The Assimilation of Amino-acids by Bacteria

5. The Action of Penicillin in Preventing the Assimilation of Glutamic Acid by *Staphylococcus aureus*

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SUMMARY: The effect on glutamic acid assimilation of the addition of penicillin to growing cultures of *Staphylococcus aureus* is described. When *Staph. aureus* is grown in media containing glutamic acid this substance accumulates in steadily increasing concentration in the cells. The addition of penicillin to the medium is followed after an interval by rapidly decreasing concentration of glutamic acid within the cells.

The assimilation of glutamic acid by normal washed cells is not affected by penicillin in high concentration. The assimilation of glutamic acid by cells which have grown in the presence of penicillin is impaired and may be completely inhibited. Complete inhibition of assimilation is brought about by bactericidal concentrations of penicillin, low concentrations requiring a longer time to become completely effective than high ones. The loss of assimilatory power can be correlated with loss of viability.

Comparison of the general properties of normal and penicillin-inactivated cells show that the respiration, glucose oxidation, glucose fermentation and lysine assimilation of the latter are normal. The internal metabolism of glutamic acid is normal in penicillin-treated cells, but, since the passage of glutamic acid across the cell wall is blocked, is limited by the existing internal concentration.

Previous papers of this series (Gale, 1947*a*; Taylor, 1947) have shown that Gram-positive bacteria are able to assimilate certain amino-acids from the external environment and to concentrate these amino-acids in their internal environment so that, at equilibrium, the internal concentration is greater than the external. Lysine passes across the cell wall of *Streptococcus faecalis* by a process of diffusion, but the migration of glutamic acid requires energy which can be supplied by metabolic processes such as glucose fermentation. The maintenance of a concentration of glutamic acid within the internal environment higher than that in the external medium is dependent upon an intact cell wall (Gale & Taylor, 1947). After glutamic acid has passed through the cell wall, a portion of it undergoes metabolic change, and the level of glutamic acid concentration measured inside the cell represents the balance between the rate of entry of that amino-acid into the cell and the rate of its metabolism within the cell (Gale & Mitchell, 1947).

Since the ability to concentrate amino-acids within the internal environment is a property of Gram-positive organisms, the effect on this property of various chemotherapeutic agents has now been tested. A preliminary note on the effect of penicillin in preventing the assimilation of glutamic acid by *Staphylococcus aureus* has been published (Gale & Taylor, 1946). Penicillin is highly effective as a bactericidal agent against most Gram-positive organisms, while many Gram-negative organisms are either unaffected or affected by comparatively

large concentrations (Fleming, 1929); however, the Gram-negative meningococci and gonococci are very sensitive, and the Gram-positive enterococcus, *Strep. faecalis* ST, is comparatively resistant. Penicillin does not affect the respiration of washed suspensions of susceptible staphylococci and was consequently thought to be bacteriostatic (Abraham, Chain, Fletcher, Florey, Gardner, Heatley & Jennings, 1941) but later work demonstrated that penicillin is bactericidal when it is allowed to act on cells which are growing or in a condition where multiplication is possible (Hobby, Meyer & Chaffee, 1942). Gardner (1940) found that bacteria will increase in size in the presence of penicillin but do not divide, with the result that enlarged and abnormal cells are produced. Hobby & Dawson (1944) showed that the action of penicillin can be enhanced by the presence of substances promoting bacterial growth and inhibited by substances preventing growth; this conclusion was confirmed by Chain & Duthie (1945), who showed that the bacteriostatic agent helvolic acid would protect staphylococci from the bactericidal action of penicillin, while sulphonamides, which allow several divisions to take place before inhibiting growth, had no such protective action.

Although penicillin has no action on washed suspensions of *Staph. aureus*, Hirsch (1943–4) and Chain & Duthie (1945) found that when penicillin was added to growing cultures then, after a lag period during which respiration was normal, a progressive inhibition of respiration took place which eventually resulted in complete cessation of oxygen consumption. Chain & Duthie (1945) found that when the penicillin was added in the early logarithmic phase of growth, the cells continued to grow for some time after the addition, but not more than one division per cell occurred and that abnormally large cells were produced. A loss of viability then took place which ran approximately parallel to the respiratory failure, and lysis of the non-viable cells resulted in a steady decrease in the total, as well as the viable, count. There have been few published studies of the biochemical action of penicillin, although Krampitz & Werkman (1947) have found that high concentrations (2000 Oxford units/ml.) inhibit the dissimilation of cellular ribonucleic acid and sodium ribonucleate when employed as substrate for *Staph. aureus*. Atkinson & Stanley (1943) found that the action of penicillin could be antagonized by cysteine, but further investigation seems to indicate that the penicillin molecule is inactivated by chemical reaction with a number of amino-thiol compounds (Chow & McKee, 1945; Cavillito, Bailey, Haskell, McCormick & Warner, 1945).

