II. THE PRODUCTION OF CARBON MONOXIDE BY THE ACTION OF ALKALINE HYPOHALOGENITES ON UREA.

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R. G. Canti [1916] has used the hypobromite method for obtaining approximate values for the amount of urea in the cerebro-spinal fluid of patients suffering from a variety of diseases. Being desirous of controlling this method by others more reliable he asked R. L. M. Wallis to determine the urea by the urease method in some cases, and G. Graham by the Folin method in others, and the writer to determine the total nitrogen by the Kjeldahl method in other cases.

Here are some typical results:

No. of case	Disease	% urea by			Total nitrogen calculated to urea
		NaOBr	Urease	Folin	
48	Uraemia	0.88	0.63	—	—
114	"	0.41	0.10	—	—
115	Carcinoma	0.28	0.18	—	—
75	Uraemia	0.41	—	0.32	—
76	"	0.45	—	0.32	—
97	Chronic nephritis	0.02	—	0.04	—
134	Uraemia	0.75	—	—	2.05
137	"	0.77	—	—	0.75
141	"	0.42	—	—	0.54
157	"	0.45	—	—	0.94

From the table it appears that the volume of gas evolved by acting upon cerebro-spinal fluid with hypobromite, particularly in cases of uraemia, far exceeds the nitrogen contained in the urea of the fluid, but does not exceed the total nitrogen of the fluid. A similar phenomenon is well known in the determination of urea in urine by the hypobromite method. K. H. Mörner [1903] has shown that the nitrogen evolved from urine by the action of hypobromite upon it may exceed the urea nitrogen by more than four times.

Canti and the writer then sought for a constituent other than nitrogen in the gas evolved from cerebro-spinal fluid and hypobromite, a gas which must be practically insoluble in water, and in a solution of sodium hydroxide. Oxygen was shown to be absent. In Canti's case 134 a portion of the gas was shaken up with part of a dilute solution of blood, the other part being kept as a control. To our surprise we obtained the characteristic colour of

carboxyhaemoglobin. On adding yellow ammonium sulphide to one portion of the COHb solution it was not reduced while the same reagent reduced a similar quantity of the diluted blood in the usual way. Another part of the COHb solution was not reduced by sodium hyposulphite while a similar amount of the control solution was instantly reduced. Precisely similar results were obtained in Canti's case 141. These two were cases of uraemia, but the same result was obtained in a case of diabetes and in a case of cerebral softening. These results caused us to test the nitrogen obtained from urea by the action of hypobromite upon it for carbon monoxide, and we found that the latter gas is always present in the nitrogen when hypobromite of the ordinary strength acts upon urea. To be quite certain that the sodium hydroxide used, namely sodium hydroxide "pure by alcohol," had no effect on the result a quantity was prepared direct from sodium, and hypobromite made with it gave with urea nitrogen containing carbon monoxide.

Marie Krogh [1913] had shown that the gas liberated from urea by hypobromite contained carbon monoxide; but when Canti and I discovered it in 1916 we did not know of her paper. By acting upon 1 cc. of a solution of urea containing 9.19 mg. N with hypobromites of different strengths Krogh once more showed that the volume of gas evolved varied with the strength of the hypobromite and she made the important observations (a) that the nitrogen contained carbon monoxide; (b) that the amount of the carbon monoxide increased with the alkalinity of the hypobromite. For example 1 cc. of the urea solution acted on by 15 cc. of a hypobromite containing 0.193 g.-mol NaOH and 0.0416 g.-atom Br (4.625 : 1) gave total gas 96.2 % of the total nitrogen in the urea and it contained 0.3 % CO; while the same amount of urea with hypobromite containing 0.747 g.-mol NaOH and 0.0203 g.-atom Br (36.86 : 1) gave total gas 99.4 % of the total nitrogen and it contained 0.62 % CO. Krogh was led to suspect the presence of carbon monoxide in the nitrogen by the fact that when she acted upon 1 cc. of urea of the afore-mentioned strength and 1 cc. of a 25 % solution of glucose with hypobromite of the concentrations just given the total gas evolved was 101.1 and 101.7 % respectively of the urea nitrogen. This being the case the gas from urea and that from urea together with glucose was analysed in an apparatus which appears to have been similar to that of Haldane; and after combustion a contraction was observed which was half that obtained on submitting the product to the action of sodium hydroxide. Krogh therefore concluded that the gas must contain a volume of carbon monoxide equal to the contraction after treatment with sodium hydroxide. No qualitative tests such as the blood test are described, nor is it stated that oxygen was added to burn the carbon monoxide. If the oxygen required for combustion was supplied by the air of the generating vessel then the results would be low. Canti and I estimated the carbon monoxide in the gas from the cerebro-spinal fluid of case 142 and in that from a solution of pure urea. To collect the gas without mixing it with air a Lunge nitrometer was filled with hypobromite

