CLXV. THE ADAPTABILITY OF GLUCOZYMASE AND GALACTOZYMASE IN *BACTERIUM COLI*

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(*Received 24 June 1937*)

It has previously been shown that, in the case of *S. cerevisiae* galactozymase is an adaptive enzyme, i.e. that its formation depends on an adaptation of the cell due to the presence of the substrate and that this adaptation is independent of cell multiplication and is inhibited by glucose [Stephenson & Yudkin, 1936]. The present paper is concerned with a similar study with *Bact. coli*.[1]

Technique

The organism was grown for 18–20 hr. in varying conditions; the crop was centrifuged and washed twice and made up so as to contain 1–2 mg. dry weight of bacteria per ml.; the concentration of the cell suspension was determined either by a micro-Kjeldahl estimation of nitrogen or (more conveniently) by a photo-electric turbidimeter [Clifton *et al.* 1935], calibrated for the organism used.

It is not possible to use a manometric estimation for glycolysis because gas evolution by *Bact. coli* varies with growth conditions independently of glycolysis; we therefore measured glycolysis directly by the method of Hagedorn & Jensen as this permits of estimations of 0·4–0·1 mg. The details and necessary data for the use of this method with galactose have already been published by one of us [Gale, 1937]. For each determination 1 ml. buffer $M/10$ *p*H 6·24, 1 ml. $3M/500$ glucose or galactose and 1 ml. of bacterial suspension (1–2 mg. dry wt./ml.) were incubated together anaerobically and 0·5 ml. was removed for estimation according to the method mentioned. The results are recorded as $Q_{glucose}$ or $Q_{galactose}$, that is γ hexose fermented per mg. dry wt. bacteria per hour. Fig. 1 shows that in these circumstances the action is linear with time for 40 min. and complete in 90 min. when the $Q_{glucose}$ is 660 and the organism is 1·0 mg./ml.; at higher values of $Q_{glucose}$ it is necessary to dilute the organism. The broth used is a tryptic digest of casein of 1 mg. N/ml., *p*H 7·2.

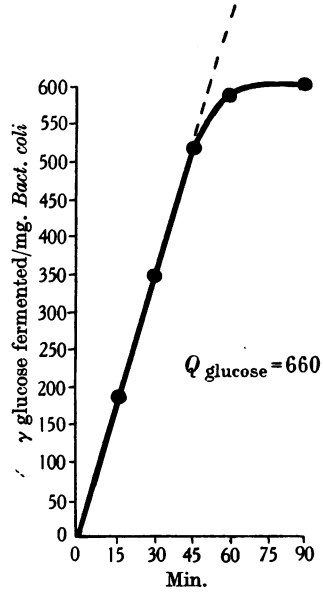

Fig. 1.

[1] A preliminary paper on this subject was communicated to the Second International Congress of Microbiology by Stephenson & Lutwak-Mann.

The influence of the growth medium on glucozymase

Fig. 2 shows that glucozymase is not a strictly adaptive enzyme since it occurs in cells grown in the absence of glucose; its activity is, however, favourably influenced by two factors, anaerobiosis and the presence of hexose in the medium. Anaerobic conditions in the absence of glucose raise the $Q_{glucose}$ from

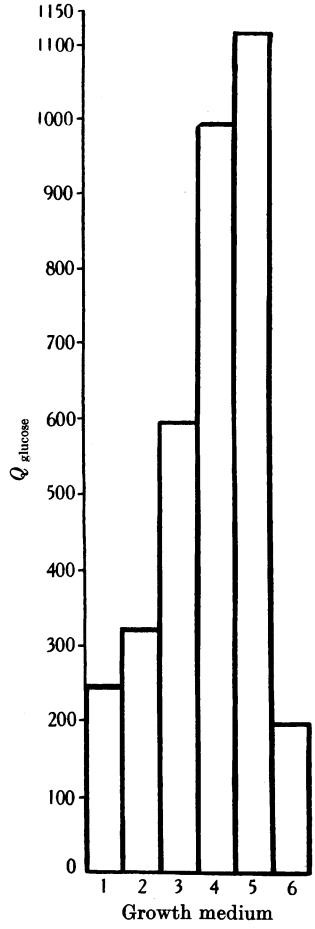

Fig. 2.

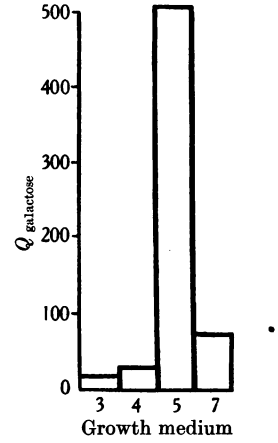

Fig. 3.

Figs. 2 and 3. Growth media: 1, Broth agar. 2, Broth agar + 1 % glucose. 3, Broth anaerobic. 4, Broth anaer. + 1 % glucose. 5, Broth anaer. + 1 % galactose. 6, Lactate inorganic aerobic. 7, Broth anaer. + 1 % glucose and galactose.

250 to 600, the addition of 1 % of either glucose or galactose approximately doubles the latter figure. Fig. 3 gives a corresponding figure for galactozymase. Here we have a very low but definitely appreciable fermentation by organisms grown on plain broth or on glucose broth ($Q_{galactose} = 12$ and 30).[1] The presence of 1 % galactose raises this to 510. In the case of *S. cerevisiae* it was found that

[1] In many cases the value found was less on glucose broth than on plain broth so the differences are not significant.

an organism adapted to galactose lost its galactozymase as a result of fermenting glucose. Probably this has its analogy in the case of *Bact. coli* in the action of glucose in suppressing the action of galactose in the growth medium (Fig. 3 (7), $Q_{\text{galactose}} = 75$).

