From Table 2 it will also be seen that the diabetogenic dose of alloxan in normal animals, as found in this laboratory, ranges between 130 and 140 mg./kg. and the lethal dose is observed to be in the neighbourhood of 225 mg./kg. It is interesting to note that the diabetogenic and lethal doses of alloxan in acetoacetate-treated animals, having blood GSH in the neighbourhood of 3 mg./100 ml., are only 75 and 100 mg./kg., respectively. Continued injection of acetoacetate for a few weeks has thus a definite effect in making the animals susceptible to alloxan.

It may be mentioned here that the diabetogenic and lethal doses of alloxan in normal animals, as quoted by Lukens (1948) and others, are 150–200 and 300 mg./kg., respectively, which are somewhat higher than the values observed by us. The disparity may be explained by the difference in diet, climate and breed of the animals.

SUMMARY

1. Rabbits injected subcutaneously with 150 mg./kg. sodium acetoacetate daily for 5 weeks show a slight rise in blood sugar, and an enormous fall in reduced glutathione in blood.

2. The susceptibility of these treated animals to alloxan is increased to a great extent, 75 mg./kg. being the diabetogenic dose and 100 mg./kg. the lethal dose, as compared with 130–150 and 225 mg./kg., respectively, for controls.

The authors record their grateful thanks to the Indian Council of Medical Research for the award of a Research Fellowship to one of them (V.G.H.).

REFERENCES

Archibald, R. M. (1945). *J. biol. Chem.* **158**, 347.
Griffiths, M. (1948). *J. biol. Chem.* **172**, 853.
Griffiths, M. (1950). *J. biol. Chem.* **184**, 289.
Grunert, R. R. & Phillips, P. H. (1949). *J. biol. Chem.* **181**, 821.
Hagedorn, H. C. & Jensen, B. N. (1923). *Biochem. Z.* **135**, 46.
Hirano, Y. (1934). *Orient. J. Dis. Inf.* **16**, 25.
Kass, E. H. & Waisbren, B. A. (1945). *Proc. Soc. exp. Biol., N.Y.*, **60**, 303.
Lazarow, A. (1946). *Proc. Soc. exp. Biol., N.Y.*, **61**, 441.
Lazarow, A., Patterson, J. W. & Levy, S. (1948). *Science*, **108**, 308.
Lukens, F. D. W. (1948). *Physiol. Rev.* **28**, 304.
Nath, M. C. & Brahmachari, H. D. (1944). *Nature, Lond.*, **154**, 487.
Nath, M. C. & Brahmachari, H. D. (1946). *Nature, Lond.*, **157**, 336.
Nath, M. C. & Chakrabarti, C. H. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 326.
Nath, M. C. & Chakrabarti, C. H. (1951). *Proc. Soc. exp. Biol., N.Y.*, **78**, 369.
Nath, M. C. & Hatwalne, V. G. (1950). *Nature, Lond.*, **166**, 692.
Nath, M. C., Hatwalne, V. G. & Gadgil, J. S. (1953). *Biochem. J.* **53**, 479.
Prunty, F. T. G. & Vass, C. C. N. (1943). *Biochem. J.* **37**, 506.
Woodward, G. E. & Fry, E. C. (1932). *J. biol. Chem.* **97**, 465.

The Assimilation of Amino-acids by Bacteria

14. NUCLEIC ACID AND PROTEIN SYNTHESIS IN *STAPHYLOCOCCUS AUREUS*

By E. F. GALE and JOAN P. FOLKES
Medical Research Council Unit for Chemical Microbiology, Biochemical Laboratory, University of Cambridge

(*Received* 22 *May* 1952)

If washed suspensions of *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) are incubated in buffered salt solution containing glucose and glutamic acid, free glutamic acid accumulates to a high concentration within the cell (Gale, 1947*a*, *b*). If certain other amino-acids, such as cysteine or alanine, are added singly to the incubation mixture, the accumulation of free glutamic acid within the cells is reduced while peptides of glutamic acid appear in the external medium (Gale & Van Halteren, 1951). If a complete mixture of amino-acids and glucose is present, then the cells no longer accumulate free glutamic acid but the combined glutamate of the cellular substance increases, glutamic acid from inside and outside the cell becoming directly incorporated into this combined glutamate fraction (Gale, 1951*b*).

In the investigations so far described in this series, no account has been taken of the nucleic acid content of the cells or of its variation. In the present work, the nucleic acid content has been studied and the effect of the addition of purines and

pyrimidines to the incubation mixture tested on the various stages of glutamic acid assimilation. Malmgren & Heden (1947) investigated the variation of 'nucleotide' content of bacterial cells during the period of growth; they used the absorption by the cells of light of wavelength 257 mμ. as a measure of 'nucleotide' content and found that the synthesis of this 'nucleotide' was greatest during the first generation time, the content falling again during the later stages of growth. Comparison of the curves obtained for 'nucleotide' synthesis and rate of growth for a wide variety of bacteria suggested that the 'nucleotide' content of the cell played a controlling role in its rate of protein synthesis. Caldwell, Mackor & Hinshelwood (1950) found that the deoxyribonucleic acid content of *Bacterium lactis aerogenes* (presumably *Aerobacter aerogenes*) was an approximately constant component of the cell, whereas the ribonucleic acid content varied over a wide range, and that the rate of growth of the cell was proportional to the ribonucleic acid content. Caldwell & Hinshelwood (1950) put forward theoretical considerations suggesting that nucleic acid guides the order in which amino-acids are laid down during protein synthesis by bacteria; they further suggest that protein, in turn, guides the order in which nucleotide units are combined in nucleic acid synthesis. Evidence and theories concerning the role of nucleic acid in protein synthesis have recently been reviewed by Chantrenne (1952).

METHODS

Organism. The organism used throughout these studies was *Staph. aureus* Duncan, the strain used for earlier studies in this series and originally isolated from pus.

