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Aminoacetone Formation by *Staphylococcus aureus*

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It has been reported (Elliott, 1958) that washed cell suspensions of *Staphylococcus aureus* are capable of producing a previously unknown amino compound which, provisionally identified as aminoacetone, was found to arise by two apparently distinct metabolic pathways. The first of these required the presence of threonine and oxygen whereas the second required the presence of glycine, glucose and oxygen. The present paper describes in detail the formation of the new metabolite, its isolation as a crystalline derivative and identification as aminoacetone. The possible role of the compound in metabolism is also discussed.

A preliminary account of this work has already been published (Elliott, 1959).

MATERIALS

δ -Aminolaevulinic acid hydrochloride was a Parke, Davis and Co. Ltd. product, kindly given by Dr J. E. Falk of C.S.I.R.O., Canberra. Aminoacetone hydrochloride and aminoacetone toluene-*p*-sulphonic acid salt were synthesized as described by Elliott (1960). DL- β -Hydroxy-

glutamic acid, DL-homoserine and DL-allothreonine were L. Light and Co. Ltd. products.

Phosphate buffers were mixtures of Na_2HPO_4 and KH_2PO_4 . Phosphotungstic acid was purified by the method of Van Slyke, Hiller & Dillon (1942).

METHODS

Estimation of aminoacetone

The picrate-reaction method of Shuster (1956) as modified by Elliott (1960) was used. In experiments in which aminoacetone formation from threonine was measured, determinations were carried out directly on the incubation media after removal of the cells by centrifuging. When aminoacetone formation from glycine was measured it was necessary to concentrate the metabolite by treatment on Dowex 50 columns as described previously (Elliott, 1960).

Paper chromatography

Whatman no. 3 papers were used throughout and washed chromatographically by allowing water to drip off the bottom of the sheets for 3 days.

Solvent systems. (I) 2-Methylpropan-2-ol-methyl ethyl ketone-formic acid-water (120:50:40:40); (II) ethanol-

m-acetic acid-pyridine-water (95:10:3:3); (III) redistilled mesityl oxide-85% formic acid-water (1:1:2) (Lugg & Overell, 1948); (IV) butan-1-ol-acetic acid-water (4:1:5). The upper layers of mixtures (III) and (IV) were used.

Colour reactions on paper

The following colour reactions were used for the detection of aminoacetone.

Ninhydrin. Papers were dipped in 0.2% ninhydrin in acetone solution and then heated in an oven at 80°.

Copper-ninhydrin. Papers were dipped in a solution containing 0.4 ml. of aqueous saturated solution of $\text{Cu}(\text{NO}_3)_2$ in 100 ml. of acetone. After drying, ninhydrin was applied as described above. α -Amino acids do not give ninhydrin colours after copper treatment whereas other amino compounds still react.

Picrate spray. Chromatograms were sprayed on both sides of the paper with a mixture containing picric acid (saturated solution), water and NaOH (10N), in the proportions of 10:50:20. After 1 min. the papers were sprayed lightly with 5N-HCl. Aminoketones give orange-brown spots.

Modified Elson-Morgan reaction. Papers were sprayed with a mixture containing acetate buffer (M, pH 4.6) and acetylacetone (10:1, v/v), heated in an autoclave for 3 min. at 15 lb./in.² pressure and sprayed with the modified Ehrlich reagent of Mauzerall & Granick (1956). Aminoketones give purple colours.

Growth of bacteria

Staphylococcus aureus (Duncan) was grown with constant shaking for 17 hr. at 37° in the 'deficient medium B' of Gale & Van Halteren (1952), except that the Marmite was replaced by Difco yeast extract. The cells were harvested and washed by suspension in 0.02M-phosphate buffer, pH 7.0, containing 0.1% of NaCl. For large-scale production of *S. aureus* the following procedure was adopted.

Batches of non-sterile medium (14 l.) were warmed to 37° in 20 l. flasks and each was inoculated with 1 l. of a fully grown culture of *S. aureus* obtained as above. Pre-warmed, water-saturated air was forced into the culture and growth allowed to continue for 6 hr. at 37°. The cells (from 30 l. of medium) were harvested on a Sharples centrifuge, washed by resuspension in 5 l. of water and again centrifuged. These cells were used in the experiments on the isolation of the new metabolite described later. Microscopic examination showed that the *S. aureus* preparations grown in this way were free from significant contamination by other micro-organisms.

