JOURNAL OF BACTERIOLOGY, Aug. 1975, p. 570-579

Vol. 123, No. 2
Printed in U.S.A.

Regulation of Catalase Synthesis in *Salmonella typhimurium*

GERARD J. FINN AND SEAMUS CONDON*
Department of Dairy and Food Microbiology, University College, Cork, Ireland

Received for publication 18 February 1975

The specific activity of catalase in *Salmonella typhimurium* and other enteric bacteria decreased during the logarithmic phase of growth and increased at the onset and during the stationary phase. The increase in catalase synthesis at the end of the exponential phase in *S. typhimurium* cells coincided with the lowest pH value reached by the culture. Maintenance of the pH at a constant neutral value did not alter the typical pattern of synthesis in contradiction of the results previously reported (McCarthy and Hinshelwood, 1959). A sudden decrease in the pH value of an *S. typhimurium* culture during exponential growth by addition of HCl did not cause an alteration in the catalase synthesis pattern. Addition of hydrogen peroxide to *S. typhimurium* cultures within the range 1 μM to 2 mM during the exponential growth phase stimulated catalase synthesis. The extent of catalase synthesis depended on the concentration of hydrogen peroxide; the maximum stimulation was observed at 80 μM. Increased catalase synthesis was not detected for 10 to 15 min after hydrogen peroxide addition. Hydrogen peroxide was produced by *S. typhimurium* cultures during the exponential and stationary growth phases. However, no direct relationship between hydrogen peroxide accumulation and synthesis of catalase was observed.

The specific activity of catalase (H_2O_2:H_2O_2 oxidoreductase EC 1.11.1.6) decreases in the logarithmic growth phase of *Enterobacter aerogenes* (14), *Salmonella typhimurium* (11), *Serratia marcescens* (P. M. Dempsey and S. Condon, 1971, Irish J. Ag. Res. **10**:iv), *Haemophilus parainfluenzae* (21), *Rhodopseudomonas spheroides* (2), and *Saccharomyces cerevisiae* (10) and in all cases increases at the onset of, and during, the stationary growth phase.

McCarthy and Hinshelwood (14) noted that, under their conditions, a sharp decrease in the pH of the growth medium occurred at the end of the logarithmic phase and reported that, if the pH was maintained at neutrality, the characteristic increase in the specific activity of catalase in *E. aerogenes* was not observed. They suggested that the synthesis of catalase at the onset of the stationary phase was associated, though probably indirectly, with the decrease in pH.

McCarthy and Hinshelwood (14) also suggested that the synthesis of catalase might be due to induction by hydrogen peroxide. However, they were unable to detect hydrogen accumulation in *E. aerogenes* cultures at any stage of the growth cycle. Several workers (3, 4, 7, 19) have shown that the addition of hydrogen peroxide to anaerobic cultures of *R. spheroides* resulted in a stimulation of catalase synthesis. In addition, an increase in catalase synthesis has been reported on aeration of anaerobically grown cells of *R. spheroides* (2) and *S. cerevisiae* (20). It is possible that these stimulations are also due to hydrogen peroxide formed as a result of microbial oxidase (1) and/or superoxide dismutase activities (15).

As part of an overall investigation into the regulation of catalase synthesis in non-photosynthetic bacteria, the role, if any, of environmental pH variations and the possible stimulatory effects of hydrogen peroxide on catalase synthesis in *S. typhimurium* were examined.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. typhimurium* LT2, *E. aerogenes* N.C.B. 418 (syn. *Aerobacter aerogenes*) and *Escherichia coli* K-12 were used. The cultures were usually grown in 0.2% (wt/vol) glucose minimal medium containing per liter of 0.05 M sodium-potassium phosphate buffer, pH 7.0: 1 g of NH_4Cl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 10 mg of $FeSO_4 \cdot 7H_2O$, and 10 mg of $CaCl_2 \cdot 5H_2O$.

The salts portion of the medium specified by McCarthy and Hinshelwood (14) was used in the simulation of their experiment.

Inocula consisted of cells pregrown at the experimental temperature in the experimental medium but with a reduced amount of glucose (0.02% wt/vol). Cultures were usually grown in 100-ml volumes in 250-ml Erlenmeyer flasks incubated in rapidly (200 oscillations/min) shaking water baths. Growth was

followed by measurements of optical density at a wavelength of 660 nm using a Unicam S.P. 500 spectrophotometer. The temperature of growth was thermostatically regulated to within 0.1 C of the stated temperature. In experiments which required a constant pH, 1 M NaOH was automatically added on demand from a burette utilizing a radiometer automatic titrator unit.

Assay of catalase. The polarographic method of Rorth and Jensen (18) as modified for whole cells (P. M. Dempsey, M.Sc. thesis, University College, Cork, Ireland, 1970) was used. Catalase activity was related to the increase in dissolved O_2 level on addition of the enzyme to aqueous liquids containing hydrogen peroxide. The assay mixture consisted of 0.88 mmol of hydrogen peroxode in 0.05 M sodium-potassium phosphate buffer (pH 7.0) and untreated bacterial culture in a final volume of 3 ml. The assay was started by the addition of substrate in a volume of 0.1 ml as soon as a dissolved O_2 equilibrium was established.

A unit of catalase (e.u.) is defined as the amount of enzyme which decomposes 1.0 μmol of H_2O_2 per min at 25 C under the specified conditions of the assay.

Hydrogen peroxide assay. The method used was a modification of that of Dempsey et al. (5). A Beckman oxygen analyzer (model 1008) was used to measure the increase in dissolved O_2 content of media containing H_2O_2 on addition of catalase. A 3.95-ml portion of membrane-filtered medium containing H_2O_2 was added to a specially constructed glass cell in which the electrode of the oxygen analyzer was mounted. On establishment of a steady dissolved O_2 reading, 300 U of purified beef liver catalase (Sigma London Chemical Co.) in a volume of 0.05 ml was added. The immediate increase in dissolved O_2 content was recorded, and the molarity of the H_2O_2 was calculated from a standard curve relating the concentration of H_2O_2 broken down to the increase in dissolved O_2 content of similar media. Concentrations of H_2O_2 as low as 1 to 2 μM are detectable by this modification.

