

shows the actions of sodium azide, dinitrophenol and crystal violet on the accumulation of free glutamate in *Staph. aureus* and *Strep. faecalis* exposed to glutamate and glucose. The three substances produce an inhibition of the passage of the amino-acid into *Staph. aureus* but an increase in the internal concentration attained in *Strep. faecalis* although, in high concentrations, they appear to have an inhibitory action on the passage of the amino-acid into the cells in this case as well. The latter inhibition can, in the case of crystal violet, be correlated with interference with the energy-supplying fermentation system in *Strep. faecalis* (Gale & Mitchell, 1947), although this is not the explanation for the inhibition of the passage into *Staph. aureus*. The increase in the internal concentration of free glutamate in *Strep. faecalis* can be ascribed to an inhibition of metabolism of the amino-acid within the cell, and it is interesting to note that concentrations of the three substances which produce optimal inhibition of internal metabolism in *Strep. faecalis* are approximately the same as those producing almost complete inhibition of passage into *Staph. aureus*.

SUMMARY

1. The accumulation of free glutamic acid within *Staphylococcus aureus* requires energy supplied, under the experimental conditions used, by the fermentation of glucose. Sodium azide and 2:4-dinitrophenol inhibit the accumulation of free glutamate without inhibiting the fermentation reactions.

2. Sodium arsenate accelerates the rates of fermentation and of glutamate accumulation.

3. Sodium azide and 2:4-dinitrophenol increase the amount of free glutamate accumulating within *Streptococcus faecalis* incubated in the presence of glutamic acid and glucose. The effect can be correlated with the inhibition, by these substances, of internal metabolism of glutamic acid in these cells.

4. Concentrations of sodium azide and 2:4-dinitrophenol producing optimal increase in the internal glutamate concentration in *Strep. faecalis* are approximately the same as those producing complete inhibition of glutamate accumulation in *Staph. aureus*.

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The Assimilation of Amino-acids by Bacteria

11. THE RELATIONSHIP BETWEEN ACCUMULATION OF FREE GLUTAMIC ACID AND THE FORMATION OF COMBINED GLUTAMIC ACID IN *STAPHYLOCOCCUS AUREUS*

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Staphylococcus aureus (*Micrococcus pyogenes* var. *aureus*), in common with certain other Gram-positive bacteria, possesses the ability to concentrate certain amino-acids in the internal environment (Gale, 1947*a, b*; Taylor, 1947). In the case of glutamic acid, passage into the cell only occurs when some exergonic metabolism such as glycolysis is also taking place. If cells containing little free glutamic acid are incubated in a medium consisting of buffered saline, glucose and glutamic acid, the

amino-acid enters the cell until the internal concentration is many times that in the external medium. The passage of the amino-acid into the cell is prevented by any substance which inhibits glycolysis and is also inhibited, at concentrations ineffective against glycolysis, by sodium azide, 2:4-dinitrophenol and 8-hydroxyquinoline (Gale, 1949, 1951). All the studies so far reported on the passage of glutamic acid into *Staph. aureus* and other Gram-positive cells have referred to the process which takes

place when 'deficient' cells are incubated in a simple buffered salt and glucose medium with glutamic acid as the only amino-acid present. Under these conditions the passage into the cell and accumulation therein of free glutamic acid are accompanied by metabolism of the glutamic acid but no glutamyl-peptides or related substances are formed within the cell (Gale & Mitchell, 1947). Hotchkiss (1947) has reported that washed suspensions of *Staph. aureus* incubated with a mixture of amino-acids and glucose assimilate amino-acids and synthesize protein without cellular proliferation. It seemed possible, therefore, that the addition of other amino-acids to the usual reaction mixture used in these studies would lead to protein synthesis and enable the relationship, which exists between the accumulation of free glutamic acid within the cell, and the condensation of glutamic acid into cell protein, to be determined. Previous studies (Gale, 1947*b*) on the concentration of free glutamic acid within growing cells suggested that condensation into protein followed the concentration within the cell of free amino-acids, but the evidence was indirect. In the experiments reported here, the free glutamic acid content of the cells and the combined glutamic acid liberated by acid hydrolysis have been estimated; the combined glutamate fraction will therefore include the glutamic acid of cell proteins and peptide structures within the cell.