Growth medium and preparation of suspensions. The organism was grown overnight at 30° in the 'deficient medium *B*' previously described (Gale, 1951*a*) which consists of a salt medium with glucose, Marmite and arginine added. The organisms were harvested on the centrifuge, washed once with distilled water, and made up in distilled water to a suspension density of approximately 20 mg. dry wt. of cells/ml. Dry weights were determined turbidimetrically on a Hilger absorptiometer previously calibrated against the organism used. The cell suspension was diluted 1:10 during incubation with amino-acid mixtures, etc., washed and concentrated on the centrifuge to the original strength for estimation of glutamic acid, etc.

Growth tests. The nutritional requirements of *Staph. aureus* Duncan were determined in synthetic media according to the general method of Gladstone (1937). A complete medium was prepared containing twenty amino-acids (as below), thiamine, nicotinamide and glucose; further tubes of media were prepared to test the effect of the omission of each amino-acid one at a time; all tubes were inoculated with a standard inoculum of $3–10 \times 10^8$ cells/10 ml. medium and growth inspected at 24 hr. intervals during incubation at 37°.

Amino-acid solutions. For experiments designed to test the effect of various combinations of amino-acids, 0·04 M solutions of glycine and of the L isomers of the following were prepared: aspartic acid, asparagine, glutamic acid, tyrosine, tryptophan, phenylalanine, histidine, lysine, arginine, methionine, proline, hydroxyproline, serine, cysteine, cystine, alanine, threonine, leucine, isoleucine and valine. Each solution was adjusted to pH 7·0 and diluted 1:30 in the incubation mixture unless otherwise stated. When a complete mixture was required, a solution (*A*) was prepared containing all the above amino-acids, each at a concentration of 2·0 mg./ml., the pH adjusted to 7·0 and, except where otherwise stated, diluted 1:10 in the incubation mixture.

Purine and pyrimidine mixture. Solutions of each of the following purines or pyrimidines at a concentration of 1·0 mg./ml. were prepared: adenine, xanthine, hypoxanthine, guanine, thymine, cytosine and uracil. A mixture (solution *P*) was also prepared containing all these substances, each at a concentration of 1·0 mg./ml.; only a small amount of cytosine was available and this was omitted from some experiments where it was found to have no significant effect, in such cases the purine-pyrimidine solution being noted as mixture *P'*. All these solutions were diluted 1:50 in the incubation mixture.

Amino-acid estimations. Free glutamic acid, arginine and lysine were estimated manometrically by use of the corresponding specific decarboxylase preparations (Gale, 1945*a*, *b*). Total amino-acid contents of cells and precipitates were determined by the same method applied to hydrolysates obtained after 18 hr. hydrolysis in boiling 6 N-HCl, excess acid being removed *in vacuo*. In preparations such as cell suspensions which contained both free and combined glutamic acid, the latter was calculated from the difference between the free and total contents.

Accumulation of free glutamic acid within cells, increase in combined glutamate fraction, rates of fermentation and respiration. These have been studied by the methods described in previous papers of this series (Gale, 1947*a*; 1951*a*, *b*).

Precipitation and estimation of protein. Suspensions of cells, after washing, were precipitated in the cold with trichloroacetic acid (TCA) to a final concentration of 5% (w/v). After standing for not less than 2 hr. in the cold, the precipitates were centrifuged down, washed once with cold 5% TCA, suspended in water and hydrolysed for 18 hr. in boiling 6 N-HCl. The excess acid was removed *in vacuo* and the hydrolysates made up to a known volume after the pH had been adjusted to approx. 5. The combined glutamate content was taken as a measure of protein present (see below).

Nucleic acid content of cells. Mitchell (1950) has described a method for the estimation of 'nucleic acid' in suspensions of bacteria. This involves estimation of the absorption at 260 mμ. and calculation of the correction for light scattering from the absorption at 350 mμ. The method estimates all substances having an absorption at 260 mμ. and thus includes free purines, nucleotides, etc. Salton (1951) has shown that organisms may contain free purines and pyrimidines, and that these are released by treatment of the cells with cetyltrimethylammonium bromide (CTAB). Since we wished to estimate the nucleic acid content of the cells free from purines or nucleotides, we tested the application of Mitchell's method to cell suspensions which had been treated with CTAB under the conditions described by Salton (1951), followed by washing and resuspension in water. It became evident during the course of the work that the calculated scattering correction introduced inaccuracies,

especially when inhibitors were used which had a differential action on protein and nucleic acid synthesis. Consequently, a more direct method of estimation was sought.

The cell suspensions were precipitated in the cold with 5% TCA as for the estimation of protein. After washing with cold 5% TCA, a portion of the precipitate, equivalent to approx. 20 mg. dry weight of original cell suspension, was extracted three successive times with 2·0 ml. 5% TCA at 90° for 10 min. 90–95% of the nucleic acid was extracted in the first two treatments and no further extraction took place after the third treatment. The extracts were collected, combined and made up to 10·0 ml. with water. 1·0 ml. of the extract was then diluted to 10·0 ml. with water and the absorption at 260 mμ. determined on a Beckman spectrophotometer against a blank containing an equivalent amount of TCA. Caspersson (1936) and Malmgren & Heden (1947) used an extinction coefficient of 22 for 0·1% nucleic acid, but Magasanik & Chargaff (1951) found that hydrolysis of the nucleic acid to nucleotides resulted in an increase in absorption of 20–30%. The extraction procedure used in this work results in hydrolysis of the nucleic acid, and the extinction coefficient used by Caspersson is clearly too low. A value of 28 for 0·1% nucleic acid has been adopted as the result of calculations based upon the base analysis of a number of preparations of nucleic acid from *Staph. aureus* grown and treated in various ways described in this paper and the next of the series (Gale & Folkes, 1953).

Quantitative estimation of purines and pyrimidines. An amount of TCA extract containing approx. 3 mg. of nucleic acid was evaporated to dryness, taken up in 20 μl. 72% (w/v) $HClO_4$ and heated in a sealed tube at 100° for 2 hr. The hydrolysate was diluted with 20 μl. water, centrifuged to remove carbon, and 20 μl. taken for separation and estimation of the bases by the method of Smith & Markham (1950) as modified by Wyatt (1951).