of the usual strength (2-5 cc. Br to 25 cc. of a solution of sodium hydroxide containing 40 g. NaOH in 100 cc.) and the fluid or the urea solution was added from the cup. A small Haldane's apparatus was used for the analysis; a suitable volume of gas having been admitted to the apparatus and measured about 2 cc. oxygen were added, the reading taken and the analysis made.

	Vol. of gas admitted cc.	Contraction on combustion cc.	Contraction after KOH cc.	CO %
Case 142 (x. 1916)	7.16	0.05	0.10	1.4
Pure urea (x. 1916)	7.07	0.025	0.07	0.99

Subsequent determinations of the carbon monoxide by the writer have given a smaller percentage than this early one. In the following determinations about 120 cc. of gas were liberated from urea as just described and of this gas 100 cc. were mixed with 20.4 cc. of oxygen. This mixture was analysed in the Haldane's apparatus:

Vol. of mixture cc.	Contraction on combustion cc.	Contraction after KOH cc.	CO %
9.74	0.05	0.06	0.74
9.68	0.02	0.06	0.75

In the following determinations a flask holding 255 cc. to the bottom of the neck was charged with alkaline hypobromite previously made from 20 cc. bromine and 200 cc. of sodium hydroxide: the flask was fitted with a rubber stopper carrying a small graduated tap funnel and a short delivery tube attached to a nitrometer. To displace the air from the flask and delivery tubes 20 cc. of 2 % urea solution were run into the flask. This gas having been expelled from the nitrometer, more urea was added to the flask and the next 150 cc. of gas collected; 40 cc. of it were mixed with 10 cc. of oxygen and the mixture analysed. To control these results a mixture of air and carbon monoxide containing about 2 % of the latter gas was made and stored over water. Three analyses of this mixture were made; then 25 cc. of the mixture were diluted with 25 cc. of air and two analyses of this were made. The table gives the results of two analyses of the gas from urea, and the analyses of the air containing carbon monoxide.

Vol. of mixture cc.	Contraction on combustion cc.	Contraction after KOH cc.	CO %
9.89	0.04	0.05	0.63
9.96	0.035	0.055	0.69
9.93	0.09	0.16	1.61
9.89	0.07	0.175	1.77
9.92	0.07	0.16	1.61
9.84	0.045	0.075	0.76
9.90	0.040	0.090	0.91

From these results it may be concluded that the nitrogen evolved from a 2 % solution of urea, when acted upon by hypobromite of the usual strength,

contains 0.7 % of carbon monoxide. Since this percentage of carbon monoxide is easily detected by shaking the gas with diluted blood its presence can easily be demonstrated in class or in lecture. For this purpose the gas from 10 cc. of a 2 % solution of urea and 50 cc. of hypobromite generated in the usual bottle, may be collected in a nitrometer. From this apparatus the gas can be driven into a 150 cc. narrow necked measuring cylinder filled with suitably diluted blood and inverted in an evaporating basin containing the same. The gas collected, the stopper is inserted, and the cylinder is well shaken. A 50 cc. Nessler glass is now filled to the mark with blood from the cylinder and a similar glass is filled to the mark with some of the original diluted blood. The difference in colour is very marked, and on the addition of a trace of solid sodium hyposulphite to each glass it will be seen that the COHb remains unchanged while the control is instantly reduced on shaking.

The Origin of the Carbon Monoxide.

Krogh [1913] showed that, when alkaline hypobromite rich in hypobromite was employed to act upon urea, less nitrogen and also less carbon monoxide were produced than when the alkaline hypobromite was poor in hypobromite and rich in sodium hydroxide (see her figures quoted above). She assumes that in the former case more carbon is oxidised to carbon dioxide and more nitrogen is oxidised to some oxide of nitrogen, which remains combined with base as nitrite or nitrate, than in the latter case and she makes experiments to prove that the residual nitrogen is present as nitrate. Nitrate had been previously found under certain conditions in the residue from this action of hypobromites on urea by Fauconier [1880] and by R. Luther [1889].

