Gratuitous Synthesis of β-Lactamase in Staphylococcus aureus

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The synthesis of β-lactamase in response to 2-(2'-carboxyphenyl)-benzoyl-6-amino-penicillanic acid as inducer was studied in Staphylococcus aureus. The inducer was not detectably hydrolyzed by β-lactamase and had minimal antibacterial activity. The kinetics of induction showed a lag of 4 to 6 min in a nutrient broth medium and 8 to 12 min in a defined medium, followed by constant differential rates of synthesis of β-lactamase. The differential rate of β-lactamase synthesis in nutrient broth was unaltered by supplementing the medium with glucose, galactose, lactose, arabinose, glycerol, or sucrose. Variations in the partial pressure of oxygen did not alter the differential rate of synthesis of β-lactamase over the range 18 to 50% oxygen in nitrogen. Even when the rate of growth was considerably reduced by high-oxygen tension, the differential rate of synthesis of the enzyme remained the same. The differential rate of β-lactamase synthesis at low inducer concentration increased after a shift down in growth rate. The effect was observed with several inducers and under different nutritional conditions, but was always preceded by a change in growth rate. It is suggested that the change in growth rate itself causes the increase in differential rate of β-lactamase synthesis.

β-Lactamase hydrolyzes the β-lactam ring of benzylpenicillin and other penicillin derivatives. It is responsible, in large part, for the clinical resistance of Staphylococcus aureus to penicillin. The synthesis of β-lactamase was shown to be inducible in S. aureus by Geronimus and Cohen (5). However, the study of the induction of the enzyme has been hampered by the fact that available inducers were antibacterials and most were hydrolyzed by the enzyme. Pollock (11) developed a method for the induction of β-lactamase in Bacillus cereus in the absence of free penicillin. Cells of B. cereus irreversibly bind benzylpenicillin at 0°C, and subsequent growth of the cells in inducer-free medium results in the synthesis of β-lactamase. Such studies have shown that the lag before enzyme is synthesized is 15 min in B. cereus. Steinman (13) examined the conditions of induction of β-lactamase with benzylpenicillin in S. aureus. He showed that addition of benzylpenicillin to suspensions of cells in a Warburg apparatus stimulated the synthesis of β-lactamase. His experiments showed that, because of the strong antibacterial action of the inducer, there was an upper limit to the concentration of benzylpenicillin which would permit β-lactamase induction. There was also an upper limit to the concentration of cells which would allow induction of the enzyme.

Leitner et al. (8) used a system analogous to Pollock's cold pretreatment method to study the induction of β-lactamase in S. aureus. These authors added benzylpenicillin to growing cultures and destroyed it 10 min later either by addition of β-lactamase or by filtration of the cells and resuspension in fresh medium. By the use of such techniques, they were able to study the influence of pH and concentration of ferrous ion on the synthesis of β-lactamase. They were able to establish that the lag before enzyme is synthesized is 3 to 4 min in S. aureus and that it is not affected by pH. However, β-lactamase was shown to be synthesized for a much longer period of time in acidic medium than in neutral medium after inducer had been removed.

These studies suffer from the disadvantage that inducer is not present during the whole of the experiment. It was either progressively destroyed (13) or deliberately removed (8).

A new derivative of penicillanic acid has become available, which, although a good inducer of β-lactamase, has little antibacterial

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activity and is not hydrolyzed by the enzyme. This derivative, 2-(2’-carboxyphenyl)-benzoyl-6-amino penicillinic acid (CBAP), has allowed us to study the synthesis of β-lactamase in an inducible strain of \textit{S. aureus} under completely gratuitous conditions.

This paper describes the kinetics of gratuitous β-lactamase induction in \textit{S. aureus}, the effect of growth conditions on the differential rate of synthesis of the enzyme, and the effect of removal of inducer from growing cultures.

We have found that the differential rate of β-lactamase synthesis in cultures growing in low inducer concentration increases markedly at high cell densities. The conditions which bring about this increase are investigated and discussed. A preliminary report of some of our observations has already appeared (7).

**MATERIALS AND METHODS**

**Organism.** The strain of \textit{S. aureus} used is C23/19. This strain was isolated from a fatal case of staphylococcal pneumonia in Manchester Royal Infirmary, but has been kept for many years in the laboratory. It is resistant to several antibiotics, including benzylpenicillin, and is phage type 80/81.

**Growth media.** Nutrient broth and Robertson’s cooked meat medium were made up from dehydrated material (Oxoid) and autoclaved at 109 C. Glucose or other supplementary carbon source was autoclaved separately at 109 C and added to a final concentration of 0.2% (w/v).