RESULTS

Conditions affecting protein synthesis

Amino-acid requirements of Staph. aureus *Duncan.* Full growth of the organism takes place in 10–12 hr. at 37° in a synthetic medium containing nineteen naturally occurring amino-acids. Omission of the amino-acids one at a time from the medium shows that arginine, lysine, methionine and hydroxyproline have no effect on growth, whereas the remaining fifteen amino-acids are essential for growth to occur within 24 hr. of inoculation. No growth occurs up to 128 hr. in the absence of histidine, aspartic acid, valine, leucine, isoleucine, tyrosine, phenylalanine, tryptophan or threonine, whereas delayed growth occurs in the absence of alanine, glycine, proline, glutamic acid or serine. It is probable that growth occurring after 24 hr. and before 128 hr. is due to the selection of mutants which are able to synthesize the missing amino-acid in each case. The delay caused by omission of glutamic acid is approximately 40 hr. and it is possible that sufficient glutamic acid for growth may be produced by transamination from aspartic acid but the latter is completely essential even after 128 hr. incubation. The salt mixture used as a basal medium was free from ammonium salts; the addition of ammonium salts resulted in markedly slower growth in all cases.

Effect of amino-acid mixtures on combined glutamate formation. Table 1 summarizes the changes which occur in the free and combined glutamic acid contents of cells and supernatant when incubation takes place for 30 min. in buffered salt solution containing glucose and various combinations of amino-acids. It can be seen that, as the complexity of the incubation mixture increases, the amount of free glutamic acid accumulating within the cells progressively decreases, as does the amount withdrawn from the medium. The combined glutamate content of the cells decreases when incubation takes place in mixtures of a few amino-acids, and it is not until all the essential amino-acids are present in the medium that a significant increase in combined

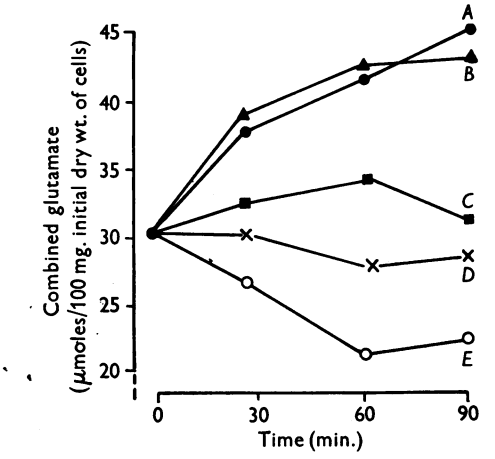

Fig. 1. Changes in the combined glutamate of cells incubated in buffered salt solution containing 1·0% glucose and additions. Additions: curve *A*, complete mixture of amino-acids; curve *B*, as (1) less lysine, arginine, methionine and proline; curve *C*, as (1) less aspartic acid; curve *D*, as (1) less cysteine; curve *E*, glutamic acid only. Conditions as for Table 2; incubation at 37°.

glutamate occurs. The further addition of the non-essential amino-acids makes little difference to the rate of increase of combined glutamate, but this may be due to a slightly inhibitory action of methionine since omission of this amino-acid from the complete mixture results in a significantly increased accumulation of combined glutamate. Table 1 also shows the effect of the omission from the complete amino-acid mixture of one or two of the amino-acids essential for growth. Omission of aspartic acid decreases but does not abolish the increase in combined glutamate obtained in 30 min., but progress curves carried out for a longer period (Fig. 1) show that such increases only occur during

Table 1. *Effect of amino-acid mixtures on the accumulation of free and combined glutamic acid by washed suspensions of* Staphylococcus aureus

(Washed suspensions of *Staph. aureus* Duncan, at final suspension density of 2·0 mg. dry wt./ml., incubated for 30 min. at 37° in buffered salt solution containing 1% glucose and each amino-acid (marked +) to a final concentration of 1·34 μmoles/ml. In cases marked (3) final amino-acid concentration is 4·02 μmoles/ml. After incubation, cells harvested and washed on the centrifuge, and free and combined glutamic acid content determined for cells and supernatants. Experiments carried out with eight incubation mixtures on each occasion, one of these mixtures containing glucose and glutamic acid only; results expressed in terms of this control for comparative purposes.)

Incubation mixture																			Relative increase in internal free glutamate of cells (100 = 34·5 μmoles/30 min./100 mg. dry wt. of cells)	Change in combined glutamate of cells (μmoles/30 min./100 mg. dry wt. of cells)	Relative change in free glutamate of supernatant (100 = 50·5 μmoles/30 min./100 mg. dry wt. of cells)
Glucose	Glutamic acid	Aspartic acid	Cysteine	Alanine	Glycine	Histidine	Valine	Leucine	Tyrosine	Phenylalanine	Tryptophan	Threonine	Serine	Isoleucine	Arginine	Lysine	Proline	Methionine			
+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ 6·5	− 1·6	+ 4
+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+100	− 5·8	−100
+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ 50	− 5·2	− 33
+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ 77	− 5·8	−102
+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ 37	− 5·2	− 34·5
+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+106	− 6·5	−109
+	+	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0	+110	− 6·3	−103
+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+ 92	− 5·6	− 96
+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ 34	− 8·3	− 35
+	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	+ 22·5	− 4·3	− 39
+	+	+	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	+ 25	− 3·1	− 62·5
+	+	0	+	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	+ 66	− 5·8	− 80
+	+	+	0	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	+ 38	−10·9	− 70
+	+	+	+	+	+	0	+	+	0	0	0	0	+	0	0	0	0	0	+ 35	− 6·8	− 54
+	+	+	+	+	+	0	+	+	+	+	0	0	+	0	0	0	0	0	+ 34	− 3·7	− 47
+	+	+	+	+	+	0	+	+	+	+	+	0	+	0	0	0	0	0	+ 35	− 5·2	− 35
+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	0	0	0	+ 21	− 0·8	− 43
+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	0	0	0	0	+ 22	+ 0·6	− 35
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0	+ 4·3	+ 2·7	− 15
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	+ 3·2	+ 2·8	− 14
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	− 6·0	+ 3·4	− 7
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	− 2·5	+ 3·9	.
+	+	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+	− 0·6	+ 3·5	− 7
+	+	+	+	+	+	+	0	0	+	+	+	+	+	0	+	+	+	+	+ 10	+ 2·7	− 5
+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+ 34	+ 2·2	− 47
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+ 2	+ 2·1	− 27
+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+ 13	+ 1·4	.
+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+ 18	+ 0·2	− 8
+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+ 9	− 0·3	− 15
+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+ 15	− 3·2	− 7
+	+	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	− 16	+ 3·7	− 21·5
+	+	+	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	− 14	+ 4·5	− 12·6
+	+	+	+	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	− 14	+ 4·5	− 14

