Inhibition of galactosidase by carbohydrates.

Sept. 15, 1948

M/50 citrate buffer 7.5 M/200 ONPG
20 m. 37° M/10 (ca.) Sugars

		% inhibition	
K-12	0	0	Control
		− 80	Maltose
		− 83	Galactose
		+ 15	Gluconate
		76	d-Cellobiose
		85	l Arabinose
		55	d Xylose
W211		—	
		87	Maltose
W33		—	
		82	Maltose
Y10		—	
		85	Maltose

PM.	0	.282	—
	glucose	.058	79
	fructose	.133	53
	mannose	.251	11
	raffinose	.000	100
	trehalose	.269	5
	dulcitol	.060	79
	sorbitol	.199	29
	melibiose	.000	100

Qualitative inhibition tests.

Sept. 15, 1948.

Due to paucity of material, the following tests were done in 1.0 ml volumes. 100 μM was dissolved in .9 ml bacterial suspension in buffer as above, then .1 ml M/100 ONPG was added after temp. equilibrium. Color read as + or − :

K-12

	Color	
—	+	
Maltose	−	
Galactosan	+	
Lactitol	−	6±
d-arabinose	+	
Ca Lactobionate	+	
Ca Maltobionate	±	Original color makes this reading doubtful

Adaptation Expt: Preliminary

3502

Sept 17, 1948

		λ420 vs —	λ650 vs —	λ650 vs water	Y20 H_2O	3 hour exposure opt. color
L	—			.121	.192	
glucose	1	[illegible]	.015			+
"	2	.100	.01[illegible]			+
lactose	3	[illegible]	.014			+
"	4	[illegible]	.019			+
Lac + Azide M/100	5	[illegible]	[illegible]			+
"	6	[illegible]	[illegible]			+
Glu + Az	7	[illegible]	[illegible]			+
"	8	[illegible]	[illegible]			+
water	9	[illegible]	[illegible]			+
G	—			.159	.246	
glu	1	[illegible]	[illegible]			—
"	2	.046	[illegible]			—
lac	3	.061	[illegible]			+
"	4	[illegible]	[illegible]			+
lac azide	5	[illegible]	[illegible]			+
lac ATP 5 mg	6	[illegible]	.038			—
" " + azide	7	[illegible]	[illegible]			—
Bz Im M/1000	8	[illegible]	.013			+
SMT M/2000	9	[illegible]	[illegible]			—
water	10	[illegible]	[illegible]			—

Concentrate cells from Y2 Lac (L) and Y2 Glu (G) 5:1

Adaptation system: 2 ml cells: 2 ml 4% sugar in M/5 buffer + 1 ml (supplement if any. Centrifuge once + resuspend in 4 ml H_2O Test c̄ ONPG in M/10 citrate buffer as above., 1 ml: 9 ONPG + buffer.

SMT, Azide apparently inhibit adaptation, benzimidazole does not at this concentration.

Conditions for gal. adaptation

9/18/48.

Conc 100 ml H-12 from Y2 Glu to 20 ml (5:1)

Add 2 ml cells to 2 ml sugars 4% in M/5 buffer pH 7.0. Add H_2O or suppl. to 5 ml volume 1130 A18. Incubate 5 shaking at 37°.

	Sugar	Suppl.
1.	Lactose	—
2	"	—
3	"	Peptone 1%
4	"	Y. Extr. 1%
5	"	Glucose 1 mg
6	Glucose + Galactose ½ ½	
7	—	—
8	Lactose	Hydol. Casein 1%

ONPG as above, but use total volume of 9 ml. rather than 10, and use 8/9 ONPG previously.

Read tubes a) against water suspensions of same cells, and b) the latter against water, all at 420.

	a (activity)	b (cell dens.)	R.A.	% L.
1	.160	.207	.77	100
3	.499	~~.334~~ .279	1.79	233
4	.551	~~.279~~ ~~.334~~ .310	1.78	231
5	.022	.200	.11	14
6	T=101	.230	0	0
<.005 7	.00[illegible]	.200	0	0
8	.519	.334	1.55	202

Y. Extr., Peptone + H.C are definitely stimulatory to adaptation.

N source: Conditions of adaptation

Sept. 20, 1948

System as above (except 2x for anaerobic expts.)
All tubes contain lactose exc. 1.

