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THE SUPPOSED OCCURRENCE OF METHYLGUANIDINE IN MEAT, WITH OBSERVATIONS ON THE OXIDATION OF CREATINE BY MERCURIC ACETATE.

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The experiments to be reported grew out of an attempt to investigate the nature of the toxic agent in "meat poisoning." Methylguanidine is one of the most toxic substances claimed to have been obtained from meat, or extract of beef, and it seemed possible that this compound might be a factor in such poisoning. The investigation is not complete, but sufficient evidence has been accumulated to indicate that methylguanidine is not present in meat, save, possibly, that which has undergone very extensive decomposition. The work is presented in its present form because it confirms the recently published work of others,[1] and because its resumption in the near future is not likely.

Methylguanidine was first obtained from physiological material by Brieger,[2] who used horse meat that had been allowed to decompose for 4 months, and by his pupil Hoffa,[3] and from the flesh of rabbits infected with "rabbit septicemia." They claimed that by their method they were unable to find any methylguanidine in fresh or normal meat. Their procedure was quite complicated and apparently was not used by other investigators. All other reports on the presence of methylguanidine in meat or in extract of beef are based on the use of either or both of the following methods of precipitation: (*a*) silver nitrate and barium hydroxide, (*b*) mercuric chloride and sodium acetate.

In 1916, Ewins[4] showed that creatine was oxidized to methylguanidine by means of silver oxide, and, that, consequently, all reports as to the presence of the latter which were based upon precipitation with silver nitrate and barium hydroxide were of little value. However, Ewins regarded precipitation with mercuric acetate as free from the danger of oxidation.

In the description of the method for the isolation of methylguanidine by means of precipitation with mercuric chloride and sodium acetate, there is mentioned a heavy white precipitate which is insoluble in hot, dil. hydrochloric acid. It seemed to the author that this could only be mer-

[1] Baumann and Ingvaldsen, *J. Biol. Chem.*, 35, 277 (1918).

[2] Brieger, *Untersuchungen über Ptomaine*, III, 1888, p. 33.

[3] Hoffa, *Sitzungsb. physikal. medizin. Ges. Würzburg*, 1889, p. 96; *Jahresb. Tierchem.*, 19, 472.

[4] Ewins, *Biochem. J.*, 10, 103 (1916).

curous chloride, and that it was probably formed as a result of the oxidation of creatine.

Experiment proved that this was the case. Both methylguanidine and oxalic acid were isolated and identified as products of oxidation. The existence of an intermediate substance was suspected, but attempts at its isolation were not immediately successful. About 20% of the nitrogen of the creatine was obtained as ammonia (or other amine that could be completely distilled in a current of air at ordinary room temperature), indicating either that some methylguanidine was further oxidized, or that there was some other reaction in progress.

The work had reached this stage when the author was informed in a personal communication that Baumann and Ingvaldsen had also observed the oxidation of creatine by means of mercuric acetate and had isolated the substance intermediate between creatine and methylguanidine and oxalic acid. That work has since been published.[1] Baumann and Ingvaldsen called their new substance methylguanido-glyoxylic acid.

$$CH_3NHC(:NH)NHCOCOOH.$$

Its constitution was established by (1) derivation from creatine, (2) nitrogen content, and (3) hydrolysis in a solution of sodium acetate, precipitation with calcium chloride, filtration and ignition. The calculated quantity of calcium oxide was obtained.

The substance is unstable, decomposing readily upon evaporation of its solutions at ordinary pressures. Using diminished pressure, the substance was readily obtained and was found to have the properties described by Baumann and Ingvaldsen.

An attempt was made by the author to determine more completely its constitution. The substance which forms the insoluble calcium compound after hydrolysis was identified as oxalic acid by weighing the air-dried precipitate, then igniting and weighing again. The weights observed were those calculated from $CaC_2O_4.H_2O$ and for CaO.

TABLE I.

Analysis of α-Methylguanido-glyoxylic Acid.

Preparation No.	Substance taken. G.	$CaC_2O_4.H_2O$. Found. G.	$CaC_2O_4.H_2O$. Calculated. G.	CaO. Found. G.	CaO. Calculated. G.
I.....	0.0906	0.0935	0.0915	0.0352	0.0350
	0.1916	0.1930	0.1988	0.0745	0.0741
II.....	0.1804	0.1840	0.1814	0.0710	0.0698
	0.1866	0.1885	0.1875	0.0734	0.0721
III.....	0.1770	0.1810	0.1782	0.0690	0.0684
	0.1608	0.1660	0.1619	0.0634	0.0621
IV.....	0.2231	0.2252	0.2246	0.0867	0.0862
	0.2392	0.2412	0.2409	Lost	..