RESULTS

Pattern of catalase specific activity during growth. The characteristic pattern of catalase specific activity of *S. typhimurium* LT2 growing in a minimal glucose medium from an overnight inoculum is shown in Fig. 1. During the logarithmic growth phase, the activity fell rapidly from an initial level of 10 enzyme units to reach a minimum of 0.25 towards the end of the log phase. During the transition period between the end of the log phase and establishment of the stationary phase, a rapid increase in activity was observed which continued into the stationary phase. Addition of chloramphenicol (200 μg/ml) to the culture at the onset of the stationary phase prevented the increase in catalase activity, indicating that the increase is due to de novo synthesis of enzyme. The stationary phase in this experiment was due to carbon source limitation. The initial high catalase activity is due to the use of overnight cultures as inocula which, invariably, were in the stationary phase of growth. Similar patterns of catalase synthesis were observed in cultures of *E. coli* (see Fig. 5) and *E. aerogenes* (see Fig. 6).

Coincidence of the increase in catalase synthesis with a decrease in the pH of the growth medium. The coincidence of the increase in catalase synthesis with a decrease in the pH of the growth medium is shown in Fig. 2. The time of initiation of catalase synthesis coincided approximately with the time that the pH of the culture medium reached its lowest value, 6.65. When a medium with reduced buffer capacity (0.01 M instead of 0.05 M) was used, a sharp fall in pH at the onset of stationary phase from 6.5 to 4.1 was observed. The increase in catalase synthesis coincided with the decrease in pH of the medium. Similarly, when the initial pH of the medium was adjusted from 7.0 to 6.6, a sharp pH decrease was noted from 6.45 to 5.5 during the transition from logarithmic to stationary phase. Again, the increase in catalase synthesis occurred at this time. It is clear, therefore, that the increased synthesis of

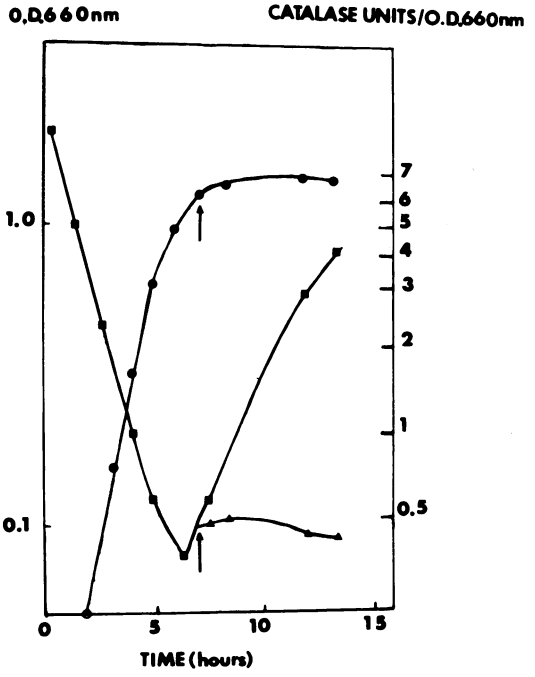

FIG. 1. *Pattern of catalase activity of S. typhimurium LT2. Chloramphenicol was added to a portion of the culture at the time indicated by the arrow. Symbols:* ●, *growth at optical density (OD) of 660 nm;* ■, *catalase specific activity (units/OD_{660});* ▲, *catalase specific activity (units/OD_{660}) after chloramphenicol addition.*

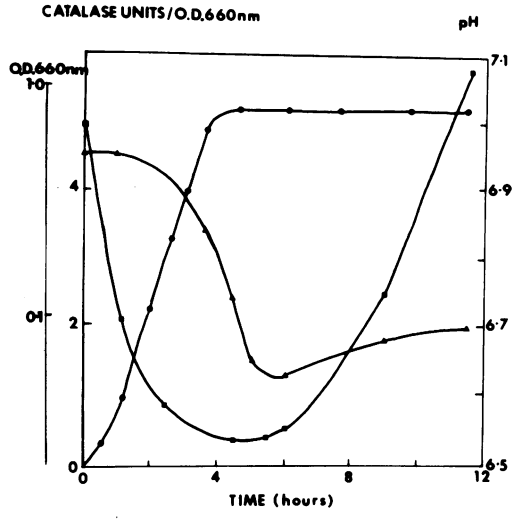

FIG. 2. *Coincidence of the increase in catalase synthesis with a decrease in pH of the culture of S. typhimurium LT2. Symbols:* ●, *growth at optical density (OD) of 660 nm;* ■, *specific activity of catalase (units/OD_{660});* ▲, *pH.*

catalase coincided with the lowest pH value reached by the culture irrespective of the actual pH value itself.

Effect of a sudden decrease in pH during the exponential growth phase on catalase synthesis. If the synthesis of catalase at the end of the log phase were stimulated by the fall in pH, one might expect that the addition of acid during the exponential phase would stimulate synthesis of the enzyme. The pH of a culture of *S. typhimurium* in the exponential phase was decreased sharply, by the addition of HCl, from 6.8 to 6.18. The effect of the fall in pH on growth rate was negligible (Fig. 3). The pH pattern of the HCl culture was similar to that of the control except that it occurred at a lower pH. No stimulation of catalase activity occurred on shift to the lower pH even though the pH immediately after HCl addition (6.18) was almost identical to the lowest pH reached by the control culture. The increase in specific activity of catalase in the HCl culture did not occur for more than 2 h after the pH shift, when the culture entered the stationary phase at a pH of 5.44.

