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The Assimilation of Amino Acids by Bacteria

20. THE INCORPORATION OF LABELLED AMINO ACIDS BY DISRUPTED STAPHYLOCOCCAL CELLS

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In a previous paper (Gale & Folkes, 1953*a*) it was shown that washed suspensions of *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*) will incorporate [^{14}C]glutamic acid into the protein portion of the cells if these are incubated with glucose and the labelled amino acid either alone or as part of a complete mixture of amino acids. The sensitivity of the incorporation process differed according to the incubation conditions; when a complete mixture of amino acids was present the incorporation was highly sensitive to chloramphenicol and aureomycin but insensitive to penicillin, whereas when glutamic acid was the only amino acid present, the incorporation was much less sensitive to chloramphenicol but markedly affected by penicillin. Studies of the effects of varying the composition of the amino acid mixture and of adding inhibitors on the rate of incorporation of [^{14}C]glutamic acid or [^{14}C]phenylalanine compared with the effects on the rate of protein synthesis showed that the former rate was no measure of the latter (Gale & Folkes, 1953*a, b*). When glutamic acid is the only amino acid present in the incubation mixture, the incorporation process appeared to take place as the result of an exchange between glutamic acid residues in the protein and the free glutamic acid accumulated within the cells. If this were the case, it should be possible to demonstrate a removal of labelled residues from the cell protein (after incorporation) by exchange with non-labelled free glutamic acid; this could not be demonstrated with intact cells since it proved impossible to remove

free labelled glutamic acid from the internal medium of the cells after incorporation experiments. In the present paper, a reversal of incorporation has been demonstrated by the use of disrupted cells which possess no internal medium.

Incorporation of labelled amino acids into a variety of tissue preparations has now been demonstrated by many workers (see reviews by Borsook, 1953; Gale, 1953) and in many cases it has been proved beyond reasonable doubt that the incorporated material has become part of the tissue protein, although protein synthesis as such has not been demonstrated during the experiments. Typical investigations of this point have included studies of the effect of prolonged and repeated washing of the protein precipitates with hot and cold trichloroacetic acid (TCA), solution and reprecipitation of the protein fraction, demonstration that the rate of release of labelled amino acid is the same as that of non-labelled amino acid during hydrolysis of the protein, and detection of the labelled residue in peptide fragments of the protein after mild hydrolysis. Incorporation requires a source of energy which can be provided by glucose for intact staphylococci (Gale & Folkes, 1953*a*) or by adenosine triphosphate (ATP) for disrupted cells (Gale & Folkes, 1953*c*) or microsome preparations (Zamecnik & Keller, 1954). Holloway & Ripley (1952) found that the incorporation of labelled leucine into rabbit reticulocytes could be correlated with their ribonucleic acid content and it is shown below that the incorporation of [^{14}C]glutamic acid

or glycine in disrupted staphylococci is dependent upon the presence of nucleic acid. Following a preliminary note on this by Gale & Folkes (1953c), Lester (1954) showed that incorporation of labelled leucine in lysozyme-treated preparations of *Micrococcus lysodeikticus* is greatly decreased by treatment with ribonuclease, and Zamecnik & Keller (1954) found a similar effect of ribonuclease treatment of microsomes.

METHODS

The organism, methods of growth and preparation of suspensions, conditions of incubation, washing and preparation of material for counting were the same as those described in the previous paper (Gale & Folkes, 1953a).

Incubation mixtures. Incubations were carried out in 15 ml. centrifuge tubes containing the following: 0.1 ml. 0.06M ATP; 0.4 ml. hexose diphosphate (HDP) solution containing the equivalent of 100 mg. barium salt/ml.; 0.5 ml. disrupted cell preparation; 0.4 ml. amino acid mixture A as below; 1.5 ml. buffered saline (Gale, 1947) pH 6.25 containing a known amount of radioactive amino acid; water or other additions to 3.5 ml. Amino acid mixture A contained the following components at a concentration of 4 mg. L isomer/ml.: L-asparagine, L-aspartic acid, DL-alanine, L-arginine dihydrochloride, L-cysteine hydrochloride, L-glutamic acid, glycine, L-cystine, L-histidine, DL-isoleucine, L-leucine, L-lysine dihydrochloride, DL-methionine, DL-phenylalanine, L-proline, DL-serine, DL-threonine, L-tyrosine, DL-tryptophan, L-valine; final pH adjusted to 6.25. In experiments designed to estimate the incorporation of a labelled amino acid in the presence of the mixture, the corresponding unlabelled amino acid was omitted from mixture A. ^{14}C -labelled amino acids were obtained from the Radiochemical Centre, Amersham; glutamic acid and glycine were labelled in the α -COOH group, other amino acids were uniformly labelled. A solution (0.1 ml.) containing 100 μg . diphosphopyridine nucleotide (DPN) was added in some experiments but was found to be without significant effect in the experiments described in this paper.

Nucleic acid preparations. Staphylococcal nucleic acid was prepared by the method of Jones (1953) and also by that of Sevag, Lackman & Smolens (1938) from the soluble portion of cells disintegrated as below for the preparation of disrupted cells. Ribonucleic (RNA) and deoxyribonucleic (DNA) acid fractions were prepared from the whole nucleic acid by the method of Dutta, Jones & Stacey (1953); in all cases the RNA fraction was treated with deoxyribonuclease and the DNA fraction with ribonuclease to destroy traces of nucleic acid impurity remaining after fractionation. The sodium salts of the nucleic acids were then precipitated with ethanol (Jones, 1953), dissolved in water and dialysed against distilled water for 18–20 hr., the dialysed solutions were then kept at -15° until required. The effectiveness of the separation was periodically checked by the Schmidt & Thannhauser procedure (1945). The extinction coefficient at 260 μm . was 24 for 0.1% nucleic acid and the strength of solutions was routinely calculated from their extinction at 260 μm . measured in a Beckman spectrophotometer. Analysis of typical sodium salt preparations gave: RNA, C, 30.65; H, 4.46; N, 14.0; P, 8.99%; DNA, C, 29.37; H, 4.90; N, 13.95; P, 7.96% (Weiler and Strauss, Oxford).

We are indebted to Dr P. W. Kent for preparations of DNA from thymus, wheat germ and herring roe, and of RNA from ox liver.

Protein-nitrogen determinations. Protein fractions of disrupted cell preparations were precipitated with TCA and nucleic acid extracted therefrom as previously described (Gale & Folkes, 1953a). Nitrogen content was determined by digestion in a micro-Kjeldahl apparatus according to Chibnall, Rees & Williams (1943), followed by distillation of the ammonia and colorimetric estimation after nesslerization.

Disintegration of cells. Suspensions of washed cells, at a density of approx. 40 mg. dry wt./ml., in 10% (w/v) sucrose solution buffered in 0.05M phosphate buffer pH 6.25 containing 0.02M-NaCl and 0.003M-MgSO₄, were subjected to supersonic vibration of 25 kcyc./sec. generated by a 500 w Mullard magnetostrictor oscillator, the transducer-cup assembly being cooled in ice throughout the exposure. The duration of exposure varies with the efficiency of the transducer-cup assembly and with the degree of disintegration required and was assessed as described below.