METHODS

Organisms. *Staphylococcus aureus* Duncan (Gale & Taylor 1947) was used throughout the work described in this paper.

Growth medium. The organism was grown for 16 hr. at 30° in the 'deficient' medium previously described (Gale, 1947*a*, 1951). The cells were harvested on the centrifuge and washed once with distilled water. Suspensions were prepared containing approximately 20 mg. dry wt. of cells/ml. and these were diluted 1:10 in the reaction solutions for the experiments described below.

Estimation of glutamic acid and its accumulation by Staphylococcus aureus. Free glutamic acid was estimated manometrically with glutamic acid decarboxylase preparations (Gale, 1945). The accumulation of free glutamic acid by the washed cells was studied as previously described (Gale, 1947*a*).

Combined glutamic acid. For studies of the formation of combined glutamic acid, a mixture of amino-acids, other than glutamic acid, was made up to contain 2.0 mg./ml. of the natural isomers of each of the following: (A) aspartic acid, tyrosine, tryptophan, phenylalanine, asparagine; (B) histidine, lysine, arginine; (C) proline, serine, alanine, threonine, methionine; (D) leucine, isoleucine, cystine, glycine, valine. In some experiments the mixtures A, B, C and D were made up separately. Vitamin-free casein hydrolysate was prepared by acid hydrolysis of vitamin-free casein (Glaxo Laboratories) followed by treatment of the hydrolysate with activated charcoal. All amino-acid mixtures were adjusted to pH 7.0 before use.

The total glutamic acid content of cell suspensions was estimated after hydrolysis of the cells in boiling 5*N*-HCl for

18 hr. The combined glutamate was then calculated from the difference between total and free glutamic acid content. Supernatant solutions obtained after centrifuging down the cells at the end of the experiments were also examined for the presence of combined glutamate in a similar manner.

RESULTS

Preliminary work. During some studies on the effect of the constituents of the growth medium on the ability of washed *Staph. aureus* to accumulate free glutamic acid, it was found that the rate of accumulation was reduced by approximately 85% in the presence of 1% marmite solution. This effect could be reproduced by substitution of the marmite solution by hydrolysed marmite, casein digest, vitamin-free casein hydrolysate or a mixture of amino-acids. Estimation of the combined glutamate of the cells showed that this increased during incubation in the presence of such amino-acid mixtures and glucose, but that no increase occurred during incubation with glutamic acid and glucose alone. The concentration of free glutamic acid within the metabolizing cell has been previously shown (Gale & Mitchell, 1947) to be determined by the balance between the rate at which it enters the cell and the rate of internal metabolism. Consequently it seemed probable that the decreased rate of accumulation of free glutamic acid when other amino-acids were present was due to an increased rate of internal metabolism consequent upon the glutamic acid entering into combination with other amino-acids. If the rate of entry of glutamic acid into the cell were unaffected by the presence of other amino-acids, and combination with other amino-acids occurred after glutamic acid had accumulated in the free state within the cell, then the rate of uptake of glutamic acid from the external medium should be approximately the same, whether the mixture of other amino-acids were present or not. Experimental test showed that this was not the case but that the rate of uptake of glutamic acid was markedly decreased by the presence of amino-acid mixtures giving rise to the formation of combined glutamate within the cells.

Effect of casein hydrolysate on the accumulation of free glutamic acid. Fig. 1 shows the accumulation of free glutamic acid within *Staph. aureus* when the cells are incubated in buffered saline containing glucose and glutamic acid with and without the addition of vitamin-free casein hydrolysate. The rate of accumulation is almost nil in the presence of 0.7% casein hydrolysate, and Table 1 shows that an increase in the combined glutamate of the cells is occurring in the presence of the hydrolysate but not in its absence. Such an increase in the combined glutamate of the cells occurs when a mixture of many amino-acids is present, and it seemed probable that restricting the amount of hydrolysate would limit

the duration of the combining process. Fig. 1 shows that the addition of 0.08 or 0.16% casein hydrolysate causes a break in the course of free glutamic acid accumulation. It is not possible to carry out accurate detailed analyses over the short periods covered by these breaks, but estimations show that an increase in the combined glutamate of the cells occurs during