Synthesis of catalase by cultures maintained at constant pH. The effect of maintaining the pH of the medium constant at 7.0 during the growth of *S. typhimurium* LT2 was determined by dividing a culture after inoculation into two parts. Neutrality was maintained at 7.0 in one by addition on demand of NaOH from an automatic titrator. The other part served as a normal uncontrolled pH culture. Despite maintaining neutrality, the normal pattern of catalase specific activity was obtained (Fig. 4). Synthesis of catalase occurred in the stationary growth phase. The activity followed a similar pattern to that of the control culture. As these results differed completely from those obtained by McCarthy and Hinshelwood (14) working with *E. aerogenes*, the experiment was repeated using a strain of *E. coli* K-12 (Fig. 5) and the strain of *E. aerogenes* used in the McCarthy and Hinshelwood (14) experiments (Fig. 6). In both cases, the pattern of catalase synthesis in the constant pH culture was similar to that of the uncontrolled pH culture. Maintaining the pH at neutrality did not prevent catalase synthesis at the end of the logarithmic growth phase. In the experiment with *E. aerogenes* (Fig. 6), the cultivation conditions (medium, aeration, temperature, etc.) used by McCarthy and Hinshelwood (14) were followed as closely as possible.

Effect on catalase synthesis of the addition

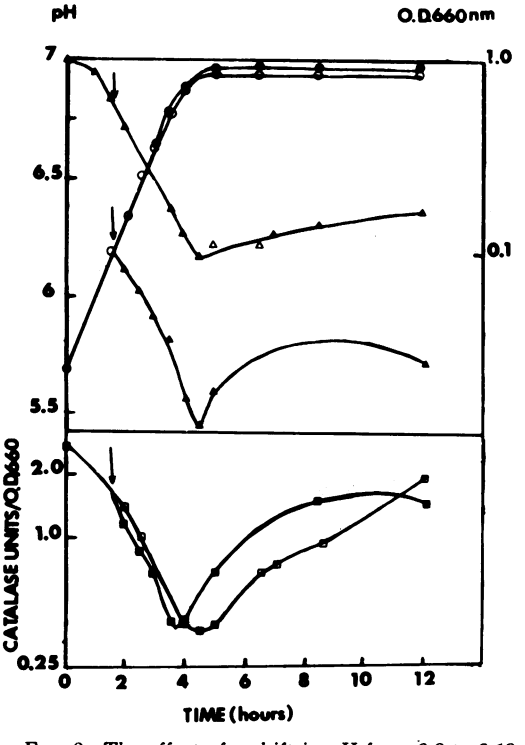

FIG. 3. *The effect of a shift in pH from 6.8 to 6.18 on catalase synthesis during exponential growth of a culture of S. typhimurium LT2. The addition of HCl is indicated by the arrows. Symbols: growth at optical density (OD) of 660 nm of control* (○) *and of pH shift culture* (●); *pH of control* (△) *and of pH shift culture* (▲); *specific activity of catalase (units/OD_{660}) of control* (□) *and of pH shift culture* (■).

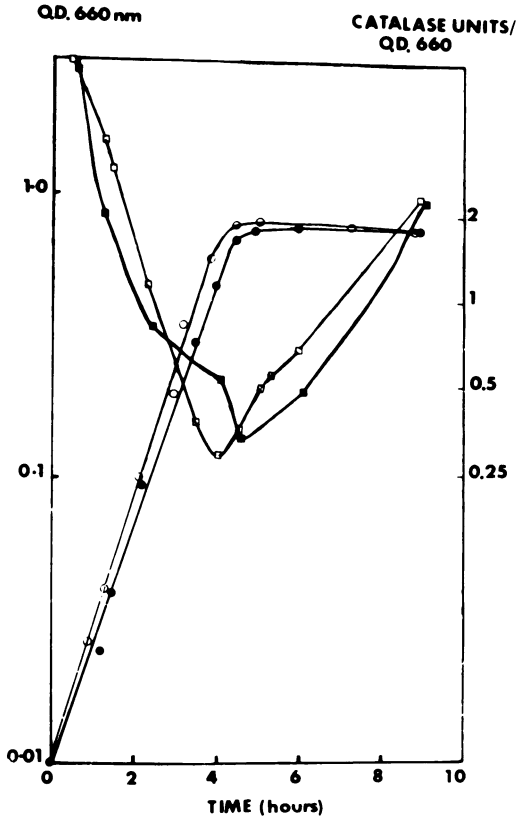

FIG. 4. *The effect on catalase synthesis of maintaining the pH at 7.0 during the growth of S. typhimurium LT2. The pH was maintained constant in one portion of the culture by the addition of NaOH on demand. Symbols: growth at optical density (OD) of 660 nm of control* (○) *and of neutralized culture* (●); *specific activity of catalase (units/OD_{660}) of control* (□) *and of neutralized culture* (■).

of H_2O_2 to exponentially growing cultures of S. typhimurium LT2. To test the hypothesis that possible H_2O_2 accumulation at the end of the logarithmic growth phase is the stimulus needed for catalase synthesis, H_2O_2 was added during the logarithmic phase of cultures of *S. typhimurium* LT2 growing in a minimal glucose medium at 37 C. Cultures in the exponential growth phase were divided into two parts: H_2O_2 being added to one part and the other part serving as a control. A range of H_2O_2 concentrations from 0.1 μM to 10 mM were used in this series of experiments. A typical result of such an experiment is shown in Fig. 7a, where H_2O_2 was added at a concentration of 80 μM and caused a stimulation of catalase synthesis. Concentrations of H_2O_2 from 1 μM to 2 mM (e.g., Fig. 7a, b, and c) stimulated synthesis of catalase in the logarithmic phase of growth. A concentration of 0.1 μM had no detectable effect, whereas an addition of 10 mM resulted in rapid lysis of the culture without an increase in the specific activity of catalase (Fig. 7d). The patterns of catalase synthesis on addition of H_2O_2 from 1 μM to 2 mM were essentially similar. Shortly after addition, the specific activity of catalase increased sharply and reached a maximum level, which varied with the concentration of H_2O_2 and then fell sharply again. In each case, where the cultures were assayed into the stationary phase, the usual increase associated with the start of stationary phase was obtained. Depending on the concentration, H_2O_2 also affected the growth of the culture. In the range 1 μM to 30 μM a slight increase in growth rate was observed, as can be seen in Fig. 7b where 10 μM