Separation of disrupted cell fraction. The disintegrated material was centrifuged for 8 min. at 800 g; the turbid supernatant was taken and again centrifuged at 4000 g for 20 min.; the sediment obtained on this occasion was washed once in buffered saline and finally resuspended in buffered saline at a suspension density of approx. 3–4 mg. dry wt./ml.; 0.5 ml. of this suspension was used for each experimental tube. Suspension densities were determined by direct weighing of samples after drying for 2 hr. at 100° .

RESULTS

Preparation of a non-viable fraction from Staphylococcus aureus capable of incorporating [^{14}C]glutamic acid

The work was started in an attempt to obtain a subcellular fraction of staphylococcal cells which would incorporate [^{14}C]glutamic acid. Suspensions of cells at various densities and in various suspension media were subjected to supersonic vibration and tests carried out on the viability and ability of the resulting materials to incorporate glutamic acid. With the transducer assembly then in use it was found that thick suspensions (20–50 mg. dry wt. of cells/ml.) lost 99% of their viability after 15–20 min. exposure and underwent complete disintegration in 30–40 min. exposure. Incorporation of glutamic acid occurred in material obtained after 15–30 min. exposure, but this property was lost as disintegration continued. If the active material was centrifuged at 16 000 g it was found that the sediment retained the ability to incorporate glutamic acid but the clear supernatant extract was completely inactive. The sediment was next fractionated to give (1) material centrifuged down in 10 min. at 800 g and (2) material centrifuged down in 20 min. at 4000 g, and (3) a 'small particle' fraction centrifuged down in 30 min. at 16 000 g. Each fraction was then incubated for 1 hr. at 37° in the presence of [^{14}C]glutamic acid, phosphate buffer pH 6.25 and

Electronmicrographs of intact and disrupted preparations of *Staphylococcus aureus* after various periods of exposure to supersonic vibration. (1) Intact cell, stage A ($\times 16800$). (2) Disrupted cell preparation, fraction 2 stage B (see Table 1) ($\times 16800$). (3) Disrupted cell preparation, fraction 2 stage C ($\times 11400$). (4) Disrupted cell preparation, fraction 2 stage D ($\times 10800$).

(a) ATP + HDP, (b) ATP, HDP and amino acid mixture A less glutamic acid, and (c) no additions; the specific activity (counts/min./mg. dry wt.) was determined after incubation in each case. It was found that some incorporation occurred in fractions 1 and 2, fraction 2 having the higher specific activity, while fraction 3 had negligible activity. In fraction 2 the amount of incorporation was greater under condition (a) than either (b) or (c). Since the presence of amino acids apparently decreased the activity of fraction 2 (the effect was later shown to be one of rate, not of amount, of incorporation as shown in Fig. 6) and the disintegrated material contained amino acids liberated from the internal medium of the cells, the sedimentable material was washed in buffered saline for further experiments. When the cells, before disintegration, were suspended in water or buffered saline the yield of fraction 2 was found to be small; tests were therefore made to find a suspension medium which would give better yields of the fraction. It was found that addition of sucrose to a final concentration of 10% to the buffered saline resulted in markedly improved yields and that the fraction so prepared was 10–12 times as active in incorporating glutamic acid as fractions prepared in the absence of sucrose. The procedure described above was then adopted for the preparation of fraction 2; if the fraction was re-suspended in buffered saline and subjected to a further 15 min. exposure to supersonic vibration it could then be separated into two fractions by centrifuging first at 4000 g and then at 16 000 g; the heavier portion retained incorporating activity but was 10–20% less active than the original fraction, whereas the lighter portion was devoid of activity.

The various fractions have been examined in the electron microscope and Pl. 1 shows preparations of fraction 2 at various stages of disintegration. All the preparations show the presence of pieces of cell-wall but the majority of the 'particles' are complex organized structures consisting of cells in which the cell-wall has been ruptured and the greater part of the contents lost leaving a small amount of electron-dense material in the cell centre. Shadowed micrographs show that, in contrast to intact cells, the units of fraction 2 throw little shadow but appear to be collapsed on the grid. Fraction 1 consists of some intact cells and a mixture of slightly damaged ones, while fraction 3 consists almost entirely of fragments of cell-wall; the action of a second exposure of fraction 2 to supersonic vibration is to remove pieces of cell-wall from the disrupted cells.

Effect of time of exposure to supersonic vibration. Much of this work has been carried out while attempts have been made to improve the design of the disintegrator and to stabilize the performance of the transducer-cup assembly. The disintegration procedure is not at present capable of exact control

and variable results are obtained, especially with regard to the time of exposure required to give optimally active fraction 2. When the transducer is freshly assembled and firmly soldered to the disintegration cup, it is found that cells are rapidly broken and that a good yield of fraction 2 is obtained after 12–15 min. exposure, whereas further exposure results in loss of activity of the fraction. With continued use the efficiency of the transducer-cup assembly decreases and it is necessary to increase the length of exposure; however, less efficient performance is also associated with decreased inactivation and some of the most satisfactory results have been obtained in an old assembly in which 45–60 min. exposure resulted in the production of very active fraction 2 unassociated with any detectable intact cells. As explained below, continued exposure results in progressive depletion of the fraction with regard to nucleic acid and consequent increased stimulation of glutamic acid or glycine incorporation by added nucleic acid. Such stimulation therefore provides a measure of the disruption and is now used to check the performance of the transducer assembly. For studies involving nucleic acid, as described in this and the following paper, it is desirable to continue exposure until the stimulation of glycine incorporation, under exchange conditions, by nucleic acid is of the order of 300–500%. The yield of fraction 2 under these conditions may be less than 1% of the initial cell suspension used.

Table 1 shows the variation in composition and viability of fraction 2 preparations obtained after exposures of 25, 35 and 45 min. in a typical experiment with a transducer assembly of low efficiency; Pl. 1 shows representative fields in the electron-microscope of the corresponding preparations. Most of the work described in this paper has been carried out with preparations corresponding to stage C of Table 1.

Properties of fraction 2. It has not been possible, except on rare occasions, to prepare fraction 2 entirely free from intact cells; on a basis of the number of viable cells/mg. dry wt. of preparation, fraction 2 possesses 0.015–0.3% of the viability of the original cell suspension. It is therefore necessary to devise methods of study which will not be invalidated by the presence of this small proportion of intact cells. The incorporation of glutamic acid into the protein fraction of either intact or disrupted cells requires a source of energy; Table 2 shows the ability to utilize either glucose or ATP + HDP. Intact cells can attack glucose either anaerobically or aerobically, while fraction 2 is unable to oxidize glucose but retains ability to ferment it with the production of acid; fraction 2 can utilize ATP + HDP as energy source for the incorporation of glutamic acid, while intact cells are unable to do so. Although

Table 1. *Properties of disrupted cell preparations*

Washed suspension of *Staph. aureus* in buffered sucrose exposed to supersonic vibration and fractionated as described in the text. Fraction 2 separated and, where indicated, incubated for 1 hr. at 37° in M-NaCl, and resuspended in water before test.