Methods and organisms used

Most of the present work was carried out with *Staph. aureus* strain D which was noted by Taylor (1947) to be capable of effecting a very high concentration of glutamic acid within the internal environment. The main results were checked by using another strain which effects a comparatively low internal concentration of glutamic acid and against *Strep. faecalis* ST used previously. The organisms were grown in two media: medium A being casein digest medium containing 1 % (w/v) glucose and 0·1 % (w/v) Marmite; medium B ('deficient

medium') consisting of a salt mixture with 1·0 % (w/v) glucose and 0·1 % (w/v) Marmite. The preparations of penicillin used were commercial preparations (Roche Products Ltd.) of purity 500 or 800 Oxford units/mg.

The estimations of amino-acids and of assimilation were carried out as previously described (Gale, 1947*a*). In general, the organisms were grown in medium B, washed and their internal amino-acid assayed; they were then incubated in a salt solution containing glutamic acid (200 μl./ml.) and 0·5 % (w/v) glucose for 1 hr. at 37°, washed and the new internal glutamic acid level assayed.

Quantities of glutamic acid and lysine are expressed in terms of μl. (Gale & Mitchell, 1947); 22·4 μl. glutamic acid = 1 μmol.

Assimilation of lysine and glutamic acid by Staphylococcus aureus

The studies on assimilation so far described in this series were carried out with a strain of *Strep. faecalis* which is unsuitable for the investigation of the action of many chemotherapeutic agents as it is resistant to penicillin, the sulphonamides, etc. Table 1 summarizes the results of experiments carried out to determine whether *Staph. aureus* assimilates lysine and glutamic acid under

Table 1. *Assimilation of lysine and glutamic acid by* Staphylococcus aureus

Staph. aureus grown in medium B, cells centrifuged down and internal amino-acid assayed. Cells suspended in salt solution containing either 200 μl. lysine or 200 μl. glutamic acid/ml. as below, left for 1 hr., centrifuged out of suspension, washed and internal environment re-assayed. Assimilation expressed as μl. increase in amino-acid content of internal environment of 100 mg. dry weight of cells.

		Increase in internal content	
External medium	Temp.	Lysine (μl.)	Glutamic acid (μl.)
Lysine	4	30	—
Lysine	37	114	—
Lysine; glucose	37	66	—
Glutamate	4	—	Nil
Glutamate	37	—	Nil
Glutamate; glucose	37	—	402

conditions similar to those required by *Strep. faecalis*. The values in Table 1 show that lysine passed into the internal environment at 4°, more effectively at 37° and that the amount taken up at 37° was less when glucose was present. These results are essentially similar to those obtained in *Strep. faecalis* where it was shown that lysine passes across the cell wall by a process of diffusion, the amount assimilated being dependent upon the charge of the cell and being decreased by glycolysis. Glutamic acid did not enter the *Staph. aureus* cells at either 4 or 37° in the absence of glucose, showing that, as in the case of *Strep. faecalis*, energy was required for the migration of glutamic acid across the cell wall. In these experiments glucose has always been added as source of energy although it was shown with *Strep. faecalis* that the breakdown of arginine would also supply energy for the migration. The assimilation of glutamine by *Staph. aureus* also requires energy which can be supplied by glucolysis.

Effect of penicillin. Table 2 shows the effect of the presence of penicillin on glutamic acid assimilation by normal cells of *Staph. aureus*. Two Oxford units/ml. had no effect on assimilation, 20–50 units/ml. gave rise to *c.* 10 % decrease in the amount of glutamic acid assimilated by 100 mg. of deficient cells under the test conditions. It is doubtful whether this decrease is significant.

Table 2. *Effect of penicillin on glutamic acid assimilation by washed normal cells*

Deficient cells incubated for 1 hr. at 37° in presence of 0·5 % (w/v) glucose, 200 μl. glutamic acid/ml., and penicillin as below.

