ENZYMES CONCERNED IN THE PRIMARY UTILIZATION OF AMINO ACIDS BY BACTERIA

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CONTENTS

The history of our acquisition of knowledge concerning the utilization of amino acids by bacteria is, in effect, an account of the advancement of methods of bacterial chemistry. The earliest observations of significance were obtained by a chemical study of the products of putrefaction of undefined media by mixed cultures of organisms (3, 4, 5, 84, 14). Such work yielded information concerning the types of compound that could be produced biologically under completely undefined conditions. A step forward was made by the use of pure cultures of organisms (74) and conditions became less obscure with the introduction of synthetic media. A large number of papers deals with the various products of amino acid breakdown that have been obtained by the inoculation of a determined medium, containing a known amino acid, with a pure strain of a known organism, and these have been

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collected and tabulated by Stephenson (86). In much of this work the period of incubation has been long, even as long as three or four months in some cases, and the products isolated chemically at the end of this period, when it is inevitable that conditions should have altered very considerably. Also, it is by no means certain that the organisms have maintained the same enzyme make-up throughout the experiment. Thus we may have recorded as products of amino acid breakdown by certain organisms, substances that are really produced in stages at different times during the incubation period, under different environmental conditions and by what are, biochemically speaking, different organisms.

The next advance was made when it was realized that it is necessary to study the metabolism of an organism, not as an integral whole, but as a collection of reactions catalyzed by specific enzymes contained in the bacterial cell. Such a study is impossible while organisms are growing in culture, as any change studied in the growth medium may be due to catabolic or anabolic reactions, or to both. To avoid this ambiguity in interpretation, the technique of the washed suspension of bacteria was evolved. By this method, experiments several hours in duration can be carried out in the absence of cell growth. This technique was first used for suspensions of *Escherichia coli* by Quastel and Whetham in 1925 (76, 77). By the use of washed suspensions together with the methylene blue, manometric and other techniques of biochemistry, not only can an idea of the relative rates and of the quantitative nature of bacterial attack on amino acids be obtained, but also the conditions of pH and temperature under which the enzymes involved are active can be investigated, as can also the growth conditions necessary for the optimal formation of such enzymes. Examples of such advances made in the field of amino acid metabolism are detailed below.

The washed suspension technique allows of investigation of the properties of enzymes as they exist in the bacterial cell but such investigation is necessarily restricted by the presence and action of other enzymes in the cell, by the permeability of the cell membrane towards the enzyme substrate, etc. A detailed ac-

count of the properties of any particular enzyme can only be given after it has been obtained in a cell-free condition. Until recently bacterial chemistry has lagged behind other branches of biochemistry in this respect owing to the difficulty of breaking up the bacterial cell with the general liberation of its enzymes; but since the production of the wet-crushing bacterial mill of Booth and Green (18) an increasing number of cell-free bacterial enzymes are being studied (38, 44). Several enzymes involved in the metabolism of amino acids have been obtained in a cell-free state by this and other methods and will be discussed below. The ideal of a crystallized bacterial enzyme of this nature has not, up to the time of writing, been realized.

Bacteria are known to utilize amino acids as (1) a source of nitrogen (and possibly carbon) for cell-growth and multiplication and (2) as a source of energy. Such utilization involves both catabolic and anabolic changes, and most of our more exact knowledge up to the present is related to the former, so that it will be chiefly with the catabolic type of change that this paper will deal.

The Breakdown of Amino Acids by Bacterial Enzymes

If we consider the immediate degradation of the amino acid molecule by bacterial enzymes, four ways of attack appear available:

1. Removal of the amino group in the α-position, or deamination.
2. Removal of the carboxyl group, or decarboxylation.
3. Deamination accompanied by decarboxylation.
4. Splitting of the molecule into smaller molecules other than by deamination or decarboxylation.

A possible product of reaction 4 is an amino acid of smaller molecular weight but, in general, the products of these methods of attack are no longer amino acids and hence need not be followed further in a study of enzymes whose substrates are specifically amino acids. Thus it is intended in this review to restrict the discussion to those enzymes immediately concerned

with the initial attack on amino acids and to omit further consideration of the fate of the products unless such consideration is essential for the understanding of the primary reaction.

1. REMOVAL OF THE AMINO GROUP FROM THE α-POSITION: DEAMINATION

The enzymes involved in this type of breakdown are usually termed "deaminases" but it must be realized that the reaction catalyzed by a "deaminase" may actually consist of more than one step, each catalyzed by a separate enzyme, as in the case of the "glutamic acid deaminase" discussed below. Deamination may be brought about in a variety of ways, each resulting in a different product:

a. Oxidative deamination to give a keto acid

$$R\cdot CHNH_2\cdot COOH + \tfrac{1}{2}O_2 = NH_3 + R\cdot CO\cdot COOH$$

b. Desaturation deamination to give an unsaturated fatty acid

$$R\cdot CH_2\cdot CHNH_2\cdot COOH = NH_3 + R\cdot CH{:}CH\cdot COOH$$

c. Hydrolytic deamination to give a hydroxy acid

$$R\cdot CHNH_2\cdot COOH + H_2O = NH_3 + R\cdot CHOH\cdot COOH$$

d. Reductive deamination to give a saturated fatty acid

$$R\cdot CHNH_2\cdot COOH + 2H = NH_3 + R\cdot CH_2\cdot COOH$$

e. Mutual oxidation-reduction between pairs of amino acids

$$H_2O + R\cdot CHNH_2\cdot COOH + X\cdot CHNH_2\cdot COOH = 2NH_3 + R\cdot CO\cdot COOH + X\cdot CH_2\cdot COOH$$

The occurrence of each of these types of reaction, together with others (*f*) which have not been fully classified, in the metabolism of bacteria will be dealt with separately. These will be followed by (*g*) a consideration of the factors influencing bacterial deamination.

(*a*) *Oxidative deamination*

The work of Krebs (68, 69, 70, 71, 72) has established that mammalian kidney and liver oxidatively deaminate the majority

of amino acids that are metabolized by these tissues. The demonstration of oxidative deamination involves showing (*a*) the liberation of NH_3 in the presence of O_2 or some oxidizing agent only, (*b*) the isolation of the keto acid formed and, if possible (*c*) that the consumption of O_2 is equivalent to the NH_3 evolved when the metabolism is stopped at the keto acid stage.

Stephenson and Gale (87) showed that washed suspensions of *E. coli* will deaminate glycine, *dl*-alanine and *l*(+)-glutamic acid aerobically but not anaerobically. As the work was designed for another purpose, no attempt was made to isolate the deamination product, but Janke and Tayenthal (60) studied the reaction with glycine in detail. They showed that the deamination is carried out by *E. coli*, *Proteus vulgaris*, *Pseudomonas fluorescens*, and *Bacillus mycoides* aerobically but not anaerobically unless some oxidizing agent such as *m*-dinitrobenzene is present. The aerobic deamination was performed in the presence of 2,4-dinitrophenylhydrazine which acts as a fixative for keto acids, and a substance was isolated having the color reactions and melting point of the glyoxylic acid derivative. Thus the deamination of glycine by the bacteria studied may be described by the equation:

$$NH_2 \cdot CH_2 \cdot COOH + \tfrac{1}{2}O_2 = CHO \cdot COOH + NH_3$$

The pH optimum for the reaction was between 7.5 and 8, and this appears to be a general finding for all enzymes of this class so far studied (43, 87). (See fig. 1.)