Preparation	Exposure time (min.)	Viable count (cells/mg. dry wt.)	Nucleic acid (% dry wt.)	RNA (% dry wt.)	DNA (% dry wt.)	Percentage activation of glycine incorporation by nucleic acid
Intact cells	—	7×10^8	10.5	8.5	2.0	0
Disrupted cells, fraction 2:						
Stage B	25	6×10^7	4.6	—	—	15
Stage B, salt extracted	—	—	1.5	1.15	0.35	130
Stage C	35	8×10^6	—	—	—	—
Stage C, salt extracted	—	—	—	0.4	0.25	305
Stage D	45	1×10^6	—	—	—	—
Stage D, salt extracted	—	—	—	0.3	0.16	565

Table 2. *Metabolic properties of intact and disrupted cells*

Disrupted cell preparation corresponds to fraction 2, stage B in Table 1; oxidation (Q_{O_2}) and fermentation ($Q_{CO_2}^{acid} = \mu$ l. CO_2 liberated from bicarbonate buffer system/hr./mg. dry wt. of preparation) at pH 7.0 studied by conventional Warburg methods; rate of glutamic acid incorporation studied in presence of energy source and 0.1 μ mole [^{14}C]glutamic acid/ml., specific activity 0.9 mc/m-mole, either alone (condition 1) or with amino acid mixture A (condition 2); counts carried out on protein fraction after drying and values converted into glutamic acid incorporated.

	Disrupted cells (a)	Intact cells (b)	a/b
Viable count, cells/mg. dry wt.	22×10^6	75×10^8	0.003
Glucose present			
Oxidation Q_{O_2}	0	74	0
Fermentation Q_{CO_2}	18	159	0.1
Glutamic acid incorporation (μ m-moles/hr./mg. dry wt.):			
Condition 1	3.95	8.2	0.45
Condition 2	9.6	18.8	0.5
ATP + HDP present			
Glutamic acid incorporation (μ m-moles/hr./mg. dry wt.):			
Condition 1	1.55	0.012	120
Condition 2	1.76	0.05	35

the proportion of intact cells in the fraction used to obtain the results in Table 2 amounted to 0.3 %, the incorporation of glutamic acid by the fraction in the presence of glucose amounted to 45–50 % of that obtained in intact cells. Consequently fraction 2 can use glucose as energy source but, since the proportion of intact cells is likely to vary widely between different preparations, it was felt that interpretation of incorporation results in the presence of glucose would be difficult and that experiments should be carried out with ATP + HDP as energy source despite the smaller rates of reaction then obtained.

Incorporation of [^{14}C]glutamic acid by disrupted cell preparations

Requirement for energy source. Fig. 1 shows the effect on the course of incorporation of adding sources of energy to the incubation mixture. Some

incorporation occurs in the absence of any such addition, and this is not significantly increased by the addition of ATP alone; addition of HDP leads to a marked increase in incorporation and this effect is made greater by the further addition of ATP. It seems probable that the preparation is able to destroy ATP and that continued generation from HDP by glycolytic enzymes is an effective way of ensuring an energy supply throughout the experimental procedure. A similar procedure has been used by Bloch, Snoke & Yanari (1952) and by Samuels (1953) for supplying energy for the biological synthesis of glutathione.

Effect of temperature. Fig. 2 shows the effect of temperature on incorporation in the presence and absence of added ATP and HDP; at 40° a rapid incorporation takes place in the presence of ATP and HDP while the 'endogenous' incorporation is almost abolished. The process does not occur in

Fig. 1. Effect of energy source on incorporation of [^{14}C]-glutamic acid by disrupted cell preparation. Cell preparation: fraction 2, stage B. Preparation incubated in buffered saline, pH 6.25, containing $0.1 \mu\text{mole DL-}[\alpha\text{-carboxy-}^{14}\text{C}]\text{glutamic acid}$ (specific activity 0.9 mc/m-mole)/ml. with additions as follows: curve 1, no addition; curve 2, $0.1 \text{ ml. } 0.06 \text{ M ATP}$; curve 3, 0.2 ml. HDP ($=20 \text{ mg. Ba salt}$); curve 4, $\text{ATP} + \text{HDP}$. Counts determined on dried protein fractions.

Fig. 2. Effect of temperature of incubation on rate of incorporation of [^{14}C]glutamic acid. Conditions as for Fig. 1. Curve 1, no addition of energy source; curve 2, $\text{ATP} + \text{HDP}$ added.

Fig. 3. Effect of pH on rate of incorporation of [^{14}C]-glutamic acid. Preparation: fraction 2, stage C, extracted with m-NaCl . Preparation incubated at 37° for 1 hr. in saline buffered as above, containing ATP , HDP and [^{14}C]glutamic acid alone (curve 1) and with $0.1 \text{ mg. nucleic acid/ml.}$ (curve 2).

Fig. 4. Variation of velocity of incorporation with concentration of [^{14}C]glutamic acid; velocity and concentration plotted as reciprocals. Conditions as for Fig. 1; incubation for 1 hr. at 37° in presence of $\text{ATP} + \text{HDP}$ with glutamic acid only (condition 1) or in presence of amino acid mixture A (condition 2). $S = \mu\text{moles } [\alpha\text{-carboxy-}^{14}\text{C}]\text{glutamic acid}$ (specific activity 0.9 mc/m-mole)/ml. $v = \mu\text{m-mole glutamic acid incorporated/mg. protein of preparation/hr.}$

either case at temperatures of 60° and over; consequently it is not similar to that described by Brunish & Luck (1952) for amino acid incorporation in deoxyribonucleohistone preparations.

Effect of pH. Fig. 3 shows that glutamic acid incorporation at 37° in the presence of ATP and HDP occurs optimally at pH 6.0; this pH was used for the remainder of the investigations reported here. Fig. 3 also shows that the pH optimum is not altered by the presence of added nucleic acid (see below).

Effect of glutamic acid concentration. Fig. 4 shows the reciprocal of the rate of incorporation plotted against the reciprocal of glutamic acid concentration; calculation of the 'apparent Michaelis constant' for the incorporation process (Lineweaver & Burk, 1934) gives a value of 0.02 μ mole glutamic acid/ml. under condition 1 and 0.08 μ mole/ml. under condition 2, both values being markedly less than that (0.21 μ mole/ml.) obtained with intact cells (Gale & Folkes, 1953a).

Fig. 5 shows the course of incorporation when glutamic acid is present at three different initial concentrations, and it can be seen that the process ceases when the incorporation reaches a value which increases with the glutamic acid concentration. In no case has the added radioactive glutamic acid been exhausted when incorporation ceased. The shape of the curves suggest that an equilibrium is

Fig. 5a. Effect of concentration on incorporation of [14 C]glutamic acid under condition 1. Incubation mixture: buffered saline pH 6.25; ATP, HDP and [14 C]glutamic acid at initial concentration 0.03 μ mole/ml. (curve 1), 0.06 μ mole/ml. (curve 2), 0.1 μ mole/ml. (curve 3); specific activity 0.9 mc/m-mole in all cases.