Penicillin present (units/ml.)	Increase in internal glutamic acid concentration (μl./100 mg.)
0	618
2	627
20	560
50	536

The action of penicillin on glutamic acid assimilation by growing cultures

Action of penicillin on growth. The growth of an inoculum of *Staph. aureus* strain D in medium A was prevented by the presence of 0·08–0·1 Oxford units penicillin/ml. For assimilation measurements it is necessary to have reasonably large amounts of cells, and so the effect was tested of adding penicillin to growing cultures of the organism. Fig. 1 shows the effect of adding various concentrations of penicillin to growing cultures of *Staph. aureus* D, the additions being made after growth had taken place for 3½ hr. at 37° in medium B. Growth was followed turbidimetrically; it does not follow that an increase in turbidity after the addition of penicillin is accompanied by a corresponding increase in cell numbers. It can be seen from Fig. 1 that the turbidity of the cultures continued to increase normally for 30 min. after the penicillin additions. The turbidity continued to increase for 1–2 hr. according to the penicillin concentration added, smaller concentrations taking longer to bring the turbidity to a steady level than higher ones. Ten units penicillin/ml. bring about cessation of growth in about 1 hr., but 0·1 unit/ml. takes nearly 3 hr. to become completely inhibitory. Viable cell counts were carried out on many of the samples. It was found that approximately one division per cell takes place within the 30 min. following the addition of penicillin in any of the concentrations tested. There was then a steady loss of viability lasting over a period of hours and depending upon the penicillin concentration. The viable count had fallen to about 4 % of its value at 3½ hr. within 2 hr. of the addition of 10 units/ml., and to about 80 % within 2 hr. of the addition of 0·1 unit/ml. Lysis of the cells set in 3–4 hr. after penicillin addition, but general lysis did not occur with this organism for 24–30 hr. after penicillin addition. The findings described here for the effect of penicillin on growth are in general agreement with those of previous authors (Chain & Duthie, 1945).

Effect of penicillin on internal accumulation of glutamic acid. The accumulation of glutamic acid and lysine within the internal environment of *Staph.*

aureus cells growing in medium A before and after penicillin addition was next tested. The organism was inoculated into media at 37° and cells harvested at intervals throughout the growth period for assay of their internal amino-acids as previously described (Gale, 1947*a*). Penicillin (4 units/ml.) was added to half the culture at 4 hr. Fig. 2 shows the effect of the penicillin on growth and on the accumulation of glutamic acid within the cells. In the normal culture the amount of free glutamic acid within the cells increased during growth, reaching

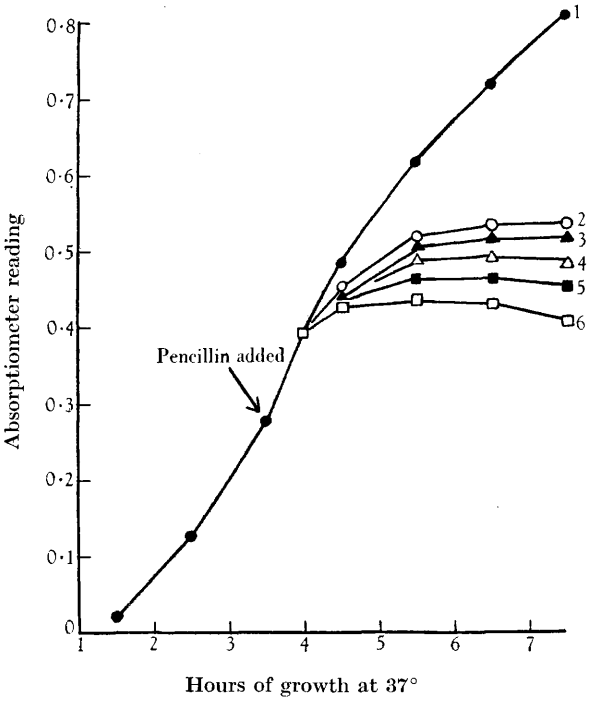

Fig. 1. Effect of addition of penicillin to growing cultures of *Staph. aureus*. Medium: salt mixture + 0·1% Marmite + 1·0% glucose. Penicillin concentrations added at 3½ hr. Curve 1, no penicillin. Curve 2, 0·1 Oxford unit/ml. Curve 3, 0·5 Oxford unit/ml. Curve 4, 1·0 Oxford unit/ml. Curve 5, 5·0 Oxford units/ml. Curve 6, 10·0 Oxford units/ml.