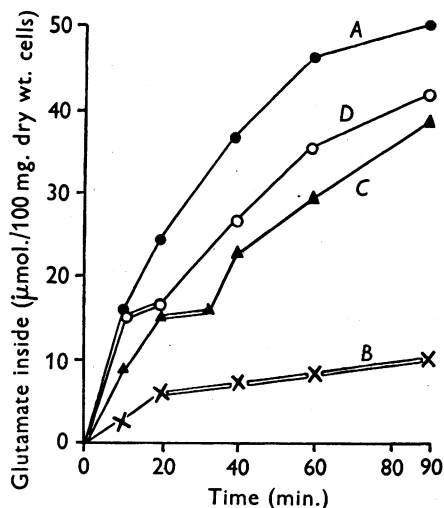


Fig. 1. Rate of accumulation of free glutamic acid in *Staphylococcus aureus* incubated with glucose, glutamic acid and casein hydrolysate. Deficient cells incubated at 37° in buffered salt solution containing 1% glucose, 10 μmol. sodium glutamate/ml. and casein hydrolysate as indicated below. Samples taken at intervals, rapidly cooled, washed and the free internal glutamate determined. Final concentration of casein hydrolysate added: A, none; B, 0.07%; C, 0.16%; D, 0.08%.

the periods covered by the breaks. In the presence of these low concentrations of casein hydrolysate, the accumulation of free glutamic acid starts at approximately the normal rate; it then almost ceases during

the period when combined glutamate is being formed, and is resumed at the normal rate again when combination ceases.

Table 1 gives a detailed analysis of the changes in free and combined glutamate of the medium and cells occurring during 1 hr. incubation under conditions corresponding to those of curves A and B of Fig. 1. In the absence of casein hydrolysate, free glutamic acid accumulates in the cells and approximately twice the amount that accumulates is removed from the external medium; there is no significant increase in the combined glutamate of cells or medium. In the presence of casein hydrolysate, the amount of free glutamic acid accumulating within the cells is equal to 9% of that accumulating in the absence of hydrolysate, and the amount of glutamate withdrawn from the medium lies within the experimental error of the estimations. The amount of glutamic acid added in the casein hydrolysate was so great that accurate estimation of the changes in the medium was not possible; attempts were therefore made to add glutamate-free casein hydrolysate by prior treatment of the hydrolysate with glutamic acid decarboxylase preparations. However, when complete glutamic acid balances were attempted, including determinations of any combined glutamate in the medium before and after incubation, it was found that treatment with the decarboxylase preparation frequently resulted in the addition of protein to the hydrolysate and that this addition could neither be controlled nor standardized. Some results obtained with this material are quoted in Table 4, but it was decided that the hydrolysate must be replaced by a synthetic mixture of amino-acids.

Effect of amino-acid mixtures on the accumulation of free glutamic acid. Table 2 shows the effect on the accumulation of free glutamic acid within the cells of adding various mixtures of amino-acids to the glucose, glutamate and salt solution comprising the external

Table 1. Changes in glutamic acid distribution during incubation of *Staphylococcus aureus* in the presence of glutamic acid and glucose with and without casein hydrolysate

(Cells grown for 16 hr. at 30° in deficient medium, harvested and made into washed suspension. Incubated at final suspension density = approx. 2 mg. dry wt. cells/ml. in buffered salt solution containing 1% glucose, 1.34 μmol. sodium glutamate/ml. and 1.0% vitamin-free casein hydrolysate. Free and combined glutamate of cells and supernatant determined before and after 1 hr. at 37°; results expressed as change (μmol.) in glutamate/100 mg. dry wt. of cells.)