Cell densities were measured in a Spekker absorptiometer with an Ilford 608 filter and dry weights were calculated from a calibration curve for this organism.

RESULTS

Formation of new substance from threonine

It was observed that when washed cell suspensions of *S. aureus* were incubated in buffered 0.9% NaCl in the presence of Difco yeast extract a small amount of an unidentified amino compound was seen on chromatograms of the incubation medium run in solvent I. The compound had R_f approx. 0.4. Increasing yields of the new substance were obtained as a result of increasing the yeast extract, up

to a concentration of 0.1%, suggesting that the yeast extract was a source of substrate rather than of a cofactor. The component of the yeast extract responsible for this effect was found to reside entirely in the fraction absorbed by Dowex 50 (H^+ form), indicating that it was probably an amino acid, and this was confirmed by the finding that casein hydrolysate efficiently replaced the yeast extract. Each of 20 amino acids known to occur in casein was then tested for ability to promote synthesis of the new substance by washed suspensions of *S. aureus*, and, of these, threonine alone was found to be active.

Preliminary identification of the threonine metabolite

Washed cell suspensions of *S. aureus* were shaken for 3 hr. at 37° in the presence of 0.01 M-L-threonine. The cells were removed by centrifuging and the supernatant was used for the preliminary determination of the properties of the metabolite.

Stability. The compound was apparently stable when solutions were heated in 6N-HCl at 105° for 16 hr. before chromatography. It was, however, completely destroyed when a solution was made alkaline with aq. NH_3 soln. or NaOH and shaken in air at room temperature for 6 hr. The compound was also destroyed when chromatographed in ammoniacal solvents.

Chemical reactions. A number of chromatograms were run and various sprays applied to the papers. The compound gave, with ninhydrin, an initial bright yellow which turned brown and finally deep purple at room temperature; it reacted with ninhydrin, after copper treatment, to give a permanent yellow, showing that it was not an α -amino acid; with ammoniacal AgNO_3 a reduction was obtained after 5 min. at room temperature. These properties suggested that the substance had an aminoketone structure, for such compounds undergo a Knorr type of condensation in alkaline solution and yield disubstituted pyrazines in the presence of air. This conclusion was confirmed by the finding that the material gave a positive reaction with the picrate spray and with the modified Elson-Morgan reaction when these were applied to chromatograms.

Chromatography. It was found that the new substance co-chromatographed with both of the two aminoketones, δ -aminolaevalulinic acid and aminoacetone, when solvent I and IV were used. In solvent III, however, the substance ran ahead of δ -aminolaevalulinic acid and had R_f identical with that of aminoacetone. A similar result was obtained with solvent II, when a good separation of δ -aminolaevalulinic acid and aminoacetone occurred (R_f 0.15 and 0.3 respectively). These results suggested that the substance was in fact aminoacetone.

Isolation of aminoacetone

Experiments on the large-scale production of the compound were carried out so that the material could be isolated and positively identified.

The washed cells from 30 l. of growth medium were suspended at a concentration of 4 mg. dry w./ml. in 0.02M-phosphate buffer, pH 7.0, at 37° and 0.25 vol. of 0.1M-L-threonine, also at 37°, was added. The mixture (6 l.) was incubated in a 10 l. jar at 37° and aerated with water-saturated air dispersed by a sintered-glass diffusion disk. Samples of the mixture were removed at intervals for aminoacetone determination. Fig. 1 shows the time course of its production. The incubation was stopped after 17 hr., when production was virtually maximal with the formation of approx. 1 μ mole/ml. of incubation medium. This amount corresponds to about 10% of the L-threonine added.

Fractionation on Amberlite IRC 50. The suspension was adjusted to pH 5.0 with acetic acid and the cells were removed by centrifuging. The supernatant was passed at the rate of 8–12 ml./min. through a column (55 cm. $\times$ 6 cm.) of Amberlite IRC 50, buffered at pH 4.9 with m-sodium acetate-acetic acid buffer. The column was washed with water until the effluent gave a negative ninhydrin test. The basic fraction was eluted with 2N-HCl until 1 ml. of the eluate gave a negative picrate reaction for aminoketone. Recovery of aminoacetone from the column was 80%. At this stage the eluates from three similar preparations were combined.