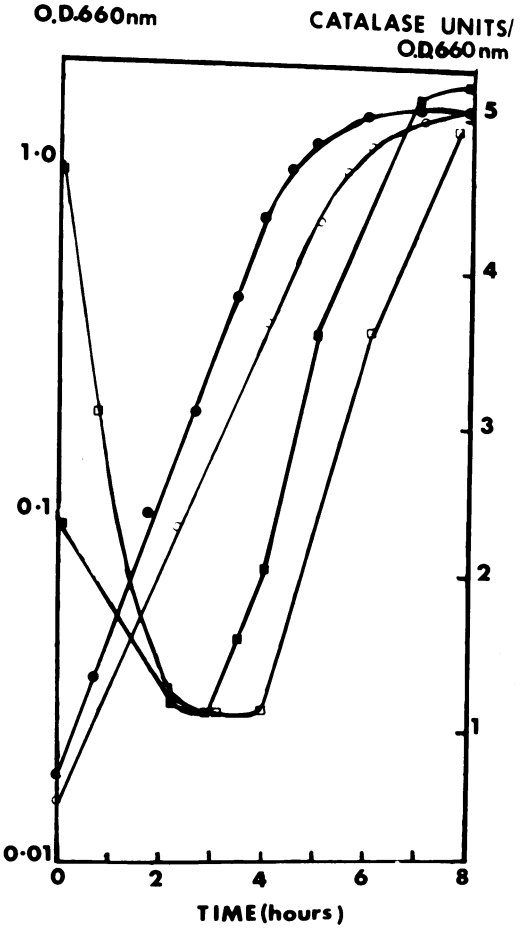

FIG. 5. *The effect on catalase synthesis of maintaining the pH at 7.0 throughout the growth cycle of a culture of E. coli. Symbols: growth at optical density (OD) of 660 nm of control* (○) *and neutralized culture* (●); *specific activity of catalase (units/OD_{660}) of control* (□) *and of neutralized culture* (■).

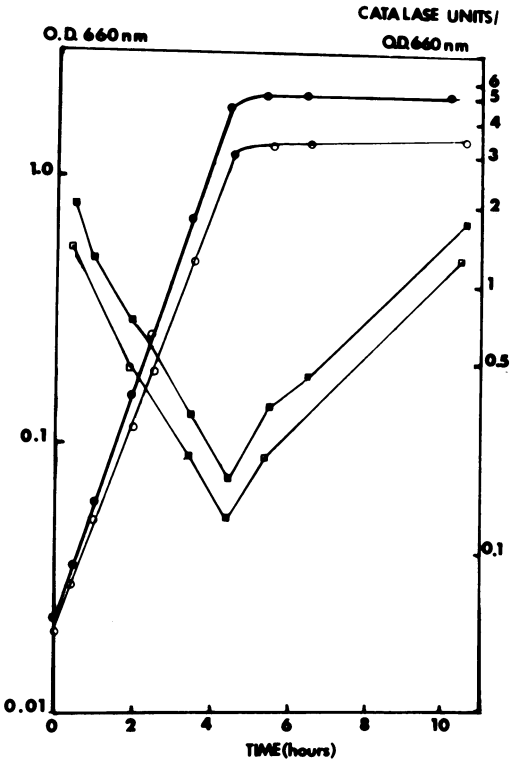

FIG. 6. *The effect on catalase synthesis of maintaining the pH at 7.0 throughout the growth cycle of a culture of E. aerogenes in the minimal medium of McCarthy and Hinshelwood (14) containing 0.2% (wt/vol) glucose at 40 C. Symbols: growth at optical density (OD) of 660 nm of control (○) and of neutralized culture (●); specific activity of catalase (units/OD_{660}) of control (□) and of neutralized culture (■).*

H_2O_2 was added. Concentrations of from 50 to 100 μM caused a slight inhibition of growth (e.g., Fig. 7a). Further increases in the concentrations of H_2O_2 had a drastic effect on the growth rate, as shown in Fig. 7c. Here 1 mM H_2O_2 was added and growth ceased for 90 min. Growth then resumed at a rate nearly five times less than that of the normal. No resumption in growth was observed after 6 h in a culture to which 2 mM H_2O_2 was added, whereas rapid death of the culture resulted when the H_2O_2 concentration used was 10 mM (Fig. 7d).

In Fig. 8, the maximum specific activity of catalase detected, on addition of H_2O_2, in the exponential growth phase is plotted as a function of the concentration of H_2O_2 used. The highest specific activity was reached with 80 μM H_2O_2.

Time interval between the addition of H_2O_2 in the exponential growth phase and the detection of the increased level of catalase. An attempt was made to estimate the time it takes for the H_2O_2 stimulation to cause an increase in catalase synthesis. Samples of a growing culture of *S. typhimurium* LT2 in minimal glucose medium at 37 C were assayed 2, 5, 10, 15, 20, and 25 min subsequent to the addition of H_2O_2 to a concentration of 80 μM, the level which causes the maximum amount of stimulation (Fig. 8). The results in Fig. 9 show that the specific activity of catalase continued to fall 10 to 15 min after addition of H_2O_2; the first increase in activity over the control culture was detected 15 min after the addition.

