THE KINETICS OF THE LYSIS OF *BACTERIUM COLI* BY GLYCINE

By J. GORDON, R. A. HALL and L. H. STICKLAND
From the School of Medicine, Leeds 2

(With 4 Figures in the Text)

INTRODUCTION

Maculla & Cowles (1948) showed that suspensions of *Bacterium coli* were lysed if they were incubated at 37° C. with concentrated glycine solutions. We found (Gordon, Hall & Stickland, 1949) that this lysis was absent, or very much reduced in extent, in strains of *Bact. coli* which had been rendered resistant to glycine. In the course of this study a number of interesting observations were made, which made it desirable to investigate more closely the details of the lysis of normal strains. The present paper deals with some of the kinetics of this lysis.

EXPERIMENTAL

Two freshly isolated strains of *Bact. coli*, numbered 7 and 8, were used. A large number of strains were tested, which showed a wide range of susceptibility to lysis by glycine; these two were chosen because they were very readily lysed. Maculla & Cowles (1948) recommended the use of very young cultures, in order to obtain a high degree of lysis. We find that with suitably chosen strains a consistently high degree of lysis may be observed with cultures up to 24 hr. old, and that the use of a solid medium is not inimical to that lysis.

Our strains were grown on the surface of agar usually for 15 hr. at 37° C. The growth was washed off with distilled water, and the suspension filtered through glass-wool to remove pieces of agar. The bacteria were centrifuged out, washed once with distilled water, and finally resuspended in a suitable volume of water, usually about 10–15 times the volume of the packed cells.

Equal volumes of this cell suspension were measured into a number of $4 \times \frac{1}{2}$ in. Pyrex test-tubes, treated with the appropriate solution of glycine, or water in the controls, and incubated at various temperatures and for various periods, as will be described later.

At the end of the incubation, the bacteria were removed by centrifuging, and the supernatant fluid treated with 25% trichloracetic acid to give a final concentration of 5%. The protein precipitate was sometimes very slow in flocculating, and the precipitation was therefore usually completed by the addition of alcohol to a concentration of about 30%.

The protein precipitate was then centrifuged out, washed with 5% trichloracetic acid and then dissolved in NaOH and treated with $CuSO_4$, as in the method of Robinson & Hogden (1940). The biuret colour that developed was read on a Spekker absorptiometer, and the total protein in the original suspension was similarly determined by the method described by Stickland (1951). The amount

of protein liberated in the supernatant fluid after the incubation was expressed as a percentage of the protein content of the suspension as determined by the direct biuret reaction, and called the 'degree of lysis'. With each experiment a blank was included, in which the cell suspension was incubated with water instead of glycine; in no case was any lysis observed in incubations up to 16 hr. at 37° C.

Using this method, the following factors in the lysis were investigated: (*a*) the effect of time, (*b*) the concentration of glycine, (*c*) the concentration of bacteria, (*d*) the pH and (*e*) the temperature.

(*a*) *The relationship between the time of incubation with glycine and the degree of lysis*

Using the technique described, samples of bacterial suspensions were incubated with 1·0M-glycine at 37° C. for varying periods of time. The degree of lysis followed the course shown in Fig. 1 for this concentration of glycine.