Aubel and Egami (12) demonstrated the deamination of *dl*-alanine by an unidentified soil organism and showed that the reaction will occur aerobically but not anaerobically except in the presence of nitrate. Simon's color test for pyruvic acid proved positive when applied during the reaction and from this fact together with the effects of various inhibitors, these workers assume that the deamination process is an oxidative one, involving dehydrogenation followed by hydrolysis of the imino acid so formed. The proof is not rigid, but some unpublished work carried out in this department with washed suspensions of *E. coli* shows that this organism carries out oxidative deamination of *dl*-alanine. It has already been shown (87) that a washed sus-

pension prepared from the organism grown on the surface of broth-agar (tryptic digest of casein) will deaminate *dl*-alanine aerobically at pH 7.5. If the suspension is first treated with toluene and the toluene then removed, the deamination rate is halved and a positive Simon's nitroprusside test shows the accumulation of pyruvic acid. A large-scale experiment was set up

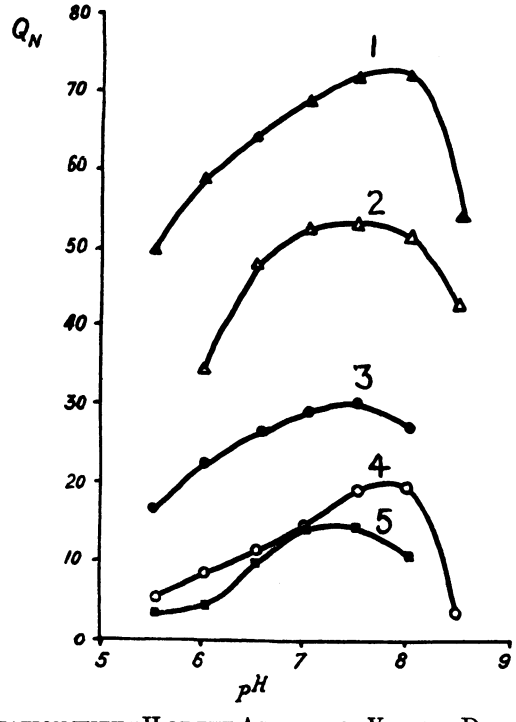


FIG. 1. VARIATION WITH pH OF THE ACTIVITY OF VARIOUS DEAMINASES IN WASHED SUSPENSIONS OF ESCHERICHIA COLI

1, *dl*-Serine; 2, *l*-aspartic acid; 3, *dl*-alanine; 4, *l*-glutamic acid; 5, glycine (38, 43, 87)

with toluene-treated organisms, alanine and phosphate buffer at pH 7.5 and incubated aerobically until a manometric control showed that about 60 per cent of the alanine was oxidized (on the assumption that ½ mol. O_2 would be taken up for each mol. NH_3 liberated). The organism was centrifuged off and the centrifugate analyzed. Pyruvic acid was isolated as its 2,4-dinitro-

phenylhydrazone and estimated by the use of a carboxylase preparation from brewer's yeast. Also the ammonia-N was estimated by the Parnas technique and the amino-N by the van Slyke method. Table 1 gives the full analysis. These results prove that the deamination of *dl*-alanine by *E. coli* is accomplished according to the equation:

$$CH_3 \cdot CHNH_2 \cdot COOH + \tfrac{1}{2}O_2 = CH_3 \cdot CO \cdot COOH + NH_3$$

Attempts to demonstrate the presence of an alanine-dehydrogenase in washed suspensions of *E. coli* or in crushed cells have not so far been successful so that no evidence has as yet been adduced for the existence in bacteria of an alanine-dehydrogenase-coenzyme as demonstrated for the kidney *d*-amino-acid-oxidase (25, 90, 98, 99).

TABLE 1

Products of deamination of dl-alanine by toluene-treated suspensions of E. coli

Total theoretical N	342μl	
Amino-N	197μl	347μl
Ammonia-N in centrifugate	150μl	
Pyruvic acid	153μl	

In the case of the *l*(+)-glutamic acid deaminase of *E. coli*, Adler and his co-workers (9) have been able to extract the "enzyme" from the cell by a modification of the method used by Stephenson (85) for the extraction of lactic dehydrogenase. The extracted enzyme reduces methylene blue in the presence of glutamic acid, requiring the presence of coenzyme II for its activity. The reduction time is increased some 3 to 5 times by the presence of either ammonium chloride or ketoglutaric acid, while the combined presence of these substances increases the reduction time 7 to 8 times. These results suggest that the enzyme involved is a dehydrogenase and that the reaction is reversible. This is confirmed by spectrometric studies of the reduction of coenzyme II in the presence of the enzyme and *l*-glutamic acid, and of the oxidation of dihydro-coenzyme II in the presence of the enzyme and ketoglutaric acid together with

ammonia,—neither being effective alone. The general scheme that has been worked out as a result of these studies is

(a) Glutamic acid + coenzyme $\rightleftharpoons$ Iminoglutaric acid + dihydro-coenzyme;

(b) Iminoglutaric acid + $H_2O \rightleftharpoons$ Ketoglutaric acid + NH_3

(c) Dihydro-coenzyme + $\frac{1}{2}O_2$ = Coenzyme + H_2O

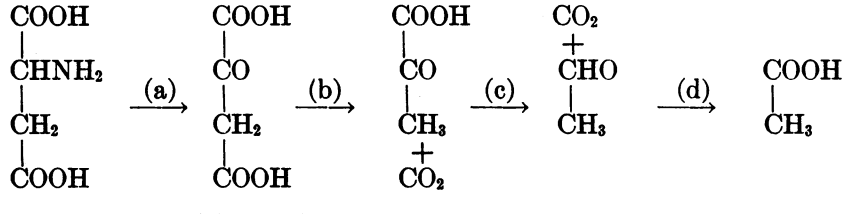
Thus the amino acid is deaminated in two steps, each being reversible, and oxygen is taken up for the reoxidation of the dihydro-coenzyme formed in the first step. Adler and his co-workers (7, 8, 9) believe this reversible reaction is of importance in shedding light on the problem of amino acid synthesis in various tissues.

Klein (64) in a recent paper has shown that washed suspensions of *Hemophilus parainfluenzae* oxidize *l*(−)-aspartic and *l*(+)-glutamic acids with the liberation of ammonia and the formation of acetic acid, according to the equations:

$$\text{Glutamic acid} + 2\tfrac{1}{2}O_2 = CH_3 \cdot COOH + NH_3 + 3CO_2$$

$$\text{Aspartic acid} + O_2 = CH_3 \cdot COOH + NH_3 + 2CO_2$$

By a study of the metabolism of possible intermediate compounds, Klein has established the probable course of the oxidation. For aspartic acid, the reaction proceeds:



The reactions (a) and (d) require the presence of either coenzyme I or II, the exact requirement not being established. Thus it appears from this work that the first step in the breakdown of the dicarboxylic amino acids by *H. parainfluenzae* is an oxidative deamination, probably proceeding in stages similar to those elucidated by Adler (9).

Woods and Clifton (104, 105) have shown that *Clostridium*

tetanomorphum breaks down certain amino acids with the evolution of H_2, and it may be that this is a form of oxidative deamination without the use of either oxygen or a hydrogen acceptor. However, in most cases, the other products have not been determined and in no case as yet is it possible to state by what particular process the ammonia is primarily liberated from the amino acid molecule.

Bernheim, Bernheim and Webster (15) extended their work on deamination by animal tissues to the deamination and oxidation of amino acids by resting *Proteus vulgaris*. They showed an oxygen uptake in the presence of the washed suspension and the natural isomers of leucine, phenylalanine, methionine, serine, alanine, proline, tyrosine and tryptophane and this O_2 uptake was accompanied by the evolution of ammonia. No attempt was made to isolate the immediate products of the deamination process or to check the oxidation process at this point so that it is impossible to tell from their results whether the oxygen taken up is involved in an oxidative deamination or in the oxidation of the deamination products. In some cases more than 1 atom of O_2 was taken up per mol. amino acid oxidized so that some of this gas at least must be involved in the later oxidative changes. Also one or two similar experiments carried out with washed suspensions of *E. coli* showed that this organism deaminates aerobically serine, alanine and glycine, but it has later been shown (43) that the deamination of serine will also take place anaerobically. Thus this work, and similarly that of Webster and Bernheim (100) on *Pseudomonas aeruginosa* does not indicate the exact nature of the deamination process but yields useful information concerning the general utilization of amino acids under aerobic conditions by the organisms studied.

(*b*) *Desaturation deamination*

Quastel and Whetham (76) showed that *E. coli* brings about the reduction of fumaric acid to succinic acid and also sets up an equilibrium between fumaric and *l*-malic acids. Thus the presence of malic acid retards the reduction of methylene blue by succinic acid in the presence of the washed suspension. As-

partic acid also retards this reduction (77) and this observation led Quastel and Woolf (78) to investigate whether aspartic acid is deaminated by *E. coli* to fumaric acid. The first suggestion that some such reaction takes place was given by Harden in 1901 (51) who showed that *E. coli* grown in glucose broth will deaminate aspartic acid with the final formation of succinic acid. Quastel and Woolf (78) found that the deamination of *l*-aspartic acid is carried out by washed suspensions of *E. coli*, the product in the absence of any inhibitor being succinic acid. If however an inhibitor such as toluene is present, then the rate of the deamination process is not affected in the initial stages; but the process does not go to completion, reaching, instead, an equilibrium mixture of aspartic acid, fumaric acid and ammonia. Further, if the correct proportions of fumaric acid and ammonia are incubated in the presence of the cells and toluene, then aspartic acid is synthesized until the same equilibrium mixture is produced. Thus we have the reversible deamination process:

$$HOOC \cdot CH_2 \cdot CHNH_2 \cdot COOH \rightleftharpoons HOOC \cdot CH{:}CH \cdot COOH + NH_3$$

In the absence of inhibitors, the fumaric acid is reduced to succinic acid and the equilibrium conditions are upset. Woolf (107) also showed that fumarase present in the organism produces malic acid from the fumaric acid and that the final equilibrium mixture is not a simple aspartic acid-fumaric acid-ammonia system but one containing malic acid as well.