Fractionation with phosphotungstic acid. The combined eluates were concentrated *in vacuo* until

Fig. 1. Aminoacetone formation by washed *S. aureus* suspensions in large-scale experiment. A portion (6 l.) of washed *S. aureus* suspension (3.2 mg. dry wt./ml.) containing 20 mM-DL-threonine and 16 mM-phosphate buffer, pH 7.0, was incubated as described in the text. Samples were removed at intervals for aminoacetone determination.

a moist solid, consisting mainly of NaCl, was obtained. This material was extracted with 400 ml. of absolute ethanol, filtered and the filter cake washed with ethanol. To the combined filtrate and washings (800 ml.) was added 100 ml. of water and the solution freed from ethanol by concentration *in vacuo* to about 20 ml. The concentrate was diluted to 400 ml. and 180 ml. of 10% (w/v) purified phosphotungstic acid in 0.25N-HCl solution was added. (The solution of aminoacetone was required to be 0.25–0.4N with respect to HCl; a small sample was titrated and adjustments were made, if necessary, before phosphotungstic acid was added.) A voluminous precipitate formed and, after standing overnight at 0°, was removed by centrifuging. The supernatant was extracted with a mixture of amyl alcohol-ether (1:1, v/v) until free from phosphotungstic acid (Van Slyke *et al.* 1942).

Paper chromatography. The aqueous solution was concentrated to a syrup *in vacuo* to remove excess of HCl and diluted with 100 ml. of water. It was adjusted to pH 3.0–4.0 by the addition of N-NaOH and freeze-dried. The solid was extracted with 50 ml. of anhydrous ethanol and the insoluble NaCl filtered off. The filtrate was acidified by the addition of 0.1 ml. of 4N-HCl and concentrated *in vacuo* to 15 ml., after which it was applied as continuous bands across 18 washed Whatman no. 3 papers (58 cm. wide). After development in butanol-acetic acid for 24 hr., the papers were dried at 37°. To locate the aminoacetone bands, use was made of the facts that the chromatograms always had a number of zones visible in u.v. light and that all bands on a given chromatogram had the same contours. The bands visible in u.v. were outlined in pencil and the aminoacetone band was located at one point by applying a narrow streak of ninhydrin down the paper and developing the colours at 30°. The aminoacetone band was then pencilled in, parallel to the other bands visible in u.v., and cut out. This procedure made possible accurate location of the bands even when they were irregular in shape. The aminoacetone was eluted chromatographically with 0.2N-HCl and the solution concentrated *in vacuo* to a small volume of thick brown oil. This was dissolved in 6.5 ml. of anhydrous ethanol, left at –10° overnight and the resulting flocculent precipitate removed by centrifuging. Chromatography of the supernatant showed that only one ninhydrin-reacting component was present but there was also some u.v.-absorbing material.

Crystallization as the toluene-p-sulphonic acid salt. The clear-yellow supernatant (6 ml.) was mixed with an equal volume of ethanol containing 2 g. of toluene-p-sulphonic acid. Ether was added (approx. 1 ml.) until a dense precipitate formed. The mixture was left at 0°, after which the

precipitate was removed by centrifuging and discarded. More ether (about 15 ml.) was added to the supernatant until a faint turbidity appeared. Needle-shaped crystals formed after 5 min. at room temperature. The flask was left at 0° and the crystals were collected on a Büchner funnel and washed in ether-ethanol (9:1, v/v). A second crop of crystals was obtained by the addition of more ether and the two lots were combined. The crystals were dried over P_2O_5 *in vacuo*; yield, 0.39 g.

The product was recrystallized twice by dissolving it in ethanol and adding ether as before, and finally obtained as fine white needles, m.p. 130–131° (uncorr.) (Found: C, 49.2; H, 6.3; N, 5.9; S, 13.0. $C_{10}H_{15}O_4NS$ requires C, 49.0; H, 6.2; N, 5.7; S, 13.1 %).

Synthetic aminoacetone toluene-*p*-sulphonic acid salt was recrystallized twice and obtained as thin plates with m.p. 130–131° (uncorr.) (Found: C, 49.1; H, 6.4; N, 5.9; S, 12.9 %). The melting point was unchanged by admixture with the salt of the natural compound. The identity of the natural and synthetic salts was confirmed by infrared spectroscopy.

