LXXVII. THE CHEMISTRY OF AMINO-ACID DEAMINATION.

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THE final phase of amino-acid metabolism, involving the removal of nitrogen from the amino-group and the production of urea, is generally regarded as involving two distinct processes: (1) deamination, with the liberation of ammonia, (2) dehydration of ammonium carbonate, with formation of urea.

The change being represented as:

Now, while there is no doubt as to the general character of the transformation, the chemical changes involved in each intermediate process are somewhat obscure. The present paper is concerned chiefly with the first process, but it will be seen that the conclusions arrived at afford an indication as to the mechanism of the formation of urea from ammonium salts.

DEAMINATION BY α -OXIDATION.

Neubauer [1908] has indicated the three most direct methods by which an amino-acid may undergo deamination. These are: (1) oxidation, with formation of the corresponding α -ketonic acid; (2) reduction, with formation of the corresponding saturated acid; (3) hydrolysis, with formation of an α -hydroxy-acid.

Chemical and physiological evidence, as summarised by Dakin [1922], supports strongly the belief that in the animal organism deamination is chiefly, if not entirely, brought about by oxidation; and such observations as those of Fiske and Karsner [1914], and Jansen [1915] that aeration of the perfusion fluids is necessary for the removal of ammonia and amino-acids is of interest in this connection. Quite apart from the subject of protein metabolism, there is ample proof that when certain amino-acids are oxidised in neutral or alkaline solution the amino-groups ultimately appear as ammonia.

If the group $-NH_2$ be assumed to be converted directly into NH_3 , the general difficulty arises as to the liberation of ammonia by oxidation in alkaline solution. Ammonium salts are very resistant towards oxidation when in strongly acid solutions, as, for example, in the familiar Kjeldahl process.

In alkaline solution, and in presence of carbon compounds, ammonia readily undergoes oxidation, as emphasised by the extensive investigations of Fosse [1919, 1920, 1921, 1922]. This difficulty of accounting for the production of ammonia by oxidation has already been raised by Werner [1923] in connection with the origin of urea in the animal body, and has been anticipated by Lippich [1908] and by Dakin [1909], both of whom have considered the possibility of deamination without liberation of ammonia, but with the formation of intermediate uramido-acids.

As Fiske and Sumner [1914] have observed: "It cannot be stated with certainty that ammonia is an obligatory intermediate product in the deamination in mammals."

A second and more special difficulty arises in connection with the theory of α -deamination. It is necessary to account for the removal of the amino-group by oxidation without breaking the carbon chain. Knoop [1910] has suggested that this is accomplished through the formation of a hypothetical intermediate body, a hydrated amino-acid, but this suggestion does not meet the general difficulty of accounting for the direct formation of ammonia by oxidation.

The formation of cyanic acid by oxidation.

Cyanic acid is so intimately associated with urea and its various syntheses outside the organism that Werner [1923] has been led to conclude that the mere occurrence of urea in the animal body implies the formation of cyanic acid at some stage in protein metabolism, which is supported by the detection of cyanic acid in dog's blood by Nicloux and Welter [1922].

Cyanic acid has been identified by Fosse [1921] during the oxidation of a great variety of organic nitrogen compounds, and appears to be of much more general occurrence than is at present realised. The discovery by Walters and Wise [1917] of cyanuric acid, the polymer of cyanic acid, in soil may be noted in this connection.

With the object of investigating the possible relationship between deamination by oxidation and cyanic acid formation, the behaviour of four aliphatic amino-acids on oxidation in alkaline solution was studied. The results obtained support the view that deamination may be brought about by the intermediate formation of an unstable cyanate.

Conditions of deamination.

The conditions which may be expected to determine the course of deamination both inside and apart from the organism are as follows:

1. *The nature and concentration of the amino-acid.*
2. *The reaction of the solution.* In acid solutions cyanic acid is very readily hydrolysed. For purposes of investigation many of the solutions were kept in the region of p_H 8 to approximate to conditions of the animal body and to stabilise any cyanic acid formed by fixing it as a cyanate.

3. *The oxidation mechanism.* It is here that the reproduction of biochemical oxidations presents the greatest difficulty. The important part played by the tissues is very hard to define and to imitate. Dakin [1922] has summarised the reasons for considering that oxidation by hydrogen peroxide most closely resembles the oxidations effected by the organism.

Accordingly, hydrogen peroxide was first employed in the present investigation. In strengths from 3 to 20 %, in varying proportions, with and without a catalyst, the action was found to be feeble at room-temperature and at 45°. The results obtained by Dakin [1906, 1] are probably due to his procedure of distillation in presence of undecomposed peroxide, a method which is not suitable for a solution which is later to be tested for cyanate. For these reasons, potassium permanganate was employed in the later experiments.

4. *The presence of glucose.* It is worthy of note that in the organism deamination occurs in presence of other substances which may affect the course of the reaction. Glucose, the normal constituent of blood, appears to be the most important of these possible accessories. By the addition of an equimolecular portion of glucose to glycine undergoing oxidative deamination the production of cyanate was increased ten times and the liberation of free ammonia was reduced from 48 % of the theoretical to 13 %.

