Cross-Adaptation Experiment.

January 31, 1948.

Grow cells of Y10 in 50 ml:

YP+ ↓ Tested on:	glucose a	galactose b	glucose + galactose c	lactose d	
Inoculum					
A glucose 1%	+++	− ±	+++ ✓	−	−
B galactose 1%	+++	+++	+++ ✓	(±)	(++)
C glu + gal 1%	+++	++±	+++ ✓	(−)	−
D. lactose 1%.	+++	(++±)	+++ ✓	+++ ✓	

Harvest, conc. to 5 ml and
Test in corresponding substrates
in azide buffer.

2 hours. 1/2

Noting that lactose-adapted cells are also galactose-adapted, but galactose-adapted are not lactose adapted.. Galactose is probably an intermediate in lactose utilization.

of azide (M/200). ~~Adaptation~~ is not completely inhibited by this concentration, use (M/100) in future.

Feb. 11, 1948.

Harvest 2 batches (A.B) of W-108 grown in 50 ml. 1% YP-gluconate broth overnight. Test sample for genetic purity.

A. (10 AM) Conc. to 12 ml. Use 1 ml cells per tube, with ½ ml. 10% sugar and phosphate-indicator. (No azide!)

	gna	gna/gl	gl	gal	gal/gl	Bu-gal.	Bugal/glu	Aa
11 AM	+++	+++	++	—	—	—	—	—
12 N	+++	+++	++	—	—	—	—	—

must be minor y. below

Aa: 4 ml. cells / 1 ml. gal. / ½ ml. phosphate-indicator for adaptation, to galactose.

B. 11 AM As above. Conc to 10 ml. 1 ml. cells/tube

	glu	glu	gna	gna-glu	gal	gal	gnagal	glgal	Megal.
11:15	—	—	±	±	—	—	±	—	—
12 N	—	—	+++	+++	—	—	+++	—	—
1:30	—	—	✓	✓	—	—	✓	—	—

① glucose does not inhibit gluconate dissimilation.

C. Cells Aa. Wash and test as:

1:30 PM.	Gna	Glu	Gal	Xyl	Xyl + Gl	Arab.
4:30	—	—				

W-108 C-source utilization. 118x

February 13, 1948.

Harvest from 100 ml gluconate broth. Conc. to 7 ml. Use ½ ml/tube. contg. ½ ml 10% sugar, 1 ml buffer-indicator solns. ± ½ ml H_2O.
Set up 9:45 AM. Inc 37°

	Glu	Glu/ml	Dalac	Glu+Gal	Ara	Ara+Glu	Xyl	Xyl+Glu	Gna.
10:20	−	−	−	−	−	−	−	−	+++
11:30	+	+	−	±	−	±	−	+	✓ *
12:30	+	+±	±	+±	+	++	−	+	−
2 PM	+ *	+±	±	++±	+++	+++	−	++	✓
5 PM	+++	+++	+++	+++	+++	+++	+±±	+++	+++.
	1:100	1:100		all −					all −

Me Gal	Gal + Me Gal	Glu + Me Gal.
−	−	−
−	−	−
−	−	±
−	±	+±
+±	+++	+++

(all −).

Streaked out on Glucose plates: —

March 15-16, 1948.

Grow Y-10 & W-254 into YP 1% Lactose, 2x50 ml. each.
Y-10 & W-327 into YP 1% Maltose, do.

Harvest each, and concentrate in 10 ml volumes in sugar .5%, phosphate M/100. At same time set up no-cells blanks. To 1 ml test samples.

Incubate at 37° 9A-1P 16. Add 4 ml. Barfoed's reagent to clarify. Boil supernatants 10 mins. Cool. Add 1 drop dil. Aerosol OT to wet Cu_2O ppt, and sediment and wash in H_2O. Take up sediment in acid ferric solution and titrate against .0200 N permanganate.

1. Y-10	Lac		0.10
2.Y-10	Mal		0.10
3.327	Mal		0.30
4.254	Lac		4.24
5.---	Glu		9.40
6.----	Mal	0.98	0.98
7.----Lac			0.28

There is therefore an almost equimolar accumulation of monose by 254, but none by 327 on lactose and maltose respectively.

The blanks contain 5 mg. sugar each. Note approximately 10% recovery pf maltose, but negligible recovery of lactose.

Keep remainders of suspensions 1 and 4 for further characterization of the accumulated material.

Take 1 ml Exp. suspensions & controls of same carb. comp.

Clarify by 5 ml Cu solution, ppt., and boil supernatant 10 min.

Sediment Cu_2O ppt., wash + dissolve in ac. Ferric sulf.

Titrate vs. N/100 $KMnO_4$.

1. Glucose + Phosphate	22.60 - 12.71	
2. Maltose + Phosphate	23.55 - 22.60	
3. Y10 culture	2.3.55 - 1 drop.	No glucose.
4. W327	23.69 - 23.91.	< maltose control.
5. - Phosphate.	23.91 - < 1 drop.	

Fractionation of Coli Lactase

March 20-22, 1948.

X. Ca 20 g. ~~to~~ Sharples paste W-254 ground with Pyrex. Extract overnight in cold with NaCl .9%. Sediment. + dilute to ca. 100 ml

3/24/48. Test extract on lactose c̄ Barfoed's method:

1 ml extract, 1 ml 5% lactose + make up to 3 ml.
Incubate 3h. at 37°. # cc .01N $KMnO_4$ to equal Cu_2O pptd.

XL	>17 cc. (Beyond method)
X	0.23 cc
L	1.18 cc
X+L (added just before Cu pptn.)	2.34 cc.

V. High activity thus indicated

Y. Ca 10 g. Autolyze 48 h. 37° under toluene. Remove toluene + clarify. Make up to ca. 50 cc. Appreciable yellow color, deeper than X.

include lactose??

Pool Autolysate + Extract. Add 4 vols. Acetone & Collect Sediment. Wash in anh. Acetone. Dry. → 1.6 gms. Acetone Powder.

3/22: Work in cold.

① 2 ml X + 8 ml acetone. Collect ppt + resuspend in 2 ml

③ Do. ⊂ 95% alcohol.

② 5 ml X + 1.8 g Am Sulf. (AS) Collect ppt. Supernatant ↓
Heavy ppt. and resuspended.

④

⑤ 5 ml Y as ② ↑.
Heavy ppt

⑥ See 2S. Add .9 g AS. Collect ppt & resuspend. S ↓
Moderate ppt. leaves v. opalescent solution.

⑦ See 5S. Do. ↓
leaves clear solution.

⑧ See 6S. Add .9 g AS (to saturation + drops H_2O) No ppt. But v. opalescent solution.

⑨ See 5S. Do. Collect + resuspend ppt.

⑩ Supernatant of 9.