the early part of the incubation, the complete mixture of essential amino-acids being necessary for continued production of combined glutamate. On incubation for 90 min. or more, a mixture of all the natural amino-acids is slightly more effective than a mixture of the essential amino-acids only.

The amino-acids found by Gale & Van Halteren (1951) to decrease the accumulation of free glutamic acid within the cells included aspartic acid, cysteine and alanine. Table 1 shows that increasing the concentration of cysteine, aspartic acid or alanine in the incubation mixture results in an increase in the rate of accumulation of combined glutamate in the cells.

Nature of the combined glutamate fraction. Table 2 shows that 90 % of the combined glutamate of the cells, before and after incubation with glucose and a complete mixture of amino-acids, is precipitated by 5 % TCA in the cold. Analysis of the material obtained by disintegrating the cell suspension on a Mickle vibrator (Mickle, 1948) shows that at least 92 % of the TCA-precipitable combined glutamate resides in the 'soluble' portion of the cells that is not centrifuged down at 4000 *g* in 20 min.

It is possible that an increase in combined glutamate of protein-like materials could occur without a concomitant increase in whole protein. Consequently, the TCA precipitate was analysed for lysine, arginine, amino nitrogen and total nitrogen in addition to glutamic acid. Table 3 shows a series of such determinations for TCA precipitates obtained from cells treated in various ways described below and in the next paper (Gale & Folkes, 1953). The precipitates obtained by treatment of cells with cold 5 % TCA contain nucleic acid as well as protein and some of the treatments listed in Table 3 (incubation in the presence of chloramphenicol in particular (Gale & Folkes, 1953)) result in an increase in nucleic acid but not in protein content; consequently total nitrogen values will not show variations which can be correlated with protein content only. Table 3 shows that combined glutamate estimations give a good indication of variations in protein content, and that the total nitrogen values correlate least well, of the quantities measured, with the combined glutamate values. In general, increases in protein content have been

Table 2. *Nature of combined glutamate fraction of* Staphylococcus aureus

(Cells harvested from deficient medium; estimations carried out on (*a*) cells as harvested; (*b*) cells after incubation for 1 hr. at 37° in buffered salt solution containing glucose and amino-acid mixture *A*. Cells were washed after incubation, and free and combined glutamic acid estimations carried out on the cell suspensions and on precipitates obtained therefrom by treatment with 5 % TCA in the cold. 'Soluble' and insoluble fractions of cells obtained by centrifuging cell debris at 4000 *g* for 20 min. after disintegration of cells on the Mickle machine (Mickle, 1948).)

Exp. no.	Preparation	Combined glutamate (μmoles/100 mg. dry wt. of cell) (*a*)	(*b*)	Increase (*b* − *a*)
1	Intact cells	28·8	37·6	8·8
	(i) TCA precipitate	25·6	34·1	8·5
	(ii) TCA-soluble fraction	3·2	3·8	0·6
2	Mickle disintegrate			
	(i) Insoluble fraction	1·8	2·2	0·4
	(ii) 'Soluble' fraction	19·9	23·6	3·7
	(iii) TCA precipitate of 'soluble' fraction	19·8	23·4	3·6

Table 3. *Analyses of protein precipitates from* Staphylococcus aureus

(*Staph. aureus* Duncan grown in deficient medium, harvested and made into washed suspension. Cells incubated for 1 hr. at 37° in buffered salt medium with additions as below. After incubation, cells washed in water and precipitated with 5 % TCA for 2 hr. in the cold; precipitates washed with cold 5 % TCA and then hydrolysed for 18 hr. in boiling 6 N-HCl; excess acid removed *in vacuo* and amino-acid determinations, etc. carried out on neutralized hydrolysates. Results expressed as percentage initial suspension. Abbreviations: *G*, glucose; *A*, complete mixture of amino-acids; *A* − *g*, complete mixture of amino-acids less glutamic acid; *P*, mixture of purines and pyrimidines; *CAP*, 30 μg. chloramphenicol/ml.)

	Initial suspension (mg. N/100 mg. dry wt. of cells)	Additions to incubation mixture: *G*	*G*, *A*	*G*, *A*, *P*	*G*, *A* − *g*, *P*	*G*, *A*, *P*, *CAP*
Glutamic acid	0·414	93	121	134	100	96
Arginine	0·441	96	125	136	108	101
Lysine	0·910	103	118	133	108	104
Amino-N	5·95	99	123	131	106	106
Total-N	8·86	98	120	134	103	115
Nucleic acid	—	95	112	148	113	178

estimated by determination of the combined glutamate content of the TCA precipitates obtained from the cells.