Experimental.

Glycine (Kahlbaum), alanine (Kahlbaum), leucine, and sarcosine (Merck), were oxidised at room-temperature, 30° and 45°, in $M/10$ and $M/50$ concentrations, by means of neutral hydrogen peroxide, 3 % and 20 %, with a trace of ferrous sulphate, and by potassium permanganate, in N alkali or in $N/15$ disodium phosphate. The periods of oxidation were 24 hours, and for leucine 48 hours.

The excess of oxidising agent was then removed, and the extent of deamination determined by aspiration of the solution. The solution was then neutralised, and divided into equal portions. In one the urea was determined by evaporating to dryness, extracting with alcohol, and applying the xanthydril reagent to the concentrated alcoholic extract according to the method of Fosse [1907].

The cyanic acid in the second portion was determined by incubation overnight with 1 g. of ammonium chloride, which interacted with the cyanate present to form urea; this was found to be more satisfactory than the method first adopted of evaporating the second portion of the extract together with the ammonium chloride to dryness on the water-bath, which led to loss of some of the cyanate by hydrolysis.

The urea formed from the cyanate was determined quantitatively by the xanthydril method; the difference between the two urea values gave the amount of cyanate present in the solution.

The identity of the precipitate of dioxanthylurea was established by microscopic appearance and melting-point in the vapour of diphenyl ether.

Control experiments showed that the amino-acids produced no urea or cyanate under the above conditions in the absence of oxidising agents.

It is necessary to free the solution completely from any unchanged peroxide or permanganate before applying the xanthydril test, otherwise a precipitate of xanthone is formed which obscures the reaction.

Table I. *Glycine oxidation. 24 hours.*

	Weight of glycine g.	Oxidiser	Temperature	Ammonia	Cyanic acid	Urea
				(percentage of theoretical)		
1	0.75	H ₂ O ₂ 20 cc. = 32 g. O	Air	1.6	Present	0
2	"	H ₂ O ₂ 40 cc. = 64 g. O	45°	4.5	"	Present
3	"	KMnO ₄ 1 g., 0.1 N NaOH 20 cc.	Air	3.4	"	"
4	"	KMnO ₄ 1 g., N NaOH 20 cc.	"	3.4	0.13	"
5	"	KMnO ₄ 1.5 g., N NaOH 20 cc.	"	—	0.95	"
6	"	KMnO ₄ 1.5 g., N NaOH 20 cc.	45°	48.2	1.4	"
7	0.15	KMnO ₄ 0.36 g., 0.1 N KOH 20 cc.	"	14.0	2.6	—
8	0.15 + 0.036 glucose.	KMnO ₄ 0.36 g., 0.1 N KOH 20 cc.	"	6.5	6.6	Present
9	"	KMnO ₄ 0.6 g., 0.2 N KOH 20 cc.	"	13.0	10.5	"

THE SIGNIFICANCE OF CYANIC ACID IN DEAMINATION.

The cyanic acid identified in the experiments here recorded may arise directly by a process of oxidative deamination, or as a secondary oxidation (in presence of a hydrocarbon residue) of the ammonia liberated by deamination. That this latter possibility must not be ignored is shown by the observations of Fosse [1919] who obtained a 70 % yield of urea by the oxidation of small quantities of glucose in presence of excess of ammonia, and who also detected cyanic acid as the intermediate product. That a secondary reaction completely accounts for the cyanate produced when glycine is oxidised does not appear likely, since cyanate has been detected during the mild oxidation of glycine when the ammonia liberated was only 1½ % of the theoretical value for complete deamination.

Furthermore, in a solution of glycine which was slowly oxidised at 45° for a period of 48 hours, the resulting rise in ammonia was not accompanied by a corresponding rise in cyanate, which suggests that the ammonia is produced by the continuous hydrolysis of the cyanate at a rate depending on the conditions of the experiment.

A formation of cyanate from ammonia by oxidation in presence of a hydrocarbon residue is not necessarily impossible in the present experiments, but by itself it is inadequate to explain the mechanism of deamination, although it may account for one of the methods whereby ammonia is removed from the circulating blood.

To explain a formation of cyanic acid by oxidative deamination without rupture of the carbon chain, two possibilities must be considered, namely: oxidation of the amino-acid when in the cyclic form, or oxidation of the amino-acid in presence of another carbon compound.

In neutral solution, amino-acids may assume a closed-chain structure:

Such a configuration might conceivably undergo direct oxidation to form cyanic acid and the aldehyde corresponding to the amino-acid:

This was investigated experimentally by oxidising glycine in almost neutral solution and in definitely alkaline solution, under which latter condition the amino-acid would exist entirely in the open-chain or salt form:

The yield of cyanate was greater in this condition, which indicates that the cyclic configuration is not essential for the production of cyanic acid.

