ON THE FORMATION OF LACTIC ACID AND CARBONIC ACID DURING MUSCULAR CONTRACTION AND RIGOR MORTIS

By P. W. LATHAM, M.D., *Downing Professor of Medicine in the University of Cambridge* (1874-1894).

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When living muscular tissue is at rest its reaction to test paper is faintly alkaline or neutral. When it contracts, and during the period of rigor mortis, carbonic acid is set free, and the reaction of the muscle to test paper becomes distinctly acid. This acidity is supposed to result from the liberation of lactic acid; but in what way this acid and carbonic acid are produced, or from what proteid constituent they arise has not hitherto been determined.

Carbonic acid cannot be directly combined with lactic acid $\text{CH}_3 \cdot \text{CH}(\text{OH}) \cdot \text{COOH}$; the synthesis, however, may be effected by adopting the following method.

Pour ten grammes of pyruvic acid $\text{CH}_3 \cdot \text{CO} \cdot \text{COOH}$ very slowly and gently over some finely powdered potassium cyanide, then add slowly 12 c.c. of concentrated hydrochloric acid; pour the whole into a flask containing a hot solution of twenty grammes of barium hydrate in 250 c.c. of water; attach a reversible condenser and boil for two hours, neglecting any immediate precipitate which may be formed. After cooling collect the white crystalline salt on a filter. This salt, which is barium methyl tartronate, when dried at 100°C ., weighs about 16 grammes and contains only a very small quantity of barium carbonate.¹

The following is the reaction :—

1. C. Böttinger, *Berichte d. deutsch. chem. Gesellsch.*, Bd. XVII, s. 144.

On carefully adding to the barium methyl-tartronate the molecular equivalent of dilute sulphuric acid, methyl-tartronic acid, $\text{CH}_3\cdot\text{C}(\text{OH})\cdot(\text{COOH})_2$ or iso-malic acid is liberated, and on evaporating the solution, rhombohedral crystals are obtained similar in appearance to those of Iceland spar.¹

On heating methyl tartronic acid with hydrochloric acid it is resolved into carbonic acid and fermentation or α lactic acid ;

Methyl-tartronic acid, however, is not one of the constituents of proteid matter, but I suggest that its antecedents or generators are ; and I shall endeavour to show that this hypothesis is true by considering :—

First, what products are formed in plants in the earliest stages in the synthesis of organic matter, and

Secondly, what products are furnished by the hydrolytic decomposition of proteid matter.

THE GENESIS OF ORGANIC MATTER IN PLANTS

The view which is now most generally accepted is that in plants CO_2 entering by the leaves combines with H_2O absorbed by the roots and from these glucose is said to be formed, a volume of oxygen equal to that of the CO_2 absorbed being exhaled by the plant ; the initial product being formaldehyde. This hypothesis was first advanced by A. von Baeyer.² MM. Loew and Bokorny and Pringsheim have shown that in living plasma there is a substance which has the property of reducing silver salts, and for this reason is regarded as an aldehyde ; the aldehyde resulting from the combination in the plant of CO_2 and H_2O . Pringsheim gives the following as the reaction :—

1. Böttinger, *loc. cit.*, p. 148.

2. Berichte, Bd. III, s. 163.

It has long been a subject of interest to discover how, by purely chemical methods, this reduction could be carried out, and various investigators have from time to time endeavoured, but unsuccessfully, to solve the problem. Maly¹ in 1865, Lieben,² Ballo,³ and others attempted it and were able to reduce the carbonates to formates, but no further. Recently, however, Dr. H. J. H. Fenton⁴ has succeeded by means of magnesium in reducing carbon dioxide to formaldehyde at a low temperature in aqueous solutions, demonstrating the truth of the above assumption. Acted upon by the contents of the living cell⁵ condensation of the aldehyde takes place, as is the case when it is acted upon by alkalies, and glucose is formed :

which by dehydration is converted according to circumstances into starch, cellulose or glucoside :

If, on the other hand, formaldehyde is oxidised in the leaves of the plant it is converted into formic acid :

which now combines with ammonia absorbed by the roots to form ammonium formate, which then, by dehydration, is converted into hydrocyanic acid :

1. *Liebig Annalen*, Bd. CXXXV, s. 119.
2. *Monatschr.*, Bd. XVI, s. 211, and Bd. XVIII, s. 582.
3. *Berichte*, Bd. XVII, s. 6.
4. *Transact. of the Chemical Society*, Vol. XCI, p. 687, 1907.
5. Adolph Baeyer, *Berichte*, Bd. III, s. 68.