Effect of addition of purine and pyrimidine mixtures. The synthesis of cellular protein described above takes place when the cells are incubated in the presence of glucose and a mixture of amino-acids. Fig. 2 shows the effect of adding the purine-pyrimidine mixture P' to the incubation medium; the rate of protein synthesis is significantly accelerated (see Table 3 also). In a series of twelve observations under the conditions holding for the

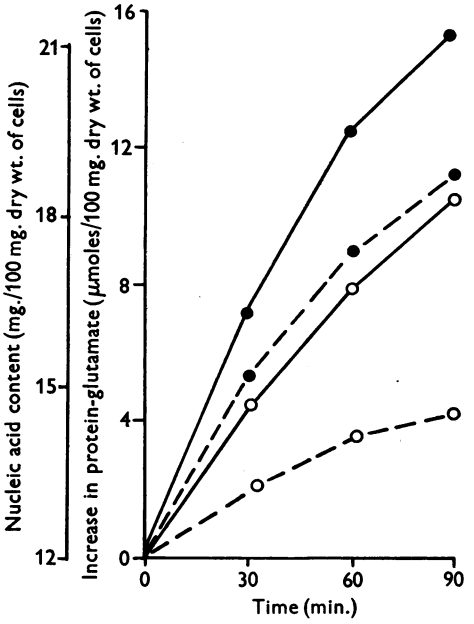

Fig. 2. Increase in nucleic acid (broken lines) and protein (full lines) of *Staph. aureus* when incubated in buffered salt solution containing 1% glucose and the complete mixture of amino-acids A with (●) and without (○) the purine-pyrimidine mixture P'.

experiment shown in Fig. 2, the mean acceleration of protein synthesis resulting from the addition of mixture P' has been 49·5 % of the control without P' (range 18–112 %).

Effect of concentration of amino-acid mixture. In the experiments described above, the concentration of the amino-acid mixture solution A was such as to give a final concentration of 0·2 mg. of each amino-acid/ml. medium. Fig. 3 shows the effect of increasing the concentration of the mixture A on the rate of protein synthesis with and without the addition of mixture P to the medium. It can be seen that the protein-synthesizing system is saturated at a much lower amino-acid concentration in the absence of P than in its presence, and that, at a high amino-acid concentration, the presence of P may increase the rate of protein synthesis by as much as 300 %.

Conditions affecting nucleic acid synthesis

Effect of amino-acids. Fig. 4*a* shows that there is little or no increase in the nucleic acid content of cells if these are incubated in the presence of glucose alone, mixture P alone, or glucose and mixture P. However, if amino-acids are added to the incubation mixture, a significant increase in nucleic acid occurs. Glycine has a marked effect, but most amino-acids added alone have only a small effect; simple mixtures of four or five amino-acids have some effect but, in general, the larger the number of amino-acids present, the greater the stimulation of

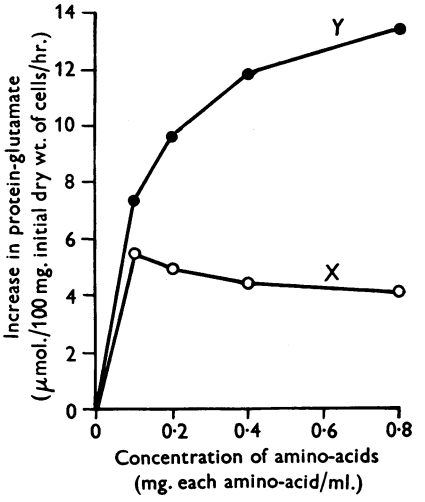

Fig. 3. Effect of amino-acid concentration on rate of protein synthesis by *Staph. aureus* Duncan in presence (●) and absence (○) of purine-pyrimidine mixture P. Cells grown for 18 hr. at 30° in deficient medium; made into washed suspension and incubated for 1 hr. at 37° in buffered salt solution containing 1·0% glucose and amino-acid mixture A at concentrations shown, with (curve Y) and without (curve X) purine-pyrimidine mixture P at final concentration of each component = 0·02 mg./ml. Protein assayed by combined glutamate content of TCA precipitate.

nucleic acid formation. A mixture of all the amino-acids essential to growth gives a marked stimulation, which is slightly increased by the further addition of the non-essential amino-acids. Increasing the concentration of the complete amino-acid mixture beyond that quoted in Fig. 4 does not further increase the formation of nucleic acid. Omission of one essential amino-acid, such as aspartic or glutamic acid, from the complete mixture results in a greatly decreased effect. Comparison of the effects shown in Table 1 and Fig. 4 suggests that the action of

amino-acids on nucleic acid formation can be correlated with their ability to promote protein synthesis.

Effect of purines and pyrimidines. Fig. 2 shows progress curves for the increase in the nucleic acid content of cells incubated with a complete amino-acid mixture with and without mixture *P'*. There is a small increase in nucleic acid in the absence of added *P'* and this is greatly increased when *P'* is added to the mixture. The growth of *Staph. aureus* Duncan is not nutritionally exacting towards purines or pyrimidines under aerobic conditions, and Salton (1951) has shown that Staphylococci contain free purines and pyrimidines within the cell, so that the small increase in nucleic acid that occurs on incubation with amino-acids and glucose may take place at the expense of purine derivatives already present within the cell or synthesized during the incubation.

Fig. 4*b* shows that the nucleic acid synthesis occurring on incubation with glucose and amino-acids is not greatly stimulated by the addition of any one purine or pyrimidine, whereas the complete mixture *P* has a marked effect. Omission of adenine, guanine, cytosine or thymine singly from the mixture *P* has little effect, whereas omission of uracil or xanthine results in a marked decrease in nucleic acid formation. The complete mixture without uracil has little more effect than that of the amino-acid mixture alone, and this can be correlated with the finding by Richardson (1936) that *Staph. aureus* strains are unable to synthesize uracil during growth under anaerobic conditions. Since cytosine was available in small quantities only, and its omission from mixture *P* had little or no effect on nucleic acid synthesis, it was not added in many of the later experiments (Gale & Folkes, 1953).