		Suppl.	Relative activity
1.	—		03
2.	Lac		35
3.	"	Glucose 1mg	07
4.	"	$(NH_4)_2SO_4$ 1ml 1%	32
5.	"	" , glucose	11
6.	"	asparagine	43
7.	"	TL	75
8.	"	4, anaerobic	28
9.	"	5, anaerobic	21
10.	"	4, Vits. .1ml	33
11.	"	2, Am. Ac.	125
12		M.C.	120

	D^i_{420}	D^t_{420}	$D^i \times \frac{230}{251}$	Δ	$\frac{\Delta}{D^i_{corr}}$
1	.251	.237	230	007	03
1r	.230				
2	229	.282	209	073	35
3	236	.231	215	016	07
4	220	265	201	064	32
5	221	225	202	023	11
6	176	230	161	069	43
7	220	351	201	150	75
8	180	.210	164	046	28
9	215	.238	196	042	21
10	213	.260	195	065	33
11	297	610	271	339	125
12	309	620	282	338	120

Sept. 11, 1948.

Effects of amino acids on adaptation.
K-12 harvested from 1/2 Glu as above.
Irradiated supplements ca 1 mg. eq. in 1 ml.

	A	B	C	D	overnight
O	242	224			–
Lac	230	218			–
Megal	246	237			+++
Bugal	319	310			+++
ONPG	240	219			–

A = D420 bact. susp. V = 9 mL
B = D420 + substrate chemic. 10 mL
C = Avg (x .90)
D = B – C = Δ
E = D/C = relative activity

		A	B	C	D	E	% bac ~~inter order~~	
A12	1	224	297	202	95	47	–	69
A3	2	246	370	216	104	48	–	71
A4	3	249	333	224	109	49	–	72
A5	4	273	429	246	173	70 ~~64~~	+	103
A6	5	249	380	224	156	70	+	103
Arcine	6	239	291	215	76	35	–	52
Glutam	7	263	400	237	163	69	+	103
Leuc	8	258	356	232	124	53	–	79
Φalan	9	230	348	207	141	68	+	102
prol	10	258	371	232	139	60	–	90
lys	11	246	366	222	144	65	+	97
arg	12	238	409	214	195	89	+	133
meth	13	231	383	208	175	84	+	125
+Lac	14	251	377	226	151	67	—	100
–Lac	15	230	217	207	10	5	–	7.5
H.C.	16	351	870	316	554	176	++	263
H.C.+Tryp.	17	347	860	312	548	178	++	266
Tr+Leuc	18	263	409	237	172	73	+	109.

only arginine and methionine showed significant stimulatory effect for K-12 adaptation.

Sept. 22, 1948.

5 ml system for adaptation as above. All Lac. K-12.

	A	B	C = A_{cor}	D (B-C)	E $\frac{B-C}{C}$	% of Lac (1)
1. —	228	305	205	100	56	100
2. HC	310	700	279	421	151	
3. AA of HC	296	650	266	384	144	
4. Σ AA	271	520	244	276	118	
5. AA − A12	229	309	206	103	50	
6. " A3	249	479	222	257	116	
7. " A4	259	520	233	187	80	
8. " A5	241	479	217	212	98	
9. " A6	250	460	225	235	104	
10. Arg + Meth.	239	371	215	156	72	130

.2 ml each AA group in 4–9.

.5 ml ca. 10.

.1 ml HC 10% 2.

1 ml ≙ 1% HC 3.

Sept 25-26, 1948.

K-12 grown 24 hours in Synthetic + Lactose 1%, 10 liters.

25g. cell paste recovered. Ca 24g. + 10cc 7.5 PO_4 buffer shaken 24h. under toluene. Remove debris & collect supernatant in

(A). ca 30cc buffer. deep yellow green fluorescence. Ca 1ml/gram bacteria.

(B). Ca 1g. washed c̄ acetone and dried at room temperature. Considerable loss by spattering allows calculation only of final product.

See 316

See 325 for assay.

Sept. 25, 1948.

K-17 grown in 200 ml 1/2 lactose. Harvest to 5 cc. 7.5 buffer & autolyze under toluene & shaking 24h & 48h.

(A) 24h. 1 ml withdrawn, debris sedimented & supernatant diluted to 1/2 ml.

(B). 48h. Remainder (4/5) removed, etc. dilute to 16 ml

each ml corresponds to 10 ml original culture & should have an activity of ca. 10 × bacterial suspensions. (i.e. .05 ml should give ca 100% hydrolysis of 10 ml M/5000 ONPG in 20 minutes). I.E., calculating 2g/liter, corresponds to 20 mg/ml

See 316

Sept. 27, 1948.

K-12 grown 36 hours in 10 liters S(Lac). 9.4 liters of supernatant were removed leaving 31 grams wet Sharples paste. Make up to ca 45 ml c̄ PO_4 buffer pH 7.5 and grind 75 minutes in Booth-Green mill. Combine efflux c̄ washings. An ~~opaque~~ milky opalescent supernant is obtained, in ca. 100 ml, i.e. .31 grams/ml

(A).

(B). 10 ml sample of culture was taken. Resuspend in eq. H_2O + measure turbidity at 1:20 D_{420}.

1:50 dilutions.