The isolated salt and the synthetic material had the same R_f when chromatograms were developed in solvents I and II. Ionophoretic examination at pH 5.0 showed that both the natural and synthetic compounds migrated towards the cathode at the same speed.

Formation of 2:4-dimethyl-3-acetylpyrrole. A portion (50 mg.) of the isolated salt was dissolved in 4 ml. of *m*-acetate buffer, pH 4.6, 0.1 ml. of acetylacetone was added and the solution heated at 100° for 15 min. The solution was cooled and, on adjustment to pH 8.0 with NaOH, crystals of 2:4-dimethyl-3-acetylpyrrole appeared. The crystalline material was filtered off, washed with a little ice-cold water and dried *in vacuo* over P_2O_5 ; m.p. 138–140° (corr.) (cf. 137°, Knorr & Lange, 1902). The m.p. was unchanged by admixture with the substituted pyrrole prepared in the same way from synthetic aminoacetone toluene-*p*-sulphonic acid salt.

The u.v. spectrum of the pyrrole formed from the natural aminoacetone salt had a maximum at 251 $m\mu$, a shoulder at 275–286 $m\mu$ and a minimum at 227 $m\mu$, in agreement with the data reported by Cookson (1953) for this compound.

The pyrroles from the salts of both the synthetic and natural aminoacetone gave immediate reactions with Ehrlich reagent and the coloured complexes derived from the two samples had identical absorption spectra.

Factors affecting aminoacetone formation from threonine

Table 1 shows that aminoacetone formation is dependent on the presence of unboiled *S. aureus*

cells. None is formed if the cells are first boiled and a negligible amount is formed in the absence of threonine. When the incubation was carried out under anaerobic conditions the yield was decreased by 81 %.

Cell density. Fig. 2 shows that aminoacetone formation is proportional to cell density up to a value of 2 mg. dry wt./ml., after which some factor, possibly oxygen supply, becomes limiting.

Age of cells. No significant difference was observed in the rate of aminoacetone formation from threonine with cells harvested in the logarithmic phase of growth and those harvested at the end of the rapid-growth phase.

Table 1. *Aminoacetone formation from threonine by washed cell suspensions of Staphylococcus aureus*

The complete system contained 10 mM-phosphate buffer, pH 7.0, 5 mM-L-threonine and washed *S. aureus* suspensions (30 mg. dry wt./ml.); volume, 10 ml. Flasks were shaken at 37° for 3 hr. In experiment 4 the flask was evacuated and the air replaced with nitrogen.

Experiment	Aminoacetone	
	μ moles/10 ml.	Percentage of that in complete system
1 Complete system	8.15	—
2 Stopped at zero time	0.10	1
3 Cells boiled	0.30	4
4 Incubated under N_2	1.55	19
5 Threonine omitted	0.25	3

Fig. 2. Effect of cell density on aminoacetone formation from threonine. Washed *S. aureus* suspensions were incubated for 3 hr. at 37° with shaking in the presence of 40 mM-DL-threonine and 20 mM-phosphate buffer, pH 7.0.

pH. As might be expected with intact cells, there was a broad pH optimum centred around pH 7.1, the activity falling by only 20% at pH 5.2.

Threonine concentration. The system is saturated with L-threonine at 0.01M. The apparent K_m is approx. 1.5 mM.

Specificity. No aminoketone formation could be detected by the picrate reaction when threonine was replaced by DL-allothreonine or by DL- β -hydroxyglutamic acid. It is possible, however, that these substances did not penetrate the cell wall of the organism. The yield from DL-homoserine, the structural isomer of threonine, was only 23% of that obtained from DL-threonine, but it remains to be determined whether this was due to homoserine alone or to contamination of it by threonine. L-Serine did not increase the yield of aminoacetone, nor did it give rise to the formation of a carbonyl-amino compound as judged by the picrate reaction, the modified Elson-Morgan reaction and by paper chromatography.

Effect of carbon dioxide. Aminoacetone formation from threonine must involve decarboxylation at some stage and it was therefore tested if there was any inhibition of its formation by CO_2 . No such effect was observed. Flasks gassed with O_2 or with $\text{O}_2 + \text{CO}_2$ (95:5) in the presence of 3.7 mM- NaHCO_3 gave identical yields.