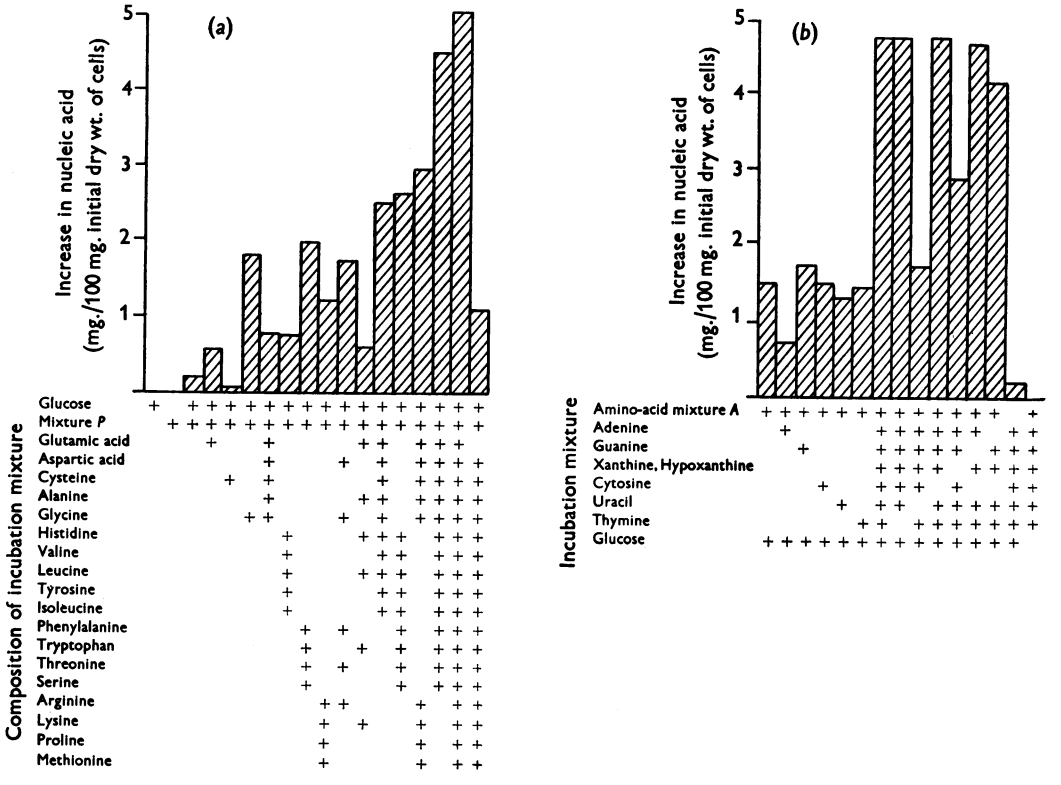

Fig. 4. Nucleic acid synthesis in *Staph. aureus* Duncan showing effect of addition of (*a*) amino-acids and (*b*) purines and pyrimidines to the incubation mixture. Cells grown for 16 hr. at 30° in 'deficient' medium; made into washed suspension and incubated, at final suspension density = 2·0 mg./dry wt./ml., for 1 hr. at 37° in buffered salt medium containing 1% glucose, amino-acids (final concentration of each component = 1·34 μmoles/ml.), purines and pyrimidines (final concentration of each component = 0·02 mg./ml.); the presence of each component in the incubation mixture is indicated by +. After incubation, cells precipitated with 5% TCA in the cold, nucleic acid fraction extracted with hot 5% TCA and estimated spectrophotometrically.

No increase in nucleic acid has been observed in the absence of glucose in the incubation medium.

Table 4. *Composition of nucleic acid fraction of* Staphylococcus aureus

((*a*) *Staph. aureus* Duncan grown for 6 hr. at 37° in 'deficient' medium, harvested and made into washed suspension. (*b*) *Staph. aureus* Duncan grown for 16 hr. at 30° in 'deficient' medium, harvested and made into washed suspension. (*c*) Portion of suspension (*b*) after incubation for 1 hr. at 37° in buffered salt solution containing glucose, amino-acid mixture *A* and purine-pyrimidine mixture *P'*. In all cases, cells precipitated with 5% TCA in the cold, precipitates washed with cold 5% TCA, extracted three successive times with 5% TCA at 90° for 10 min., extracts collected and analyses performed on combined extracts. Results expressed as % dry wt. of initial cells.)

	(*a*)	(*b*)	(*c*)
Total nucleic acid	14·1	10·3	13·0
Adenine	1·34	0·86	1·07
Guanine	1·68	1·02	1·29
Cytosine	0·90	0·66	0·83
Uracil	0·625	0·535	0·675
Thymine	0·22	0·25	0·27

Composition of the nucleic acid fraction. Table 4 gives analyses of the purine and pyrimidine bases determined on hydrolysates of nucleic acid fractions obtained from *Staph. aureus* before and after incubation with the complete incubation mixture containing glucose, amino-acids and mixture *P'*, and also from cells harvested at two different 'ages' of culture. The major part of the changes in the nucleic acid would appear to reside in the ribonucleic acid fraction; this is in accordance with the findings of Caldwell *et al.* (1950) for *Bact. lactis aerogenes*.

Relationships between nucleic acid content of Staphylococcal cells and their ability to synthesize cellular protein or to accumulate free glutamic acid

Previous studies in this series have suggested that there is a metabolic connexion between the processes of free glutamic acid accumulation and of protein synthesis (Gale, 1951*b*; Gale & Van Halteren, 1951). A number of workers have suggested a connexion between protein synthesis and nucleic acid metabolism (Jeener & Brachet, 1944; Caspersson, 1947; Malmgren & Heden, 1947; Caldwell & Hinshelwood, 1950; Swensson, 1950; Jeener & Jeener, 1952; Chantrenne, 1952). In the course of the work described here and in the next paper (Gale & Folkes, 1953), it became clear that a number of methods had been obtained whereby the nucleic acid content of washed *Staph. aureus* cells could be altered; some of these are summarized in Table 5. Malmgren & Heden (1947) have described how the 'nucleotide' content of bacteria varies with the age of the culture so that cells with different nucleic acid contents can presumably also be obtained by harvesting the organisms at different phases of growth. It was therefore decided to use these various methods to test what relationship, if any, could be found between the nucleic acid content of the cells and their ability either to synthesize protein or to accumulate free glutamic acid.