The existence of the desaturation deaminase was proved by Virtanen and Tarnanen (97) who obtained from *Pseudomonas fluorescens* in a cell-free state (by extracting the dried cells with toluene-water) the aspartase responsible for the production of fumaric acid from aspartic acid. The filtered extract contained fumarase and aspartase, but the former was removed by tryptic digestion, leaving a preparation with which the properties of the cell-free aspartase could be studied.

Gale (38) investigated the deamination of *l*-aspartic acid by washed suspensions of *E. coli* and by a study of the loss and recovery of activity of the suspensions on standing under various conditions, discovered that the rate of deamination is greatly

increased by the addition of small amounts of adenosine or inosine. This effect had not been noticed for the aspartic acid enzymes previously studied. The enzyme was obtained in a cell-free condition by the use of thick washed suspensions crushed in the mill of Booth and Green (18). It soon became obvious that there were at least two enzymes in the cell-free juice so obtained which would deaminate aspartic acid anaerobically. Simple ammonium sulphate fractionation led to the separation of two enzymes: one stable to incubation with toluene and unaffected by the presence of adenosine; the other, inactive in the absence of some coenzyme which could be replaced *in vitro* by adenosine, and completely inactivated by toluene treatment. The first of these enzymes was presumably the aspartase of Quastel and Woolf (78) and was therefore renamed "aspartase I" while the other, hitherto unstudied, enzyme was named "aspartase II." Both enzymes are optimally active at pH 7.5. Since however the simple fractions containing the separate enzymes contained fumarase, and no method of separating aspartase II and fumarase could be found, owing to the greater sensitivity of the former, it is impossible to state the immediate product of the deamination accomplished by aspartase II,— a mixture of fumaric and malic acids being obtained from aspartic acid by the action of the fraction containing the latter enzyme.

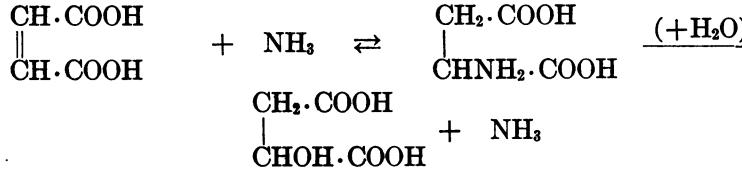
An earlier case of desaturation deamination is recorded by Raistrick (79) who obtained urocanic acid from *l*-histidine:

$$C_2H_3N_2 \cdot CH_2 \cdot CHNH_2 \cdot COOH = C_2H_3N_2 \cdot CH{:}CH \cdot COOH + NH_3$$

The urocanic acid was obtained by the action of *E. coli, Eberthella typhosa, Salmonella paratyphi, S. enteritidis* and *Shigella dysenteriae* respectively on histidine in Ringer's solution. The author states that growth in this medium was poor and that this difficulty was overcome by using a heavy inoculum from several agar slopes. Since however the organisms were in culture over a period of 30 to 40 days, it is not possible to state with certainty whether the urocanic acid is a primary product of desaturation deamination or is produced by a secondary action upon the product of deamination by some other process.

(c) *Hydrolytic deamination*

Woolf (107) showed that the deamination of aspartic acid by *E. coli* suspensions in the presence of toluene results in an equilibrium mixture of aspartic, fumaric and malic acids, the malic acid being produced by the action of fumarase on the fumaric acid produced from aspartic acid by aspartase I. Following the fractionation by Gale (38) of the aspartases of *E. coli* into two separate enzymes, Virtanen and Erkama (92) reported the separation of two aspartases from *Pseudomonas fluorescens* using a method of fractionation involving acetic acid. Their preparation did not contain fumarase as it would not carry out the formation of malic acid from fumaric acid in the absence of ammonia. However, in the presence of ammonia, malic acid was formed from fumaric acid and Virtanen explains this as follows: The preparation contains two enzymes acting upon aspartic acid, one deaminating it to form fumaric acid and one to form malic acid, the former being reversible but not the latter. Thus when fumaric acid is incubated with the preparation in the presence of ammonia it is first converted to aspartic acid by reversal of "aspartase I" and the aspartic acid is then irreversibly deaminated to malic acid by the other enzyme present.



Both enzymes are active in the presence of toluene. It seems probable that the postulated reversible enzyme in this work is aspartase I but whether the other enzyme bears any relation to aspartase II is sheer conjecture. In any case, it is doubtful whether it is legitimate to apply results obtained with one organism to any other. If Virtanen has established the existence of an enzyme forming malic acid directly from aspartic acid, this will be the first positive demonstration of a hydrolytic deaminase.

There are several references to hydroxy acids being isolated from growth experiments involving certain amino acids (11, 54,

82, 83) but there is no evidence in these cases that the acid is a product of hydrolytic deamination.

(*d*) *Reductive deamination*

Cook and Woolf (24) carried out an investigation designed to determine the distribution of the enzymes responsible for the production of (a) fumaric acid, and (b) succinic acid from aspartic acid. They used 11 organisms representative of the strict aerobes, strict anaerobes and facultative anaerobes. Washed suspensions of all 11 deaminated *l*-aspartic acid to succinic acid when incubated at 37° anaerobically. This reaction was completely inhibited by the presence of toluene. The four facultative anaerobes all effected the reaction:

$$\text{Aspartic acid} \rightleftharpoons \text{fumaric acid} + \text{ammonia}$$

giving the same equilibrium constant as that obtained for the aspartase of *E. coli*. However, none of the strict aerobes or strict anaerobes showed any trace of this reaction; and it must be assumed that these latter organisms carry out a simple reductive deamination:

$$HOOC \cdot CH_2 \cdot CHNH_2 \cdot COOH = HOOC \cdot CH_2 \cdot CH_2 \cdot COOH + NH_3$$

No evidence could be obtained for the reversibility of this reaction.

Hopkins and Cole (58) showed the production of β-indole-propionic acid from tryptophane by *E. coli* growing in culture. Woods (101) studied the production of indole from tryptophane by thick washed suspensions of *E. coli* and found that the reaction only took place under strongly aerobic conditions. The product of the anaerobic attack proved to be β-indole-propionic acid and was formed quantitatively.

$$C_8H_6N \cdot CH_2 \cdot CHNH_2 \cdot COOH + 2H = C_8H_6N \cdot CH_2 \cdot CH_2 \cdot COOH + NH_3$$

The hydrogen necessary for this reaction comes presumably from the endogenous hydrogen-donators of the cell. No indole is formed from β-indole-propionic acid in the presence of washed suspensions under otherwise optimal conditions for indole pro-

duction, from which it is concluded that the acid does not form an intermediate in the aerobic production of indole from tryptophane.

The production of saturated fatty acids from corresponding amino acids by organisms growing in culture has been reported by various authors (19, 20, 65).

(e) Mutual oxidation-reduction of pairs of amino acids

Stickland (88, 89) showed that washed suspensions of *Clostridium sporogenes* activate certain amino acids as hydrogen-donators and others as hydrogen-acceptors so that coupled reactions take place between pairs of them, resulting in their deamination. The examination of the function of the amino acids in this respect was continued by Woods (103); and the results of these two workers show that the following amino acids act as H-donators: the natural isomers of alanine, valine, leucine, phenylalanine, cysteine, serine, histidine, aspartic acid, and glutamic acid; and the following as H-acceptors: glycine, proline, hydroxyproline, ornithine, and arginine. The general type of reaction is probably of this nature:

$$R\cdot CHNH_2\cdot COOH + X\cdot CHNH_2\cdot COOH + H_2O = R\cdot CO\cdot COOH\cdot + X\cdot CH_2\cdot COOH + 2NH_3$$

The products of the complete reaction have not been identified in all cases. However, the reaction between alanine and ornithine probably follows this scheme as the products have been identified as δ-aminovaleric acid, ammonia, acetic acid and CO_2, the latter two arising from the alanine probably by way of the intermediate production of pyruvic acid:

$$NH_2CH_2\cdot(CH_2)_2\cdot CHNH_2\cdot COOH + CH_3\cdot CHNH_2\cdot COOH + H_2O \rightarrow NH_2CH_2\cdot(CH_2)_2\cdot CH_2\cdot COOH + CH_3\cdot CO\cdot COOH + 2NH_3$$

Similarly the reaction between glycine and alanine gives rise to 2 molecules of acetic acid, CO_2 and NH_3. There are presumably several enzymes involved in this type of reaction, one of which is an amino acid dehydrogenase similar to that postulated in the oxidative deamination discussed above but, whereas in that case

the "activated" hydrogen is taken up by the coenzyme, in this case the H-acceptor is another amino acid molecule.