The prussic acid may now react in various ways, as follows :—

A.—By oxidation it is converted into cyanic acid

B.—By reduction it is converted into methylamine

C.—It may combine directly with formic aldehyde and produce methyl cyan-alcohol or glycollic nitrile

which combined with $2\text{H}_2\text{O}$ leads to glycollic acid and ammonia

By means of a reducing agent this acid may be converted into acetic acid¹

the latter in combination with formic acid giving rise to acetic aldehyde

or by dehydration to acetic anhydride

Acetic aldehyde and acetic anhydride may also be obtained by another method from glycollic nitrile

By a similar series of changes we can then proceed to the formation of higher aldehydes, propionic, butyric, valerianic, etc., their acids and anhydrides.

1. Claus, *Annalen d. Chemie*, Bd. CXLV, s. 256.

By combining glycollic nitrile with NH_3 and subsequent hydration we obtain glycocoll¹

In the same way by combining it with methylamine we obtain sarcosine

which combined with cyanamide forms kreatin.

D.—The most interesting and important action, however, is the formation of amino-malonic nitrile from the condensation of three molecules of prussic acid

Little attention has been paid to this substance, but I have reason to suppose that it plays an important part in the synthesis of the various proteids and of their derivatives. It is prepared in the following manner :—

If a fragment of potassium cyanide be added to anhydrous hydrocyanic acid, condensation of three molecules gradually takes place,² forming amino-malonic nitrile, a crystalline body

a black insoluble substance being produced at the same time. The nitrile may be extracted with ether; this, on evaporation, yields the crystals, which may then be dissolved in water, decolourised with animal charcoal, and recrystallised. The yield by this method, however, is small. A much larger result, not less than 50 per cent. of the theoretical amount, is obtained by the following process.³

1. Eschweiler, *Annalen d. Chemie*, Bd. CCLXXVIII, s. 655.

2. Lescoeur, Regault, *Bulletin de la Société Chimique de Paris*, t. XLIV, p. 473.

3. Eug. Bamberger, Leo. Rudolph, *Berichte*, Bd. XXV, s. 1083.

Add 2.6 grammes of pure dimethyl-aniline-oxide to 0.6 gramme of anhydrous prussic acid. Let the pale yellow mixture remain in the dark for twenty-four hours at the ordinary temperature. Extract with boiling water and separate the nitrile from the aqueous extract by shaking it several times with ether. It must then be recrystallised from boiling water and decolourised with animal charcoal.

Warmed with barium hydrate, amino-malonic nitrile is resolved into glycocoll, ammonia, and carbonic acid

the carbonic acid and ammonia being liberated in the same proportions as result from the decomposition of urea when heated with barium hydrate.

The intermediate stages may be represented as follows :—

which with a further molecule of H_2O

or, if we suppose that the NH_3 and CO_2 are discharged separately we may assume that they combine to form ammonium carbamate since the two gases if passed in a perfectly dry state into cold absolute alcohol are known to combine, the product separating as a copious crystalline precipitate. This, if filtered off and then heated with absolute alcohol in a sealed tube to 100° , on cooling appears as large crystalline laminae of ammonium carbamate.¹ Under certain conditions this is converted into urea and ammonium carbonate

1. Kolbe and Basaroff, *Chem. Soc. Journal*, [2] 6, 194.

If glycocoll be heated with acetic anhydride it is converted into acetyl-glycocoll.¹

If it be heated in a sealed tube to 160° C. with benzoic acid it is converted into hippuric acid²

which is a normal constituent of the urine of herbivorous animals, and which appears also in human urine after the administration of benzoic acid.³

In the *Lancet* for December 16, 1905, and December 8, 1906, I described a new method for the synthesis of tyrosine from amino-malonic nitrile (that is from prussic acid) and oxy-benzaldehyde, the following being the reactions :—

This heated in a sealed tube with 2KCN to 175°-182° is converted into

the 2KCN acting as the reducing agent ; when heated with 4H₂O being converted into

1. Curtius, *Berichte*, Bd. XVI, s. 757, and Bd. XVII, s. 1663.
2. Dessaignes, *Jabresbericht der Chemie*, s. 367, 1857.
3. Bouis, Ure : *Berz. Jabresbericht*, Bd. XXII, s. 567.