It seemed desirable to ascertain whether sodium hypochlorite when it acts upon urea also gives carbon monoxide. H. J. H. Fenton [1878], using sodium hypochlorite whose strength he does not give, obtained according as he used hypochlorite alone or mixed with sodium hydroxide 49.72 % of the nitrogen in urea; but when the same hypochlorite was used with sodium carbonate he obtained 92.51 %. The writer obtained the results embodied in the following table. Washed chlorine was passed into sodium hydroxide (40 g. NaOH in 100 cc.) cooled in ice, until the solution was of such a strength that 1 cc. of the clear liquid was equivalent to 62.8 cc. *N*/10 sodium thio-sulphate; the usual bottle and tube was used for the reaction and the gas was collected over water in a Lunge nitrometer.

Exp.	Urea 2 g. in 100 cc.	NaOCl solution	NaOH 40 g. in 100 cc.	Na ₂ CO ₃ saturated	NaOCl cc. Normal	% of total <i>N</i> in urea
1	5 cc.	25 cc.	—	—	157	61.3
2	"	25	—	25 cc.	157	54.8
3	"	25	25 cc.	—	157	77.8
4	"	10	40	—	62.8	84.5
5	"	5	45	—	31.4	81.6
6	"	2	48	—	12.5	78.2
7	"	10	—	40 cc.	62.8	53.8

Here it is seen that a concentrated alkaline hypochlorite gives much less gas than a hypochlorite of one-fifth the strength but containing far more alkali. Experiment 4 was repeated on a large scale in order to collect a quantity of the gas free from air and to examine it for carbon monoxide. In 50 cc. of the gas carbon monoxide could be detected, but 100 cc. were required to give a really good test. The amount was too small for estimation in my Haldane's apparatus (capacity 10 cc.). It is certain that although carbon monoxide appears over a wide range of concentrations of hypochlorite it is always less in amount than in the gas from hypobromite. Fenton [1878] showed that cyanate was present in the residual liquid from the action of hypochlorite. Nitrate had been shown to be present by Fauconier [1880] and Luther [1889].

It is certain from what precedes that the action of hypohalogenite upon urea is a complex occurrence. In this paper the question of residual nitrogen is not discussed; only an attempt will be made to explain the formation of carbon monoxide. Schestakov [1905] showed that when an alkaline hypobromite or hypochlorite acts upon urea the following compounds can be isolated from the residue in the form of their benzaldehyde derivatives; hydrazine, semicarbazide, hydrazinecarboxylic acid—the first of these in a yield of 60 %. Chattaway [1909, 1, 2] showed that when chlorine is passed into a strong aqueous solution of urea, or better into such a solution in presence of zinc oxide, a good yield of dichlorocarbamide is obtained and that this compound when acted on by ammonia forms *p*-urazine¹

This compound on hydrolysis by acids yields salts of hydrazine. Linch [1912] showed that when sodium hypobromite acts upon semicarbazide an 80 % yield of *p*-urazine is obtained, and in this reaction he believes that hydrazodicarbonamide is an intermediate product for he shows that hydrazodicarbonamide itself when acted upon by hypobromite also yields *p*-urazine. He further showed that acetylcarbamide acted upon by hypobromite gave diacetylhydrazodicarbonamide which was partly hydrolysed by the alkaline liquid to hydrazodicarbonamide. Thiele [1892] showed that hydrazodicarbonamide is oxidised by chromic acid to azodicarbonamide and that the latter can be hydrolysed to potassium azodicarboxylate. This salt gives a bright yellow solution in water, its aqueous solution is unstable but is stabilised by alkali, and the salt when heated a little above 100° gives carbon monoxide.

¹ Busch regards the *p*-urazines as urazoles and on his view this compound would be amino-

urazole. $\begin{array}{c} \text{HN}-\text{CO} \\ | \\ \text{HN}-\text{CO} \end{array} \text{N} \cdot \text{NH}_2$. I am not convinced that his arguments apply to this compound.

When the salt is decomposed by water alone only nitrogen is evolved. All these reactions are summarised in the following scheme:

In order to trace the origin of the carbon monoxide formed by the action of hypohalogenite upon urea the action of alkaline hypobromite upon many of the substances in the above table has been examined.

Hydrazocarboxylic acid and its hydrazine salt. As recommended by Stolle and Hofmann [1904] carbon dioxide was passed into a well cooled solution of hydrazine hydrate, and a part of the solution was acted on by hypobromite of the usual strength. No carbon monoxide could be detected in the nitrogen evolved. Another part of the solution was evaporated in a vacuum over sulphuric acid and the deliquescent crystals of the hydrazine salt of the acid also failed to give any carbon monoxide with hypobromite.