The defined medium was developed from those of Fildes et al. (4) and Bonieci (2). It contained L-cystine and L-tryptophan (10^{-4} M); L-arginine, L-histidine and L-phenylalanine (2.5 \times 10^{-4} M); L-valine, L-leucine, glycine, L-glutamic acid, L-aspartic acid and L-proline (6.67 \times 10^{-4} M); KH2PO4 (5 \times 10^{-2} M) adjusted to pH 7.4 with NaOH; thiamine HCl, nicotinamide (400 μg each per liter); biotin (2 μg/ liter); glucose (1.2 \times 10^{-2} M); MgSO4 \cdot 7H2O (2.0 \times 10^{-4} M); Fe(NH4)2(SO4)3 (8 \times 10^{-4} M). The amino acids were autoclaved together with the phosphate buffer at 109 C. The other components were autoclaved together as a separate solution at 109 C. The two parts were combined when the solutions had cooled and the medium was used within 2 days.

**Storage of organism and preparation of inocula.** The culture was stored in Robertson’s cooked meat medium at 4 C. Once a month, a working stock culture was prepared in 8 ml of nutrient broth with no supplementary carbon source at 37 C without shaking. The cultures were stored at 4 C and all inocula were prepared from them.

For an experiment in nutrient broth-glucose, 0.5 ml of working stock culture was added to 100 ml of nutrient broth, with no added glucose, in a 500-ml conical flask. This was grown for 7 hr on an orbital shaker (L. H. Engineering, England) at 37 C, stored overnight at 4 C, and used the next day.

**Inocula for an experiment in defined medium were prepared by inoculating 0.5 ml of working stock culture into 100 ml of defined medium in a 500-ml conical flask. This was grown overnight (16 hr) on the orbital shaker at 37 C. Two further overnight passages from 0.5-ml inocula were carried out before the culture was used in an experiment. Between passages, the cultures were stored at 4 C.

**Growth of cells for enzyme studies.** Cells were grown under conditions of vigorous aeration in an apparatus similar to that described by Schlegel et al. (12), as modified in this department by Harvey et al. (6). The medium (500 ml) was contained in a 1-litre flask fitted with a side arm to facilitate sampling. Magnetic stirring drive assemblies were arranged to accommodate 5 or 10 flasks in a water bath held at constant temperature by Cricotherm IIA units (Shandon Scientific Co. Ltd., London). Aeration was effected by the stirring action of 43-mm magnetic bars encased in polypropylene (Gallenkamp, London). In all experiments, the vortex produced by stirring extended to the bottom of the flask and was there broken by the stirring bar, so that bubbles were introduced into the body of the medium (12). This arrangement allowed a number of cultures to be grown under constant and identical conditions of aeration, agitation, and temperature.

Samples (10 ml) were removed at intervals and pipetted onto 0.1 ml of chlorhexidine digluconate (1 mg/ml). After thorough mixing, the extinction at 590 nm for nutrient broth or 350 and 500 nm for defined medium was measured on a Spectronic 20 (Bausch & Lomb, Rochester, N.Y.) or an optically matched test tubes (105 × 13 mm). A portion (5 ml) of this sample was used for assay of β-lactamase. One unit, at optical density 500 nm, is equivalent to 100 μg of protein per ml of culture and, at 350 nm, is equivalent to 55 μg of protein per ml of culture. These relationships were derived by using cells grown in defined medium.

**Induction of β-lactamase.** Inducer was added to the cultures immediately after inoculation. CBAP was used at 7.25 μM for measurement of the differential rate of synthesis under different nutritional conditions, and at 7.25 \times 10^{-4} μM in experiments where a suboptimal inducer concentration was studied. CBAP concentrations were corrected for a relatively high content of penicilloic acid in the preparation. Concentrations of CBAP are taken as the difference between the iodine-reacting material before and after alkaline hydrolysis. Meticillin was used at a concentration of 2.5 μM, at which the cells showed considerable growth. Cephalosporin C was used at a concentration of 100 μM. At this concentration, growth was not inhibited, but cephalosporin C did not induce high levels of enzyme.