a steady value as growth ceased. The total assimilation of glutamic acid/100 mg. cells was approximately constant throughout the growth period (Gale & Mitchell, 1947), but since the level measured inside the cell represents a balance between the amount withdrawn from the external environment and the amount metabolized, this level will be lowest when the internal metabolism is highest, i.e. when growth is taking place most rapidly (Gale, 1947*b*). After the addition of penicillin to the external medium, the accumulation of free glutamic acid within the cells increases normally for approximately an hour and then begins to decrease rapidly although growth has not ceased. It can be seen from Fig. 2 that the fall in the internal glutamic acid level continued until the culture ceased to grow. The cells were assayed for lysine content at the same time as their glutamic acid content, but, although the accumulation of lysine in

the normal cells shows a rising curve of the same shape as that shown in Fig. 2 for glutamic acid, the addition of penicillin had no significant effect upon the accumulation of lysine within the cells. These results suggest that the cells ceased to assimilate glutamic acid, but not lysine, from the external medium shortly after the addition of penicillin to the medium.

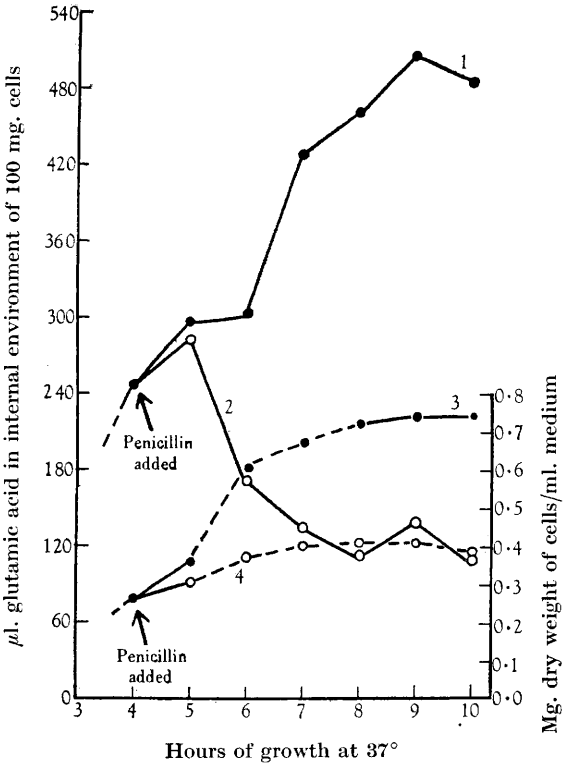

Fig. 2. Effect of addition of penicillin to growing cultures of *Staph. aureus* on the accumulation of free glutamic acid in the internal environment of the cells. Medium: casein digest + 0·1% Marmite + 1% glucose. Curve 1, accumulation of free glutamic acid in 100 mg. cells during normal growth. Curve 2, accumulation of free glutamic acid in 100 mg. cells during growth when penicillin (4 Oxford units/ml.) added at 3½ hr. Curves 3 and 4, growth curves normal (3) and penicillin added at 3½ hr. (4) to compare with curves 1 and 2 respectively.

Effect of penicillin on glutamic assimilation by growing cells. The assumption that glutamic acid assimilation is impaired after the addition of penicillin to the culture was first tested on growing cells. The experiment recorded in Table 3 shows that in the normal culture, the internal content of glutamic acid was 387 µl. higher in the cells (100 mg.) which had grown in the presence of added glutamic acid than in the cells grown in the 'deficient' medium B. In the cultures to which penicillin was added 1 hr. before glutamic acid, the difference between the two groups of cell was decreased to 116 µl. glutamic acid, and this difference was still further diminished to 49 µl. in the cultures to which

penicillin was added 2 hr. before the glutamic addition. This again suggests that the capacity to assimilate and concentrate glutamic acid is impaired in cells growing in the presence of penicillin.

Table 3. *Effect of penicillin on glutamic acid assimilation by growing culture of* Staphylococcus aureus *D*

Staph. aureus D was inoculated into deficient medium B incubated at 37°. After 5 hr. (B) or 4 hr. (C) the culture was divided into two and penicillin (10 units/ml.) added to one half; after a further 1 hr. (B) or 2 hr. (C) each batch of culture was again divided into two and glutamic acid (200 μl./ml.) added to half of each batch; all cultures were then allowed to grow for a further hour and all were harvested 7 hr. after inoculation.