Incubation mixture ...	Glucose (a)	Glucose + glutamate (b)	Glucose + glutamate + casein hydrolysate (c)	Initial value
Internal free glutamate of cells	-4.46	+34.2	+2.9	19.0
External free glutamate of medium	+2.4	-63.0	-1.8*	(b) 72.0
				(c) 398.0
Combined glutamate of cells	0	+ 0.13	+5.7	36.8
Combined glutamate of medium	0	0	0	0
Balance	-2.06	-28.6	+6.8*	

* Query experimental error owing to large amount of free glutamate in mixture (c).

Table 2. *Effect of presence of amino-acids on accumulation of free glutamic acid*

(*Staph. aureus* Duncan grown for 16 hr. at 30° in deficient medium, cells harvested and made into washed suspension; incubated for 1 hr. at 37° in buffered salt solution containing 1% glucose, 9 μmol. sodium glutamate/ml. with or without the addition of various mixtures of amino-acids, as below, each at a final concentration of 0.2 mg. L-isomer/ml.)

Amino-acids present (other than glutamic acid)	Increase in internal free glutamic acid (μmol./100 mg. dry wt. cells)	Reduction in rate of free glutamate accumulation (%)
None	32.6	—
Mixture A (aspartic; tyrosine, tryptophan, phenylalanine, asparagine)	16.4	50
Mixture B (histidine, lysine, arginine, methionine)	24.3	25
Mixture C (proline, serine, alanine, threonine)	16.7	48
Mixture D (leucine, cystine, isoleucine, glycine, valine)	15.8	52
Mixtures A + B	12.1	63
Mixtures A + B + C	6.9	79
Mixtures A + B + C + D	0.27	92

Table 3. *Changes in glutamic acid distribution during incubation of Staphylococcus aureus in the presence of glutamic acid and glucose with and without the addition of a mixture of eighteen other amino-acids*

(Conditions as for Table 1 with substitution of the amino-acid mixture (final concentration of each amino-acid = 0.2 mg./ml.) for casein hydrolysate. Results expressed as change (μmol.) in glutamate/100 mg. dry weight of cells.)

Incubation mixture ...	Glucose	Glucose + glutamate	Glucose + glutamate + amino-acid mixture	Glutamate + amino-acid mixture	Initial value
Internal free glutamate of cells	+0.2	+29.8	- 6.9	- 1.5	14.1
External free glutamate of medium	+1.48	-48.0	- 7.5	+11.4	69.0
Combined glutamate of cells	-0.56	- 0.2	+11.2	-13.4	32.5
Combined glutamate of medium	+0.9	+ 1.3	0	0	0
Balance	+2.02	-17.1	- 3.4	- 3.5	115.6

Table 4. *Changes in glutamic acid distribution during incubation of Staphylococcus aureus in the presence of glutamic acid and glucose with and without a source of other amino-acids*

(Conditions as for Tables 1 and 3. Control = glucose, glutamate, buffered salt solution.)

Dry wt. cells (mg.)	Glutamate removed from medium		Change in internal free glutamate		Increase in combined glutamate		Loss of glutamate on balance	
	Control (μmol.)	Amino-acids added (μmol.)	Control (μmol.)	Amino-acids added (μmol.)	Control (μmol.)	Amino-acids added (μmol.)	Control (μmol.)	Amino-acids added (μmol.)
A. Casein hydrolysate as amino-acid source								
123	63.0	1.8	+34.2	+ 2.9	0	5.7	17.2	3.45
98	44.1	9.8	+34.2	+ 2.0	0	11.1	17.8	—
112	35.5	15.6	+24.1	- 3.3	0	10.0	10.7	—
87	46.9	12.5	+32.9	+ 6.1	0	13.0	6.7	—
B. Complete amino-acid mixture as amino-acid source								
106	48.0	7.4	+29.8	- 6.9	0	11.2	17.2	3.45
116	56.8	5.35	+32.2	- 5.8	0	6.35	28.4	4.8
100	55.1	8.5	+30.1	-10.2	0	6.7	27.5	7.18
103	54.9	6.95	+30.5	- 8.05	0	8.15	31.0	8.6
111	55.0	0.7	+33.8	-12.3	0	6.9	25.7	4.7