Attempt to find a decarboxylation product of threonine

The initial attack on the threonine molecule by the *S. aureus* system might be expected to involve either an oxidation or a decarboxylation step. If the latter were the case it is possible that there would be some accumulation of 1-amino-2-propanol when aminoacetone formation was prevented by incubation of the system under anaerobic conditions. To test for this, experiments were carried out as follows. Incubation mixtures containing 20 mM-L-threonine were shaken with washed *S. aureus* suspensions at 37° for 3 hr., the gas phase in the flasks being air in one case and N_2 in the other. After incubation, the mixtures were acidified by the addition of 1.5 ml. of N-HCl and boiled for 2 min. to release cell contents. The supernatants, after centrifuging, were adjusted to pH 5.0 and the bases absorbed on to Amerlite IRC 50 columns. After washing, the columns were eluted with HCl, the eluates were evaporated to dryness *in vacuo* and the residues dissolved in water. The salts present were precipitated by the addition of 4 vol. of ethanol, removed by centrifuging and the supernatants were used for chromatography in solvent II.

It was found that in the aerobic experiment the major ninhydrin-reacting component present was aminoacetone. In the anaerobic experiment there was only a trace of aminoacetone and there was no evidence of any accumulation of an amino alcohol.

Although a marker of 1-amino-2-propanol was not available, ethanolamine, the lower homologue, ran almost as fast as aminoacetone, and in both the anaerobic and aerobic experiments the only fast-moving component was aminoacetone itself. There was thus no detectable accumulation of a decarboxylation product of threonine under anaerobic conditions.

Aminoacetone synthesis from glycine

The preceding results show that aminoacetone is formed from threonine by *S. aureus*. Although the presence of glucose is not obligatory for this reaction, it was observed that when suspensions of the organism were incubated in the presence of

Fig. 3. Aminoacetone synthesis from glycine by washed *S. aureus* suspensions. Washed *S. aureus* (2 mg. dry wt./ml.) were incubated with shaking at 37° in the presence of 33 mM-phosphate buffer, pH 7.0, 10 mM-glycine and 1% glucose. Volume, 20 ml. ●, Complete system; ○, glycine omitted.

Table 2. Synthesis of aminoacetone from glycine by washed *Staphylococcus aureus* suspensions

Washed *S. aureus* suspensions (4 mg. dry wt./ml.) were incubated, with shaking, at 37° for 3 hr. in the presence of 33 mM-phosphate buffer, pH 7.0, 10 mM-glycine and 1% glucose. Volume, 20 ml.

Experiment	Aminoacetone	
	μmoles/20 ml.	Percentage of that in complete system
1 Complete system	1.23	—
2 Stopped at zero time	0.08	7
3 Glycine omitted	0.40	34
4 Glucose omitted	0.33	28
5 Complete, incubated under N_2	0.43	36

Table 3. *Retention of glycine metabolite on Amberlite IRC 50*

Experimental details are described in the text.

Experiment	Fraction	Aminoacetone	
		$\mu\text{moles/10 ml.}$	Percentage of that in complete system
1 Complete system	Total aminoketone	0.46	—
2 Complete system	Non-basic aminoketone	0.02	4
3 Glucose omitted	Total aminoketone	0.13	28

glucose alone, a small amount of a compound displaying the properties of an aminoketone was produced. The chromatographic behaviour and chemical reactions of the product were identical with those of aminoacetone. The addition of glycine caused a stimulation of the rate of synthesis of the material which proceeded linearly for 2 hr. (Fig. 3), though the maximum rate was only about 6% of that with threonine.

Maximum formation of the metabolite from glycine is dependent on the presence of glucose (Table 2), and omission of this substrate diminished the yield by 72%. It is probable that sufficient oxidizable substrate is always present to permit some synthesis and it is noteworthy that *S. aureus* cells invariably contained some glycine. As with aminoacetone formation from threonine, O_2 is necessary for aminoketone formation from glycine. Replacement of air in the incubation flask by N_2 caused a 64% inhibition of aminoketone synthesis from glycine (Table 2). In this experiment no attempt was made to remove dissolved O_2 from the system.

Specificity. Glycine could not be replaced by alanine or by a mixture of alanine and ammonia. It can therefore be concluded that the glycine is not merely a source of amino groups or of ammonia. Glucose could not be replaced by sodium pyruvate, although this result may be due to a failure of the substrate to penetrate the cell.