Table 5. *Variation of rates of protein synthesis or accumulation of free glutamic acid with nucleic acid content of washed suspensions of* Staphylococcus aureus

(Suspensions of *Staph. aureus* Duncan incubated in buffered salt solution with additions as below (first treatment) for 45 min. at 37°. Cells centrifuged down, washed and estimations made of nucleic acid and protein-glutamate content. The remainder of each batch divided into two and incubated in buffered salt solution containing 1% glucose and (*a*) complete amino-acid mixture *A* for 1 hr. at 37°, or (*b*) 2·7 μmoles sodium glutamate/ml. for 15 min. at 37°. Increases in protein-glutamate and internal free glutamic acid estimated. Results expressed on basis of (i) 100 mg. dry wt. of cells as initially sampled, and (ii) 100 mg. dry wt. of cells allowing for the increase in protein content during the first treatment.)

	First treatment			Second treatment			
				(*a*) Increase in protein glutamate (μmoles/100 mg. cells)		(*b*) Increase in free glutamic acid content (μmoles/100 mg. cells)	
Additions to salt solution	Protein-glutamate at end of treatment (μmoles/100 mg. initial dry wt. of cells)	Nucleic acid content at end (mg./100 mg. cells) (i)	(ii)	(i)	(ii)	(i)	(ii)
(1) Glucose, complete amino-acid mixture *A*, purine mixture *P'* + 30 μg. chloramphenicol/ml.	28·8	17·1	17·4	6·91	7·08	12·1	12·4
(2) As (1) with glutamic acid omitted from mixture *A*	27·0	16·45	17·1	6·0	6·52	12·7	13·8
(3) As (1) without chloramphenicol	35·1	15·05	12·75	5·6	4·7	(19·7	16·4)
(4) As (3) + 3000 units penicillin G/ml.	32·4	13·75	11·75	6·2	5·68	15·2	13·8
(5) Glucose, amino-acid mixture *A*	32·4	12·7	11·5	6·9	6·3	16·5	14·1
(6) Glucose only	29·0	10·7	10·8	4·8	4·85	20·0	20·3
(7) Initial suspension (first treatment omitted)	29·4	10·5	—	4·04	—	21·2	—

Alteration of nucleic acid content of washed cells. A large batch of *Staph. aureus* was grown and harvested in the usual manner. The nucleic acid content and rates of fermentation, free glutamic acid accumulation and protein synthesis (in the absence of added purines or pyrimidines) were measured. Various portions of the suspension were then incubated for 45 min. under the conditions set out in Table 5, with the consequent changes in nucleic acid content also shown. Each portion of the suspension was then washed, divided into two, and the rates of protein synthesis or free glutamic acid accumulation determined under the appropriate conditions. The

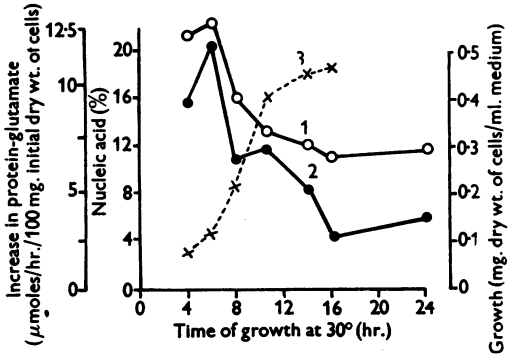

Fig. 5. Variation with age of culture in *Staph. aureus* Duncan of nucleic acid content (○), rate of protein synthesis (●) and growth (×). Growth took place in deficient medium at 30°. Samples of culture taken at intervals as shown, washed suspensions prepared and divided into two portions. Nucleic acid content determined on one; rate of increase in protein glutamate determined on other by incubation for 1 hr. at 37° in buffered salt solution containing 1% glucose and amino-acid mixture *A* (final concentration of each component =0·2 mg./ml.).

results are set out in Table 5 in order of decreasing nucleic acid content at the end of the first incubation. Inspection shows that, as the nucleic acid content has increased, the rate of accumulation of free glutamic acid has decreased. There is no clear-cut correlation between nucleic acid content and rate of protein synthesis, but this might be masked by the considerable error in the measurement of rates of protein synthesis in some of these examples. It is interesting to observe that the inhibitory action of chloramphenicol on protein synthesis (Gale & Folkes, 1953) during the first incubation does not persist when the cells are washed and resuspended in an amino-acid mixture. There is an increase of approximately 65% in the nucleic acid content of cells incubated with glucose, amino-acids and mixture *P'* in the presence of chloramphenicol; this increase is associated with an increase of 71% in the rate of protein synthesis by the washed cells while their rate of accumulation of free glutamic acid has decreased by 43%.

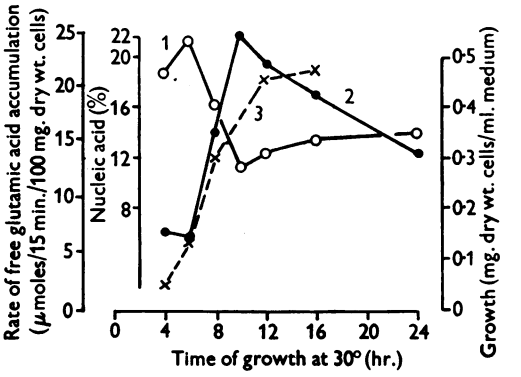

Fig. 6. Variation with age of culture in *Staph. aureus* Duncan of nucleic acid content (curve 1), rate of accumulation of free glutamic acid (curve 2) and growth (curve 3). Conditions as for Fig. 5 with the exception that the suspension was incubated for 15 min. at 37° in buffered salt solution containing 1% glucose and 2·7 μmoles sodium glutamate/ml. for determination of rate of accumulation of free glutamic acid.

