OBSERVATIONS

ON SOME OF THE PRODUCTS OF THE PUTREFACTION OF Vegetable and Animal Substances, AND THEIR RELATION TO PATHOLOGY.

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Observations on some of the Products of the Putrefaction of Vegetable and Animal Substances, and their relation to Pathology. *By* William K. Sullivan.

A BRIEF summary of the observations which form the subject of the following communication, was appended as a note to a paper which I read at the Dublin Meeting of the British Association, and which is printed in the present number of this journal ("On the formation of several of the acids of the series $C_nH_nO_4$, by the destructive distillation of peat"). It had already been put into type in its original shape, when it struck me that the subject being of so much importance, especially in a physiological point of view, my observations might not prove uninteresting, if somewhat extended, and the analytical details added. Such an extension was incompatible with the limits of a note, and I have accordingly made a distinct communication of it.

Mass of wheaten flour set to putrefy several years ago.

Several years ago, I made between thirty and forty pounds of wheaten flour into dough with distilled water, placed it in an earthen pan, covered it with distilled water, and placed in the water and over the mass of dough an inverted funnel, the neck of which communicated by means of a narrow glass tube with the top of a tall narrow bell glass provided with a stop-cock, and inverted over some mercury in a basin. The whole apparatus was laid aside in a cold damp cellar for one year; during this period the mass swelled up, bubbles of gas where evolved which collected in the funnel, and could be made to pass into the bell glass from time to time by opening the stop-cock. The gas collected in this way consisted almost entirely of carbonic acid, but small quantities of marsh gas C_2H_4 and uncombined hydrogen were obtained. Sulphide of hydrogen did not appear to have been given off at any period of the putrefaction; a piece of paper dipped into acetate of lead, suspended in the bell glass for days, did not exhibit the slightest blackening. Protochloride of mercury or basic acetate of lead was not precipitated black by the water standing over the dough.

Gases evolved during putrefaction.

Changes produced in mass

Very soon after the dough was placed in the water the putrefaction set in, but its energy gradually diminished according as the liquor became acid. At the end of the by putrefaction.

year the greater part of the mass appeared to have undergone but little true decomposition. Nevertheless, the gluten throughout the whole mass had undergone considerable physical change. It had lost much of its tenacity, the external portion especially forming a soft slimy mass in which quantities of starch globules more or less unaltered were imbedded. A portion of the gluten taken from within about an inch of the surface of the mass, and freed from all adhering starch, was digested with water slightly acidulated with hydrochloric acid at a temperature of 22° C. until it dissolved. This solution examined with a polarimeter presented the power of left-handed deviation in a very feeble degree, compared with a solution of an equal degree of strength made with fresh gluten.

Slowness of putrefaction shown by a second experiment to be owing to the formation of acids in the liquor.

At the end of the second year the appearances were but little altered, and it was quite remarkable how little the putrefaction had progressed. That this retardation was caused by the presence of acids in the liquor, was very clearly demonstrated by another experiment made with flour mixed up with ground chalk, and then made into dough and placed in water beside the other mass. In the course of a few months the mass had putrefied more than the other had done after the lapse of two and a half years. In this case a little sulphide of hydrogen appeared to have been formed. Circumstances prevented me from continuing the experiments after this period. But being anxious to determine the nature of the substances dissolved in the water of the flour putrefied without chalk, I placed the whole mass upon a linen strainer, and when it had thoroughly drained, I mixed the mass on the filter with a large quantity of distilled water, allowed the solid part to subside, and decanted off the supernatant liquor, which was then added to that which had drained from the mass. This liquor was mixed with baryta water and distilled until the greater part of the liquor had passed over. The latter was very weakly alkaline; it was neutralized with hydrochloric acid and evaporated to dryness, at first on a sand bath, and lastly over a water bath. This dried mass was then preserved in tubes. The residue in the retorts was then removed, some carbonic acid passed through it, boiled, filtered, and evaporated to dryness, and the dry mass preserved in tubes.

Experiments not being proceeded with, the chlorides of bases and baryta salts of acids preserved.

Experiment on

While these experiments were in progress I also allowed a mass of brain to putrefy under exactly similar putrefaction of brain.

circumstances. In this case the putrefaction set in with great rapidity, and continued uninterruptedly for several months, because here the liquor never became decidedly acid. Both sulphide and phosphide of hydrogen were evolved during the decomposition of the mass.

Several bases formed during putrefaction of brain.