Culture	Penicillin 10 units/ml. added after time indicated	Glutamic acid (200 μl./ml.) added after time indicated	Internal glutamic acid: μl./100 mg. cells	Internal glutamic acid: Increase due to added glutamic acid (μl.)
A	—	—	365	387
	—	6 hr.	752	
B	5 hr.	—	202	116
	5 hr.	6 hr.	318	
C	4 hr.	—	206	49
	4 hr.	6 hr.	255	

Glutamic acid assimilation by cells grown in the presence of penicillin

Since the assimilation of glutamic acid by cells growing in the presence of penicillin was apparently impaired, the ability of such cells to assimilate glutamic acid after removal from the growth medium was next tested. The cells were grown as usual in medium B and penicillin (10 units/ml. medium) added at $3\frac{1}{2}$ hr. after inoculation. At $4\frac{1}{2}$ hr. the cells were harvested and suspended in glutamic acid glucose salt solution as usual and their ability to concentrate glutamic acid in the internal environment compared with normal cells grown for the same period but in the absence of penicillin. Normal cells assimilated 638 μl. glutamic acid/100 mg., while the cells grown in the presence of penicillin assimilated 42 μl./100 mg. The addition of penicillin (50 units/ml.) to the external environment during the assimilation procedure had no significant effect. Washing the cells grown in presence of penicillin with distilled water or incubation with cysteine (1 mg./ml.) did not alter the impaired assimilation of glutamic acid. The assimilation of glutamine was of the same order as that of glutamic acid and was impaired to the same extent in the cells grown in penicillin.

Effect of penicillin concentration and time of contact. Reference to Fig. 1 shows that the speed with which penicillin brought about cessation of growth varied with the concentration of penicillin added to the culture. Table 4 shows the glutamic acid assimilated by cells harvested at intervals after the addition of various concentrations of penicillin to the cultures. The ability of these cells to assimilate glutamic acid was investigated as usual. Cells taken from cultures

to which no penicillin had been added during growth assimilated 560–700 μl. glutamic acid/100 mg. under the standard conditions of test. Within 30 min. of the addition of penicillin (10 units/ml.) to the culture, the assimilatory power of the cells had fallen to 14 % of that of the control; within 1 hr. the assimilation had fallen to 4 % that of the control, and after 90 min. assimilation was no longer possible. Lower concentrations of penicillin produced the same failure of assimilatory power but took longer to make this complete; thus penicillin at 1 unit/ml. took over 2 hr. to prevent glutamic acid assimilation completely. If these values for assimilation are compared with the curves shown in Fig. 1, it can be seen that there is a correlation between the cessation of cell growth and the inhibition of glutamic acid assimilation.

Table 4. *Effect of the presence of penicillin during growth on the assimilation of glutamic acid by* Staphylococcus aureus

Cells grown in medium B and penicillin added in all cases after 3½ hr. growth at 37°. Cells harvested at various times after the penicillin addition, incubated for 1 hr. at 37° in glutamic acid (200 μl./ml.) and glucose (0·5 % w/v), and the increase in the internal glutamic acid content assayed as usual.

Penicillin concentration (units/ml. medium)	Glutamic acid assimilated (μl./100 mg. cells) Time of harvesting after penicillin addition				
	30 min.	1 hr.	1½ hr.	2 hr.	3 hr.
0	561	702	602	590	614
0·1	—	—	—	130	—
0·5	—	—	—	113	—
1·0	—	—	87	—	0
5·0	—	—	0	—	0
10·0	82	31	0	0	0

Effect of penicillin on general metabolic activity of cells

Table 5 shows the general metabolic activities of normal cells compared with those of cells harvested after 90 min. growth under the usual conditions in the presence of penicillin at 10 units/ml.

Table 5. *Metabolic activities of normal* Staphylococcus aureus *cells and of cells grown in presence of penicillin*

'Penicillin cells' grown for 90 min. in medium containing 10 units penicillin/ml.