Hoogerheide and Kocholaty (57, 66) have shown that *C. sporogenes* can activate and utilize H_2 and, using this as a method for the investigation of H-acceptors, claim that some substrates can act as either H-donators or -acceptors so that intermolecular reactions occur in which one molecule is oxidized and another reduced. Clifton (23) has shown that *Clostridium botulinum* also utilizes amino acids by means of the Stickland reaction.

(*f*) *Unclassified deamination processes*

The work of Bernheim and his co-workers (15, 100) on the aerobic breakdown of amino acids by washed suspensions of various organisms has already been discussed. This work is of interest in particular for the light it gives on the optical specificity of the bacterial attack on amino acids. The organisms used were *Proteus vulgaris* and *Pseudomonas aeruginosa* and their action in washed suspension on 13 amino acids was studied. Both organisms attack both isomers of alanine and serine, and the latter deaminates also both isomers of tyrosine and proline; in all other cases only the natural *l*-isomer is attacked. Gale and Stephenson (44) have shown that washed suspensions of *E. coli* will deaminate *dl*-serine anaerobically at an optimum pH of 7.5. The isomers are attacked at different rates but the product of the deamination process has not been identified.

A series of papers by Desnuelle *et al.* (26, 27, 28) has dealt with the deamination of cysteine and cystine by washed suspensions of *E. coli*. Cysteine is attacked anaerobically with the liberation of NH_3 and H_2S in equimolecular quantities, the enzyme responsible for the liberation of the H_2S ("cysteinase") being strictly adaptive in the sense of Karström (61). Cystine is attacked in the presence of a reducing agent, or during the cofermentation of glucose, so that it is first reduced to cysteine. The other products of the breakdown have not yet been reported. A further paper (29) deals with the anaerobic degradation of cysteine and cystine by *Propionibacterium pentosaceum* and reveals marked differences between the modes of attack on these amino acids by this organ-

ism and *E. coli*. For instance whereas the cysteinase of *E. coli* is strictly adaptive, partially inhibited by the presence of glucose and specific for the natural isomer of cysteine, the enzyme of *Propionibacterium pentosaceum* is not adaptive, is accelerated by the presence of glucose and shows no optical specificity. Tarr (91) has shown that cystine is disrupted anaerobically at pH 7.8 by washed suspensions of *Proteus vulgaris* with the formation of ammonia, hydrogen sulphide, hydrogen, carbon dioxide and acetic acid. Washed suspensions of *Clostridium tetanomorphum* attack *l*-cystine with the liberation of NH_3, H_2S, CO_2 and unidentified products (Woods and Clifton, 101, 102).

The breakdown of tryptophane by bacteria has been the subject of many papers. It has been mentioned above that Woods (101) has shown the reductive deamination to β-indolepropionic acid by *E. coli* under anaerobic conditions and this has been confirmed by Majima (73). Under aerobic conditions, tryptophane is converted quantitatively to indole (52, 101) but, up to the time of writing no attempt to identify the intermediate products has met with success. Woods (101, 102) showed that ammonia is liberated quantitatively and 5 atoms of oxygen are taken up during the complete conversion to indole by washed suspensions of *E. coli*. He was unable to show indole formation under similar conditions from β-indolealdehyde, β-indolecarboxylic acid, β-indoleacetic acid, β-indolepropionic acid or β-indoleacrylic acid. On the other hand, β-indolepyruvic acid gave a small (10%) production of indole in the presence of ammonia, which suggests that there is first a resynthesis to tryptophane. Similar conclusions have been drawn by other workers (37, 73, 81). Happold and Hoyle (49) obtained a non-viable "tryptophanase" from *E. coli* cells which forms indole from tryptophane under aerobic conditions but will not react with β-indolealdehyde, β-indolepropionic acid, β-indoleacetic acid, or β-indolepyruvic acid. A later paper by these workers (13) deals with the groups in the side-chain of the tryptophane molecule essential for the action of the tryptophanase. Their results indicate that the breakdown to indole requires (a) a free carboxyl group, (b) an unsubstituted α-amino group and (c) a β-carbon atom capable of oxidative

attack. They suggest a tentative scheme for the breakdown which involves a reductive fission of the molecule, but this awaits experimental confirmation.

Woods and Clifton (101, 102) have described an anaerobic breakdown of certain amino acids by washed suspensions of *Clostridium tetanomorphum* which results in the liberation of ammonia and hydrogen; this work has been mentioned above as demonstrating a possible form of anaerobic oxidative deamination

(*g*) *Factors influencing bacterial deamination*

"Age of culture." Wooldridge and co-workers (106) first showed that the activity of certain bacterial dehydrogenases varies markedly with the age of the culture from which the cells have been harvested. This applies also to many of the deaminases: Gale and Stephenson (43) showed that serine-deaminase may vary in activity from $Q_N = 200$ for a 6-hr. culture to $Q_N = 1100$ for a 12-hr. culture, the period of maximum activity roughly coinciding with the cessation of active cell-division. In the case of aspartase (38) this variation in activity with "age of culture" is due to a change in the properties of the growth medium brought about by the metabolic activities of the cells.

Anaerobiosis and aerobiosis. Stephenson and Gale (38, 43, 87) have investigated the effect of growing cultures of *E. coli* under varying conditions of aerobiosis on the deaminase activities of the washed suspensions. Generally speaking, their results show that the oxidative enzymes are formed best under strongly aerobic conditions while the formation of the anaerobic deaminases is favored by anaerobic growth conditions.

Effect of the presence of glucose during growth. Kendall and his co-workers, in a series of papers from 1912 to 1922, studied the production of ammonia by several bacterial species when growing in protein digests and showed that this production is greatly checked and, in some cases, completely inhibited by the presence of glucose in the growth medium. They interpreted this as due to a "sparing" action exerted by the carbohydrate on the deamination of proteins, believing that in the presence of a readily available source of carbon and energy, the organism decomposed

less nitrogenous material. Raistrick and Clark (80) pointed out that ammonia is not only a product of the decomposition of proteins but also a source of nitrogen for growth, so that while, in the protein digest medium, the ammonia produced is in excess of that required for cell synthesis, it may be possible that in the presence of additional carbohydrate, this excess is used up in increased cell production. In investigating this point they followed the growth of various bacterial species in synthetic media containing tryptophane or tyrosine with and without glycerol. They estimated the ammonia-N, amino-N, total-N and "synthesized-N" (= cell-N) after periods of 19 to 51 days. They showed that the presence of glycerol in the medium (a) increases the amount of cell-N formed and (b) decreases the amount of ammonia-N formed, and stated that "we believe that carbohydrate, far from having a protein-sparing effect, actually enables the bacteria to utilize more protein or protein products than they would in the absence of carbohydrate." A detailed investigation and discussion of this point has been carried out by Waksman and Lomanitz (97a) who come to much the same conclusions but point out that "a living being derives its energy from a substance which is most available to it and which may be specific for a particular organism."

Happold and Hoyle (49, 50) investigating the production of indole from tryptophane by *E. coli* (see above) have described the preparation of a non-viable "tryptophanase" from cultures of the organism and report that such preparations made from cultures grown in the presence of glucose are inactive, whereas active preparations are not inhibited by the presence of glucose. These workers adduced evidence that tryptophanase is an adaptive rather than a constitutive enzyme, and Fildes (35) reports that, although the organism grown in the absence of tryptophane contains a small amount of tryptophanase, the presence of this amino acid in the growth-medium considerably increases the amount of enzyme in the cells. He further found that it was this "adapted" portion of the tryptophanase which is inhibited by the presence of glucose in the growth medium,—the constitutive part remaining unaffected. Happold and Hoyle (50) con-

sider that the "carbohydrate affords a readier source of energy for *B. coli* than does the tryptophane so that the bacillus does not require to elaborate a mechanism which it has evolved for normal life."

