Inhibition of galactosidase by carbohydrates.

Sept. 15, 1948

M/50 citrate buffer 7.5 M/300 ONPG
20m. 37° M/10(ca.) Sugars

	% inhibition	
K-12	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/500 ONPG was added after temp. equilibrium. Color read as + or − :

N-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

		λ 420 red	λ 650 vs 9	λ 650 vs water	420 H_2O	3 hour exposure opt. color
L	—			.121	.192	
glucose	1	.09	.015			+
"	2	.10	.017			+
lactose	3	.05	.014			+
"	4	.21	.019			+
Lac + Azide M/100	5	.05	.000			+
"	6	.03	.011			+
Glut A2	7	.04	.1			+
"	8	.1	.0			+
water	9	.18	.1			+
G	—			.159	.216	
glu	1	.09	.011			—
"	2	.04	.0			—
lac	3	.06	.008			+
"	4	.13	.03			+
lac azide	5	.1	.02			+
lac ATP 5 mg	6	.03	.030			—
" " + azide	7	.035	.002			—
Bz Im M/1000	8	.04	.013			+
SMT M/2000	9	.11	.005			—
water	10	.1	.0			—

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 = 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 as 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 Hydrol. Casein 1%	

ONPG as above, but use total volume of 9 ml. gallon 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	Y. Extr., Peptone + H.C
3	.499	~~.334~~ .279	1.79	233	are definitely stimulatory to adaptation.
4	.551	~~.279~~ ~~.334~~ .310	1.78	231	
5	.022	.200	.11	14	
6	T=101	.230	0	0	
7	.006	.200	0	0	<.005
8	.519	.334	1.55	202	

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/mg	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, V, Ts. .1ml	33
11.	"	2, Am. Ac.	125
12		M.C.	120

	D^i_{420}	D^t_{420}	$D^i \times \frac{230}{251}$	Δ	$\frac{\Delta}{D^i_{420}}$
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 42 Flu as above.

Indicated supplements ca 1 mg. eq. in 1 ml.

	A	B	C	D. overnight
O	242	224		-
Lac	280	218		-
Megal	246	237		+++
Bugal	319	310		+++
CNPG.	240	219		-

A = D_{420} bact. susp. V = 9 mC
B = D_{420} + substrate chrom. 10 mC
C = 4 cm (x .9 C)
D = B - C = Δ
E = D/C = relative activity

		A	B	C	D	E		% Lac
A12	1	224	311	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	+	103
A6	5	241	380	224	156	70	+	103
Leucine	6	239	291	215	76	35	-	52
Valine	7	263	400	237	163	69	+	103
Isoleuc	8	288	356	232	124	53	-	79
Phalen	9	280	348	207	141	68	+	102
prol	10	256	371	232	139	60	-	90
lys	11	246	366	222	144	65	+	97
arg	12	235	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	326	584	176	++	263
H.C.+Trp.	17	347	860	312	548	178	++	266
T+Leuc	18	263	401	237	172	73	+	109.

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

Amino Acids & Adaptation

Sept. 22, 1948.

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

		A	B	C = A_con	D (B-C)	E B-C / C	% of Lac (1)
1.	—	228	305	205	100	56	100
2.	HC	310	710	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.	" A_5	249	471	222	257	116	
7.	" A4	257	520	233	287	80	
8.	" A5	241	477	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 ea. 10.

.1 ml HC 10% 2.

1 ml ≙ 1% HC 3.

Sept 25-26, 1948.

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

25 g. cell paste recovered. Ca 2½ g. + 10 cc 7.5 PO4 buffer shaken 2½ h. under toluene. Remove debris & collect supernatant in

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

(B). Ca 1 g. washed ē 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 Y2 lactose. Harvest to 5 cc. 7.5 buffer & autolyze under toluene & shaking 24h & 48h.

(A) 24h. 1 ml withdrawn, debris sedimented & supernatant diluted to 4 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 suspension. (i.e. .05 ml should give ca 100% hydrolysis of 10 ml M/5000 ONPG in 20 mins). I.E., calculating 2g/liter, corresponds to 20 mg/ml

See 316

Sept. 27, 1948.

N-12 grown 36 hours in 10 liters S(Lac). 9.4 liters of supernatant were removed leaving 31 grams wet Shapiro paste. Make up to ca 45 ml $\bar{c}$ PO_4 buffer pH 7.5 and grind 75 minutes in Booth-Dienert mill. Combine efflux $\bar{c}$ 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 D_{420}.

1:20

1:50 dilutions.

1 Unit = A of .100 in D_{420}. for calf fin pups.

Assays:	A	B	C	D	Act./ml.		
1	008	290		283	14	315A	.2 ml
2	002	205		205	~~100~~ 10	B	.2 ml
3	007	260		254	25	314A	.1 ml
4	001	043		042	40		.01 ml
5	010	020		021	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 (acid) hydrolysis!