[1] Baumann and Ingvaldsen, *J. Biol. Chem.*, 35, 277 (1918).

It was also attempted to isolate methylguanidine from the products of hydrolysis of the substance. 0.33 g. was dissolved in water and evaporated to dryness after adding 3 cc. conc. hydrochloric acid. The residue was dissolved in water and treated with an excess of sodium picrate. There was obtained 0.243 g. of a substance having the characteristic crystal form of methylguanidine picrate and melting at 195° (uncorr.). The small yield is noteworthy. The theoretical yield is 0.665 g. It is probable that some other form of decomposition occurs. This may partly explain the failure to secure more than 50% of methylguanidine from creatine.

A typical protocol is presented. The methylguanido-glyoxylic acid was identified by melting point, nitrogen content and by hydrolysis and precipitation with calcium chloride, weighing as $CaC_2O_4.H_2O$ and as CaO. Methylguanidine was weighed as the picrate, which was identified by crystal form, melting point and yield of picric acid and of methylguanidine hydrochloride. For this purpose a weighed quantity of the picrate was dissolved in hot water, transferred to a continuous extraction apparatus, treated with hydrochloric acid and then extracted with benzene, using a weighed flask. After complete extraction, the benzene was distilled off, the flask dried at 105°, cooled and weighed. The acid solution was also evaporated, at first in a beaker, then in a small glass evaporating dish, dried at 105° and weighed. The calculated quantities of picric acid and methylguanidine hydrochloride were obtained.

13.1 g. creatine (4.2 g. nitrogen) was dissolved in 800 cc. water, and 225 g. mercuric acetate added. The mixture was allowed to stand at room temperature for 8 days and was then filtered. Ten cc. of this filtrate was used for a determination of creatine, and found to contain less than 0.1 mg. of this substance. The precipitate was then washed with cold water. The precipitate is known as Fraction I; the filtrate as Fraction II.

Fraction I.—The precipitate was suspended in water and decomposed with hydrogen sulfide. The mercuric sulfide was filtered out and the filtrate set aside. The precipitate was then washed with hot water and the washings allowed to cool, whereupon white crystals separated. These were kept, and the mother liquid and the filtrate previously obtained evaporated at about 15 mm. pressure to a small volume. The distillate was collected and tested for nitrogen. None was found. The liquid in the distilling flask yielded a second crop of crystals which was added to the first. After filtering, washing with cold water and drying at 115°, they weighed 5.133 g. They were identified as α-methylguanido-glyoxylic acid, as described previously. The filtrate was evaporated and yielded 1.058 g. of a substance containing 16.2% of nitrogen and melting at 135°. After recrystallizing from hot water, the melting point was

202°. The substance which melted at 135° was probably a mixture of methylguanido-glyoxylic acid (m. p. 203°) and oxalic acid.

The filtrate from these crystals contained 0.900 g. of nitrogen. Upon treatment with picric acid, methylguanidine picrate was obtained. The amount, however, accounted for only 0.495 g. of nitrogen.

Fraction II.—This was treated with hydrogen sulfide and mercuric sulfide filtered out and washed with hot water. No crystals were obtained on cooling or after evaporation *in vacuo*. The distillate was nitrogen free. The liquid in the distilling flask contained 1.68 g. nitrogen, of which 1.32 g. was accounted for as methylguanidine upon treatment with picric acid.

TABLE II.

Distribution of Nitrogen after Oxidation of 13.1 g. Creatine (4.20 g. Nitrogen) with Mercuric Acetate.

	Fraction I. G.	Fraction II. G.
Methylguanido-glyoxylic acid	1.490	...
Crystals melting at 135° (impure methylguanido-glyoxylic acid)	0.170	...
Methylguanidine	0.495	1.320
Nature unknown	0.405	0.360
Total	4.24 g.	

It is clear that precipitation with mercuric salts is not suited to the isolation of methylguanidine, not only because these oxidize creatine but also because they do not completely precipitate methylguanidine, as is evident from the preceding protocol. Nor is the matter helped by making the mixture alkaline. In one experiment, 0.934 g. of methylguanidine hydrochloride (equivalent to 2.58 g. of the picrate) was dissolved in water and treated with mercuric chloride and sodium hydroxide, keeping the mixture just alkaline to litmus. The precipitate was filtered out and dissolved in hydrochloric acid, the mercury removed with hydrogen sulfide, and the filtrate from the mercuric sulfide evaporated. Upon treatment with sodium picrate, only 1.101 g., or less than half of the calculated quantity of methylguanidine picrate, was obtained. It is evident that the method offers no possibilities, even if creatine could be quantitatively removed before treatment with mercuric salts.