Fig. 5b. Reversibility of incorporation of [14 C]glutamic acid. Preparation incubated for 120 min. as curve 3 in Fig. 5a, washed twice in buffered saline and then incubated in buffered saline with additions as follows: curve 3a, ATP + HDP; curve 3b, ATP, HDP, 0.1 μ mole [14 C]glutamic acid/ml.; curve 3c, ATP, HDP, 0.1 μ mole [12 C]glutamic acid/ml. Incubation with [12 C]glutamic acid in absence of ATP and HDP, or with ATP, HDP and either [14 C]glycine or [14 C]aspartic acid gives results significantly the same as curve 3a.

set up between the glutamic acid residues of the protein and the glutamic acid of the medium. Evidence has been presented with intact cells (Gale & Folkes, 1953a) that incorporation under this condition (condition 1, glutamic acid being the only amino acid present in the incubation mixture) takes place as a result of an exchange between protein residues and the free glutamic acid of the medium. If this is so, it should be possible to demonstrate a removal of 14 C-labelled residues from the protein by incubation in the presence of [12 C]glutamic acid. Fig. 5 also shows the results of an experiment designed to test this point. The disrupted cell fraction was first incubated with 0.1 μ mole [14 C]glutamic acid/ml. in the presence of ATP and HDP as usual; after 120 min. incubation, the fraction was centrifuged down and washed twice in buffered saline to free it from labelled glutamic acid. The washed radioactive fraction was then divided into a number of equal portions which were incubated under the conditions shown in Fig. 5. Incubation with ATP and HDP gave no significant alteration in radioactivity; incubation with ATP, HDP and 0.1 μ mole [14 C]glutamic acid/ml. gave a small increase in radioactivity; incubation with ATP, HDP and 0.1 μ mole [12 C]glutamic acid gave a progressive loss in radioactivity. This loss was not obtained if incubation took place with [14 C]glutamic acid in the absence of ATP and HDP, or with [14 C]glycine or aspartic acid in the presence or absence of ATP and HDP.

The glutamic acid incorporated after incubation with 0.1 μ mole glutamic acid/ml. corresponds to less than 2% of the total glutamic acid residues in the protein of the preparation; increasing the concentration of glutamic acid in the incubation mixture does not lead to parallel increase in the amount of incorporation and the highest incorporation measured (in the presence of 1.2 μ moles glutamic acid/ml.) has corresponded to 5.6% of the protein glutamic acid residues present.

Effect of adding amino acid mixture A. Fig. 6 shows the time course of [14 C]glutamic acid incorporation in the presence of ATP and HDP when glutamic acid is the only added amino acid (condition 1) and when it forms one component of the complete mixture A (condition 2). Under condition 1 the rate of incorporation steadily decreases until the equilibrium position is reached. The effect of adding the amino acid mixture A to the incubation mixture is to decrease the initial rate of incorporation but this rate remains linear for at least 3 hr. if the concentration of A is sufficiently high (greater than 0.3 ml. A/3.5 ml. incubation mixture). Lower concentrations of A give results intermediate between that obtained under condition 1 and the linear incorporation obtained with high concentrations, as shown in Fig. 6.

Table 3 shows the changes in protein-nitrogen of the disrupted cell fraction which occur during incubation under conditions 1 and 2. Under condition 1, or when the fraction is incubated with ATP and HDP alone, there is no increase in protein-nitrogen while frequently, as in the case quoted in Table 3, a small decrease is observed. Under condition 2 a significant increase in protein-nitrogen takes place and this increase agrees with

that which would be expected if the incorporated glutamic acid formed part of new protein. It is shown in the following paper (Gale & Folkes, 1955) that synthesis of enzymes can be demonstrated under this condition.

Attempt to obtain incorporation in soluble protein fraction. During the preliminary studies it was found that no incorporation took place into the proteins of the clear supernatant obtained by high-speed centrifuging of the disintegrated cell material. An attempt was made to obtain incorporation into the soluble protein fraction by adding it to fraction 2 in the presence of ATP, HDP and [^{14}C]glutamic acid. After incubation for 1 hr. at 37° the protein of the combined fractions was precipitated with 5% TCA in the cold and prepared for counting as usual. It was found that the radioactivity of the precipitate was significantly greater (in one case, 216% greater) than that of the control precipitate from fraction 2 incubated in the absence of the soluble fraction. If, however, fraction 2 was separated, after incubation, from the soluble fraction and the protein of the two fractions precipitated separately, it was found that the increase in radioactivity was entirely in fraction 2 and that no incorporation had taken place into the protein of the soluble fraction.

It was clear that the presence of the soluble fraction during incubation resulted in increased incorporation in fraction 2. The stimulating substance in the soluble fraction was non-dialysable and was precipitated by saturation with ammonium sulphate or addition of ethanol to 85% final concentration. Fractional precipitation of the soluble fraction with ethanol was carried out, and the material precipitated by 0-35, 35-45, 45-55 and 55-85% ethanol was dissolved in water and dialysed free from ethanol; all four precipitates proved to contain stimulating material and no one precipitate was markedly more active than the

Fig. 6. Effect of presence of amino acid mixture A on course of incorporation of [^{14}C]glutamic acid by disrupted cell preparation. Disrupted cell preparation: stage B, incubated in buffered saline pH 6.25 with ATP, HDP and 0.1 μmole [^{14}C]glutamic acid (specific activity 0.9 mc/m-mole)/ml. in presence of following amounts of amino acid mixture A (less glutamic acid)/3.5 ml.: curve 1, none; curve 2, 0.1 ml.; curve 3, 0.2 ml.; curve 4, 0.4 ml.

Table 3. Changes in protein-nitrogen content of disrupted cell fraction during [^{14}C]glutamic acid incorporation

Incubation mixtures contain ATP, HDP, DPN and buffered saline in all cases; temperature 37°; time of incubation 90 min. Concentration of [^{14}C]glutamic acid (specific activity 0.25 mc/m-mole) 1 μmole /ml.; amino acid mixture A at final concentration 0.45 mg. each component/ml. After incubation, preparation precipitated with 5% TCA in cold, nucleic acid extracted from precipitate with hot TCA, residue divided into portions for determination of radioactivity and protein-nitrogen. Radioactivity converted into glutamic acid incorporated by calibration of counting equipment; calculated increase in protein-nitrogen on basis that glutamic-nitrogen represents 5.0% protein-nitrogen (Freeland & Gale, 1947).

Additions to incubation mixture	Glutamic acid incorporated ($\mu\text{g. N}$)	Protein-nitrogen		
		Content found ($\mu\text{g.}$)	Change found ($\mu\text{g.}$)	Calculated change ($\mu\text{g.}$)
Initial preparation	—	49.0	—	—
No addition	—	46.5	-2.5	—
[^{14}C]Glutamic acid	0.115	44.5	-4.5	+2.3
[^{14}C]Glutamic acid + amino acid mixture	0.371	58.0	+9.0	+7.4

others. The precipitates were then combined and the material taken through the procedure of Sevag *et al.* (1938) for the separation of nucleic acid. The full stimulating activity of the preparation was found in the nucleic acid fraction.

Effect of nucleic acids on [^{14}C]glutamic acid incorporation into the protein of the disrupted cell fraction

Preparation of fraction 2 to obtain nucleic acid effect. Nucleic acid, prepared from the staphylococcal disintegrate by the method of Sevag *et al.* (1938) or that of Jones (1953), increased the rate of incorporation into the disrupted cell fraction. The degree of activation was found to vary within wide limits from day to day with various preparations of fraction 2 and a method of treating the latter in order to obtain more consistent activating effects was sought. Table 4 shows results of an experiment with a preparation of fraction 2 which did not, as initially prepared, respond markedly to nucleic acid. Incubation of the fraction with ribonuclease resulted in a decrease in the rate of incorporation in the absence of added nucleic acid but a well-marked response to added nucleic acid. Similar effects were obtained by incubation of the fraction with deoxyribonuclease or with m-NaCl ; on grounds of economy the treatment with m-NaCl has been the one generally adopted for the remainder of the investigations of nucleic acid effects. Estimation of the nucleic acid content of the fraction before and after treatment shows that either ribonuclease or deoxyribonuclease removes approx. 80% of the nucleic acid, whereas salt extraction removes 55–60%. As also shown in Table 4, good nucleic acid effects can be obtained with fraction 2 prepared from cells disintegrated in m-NaCl containing 10% sucrose but the yield of fraction 2 is small under these conditions and the method has not been generally adopted.