In prep. 316., 1 ml being = culture medium 10 liters/100 [illegible] cells.

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

Sept 28, 1948.

K-12 grown on 100 ml T(0) glucose (A) & do. + H.C. (B) (½ ml/100) shaken 16 hours. Adjust densities:

(A) 1:10 dilution o D_{650} 259

(B) HC. 319

ratio of 1.23 : 1.

Re-suspend ~~dilute~~ HC ~~culture~~ (0) culture ~~in~~ 20 ml H_2O; the HC culture in 24.6 ml H_2O to adjust initial densities.

The adaptation system consists of 1 ml cells + 3 ml T(0) + lactose + H [illegible] supplement. Adapt 3 hours, in duplicate. Resuspend in 1 ml [illegible] 8 ml buffer for A., Add 1 ml ONPG 20 minutes for B.

		A	B	C	D	E
1	[illegible] pH 7.0 M/50.	.201	745			
		.196	641			
2.	T/0 [illegible]	.248	669			
12		.260	720			
3.	T(0) lac + .1 ml H.C.	.256	710			
13		.260	731			
(B) 4.	Lac buffer	.177	169			
14		.184	175			
5.	T(0) lac	.187	171			
15		.188	170			
6.	T(0) 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 100cc or 2.9×10^5 units all together. To fractionate remove 50ml and dilute ~ 50ml H_2O. (1.5×10^5 units; ~~1.5×10^3~~ 1500/ml). "316" is fraction 0. Add Am Sulf in 4 aliquots of 17.5g. each in ice bath to give 1/4 sat'd fractions. Take up sediments in 10ml 4/50 $PO_4^=$ except for the final fractions.

	Prop. fract.	Act.	Prop. Act.	Assay .01	.001
0	1.00.		1.00.	615	089
1 (1/4sat).	5.00			129	019
2 (1/2sat)	5.00			390	055
3 (3/4sat)	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. 1ml m/5000 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 (40ml) and add 24 gms AS (3/4sat.). Take up ppt in M/50 citrate buffer.) 0 (1A

P30. To remaining 50ml (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 10ml 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 needed. 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.

Nutrition - Adaptation

Sept 30, 1948.

K-12 in shake overnight. A) T(0) 1:100 dilution. B) T(Pro) C) T(AA), 5ml 1mg/ml. 5ml 1% HCl. 2ml Resuspending in 5 ml H_2O. Turbidity at

	Dose
A	119
B	119
C	252
	050

Dilute A and B to 11.9 ml to equalize C.

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

A. B. 1 ml cells, 3 ml substrate.

① Phosph M/50 7.5 + 2% lac
② T. (2%) Lac.
③ ② + ~~supplement~~ proline 1mg% .2ml
④ ② + ~~HCl~~ AA. ~~H2O~~ 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(0) cells did not adapt!! T(AA) cells were stimulated by T(0). & further by amino acids.

Urine

	A	B	C = .9A	D = B − C	E = D/C	% Lac − Suppl.		
1	25.7	36.8	231	137	59	120	Σ AA	
2	24.8	32.7	223	106	48	98	AA − Arg + arg	
3	24.1	33.5	217	118	54	110	" lys	
4	24.2	32.9	218	141	51	104	" meth	
5	24.1	33.0	217	83	38	77	" cyst	INHIBITORY!
6	25.9	40.2	233	169	73	149	AA − arg	
7	24.2	39.6	218	152	70	143	− lys	
8	24.1	39.7	217	145	65	132	− meth	
9	24.9	38.2	224	148	66	135	− cyst	
10	25.0	41.27	225	202	54	110	AA − AA + dal	
11	26.0	41.10	234	176	75	153	+ tyr	
12	27.0	41.51	243	208	85	174	+ thyp	
13	26.9	44.89	242	247	102	208	AA − dal	dal inhib? thyp stimulatory.
14	24.1	41.52	244	208	85	173	− tyr	
15	24.1	44.41	242	199	82	167	− thyp	
16	23.0	41.22	207	102	49	—	0	
17	31.1	76.75	287	508	177	362	M.C.	
~~18~~								

Sept. 30, 1918.

A. Phosphate vs. citrate. EDC 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.

1 Add —	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

	D 420.
1. Phosphate	310
2. Glycerophosphate	488
3. " + Phosph.	477
4. Barbital	513
5. " + "	494

Deficiency in phosphate was visibly apparent. A Na Cl 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 32½.

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 .02ml Phosphate M/50+:

1.	—	2.75
2.	NaCl	395
3.	KCl	2.59
4.	Na_2SO_4	5.14.

M/50. Repeat

1.	~~[illegible]~~	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	.10
M/50+ 2.	.1	405	
3.	.5	388	
4.	1.0	↓	
5.	5.0		

Lactase.

Sept 30, 1918.

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

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

Dilution	A.	B
		621

Also, other assays:

325

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

→ 3.2 grams dry powder obtained: Lactase 325A.