A very superficial examination of the acid, basic, and other products contained in the liquid in which the brain had putrefied, made at the time, and among which I found valeric acid in comparatively large proportions, and what I believed to have been trimethylamine and some peculiar compound containing phosphorus, led me to believe that a considerable number of the ammonia bases are produced during the slow decay of vegetable and animal substances.

Experiments resumed during the past year.

Having been engaged during the past year in searching for similar bodies among other products, I determined to examine the chlorides and baryta salts from the putrefaction of the flour, which I had carefully preserved in tubes, as above mentioned, but which I had not had time to analyze. The following is a summary of the results which I obtained.

Fractional distillation of mixed chlorides into three portions.

The dried mass presumed to contain the mixed chlorides of several bases, was treated with absolute alcohol, which dissolved a portion of it; the residue was common chloride of ammonium, as was fully established by a determination of the platinum in the precipitate formed by the addition of chloride of platinum and alcohol to a solution of it. The alcoholic solution separated from the chloride of ammonium was evaporated to dryness; the dry mass was introduced into a retort and a quantity of baryta dissolved in water added, equivalent to what would be required to saturate one-third of the acid contained in a quantity of common chloride of ammonium equal in weight to the dry mass put into the retort. The mass was then heated in a water bath, the product of distillation received into water acidulated with hydrochloric acid, the solution thus obtained evaporated, and the resulting dry mass, which I shall call A, preserved for further examination. As much more baryta was added to the residue in the retort and a second product, B, obtained. A third portion of baryta was then put into the retort and a third product, C, found.

Fractional distil-

The product called A was fractionated in exactly the same manner that the whole mass had been, the distillate being subdivided into three portions, *a*, *b*, *c*. The porlation of portion A.

tion *a* was dissolved in the smallest possible quantity of water; bichloride of platinum was then added, and a mixture of alcohol and ether which threw down a double salt possessing all the properties of the ordinary platinochloride of ammonium. A determination of the platinum in this salt verified the supposition that the substance operated upon was almost wholly chloride of ammonium.

Preparation of salts of palladium and gold with the product *b*.

Mode of determining the amount of gold and chlorine in gold salt.

A portion of the product *b* was dissolved in water and treated with bichloride of platinum and alcohol, but scarcely any precipitate could be obtained. The whole was then evaporated to dryness in a water bath, but it was found so difficult to effect a separation of the pure compound, that I had to have recourse to the combinations with other metals. Another portion was treated with protochloride of palladium, but although a beautiful salt was formed, considerable difficulty was experienced in separating it in a pure state. Having dissolved the remainder of the product in the smallest possible quantity of water, a very concentrated solution of terchloride of gold was added, which threw down a yellow precipitate that dissolved on the addition of a mixture of ether and alcohol. The solution thus formed was exposed to spontaneous evaporation in a warm place, but shaded from the light. A number of small short rectangular prisms separated, which dissolved with difficulty in cold water; but freely in boiling water, though not without suffering slight decomposition if boiled for some time. This difference of solubility afforded a means of obtaining the salt in a comparatively pure state; the crystals were accordingly dissolved in a small quantity of boiling water; on cooling the compound crystallized out. The proportions of gold and chlorine in these crystals were determined by introducing a weighed quantity of them into a small flask, adding some granulated zinc and one drop of diluted sulphuric acid, and allowing the whole to digest for some time. When all the gold was precipitated, the supernatant clear liquid was decanted off, and the residue repeatedly washed by decantation. The precipitated gold was treated with dilute nitric acid in order to dissolve the excess of zinc; the solution was decanted off and added to the previous liquor, and the gold repeatedly washed by decantation as before. When fully washed it was transferred to a small porcelain cup, dried in a water bath, gently ignited, and weighed. All the liquids obtained in the washing of the gold were then placed in an evaporating basin, and a little milk of lime added, and the whole evaporated in a water bath to dryness to expel the volatile base. The dried residue was boiled with water, the solution filtered, and a few drops of nitric acid added so as to carefully neutralize the lime; it was then heated to boiling, and nitrate of silver added, to precipitate the chlorine. The milk of lime used, was prepared from lime made with white marble; before being used it was slacked and then mixed with a large quantity of pure water, allowed to settle, the clear liquid decanted off, and fresh water poured on, this operation being repeated three or four times so as to separate any chlorine which might be present. 0.115 gramme of the gold salt all treated in this way gave 0.056 gramme of metallic gold, and 0.165 gramme of chloride of silver. These numbers lead to the conclusion that the body under examination was the aurochloride of trimethylium, $N(C_2H_3)_3,HCl,AuCl_3$, as will be evident from the following comparison:

