GLYCINE AND FORMIC HYDROGENLYASE

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(With 1 Figure in the Text)

INTRODUCTION

In a recent paper Gordon (1948) described the adaptation of a strain of *Bact. coli* to grow in high concentrations of glycine. This new variant-resistant strain showed the usual bacteriological properties of *Bact. coli*, with the exception that there was a complete absence of gas-formation during the fermentation of sugars.

'Gas-formation' by bacteria-fermenting sugars is still held to be brought about, at least chiefly, by the decomposition of formic acid by formic hydrogenlyase. In an attempt to throw some light on the mechanism of the resistant strain's loss of the power to produce 'gas', the following experiments were carried out: (1) the inhibition of formic hydrogenlyase by glycine was tested; (2) the absence of formic hydrogenlyase was confirmed, in washed suspensions of the adapted strain grown under such conditions that the normal strain would have contained it; (3) the presence of formic acid among the products of fermentation of sugars by the adapted strain was observed; and (4) the effect of glycine on the rate of appearance of formic hydrogenlyase in washed suspensions of *Bact. coli* was studied.

EXPERIMENTS

Bacterial suspensions. Three different kinds of washed suspensions of *Bact. coli* were required: (1) the normal strain grown for 24 hr. on broth containing 0·5 % sodium formate—this preparation contained active formic hydrogenlyase ($Q_{H_2} = 100–200$);* (2) the normal strain grown on the surface of agar—this suspension was completely devoid of formic hydrogenlyase, and was used for following the appearance of the enzyme, as described by Stephenson & Stickland (1933); and (3) the adapted strain grown for 48 hr. in broth containing 0·5 % sodium formate—the longer growth period was necessitated by the slower growth of this strain.

* $Q_{H_2} = \frac{\mu l.\ H_2}{\text{mg. dry wt.} \times \text{hr.}}$.

From the liquid media the bacteria were separated by centrifugation, washed once with distilled water, and suspended in a volume of distilled water equal to about one-fortieth of the original volume of medium. Such a suspension contained usually about 2–4 mg. dry weight of bacteria per ml. From the agar the bacteria were collected by stirring gently with distilled water, filtering the suspension thus obtained through glass-wool, centrifuging, washing once with distilled water, and finally suspending in distilled water at a concentration of about 10 mg. dry weight of bacteria per ml.

RESULTS

(1) *The inhibition of formic hydrogenlyase by glycine*

To follow the rate of evolution of hydrogen from formic acid by bacterial suspensions, Barcroft manometers were used, as described by Stephenson & Stickland (1932). The right-hand vessel contained 1·0 ml. of phosphate buffer M/15, pH 7·0, 0·5 ml. of sodium formate M and 1·0 ml. of bacterial suspension containing 1–2 mg. dry weight of bacteria (the normal strain grown on broth containing 0·5 % sodium formate). To these were added the appropriate volume of 2M-glycine solution and water to make the volume up to 5 ml. The left-hand vessel contained 5 ml. of water, and the whole apparatus was filled with nitrogen purified by passage over heated copper. Potash and filter-paper were used in the usual way to absorb CO_2. After equilibration in a bath at 40° C. for 5–10 min., readings were taken at 10 min. intervals for 30 min.

The course of hydrogen evolution was very nearly linear, and the results are given in terms of Q_{H_2} in Table 1. The enzyme was not very sensitive to glycine, and showed on the average about 50 % inhibition at molar concentration (7·5 %).

(2) *The absence of formic hydrogenlyase from the adapted strain*

A thick suspension of bacteria of the adapted strain, grown on formate broth, was tested under the conditions described in the preceding section.

Table 1. *The inhibition of formic hydrogenlyase by glycine*

Glycine concentration (M)						
0	1·0		0·4		0·2	
		Inhibition		Inhibition		Inhibition
Q_{H_2}	Q_{H_2}	(%)	Q_{H_2}	(%)	Q_{H_2}	(%)
93	25	74	58	38	82	12
122	30	75	—	—	—	—
136	47	64	—	—	—	—
150	114	24	—	—	—	—
178	80	55	—	—	—	—
165	91	45	—	—	—	—

There was a complete absence of hydrogen evolution; allowing for the dry weight of the bacteria used and the sensitivity of the apparatus, it may be said that Q_{H_2} was at any rate less than 1, compared with values of 100–200 for the normal strain.