**β-Lactamase assay.** β-Lactamase was assayed on whole cells in which growth and β-lactamase synthesis had been stopped with chlorhexidine digluconate. One unit of activity is that amount of enzyme which catalyzes the hydrolysis of 1 μmole of phenoxyc penicillin in 1 min at 37 C and pH 7.0. The assay was started by adding the sample (5 ml) of treated cells to a solution (10 ml) of phenoxyc penicillin (4 \times 10^{-2} M) in phosphate buffer, pH 7.0 (2 \times 10^{-2} M). After 20 min
of incubation, a sample of the assay mixture was taken for penicilloic acid estimation as follows. A 5-ml amount of assay mixture was filtered through a membrane filter (0.2 μm pore size; Gelman Instrument Co., Ann Arbor, Mich.). The filtrate was immediately diluted 1:4 in acetate buffer (5 × 10^-2 M), pH 4.6. β-Lactamase is predominantly intracellular in this strain under the conditions of these experiments. The combination of filtration, dilution, and change in pH stopped β-lactamase activity.

The amount of penicilloic acid formed by hydrolysis of penoxypenicillin was determined by the decolorization of a starch-iodine solution. The degree of decolorization was measured by using a Technicon autoanalyser (Technicon Instrument Co., Ltd., Chertsey, Surrey, England). Diameters of the tubing used were as follows: sample line, 0.05 cm; reagent line, 0.165 cm; air line, 0.102 cm. The sample was mixed with the starch-iodine reagent, and the mixture was passed through a series of coils, so that the extinction at 550 nm was not measured until 15 min had elapsed. The extinction was recorded on a chart recorder (Technicon Instrument Co.) and the samples were compared with standard solutions of penicilloic acid prepared from penoxypenicillin by alkaline hydrolysis.

Starch-iodine reagent was made up as follows. Solution A: 50 ml of 1 N sodium hydroxide and 100 ml of 1 N acetic acid were added to approximately 1 litre of distilled water. A 1.5-ml amount of 0.1 N iodine in 2% (w/v) potassium iodide was added. Solution B: 3 g of hydrolyzed starch (Connaught Laboratories, University of Toronto) was suspended in 20 ml of water and added to 200 ml of boiling water; then 1.5 g of potassium iodide was added.

When the solutions had cooled, solution B was added to solution A and the volume was made up to 2 litres with distilled water.

Removal of inducer from cultures. A sample of the culture was filtered through a membrane filter (0.2 μm pore size; Gelman Instrument Co.), washed with two 5-ml samples of inducer-free medium, and sucked not quite dry. The filter was placed in a flask of fresh medium containing no inducer; vigorous stirring released the cells. Growth and β-lactamase were then measured at intervals.

Materials. All chemicals used were of Analar grade and obtained from B.D.H., Poole, England, except the following. Amino acids of Japanese biosynthetic origin were obtained from T. J. Sas and Son Ltd., London.

Penoxypenicillin, CBAP, and chlorhexidine digluconate (Hibitane) were gifts from Imperial Chemical Industries, Macclesfield, Cheshire, England.

Membrane filters and filter holders (Gelman Instrument Co.) were obtained from Camlab, Cambridge, England, and modified in this department.

Oxygen and oxygen-free nitrogen were obtained from British Oxygen Co. Ltd., Glasgow.

RESULTS

Resistance of the inducer, CBAP, to enzymatic hydrolysis. CBAP did not alter the rate of hydrolysis of penoxypenicillin by an induced culture of S. aureus (Table 1). In addition, CBAP was not hydrolyzed by an induced culture of S. aureus, even after 6 hr of incubation at 37 C.

Effect of CBAP on the growth of S. aureus. Increasing concentrations of CBAP reduced the yield of cells slightly (Table 2), but did not affect the rate of growth of the cells appreciably. The differential rate of β-lactamase synthesis during logarithmic growth increased to a maximum with increasing concentrations of CBAP (Table 2). The response follows typical saturation kinetics. At 7.25 μM, the concentration used routinely, the differential rate was maximal. At this concentration, there was little effect on the growth rate of S. aureus.

Differential rate of synthesis of β-lactamase under different growth conditions. Growth of S. aureus in the presence of CBAP in nutrient

<table>
<thead>
<tr>
<th>TABLE 1. Rate of enzymatic hydrolysis of penoxypenicillin and CBAP[a]</th>
<th>Substrate</th>
<th>Penicillioic acid produced (μmole/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoxypenicillin (20 mM)</td>
<td>0.39</td>
<td>Nil</td>
</tr>
<tr>
<td>CBAP (3.5 mM)</td>
<td>Nil</td>
<td>0.40</td>
</tr>
<tr>
<td>Phenoxypenicillin (20 mM) +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBAP (3.5 mM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[a] The incubation mixture contained phosphate buffer, pH 7.0 (20 mM), chlorhexidine digluconate (10 μg/ml), and induced cells (100 μg of protein/ml); hydrolysis was allowed to proceed at 37 C. CBAP concentrations were measured by its capacity to react with iodine after alkaline hydrolysis.