The base in the portion *b* **was trimethylamine.**

	Calculated.	Found.
$N(C_2H_3)_3H$ . . .	15.074 . .	
Cl_4	35.600 . .	35.441
Au	49.326 . .	48.695
	100.000	

The dried mass B was fractionated in the same manner as A, into two portions. The first portion consisted chiefly of chloride of ammonium and chloride of trimethylium. The second portion was dissolved in a small quantity of water; a few drops of alcohol were added, and then a solution of bichloride of platinum, as long as any precipitate was formed. The whole was allowed to digest in the liquid for about ten minutes, and was then filtered to separate the precipitate. The latter was treated with boiling water, which dissolved it. When the solution cooled it crystallized out as golden scales of great brilliancy. 0.152 gramme gave on ignition 0.051 gramme of metallic platinum; 0.90 gramme digested with granulated zinc, and then boiled with lime-water, filtered, a few drops of nitric acid added, and the chlorine precipitated by nitrate of silver, gave 0.133 gramme of chloride of silver. These numbers correspond to platinochloride

Examination of B.

Preparation of platinum salt.

The base

was amylamine.

of amylium, $N\left\{\begin{matrix} C_{10}\ H_{11} \\ H \\ H \end{matrix}\right\}HCl.\ PtCl_2$, as the following comparison shows:—

	Calculated.	Found.
$N(C_{10}H_{13})H$ . . .	30.046	
Cl_3	36.290	36.503
Pt.	33.664	33.552
	100.000	

Treatment of the mother liquor from which the platinum compound of amylamine was separated.

The solution from which the foregoing salt crystallized out, was still further concentrated at a very gentle temperature and shaded from the light; a very concentrated solution of bichloride of platinum was then added, and the whole set aside in the dark for some days. During this time a crystalline precipitate separated, which was dissolved in boiling water; on being set aside until next day, a quantity of deep orange yellow tabular crystals deposited, but mixed with some of the golden scales of the last salt, and what appeared to be the platinum compound with common ammonia. The whole mass was accordingly digested with zinc, and the solution boiled with baryta water in a small retort connected with a bottle containing water kept cool by ice. The weak alkaline solution thus obtained was about one-third neutralized with hydrochloric acid, and distilled at a very moderate temperature; the product thus obtained was then about two-thirds neutralized with hydrochloric acid, and evaporated to a very small quantity; this was then divided into two portions, to one a strong solution of bichloride of platinum was added, and a few drops of alcohol, a slight precipitate was thrown down, which was separated by filtration; re-dissolved in water and set aside, it crystallized in fine scales. The mother liquor was set aside for some days, when the orange yellow tabular crystals again formed, but still mixed with a small quantity of some other compounds. The whole quantity, amounting 0.125 gramme, was burned, and gave 0.049 gramme of metallic platinum = 39.200 per cent. The body having the nearest composition to this would be platinochloride of ethylium, $N\left\{\begin{matrix} C_4\ H_5 \\ H \\ H \end{matrix}\right\}HCl,\ Pt\ Cl_2$, which contains 39.300 per cent. of platinum.

The base present in largest quantity was ethylamine.

Gold salt of ethylamine.

The gold salt was prepared with the other portion of the chloride: it consisted of fine bundles of lustrous golden orange prismatic needles, but containing a little of another gold salt mixed with it. With the extremely small quantity of material at my disposal, I found it impossible to purify a sufficient quantity of the salt for an analysis.

C contained several bases.

The portion marked C, from the first fractioning of the chlorides, apparently contained several bases; but I had not enough substance to enable me to prepare the platinum or gold salts in a state of purity.

Bases found, trimethylamine, etc.

The only bases, accordingly, whose presence I was able to determine with certainty, were, trimethylamine, ethylamine, and amylamine.

Baryta salts yielded acetic, butyric, and valeric acids.

Formic acid probably present.

The baryta salts were treated by Liebig's process of fractional distillation, and in other respects in the manner described in my other paper, already referred to; it is therefore unnecessary to describe the process here. The only acids which I could detect were acetic, butyric, and valeric; several higher members of the series are undoubtedly formed by putrefaction; but it would require to operate upon a very large quantity of material in order to be able to separate them. I am also disposed to think that formic acid is also produced by putrefaction. The results which I obtained, although rendering its existence very probable, do not entitle me to pronounce positively upon its presence.

Negative character of evidence as to the presence of propionic acid.