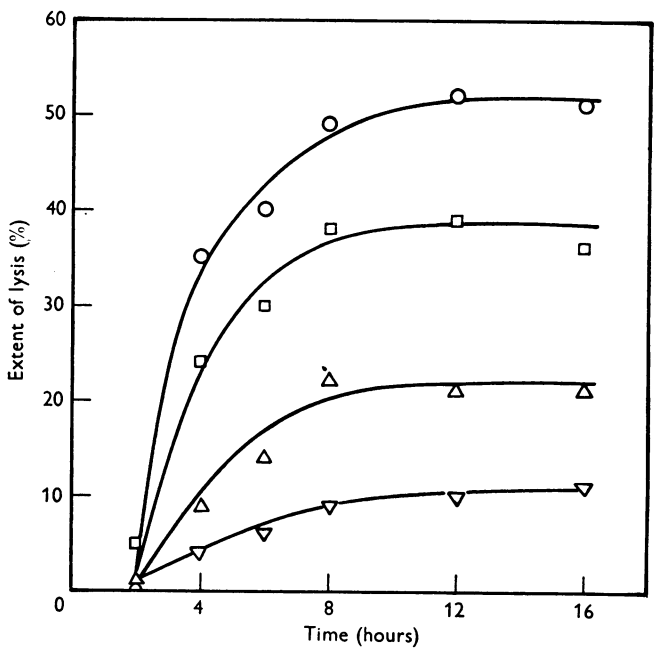

Fig. 1. The course of lysis at different glycine concentrations, at pH 7·5 and 37°. *Bact. coli*, strain 8, was used. ○——○, 1·0M-glycine; □——□, 0·5M-glycine; △——△, 0·2M-glycine; ▽——▽, 0·1M-glycine.

In this experiment there was a delay of 2 hr. during which practically no lysis occurred, followed by a rapid lysis which reached a maximum in 8 hr. Further incubation led to no further increase in the degree of lysis. The reaching of a maximum in 8 hr. is in close agreement with the findings of Maculla & Cowles (1948). The initial delay is a constant feature, but in other experiments there was sometimes a slight increase in the degree of lysis between 8 and 12 hr.

(*b*) *The relationship between concentration of glycine and lysis*

The course of the lysis at different glycine concentrations is shown in Fig. 1. Three points in these curves deserve attention: (*a*) the period of delay before the onset of lysis is roughly the same at all glycine concentrations, (*b*) the rate of the lysis between 2 and 8 hr. is roughly proportional to the glycine concentration, and lysis at each concentration reaches a maximum in 8 hr., (*c*) the limiting degree of lysis, reached in 8 hr., varies directly with the glycine concentration. Point (*c*) is illustrated better in Fig. 2, which shows that the degree of lysis tends towards a maximum as the concentration of glycine increases.

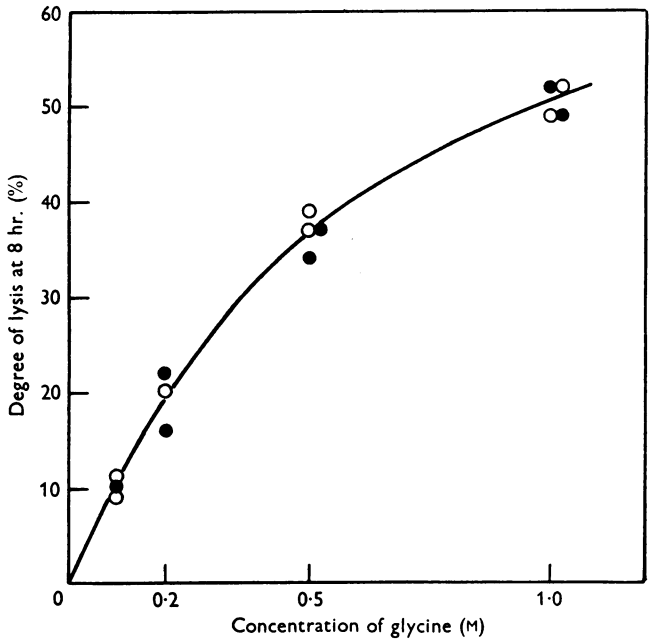

Fig. 2. Relationship between degree of lysis and concentration of glycine. ○, strain 7; ●, strain 8.

(*c*) *The relationship between concentration of the bacterial suspension and degree of lysis by glycine*

The bacteria, after washing, were suspended in about 6 vol. of distilled water. To this suspension, and a twofold, fourfold and eightfold dilution of it, was added glycine solution to give a final concentration of 1·0 M at pH 7·5, and the course of the resulting lysis at 37° C. was followed. In each case the lysis followed the usual course, and the results are given in Table 1 in terms of the degree of lysis after 8 hr.

Within the range studied, the concentration of the suspension clearly had no significant effect on the degree of lysis.