Effect of addition of nucleic acid on course of incorporation. Fig. 7 shows the time course of [^{14}C]glutamic acid incorporation under conditions 1 and 2 with and without added nucleic acid. Under both conditions the presence of nucleic acid markedly increases the rate of incorporation and, under condition 1, also increases the final radioactivity attained.

Fig. 7. Effect of nucleic acid on course of incorporation of [^{14}C]glutamic acid in nucleic acid-depleted disrupted cell preparation. Cell preparation: disrupted cell fraction 2, stage C, extracted with m-NaCl for 1 hr. at 37° before incorporation experiment. Curves marked 1, incorporation under condition 1; curves marked 2, incorporation under condition 2; curves marked A, in absence of nucleic acid; curves marked B, in presence of 0.1 mg. nucleic acid/ml.

Table 4. *Effect of treatment of disrupted cell preparation on subsequent response to nucleic acid*

Disrupted cells, stage C fraction 2, incubated for 1 hr. at 37° as below, centrifuged down, washed once in buffered saline, resuspended in water and rate of incorporation of [^{14}C]glutamic acid under condition 2 determined in presence and absence of 0.1 mg. staphylococcal nucleic acid/ml. Concentration of glutamic acid, $0.2\text{ }\mu\text{mole/ml.}$ ($0.2\text{ }\mu\text{C/ml.}$).

Disintegration medium	Pretreatment of fraction 2 for 1 hr. at 37°	Rate of [^{14}C]glutamic acid incorporation (counts/min./mg. dry wt.)	
		No addition	Nucleic acid added
Buffered saline + 10% sucrose	None	227	293
	Ribonuclease, 100 $\mu\text{g.}/\text{ml.}$ in phosphate pH 7.0	156	346
	Deoxyribonuclease, 30 $\mu\text{g.}/\text{ml.}$ in phosphate pH 7.0; 0.003 M- MgSO_4	145	540
	m-NaCl	143	446
	None	164	463
Buffered saline + 10% sucrose + m-NaCl	None		

Table 5. *Inactivation of nucleic acid preparation*

Nucleic acid preparation, initial composition 86% RNA, 14% DNA, assayed for stimulation of rate of incorporation of [¹⁴C]glutamic acid under condition 2 into protein of disrupted cells, fraction 2 stage C, salt-extracted. After treatment as below, residual nucleic acid precipitated as sodium salt and dialysed overnight against distilled water.

Pretreatment conditions			Activity remaining (%)
Medium	Time (min.)	Temp. (°)	
N-NaOH	60	25	50
N-NaOH	120	37	16
N-HCl	10	100	0
0.05M phosphate, pH 7.0 + 100 µg. ribonuclease/ml.	60	37	29
0.05M phosphate, pH 7.0 + 30 µg. deoxyribonuclease + 0.003M-MgSO ₄	60	37	65

Table 6. *Activation of nucleic acid-depleted preparations by added nucleic acids*

Disrupted *Staph. aureus*, stage C fraction 2, incubated for 1 hr. at 37° in (a) 0.05M phosphate pH 7.0 + 100 µg. ribonuclease/ml. or (b) 0.05M phosphate buffer pH 7.0 + 0.003M-MgSO₄ + 30 µg. deoxyribonuclease/ml.; centrifuged down, washed in buffered saline. Rate of incorporation of [¹⁴C]glutamic acid under condition 1 with added nucleic acid as below determined after 1 hr. at 37°.

Nucleic acid added		Rate of glutamic acid incorporation			
		Ribonuclease treated		Deoxyribonuclease treated	
		Counts/ min./mg.	Increase (%)	Counts/ min./mg.	Increase (%)
None	Concn. (mg./ml.)	55	—	53	—
RNA	0.02*	101	84	131	146
	0.08	106	93	134	152
DNA	0.015*	118	114	173	225
	0.03	123	124	191	260
Ratio	Increase due DNA*	1.35		1.38	
	Increase due RNA*				

* Values used for determination of ratio.

Fig. 8. Effect of ribonucleic and deoxyribonucleic acid on incorporation of [¹⁴C]glutamic acid in nucleic acid-depleted preparation. Cell preparation: disrupted cell fraction 2, stage C, salt extracted. Incubation conditions: condition 1, preparation used at density 2.5 mg. dry wt./3.5 ml.

Inactivation of nucleic acid preparation. Table 5 shows the results of treatment of a nucleic acid preparation with acid, alkali or nucleases. The active

material is both acid and alkali labile and partly removed by incubation with either ribonuclease or deoxyribonuclease. It can be seen, however, from Table 5 that the degrees of inactivation produced by nuclease action cannot be directly correlated with the composition of the nucleic acid preparation used and it would appear that activity resides in both RNA and DNA fractions.

Effect of ribonucleic and deoxyribonucleic acids. Fig. 8 shows that the incorporation of [¹⁴C]glutamic acid into salt-extracted disrupted cells is activated by either RNA or DNA, the latter being approximately twice as effective as the former on a dry-weight basis and giving a higher rate of incorporation at saturation concentrations. If both nucleic acid fractions are present at sub-saturation concentrations, their effects are additive but Fig. 8 also shows the effect of adding DNA in the presence of a saturation concentration of RNA; there is no additive effect under these conditions while very low concentrations of DNA decrease the normal response to RNA. Table 6 shows that activation with RNA or DNA is not affected by the method used for removal of nucleic acid from the disrupted

cells, and that there is no significant difference in the response to the two types of nucleic acid by preparations treated with ribonuclease or deoxyribonuclease.

Nucleic acid preparations from sources other than *Staph. aureus* have been tested, and the following found to be without significant effect when tested in concentrations of up to 0.3 mg./ml.: whole nucleic acid from *Ps. aeruginosa* or *Cl. welchii*; DNA from thymus, wheat germ or herring roe; RNA from *Saccharomyces cerevisiae* or ox liver. Nucleic acid from another strain of *Staph. aureus* had significantly the same activity as that from the strain Duncan used for the preparation of the disrupted cells. It is shown in the following paper (Gale & Folk, 1955) that a mixture of purines and pyrimidines is as effective as RNA in stimulating the development of certain enzymes in disrupted cell preparations; the same mixture had little or no activity (amounting to less than 5% of the effect produced by RNA) in the incorporation experiments.

If staphylococcal nucleic acid preparations are digested with the corresponding nuclease and the nucleic acid then reprecipitated and dialysed (as described above for the whole nucleic acid tests), the activity of the preparations is lost. However, if the complete digests are added to disrupted cells, the stimulating effect on incorporation remains and may even be enhanced. Consequently the material involved in stimulating incorporation cannot be the complete nucleic acid structure; the nature of the active material will be dealt with in a later communication.