medium. The glutamic acid concentration in the external medium was kept constant at $10 \mu\text{mol./ml.}$ The addition of any of the mixtures resulted in a reduction of the rate of accumulation of free glutamic acid. Increasing the total number of amino-acids added resulted in progressive inhibition, and addition of the complete mixture of nineteen amino-acids abolished the accumulation of free glutamic acid almost completely. In later experiments the concentration of glutamic acid in the medium was reduced to $1.3 \mu\text{mol./ml.}$ and the addition of the mixture of eighteen amino-acids, each at a final concentration of 0.2 mg./ml. , then resulted in a decrease in the internal glutamic acid concentration during incubation. The suppression of the rate of accumulation of the free amino-acid within the cells can be correlated with the completeness of the external amino-acid mixture regarded as protein precursor. A similar effect of the quality of the amino-acid mixture has been noted by Pollock & Wainwright (1948) in their studies of the stimulation of nitratase formation by washed organisms. Table 3 shows that the cells form combined glutamate when incubated with the complete amino-acid mixture. The incomplete amino-acid mixtures, which effect a partial suppression of the accumulation of free glutamic acid, do not necessarily promote the formation of combined glutamate within the cells, and their effects will be dealt with in a later communication.

Table 3 gives a detailed analysis of the changes occurring in the distribution of glutamic acid during the incubation of *Staph. aureus* with various components of the glucose, glutamate and amino-acid mixture system. No increase in combined glutamate of the cells occurs in the absence of the amino-acid mixture or glucose. When the cells are incubated with glucose and glutamic acid alone, a marked accumulation of free glutamic acid occurs within the cells and more glutamic acid is taken up from the medium than appears in the free state within the cells. In no case is there any significant appearance of combined glutamate in the supernatant medium, the small amounts recorded in some experiments probably being due to autolysis of some of the cells during incubation. The discrepancy between the amount of glutamic acid removed from the medium and the amount appearing as the free amino-acid within the cells corresponds to the metabolism which takes place under these conditions (Gale & Mitchell, 1947). When the amino-acid mixture is added to the external medium, the amount of glutamate removed from the medium within a given time is greatly reduced, the concentration of free glutamic acid within the cells falls, the combined glutamate of the cells increases, and the amount of glutamic acid unaccounted for in the balance sheet is greatly reduced. Table 4 summarizes several experiments, carried out

with the complete synthetic amino-acid mixture or vitamin-free casein hydrolysate as external source of amino-acids, which support these conclusions.

Effect of internal concentration of free glutamic acid on rate of formation of combined glutamate

In the experiments described above, the formation of combined glutamate in the cells was accompanied by a decrease in the concentration of internal free glutamic acid. In order to determine whether

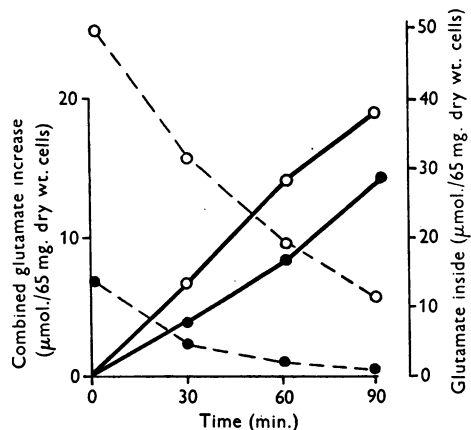


Fig. 2. Relation between internal free glutamate concentration and rate of formation of combined glutamate in *Staphylococcus aureus*. Deficient cells treated either with glucose and glutamate (○) or with glucose alone (●) and then incubated in buffered salt medium containing 1% glucose, and a mixture of 18 other amino-acids (final concn. $0.2 \text{ mg. each amino-acid}$). Samples taken at intervals and internal free glutamate determined (---); cells then hydrolysed with boiling $5N\text{-HCl}$ for 18 hr. and combined glutamate determined. —, increase in combined glutamate of cells.