(*d*) *The relationship between* pH *and rate of lysis by glycine*

The glycine solutions used in the experiments which have been described had been adjusted to pH 7·5. At this pH the buffering power of the glycine itself is

sufficient to keep the pH constant, but at lower pH values glycine hardly buffers at all. Hence in order to study the effect of pH on the rate of lysis, sodium acetate (M/5) was added to the solution of glycine (2M), and samples were adjusted

Table 1. *Effect of concentration of bacteria on degree of lysis*

Strain	Total bacterial protein (mg./ml.)	Wet weight of cells (mg./ml.)	Soluble protein liberated in 8 hr. (mg./ml.)	Lysis in 8 hr. (%)
7	4·08	66	2·17	53
	2·04	33	1·12	55
	0·90	14·5	0·56	62
	0·45	7·3	0·205	46
8	4·93	79	2·60	53
	2·46	39·5	1·20	49
	1·15	18·5	0·63	55
	0·58	9·3	0·26	45

The different dilutions of the suspension of *Bact. coli* were incubated for 8 hr. at 37° C. with 1·0M-glycine at pH 7·5.

'Wet weight of cells' is derived from 'Total bacterial protein', according to the data of Stickland (1951).

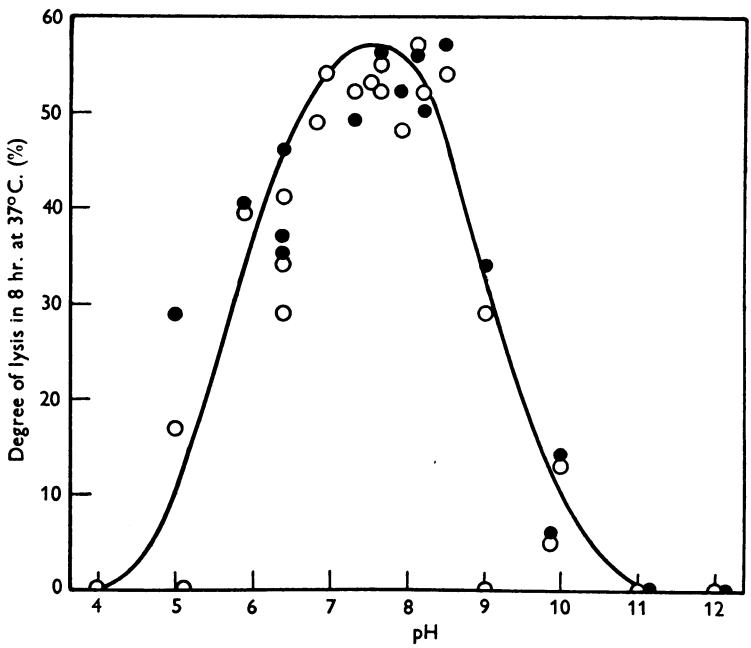

Fig. 3. The relationship between rate of lysis by glycine at 37° C. and pH. Two strains of *Bact. coli* were used, strain 7 (hollow circles) and strain 8 (solid circles). The results of four experiments are combined in the figure.

roughly to various pH values in the range 4–12 and then their exact pH measured with the glass electrode.

The suspension of *Bact. coli* was incubated with these glycine solutions for various periods, and the extent of the lysis produced was measured.

The resulting curves at each value of the pH relating the degree of lysis to time showed the usual initial phase of delay followed by lysis, which reached a maximum in about 8 hr. In the absence of a linear reaction, the rate of lysis is difficult to express numerically; therefore, as an approximation, the total lysis after 8 hr. has been plotted, and is shown in Fig. 3.

At pH 4 and 5 no lysis occurs, at 6 it is already nearly maximal, at about 7 and 8 a peak occurs and at pH 10 it has almost returned to zero.