Stephenson and Gale (87) investigated the effect with regard to the deaminases of glycine, alanine and glutamic acid in washed suspensions of *E. coli*. They showed that the presence of glucose has little or no effect upon the actual deamination process by washed suspensions grown in tryptic digest of casein, so that once the enzyme make-up of the cell has been completed, the glucose has no sparing-effect or inhibition-effect on the deamination. If, however, glucose is added to the *growth* medium, then the resulting washed suspension has less than 5 per cent of the deaminase activity of similar suspensions of bacteria grown in the absence of carbohydrate. The effect is not due to anaerobiosis produced by fermentation gases as the effect is not altered by bubbling the growth medium with oxygen. These observations were later extended to the deamination of *dl*-serine (43) and of *l*-aspartic acid (38) and it was suggested that the principal effect of glucose is to inhibit the formation of the deaminases in question during growth. Whether the glucose acts as such or whether its action is due to the acids produced from it during the fermentation, as is suggested by the work of Berman and Rettger on proteolytic enzymes, is not certain. In the above work (87) the pH of the medium was maintained near neutrality during growth by the addition of chalk to the medium, but it is doubtful whether such a device is effective in controlling the pH in the immediate vicinity of a rapidly fermenting organism. In view of the recent work, to be discussed later, on the conditions under which the amino acid decarboxylases are formed in bacteria, it appears probable that the presence of carbohydrate in the medium, by altering the pH in the vicinity of the organism and consequently the ionization of the substrate, may change the focus of the attack on the amino acid from the amino group to the carboxyl group. The question would be clarified by a study of the formation of the various deaminases during growth of organisms in non-carbohydrate media adjusted to various pH values.

Existence of co-deaminases. Mention has been made of the finding (9, 64) that the *l*(+)-glutamic acid dehydrogenase of *E. coli* and *Hemophilus parainfluenzae* requires the presence of coenzyme II for its activity. The results obtained with animal tissues (90, 98, 99) would lead us to expect that other bacterial oxidative deaminases will be resolved into dehydrogenases which will only be active in the presence of certain coenzymes, but no further evidence along these lines has yet been reported.

In the case of the anaerobic deaminases, Gale and Stephenson (43) showed by a study of the loss and recovery of activity towards *dl*-serine of washed suspensions of *E. coli* that the *dl*-serine deaminase probably requires a coenzyme. This postulated coenzyme appears to exist in a phosphorylated and non-phosphorylated form and in a reduced and an oxidized form, the phosphorylated and reduced form being active as codeaminase. Somewhat similar investigations of the activity of aspartase II of *E. coli* both in a cell-free condition and in the bacterial cell showed (38) that this deaminase requires a coenzyme which can be replaced *in vitro* by adenosine or inosine.

2. REMOVAL OF THE CARBOXYL GROUP: DECARBOXYLATION

Ellinger (32) and Ackermann (3, 4, 5) were the first to demonstrate the formation of various amines during bacterial putrefaction. Their method was to inoculate a synthetic medium containing salts, peptone, glucose and an amino acid, with decomposing pancreas and then, after an incubation of some weeks, to isolate the amine from the medium. In such a manner Ackermann demonstrated the formation of putrescine and δ-aminovaleric acid from arginine, and of histamine, cadaverine and γ-aminobutyric acid and β-alanine from their corresponding amino acids. This work is typical of the early investigations (1, 32, 33) showing the bacterial formation of amines without giving information concerning the processes, organisms or enzymes involved.

An advance was made by the use of pure strains of organisms growing in synthetic media. Thus Berthelot and Bertrand (16, 17) isolated a "Bacillus aminophilus intestinalis" (said to be

related to *Klebsiella pneumoniae*) which proved capable of forming histamine, tryptamine and tyramine. Strains of *E. coli* and *Proteus vulgaris* are reported to form tyramine from tyrosine (82) and isoamylamine from leucine (11). The formation of putrescine by *E. coli* growing in a medium containing arginine as sole source of nitrogen has been claimed by Akasi (10) and Hirai (56), while the formation of histamine has been the subject of many papers (55, 62, 63, 75). In all this work the general technique has been the inoculation of a synthetic medium containing inorganic salts, the amino acid in question and either glycerol or carbohydrate, with a pure strain of the organism. This is followed by a lengthy incubation and chemical isolation of the amine product. This method is usually not quantitative, and the long incubation may entail considerable variations in the enzyme make-up of the organism as the constitution of the medium alters.

Koessler and Hanke (65) studied the formation of histamine by a "colon bacillus" isolated from a case of cystitis and used an extraction method to remove histamine from the culture medium followed by colorimetric estimation. They showed that whenever the amine is produced, the medium first becomes distinctly acid and that "histamine is never produced except in the presence of an easily available source of carbon such as glycerol or glucose." This conclusion was later (46, 47, 48) found to apply to many strains of "colon bacilli" (isolated from feces) which were able to form histamine from histidine. Some strains of these organisms also produced tyramine when grown in a medium containing tyrosine, but it was found that the organisms which formed tyramine would not form histamine and *vice versa*. After an improvement of the method (31) of extraction of histamine from culture media, Eggerth (30) carried out a detailed investigation of the production of histamine by many strains of *E. coli*, *Salmonella spp.*, *Eberthella spp.*, *Aerobacter spp.*, etc. The organisms were grown in various determined media containing inorganic salts, glucose and, in some cases, asparagine or peptone to assist growth. In favorable conditions histamine production began within 24 hours and continued for 4 or 5 days. Experiments in which the pH of the medium was adjusted during growth

showed that histamine is produced most rapidly, in most cases, between pH 5.0 and 5.5 and, for most organisms, not at all at reactions more alkaline than pH 6.5. The work showed also that the temperature of incubation markedly affects the histamine formation, some organisms having an optimal temperature below 30° and others around 37°. The results reveal the large number of intestinal organisms capable of producing histamine; but as they deal with organisms growing in culture, they do not indicate what enzymatic processes are involved, under what conditions the amine-forming enzymes are produced by bacteria or what exact conditions are necessary for such enzymes to be active once formed. Attempts were made to produce histamine by washed suspensions but as the organisms were obtained from the surface of agar and buffered at pH 5.2, it is not surprising in view of more recent work (39) that the results were unsatisfactory,—as the organisms grown in this manner would contain little, if any, of the necessary histidine decarboxylase and also the pH of 5.2 is about 1 pH unit too alkaline for optimal activity. This will be dealt with below.

Virtanen *et al.* (93, 96) have shown the formation of β-alanine and γ-aminobutyric acid from aspartic and glutamic acids respectively by the root-nodule bacteria and have also shown the quantitative formation of cadaverine from lysine by *E. coli.* They have avoided the difficulty of interpreting experiments in which growth is occurring by using thick suspensions of the organism and incubating these in a non-nutrient medium containing phosphate and lysine only, estimating the cadaverine formed by isolation as the picrate.

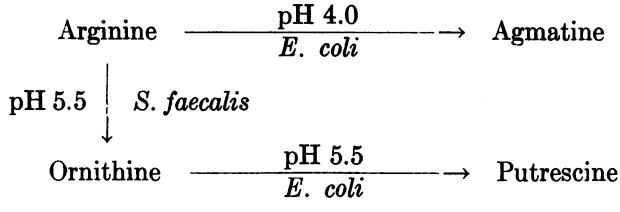
The first study of the enzymes involved in the formation of amines has been made by Gale (39) using washed suspensions of *E. coli* and investigating their power to decarboxylate amino acids by the Warburg manometric technique. Of 14 strains of *E. coli* investigated, 12 decarboxylated arginine to form agmatine; 12, histidine to histamine; 13, lysine to cadaverine; 12, ornithine to putrescine; and 9, glutamic acid to γ-aminobutyric acid. The decarboxylases involved are active over a restricted range of pH having optimal levels at the unusually acid values of 4.0 for arginine, histidine and glutamic acid, 4.5 for lysine and 5.0 for

ornithine. The enzymes proved very sensitive to temperatures above 20° to 25°, and washed suspensions grown at 27° proved markedly more active than those prepared from cultures grown at 37°. It is doubtful whether this thermolability is a property of the enzymes themselves or of the enzymes as they function in washed suspension, since the glutamic acid decarboxylase is far less thermolabile in *Proteus vulgaris* (42) and the histidine enzyme is formed better at 37° than at 27° in *Clostridium welchii* (42). At the optimum pH and at an experimental temperature of 30°, the washed suspensions of *E. coli* carry out a simple quantitative decarboxylation of the amino acids mentioned (without any coincident deamination) thus producing the corresponding amines which have been isolated and identified in each case.

The organisms contain only the decarboxylases if they are grown under certain specific conditions. The early work on growth experiments in which amines were produced had all been carried out in media containing fermentable carbohydrate or glycerol, so in this work the organisms were grown at first in 2% glucose broth without chalk so that the pH fell during incubation. Washed suspensions obtained from such cultures contained active amino acid decarboxylases of the type described above. By substitution of the glucose by other sugars, it was shown that the presence of glucose during growth has no specific effect but that any fermentable carbohydrate in the medium will give rise to a culture containing active decarboxylases. Further it was shown that the fermentable carbohydrate acts in this respect by acting as a source of acid and that the factor controlling the production of amino acid decarboxylases is the pH of the growth medium; bacteria grown at pH 5 in a non-carbohydrate medium having higher activities than those grown in a similar medium containing glucose. In all cases organisms grown at pH 7 in non-carbohydrate media have very little decarboxylase activity (hence the failure of Eggerth's experiment with washed suspensions (30)) and this activity is greatly increased (20 to 100-fold) by growing the organism at pH 5,—the lower the pH during growth, the greater the activity of the decarboxylases, within physiological limits.