*Incorporation of ^{14}C -labelled amino acids
by disrupted cell preparations*

Incorporation of [carboxy- ^{14}C]glycine. Experiments similar to those described above for glutamic acid have been carried out with glycine, incorporation into the protein fraction of disrupted cells being investigated only. The results are qualitatively similar to those found for glutamic acid: incorporation under condition 1 requires provision of an energy source and ceases when equilibrium is attained; under condition 2 incorporation continues in a linear fashion for some hours; depletion of the nucleic acid content of the cell preparation decreases the rate of incorporation and this can then be stimulated by addition of either RNA or DNA. When the investigations on nucleic acid stimulation were carried out, it was noticed that the response of any given cell preparation was greater when incorporation of glycine was measured than when glutamic acid was studied. Consequently the nucleic acid effects were tested on the rates of incorporation of a number of labelled amino acids.

Nucleic acid effects; effect of time of exposure to supersonic vibration. It is shown in Table 1 that the degree of nucleic acid depletion of fraction 2 is affected by the time of exposure to supersonic vibration. In the course of the studies described above it was also found that the degree of stimulation of the incorporation of either glutamic acid or glycine increased with the time of exposure; Table 1 gives typical figures for the stimulation of glycine incorporation. The rate of incorporation measured in the presence of a saturation concentration of nucleic acid remains approximately constant whatever the time of exposure, but the rate in the absence of added nucleic acid decreases with exposure time; if the latter rate is expressed as a percentage of the former, the figure obtained can be used as an indication of the degree of nucleic acid depletion or 'resolution' of the disrupted cell preparation. Fig. 9 shows the effect of exposure time on the subsequent 'resolution' when determined with respect to the rates of incorporation of a number of labelled amino acids.

It is clear that the degree of 'resolution' obtained varies with the amino acid whose incorporation is studied. Glycine and also aspartic acid show nucleic acid effects soon after cell rupture begins; phenylalanine and glutamic acid show marked 'resolution' only when disintegration has proceeded for some time; lysine shows a small stimulation by nucleic acid when exposure is continued until almost complete disintegration has occurred, but no effect of nucleic acid has been demonstrable on alanine incorporation under any condition yet tested. The results shown in Fig. 9 were obtained with disrupted cell preparations from which nucleic acid was removed by incubation with M-NaCl; Table 7 shows results obtained with a preparation exposed to supersonic vibration for 40 min. and then incubated with either ribo- or deoxyribonuclease. The incorporation of glycine is reduced to approx. 17% of that attained in the presence of nucleic acid while the incorporation of alanine is, if affected at all, slightly inhibited by the addition of nucleic acid. Inspection of the degree of 'resolution' obtained in different experiments and by different procedures shows that, although the general trend is the same (i.e. glycine, aspartic acid and arginine show effects under moderate treatment whereas tyrosine, lysine and alanine show little or no 'resolution' even under drastic treatment), each amino acid has to be considered separately, and results obtained with any one of the amino acids tested differ from those obtained with any other. The differing specific activities and availabilities of the various labelled amino acids used has meant that degrees of 'resolution' have not been tested in all cases under the same conditions of amino acid concentration (see Table 7); in the case of glutamic acid, however, it has been

shown that the degree of 'resolution' is not affected by the amino acid concentration over the range 0.03–0.3 μ mole/ml. Table 7 also shows the relative rates of incorporation and it would appear that there is no correlation between this rate and ease of 'resolution'.

The degree of 'resolution' towards any one amino acid is easily and quickly determined and gives a measure of the efficiency of the disintegration and nucleic acid depletion procedures. It is now customary to check the 'resolution' towards glycine at intervals and adjust the period of exposure to supersonic vibration to that time necessary to give a nucleic acid stimulation of 3–400 % or greater (stage C–D, Table 1). The yield of fraction 2 decreases with continued exposure and, at stage D, may be less than 1 % of the original suspension. In Fig. 9 stage D is reached after an exposure of 50–55 min. and these results were obtained with an old transducer assembly that shortly after became ineffective. With newly assembled and efficient apparatus, stage D may be reached in 20–25 min. exposure, further exposure resulting either in complete fragmentation of the cells or destruction of the activity of fraction 2 even in the presence of nucleic acid.

Effect of nucleic acid on the incorporation of labelled amino acids in the presence of a complete amino acid mixture. The results described above for the varying

Fig. 9. Effect of duration of exposure to supersonic vibration on subsequent 'resolution' of disrupted cell preparation with respect to incorporation of various amino acids. Cell suspension exposed to supersonic vibration for period shown, fraction 2 separated and extracted with *m*-NaCl for 1 hr. at 37°. Incorporation tested under condition 1 in all cases with concentration of added amino acid as in Table 7; radioactivity estimated after incubation for 90 min. at 37° in presence (A) of 0.1 mg. nucleic acid/ml. and in absence (B) of added nucleic acid. 'Resolution' (%) = 100 (A – B)/A.

Table 7. *Effect of nucleic acids on rate of incorporation of various ¹⁴C-labelled amino acids*

Disrupted cell preparation: fraction 2, stage C, 40 min. exposure; nucleic acid depleted by pretreatment for 1 hr. at 37° as below. Preparation incubated with ATP, HDP and labelled amino acid as below, with and without 0.1 mg. nucleic acid/ml. for 1 hr. at 37°; counts determined on dried protein fraction.

Relative total incorporation = incorporation in presence of nucleic acid corrected to specific activity of 1.0 mc/m-mole.

¹⁴ C-labelled amino acid				Rate of incorporation (counts/min./mg./hr.)			
Pretreatment of fraction		Concn. (μmole/ml.)	Spec. act. (mc/m-mole)	Fraction alone	Fraction + nucleic acid (NA)	Increase due to NA (%)	Relative total incorporation
m-NaCl	Glycine	0.03	2.7	359	1126	215	418
	Aspartic acid	0.025	4.0	520	999	92	250
	Arginine	0.02	6.0	300	573	91	94
	Phenylalanine	0.08	1.2	125	219	75	182
	Glutamic acid	0.035	0.9	178	267	50	299
	Proline	0.027	5.0	385	548	42	110
	Tyrosine	0.02	9.0	656	799	22	89
	Lysine	0.02	6.0	3758	3239	(– 14)	539
	Alanine	0.02	3.0	2018	1614	(– 20)	538
Ribonuclease 100 μg./ml.	Glycine	0.03	2.7	258	1632	471	567
	Arginine	0.02	6.0	260	658	150	109
	Glutamic acid	0.03	0.9	189	231	22	256
	Tyrosine	0.02	9.0	562	528	(– 3)	59
	Alanine	0.02	3.0	1161	973	(– 16)	327
Deoxyribonuclease 30 μg./ml.	Glycine	0.03	2.7	247	1536	520	564
	Arginine	0.02	6.0	260	705	171	117
	Glutamic acid	0.03	0.9	185	258	40	286
	Tyrosine	0.02	9.0	579	530	(– 8)	59
	Alanine	0.02	3.0	1118	800	(– 28)	274

Table 8. *Effect of nucleic acids on incorporation of labelled amino acids in the presence of a complete amino acid mixture*

Disrupted cell preparation: fraction 2, stage C, extracted with m-NaCl. Preparation incubated for 90 min. at 37° in presence of ATP, HDP and amino acid mixture A, all components other than those shown below at a final concentration of 1.6 mg. each L isomer/3.5 ml.; glycine, glutamic acid, 1 μ mole/ml.; aspartic acid, alanine, 0.5 μ mole/ml. In four parallel samples, one amino acid (as below) labelled with 14 C. In all cases, effect was studied of addition of either 0.05 mg. DNA/ml. or 0.1 mg. RNA/ml. and incorporation estimated by counts/min. of dried protein fractions.