If, instead of using hippuric acid in the above synthesis, acetyl glycocoll, $\text{CH}_2(\text{NH}\cdot\text{COCH}_3)\text{COOH}$, is used, the same series of reactions will ensue, ammonium acetate instead of the benzoate being among the final products.

This experiment suggests that HCN ; $\text{CH}(\text{NH}_2)\cdot(\text{CN})_2$; $(\text{CH}_3\cdot\text{CO})_2\text{O}$; together with $\text{OH}\cdot\text{C}_6\text{H}_4\cdot\text{CHO}$ (or very probably $\text{NH}_2\cdot\text{C}_6\text{H}_4\cdot\text{CHO}$) are among the earliest products formed in the synthesis of proteid.

We have now to consider in what way we can synthesise methyl tartronic nitrile and its amino compound, namely, iso-aspartic or amino-iso-succinic acid from these products.

By mixing together 16 c.c. of acetic anhydride and 74 c.c. of dry ether, placing the solution in an ice bath and after slowly adding 10 grammes of KCN , passing through the mixture a stream of HCl gas and leaving it at rest in a bath at 0° for twenty-four hours, we obtain diacetyl-dicyanide $(\text{CH}_3\cdot\text{CO}\cdot\text{CN})_2$,¹ the polymeric of pyruvic nitrile $\text{CH}_3\cdot\text{CO}\cdot\text{CN}$, or acetyl cyanide

Diacetyl-dicyanide is readily soluble in alcohol, ether and benzol. If left at rest with fuming HCl for twenty-four hours and then boiled with dilute HCl it is transformed into acetic acid and iso-malic or methyl-tartronic acid. According to L. Bouveault² the following are the reactions :

1. Brunner, *Monatsbeft. f. Chemie*, Bd. XIII, s. 835.

2. *Bulletin de la Soc. chimique* [3] t. IX, p. 557.

the latter being immediately saponified into methyl-tartronic acid and 2NH_3 .

Judging from analogy, however, methyl-tartronic nitrile should also result from the combination of diacetyl-dicyanide with 2HCN ;

which by hydration is resolved into lactic acid, CO_2 and 2NH_3

or, the CO_2 and NH_3 in the latter case being liberated in the same proportions that result from the decomposition of urea—we may have

If, when the methyl-tartronic nitrile is decomposed, the intermediate products, methyl-tartronic acid and ammonia being formed, other substances are present such as the ketonic acids or the cyan-alcohols, $\text{R} \cdot \text{CH}(\text{OH}) \cdot \text{CN}$, the liberated NH_3 will combine with them and on the decomposition of the methyl-tartronic acid, α lactic acid, and CO_2 will be alone set free. This I suggest is the action which takes place during muscular contraction, an explosive outburst of the two products being the result : the NH_3 combining with other constituents of the muscular tissue.

The same products may be formed slowly when the muscle is at rest ; or if, when at rest, NH_3 is set free from some other constituent of the muscular tissue the NH_3 will combine with the methyl-tartronic

nitrile forming a compound homologous with amino-malonic nitrile, namely, amino-iso-succinic nitrile, which by hydration can be converted into α alanine or amino-propionic acid and urea—

or the intermediate products amino-iso-succinic acid and NH_3 may be formed.

Before proceeding to prove that methyl-tartronic nitrile (or its components) are constituents of fibrin there are some extremely interesting points regarding amino-malonic nitrile, and its derivative tartronic nitrile, which are worthy of reference and consideration.