The product obtained by the decarboxylation of arginine by

E. coli proved to be agmatine, whereas the amine previously reported as the product of bacterial putrefaction of arginine is putrescine (4, 10, 56). In those cases where mixed cultures have been used (4) it was suggested (39) that an organism was present which brought about the breakdown of arginine to ornithine before the decarboxylation occurred. Hills (53) has shown that many of the streptococci possess an enzyme capable of bringing about this reaction and Gale (41) has shown that *E. coli* and *Streptococcus faecalis*, when grown in symbiosis, attack arginine, according to the scheme:



Thus the two organisms grown in symbiosis attack arginine to produce either agmatine or putrescine according to the pH. The production of putrescine from arginine by *E. coli* alone has been reported (10, 56) but in these cases it is noticeable that the organism has been grown in a synthetic medium containing arginine as sole source of nitrogen so that, presumably, the organism has to elaborate some method of obtaining nitrogen from the amino acid for growth purposes before any decarboxylases are formed or can come into play.

The studies on decarboxylation by washed suspensions have been extended, up to the time of writing, to *Streptococcus faecalis* (40), *Proteus sp.* and *Clostridium spp.* (42). *S. faecalis* does not attack any of the amino acids decarboxylated by *E. coli* but washed suspensions grown in glucose broth decarboxylate tyrosine to tyramine at an optimum pH of 5.0, the enzyme responsible again being formed in response to acid growth conditions, particularly during the cofermentation of glucose. The enzyme is strictly specific as the washed suspensions decarboxylate no other amino acids tested (namely, phenylalanine, tryptophane, serine and alanine). Six out of seven strains tested possessed the

tyrosine-decarboxylase, the more saccharolytic strains having the greater activity. Of 16 coliform strains (39), 9 of the genus *Proteus* and 17 of the genus *Clostridium* (42) that were tested, none possessed the tyrosine-decarboxylase.

Of the 9 representatives of the genus *Proteus* (42) (which included *P. vulgaris*, *P. morganii*, the strains Kingsbury, HX2, HX19, etc.), 7 decarboxylated glutamic acid to γ-aminobutyric acid and 2 produced putrescine from arginine very slowly. The glutamic decarboxylase is similar in its properties to the enzyme in *E. coli* but is much less sensitive to temperature increase. It would seem that organisms of the genus *Proteus* cannot be considered as important amine producers.

Kendall and Schmidt (63) observed that when *Clostridium welchii* is grown in a carbohydrate medium, a non-specific toxic substance is produced in the medium which has the physiological reactions of histamine. Later (62) the substance was isolated from a bulk experiment and identified as histamine but apart from showing that this amine is produced only when the organisms are growing in the presence of carbohydrate, these workers did not obtain the detailed conditions for its production. Some 72 strains of *C. welchii* were investigated and histamine was found in the majority of cases. Eggerth (30) included 4 strains of *C. welchii* amongst the organisms he investigated and found all four to produce histamine when grown in the presence of salts, glucose, meat-extract, histidine etc. Gale (42) proceeded to investigate the power of washed suspensions of *C. welchii* to decarboxylate amino acids under the general conditions found satisfactory with other organisms (39, 41). Of 10 strains investigated, 9 decarboxylated histidine to histamine, and all decarboxylated glutamic acid. The latter decarboxylase again is similar in its properties to the enzyme in *E. coli* but the histidine-decarboxylase differs in several ways from the histidine enzyme in coliform organisms. In the first place, it has a pH optimum of between 2.5 and 3.0 compared with 4.0 in coliform bacteria. Why this should be is not clear. In the coliform organisms, the pH-activity curve for histidine-decarboxylase falls steeply on the acid side of 4.0 and the enzyme is almost inactive at pH 3.5;

and it has been suggested that this is due to denaturation of the enzyme protein, and, in actual practice, it is often found that the organisms coagulate below pH 4.0. This is not the case with *C. welchii* where coagulation of the organisms does not take place even at pH 1 and the pH-activity curve for the histidine-decarboxylase is approximately symmetrical. Thus the difference in the apparent properties of the two enzymes may be due to the greater resistance to denaturation of the proteins in *C. welchii* over those of coliform organisms. (See dotted curves, fig. 2.)

As a result of the lower pH optimum of the *C. welchii* histidine-decarboxylase, this enzyme requires more acid conditions for its formation than are necessary with *E. coli*. Thus in glucose broth, the histidine enzyme only appears late in the growth period when the general medium pH has fallen below 5. When the organism is grown in non-carbohydrate media, it is found as before that the lower the growth pH, the more active the decarboxylases, but in the case of the histidine enzyme the activity of a culture grown at pH 5 is $Q_{CO_2} = 10$ compared with a figure of 60–70 for the same organism grown in the presence of glucose. It is not possible to obtain cultures from non-carbohydrate media at pH values much below 5 and it appears that, in the case of this organism, histamine is only produced rapidly *in vivo* during the cofermentation of carbohydrate as it is only by this means that a pH sufficiently low for the formation of the enzyme during growth can be obtained. It is probable that a very saccharaolytic organism like *C. welchii* may, during the fermentation of sugar, especially when in a heterogeneous medium such as an infected muscle, produce a pH in its immediate vicinity below that of the medium in general. Washed suspensions of *C. welchii* are, of course, fully active in the absence of sugar, but it is unlikely that much histamine will be formed in infective conditions unless the organism has the opportunity to ferment carbohydrate. This is of importance in gas gangrene as *C. welchii* ferments muscle glycogen with the production of acid and gas, and washed suspensions obtained from cultures grown in the presence of glycogen are active histamine-producers. The fermentation of glycogen is strictly adaptive.

Table 2 shows the distribution of the amino acid decarboxylases

in organisms that have been investigated so far by the washed suspension technique. In each case a representative organism has been chosen; but it has been shown that there are marked strain differences, as, for example, a representative *E. coli* possesses decarboxylases for arginine, ornithine, lysine, histidine and glutamic acid but some strains of coliform organisms possess only one or two of these enzymes when grown under the appropriate conditions. In all the work discussed, values of Q_{CO_2} (= μl. CO_2 liberated from the specific substrate per hour per mg. dry weight of organism) less than 1 have been regarded as insignificant so that certain enzymes of very weak activity may have

TABLE 2

Distribution of amino acid decarboxylases

ORGANISM	ENZYME-SUBSTRATE					
	Arginine	Ornithine	Histidine	Lysine	Tyrosine	Glutamic acid
Escherichia coli	+	+	+	+	−	+
Klebsiella pneumoniae	−	+	+	+	−	−
Streptococcus faecalis	−	−	−	−	+	−
Proteus vulgaris	−	+	−	−	−	+
Proteus morganii	−	+	−	−	−	+
Clostridium welchii	−	−	+	−	−	+
Clostridium sporogenes	−	−	−	−	−	−
Clostridium septicum	−	+	−	−	−	−
Clostridium aerofoetidum	−	−	−	−	+	+

Presence of activity is indicated by +.

been disregarded, when their presence might show up in prolonged growth experiments. Thus strains of *E. coli* and *Proteus vulgaris* have been reported to produce tyramine from tyrosine (82) and isoamylamine from leucine (11) whereas no sign of such activity was found in work with washed suspensions of these organisms (39, 42). It is also possible, of course, that these enzymes are confined to strains which were not represented in the work with washed suspensions. Thus the enzymes shown in table 2 cannot be taken to represent the maximum decarboxylating powers of the organisms concerned.

The distribution of the decarboxylases indicates that each enzyme is specific for the decarboxylation of one amino acid and

probably (39, 41) for the amino acid molecule itself. A surprising thing is the frequency of the occurrence of the glutamic acid decarboxylase which has been found in the majority of strains of *E. coli*, *Proteus spp.*, *C. welchii* and *C. aerofoetidum* investigated; moreover it is usually present in a very active state (42), and it would seem that the metabolism of γ-aminobutyric acid in bacteria should repay study. The enzymes are not produced if growth occurs in an aminoacid-free medium (39, 41).