With regard to the presence of propionic acid, my experiments are rather of a negative character, and certainly do not lead to a definite result. On distilling one of the soda residues (resulting from the partial neutralization of the mixed acids, with a view of separating acetic acid) with phosphoric acid, neutralizing the distillate with baryta, and crystallizing the baryta compound two or three times, which was a matter of considerable difficulty, a salt was obtained having the exact appearance of propionate of baryta. When a solution of this salt was decomposed by carbonate of potash, the solution filtered, evaporated to dryness, and the residue treated with absolute alcohol, no crystalline salt could, however, be separated by the addition of ether, which, it is to be presumed, would be the case if propionic acid was present.

Propionic acid

It is probable that the acid which has been obtained by the putrefaction of lentils, peas, etc., by Bœhme,[1] and

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[1] Jour. für Prakt. Chem., xli, 278, (as quoted in Gerhardt's *Traite de Chim.*, *t.* ii., *p.* 440).

not readily formed during putrefaction.

Keller states that he obtained it.

which Dessaignes and Chautard[2] believe to be present in spent tanners' bark, may be the butyroacetic, and not true propionic acid, which does not appear to be readily formed in processes of putrefaction. When mangel wurzel or other varieties of beet are stored in heaps, and happen to heat and decay, a good deal of butyric acid is sometimes formed; but I was never able to detect propionic acid, nor could I in putrefied beet juice, although I have operated on a large quantity. F. Keller, nevertheless, states[3] that propionic acid is the predominating acid formed when bran is fermented with animal tissues; indeed, he says the only acids he obtained were acetic and propionic acids. According to him, propionate of baryta contains nine atoms of water of crystallization, which it loses on being heated to 140°. This statement is opposed to the usual opinion that propionate of baryta is an anhydrous salt. Frankland and Kolbe distinctly state[4] that propionate of of baryta dried at 100° has the formula, $BaO, C_6H_5O_3$.

Strecker thinks the mixed salts of acetic and butyric acids could be mistaken for propionates.

He obtained it himself however.

Strecker[5] observes, that the mixed salts of acetic and butyric acids could be mistaken for propionates, and that the formation of propionic acid in the fermentation of bran, as mentioned by Keller, is by no means proved. He, however, obtained a large quantity of that acid under circumstances which certainly show that it can be formed during putrefaction, if not in the first stages, at least as the result of secondary reactions. In making lactic acid by the modified process proposed by Bensch, that is, by mixing together water, sugar, sour milk, and cheese, the mixture was left for two or three months in a place in which the temperature varied from 20° to 0°. There was formed at the end of this time, besides lactate of lime, a considerable quantity of mannite (as much as 1lb. from 10lbs. of sugar). On allowing such a mixture of lactate and mannite to remain during a summer at a temperature reaching to 20° or 22°, the lactate of lime gradually dissolved, and the evolution of gas continued. After standing for a year, the mass was treated according to Bensch's process for separating butyric acid, but none could be found; the only acids obtained being a large quantity of propionic acid, a small quantity of valeric acid (derived from the cheese), and acetic acid.

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[2] Journ. de Pharm., xiii., 244.

[3] Annal. der Chem. u. Pharm., lxxiii., 205.

[4] Annal. der Chem. u. Pharm., xcii. 80.

[5] Annal. der Chem. u. Pharm., lxv. 288.

Great interest in a pathological point of view, which the formation of compound ammonia by putrefaction presents.

The formation of compound ammonia bases in the process of putrefaction is of great interest in a pathological point of view. Indeed I was originally led to institute the foregoing experiments from the belief that such investigations would throw light upon many pathological changes, and from the conviction that the ammonia which is said to exist in the blood in typhus, scarlatina, variola, cholera, and other diseases, is a product of decomposition, and would be found to be oftentimes a mixture of methylamine and other compound bases with common ammonia. That this is the case in the last stages of yellow fever, when the quantity of ammonia (part, at least, being in the state of chloride of ammonium, a body which could scarcely be the result of the decomposition of urea), becomes very considerable, I have not the least doubt.

Discovery that leucine can be formed in the living body.

In the year 1848, I showed[6] (I believe for the first time) that leucine could be formed in the living system, and that, too, without scarcely any organic disease. That body is always a product of the putrefaction of substances containing nitrogen, especially when it takes place under water, and I am consequently inclined to look upon its presence in animal secretions as a proof of putrefactive changes having set in. It is also probable that in every case where leucine is formed during putrefaction, compound ammonia bases will also be found if sought for. Whether this was the case in the liquid in which I observed the leucine, I could not, of course, determine; but it is worthy of remark that creatine was found in comparatively large quantity, while there was but little urea present. Since my discovery of this remarkable substance in an animal secretion, it has been found by several other observers. Frerich and Staedeler, for example, observed it in the human liver, after acute atrophy of that organ, after typhus, etc.;[7] Scherer also found it in the liver of a drunkard who had died of typhus.[8] Gorup-Besanez observed it in normal ox-liver, and also in the pancreas of that animal;[9] a similar observation has been made by Wolff,[10] who, in addition to a large quantity

Fact confirmed by other observers.