(3) *The presence of formic acid among the products of fermentation of sugars by the adapted strain*

Fermentation experiments were carried out on 200 ml. lots of peptone broth containing 1 % of the sugar in flasks, with both the normal and the adapted strain. After 3 days' incubation the cultures were autoclaved and formic acid determinations carried out on aliquot portions by the method of Dakin (1913).

Table 2. *Formic acid as a product of fermentation by the adapted strain*

	Formic acid (mg./100 ml. medium)	
Sugar fermented	Normal strain	Adapted strain
Glucose	0·5	53
Lactose	14	61
Mannitol	1·0	71

The results (Table 2) showed that the products of fermentation by the adapted strain contained far more formic acid than with the normal strain; with the latter the exceptionally low yield of formic acid must be attributed no doubt to the destruction of the acid, either by formic hydrogenlyase or perhaps partly aerobically by formic dehydrogenase, during the later stages of the very long incubation period.

(4) *The effect of glycine on the rate of appearance of formic hydrogenlyase*

It was shown by Stephenson & Stickland (1933) that if a suspension of *Bact. coli* grown under such conditions that it was devoid of formic hydrogenlyase, was incubated with sodium formate and broth at 40° C., then after a latent period of only about 1 hr. there started a rapid development of formic hydrogenlyase activity. The rate of increase of activity was constant, and the activity reached a maximum some 3 hr. from the start of the incubation. The maximum rate was maintained constant for about an hour, eventually falling off presumably on account of increasing alkalinity of the medium. During the period of the appearance of the maximal activity total cell counts could detect no increase in the number of cells present. This phenomenon, though possibly capable of more than one interpretation, is obviously related closely to the formation of formic hydrogenlyase in bacteria during their growth in a formate-containing medium. Therefore its inhibition by glycine was studied.

The manometer vessels contained 1·5 ml. of broth (concentrated *in vacuo* to five times its normal strength) at pH 7·0, 1·0 ml. of phosphate buffer M/15, pH 7·0, 0·5 ml. sodium formate M, 0·2 ml. of bacterial suspension containing about 2 mg. dry weight of cells, the requisite volume of 2·0 M-glycine, and water to make up 5·0 ml. The evolution of hydrogen was observed as before in an atmosphere of purified nitrogen, carbon dioxide being absorbed as usual.

The results of a typical series of experiments is shown in the figure, and they show that a strong inhibition of the appearance of the enzyme is observed at a concentration of glycine which does not inhibit the enzyme itself (Table 1).

DISCUSSION

The results of the *in vitro* experiments described above are quite consistent with one another. The adapted strain's loss of the power to produce 'gas', i.e. hydrogen, is not to be accounted for by any failure to produce formic acid, and must consequently be due to a loss of the power to decompose it; this loss of formic hydrogenlyase is confirmed experimentally.

The inhibitory action of glycine on the appearance of hydrogenlyase might be of three different kinds: (1) an increase in the lag period, (2) a decrease in the rate of appearance of the enzyme, or (3) a limitation of the maximum rate reached. Although under carefully controlled conditions of initial cell concentration it had been found by Stephenson & Stickland that no cell multiplication took place while formic hyrogenlyase was increasing to its maximum value in some 3 hr., it would not be safe to pursue such experiments any further, as growth would be sure to take place, which would greatly complicate the interpretation of the results. However, in the presence of high concentrations of glycine it is known that growth is impossible, so longer periods up to 7 hr. were used. These experiments (Fig. 1) showed that with increasing concentrations of glycine all three of the above-mentioned effects were observed. At the

concentration of glycine used at the beginning of the adaptation (1·0–1·5%, or 0·13–0·2M) the lag is not increased, and the rate of enzyme increase is about one-half of the normal. A culture grown under these conditions might be expected to show a 'gas-production' not easily distinguishable from the normal. At 0·32M-glycine (2·4%), the enzyme formation *in vitro* is not completely suppressed, but the lag period is increased, and the rate of appearance of the enzyme is reduced to about one-third or less. At 0·64M (4·8%) a concentration of the same order as that at which the adapted strain lost its power of gas-formation, the appearance of the enzyme is almost completely suppressed. Evidently exposure to these conditions led to an irreversible change in the bacteria, such that they could now no longer form the enzyme, even in the presence of sodium formate and in the absence of glycine.