Factors influencing bacterial decarboxylation

Gale (39, 41, 42) showed in the course of the above investigations that (a) the decarboxylase activity of a washed suspension towards any particular amino acid varies with the "age of the culture" from which the washed suspension is prepared. Thus young cultures have little activity, the activity increasing during active cell division and reaching a maximum as growth ceases. The case of the histidine-decarboxylase of *C. welchii* differs from the usual type because, when growing in the presence of carbohydrate, the enzyme does not appear until late in the growth period when the pH of the medium has fallen below 5 (42). (b) The presence of oxygen during the decarboxylation by washed suspensions has no effect on the reaction, with the exception of the glutamic acid decarboxylase of *C. welchii* which is partially inhibited by the presence of oxygen (42). (c) There is evidence that the decarboxylases require a coenzyme or coenzymes for their action (39) but these have not been identified although they cannot be replaced by aneurin diphosphate (cocarboxylase).

The effect of growth conditions on the production of the enzymes has been discussed above. In every case studied, the decarboxylases are formed in response to acid growth conditions, the lower the pH during growth, the more active the washed suspensions obtained. Since the optimum pH of these enzymes is below 5.5 in every case and below 4.5 in most cases, it is probable that the amino acid can only be attacked when it possesses an undissociated carboxyl group. As the pH of the growth medium is lowered, the proportion of the amino acids in the undissociated acid state will increase and thus the response of the organism in producing more enzyme may be a type of adaptation. Pointing

out that decarboxylation of amino acids results in the formation of alkaline material, Hanke and Koessler (47) wrote "the production of amines from amino-acids seems to be a protective mecha-

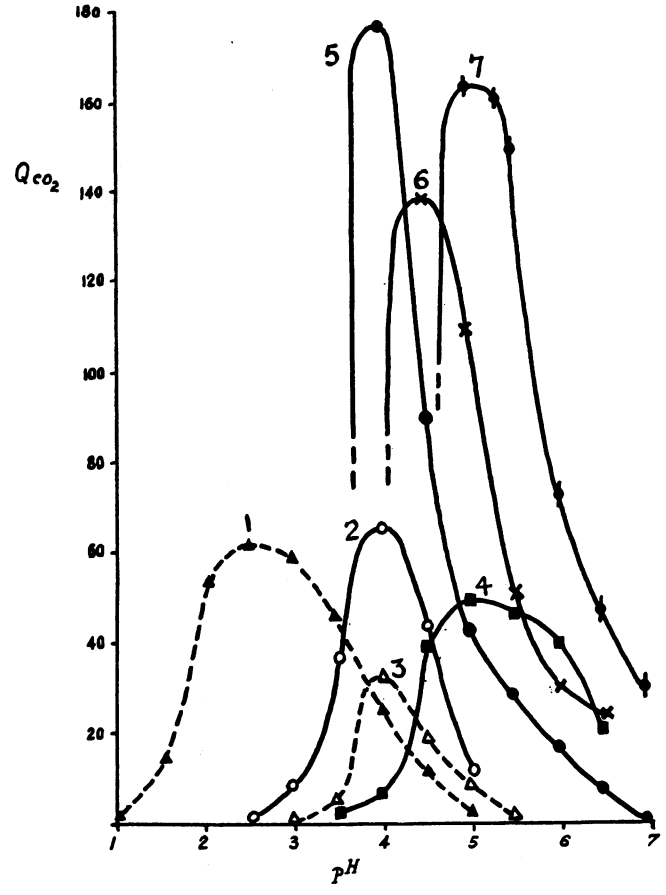


FIG. 2. VARIATION WITH pH OF THE ACTIVITY OF VARIOUS AMINO ACID DECARBOXYLASES IN WASHED SUSPENSIONS OF BACTERIA

1. Histidine-decarboxylase of *Clostridium welchii* (42). 2. Glutamic acid-decarboxylase of *Escherichia coli* (39). 3. Histidine-decarboxylase of *E. coli* (39). 4. Ornithine-decarboxylase of *E. coli* (39). 5. Arginine-decarboxylase of *E. coli* (39). 6. Lysine-decarboxylase of *E. coli* (39). 7. Tyrosine-decarboxylase of *Streptococcus faecalis* (40).

nism and is resorted to when the accumulation of H ions within the organism's protoplasm is incompatible with its normal life processes. The amines can thus be thought of as reaction

buffers." From a teleological point of view, it is difficult to assign any function to the amino acid decarboxylases at present although it must be remembered that when the reaction becomes acid, amino acids can no longer be attacked by deamination for the deaminases are inactivated and, at pH values lower than 5, carbohydrate itself is but slowly attacked, and the production of decarboxylases may be the method by which the organism extends its range of existence, utilizing amino acid decarboxylation when other substrates and methods of attack are no longer available. A further suggestion is that the decarboxylases may serve the purpose of providing carbon dioxide, which is essential for the growth of many bacteria (45), under conditions too acid for sufficient gas to be retained in normal solution in the medium.

3. DEAMINATION ACCOMPANIED BY DECARBOXYLATION

Figure 3 shows the variation with pH of the activity of glutamic acid deaminase (87) and glutamic acid decarboxylase (39) of *E. coli*. It can be seen that not only are the optimum pH values for these two enzymes of the same organism widely separated and on opposite sides of neutrality but that neither enzyme is at all active when the other is optimally active. Further, the decarboxylase is active over such a restricted range that there is no pH at which both enzymes are effectively active together. It is not possible to say whether this case, the only one for which complete data are available at present, is representative of deaminases and decarboxylases in general, although it is significant that all the deaminases so far studied are optimally active at pH 7.5 to 8.0 while all the decarboxylases have optimal pH values at or below 5.0. This may mean that for decarboxylation to occur, the —COOH group must be undissociated and, for deamination, the $-NH_3^+$ group. Hence it is probable that bacteria cannot attack amino acids by both deamination and decarboxylation *simultaneously*. Thus there is no record of alcohols (which would be formed by coincident decarboxylation and hydrolytic deamination) being produced from amino acids by bacteria, although a series of papers by Ehrlich between 1905 and 1912 has shown the production of such by yeast in culture. In culture experiments which last over several days and in which

considerable changes of pH may occur due to the metabolic activities of the growing organisms, products of deamination and decarboxylation might be expected as a result of the two processes occurring at separate times during the incubation. Thus Brasch (19, 20) has reported the isolation of propionic acid from a culture of *Clostridium putrificum* growing in a medium containing aspartic acid: similarly he has obtained butyric acid from glutamic acid. It should be of interest to investigate the action of washed suspensions of yeast upon amino acids at various pH

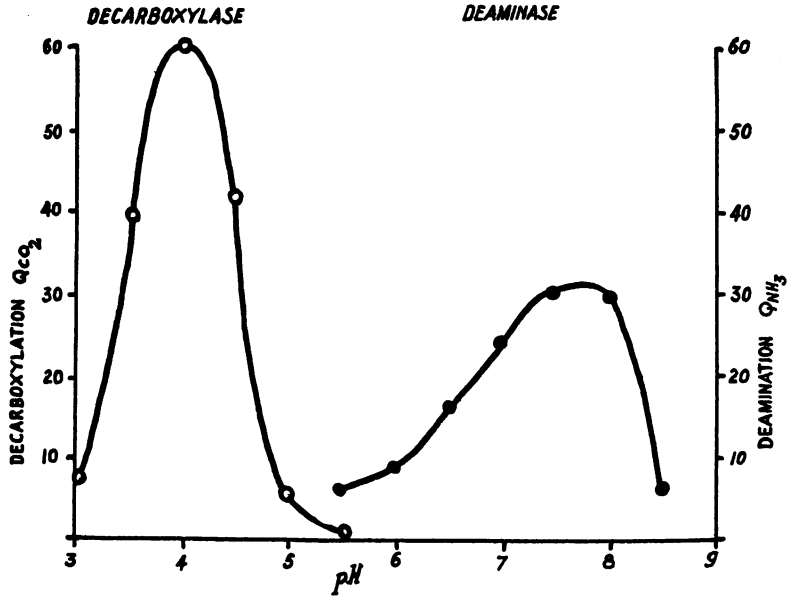


FIG. 3. VARIATION WITH pH OF THE ACTIVITIES OF THE ENZYMES OF ESCHERICHIA COLI WHICH ATTACK *l*-GLUTAMIC ACID (39, 87)

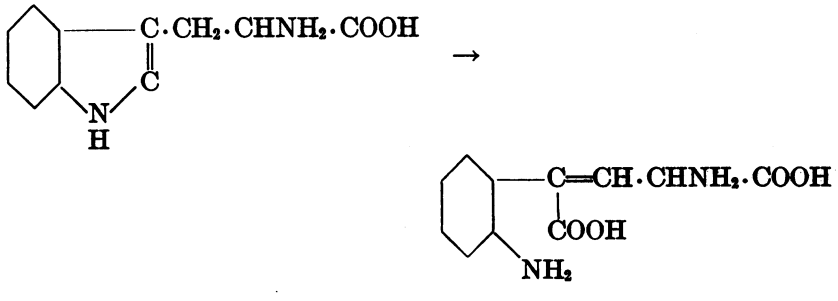
values, to determine whether the results obtained by Ehrlich are due to simultaneous deamination and decarboxylation by enzymes of different properties from those found in bacteria, or whether the alcohols are produced in two stages as the growth medium reaction alters.