Many other procedures were tried, but none was satisfactory. The best results were obtained with a technique which is based upon the same principles as is Brieger's, but differs in certain details.

The hashed meat was suspended in about twice its weight of water, and was heated to boiling and filtered through muslin. The filtrate was set aside and the residue was again extracted. This process was repeated. The extracts were separately precipitated with basic lead acetate solution. The next day the precipitate in the first extract was removed by centrifugation, and was suspended in the second extract. This was repeated the next day for the third extract, serving to economize in wash

water and in the time required to evaporate it. The liquid was then evaporated at about 10 mm. pressure to a volume of about 100 cc. per kg. of meat, and was then poured into about two volumes of alcohol. The next day the precipitate was filtered out, washed with alcohol, and the filtrate was treated with hydrogen sulfide. The lead sulfide was removed, washed with alcohol, and the filtrate treated with 2 or 3 cc. of a saturated alcoholic solution of zinc chloride to remove creatinine. After 2 or 3 days in the ice-chest, the precipitate was filtered out, and the filtrate was treated with an alcoholic solution of mercuric chloride until precipitation was complete. After standing overnight, this precipitate was filtered out, and the filtrate treated with hydrogen sulfide. The filtrate from the mercuric sulfide was evaporated to small volume and precipitated with alcohol. The insoluble portion was rejected, the filtrate and washings were again evaporated and precipitated with absolute alcohol. This was repeated until the residue was practically entirely soluble in absolute alcohol. Water was then added, the alcohol boiled away, and the bases remaining precipitated with hydrochloric and phosphotungstic acids. The next day this precipitate was washed with a dilute solution of hydrochloric and phosphotungstic acids, and then decomposed with barium hydroxide in the cold. The filtrate from the barium phosphotungstate was acidified with hydrochloric acid and evaporated to small volume. Four or five volumes of 95% alcohol were then added and the mixture allowed to stand overnight. The precipitate was filtered out, washed with alcohol, the filtrate evaporated and the treatment with alcohol repeated until all was soluble in absolute alcohol. Water was added, the alcohol boiled away, and sodium picrate added. After standing on ice overnight, the precipitate was filtered out, dissolved in hot water, filtered and allowed to crystallize.

If any considerable amount of the precipitate was obtained it was filtered out, dried and weighed. Some was taken for a melting-point determination. The remainder was dissolved in hot water, decomposed with hydrochloric acid and extracted with benzene, in a continuous extraction apparatus. The benzene was evaporated and the picric acid weighed. The acid liquid was also evaporated in a weighed dish and the residue of methylguanidine hydrochloride(?) weighed.

In all, 3 lots of beef that had been standing at room temperature for 3 or 4 days were used. With two of these, very good results were obtained. In each case, two samples were taken. To one was added a weighed quantity of methylguanidine hydrochloride (obtained from a weighed quantity of the picrate); the other was used as a control. In both cases the latter yielded a negligible amount of picrate, while the added methylguanidine was recovered, quantitatively, as the picrate and identified as already described. Protocols follow:

Expt. V. June 24, 1400 g. of beef used. 0.005 g. picrate obtained.

Expt. VI. June 25, 1400 g. of the same beef used. Added 0.808 g. methylguanidine hydrochloride (equivalent to 2.23 g. of the picrate). Obtained 1.65 g. of picrate which melted at about 195° and which yielded 1.24 g. of picric acid and 0.621 g. of hydrochloride. (Calculated for methylguanidine picrate 1.25 and 0.600 g., respectively.)

Expt. VII. July 12, 1400 g. of another sample of meat used. 0.015 g. of dark picrate obtained.

Expt. X. July 19, 1400 g. of the same sample of meat as used in Expt. VII. Added 0.475 g. methylguanidine hydrochloride (equivalent to 1.33 g. of the picrate). Obtained 1.35 g. of picrate which melted at about 195° and which yielded 1.06 g. of picric acid and 0.517 g. of hydrochloride. (Calculated for methylguanidine picrate 1.02 and 0.490 g., respectively.)