<table>
<thead>
<tr>
<th>TABLE 2. Effect of inducer (CBAP) on growth on S. aureus[a]</th>
<th>CBAP concn (μl)</th>
<th>MGT[b] (min)</th>
<th>Yield (OD 500 nm)</th>
<th>Differential rate of β-lactamase synthesis[c]</th>
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</thead>
<tbody>
<tr>
<td>Nil</td>
<td>22.5</td>
<td>1.80</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>0.07</td>
<td>22.5</td>
<td>1.80</td>
<td>0.18</td>
<td></td>
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<tr>
<td>0.29</td>
<td>22.5</td>
<td>1.80</td>
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</tr>
<tr>
<td>1.45</td>
<td>23.0</td>
<td>1.65</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>4.35</td>
<td>23.0</td>
<td>1.45</td>
<td>1.75</td>
<td></td>
</tr>
<tr>
<td>7.25</td>
<td>24.0</td>
<td>1.30</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td>14.50</td>
<td>25.0</td>
<td>1.20</td>
<td>1.83</td>
<td></td>
</tr>
</tbody>
</table>

[a] Cells of S. aureus were grown in nutrient broth containing 0.2% (w/v) glucose. Growth was followed by measuring the extinction at 500 nm in a spectrophotometer.

[b] Mean generation time, measured during the initial rapid logarithmic growth rate.

[c] β-Lactamase (units) per increase in turbidity (OD 500 nm).
broth supplemented with glucose, galactose, glycerol, arabinose, sucrose, or lactate, at 0.2% (w/v) and in nutrient broth alone gave the same differential rate of synthesis of β-lactamase in all cases. Variations in aerobiosis by alteration of the partial pressure of oxygen in the gas phase did not alter the differential rate of synthesis in defined medium. At high partial pressure of oxygen (50%), the growth rate was considerably reduced, but the differential rate of β-lactamase synthesis remained unaltered (Table 3).

**Induction kinetics.** The induction kinetics in nutrient broth-glucose (Fig. 1) showed a lag of 4 to 6 min before enzyme appeared; in defined medium, the lag was longer (about 8 to 12 min). The length of the lag, therefore, appeared to depend on the medium but, at saturating concentrations of CBAP (7.25 μM), it was followed by a high differential rate of β-lactamase synthesis. A low CBAP concentration (7.25 × 10⁻² μM) induced a low differential rate of synthesis but, as the cell density increased and growth rate slowed down, the differential rate of enzyme synthesis increased abruptly (Fig. 2). Cohen and Monod (3) have attributed similar, but not identical, kinetics to the synthesis of a permease which transports inducer into the cell. If such a permease for CBAP existed, it would presumably be induced simultaneously with β-lactamase. Accordingly, we measured the differential rate of β-lactamase synthesis in high (7.25 μM) and low (7.25 × 10⁻² μM) CBAP concentration by cells previously grown in the presence of CBAP (7.25 μM). Preinduced cells established a high differential rate of β-lactamase synthesis from immediately after addition of 7.25 μM inducer (Fig. 3) and showed only the basal rate in the absence of inducer.

These same preinduced cells established a low differential rate in 7.25 × 10⁻² μM CBAP which increased abruptly at high cell density (Fig. 3). This was exactly the same result obtained with cells which were not preinduced. Preinduction, therefore, does not affect the result.

These cultures grew logarithmically until just before the increase in differential rate of enzyme synthesis. The end of the initial fast logarithmic growth rate is indicated in Fig. 2 by an arrow on the growth curve and on the plot of enzyme versus turbidity. These growth curves are typical of the results obtained in all our experiments. There was always an initial fast logarithmic growth rate which changed to a slower growth rate at high cell density. On all subsequent plots of the differential rate of β-lactamase synthesis, the point at which the growth rate changed is indicated by an arrow.