Relation of galactozymase formation to growth

In the case of *S. cerevisiae* it was found that the organism grown in the presence of glucose could acquire galactozymase by incubation in the presence of galactose in buffer without cell proliferation; we searched for evidence of the same phenomenon in *Bact. coli*.

Exp. 1. The organism was grown 20 hr. in plain broth in a flask (semi-aerobically); the organism was washed and resuspended in 1% galactose in buffer at *p*H 7·0. At intervals samples were removed, the cells spun out and washed and the $Q_{\text{galactose}}$ measured; a control experiment was done in which the cells were incubated in buffer without galactose. No appreciable increase in galactozymase in the cells incubated with buffer and galactose over those incubated in buffer alone was found in the following intervals; 30 and 60 min., 20 and 40 hr.:

We next attempted to find out whether, if galactose were added to cells already grown in plain broth, the enzyme would then appear and whether, if so, its appearance would be accompanied by cell proliferation.

Exp. 2. The organism was grown for 20 hr. on 1600 ml. broth in a flask; 16 g. sterile galactose in 100 ml. water were then added and 200 ml. withdrawn (with

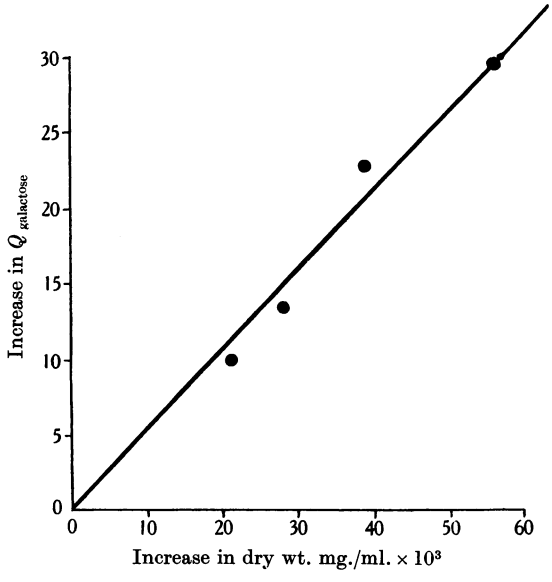

Fig. 4.

sterile precautions) at intervals for estimations of $Q_{\text{galactose}}$ and for estimation of bacteria with the turbidimeter. The results are given in Tables I and II and Fig. 4.

Table I

Hours after addition of galactose	Cells mg./ml.	Increase in cells mg./ml.	$Q_{galactose}$	Increase in $Q_{galactose}$
0	0·104	0·000	24	0
1	0·125	0·021	100	76
3	0·132	0·028	126	102
4	0·143	0·039	180	156

Table II

Time hr.	Total cells %	Old cells %	New cells %	$Q_{galactose}$	Increase in $Q_{galactose}$
0	100	100	0	24	0
1	100	83	16·8	100	76
3	100	78·4	21·6	126	102
4	100	72·8	27·4	180	156

There is thus no evidence for the adaptation of non-proliferating cells even in broth but strong evidence for supposing that the increase in galactozymase is due to the increasing proportion of new cells which have grown in the presence of galactose. We next tried to show whether (as in the case of *S. cerevisiae*) organisms which were adapted to ferment galactose lost this adaptation as a result of fermenting glucose.

Exp. 3. The organism was grown on galactose broth for 20 hr., centrifuged, washed and resuspended in buffer + 1 % glucose and incubated at 40°; a control was done in buffer without glucose:

Initial $Q_{galactose}$	540
After 3 hr. incubation with 1 % glucose	570
After 3 hr. incubation with 1 % buffer	500

With a more prolonged experiment glucozymase and galactozymase fell off at approximately the same rate in the presence of glucose and of plain buffer.

Table III

	Rate of fermentation (mg./hr.)	
	Glucose	Galactose
Initial	0·70	0·44
After 6 hr. in buffer + 1 % glucose	0·096	0·104
,, 6 ,, only	0·107	0·088
,, 22 ,, + 1 % glucose	0·056	0·063
,, 22 ,, only	0·059	0·056

There is thus no more destruction of galactozymase on incubating with glucose than on incubating with plain buffer.

Summary

Washed suspensions of *Bact. coli* ferment glucose at a rate which varies greatly according to the conditions in which the organism is grown. The rate of glycolysis ($Q_{glucose}$) is approximately doubled by changing from highly aerobic to anaerobic growth conditions. An increase of similar magnitude is obtained by adding glucose 1 % (or galactose 1 %) to the growth medium. Galactose is fermented at an extremely low rate as compared with glucose; this rate is not raised

by adding glucose to the medium but is increased about 40-fold by the presence of 1% galactose; this increase is not attained when glucose and galactose are simultaneously present.

Adaptation to galactose in the case of this organism is, so far as our experiments show, invariably accompanied by growth; no evidence of adaptation in the absence of cell multiplication in the presence of galactose can be shown and the increase in $Q_{\text{galactose}}$ is proportional to the number of cells which have multiplied in the presence of the specific substrate.

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