The latter experiments were much less satisfactory. A considerable amount of insoluble picrate was obtained, but analysis showed that this was not methylguanidine picrate but some other substance. The protocols follow:

Expt. XI. July 24, 1400 g. meat used. Obtained 0.6445 g. picrate decomposing at about 190°. This yielded 0.4244 g. picric acid and 0.2755 g. of hydrochloride. (Calculated for methylguanidine picrate 0.488 g. and 0.233 g., respectively.) The hydrochloride was dissolved in water, and aliquots taken for nitrogen determinations. The total was 0.0642 g. If the original substance had been methylguanidine picrate 0.0895 g. was to be expected. The remainder of the solution was treated with sodium picrate. A picrate melting at 205° was obtained. Since this is considerably higher than the melting point (198°) of the purest methylguanidine picrate which had been obtained, it is obvious that the two are not identical.

Expt. XII. July 25, 1400 g. of the same meat used, with the addition of one g. creatine. Obtained 0.6545 g. picrate, decomposing at about 190°. Analysis lost.

Expt. XIII. July 27, 750 g. of the same meat used. Added 0.095 g. methylguanidine hydrochloride (equivalent to 0.266 g. of the picrate). Obtained 0.9066 g. of the picrate decomposing at 205° and yielding 0.6553 g. picric acid and 0.3372 g. hydrochloride. (Calculated for methylguanidine picrate 0.687 and 0.337 g., respectively.) The hydrochloride was treated as in Expt. XI. Calculated 0.126 g. nitrogen. Found 0.078 g. The remainder of the solution was treated with sodium picrate. A picrate melting at 210° was obtained.

It is probable that the poor results of these last experiments were due to faulty technique. However, it is believed that sufficient evidence has been presented to demonstrate that methylguanidine is not present in meat that is in a fair state of preservation. Whether or not it is present in badly decomposed meat has not been determined. That may depend upon the nature of the organisms responsible for the decomposition. Falk, Baumann and McGuire[1] have found that the creatine of meat is resistant to the action of the bacilli of the paratyphoid group, which is the type commonly found in "meat poisoning."[2,3] In any event,

[1] Falk, Baumann and McGuire, *J. Biol. Chem.*, 37, 525 (1919).

[2] Jordan, *Food Poisoning*, Univ. Chicago Press, 1917.

[3] Hübener, *Fleischvergiftungen und Paratyphusinfektionen*, G. Fischer, Jena, 1910.

methylguanidine can scarcely be regarded as playing an important part in the mechanism of "meat poisoning" for such poisoning is due to meat that is not badly decomposed. The nature of the toxic agent will be considered in another paper.

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THE CONSTITUTION OF CAPSAICIN, THE PUNGENT PRINCIPLE OF CAPSICUM.

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The pungent principle of Capsicum was first isolated by Thresh,[1] who assigned to it the name capsaicin. Thresh, however, did not throw any light on the constitution of the substance and overlooked the presence of nitrogen, giving the formula $C_9H_{14}O_2$.

Micko[2] improved the method of extracting capsaicin and obtained it as a crystalline substance of extreme pungency, melting at 63.5° and possessing the properties of a phenol. On the basis of analyses and molecular-weight determinations, he ascribed to it the formula $C_{18}H_{28}NO_3$. This is an impossible formula. His analyses agree well with the formula $C_{18}H_{27}NO_3$. Micko found one hydroxyl and one methoxyl group. He prepared a benzoyl derivative, melting at 74°, which was not pungent. On treating an alcoholic solution of capsaicin with platinic chloride and hydrochloric acid and allowing the mixture to evaporate spontaneously he noted a vanilla-like odor. This suggests the presence of a vanillin residue in capsaicin.

Micko isolated capsaicin from paprika (*Capsicum annuum*) and from cayenne pepper (*Capsicum fastigiatum*). From one kg. of cayenne pepper he extracted 5.5 g. of crude capsaicin, which was 20 times the amount he found in paprika.

Nelson[3] extracted capsaicin by Micko's method and obtained 2.13 g. of the pure crystalline substance, melting at 64.5°, from 1.5 kg. of African capsicum.

Preparation of Capsaicin.

In order to prepare material for this investigation Micko's method of extraction was used. A considerable difference was observed in the amounts of capsaicin recoverable from different lots of cayenne pepper obtained on the market. In one case, only 12 g. of crude capsaicin was obtained from 40 pounds of the pepper. From 50 pounds of a very hot

[1] *Pharm. J. and Trans.*, [3] **7**, 21, 259, 473 (1876–77); **8**, 187 (1877–78).

[2] *Z. Nahr. Genussm.*, **1**, 818 (1898); **2**, 411 (1899).

[3] *J. Ind. Eng. Chem.*, **2**, 419 (1910).