30 = 1 hour. 3:30 2 hr.

[As 34° [illegible] for effect on glucose [illegible]

Summary of ~~#~~ p. 195

Tested for stability at 40°.

W382. + W 340 gave identical results.

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

at 34°

① Glucozymase in glucose adapted cells is unstable at 40° in absence of substrate, but in galactose and lactose adapted cells is stable.

② Glucozymase is adaptive at 34°.

③ Lactase is unstable at 40°.

Suggested.

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

[Does substrate protect stability?]

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 substrate added (minutes).	Aa +	Aa −	Ab +	Ab −	Ba +	Ba −	Bb +	Bb −
Y-10 cells. 0	+15	++30 45	++15	+++30	++15	++30 30	+15	++30 45 +++15 30
30	+45	++60 45			++45	++60 75	++45	++60 75 ++45 60
60	+75	90 75			+75	90	++75	90 ++75 90
120		160					++160	160
W382 cells. 0	±15	±30 +75 ±120	−15	−30 +75 ±120	−	−	+15	++30 45 60 +15 30
30	−45	−45					+45	++60 90 +45 60
60	−75	−75					+75	++90 120 ±75 90
120							++160	160 −160 ++160

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

Time Required to ferment:

Cells dissimulated 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-382.

W-382.

Cf. 195.

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

W-382 glucozymase in glucose adapted cells is very unstable compared to the corresponding ~~bb~~ Y10 cells or to glucozymase in lactose adapted cells of W 382. Azide does not prevent this instability.

No indication at this time of lactase instability

Check on possible temperature-sensitive Lac-

May 15, 1918.

Anac Lac-N2 acc. BCP fermentation tubes amply from 3d. slants of:

5P15

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

① N16. ~~AA~~ = 19 hours.

② 7P16 = 25h.

③ 9H17 = 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 50 ml ½ Lac broth, cells harvested in 10 ml H_2O. Successive 10 fold dilutions in 10 ml M/50 citrate buffer pH 7.5 at 37°, ONPG M/5000. 10 H_{1H2} Incubate 10 mins, then boil.

(1) ~~Two~~ Preliminary tests:

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

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

(2) ~~Use .2 ml cells~~. ~~Vary substrate conc~~. 10 mins tests 5 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
0						
~~.1~~	.066	.0411	.140	.038	.060	.080
.2	.127	.084	.276	.073	.115	.161
~~.3~~						
.4	.250	.[illegible]	.520	.112	.225	.295
~~.8~~						
.6	.[illegible]	.2[illegible]	.740	.109	.315	.425
.8	.450	.[illegible]	.935	.278	.405	.53
1.0	.540	.270	1.05	.339	.486	.56

after 1 hr .14 .690 .143 .465

2 hr .750 .525

ONP. CT.

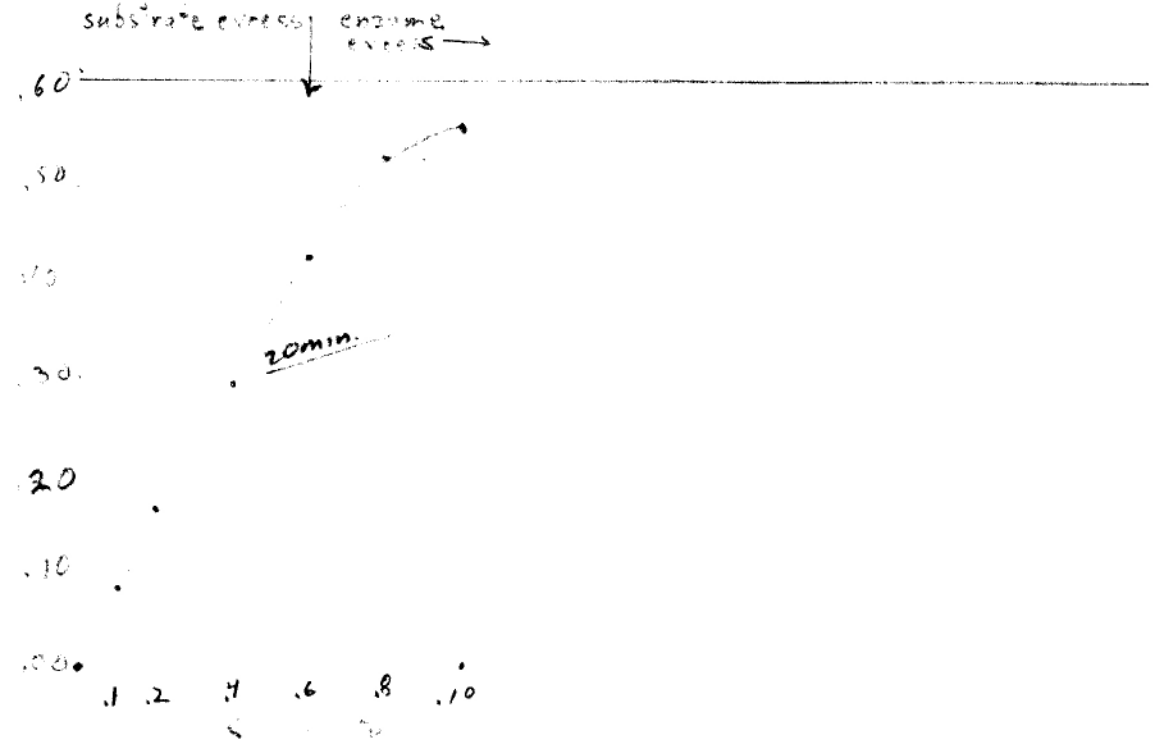
Mx ~~10~~ / 59000 Citrate buffer pH 7.5 M/50. $\lambda = 420$.

replicates.

	C	D.
A.	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 NPO system.


substrate excess
enzyme excess
.60
.50
.40
.30
20
.10
.05
.1 .2 .4 .6 .8 .10
20min.

Inhibition by maltose.

2	.032	—
3	[illegible]	.019
4	.062	—
5	.290	.015

Cell Heule

1	.249	.161

M/10

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

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

20 min readings at 37°.

1 is Heule.

	D_{420}	D_{650}
2	.032	0
3	.080	.019
4	.062	0
5	.290	.015
Heule 1	.249	.161

Note inhibition by maltose and glucose

Repeat using Sucrose + Maltose.

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

Note inhibition by maltose but not by sucrose