the rate of combination of glutamate is affected in any way by the initial concentration of free glutamic acid within the cells, experiments were carried out in which the suspension of *Staph. aureus* was divided into two portions, one of which was first incubated in buffered salt solution containing glucose and glutamic acid, while the other was incubated in a similar solution without glutamic acid; the two suspensions were then washed, incubated with the complete amino-acid mixture (less glutamic acid) and glucose, and their rates of formation of combined glutamate compared. Table 5 gives a detailed analysis of the glutamic acid changes in such an experiment, and it can be seen that the cells which had been previously saturated with internal free glutamic acid form combined glutamate at a rate approximately 340% greater than that attained in the cells with low internal free glutamic acid. The concentration of free glutamic acid within the pretreated cells falls very rapidly on incubation with the amino-acid mixture

Table 5. *Changes in glutamic acid distribution: effect of initial concentration of internal glutamic acid in Staphylococcus aureus*

(Cells grown for 16 hr. at 30° in deficient medium; made into washed suspension and divided into portions *A* and *B*; each portion incubated for 1 hr. at 37° in buffered salt solution, *A* with 1% glucose, *B* with 1% glucose and 10 μ mol. sodium glutamate/ml. Organisms centrifuged down, washed once in distilled water and resuspended for treatment as in Table 3. Results expressed as change (μ mol.) in glutamate/116 mg. dry wt. of cells.)

Initial concentration of internal free glutamic acid (μ mol./116 mg. dry wt. of cells)	Cells pretreated with glucose			Cells pretreated with glucose + glutamic acid	
	13.2	13.2	13.2	54.5	54.5
Incubation mixture	Glucose + glutamate	Glucose + glutamate + amino-acid mixture	Glucose + amino-acid mixture	Glucose	Glucose + amino-acid mixture
Internal free glutamate of cells	+32.2	-5.8	-10.7	-17.0	-32.9
External free glutamate of medium	-56.6	-5.35	+3.8	+1.8	+13.0
Combined glutamate of cells	-7.1	+6.35	+4.9	+2.05	+16.5
Balance	-28.4	-4.8	0.0	-13.8	-0.4

Table 6. *Effect of concentration of internal free glutamic acid on rate of increase in combined glutamate in Staphylococcus aureus*

(Collected results on batches of cells pretreated with glucose or glucose + glutamic acid (as in Table 5) and then incubated with glucose and a mixture of eighteen amino-acids (without glutamic acid) as in Table 3. Decrease in internal free glutamate and increase in combined glutamate determined during 1 hr. at 37° and results expressed as change in μ mol. glutamate/100 mg. dry wt. of cells.)

Exp.	Internal free glutamic acid		External glutamate concentration (μ mol./ml.)	Increase in combined-glutamate (μ mol./hr./100 mg. cells)
	Initial (μ mol.)	Decrease (μ mol.)		
<i>a</i>	11.3	9.2	0	5.1
	—	5.05	1.34	5.45
	47.0	28.4	0	14.3
<i>b</i>	13.2	11.0	0	3.4
	—	6.3	1.34	6.5
<i>c</i>	15.7	9.45	0	14.3
	—	5.37	1.34	12.1
	61.0	26.5	0	19.2
<i>d</i>	51.0	22.4	0	14.0

and glucose. Consequently, the difference between the free glutamic acid concentrations within the previously saturated and the control cells decreases rapidly on incubation. Fig. 2 shows that the rate of combination of glutamic acid in the cells initially saturated with free glutamic acid is higher than that of the control, but that the rate falls as the internal free glutamic acid concentration falls.

Table 6 shows collected results relating the rate of increase of cell-combined glutamate to the initial concentration of free glutamic acid within the cells and to the presence or absence of external glutamic acid. It is clear that a high internal concentration of free glutamic acid can be correlated with a rapid rate of formation of combined glutamate. The rate of combination is not affected to any marked extent by the presence of external glutamic acid at the concentration used (see below), but its presence decreases the rate of disappearance of free glutamic acid inside the cells. In some cases the increase in

combined glutamate of the cells is approximately equal to the decrease in the amount of internal free glutamic acid, but in other cases it would appear that the combined glutamate can be drawn from either the internal or external medium.