14 C-labelled amino acid in mixture A	Specific activity (mc/m-mole)	Incorporation in absence of added nucleic acid (counts/min./mg.)	Percentage increase in incorporation in presence of	
			DNA	RNA
Glycine	0.34	285	180	123
Aspartic acid	0.31	257	120	81
Glutamic acid	0.11	102	94	64
Alanine	0.23	240	6	0

Table 9. *Effect of certain antibiotics on incorporation of amino acids by disrupted cells*

Disrupted cell preparation: fraction 2, stage B. Incubation at 37° for 1 hr. in presence of ATP, HDP and amino acids as below; antibiotics added at final concentration of 100 μ g./ml. Counts carried out on cold TCA (5%) precipitates of preparation after drying.

Amino acid	Concn. (μ mole/ml.)	Specific activity (mc/m-mole)	Incorporation (counts/min./mg.)	Percentage inhibition of incorporation by		
				Chloramphenicol 100 μ g./ml.	Bacitracin 100 μ g./ml.	Penicillin 100 μ g./ml.
Glycine	0.06	2.7	1541	40	57	47
Glutamic acid	0.1	0.9	153	65	48	40
Alanine	0.015	3.0	434	25	33	20
Aspartic acid	0.03	4.0	559	79	22	12
Phenylalanine	0.06	1.2	104	60	8	6
Leucine	0.02	6.0	851	83	2	0
Arginine	0.02	6.0	412	35	0	0
Lysine	0.015	6.0	1294	49	—	0
Tyrosine	0.01	9.0	164	82	—	—
Proline	0.02	5.0	186	72	—	—

Fig. 10. Effect of chloramphenicol on incorporation of various amino acids under condition 1. Disrupted cell preparation: fraction 2, stage B. Incubation carried out for 90 min. at 37° under condition 1 with concentrations of amino acids as in Table 7. Curve 1, alanine; 2, glycine; 3, glutamic acid; 4, leucine. Curve 5 shows effect of chloramphenicol on net protein synthesis under condition 2.

effects of nucleic acid on incorporation processes were all obtained under condition 1, when the amino acid studied was the only one added to the incubation medium. Fig. 7 shows that nucleic acid also stimulates the incorporation of [14 C]glutamic acid under condition 2 when increase in protein-nitrogen can be demonstrated. The question arises whether the effect of nucleic acid under condition 2 can be taken as an effect on protein synthesis, in which case it might be expected that the effect would not differ with the particular amino acid studied. To test this point, salt-extracted fraction 2 at stage C was incubated with ATP, HDP and a complete amino acid mixture of which, in separate but otherwise identical samples, either glycine or aspartic acid or glutamic acid or alanine was labelled with 14 C. The effect of addition of nucleic acid on the rate of incorporation of radioactivity into the protein was then determined; Table 8 shows that the effects again varied with the radioactive amino acid studied, glycine showing the largest nucleic acid effect and alanine showing none.

Effect of antibiotics. Fig. 10 shows the effect of chloramphenicol on the rate of incorporation of various amino acids each tested under condition 1.

In each case the incorporation is 15–25 % inhibited by 3 μ g. chloramphenicol/ml. but the inhibition ceases at a plateau level the position of which differs with different amino acids. The shapes of the inhibition curves are thus very similar to those obtained for the inhibition by penicillin of incorporation processes towards certain amino acids (Gale & Folkes, 1953*a*). Fig. 10 also shows the effect of chloramphenicol on the synthesis of protein by disrupted cell preparations (Gale & Folkes, 1955), and it can be seen that this process is significantly more sensitive to the antibiotic than any of the individual incorporation processes studied.

Inhibition curves showing plateaux over a wide range of inhibitor are typical of the effects of certain antibiotics on amino acid incorporation processes. Table 9 shows the inhibitions obtained at plateau concentrations of chloramphenicol, penicillin and bacitracin for incorporation of a number of amino acids. It is clear that the various processes again react independently of each other.

DISCUSSION

One of the fundamental questions underlying studies of protein metabolism by the use of isotopes is whether the incorporation of a labelled amino acid, that occurs when a biological preparation is incubated with a single such amino acid (condition 1 of these studies), takes place as a result of protein synthesis or an exchange reaction. In the staphylococcal preparations described in these papers, it is not possible to demonstrate net synthesis of protein unless a mixture of amino acids is included in the incubation mixture (condition 2). Under condition 1 it is frequently the case that no change, either loss or gain, is demonstrable in the protein-nitrogen of the preparation and in Fig. 5 it has been shown that no loss of incorporated [^{14}C]glutamic acid residues occurs during incubation with energy source or with amino acid in the absence of energy source. Podolsky (1953) has shown in the case of *Esch. coli* that the rate of protein degradation under a number of conditions amounts to 8–10 % in 30 hr. Nevertheless, it is possible that incorporation under condition 1 may occur by a process of protein synthesis exactly balanced by protein breakdown. The present series of studies (Gale & Folkes, 1953*a, b*; Gale, 1953; this paper) have established the following facts:

(*a*) The rate of incorporation of a given ^{14}C -labelled amino acid varies with the composition of the incubation mixture and with the presence of inhibitors in a manner not correlated with the rate of net protein synthesis.

(*b*) Under condition 2 protein synthesis can be inhibited without any corresponding change in the rate of incorporation of glutamic acid.

(*c*) The incorporation of [^{14}C]glutamic acid is less

sensitive than protein synthesis to chloramphenicol or aureomycin and more sensitive to penicillin or bacitracin.

(*d*) In intact staphylococci under condition 1, increasing specific activity of the protein is accompanied by decreasing specific activity of the free amino acid within the amino acid pool, quantities remaining unchanged.

(*e*) Under condition 1 incorporation ceases when a condition of equilibrium is established between the protein and the medium.

(*f*) In disrupted cells the rate of incorporation of [^{14}C]glutamic acid is markedly decreased by the addition of other amino acids. The data presented in Fig. 4 could be interpreted in the sense that the amino acid mixture competitively inhibits the incorporation of glutamic acid.

(*g*) Preparations rendered radioactive by incorporation of [^{14}C]glutamic acid lose radioactivity by subsequent incubation with [^{12}C]glutamic acid and an energy source but not with either alone.

(*h*) The rate of incorporation of a given amino acid is affected by the presence of nucleic acids or antibiotics in a way specific for that amino acid and not dependent upon the effects on protein synthesis.

It is clear that the rate of incorporation of a labelled amino acid cannot be taken as a measure of the rate of net protein synthesis, although some of the findings (*a, b, c, e*) could be explained on a basis of balanced breakdown and synthesis, especially if different rates of synthesis of different proteins were invoked. This explanation could also explain both the loss of radioactivity during incubation with an energy source and [^{12}C]glutamic acid, and no loss during incubation with an energy source alone (*g*), although it would then be expected that incubation in the absence of an energy source should lead to loss of radioactivity as a result of unbalanced protein breakdown. The effects of antibiotics (*c, h*) are difficult to explain on a basis of protein synthesis, although the complexity of the protein composition of a bacterial cell might be invoked as a reason for the absence of any obvious explanation. Nevertheless, all the experimental findings are in accord with the hypothesis that the incorporation of a single amino acid under condition 1 is a process involving that amino acid alone, and arises from substitution of corresponding residues in preformed protein by molecules of that amino acid, labelled or otherwise, in the medium. Since a source of energy is required before substitution will take place, it is probable that the underlying exchange reaction relates to derivatives rather than to the free amino acids themselves. Quantitative considerations indicate that only a small proportion of the total protein of the preparations used can enter into such exchange reactions, and the question arises as to the nature of these proteins.