	Normal cells	'Penicillin cells'
Respiration Q_{O_2}	21·5	19·6
Glucose oxidation Q_{O_2}	86·5	84·5
Glucose fermentation $Q_{CO_2}^{acid}$	96	108
Lysine assimilation (μl./100 mg.)	90	96
Glutamic acid assimilation	602	0
Comparative viable count	452	9

Respiration. This was measured in Warburg manometers containing 1·0 ml. suspension of cells and 2·0 ml. M/15 phosphate buffer pH 7·5 in the main cup, and 0·3 ml. 10 % (w/v) NaOH in the centre pot. The steady rate of respiration

measured over 15 min. was determined in this manner and also in the presence of penicillin (50 units/ml.). The values given in Table 5 show that the respiration of the cells grown in the presence of penicillin was slightly lower than that of normal cells; in neither case did the addition of penicillin to the Warburg cup have any inhibitory effect on respiration.

Glucose oxidation. This was also measured in Warburg manometers in parallel with the blank respiration experiment; in this case 0·2 ml. 2 % (w/v) glucose was added from the side-bulb at the beginning of the experiment and the steady rate of oxygen uptake measured. There was no significant difference between the rates of oxidation carried out by the two cultures, and again the addition of penicillin to the manometer cups had no inhibitory effect.

Glucose fermentation. Manometers were assembled containing 1·0 ml. cell suspension and 1·5 ml. M/40-$NaHCO_3$ in the main cup, and 0·5 ml. 1 % (w/v) glucose in the side-bulb. The manometers were filled with a gas mixture containing 5 % CO_2 + 95 % N_2. After equilibration at 37° the glucose was added to the main reaction compartment and the rate of fermentation determined from the CO_2 evolution. Since the organism carries out a homolactic fermentation, the Q_{CO_2} measured in this way is a direct measure of lactic acid production. There was no significant difference between the rates of fermentation of the two cultures and the addition of penicillin had no inhibitory effect. The assimilation of glutamic acid in the experiments described is dependent upon the supply of energy from glycolysis; these measurements show that the impairment of assimilation is not a consequence of inhibition of fermentation by penicillin.

Lysine assimilation. This was measured, as previously described, by standing the deficient cells in a solution of 200 μl. lysine/ml. for 1 hr. at 37° and measuring the increase in the internal lysine concentration. The amount of lysine taken up is dependent upon the electrical properties of the cells (Gale, 1947*a*) and on the intact nature of the cell wall (Gale & Taylor, 1947). Table 5 shows that the cells from the two cultures were essentially similar in their capacity to assimilate lysine and confirms the earlier finding that the accumulation of lysine within growing cells is independent of the presence of penicillin in the medium.

Comparative viable counts. Viable counts were carried out on standard volumes (of equal turbidity) of the two cell suspensions diluted 10^6 times by serial dilution. Table 5 shows that the viability of the suspension harvested from the medium containing penicillin was about 2 % of the normal cell suspension.

Penicillin is known to have four effects on *Staph. aureus* when added to growing cultures: (1) the cells become non-viable (Chain & Duthie, 1945); (2) their respiration progressively fails (Chain & Duthie, 1945; Hirsch, 1943–4); (3) lysis occurs after several hours (Fleming, 1929; Chain & Duthie, 1945), and (4) assimilation of glutamic acid is prevented. The results recorded in Table 5 show that the failure of glutamic acid assimilation preceded the failure of respiration and the onset of general lysis and would appear to take place simultaneously with or before loss of viability.

Effect of penicillin on internal metabolism of glutamic acid

In the previous paper (Gale & Mitchell, 1947) it was shown that some metabolism of glutamic acid took place after it had passed into the internal environment of the cell. This metabolism could be demonstrated since, if the amount of glutamic acid which accumulated inside the cell during assimilation was compared with the amount which was removed from the external environment, there was on balance a 'disappearance' of the amino-acid. This disappearance or metabolism of glutamic acid is inhibited by the triphenylmethane dyes. Table 6 shows the results of such balance experiments carried

Table 6. *Effect of penicillin on glutamic acid metabolism*

Organism grown in medium B. Penicillin 10 units/ml. added to half of culture 90 min. before harvesting. Cells centrifuged down, washed and suspensions incubated in glutamic acid solution + 1 % (w/v) glucose for 1 hr. at 37°. Internal and external glutamic acid assayed before and after incubation.