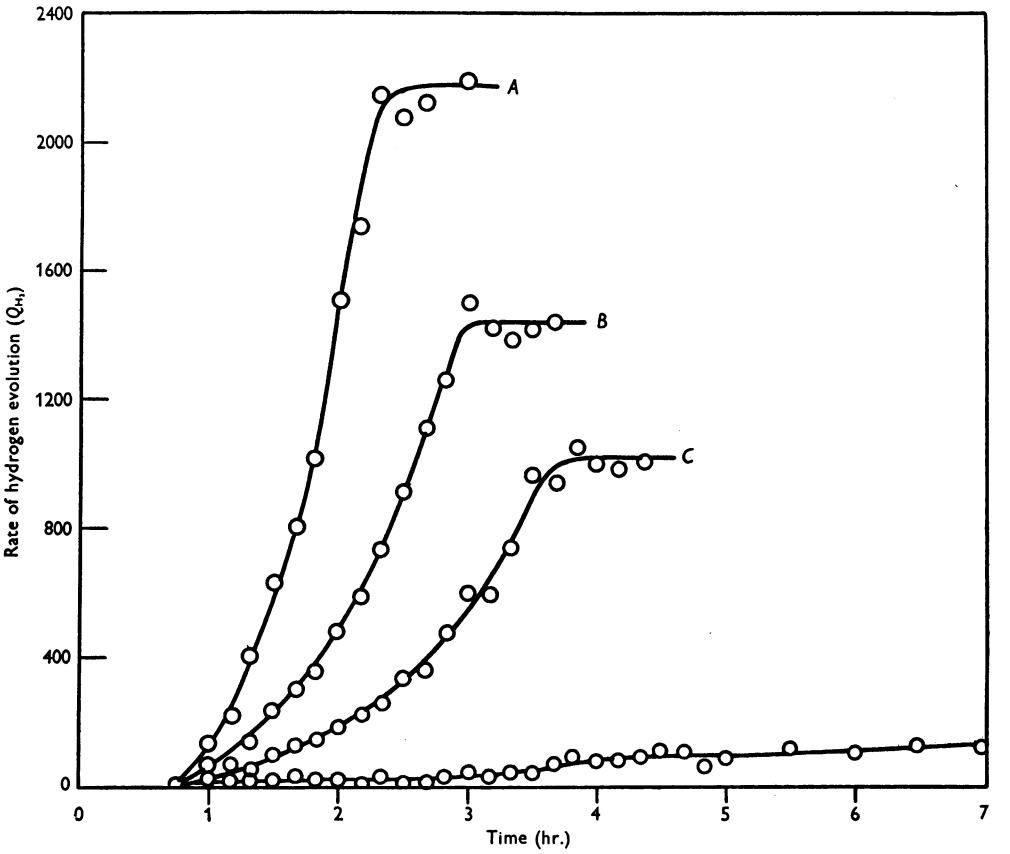

Fig. 1. The effect of glycine on the rate of appearance of formic hydrogenlyase in normal *Bact. coli*. *A*, Control (no glycine); *B*, 0·16M-glycine; *C*, 0·32M-glycine; *D*, 0·64M-glycine.

SUMMARY

1. A strain of *Bact. coli* adapted to growth in high glycine concentrations, which had lost the power of producing 'gas' from sugars, was shown to be devoid of formic hydrogenlyase.

2. Formic acid was prominent among the products of fermentation of sugars by this strain.

3. The appearance of formic hydrogenlyase *in vitro*, in bacteria grown in such a way as not to contain the enzyme, was inhibited by glycine at high concentrations.

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