1 Unit = Δ of .100 in D_{420}.
for cell free preps.

Assays:	A	B	C	D	Act./ml.		
1	008	290		283	14	315A	.2 ml
2	002	205		205	~~[illegible]~~ 10	B	.2 ml
3	007	260		254	25	314A	.1 ml
4	001	043		042	40		.01 ml
5	010	020		011	90		.001 ml
6	032	1500		1500	150+	316A	.1 ml
7	002	980		980	980		.01
8	000	290		290	2,900		.001
9	360	1900		1600	(445)	316B(cells)	1 ml
10	079	880.		809	(1100.)		.2 ml
ONPG.	012	012.		0	0.		

v. high activity!
for non-enzymatic (none) hydrolysis!

In prep. 316., 1 ml being ≙ culture medium 10 liters/100 ≙ 100 ml cells.

and .001 ml should be equivalent to .1 ml cells, which it is, very nearly. Therefore a large proportion of the cellular activity is present in extracts. Autolysates are nearly as effective with smaller volumes.

Sept 28, 1948.

K-12 grown on 100 ml T(O) glucose A & do. + H.C. (½ ml/100) B shaken 16 hours. Adjust densities:

		D650
(A)	1:10 dilution O	259
(B)	HC.	319

ratio of 1:23 : 1.

Re Suspend ~~Dilute the~~ (O) culture in ~~50~~ 20 ml H_2O; the HC culture in 24.6 ~~61.5~~ ml H_2O to adjust initial densities.

The adaptation system consists of 1 ml cells + 3 ml T(O) 2% lactose + 1 ml supplement. Adapt 3 hours, in duplicate. Resuspend in 4 ml H[illegible] in 8 ml buffer for A., Add 1 ml ONPG 20 mins for B.

		A	B	C	D	E
	1. [illegible] pH 7.0 M/50.	.201	745			
		.196	641			
	2. T(O) Lac	.248	669			
	12	.260	720			
	3. T(O) Lac + .1 ml H.C.	.256	710			
	13	.260	731			
(B	4. Lac buffer	.177	169			
	14	.184	175			
	5. T(O) Lac	.187	171			
	15	.188	170			
	6. T(O) Lac + .1 ml HC	.189	153			
	16	.172	168			

	A.	B.
A. .25/9	.232	.219
B. .25/9	.239	.200

Negligible activity of unadapted culture and of B series.

Sept 28, 1948.

(NZ) W478 x W583 on Lac B_1. 20 colonies streaked on Lac EMB: All ++.

Fractionation of galase 316.

Sept. 28-9, 1948.

Original extract (316) consisted of 2900 u/ml in 100 cc or 2.9×10^5 units all together. To fractionate remove 50 ml and dilute c̄ 50 ml H_2O. (1.5×10^5 units; ~~1.5×10^3~~ 1500/ml). "316" is fraction 0. Add Am Sulf in 4 aliquots of 17.5 g. each in ice bath to give 1/4 sat'd fractions. Take up sediments in 10 ml M/50 PO_4 except for the final fractions.

	Prop. fract.	Act.	Prop. Act.	Assay .01	.001
0	1.00.		1.00.	615	.089
1 (1/4 sat).	5.00			129	019
2 (1/2 sat)	5.00			390	055
3 (3/4 sat)	5.00			194	023
4 (sat.)	10.00			101	015
5 Supernat.	1.00.			060	015.
					.140

Assay at the equivalent of .01 and .001 ml of ~~the original~~ fraction 0. 1 ml M/500 ONPG in M/50 PO_4 buffer.

Enzyme activity is probably not quite linear. Fractions have higher total activity than the original "soup".

Pool fractions ~~1~~, 2 + 3 (40 ml) and add 24 gms AS (3/4 sat). Take up ppt in M/50 citrate buffer, 20 ml 319A

P30. To remaining 50 ml (1.5×10^5 units) add 250 ml cold acetone, let stand, and filter off 330 mg. dry powder. 319B. This should have an activity of about 500 u/mg. Take up 10 mg in 10 ml Phosphate buffer.

Effect of phosphate on lactase

Sept. 29

Lactase preparation 319A is suspended in M/50 citrate buffer. pH 7.5 (Ethylene diamine - citric acid) = (EDC buffer), and should have a potency ca. [100/20] × (.58 + .23) ×10³ u/ml. = 4000 u/ml.

Assay

.001 ml in citrate and in phosphate buffer M/50. pH 7.5.

Triplicate series.

Phosphate seemed to be melted. After 7 mins, use 7 ml EDC + 1 ml Phosph. + 1 ml enzyme + 1 ml ONPG.

1	~~EDC~~ PO_4	.371
2	"	369
3	"	390
11	~~PO_4~~ EDC	012.
12		013
13		012.