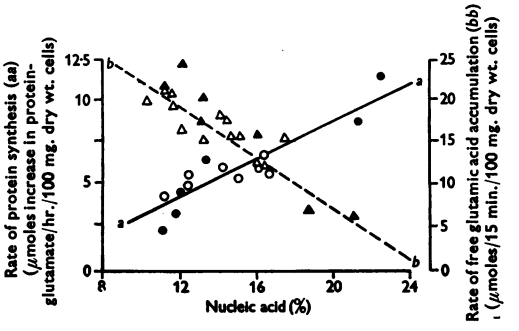

Fig. 7. Correlations between nucleic acid content of *Staph. aureus* Duncan and rate of protein synthesis (*aa*), or rate of accumulation of free glutamic acid (*bb*). *Rate of protein synthesis.* Solid circles (●) represent determinations with cells of differing age of culture as in Fig. 5; open circles (○) determinations with washed suspensions as in Table 5. $A = 0{\cdot}54y - 2{\cdot}02$ where A = rate of increase in protein glutamate in μmoles/100 mg. dry wt. of cells/hr. and y = % nucleic acid content of cells. $n = 14$, $r = +0{\cdot}875$, $P < 0{\cdot}01$. *Rate of free glutamic acid accumulation.* Solid triangles (▲) represent determinations with cells of differing age of culture as in Fig. 6; open triangles (△) determinations with washed suspensions as in Table 5. $B = 37{\cdot}2 - 1{\cdot}5y$ where B = rate of accumulation of free glutamic acid in μmoles/100 mg. dry wt. of cells/15 min. and y = % nucleic acid content of cells. $n = 20$, $r = -0{\cdot}812$, $P < 0{\cdot}01$.

Variation of nucleic acid content in growing cells. A large batch of medium was inoculated and samples of culture withdrawn at intervals through growth at 30°. Washed suspensions were made at each stage and, as before, nucleic acid content and rates of

accumulation of free glutamic acid or protein synthesis (in the absence of added purines and pyrimidines) determined. The variations described by Malmgren & Heden (1947) for 'nucleotide' content of the cells were found to hold for the nucleic acid content. Figs. 5 and 6 show the variations obtained in two experiments; in one (Fig. 5) the rate of protein synthesis was determined at each stage and it can be seen that there is a close correlation between that rate and the nucleic acid content at harvesting of the cells; in the other (Fig. 6) it can be seen that the rate of accumulation of free glutamic acid varies inversely as the nucleic acid content.

Correlations. Results from the experiments with both washed and growing cells have been plotted and analysed statistically in Fig. 7. A highly significant correlation is found between the nucleic acid content of the cell and its rate of protein synthesis; the values obtained in Table 5 accord well with the correlation obtained, especially when the experimental error is taken into account. An equally significant but negative correlation is obtained between the nucleic acid content of the cell and its ability to take up and concentrate free glutamic acid.

Discussion. The discussion of these results is reserved for the end of the following paper (Gale & Folkes, 1953).

SUMMARY

1. Increase in the protein content of *Staphylococcus aureus* cells takes place if these are incubated in the presence of glucose and a mixture of amino-acids which must include all those essential for growth.

2. Increase in the nucleic acid content of the cells takes place if they are incubated in the presence of glucose and a mixture of purines, pyrimidines and amino-acids. Little or no formation of nucleic acid occurs in the absence of added amino-acids, and optimal synthesis occurs when the amino-acid mixture is also optimal for protein synthesis.

3. The addition of purines and pyrimidines to cells incubated in glucose and amino-acids results in a marked acceleration in the rate of protein synthesis.

4. There is a strong positive correlation between the rate of protein synthesis and the nucleic acid content of the cells, the former falling to zero when the latter has fallen to about 4 %.

5. The rate at which cells, incubated with glucose and glutamic acid, accumulate free glutamic acid varies inversely with the nucleic acid content.

The authors are indebted to Dr R. Markham for a gift of cytosine and for advice on chromatographic procedures; to Dr J. Tosic for total nitrogen determinations; to Mr B. Slater for amino-nitrogen determinations and to Miss P. J. Samuels for assistance with the chromatography.

REFERENCES

Caldwell, P. C. & Hinshelwood, C. (1950). *J. chem. Soc.* p. 3156.
Caldwell, P. C., Mackor, E. L. & Hinshelwood, C. (1950). *J. chem. Soc.* p. 3151.
Caspersson, T. (1936). *Skand. Arch. Physiol.* **73**, suppl. 8.
Caspersson, T. (1947). *Symp. Soc. exp. Biol.* **1**, 127.
Chantrenne, H. (1952). *J. gen. Microbiol.* (in the Press).
Gale, E. F. (1945*a*). *Biochem. J.* **39**, 46.
Gale, E. F. (1945*b*). *Nature, Lond.*, **157**, 265.
Gale, E. F. (1947*a*). *J. gen. Microbiol.* **1**, 53.
Gale, E. F. (1947*b*). *J. gen. Microbiol.* **1**, 327.
Gale, E. F. (1951*a*). *Biochem. J.* **48**, 286.
Gale, E. F. (1951*b*). *Biochem. J.* **48**, 290.
Gale, E. F. & Folkes, J. P. (1953). *Biochem. J.* **53**, 493.
Gale, E. F. & Van Halteren, M. B. (1951). *Biochem. J.* **50**, 34.
Gladstone, G. P. (1937). *Brit. J. exp. Path.* **18**, 322.
Jeener, R. & Brachet, J. (1944). *Enzymologia*, **11**, 222.
Jeener, H. R. & Jeener, R. (1952). *Exp. Cell Res.* **3**, 675.
Magasanik, B. & Chargaff, E. (1951). *Biochim. Biophys. Acta*, **7**, 396.
Malmgren, B. & Heden, C. (1947). *Acta path. microbiol. scand.* **24**, 472.
Mickle, H. (1948). *J. R. micr. Soc.* **48**, 10.
Mitchell, P. (1950). *J. gen. Microbiol.* **4**, 399.
Richardson, G. M. (1936). *Biochem. J.* **30**, 2184.
Salton, M. R. J. (1951). *J. gen. Microbiol.* **5**, 391.
Smith, J. D. & Markham, R. (1950). *Biochem. J.* **46**, 509.
Swensson, P. A. (1950). *Proc. nat. Acad. Sci., Wash.*, **36**, 699.
Wyatt, G. R. (1951). *Biochem. J.* **48**, 584.