Coincidence of catalase synthesis with H_2O_2 accumulation. Several attempts were made to detect and measure H_2O_2 in the culture fluid of *S. typhimurium*. In normally growing cells H_2O_2 was detected but only at low transient concentrations (Fig. 10). In such experiments the concentration of H_2O_2 in the culture fluid at the end of the stationary phase was no greater than at various times during exponential growth. Occasionally, in cultures which were not growing properly, an abnormal accumulation of H_2O_2 was noted towards the end of the exponential growth phase.

DISCUSSION

The variation in catalase activity during growth of *S. typhimurium*, *E. coli*, and *E. aerogenes* reported here is similar to that observed by other workers (1, 10, 11, 14, 21; Dempsey and Condon, 1971, Irish J. Ag. Res. **10**:iv).

The increase in synthesis of catalase at the start of the stationary phase coincided with a decrease in the pH of the culture medium. This decrease is due to inadequate buffering of the medium rather than increased capacity for acid production by the cells at the end of the log phase. McCarthy (13) working with asparagine deaminase and McCarthy and Hinshelwood (14) with catalase observed a similar coincidence of enzyme synthesis with a decrease in the pH of *E. aerogenes* cultures. These workers reported that synthesis of these enzymes in the stationary phase could be indefinitely prevented by maintaining the culture pH at neutrality. Although they showed that decreased pH did not cause an increase in the specific activity of catalase of washed cells in buffered saline, they suggested that a decrease in pH might indirectly stimulate cells to produce catalase, possibly through increased hydrogen peroxide formation. However, they could not detect hydrogen peroxide formation in their

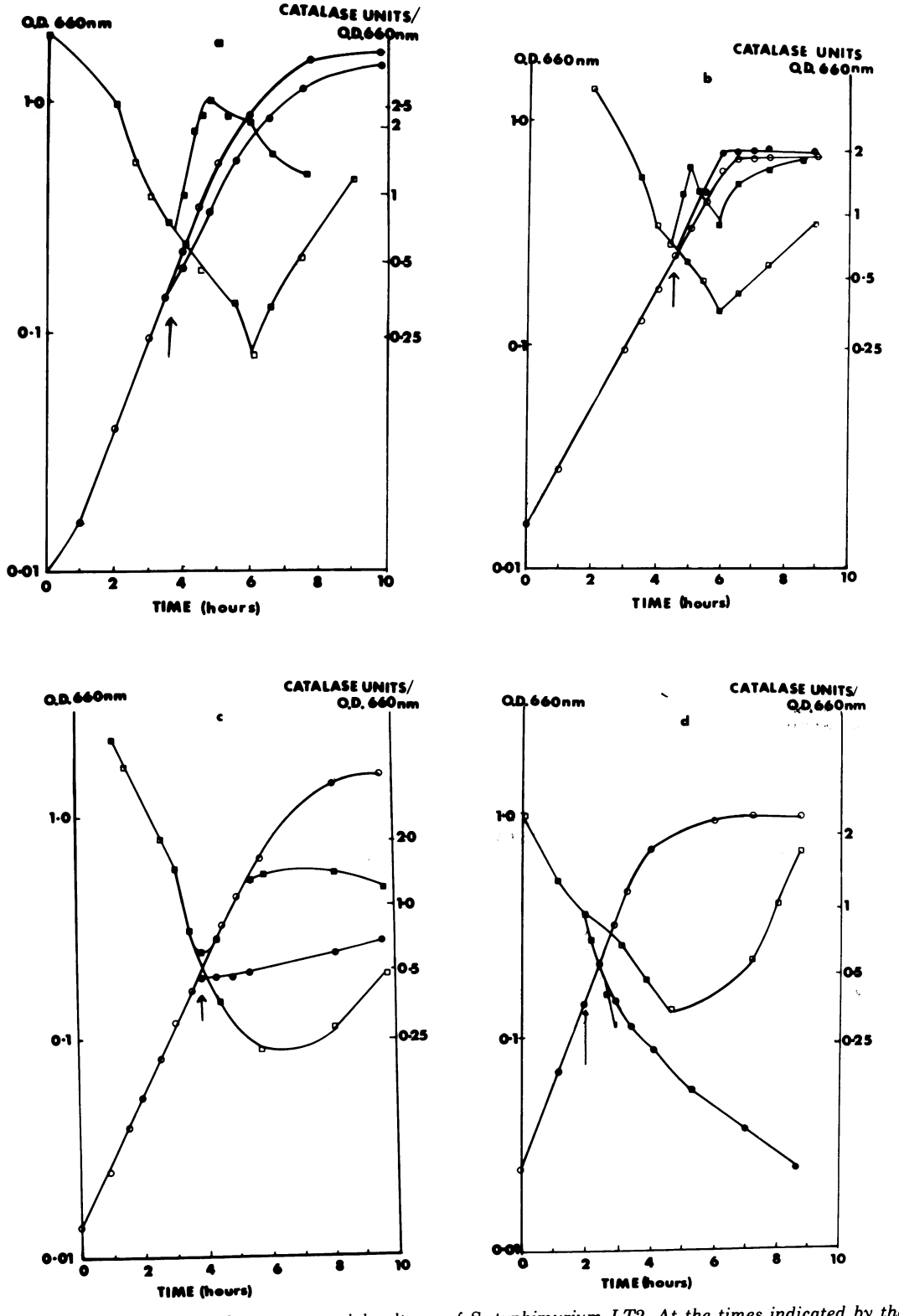

FIG. 7. *Addition of H_2O_2 to exponential cultures of S. typhimurium LT2. At the times indicated by the arrows the cultures were divided and H_2O_2 was added, to one portion, to concentrations of 80 μM (a), 10 μM (b), 1.0 mM (c), and 10 mM (d). The other portion was maintained as a control. Symbols: growth at optical density (OD) of 660 nm of control, ○; of culture receiving H_2O_2, ●. Catalase (units/OD_{660}) of control, □; of culture receiving H_2O_2, ■.*