ONPM/5000 m

21	EDC	640
22	"	640
31	PO_4	750
32	"	745

41. (7 mins later).
EDC + PO_4. 0

may be due to inhibition by citrate.

Sept 30, 1948.

.5 ml 1 mg/ml. .5 ml 1% HCl

K-12 in A) T(O) B) T(Prol) C) T(AA) 9 ml

8 hals overnight. Resuspend in 5 ml H_2O. Turbidity at 1:100 dilution:

	Dose
A	119
B	119
C	~~052~~
	050

Dilute A and B to 11.9 ml to equalize c̄ c.

Adaptation system: 5 ml. 3 hours 37°. 10^{30} A – 1^{30} P

1 ml cells
3 ml substrate.

① Phosph M, 50 7.5 + 2% Lac
② T. (2%) Lac.
③ ② + ~~supplement~~ proline 1 mg% .2 ml
④ ② + ~~HCl~~ AA. ~~1%~~ 1 ml

		A.	B.
A	1	176	220
	2	259	331
	3	162	218
	4	160	291
B	1	169	215
	2	167	206
	3	186	226
	4	174	272
C	1	150	281
	2	190	310
	3	226	589
	4.	249	778

T(O) cells did not adapt!! T(AA) cells were stimulated by T(O). & further by amino acids.

	Urine A	B	C = .9A	D = B-C	E = D/C	% Lac	Suppl.	
1	25.7	3.68	231	137	59	120	Σ AA	
2	248	3.27	223	106	48	98	AA-A12 + arg	
3	241	3.35	217	118	54	110	" lys	
4	242	3.29	218	111	51	104	" meth	
5	241	3.00	217	83	38	77	" cyst	INHIBITORY!
6	259	4.02	233	169	73	149	AA - arg.	
7	242	3.66	218	152	70	143	- lys	
8	241	3.67	212	145	65	132	- meth	
9	249	3.72	224	148	66	135	- cyst	
10	250	4.27	225	202	54	110	AA-A4 + dal	
11	260	4.10	234	176	75	153	+ tyr	
12	[illegible]	4.51	243	208	85	174	+ thyp	
13	269	4.89	242	247	102	208	AA - dal	dal inhib? thyp stimulatory.
14	211	4.52	244	208	85	173	- tyr	
15	269	4.41	242	199	82	167	- thyp	
16	230	[illegible]	207	102	49	—	0	
17	319	7.75	287	508	177	362	M.C.	
~~18~~								

Activation of Lactase.

Sept. 30, 1948.

A. Phosphate vs. EDC citrate. System is, as usual, 10 ml and M/2000 in ONPG. .001 ml of Lactase 319A used for test.

1. 1 ml M/5 Phosphate	222
2. 1 ml M/5 citrate	021
3. 1 ml each.	022

All contain 1 ml Phosphate Buffer

B.

Add	
1 —	189
2 1 ml EDC	012
3. 1 ml Na citrate M/5.	190.

The inhibition is clearly due to the ethylene diamine component of the EDC buffer!

Oct. 1. Test .002 ml of 319A in the following buffers, each at M/50 pH 7.5

	D420.
1. Phosphate	310
2. Glycerophosphate	488
3. " + Phosph.	477
4. Barbital	513
5. " + "	494

Deficiency in phosphate was visibly apparent. A NaCl effect?

Phosphate is not required for the reaction.

ONPM/5000 in: M/50

1. Phosphate	694
2. Barbital	645.
3. Glycerophosphate	725

Activation of lactase & other assays 324a.

To test influence of NaCl add 1ml of M/5 NaCl, KCl, and Na_2SO_4 respectively to a phosphate buffer system as above. 319A .002ml

Phosphate M/50+:

1. — 2.75
2. NaCl 3.95
3. KCl 2.59
4. Na_2SO_4 5.14.

M/50. Repeat

1. ~~NaCl~~ 317
2. NaCl 512
3. Na_2SO_4 592
4. KCl 298
5. LiCl 218
6. NH_4Cl 230
7. $(NH_4)_2SO_4$ 252
8. $MgSO_4$ 257

Inhibitory.

NaCl concentration series:

1. — 316 .010
M/50x 2. .1 405
3. .5 388
4. 1.0
5. 5.0

Sept 30, 1948.

17g. wetpaste K-12 harvested from 20 ~~gallons~~ litres (low yield!
S(Lac)

Add ca 50cc cold acetone to dehydrate, filter, and desiccate the residue. Assay sample of cells for activity.

	A.	B
D420.	[illegible]	621

Also, other assays:

325

314B.	1mg	1150	ca. 35 u/mg.
	.1mg	379	
	.01mg	046	
319B.	1mg	1070	ca. 190 u/mg.
	.1mg	960	
	.01mg	193	

→ 5.2 grams dry powder obtained: Lactase 325A.