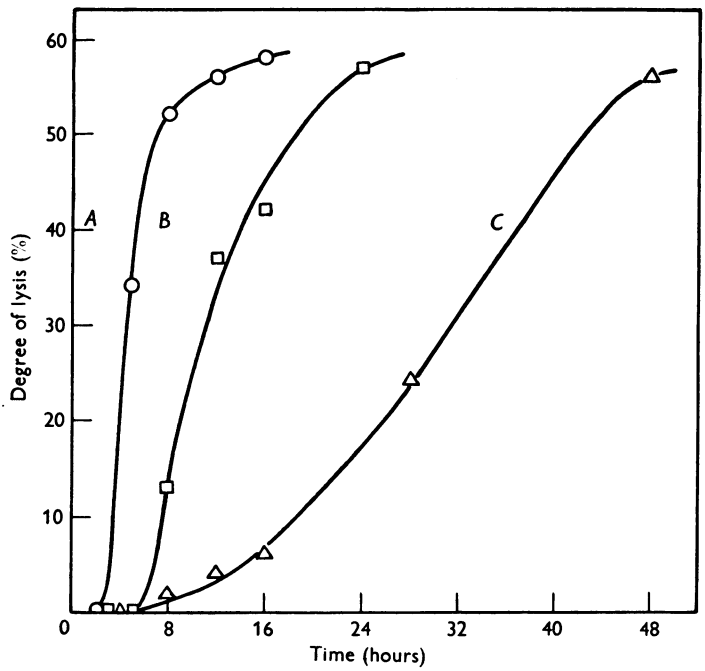

Fig. 4. The effect of temperature on the rate of lysis of *Bact. coli* (strain 8) by glycine (1·0M). ○——○, 37° C.; □——□, 32° C.; △——△, 27° C. The estimate of Q_{10} is obtained as the ratio AC/AB.

Table 2. *The temperature coefficient of lysis of* Bacterium coli *by glycine*

Exp. no.	Strain	Range of temperature (° C.)	Q_{10}
1	8	27–37	4·4
2	7	30–37	5·3
	8	30–37	5·2
3	7	27–37	4·9
	8	27–37	6·7

(*e*) *The effect of temperature on the rate of lysis*

When the course of lysis by 1·0M-glycine at pH 7·5 was followed at various temperatures, curves of the type shown in Fig. 4 were obtained. The temperature coefficient of the reaction is high, as can be seen from the figures in Table 2.

The temperature coefficients (the ratio of the velocities over a temperature interval of 10°, or Q_{10}) were estimated (*a*) by comparing the times taken at different

temperatures to reach the same degree of lysis (e.g. say, 40%) and (*b*) by comparing the slopes of the time-lysis curves at different temperatures. The data were not sufficiently exact for accurate calculations to be made, but the table shows that the Q_{10} is probably not far from 5.

In two experiments at room temperature (17–18° C.) no appreciable lysis was observed (16 hr., 1%; 24 hr., 3%).

DISCUSSION

The chief purpose of the experiments described was to discover some of the basic facts necessary for the study of the mechanism of the lysis of *Bact. coli* by glycine. Apart from this the interesting points observed were (*a*) the relationship between pH and rate of lysis, and (*b*) the temperature coefficient of the lytic process. The pH curve is similar in shape to that shown by many hydrolytic enzymes, and shows no relation to the titration curve of either glycine or bacterial protein. The high temperature coefficient suggests that the process of lysis may be a chemical reaction. As Höber (1946) says: 'Many chemical reactions have Q_{10} values between 2 and 4, while a physical process...is apt to have a temperature coefficient in the neighbourhood of 1·2–1·3.'

SUMMARY

The lysis of *Bacterium coli* suspensions brought about by glycine shows the following characteristics:

(1) There is a latent period of 2 hr., followed by a rapid lysis reaching a maximum in about 8 hr.

(2) The extent of the lysis is independent of the dilution of the bacterial suspension over a wide range.

(3) The extent of the lysis increases with the glycine concentration up to 1·0 M, but is approaching a limit at this concentration.

(4) The lysis is negligible below pH 5 and above pH 10, and shows a maximum rate in the region of pH 6·5–8·5.

(5) The rate of lysis has a very high temperature coefficient (Q_{10} of the order of 5).

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