	External environment	Internal environment	Change in external environment (μl. glutamic acid)	Change in internal environment	Glutamic acid metabolized (μl.)
Normal cells:					
Initial	1586	690	−831	+370	461
Final	755	1060			
Penicillin-treated cells:					
Initial	1646	579	−106	−263	363
Final	1540	315			

out during the assimilation of glutamic acid by washed suspensions of *Staph. aureus* cells both normal and harvested from medium to which penicillin (10 units/ml.) was added 90 min. prior to harvesting. In both cases glutamic acid disappeared during the assimilation experiment, the amount disappearing in the experiment with penicillin-treated cells being about 80 % of that disappearing with normal cells. However, with the normal cells the amount of glutamic acid in the internal environment increased by 370 μl. during assimilation while 831 μl. were withdrawn from the external medium. With the penicillin-treated cells the amount of glutamic acid withdrawn from the external medium was very much less, a decrease of only 106 μl. being measured, and the experimental error in the determination was of the order of 40 μl.; but the internal glutamic acid level decreased by 263 μl. The figures suggest that the internal metabolism of glutamic acid continued normally in the penicillin-treated cells, but since the passage of glutamic acid across the cell wall was blocked, this metabolism took place at the expense of the internal glutamic acid already present. In the normal cells the glutamic acid metabolized within the cells is balanced by assimilation from the external medium and an increase in the internal level to maintain equilibrium with the external concentration. The fall in the curve (Fig. 2) showing glutamic acid accumulation within the growing

cells after the addition of penicillin to the medium is thus explained, since the assimilation of glutamic acid is blocked shortly after the addition, and metabolism of the internal glutamic acid then produces the drop in its internal concentration. These experiments provide further proof that the metabolism of glutamic acid by these cells is an intracellular process.

Effect of penicillin on glutamic acid assimilation by Streptococcus faecalis *ST*

Strep. faecalis ST needs about 10 times the concentration of penicillin required by *Staph. aureus* in order to prevent growth. When tested under the usual conditions, normal *Strep. faecalis* cells assimilated 142 μl. glutamic acid/100 mg. cells; cells which had been grown in the presence of penicillin (10 units/ml.) medium for 90 min. prior to harvesting assimilated 57 μl./100 mg. under the same conditions. The action of penicillin was thus to impair glutamic acid assimilation in these cells as in *Staph. aureus*.

DISCUSSION

The experiments described show that one action of penicillin on growing staphylococci is to prevent the assimilation of glutamic acid. Since it has been possible to study the assimilation of only such amino-acids as can be estimated by the decarboxylase technique, it is not possible at present to say whether this impairment of assimilation extends to amino-acids other than glutamic acid. The assimilation of lysine is certainly not affected in the same way, but this amino-acid is not assimilated by the same type of mechanism as glutamic acid. The Gram-positive cocci are, in general, nutritionally exacting for a range of amino-acids including glutamic acid, and it is reasonable to suppose that a loss of viability would follow inhibition of assimilation of these amino-acids. It is difficult, however, to distinguish between cause and effect, and it is not possible to say at present whether cells rendered non-viable by penicillin acting in some other way would be able to assimilate glutamic acid or not. There is a suggestion in the data shown in Table 4 and Figs. 1 and 2 that cessation of assimilation precedes loss of viability. In Fig. 2 there is definitely an increase in turbidity after the point at which the internal concentration of glutamic acid begins to fall instead of to rise, and the internal concentration does not reach a new steady level until growth (as measured by turbidity increase) ceases. The glutamic acid which accumulates inside the cell acts as a reservoir of amino-acid for protein synthesis (Gale, 1947*b*) and for other metabolic purposes (Gale & Mitchell, 1947), and it may be that growth will continue as long as there is more than a certain limiting concentration within the cell. In that case the sequence of events would be: (i) penicillin prevents the passage of glutamic acid into the cell; (ii) the synthesis of protein, etc., proceeds at the expense of the accumulated glutamic acid (and other amino-acids) within the cell and consequently the concentration falls; (iii) the internal concentration falls to the lowest level permitting synthesis of protein and growth ceases. Once penicillin has acted on the cell wall, the further

growth is therefore limited by the amount of essential amino-acids accumulated within the cell, and it may also be the case that this suffices for sufficient growth to produce enlarged forms of the cells but not for complete division.

Penicillin has no effect on the mechanism whereby glutamic acid is assimilated and concentrated within the internal environment in normal 'resting' cells, but affects cells during growth in such a way that assimilation is prevented. This suggests that penicillin either combines with or produces a reorganization of the cell wall such that the assimilatory mechanism is blocked.

A recent paper by Shwartzman (1946) has shown that the resistance of Gram-negative organisms to penicillin is increased by the presence of certain amino-acids, aspartic and glutamic acids being very active in this respect. It is possible that a mechanism similar to that discussed above is also operative in this case.

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