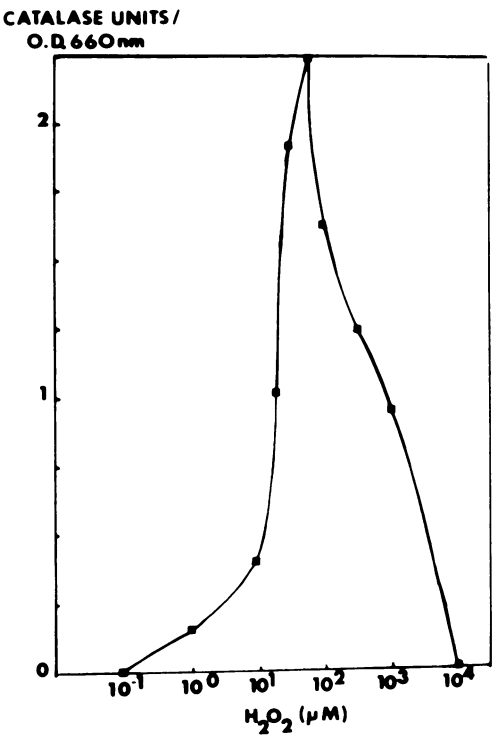

FIG. 8. *The relationship between H_2O_2 concentration added to exponential cultures of S. typimurium LT2 and the increase in the specific activity of catalase.*

cultures. Our results differ with those of McCarthy and Hinshelwood (14). Working principally with *S. typhimurium* LT2 we have not found any effect of a decrease in pH on the pattern of catalase synthesis. A sudden substantial shift in pH during exponential growth did not stimulate catalase synthesis. Neither did maintenance of the culture pH at neutrality have any significant effect on the characteristic pattern of synthesis in *S. typhimurium*, *E. coli*, or in the strain of *E. aerogenes* used by McCarty and Hinshelwood (14). In the case of the *E. aerogenes* experiment, the growth conditions, such as salts composition of the medium, and incubation temperature of McCarthy and Hinshelwood (14) were adhered to as much as possible. The concentration of the glucose used by McCarthy and Hinshelwood (14) is not clear to us. However, a series of experiments in this laboratory with *S. marcescens* (Dempsey, unpublished data) indicate that the normal pattern of catalase synthesis is obtained in cultures grown in minimal media at the expense of glucose at concentrations up to 1% (wt/vol). We must conclude that the result obtained by McCarthy and Hinshelwood (14) is due to some specific condition of cultivation which we are unable to simulate and that the regulation of catalase synthesis is independent of the pH of the culture.

It is commonly believed that the physiological function of catalase is to prevent H_2O_2 accumulation to inhibitory levels. Until recent years the absence of catalase from obligate anaerobes was favorably entertained as a reason for their sensitivity to O_2. Superoxide dismutase, which converts the O_2^- radical (an extremely reactive product of O_2 metabolism) to H_2O_2 (15), is now considered the more essential enzyme for aerobic growth. This enzyme is absent from most obligate anaerobes but (with one exception) present in aerotolerant catalase-negative bacteria such as the lactic acid bacteria (16). Ten of 17 mutants of *E. coli* which were obligately anaerobic at 42 C but facultative at 30 C were found to be deficient in superoxide dismutase (6). Although these data strongly support the necessity for superoxide dismutase for aerobic growth, they do not rule out the necessity for catalase or peroxidase to control the concentration of H_2O_2 in cells as a consequence of oxidase and/or superoxide dismutase

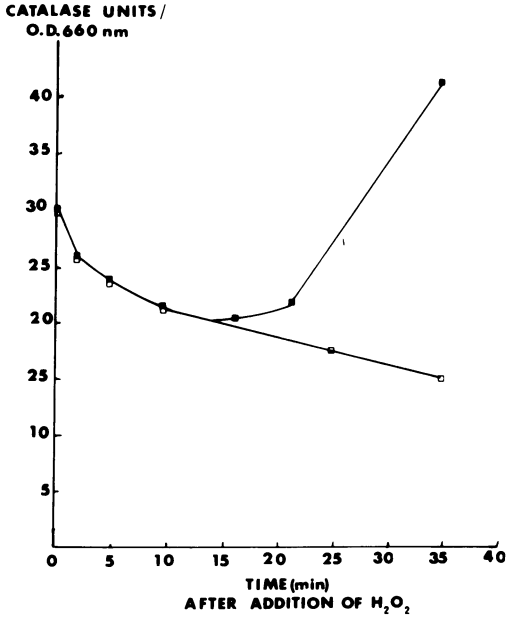

FIG. 9. *An estimate of the time interval between the addition of H_2O_2 (80 μM) to exponential cultures of S. typhimurium LT2 and the increase in catalase specific activity. A culture was divided at zero time. H_2O_2 was added to one portion (■) whereas the other was maintained as a control (□).*