Accumulation of aminoketone outside the cell. It was investigated whether or not the aminoketone was freely diffusible from the *S. aureus* cell. Incubations were carried out under the conditions described in Table 2, with glycine and glucose present. After incubation for 3 hr. in air the reaction in one flask was stopped by centrifuging the intact cells; the second flask was acidified by the addition of 0.4 ml. of 5N-HCl and the suspension heated to 100° for 1 min. to release the cell contents, cooled and centrifuged. It was found that amounts of aminoacetone in the two supernatants were 0.45 and 0.48 μmole respectively, showing that the metabolite was not concentrated to any significant extent within the bacterial cells.

Properties of the glycine metabolite

The glycine metabolite gave the same colour reactions on paper as did the threonine metabolite. Moreover, it did not separate from aminoacetone when chromatographed in any of the acidic solvents used in this work. Two-dimensional chromatography in solvents I and III showed that the glycine metabolite could be separated from δ -amino-laevulinic acid and moreover that the unfractionated eluates from Dowex 50 columns (Elliott, 1960) contained none of the latter compound. There was insufficient of the glycine metabolite formed to make its isolation feasible and, because of its low concentration relative to salts present in the incubation medium, it was not possible to determine the charge on the molecule by paper electrophoresis. It was therefore necessary to resort to ion-exchange resins to establish its basic nature.

Fractionation on Amberlite IRC 50. An incubation mixture (190 ml.) containing glycine and glucose as described in Table 2 was set up together with a control experiment in which glucose was lacking. After incubation the total aminoketone present in samples of both mixtures was estimated after Dowex 50 treatment (Elliott, 1960).

The remainder of the complete incubation mixture was adjusted to pH 5.0, the cells were removed and the supernatant was passed through a column (20 cm. $\times$ 2 cm.) of Amberlite IRC 50 buffered at pH 4.9 and washed through with water. The volume of the combined effluent and washings was measured and an appropriate sample taken for estimation of the non-basic aminoketone (i.e. that not retained by Amberlite IRC 50). Essentially all of the aminoketone was retained on the Amberlite resin (Table 3), showing that all of the aminoketone, whether dependent for its formation on glucose or not, is basic in nature. This rules out the possibility of there being formed a mixture of a basic and a non-basic component.

The basic compounds were eluted from the Amberlite columns with 2N-HCl and the eluent was evaporated to dryness *in vacuo*. The solids were extracted with ethanol, the solution was

evaporated to dryness once and extracted with 2 ml. of anhydrous ethanol. Chromatography in solvent II showed it to have the same R_f as aminoacetone.

DISCUSSION

The experimental work described shows that aminoacetone is produced by washed suspensions of *S. aureus* by two apparently distinct metabolic routes.

The conversion of threonine into aminoacetone presumably involves both the oxidation of the β -hydroxyl group and the loss of the carboxyl group, unless some more complicated pathway is postulated. Assuming the first step to be an oxidation, this would result in the formation of α -aminoacetoacetic acid (reaction 1), which would spontaneously break down to aminoacetone (reaction 2).

Laver, Neuberger & Scott (1959) have shown that synthetic α -aminoacetoacetic acid, prepared from its ester, has a half-life of very much less than 1 min. at pH 7.0 and room temperature.

On the other hand, if decarboxylation of threonine occurred first, 1-amino-2-propanol would be formed (reaction 3) and a second enzyme would be required for its further conversion into aminoacetone (reaction 4).

Krasna, Rosenblum & Sprinson (1957) found that the 1-amino-2-propanol moiety of vitamin B₁₂ was derived from threonine and it was suggested that a decarboxylation of the amino acid takes place.

Experimentally it has been found that there is no detectable accumulation of 1-amino-2-propanol when aminoacetone formation from threonine is prevented by incubating the system under anaerobic conditions. This result, together with the fact that there is no lag period in the formation of aminoacetone under aerobic conditions, favours the view that aminoacetone arises from threonine via reactions 1 and 2. Studies of the enzyme system at the cell-free level are required before this can be determined with certainty, however.