**Effect of pH.** In cultures growing in nutrient broth with glucose, the pH fell from 7.2 to 5.1 as the culture grew. To assess the effect of pH on enzyme synthesis, we compared a standard culture at low inducer level with one to which hydrochloric acid was added. Addition of acid reduced the pH to 5.3, and the growth rate immediately declined, but there was a lag before the differential rate of β-lactamase synthesis increased (Fig. 4). In the acid-treated culture, the faster rate of enzyme synthesis was established about a generation before it occurred spontaneously in the control. However, in a culture growing on nutrient broth, a similar increase in relative rate of enzyme synthesis was observed (Fig. 5), even though the pH did not fall below 7.0. A fall in pH is not, therefore, essential for an increase in differential rate of enzyme synthesis at low inducer concentration and high cell density. In all these experiments, the increased rate of enzyme

<table>
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<tr>
<th>Growth medium</th>
<th>Gas phase</th>
<th>Differential rate of β-lactamase synthesis</th>
<th>MGT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient broth</td>
<td>Air</td>
<td>1.87</td>
<td>25</td>
</tr>
<tr>
<td>Nutrient broth + glucose</td>
<td>Air</td>
<td>1.80</td>
<td>25</td>
</tr>
<tr>
<td>Nutrient broth + glycerol</td>
<td>Air</td>
<td>1.67</td>
<td>25</td>
</tr>
<tr>
<td>Nutrient broth + arabinose</td>
<td>Air</td>
<td>1.60</td>
<td>25</td>
</tr>
<tr>
<td>Nutrient broth + galactose</td>
<td>Air</td>
<td>1.67</td>
<td>25</td>
</tr>
<tr>
<td>Nutrient broth + lactose</td>
<td>Air</td>
<td>1.67</td>
<td>25</td>
</tr>
<tr>
<td>Nutrient broth + sucrose</td>
<td>Air</td>
<td>1.80</td>
<td>25</td>
</tr>
<tr>
<td>Defined medium + glucose</td>
<td>18% oxygen</td>
<td>1.60</td>
<td>69</td>
</tr>
<tr>
<td>Defined medium + glucose</td>
<td>30% oxygen</td>
<td>1.81</td>
<td>63</td>
</tr>
<tr>
<td>Defined medium + glucose</td>
<td>50% oxygen</td>
<td>1.70</td>
<td>190</td>
</tr>
</tbody>
</table>

* Expressed as units of enzyme per unit increase in turbidity as measured at 500 nm.

* Mean generation time, measured during the initial rapid logarithmic growth.
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FIG. 1. Initial kinetics of induction of β-lactamase in S. aureus grown in nutrient broth-glucose (○) and in defined medium (△). Cultures were allowed to grow until logarithmic growth was well established before inducer (7.25 μM CBAP) was added.

FIG. 2. Growth of S. aureus in nutrient broth-glucose (A). Inducer (CBAP) concentrations at 7.25 μM (○) and 7.25 X 10^-4 μM (△) were added to the cultures at zero time. (B) Differential rate of β-lactamase synthesis in the same cultures. Cells used as inoculum were grown in nutrient broth-glucose containing no inducer. Last point on the initial high logarithmic growth rate is indicated by an arrow. In subsequent figures, the differential plot only is shown; onset of the decreased rate of growth is indicated by an arrow (†).

synthesis was preceded by a shift down to a lower growth rate.

This effect is not limited to cultures induced by CBAP. We have observed the same phenomenon in other cultures induced by methicillin and cephalosporin C (Fig. 6). Again, in these experiments, the rise in differential rate of β-lactamase synthesis occurred after the growth rate had changed.

Removal of inducer from cultures of S. aureus. When cells induced at pH 7.1 were transferred to inducer-free medium, enzyme synthesis continued for much longer in acid medium (pH 5.3) than in neutral medium (pH 7.1; Fig. 7A). A similar result was obtained with cells induced at pH 5.3 (Fig. 7B). Growth at low pH (5.3) in the absence of inducer yielded only the basal level of enzyme. Therefore, the decay of β-lactamase synthesizing ability is dependent on the pH of the growth medium.

DISCUSSION

CBAP has a unique combination of properties which makes it particularly suitable as an inducer of β-lactamase in S. aureus. It is not hydrolyzed by the enzyme and has very little effect on the growth of the organism. This can be contrasted with methicillin, which, although relatively resistant to hydrolysis by β-lactamase (10), has a strong antibacterial effect which limits its usefulness as an inducer. CBAP does not affect
with inducer and where the rate of growth is virtually unaffected by the inducer (Table 2).