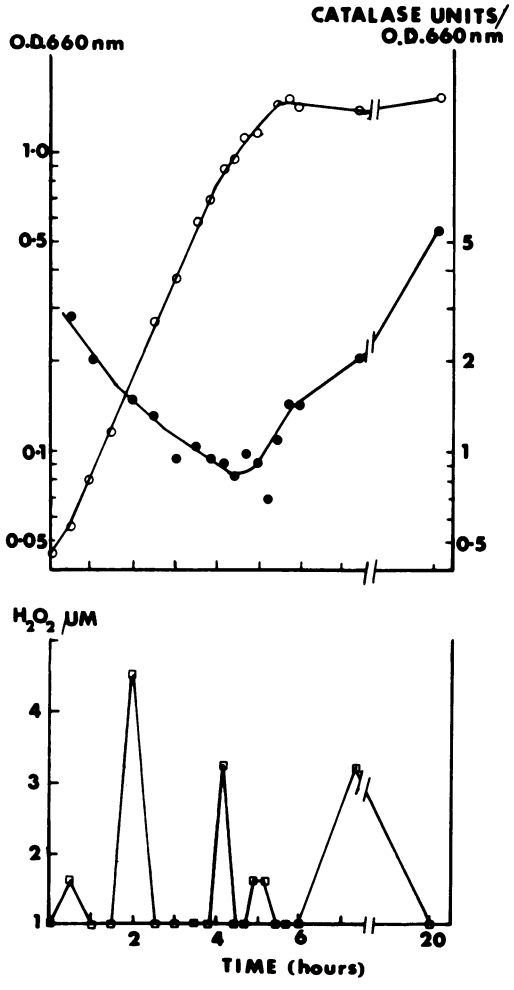

FIG. 10. *Secretion of H_2O_2 by a culture of S. typhimurium LT2. Symbols: growth at optical density (OD) of 660 nm, O; catalase units/OD_{660}, ●; H_2O_2 concentration, □.*

activity during aerobic metabolism. The accumulation of H_2O_2 in certain aerobically grown strains of group N streptococci in milk resulted in inhibition of growth and viability (1, 5), even though members of this group of bacteria have been shown to have superoxide dismutase (16). The peroxidase produced by such strains is, presumably, unable to cope with high concentrations of H_2O_2 produced during aerobic growth. It is reasonable to expect that a bacterial strain which is superoxide dismutase positive, but lacking catalase and peroxidase, would be inhibited during aerobic growth as a consequence of H_2O_2 accumulation. That catalase is synthesized in bacteria in response to H_2O_2 accumulation is therefore a plausible hypothesis.

Addition of H_2O_2, within the concentration range of 1 μM to 2 mM, in the exponential phase of growth to cultures of *S. typhimurium* stimulated catalase synthesis. Maximum levels of catalase were detected when the concentration of H_2O_2 added was 80 μM (Fig. 8). It should be remembered that these data refer to intracellular catalase content which could reflect a balance between synthesis and destruction of catalase. This is especially true at high concentrations of H_2O_2 which inhibit various growth processes including catalase activity. Failure of H_2O_2 concentrations greater than 80 μM to elicit accumulation of the maximum catalase level does not necessarily mean a specific failure to stimulate maximum catalase synthesis but may be due to general failure of metabolism or a specific destruction of catalase synthesized. From these results, it might appear that the substrate H_2O_2 is capable of direct induction of catalase synthesis. However, the addition of H_2O_2 is not the only way that logarithmic phase cells can be stimulated to produce greater catalase activity. Aeration of anaerobically grown cultures of the photosynthetic *R. spheroides* (2) and *S. cerevisiae* (20) also caused an increase in catalase activity. This might be explained by assuming that these cells have oxidase and/or superoxide dismutase enzymes and that H_2O_2 accumulates as a consequence of aeration, just as occurs in the essentially fermentative streptococci (1, 5).

Other observations of increases of catalase synthesis do not, however, readily fit a hypothesis of H_2O_2 induction. Kovacs et al. (9) showed that the catalase synthesis increase, in aerobically growing logarithmic-phase cells of *Staphylococcus aureus*, coincided with a fall in oxidation-reduction potential of the medium. A subsequent study (17) showed that glutathione additions to *S. aureus* cultures, which caused a decrease in oxidation-reduction potential of the culture, resulted in earlier increased synthesis of catalase. It is difficult to explain H_2O_2 accumulation under these reducing conditions. To reconcile these observations, at least two hypothesis should be considered: (i) that H_2O_2 is not itself the direct inducer of catalase synthesis, and that the direct inducer is synthesized after stimulation by H_2O_2 and by other circumstances (e.g., sudden drop in oxidation-reduction potential of the medium); or (ii) that H_2O_2 is only one of at least two direct inducers of catalase synthesis.

According to current concepts of induction

mechanisms, the first hypothesis is the more probable. There is a certain amount of evidence which suggests that H_2O_2 is not the direct inducer of catalase synthesis. A lag of 10 to 15 min after addition of H_2O_2 was observed in *S. typhimurium* before catalase synthesis was detected (Fig. 9). This time lag between addition of inducer and detection of new enzyme is longer than generally accepted for enteric bacteria. For example, induction of β-galactosidase takes only 80 to 90 s in *E. coli* (8, 12). About 10 min therefore could be available for synthesis of the direct inducer after addition of H_2O_2. In support of this hypothesis, Clayton (4) and Shanmugan and Berger (19) showed that, after the addition of H_2O_2 to photosynthetically grown *R. spheroides*, catalase synthesis was delayed for at least 7 min and then continued for at least another 20 to 30 min. The H_2O_2 would most likely be broken down by residual catalase and peroxidase in the first 2 min after addition (4). Contact with the inducer during the period of initiation of messenger ribonucleic acid synthesis is essential, according to current ideas on induction of enzyme synthesis. Unless the messenger ribonucleic acid for catalase is unusually stable, it is difficult to explain how H_2O_2 present for about 2 min could continue to stimulate synthesis of catalase 20 min after its destruction. Although the evidence is circumstantial, it is quite possible, as originally suggested by Clayton (4), that some compound, synthesized on addition of H_2O_2 to exponentially growing cells, is the actual inducer of catalase synthesis.