4. SPLITTING OF THE AMINO ACID MOLECULE

Few cases of such a reaction by bacterial enzymes have been reported up to the present. Ackermann (2) showed that mixed

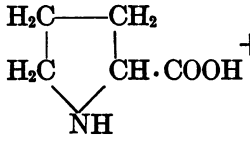
cultures of organisms will produce ornithine when growing in a medium containing arginine. Hills (53) has shown that this reaction is carried out by certain streptococci with the production of ammonium carbonate in addition to ornithine. He was unable to show the intermediate formation of citrulline, urea, etc., and the precise mechanism of the reaction is still obscure. The same change is carried out by some strains of *Lactobacillus acidophilus* in washed suspension (40) the optimum pH for the reaction being around 6.0. Again urea is not an intermediate substance in the removal of the guanidine nucleus. Ackermann (6) has also shown the formation of citrulline from arginine by mixed cultures and Horn (59) has obtained this reaction by growing *Pseudomonas aeruginosa* in a determined medium containing arginine as sole source of nitrogen. The yield was small.

It has been mentioned above that no attempt to identify an intermediate substance in the production of indole from tryptophane has met with success and it may be that this is also a case in which the amino acid molecule is split (into indole and alanine?) as a preliminary to further breakdown. Such a breakdown consisting of "reductive fission" of the tryptophane molecule has been postulated by Baker and Happold (13) although there is no experimental evidence in support of such a change as yet. A further type of attack on the tryptophane molecule that might be classed under this heading has been shown by Kotake (67): *Bacillus subtilis* produces kynurenic acid, kynurenine and anthranilic acid from tryptophane in the presence of glycerol and aluminum phosphate. It is suggested that the first step in this breakdown is the production of kynurenine from tryptophane:



followed by disruption of the kynurenine along two paths to kynurenic acid and anthranilic acid.

Stickland (89) has also shown that when *l*-proline is reduced by *l*-alanine in the presence of washed suspensions of *C. sporogenes*, the ring of the proline molecule is split to produce δ-aminovaleric acid:



$$+ CH_3 \cdot CHNH_2 \cdot COOH + H_2O \rightarrow$$

$$CH_2NH_2 \cdot (CH_2)_3 \cdot COOH + CH_3 \cdot CO \cdot COOH + NH_3$$

after which occurs the second step:

$$CH_3 \cdot CO \cdot COOH + \tfrac{1}{2} O_2 \rightarrow CH_3COOH + CO_2$$

Anabolic Changes Involving Amino Acids

While our knowledge of the catabolic changes involving amino acids is considerable, as this review has shown, and is reaching a stage when the enzymes concerned can be studied with a fair degree of exactness, this cannot be said to be the case with anabolic changes. Leaving aside the discussion of growth factors, it is true to say that some organisms such as certain strains of *E. coli* can grow in a non-amino-acid synthetic medium containing ammonia whilst others such as *Staphylococcus aureus* cannot. In the former case the organism must be able to synthesize the amino acids of its protoplasm from carbon compounds and ammonia. In many such cases the addition of amino acids hastens the rate of growth and it is generally assumed that this is due to the fact that certain of the amino acids can only be synthesized slowly by the organism so that the rate of synthesis restricts the rate of growth, with the consequence that addition of these amino acids speeds up the growth. When such an amino acid is added to the simple growth medium, it is not clear whether it is first deaminated and then rebuilt from its breakdown products or whether it is directly assimilated by the organism without such intermediate breakdown. It has been shown (38, 43) that some of the deaminases, at least, are almost

inactive in very young cultures and this may be a factor contributing to the economy of the cell during its actively anabolic stage. In those cases where the organism cannot grow on a non-amino-acid medium, this is presumably due to its inability to synthesize some "essential" amino acid from simple substances. Thus some strains of *Eberthella typhosa* and *S. aureus* cannot grow in a simple medium unless tryptophane is added (36). Fildes (34) suggests that the loss of such a synthetic function is brought about by continued growth of the organism in such an environment that the essential factor is supplied and the need to synthesize it does not arise. In this connection, Fildes and his coworkers (36) showed that a strain of *E. typhosa* which would not grow in a non-amino-acid medium without the addition of tryptophane, could be "trained" to dispense with the tryptophane by continued subcultivation into media containing progressively smaller and smaller amounts of the amino acid. If Fildes' (34) theory is correct it should be possible to demonstrate the reverse of this training process: the loss of synthetic power by an organism growing in the presence of the synthesized material.

It is generally assumed that synthesis of amino acids is accomplished by reversal of the enzymes involved in amino acid breakdown. Of all such enzymes studied, two have so far been shown to possess the property of reversal: the *l*-glutamic acid dehydrogenase studied by Adler *et al.* (9) and the aspartase studied by Quastel and Woolf (78). In animal tissues, Braunstein and Kritzmann (21, 22) have demonstrated a transamination whereby the amino group of either of the natural dicarboxylic amino acids can be transferred to a monocarboxylic keto acid with the formation of the dicarboxylic keto acid and a monocarboxylic amino acid:

$$\begin{matrix}\mathrm{CHNH_2\cdot COOH}\\ |\\ \mathrm{CH_2\cdot COOH}\end{matrix} + \mathrm{R\cdot CO\cdot COOH} \rightleftharpoons \begin{matrix}\mathrm{CO\cdot COOH}\\ |\\ \mathrm{CH_2\cdot COOH}\end{matrix} + \mathrm{R\cdot CHNH_2\cdot COOH}$$

Thus, once either of the dicarboxylic amino acids has been synthesized, then the way is open to the formation of other amino

acids once their corresponding keto acids have also been synthesized. The transamination has yet to be demonstrated in bacteria.

A further method for the synthesis of aspartic acid has been shown by Virtanen and Laine (94, 95) for the symbiotic genus *Rhizobium*. In this case, it is claimed that the organism fixes atmospheric nitrogen with the formation of hydroxylamine which then combines with oxaloacetic acid, produced by the host-plant from carbohydrate, to form aspartic acid:

$$NH_2OH + HOOC \cdot CH_2 \cdot CO \cdot COOH \rightarrow HOOC \cdot CH_2 \cdot CHNH_2 \cdot COOH$$

Virtanen and Laine (94) claim to have further demonstrated the transfer of the amino group of the aspartic acid to pyruvic acid to form alanine in the presence of the host-pea.

Summary

An account is given of our knowledge of bacterial metabolism of amino acids with especial emphasis laid on the enzymes involved in the primary breakdown of the amino acid molecule. Bacterial enzymes can attack amino acids by (*a*) removal of the α-amino-group (*b*) removal of the terminal carboxyl group (*c*) by combined deamination and decarboxylation or (*d*) splitting the molecule into simpler molecules. The third alternative probably does not occur in practice.

Amino acids may be deaminated in a number of ways. Experiments with washed suspensions of bacteria and with cell-free enzymes isolated therefrom show that the process can be carried out by (*a*) oxidation or dehydrogenation (*b*) desaturation (*c*) hydrolysis (*d*) reduction of the amino acid molecule in specific cases. Also certain strict anaerobes carry out a process of mutual oxidation-reduction between pairs of amino acids that results in their deamination. The deaminases all appear to be optimally active between pH 7.5–8.5. The deaminase activity of bacterial cells varies with the age of the culture from which the cells are taken, the composition of the growth medium, and the degree of anaerobiosis during growth. Co-deaminases have been described in certain cases.

Amino acids may be decarboxylated to give amines under acid

conditions, the specific decarboxylases being optimally active at or below pH 5.0. Such decarboxylases are only formed when the organisms are grown under acid conditions and the decarboxylase activities of the cells vary under varying conditions of growth.

A few isolated cases have been described of amino acids being split into simpler molecules before undergoing further degradation.

Little is known of the way in which bacteria synthesize amino acids although the deaminases of the two dicarboxylic amino acids are known to be reversible and it is possible that the synthesis is accomplished by the production of aspartic and glutamic acids followed by transfer of the amino group from these to keto acids with the formation of other amino acids.

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