The kinetics of induction under gratuitous conditions show the same pattern as has already been described for other inducible systems, i.e., a short lag followed by a constant differential rate of enzyme synthesis. The length of the lag in nutrient broth-glucose agrees well with the results of Leitner et al. (8), who found a lag of 3 to 4 min for staphylococcal $\beta$-lactamase in-

![Graph](http://jb.asm.org/)

**Fig. 3.** Differential rate of $\beta$-lactamase synthesis in *S. aureus* growing in nutrient broth-glucose. Cells used as inoculum had been grown in the presence of 7.25 $\mu$M CBAP. Cultures contained 7.25 $\mu$M CBAP (O), $7.25 \times 10^{-2}$ $\mu$M CBAP ($\Delta$), and no CBAP (□). The last points which fall on the initial high logarithmic growth rate are indicated by arrows, (†) and (↓).

![Graph](http://jb.asm.org/)

**Fig. 4.** Effect of step-down in growth rate, as a result of pH drop, on differential rate of $\beta$-lactamase synthesis in *S. aureus* growing in nutrient broth-glucose containing $7.25 \times 10^{-2}$ $\mu$M CBAP. Control (O) compared with a replicate culture ($\Delta$) in which the pH was lowered to 5.3 by addition of normal HCl at the arrow (†). Growth rate of this culture decreased on addition of acid. Point at which growth rate of control decreased is indicated by arrow (↓).

![Graph](http://jb.asm.org/)

**Fig. 5.** Differential rate of $\beta$-lactamase synthesis in *S. aureus* growing in nutrient broth-glucose (O) compared with that in a culture grown in nutrient broth (Δ). In both cultures, the concentration of CBAP was $7.25 \times 10^{-2}$ $\mu$M. The pH fell from 7.2 to 5.3 in the culture grown in nutrient broth-glucose, but remained at 7.2 in the culture grown in nutrient broth. Points at which growth rate decreased are indicated by arrows (↓).

![Graph](http://jb.asm.org/)

**Fig. 6.** Differential rate of synthesis of $\beta$-lactamase in *S. aureus* growing in defined medium with cephalosporin C as inducer (Δ) and in nutrient broth with methicillin as inducer (O). Points at which growth rate decreased are indicated by arrows (↓).
duced by benzylpenicillin in tryptic digest broth supplemented with ferrous ammonium sulfate. However, our data show a longer lag in defined medium (Fig. 1). The rate of growth in defined medium is two to three times slower than in nutrient broth, but from the present data it is not possible to say whether this increase is related to growth rate or to some specific deficiency in the defined medium.

Growth with different carbon sources and under different conditions of aeration did not affect the synthesis of the enzyme (Table 3). This emphasizes the gratuitous nature of $\beta$-lactamase induction by CBAP in contrast to the induction of other enzymes which interact with environmental conditions.

The only situation we have encountered in which the differential rate of $\beta$-lactamase synthesis changes is when growth takes place in the presence of suboptimal concentrations of CBAP. Under these conditions, the slowing down of growth normally found in our medium was followed by an increased differential rate of enzyme synthesis (Fig. 2). The effect was observed also with methicillin and cephalosporin (Fig. 6). It is likely that both of these inducers were present at suboptimal levels. With methicillin it is not possible to achieve a saturation curve like that obtained with CBAP (Table 2), because of the bactericidal effect of higher methicillin concentrations. Cephalosporin C never gave high levels of enzyme and, for this reason alone, we must conclude that induction mechanisms were not being fully activated.

The common factor in all our experiments is that a shift down in growth rate occurred shortly before the abrupt increase in differential rate of synthesis of $\beta$-lactamase. Whether the change in growth rate occurred spontaneously in the culture or was brought about by lowering the pH (Fig. 4) did not affect the result. Even when the change in growth rate was not accompanied by a change in pH, the same result was observed (Fig. 5). It seems then, that a shift down in growth rate caused an increase in the differential rate of $\beta$-lactamase synthesis. This implies that the rate of synthesis of this induced enzyme and of cell material are not affected to the same extent by the conditions which bring about a change in growth rate. It is unlikely that a change in a hypothetical permease for $\beta$-lactamase inducers could explain the effect, because preinduction, under conditions which give high enzyme levels, does not change the response to low inducer concentrations (Fig. 3).

Our results on the decay of the $\beta$-lactamase synthesizing system after CBAP withdrawal are in general agreement with Leitner et al. (8), who used a different inducer in tryptic digest broth. Decay is slower at low pH (5.3) than at pH 7.4 (Fig. 7). Leitner (9) subsequently suggested that the $\beta$-lactamase operator is more rapidly repressed at pH 7.4, but it is possible that the different rates of decay of enzyme synthesis reflect the degree of binding of inducer at different pH values.

Acknowledgments

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Literature Cited