To determine the influence of the presence of other carbon compounds, glycine was oxidised together with glucose, and also with formaldehyde in equimolecular proportions. In each case the yield of cyanic acid was greatly increased, rising from 1.4 % of the theoretical to 10.5 %.

This can be most readily explained by a union between the aldehyde and the amino-group previous to oxidation:

The oxidation of such a methylene-amino-acid would account for the production of a ketonic acid by oxidative deamination. Further support for this view was obtained by the oxidation of sarcosine, which gave a greater yield of cyanic acid than did glycine under similar conditions, showing that the oxidation had attacked the linkage between the nitrogen and the asymmetric carbon of the substituted amino-acid. This is in agreement with Dakin's observation [1906, 2] that methylamine is formed during the oxidation of sarcosine.

Table II. *Sarcosine oxidation. 24 hours.*

	Weight of sarcosine g	Oxidiser	Tem- perature	Ammonia	Cyanic acid	Urea
				(percentage of theoretical)		
1	0.178	KMnO ₄ 0.6 g., 0.2 N KOH 20 cc.	30°	21.5	3.0	—
2	0.178 + 0.036 (glucose)	" "	"	8.0	2.5	0

Table III. *Alanine oxidation. 24 hours.*

	Weight of alanine g.	Oxidiser	Tem- perature	Ammonia	Cyanic acid	Urea
				(percentage of theoretical)		
1	0.89	H ₂ O ₂ 20 cc. = 0.16 g. O	Air	1.5	Present	Present
2	0.178	KMnO ₄ 0.75 g., 0.1 N KOH 20 cc.	30°	19.0	0	0
3	0.178 + 0.036 (glucose)	KMnO ₄ 0.75 g., 0.1 N KOH 20 cc.	"	27.5	0	0
4	0.178 + 0.16 (CH ₂ O)	KMnO ₄ 1.2 g., 0.1 N KOH 20 cc.	"	—	1.0	0

Table IV. *Leucine oxidation. 49 hours. Temperature 30°.*

	Weight of leucine g.	Oxidiser	Ammonia	Cyanic acid	Urea
			(percentage of theoretical)		
1	0.131	KMnO ₄ 0.6 g., 0.2 N KOH 20 cc.	98	0	0
2	0.131 + 0.036 (glucose)	" "	28	0	0

Oxidation of alanine and leucine.

When oxidised with hydrogen peroxide under the conditions described, alanine is only slightly attacked at air temperature, a yield of ammonia corresponding to 1.5 % of the theoretical being obtained after 24 hours. Cyanic acid and urea were both detected in the solution. On oxidising alanine with alkaline permanganate at 45°, the yield of ammonia was increased to about 20 %, as compared with 48 % in the case of glycine under the same conditions. When the alanine was oxidised at the higher temperature, neither cyanic acid nor urea was detected in the solutions, which may be due to alanine undergoing a form of deamination different from that of glycine, although addition of formaldehyde or formic acid to the alanine before oxidation led to a production of cyanic acid.

Cyanic acid has not yet been detected during the oxidative deamination of leucine.

Whether these results may be taken to indicate that, in general, glycine undergoes a form of deamination distinct from that of alanine or leucine, or whether they are due to the experimental difficulties of correlating the conditions of effective oxidation with those favourable for the detection of the unstable cyanic acid can best be determined by perfusion experiments. For the present, all one can conclude is that the oxidative deamination of glycine and sarcosine involves the intermediate formation of cyanic acid.

THE SIGNIFICANCE OF CYANIC ACID IN THE ORGANISM.

The formation of cyanic acid as an intermediate stage in deamination not only offers an explanation of the mechanism of deamination but has several very important possibilities in connection with the origin of urea.

Cyanic acid is stable towards oxidising agents, and once formed is unlikely to undergo further oxidative changes. It is, however, very reactive in two ways of great biological significance. It may undergo hydrolysis to produce ammonia and carbon dioxide, or it may unite with ammonia to form urea.

In presence of acids, cyanic acid is completely hydrolysed to ammonia:

In aqueous solution alone, cyanic acid is in part hydrolysed to ammonia and carbon dioxide, and in part converted into urea by union with ammonia [Werner and Fearon, 1920]:

In presence of excess of ammonia, cyanic acid is ultimately completely converted into urea:

This unique property of cyanic acid, producing either a base or a neutral compound according to conditions of hydrolysis, suggests at once a more precise explanation of one of the factors for the maintenance of the neutrality of the organism. Thus may be explained the low concentration of ammonia in normal blood and the production of ammonia in conditions of acidosis, and also the formation of urea when a surviving liver is perfused with solutions of ammonium salts, as in the well-known experiments of Schröder.

CONCLUSIONS.

1. Cyanic acid is formed during the oxidative deamination of glycine, sarcosine, and alanine.
2. The α -deamination of glycine involves the intermediate formation of cyanic acid.
3. Cyanic acid must be considered as an important factor in the production of urea and in the maintenance of neutrality of the organism.

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