By des-aminating amino-malonic nitrile in the usual way, that is by means of HNO_2 , it will be converted into tartronic nitrile

with which the nitriles of malic acid $\begin{array}{c} \text{CH} \\ \diagup \text{CN} \\ \diagdown \text{OH} \\ | \\ \text{CH}_2\cdot\text{CN} \end{array}$ and of oxyglutaric

acid $\begin{array}{c} \text{CH} \\ \diagup \text{CN} \\ \diagdown \text{OH} \\ | \\ \text{CH}_2\cdot\text{CH}_2\cdot\text{CN} \end{array}$ are homologous.

In a later communication I shall endeavour to prove that these three nitriles are constituents of living proteid, being the precursors of amino-malonic, aspartic and glutamic acids, and that in dead proteid they are transformed into the anhydrides

I further suggest that amino-malonic nitrile $\text{CN} \cdot \text{CH} \begin{smallmatrix} \text{CN} \\ \text{NH}_2 \end{smallmatrix}$ is a constituent of living proteid, which in dead proteid is transformed as follows—

and that tartronic nitrile may be similarly transformed

and that if each of these is combined with 2HCN it is converted respectively into adenine and hypoxanthine

the latter when oxidised in the tissues being converted into uric acid¹

1. Burian, R., *Zeitsch. f. physiol. Chemie*, Bd. XLI.

Unfortunately he adopted an erroneous molecular formula for albumin (Lieberkühns), an error which he corrected a few years later. He also committed himself to the statement 'that the albuminous molecule contains an urea group, and represents a complex ureide,' which is not entirely borne out by the facts and has probably discouraged many from carefully considering the results which he obtained. These experiments, as recorded in his first paper, consisted in heating 100 grammes of coagulated albumin with 200 grammes of crystallised hydrate of baryta and one litre of water

(i) to a temperature of 100°C . under ordinary atmospheric pressure for 120 hours,

(ii) digesting them in an autoclave at a temperature of $140\text{--}150^{\circ}\text{C}$.

He determined in each experiment the amount of NH_3 liberated, and, from the amount of BaCO_3 formed, calculated the quantity of CO_2 .

In both cases the amounts of CO_2 and NH_3 liberated were in the same proportion as those obtained from the decomposition of urea.

If Schutzenberger had been dealing only with such substances as amino-malonic nitrile, tartronic nitrile, methylamino, and methyl-tartronic nitriles his inferences would have been correct, but in his later experiments, in which the decomposition of albumin was more profound, this relationship between CO_2 and 2NH_3 entirely disappears. On evaporating the solution of albumin, freed from the baryta, a series of crystalline compounds were obtained, to two of which I will now call attention. He says, 'I have found at least two acids' [in the solution of hydrolysed albumin] 'which by their presence prevent the complete precipitation of the baryta by means of carbonic acid, from the solution. They are either crystallisable with difficulty or not at all, are deliquescent and cannot be precipitated by nitrate of mercury. These characters make their examination and their separation extremely difficult. One of them has the composition of an isomeride of aspartic acid ($\text{C}_4\text{H}_7\text{NO}_4$), already found by Kessler in the products of the decomposition of albumin by sulphuric acid, but differs from aspartic acid by being highly soluble in water.

Another found in small quantities, gives numbers corresponding to the formula of a di-amido-citric acid.'

Unfortunately, in neither of these cases does Schutzenberger give his analyses, so that with regard to the latter substance we are left in doubt. The first substance, the isomeride of aspartic acid, is, I suggest, iso-aspartic acid or amino-iso-succinic acid

derived from the proteid constituent diacetyl-dicyanide and NH_3 , or from the combination of methyl-tartronic nitrile with NH_3 and its subsequent saponification.

The other compound 'corresponding to the formula of a di-amido citric acid' is, I suggest, $\text{C}_6\text{H}_{12}\text{N}_2\text{O}_6$, differing from diamido-citric acid $\text{C}_6\text{H}_{11}\text{N}_2\text{O}_6$ by one atom of hydrogen, and if so, it is a compound of glycocoll with the above-mentioned iso-aspartic acid

resulting from the saponification of the proteid constituents amino-malonic nitrile and methyl-tartronic nitrile

which incompletely hydrolysed with baryta in a sealed tube, give

the CO_2 combining with the baryta and the NH_3 with other constituents of the proteid.