Removal of nucleic acid from disrupted cell preparations decreases the incorporation and, although incorporation has never been completely abolished in this way, it has been reduced to 10 % of the initial and then fully restored by adding nucleic acid to the depleted preparation. It thus appears that incorporation by exchange occurs only in nucleoproteins and that nucleic acid plays a part in the process. Fig. 7 shows that nucleic acid affects not only the rate of exchange but also the amount that can occur, and it would appear that nucleic acid controls the amount of protein in which exchange takes place rather than the rate of exchange with available protein. Activation of incorporation is given by either RNA or DNA from staphylococci, although nucleic acids from other sources have proved inactive. Preliminary evidence has been obtained that it is not the whole nucleic acid structure which is active in the incorporation of a single amino acid. Disrupted cell preparations retain many synthetic abilities, including those involved in the synthesis of nucleic acid from purines, pyrimidines and amino acids (Gale & Folkes, 1955), and it is not yet possible to say whether the activity in incorporation experiments of nucleic acid digests is due to specific components within the digest or to resynthesis of complete nucleic acid therefrom. This matter is now being investigated. The differing nucleic acid effects obtained with different amino acids (Fig. 9) may be explained if the incorporation of specific amino acids is controlled by specific fragments of nucleic acid, some of which are more readily damaged than others. The plateaux obtained in inhibition curves for the effects of antibiotics on incorporation processes would suggest that each process involves at least two steps and a further connexion between these processes and nucleic acid fragments is indicated by the fact that penicillin, which inhibits incorporation of certain amino acids only, gives rise in the growing cell to the accumulation of specific nucleotide-like substances (Park, 1952).

Incorporation involves a relationship between portions of protein and portions of nucleic acid. Any hypothesis concerning the mechanism of incorporation must await characterization of those portions. A number of authors (Haurowitz, 1950; Dounce, 1951; Gale, 1953; Delbrück, 1954) have pointed out that specific combinations of nucleotides in a nucleic acid molecule could relate to specific amino acid positions in a protein molecule so that one could act as a model or template for the other. It is a part of such theories that amino acids can be adsorbed or combined at specific sites on nucleic acid as a stage in protein synthesis, and that peptide bond formation follows organization of the amino acids in this way. If the situation is a dynamic one and preformed proteins can enter into

a similar relationship with nucleic acids, then presumably a position will arise in which individual residues will be combined with their specific sites on the nucleic acid and not through peptide bonds with other amino acids. In such a case exchange of an individual residue with a corresponding one in the environment could clearly take place, and this process could occur without the net protein synthesis which would result when all the necessary amino acids were available. The properties of the exchange reaction would thus be largely determined by those of the amino acid and its specific combining site, and it would be reasonable to expect that these would differ from amino acid to amino acid. Exchange reactions may thus be a property of the protein-forming mechanism and their investigation thus lead to elucidation of the latter.

SUMMARY

1. A sedimentable fraction, consisting of disrupted cells, capable of incorporating a number of ^{14}C -labelled amino acids, has been prepared from suspensions of *Staphylococcus aureus* after exposure in a sucrose medium to supersonic vibration.

2. Incorporation of ^{14}C glutamic acid requires a source of energy which can be supplied by adenosine triphosphate (ATP) and hexose diphosphate (HDP).

3. When glutamic acid is the only amino acid added to the incubation mixture, incorporation ceases when only a fraction of the added amino acid has been incorporated; when the concentration of added amino acid is low the incorporation ceases at a level dependent upon that concentration.

4. Preparations rendered radioactive by incorporation of ^{14}C glutamic acid lose radioactivity if subsequently incubated with ^{13}C glutamic acid and ATP + HDP but not with ^{13}C glutamic acid alone, ATP + HDP alone, or ^{12}C glycine or ^{12}C aspartic acid and ATP + HDP.

5. In the presence of a complete mixture of amino acids, the incorporation of ^{14}C glutamic acid proceeds linearly for some hours and is accompanied by a significant increase in protein-nitrogen.

6. Depletion of the nucleic acid content of the disrupted cell preparation results in marked decrease in the rate and amount of incorporation; incorporation can be restored in such preparations by addition of staphylococcal nucleic acid, deoxyribonucleic acid being more active than ribonucleic acid on a dry weight basis.

7. The degree of inactivation of incorporation, and subsequent activation by nucleic acids, depends upon (a) the time of exposure of the cells to supersonic vibration during disintegration and (b) the amino acid incorporated.

8. Incorporation is inhibited by chloramphenicol, bacitracin and penicillin but the sensitivity

varies with different amino acids. In all cases studied, inhibition ceases at a plateau value which again varies with the individual amino acid concerned.

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The Assimilation of Amino Acids by Bacteria

21. THE EFFECT OF NUCLEIC ACIDS ON THE DEVELOPMENT OF CERTAIN ENZYMIC ACTIVITIES IN DISRUPTED STAPHYLOCOCCAL CELLS

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The rate of protein synthesis has been associated with the nucleic acid content of cells in a number of studies (Brachet, 1941; Caspersson, 1941, 1947; Jeener & Brachet, 1944; Malmgren & Heden, 1947; Caldwell, Mackor & Hinshelwood, 1950; Mitchell & Moyle, 1951; Jeener, 1952a, b; Jeener & Jeener, 1952; Northrop, 1952; Price, 1952; Wade, 1952; Gale & Folkes, 1953a), and a number of authors have suggested that nucleic acid plays a role, possibly as a template, in the synthesis of protein (Haurowitz, 1950; Caldwell & Hinshelwood, 1950; Dounce, 1952; Gale, 1953, 1955; Rich & Watson, 1954). In the present paper a direct effect of nucleic acids on the 'development' of certain enzyme systems in disrupted staphylococcal cells is shown.

In washed suspensions of intact *Staphylococcus aureus*, protein synthesis will occur only if a complete mixture of the amino acids essential for growth is provided; the rate of synthesis can be markedly stimulated by the addition of a mixture of purines and pyrimidines to the medium and is directly

proportional to the nucleic acid content of the cells (Gale & Folkes, 1953a). If a single amino acid is added to the incubation medium, then no synthesis of protein can be demonstrated, but accumulation of the free amino acid takes place within the cell; if the amino acid is labelled with ^{14}C , then incorporation of the label into the protein of the cell can be shown whether protein synthesis is demonstrable or not (Gale & Folkes, 1953c). In the previous paper of this series (Gale & Folkes, 1955) it has been shown that incorporation of [^{14}C]glutamic acid into the protein of disrupted staphylococcal cells is dependent upon, and activated by, nucleic acid derived from staphylococci. It was also shown that the rate of incorporation of a single labelled amino acid cannot be taken as a measure of protein synthesis and that, even under conditions in which protein synthesis is occurring, the effect of nucleic acids upon incorporation varies with the particular amino acid whose incorporation is being measured. Consequently, such studies are not suitable for the investigation of protein synthesis as such, and the