Although the aminoketone derived from glycine has not been positively identified by isolation, its

properties warrant the assumption that it is, in fact, aminoacetone. In considering the mechanism of its synthesis from glycine it should be pointed out that threonine is an essential amino acid for *S. aureus* (Gale & Folkes, 1953). It is therefore unlikely that aminoacetone formation from glycine occurs via threonine, though perhaps the possibility should not be entirely discounted. The requirements of the system responsible for the synthesis of aminoacetone from glycine are consistent with the mechanism shown in reaction 5. In this it is envisaged that acetyl-coenzyme A (CoA), derived from glucose, condenses with glycine to yield α -aminoacetoacetic acid.

Aminoacetone would arise as before by the spontaneous decarboxylation shown in reaction 2. This scheme is based on that proposed by Nemeth, Russel & Shemin (1957) for the synthesis of δ -aminolaevulinic acid from succinyl-CoA and glycine. Since the present work was done, Gibson, Laver & Neuberger (1958) and Kikuchi, Kumar & Shemin (1959) have in fact demonstrated aminoacetone synthesis from acetyl-CoA and glycine by red-blood-cell preparations. The existence of such a reaction was foreshadowed by the earlier suggestion of Nemeth *et al.* (1957) that the succinyl-glycine reaction might also apply to an acetyl derivative.

The question arises whether aminoacetone is a normal metabolite in animals and bacteria. No systematic study of the distribution of the threonine system has yet been made, though it was found in the one case tested that *Streptococcus faecalis* produced an aminoketone from threonine, although more slowly than did *S. aureus*. The glycine system appears to occur both in animals and bacteria and particularly noteworthy is the observation by Gibson *et al.* (1958) that red-blood-cell preparations form aminoacetone when incubated with glycine and pyruvic acid. It is likely therefore that the compound is a physiologically occurring metabolite. Mauzerall & Granick (1956) found that, of the total pyrrole formed by condensation of acetylacetone with the aminoketones present in normal human urine, 20–40% was of a type derived from an aminoketone lacking a carboxyl group. This observation has been confirmed by the finding (unpublished observations) that the basic fraction of human urine from normal adults gives rise to a pyrrole on condensation with acetylacetone, which is extractable into ether at pH 8.0. The material gave a coloured complex with Ehrlich reagent whose absorption spectrum was the same as that given by the pyrrole derived from aminoacetone.

It has been suggested (Elliott, 1959) that the above facts may be integrated into a metabolic cycle in which it is postulated that aminoacetone is deaminated to yield methylglyoxal, which would then be rapidly converted into D-lactic acid by the widespread glyoxalase system. The presence of an enzyme system capable of oxidizing D-lactic acid to pyruvate has been shown to be present in liver and kidney preparations of rat (Huennekens, Mahler & Fordman, 1951; Mahler, Tomisek & Huennekens, 1953) and in bacteria (Kaufman, Korkes & Del Campillo, 1951). This system, together with pyruvate oxidase, would regenerate acetyl-CoA. It was reported previously by Nemeth *et al.* (1957) that [^{14}C]aminoacetone was converted into $^{14}\text{CO}_2$ and into purines by the rat, and these authors suggested that this aminoketone might be involved in the oxidative metabolism of glycine, but they also stated that there was no evidence that aminoacetone was produced by the cell. The postulated cycle would provide a pathway for the oxidation of threonine and glycine and in the latter case would be an alternative route to the succinate-glycine cycle (Shemin, 1955).

The accumulation of aminoacetone by *S. aureus* would occur if the organism were incapable of deaminating aminoacetone. Such a metabolic defect would be in accordance with the limited nitrogen metabolism implied by the strict amino acid requirements of the organism.

It remains to be established whether or not aminoacetone is metabolized by the pathway suggested and, if so, what the quantitative importance of the mechanisms might be.

SUMMARY

1. Washed suspensions of *Staphylococcus aureus* form an aminoketone when shaken in air in the presence of threonine.

2. The threonine metabolite has been isolated in crystalline form as the salt of toluene-*p*-sulphonic acid and has been positively identified as aminoacetone.

3. A compound with properties indistinguishable from those of aminoacetone is synthesized in *Staphylococcus aureus* by what is apparently a second route requiring the presence of glycine, glucose and oxygen.

4. The mechanisms involved in the formation of aminoacetone by the two routes are discussed.

5. The possible role of aminoacetone in metabolism is discussed. It is suggested that the metabolite may be oxidized by a metabolic cycle in which the compound is converted into methylglyoxal and thence to pyruvic acid via the glyoxalase and D-lactate dehydrogenase systems.

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