The increase in catalase synthesis at the end of the logarithmic phase has still to be satisfactorily explained. If H_2O_2 or some product of H_2O_2 metabolism is a physiological inducer of catalase synthesis, one might expect an accumulation of H_2O_2 in cultures at the end of the exponential growth phase coincident with the increase in catalase synthesis. Low concentrations of H_2O_2 were detected in *S. typhimurium* cultures during this study (Fig. 10). The H_2O_2 produced was rapidly lost from the cultures indicating a balance between synthesis of H_2O_2 and its breakdown. Considering the data of Fig. 7 and 8, the low transient concentrations of H_2O_2 detected in the culture fluid would be sufficient to stimulate synthesis of small quantities of catalase. However, such small amounts of catalase synthesis would not be enough to offset the overall reduction in the specific activity of catalase as a consequence of exponential growth. Efforts to demonstrate significant accumulation of H_2O_2, coincident with the increase in catalase synthesis at the onset of the stationary phase, in general met with failure. An accumulation of approximately 30 μM was detected in a few initial experiments but on repetition such cultures were found to be growing unsatisfactorily. In cultures growing satisfactorily no significant accumulation of H_2O_2 was detected at the end of the exponential phase. There are two possible explanations. The H_2O_2 detected in these experiments is that which accumulates extracellularly. It is possible that the pattern obtained differs from that which represents intracellular synthesis of H_2O_2. H_2O_2 may accumulate to high intracellular concentrations at the end of the log phase but for some unknown reason this is not reflected in a corresponding high extracellular H_2O_2 concentration. The second possibility is that the increase in catalase synthesis at the end of the log phase does not require stimulation by H_2O_2. Further experiments are needed to decide between these two possibilities.

ACKNOWLEDGMENTS

We would like to express our appreciation to Pat Higgins and Liam Burgess for technical assistance.

LITERATURE CITED

1. Anders, R. F., D. M. Hogg, and G. R. Jago. 1970. Formation of hydrogen peroxide by group N streptococci and its effects on their growth and metabolism. Appl. Microbiol. **19**:608-612.
2. Clayton, R. K. 1960. The induced synthesis of catalase in *Rhodopseudomonas spheroides*. Biochim. Biophys. Acta **37**:503-512.
3. Clayton, R. K. 1960. Physiology of induced catalase synthesis in *Rhodopseudomonas spheroides*. J. Cell. Comp. Physiol. **55**:1-8.
4. Clayton, R. K. 1960. An intermediate stage in the H_2O_2-induced synthesis of catalase in *Rhodopseudomonas spheroides*. J. Cell. Comp. Physiol. **55**:9-14.
5. Dempsey, P. M., J. O'Leary, and S. Condon. 1975. Polarographic assay of hydrogen peroxide accumulation in microbial cultures. Appl. Microbiol. **29**:170-174.
6. Fridovich, I. 1974. Superoxide dismutase, p. 465. *In* O. Hayaishi (ed.), Molecular mechanisms of oxygen activation. Academic Press Inc., New York.
7. Gray, E. D., A. M. Haywood, and E. Chargaff. 1964. Rapidly labelled ribonucleic acids of *Rhodopseudomonas spheroides* under varying conditions of catalase synthesis. Biochim. Biophys. Acta **87**:397-415.
8. Kepes, A., and S. Beguin. 1966. Peptide chain initiation and growth in the induced synthesis of β-galactosidase. Biochim. Biophys. Acta **123**:546-560.
9. Kovacs, E., H. H. Mazarean, and A. Jaki. 1965. Untersuchungen uber den Zusammenhang zwischen Katalasesynthese und Atmungsintensitat in *Staphylococcus aureus* kulturen. Enzymologia **28**:316-321.
10. Kwiek, S., A. Gabrys, and R. Lorenc. 1970. Dependence of the activity of yeast catalase on yeast growth phases and culture conditions. Acta Microbiol. Pol. Ser. B **2**:111-114.
11. Kwiek, S., A. Gabrys, and J. Witecki. 1970. The effect of glucose and galactose on catalase activity of *Salmo-*

nella typhimurium in aerobic and anaerobic cultures. Acta Microbiol. Pol. Ser. B **2**:115-120.
12. Lacroute, F., and G. S. Stent. 1968. Peptide chain growth of β-galactosidase in *Escherichia coli*. J. Mol. Biol. **35**:165-173.
13. McCarthy, B. J. 1959. Variations in dehydrogenase and deaminase activity during bacterial growth. Proc. R. Soc. B **150**:410-422.
14. McCarthy, B. J., and C. Hinshelwood. 1959. Variations in catalase activity during a bacterial growth cycle. Proc. R. Soc. B **150**:13-23.
15. McCord, J. M., and I. Fridovich. 1968. The reduction of cytochrome C by milk xanthine oxidase. J. Biol. Chem. **243**:5753-5760.
16. McCord, J. M., B. B. Keele, Jr., and I. Fridovich. 1971. An enzyme-based theory of obligate anaerobiosis: the physiological function of superoxide dismutase. Proc. Natl. Acad. Sci. U.S.A. **68**:1024-1027.
17. Mazarean, H. H., and E. Kovacs. 1966. The effect of thiol compounds on catalase activity. Enzymologia **31**: 184-188.
18. Rorth, M., and P. K. Jensen. 1967. Determination of catalase activity by means of the Clark oxygen electrode. Biochim. Biophys. Acta **139**:171-173.
19. Shanmugan, K. T., and L. R. Berger. 1969. Mechanism of catalase induction in *Rhodopseudomonas spheriodes*: role of porphyrin excretion. Arch. Microbiol. **69**:197-205.
20. Sulebele, G. A., and D. V. Rege. 1968. The nature of the glucose effect on the induced synthesis of catalase in *Saccharomyces cerevisiae*. Enzymologia **35**:321-333.
21. White, D. C. 1962. Cytochrome and catalase patterns during growth of *Haemophilus parainfluenzae*. J. Bacteriol. **83**:851-859.