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[6] Proceedings of the Pathological Society of Dublin. Session 1847–1848, p. 29.

[7] Wien. Med. Wochenschrift, 1850, No. 30, quoted in Gmelin's Handbuch der Chemie (Fortsetzung), Bd. xiii 2te. Hälfte, S. 75.

[8] Arch. f. Path. Anat., x., S. 228—230.

[9] Annal. der Chem. u. Pharm., xviii S. 1—43.

[10] Arch. f. Path. Anat., x., S. 228—230.

of leucine, also detected the presence of a homologue of it. Virchow and Frerich have also found leucine in fresh pancreatic juice and in the glands themselves. Most of these observers believe that it is a normal product of the organism; but Virchow[11] has observed it to form and increase after death, and consequently looks upon it as a cadaverous product. That it is found in the living organism is, however, placed beyond doubt by my observation. But on the other hand it is doubtful whether it is ever produced by the healthy action of the organs. That it is a product of decay, though formed within living tissues, is, I think, supported by the circumstance that Gorup-Besanez found several of the acids of the series $C_nH_nO_4$ accompanying it in the liver; and in the mother liquor of the pancreatic tissues from which the leucine was separated, he observed the characteristic smell of the same acids on the addition of sulphuric acid. In connection with this point it would be of interest to determine whether tyrosine and hypoxanthine, which Wolff found in large quantities in the pancreas of the ox, and Scherer in the liver of the drunkard above mentioned (he finds hypoxanthine in all human livers), are always formed during putrefaction along with leucine. Gorup-Besanez found no tyrosine in the liver of the ox, and he thinks the presence of hypoxanthine doubtful.

Is probably always a product of decay.

Are tyrosine and hypoxanthine always formed during putrefaction?

Probability of presence of compound ammonias in sweat, etc.

Caproic acid probably present in sweat.

Immediately after Wurtz's discovery of methylamine and ethylamine, I sought for them in several diseased secretions, but did not get any very decided results. I obtained, however, from the sweat of a patient suffering from bromidrosis, a small quantity of ammoniacal chlorides, which yielded, with bichloride of platinum and also with terchloride of gold, crystalline compounds, which appeared when examined under the microscope, to consist of at least three different forms of crystals. I determined the amount of platinum in the mixed salts, and found that the atomic weight of the base or bases was much higher than that of common ammonia. Here also, as in ordinary perspiration, the greatest part of the organic matter consisted of volatile acids—formic, acetic, butyric; but in addition to them, I obtained crystals of baryta salt, which had what appeared to me the exact form of caproate of baryta. These acids, as Lehmann has shown,[12] are not the

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[11] Arch. f. Path. Anat., viii., S. 335—363.

[12] Lehrbuch der Physiologischen Chemie, 2te Auf. 1str Bd., S. 57.

products of the decomposition of the sebaceous substance. The fact of their occurring in normal sweat is apparently opposed to the idea that they are products of putrefaction; but in the present state of our knowledge, no decided opinion can be formed as to how far the presence of the acids of this series can be considered as an indication of putrefaction.

Other occupations have hitherto prevented me from pursuing this kind of research; and I do not know whether any one else has turned his attention to the subject. It would undoubtedly be of the greatest importance to determine the pathological conditions under which salts of ammonia are developed in the blood, etc., and whether the compound ammonias are formed in every case where common ammonia is produced. Such investigations would be very much facilitated by a good microscopical investigation of the forms of the platinum, palladium, and gold salts of the ammonia bases, and the publication of a good series of photographic views illustrative of them.

Importance of determining the condition under which compound ammonias are formed in the blood.

It is also possible that some of the phosphorus bases discovered by Paul Thenard, and which have formed the subject of a recent admirable memoir by Hofman and Cahours, may be formed by the putrefaction of the brain and nervous matter. I hope to be able to resume my experiments on the putrefaction of the latter bodies immediately, and this time on a sufficient scale to enable me to separate most of the substances formed.

Probability that phosphorus bases are formed during the putrefaction of the brain.