In the absence of external sources of glutamic acid, the rate at which the internal concentration of free glutamic acid decreases during the formation of combined glutamate is frequently greater than the rate of appearance of combined glutamate in the cells. Fig. 3 shows that the rate of disappearance is greater when incubation takes place in the presence of glucose and an amino-acid mixture (free from glutamic acid) than in the presence of glucose or buffered salt alone. When *Staph. aureus* cells are incubated in buffered salt solution, a slow diffusion of internal glutamic acid occurs and 90% of the amino-acid disappearing from within the cell can be recovered from the external medium. If incubation takes place in the presence of glucose, the outward

diffusion is greatly decreased. This effect of glucose is the opposite of that which takes place with suspensions of *Strep. faecalis* (Gale, 1947a, 1948). The glutamic acid which disappears from *Staph. aureus* in the presence of glucose and amino-acids can be recovered to the extent of approximately 50% from the external medium, while approximately 35% is

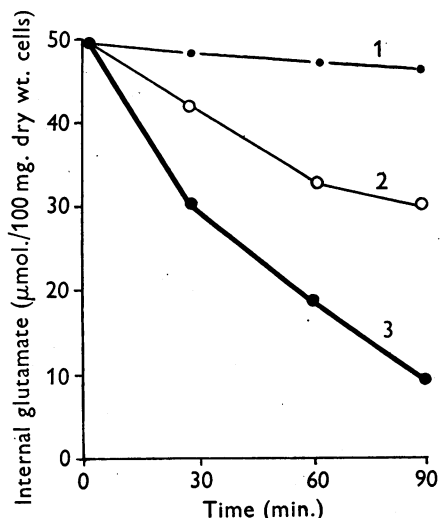


Fig. 3. Rate of decrease of internal free glutamate in *Staphylococcus aureus*. Cells incubated at 37° in (1) buffered salt solution containing 1% glucose, (2) buffered salt solution alone, (3) buffered salt solution containing 1% glucose and a mixture of 18 amino-acids (no glutamic acid) each at final concentration of 0.2 mg./ml. Samples taken at intervals, cooled rapidly and free glutamate content determined.

accounted for by increase in combined glutamate of the cells. It appears that glutamic acid passes out of the cell more easily under the conditions in which its combination occurs than when incubation takes place in a simple salt or salt and glucose medium.

Effect of external concentration of free glutamic acid on rates of accumulation of free and combined glutamic acid in the cells

It has been shown above that the formation of combined glutamate in the cells is accompanied by cessation of the accumulation of free glutamic acid. In the experiments quoted the amount of glutamic acid present in the external medium has been of the same order as the amount of each of the other amino-acids added. It has also been shown that the internal concentration of free glutamic acid plays a significant part in the rate of formation of combined glutamate. The question arises whether high external concentrations of glutamic acid would restore accumulation of the free amino-acid and what effect this would have on the rate of formation of combined glutamate. Table 7 shows results obtained when *Staph. aureus* cells were incubated with a constant concentration of the amino-acid mixture but with the external concentration of glutamic acid increased 10–100 times the value previously used. When the external concentration is low, the internal concentration of free glutamic acid falls during the formation of combined glutamate; increasing the external concentration slows down the disappearance of internal free glutamic acid and, at very high values, restores a small rate of accumulation of the free amino-acid. An increase in the external glutamic acid concentration from 1.34 to 13.4 μmol./ml. results in a significant increase in the rate of formation of combined glutamate, but higher concentrations appear to be inhibitory and no increase in the combined glutamate of the cells occurs when the external concentration is 134 μmol./ml.

DISCUSSION

In an early paper of this series (Gale, 1947b) it was shown that the free glutamic acid content of *Staph. aureus* cells in growing cultures increased as growth progressed and reached a maximum when growth

Table 7. *Effect of external glutamic acid concentration on rates of accumulation of free glutamic acid and formation of combined glutamate in Staphylococcus aureus*

(Conditions as for Table 3. Final concentration of each component of amino-acid mixture = 0.16 mg./ml. Control = glucose, sodium glutamate, buffered saline solution.)

External concentration sodium glutamate (μmol./ml.)	Rate of free glutamate accumulation (μmol./100 mg. dry wt. cells/hr.)		Rate of increase in combined glutamate (μmol./100 mg. dry wt. cells/hr.)	
	Control	Amino-acids present	Control	Amino-acids present
1.34	+39.5	-6.75	0	7.7
13.4	+43.7	+0.45	0	8.3
45.0	—	+6.4	0	6.6
1.34	+28.0	-6.7	0	5.4
13.4	—	-0.7	0	6.15
45.0	—	+2.5	0	0.93
134.0	+43.7	+4.0	0	0.0

ceased. It was also shown that the rate of accumulation of free glutamic acid within the harvested cells, tested in the absence of other amino-acids, was constant and independent of the growth phase. Since the steady state concentration of free glutamic acid within the cells is determined by a number of processes (Gale & Mitchell, 1947) and there is a reciprocal relation between the free and combined glutamic acid contents of the cells, it was deduced that the free glutamic acid accumulated within the cells before condensation into protein, and that a high rate of protein synthesis in 'young' growing cells lowered the concentration of free glutamic acid inside these cells. From the facts put forward in this communication it is clear that the relationship between accumulation of free glutamic acid and formation of combined glutamate in the cell cannot be as simple as previously suggested.

If washed cells are incubated in the presence of glucose and a mixture of amino-acids, glutamic acid enters into a combined form within the cell from which it can be released by acid hydrolysis. When these results are considered in conjunction with those of Hotchkiss (1947), it seems reasonable to suppose that the combined form is of a protein or protein + peptide nature. If glutamic acid is the only amino-acid present, it becomes concentrated in the free state inside the cell and no peptide synthesis occurs. If, during the accumulation of the free glutamic acid inside the cell, an amino-acid mixture, each component of which is in approximately the same concentration as the external glutamic acid, is added, the accumulation appears to cease and combined glutamate is formed. If, however, the external concentration of glutamic acid is greatly in excess of the concentration of the components of the amino-acid mixture, then some accumulation of free glutamic acid occurs within the cell, but the formation of combined glutamate is suppressed. There appears, therefore, to be competition between the processes involved in the accumulation of the free amino-acid and its combination into cell material, the former process taking place when glutamic acid is the major component of the external medium, the latter when a complete and balanced amino-acid mixture forms the external medium.

Comparison of the changes in glutamic acid distribution that take place in the two cases shows that, when the formation of combined glutamate (protein synthesis?) is occurring, the concentration of in-

ternal free glutamic acid decreases instead of increases, and this is accompanied by a marked decrease in the rate of withdrawal of glutamic acid from the external medium. The concentration of free glutamic acid within the cells is determined by the balance between the rate at which it enters the cell and the rate at which it is metabolized within the cell; the onset of synthesis would therefore cause a decrease in the rate of internal accumulation, but this should not be accompanied by a fall in the rate at which the amino-acid enters the cell. Two explanations appear possible: either the presence of other amino-acids in the external medium must decrease the rate of entry of glutamic acid into the cell or, alternatively, the passage of glutamic acid into the cell involves a metabolic stage which is also part of the process of peptide bond formation so that glutamic acid does not accumulate in the free state within the cell when the presence of other amino-acids renders peptide bond formation possible.

SUMMARY

1. When *Staphylococcus aureus*, in washed suspensions, is incubated in the presence of glucose and glutamic acid, free glutamic acid accumulates within the cells. A certain amount of the amino-acid is metabolized but no formation of combined glutamate occurs. In the presence of a complete and balanced mixture of amino-acids, the accumulation of free glutamic acid within the cells decreases and may cease, the amount of glutamic acid withdrawn from the medium in a given time is markedly less than that withdrawn when glutamic acid is the only amino-acid present, and an increase occurs in the amount of combined glutamate in the cells.

2. The rate at which the combined glutamate of the cells increases can be correlated with the initial concentration of free glutamic acid within the cells.

3. If the external glutamic acid concentration is raised to approximately 100 times that of the other components of the amino-acid mixture, some accumulation of free glutamic acid occurs, but the formation of cellular combined glutamate is suppressed.

4. Whether glutamic acid accumulates in the free state within the cell or enters into combination within the cell depends upon the balance between glutamic acid and the other amino-acids in the medium.

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