Dichlorocarbamide. An aqueous solution of this compound was run into strong ammonia and the gas evolved collected in a nitrometer. The yield of gas assumed to be nitrogen was 10.52 % of the dichlorocarbamide used: theory 21.73. But the gas was rich in carbon monoxide, containing as the mean of three concordant results 1.24 % CO. When sodium hydroxide was used in place of ammonia the evolved gas also contained carbon monoxide. In all cases a crystalline precipitate of *p*-urazine was obtained as described by Chattaway.

p-Urazine. This compound acted upon by hypobromite of the usual strength also gave monoxide, the amount of which has not yet been determined.

Semicarbazide. Linch's experiment on this compound (1 g.-mol semicarbazide hydrochloride, 4 g.-mols NaOH and 3 g.-atoms Br) was repeated with the modification that the evolved gas which Linch assumes to be nitrogen, was collected and examined. It was found to contain 22 % of carbon dioxide and also some carbon monoxide. The amount of the latter gas was 0.64 %—slightly less than that given by urea under ordinary conditions.

Acetylcarbamide with hypobromite of the usual strength also gave carbon monoxide—amount not yet estimated.

Hydrazodicarbonamide. 0.5 g. of the solid was placed in a dry tube which

was put into the usual bottle containing hypobromite made from 50 cc. of 10 *N* NaOH and 5 cc. Br (1 g.-mol: 119 g.-mols: 46.4 g.-atoms) and the gas collected in a nitrometer. The action was quite slow and it gave 101.45 cc. moist nitrogen at 67° and 770 mm. This is equivalent to 28.23 g. N per g. molecule, that is half the total nitrogen. It contained no trace of carbon monoxide.

Linch's experiment with this compound was repeated (1 g.-mol: 2 g.-mols: 2.33 g.-atoms), much less nitrogen was evolved but now the gas contained a little carbon monoxide. Therefore the following experiment was tried: 0.59 g. of the compound was thoroughly shaken with 50 cc. water in the bottle. Into this tube 10 cc. of 10 *N* NaOH was placed, cooled to 0°, and 0.5 cc. Br added. (1 g.-mol: 20 g.-mols: 3.9 g.-atoms.) On mixing these 66 cc. N₂ at 20° and 764 mm. were obtained = 15.22 g. N per g.-mol or rather more than 1 g. atom. This gas gave a strong reaction for carbon monoxide. Its amount has not been determined. When the hydrazo-dicarbonamide dissolves in the hypobromite it gives a clear and intensely yellow coloured solution.

Azodicarbonamide. The first experiment with this compound was made in exactly the same way as the first experiment with hydrazodicarbonamide and with similar quantities. There is however a great difference in the speed of the reaction which in this case is very high and much heat is evolved. Calculated on the g.-molecule the nitrogen evolved was 38.67 g. or 2.75 g.-atoms. No trace of carbon monoxide could be detected in this gas. This experiment was repeated as in the last experiment with hydrazodicarbonamide using 0.58 g., 5 cc. 10 *N* NaOH, and 0.8 g. Br (1 g.-mol: 10 g.-mols: 1 g.-atom). In this case the yield of nitrogen was 10.68 g. per 1 g.-mol substance, that is 0.75 g.-atom, and the gas gave good tests for carbon monoxide but the amount has not yet been determined. The remaining solution was bright yellow.

Schestakov explains the formation of hydrazine, which he isolated as benzaldazine, and of hydrazocarboxylic acid which he also isolated in small amounts as its benzaldehyde derivative by the following scheme:

and he believes that the hydrazocarboxylic acid is the compound which furnishes the nitrogen obtained by the action of hypobromite on urea. This is probably the main series of reactions; but these reactions alone fail to explain (1) the formation of semicarbazide which Schestakov isolated in small amount as benzalsemicarbazide, (2) the production of carbon monoxide. The writer would suggest provisionally that in addition to the reactions indicated by Schestakov the following occur:

That this reaction does occur is made very probable by Fenton's observation

[1878] that cyanate is contained in the residual solution when sodium hypochlorite acts upon urea. Then

From the semicarbazide, hydrazodicarbonamide is next produced as indicated by Linch, then azodicarbonamide by oxidation by hypobromite and finally carbon monoxide as outlined in the scheme on p. 16. This work is incomplete, but it is being continued in the hope of further elucidating the changes which occur when hypohalogenites act upon urea.

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SUMMARY.

Krogh has shown that carbon monoxide is produced as well as nitrogen when sodium hypobromite acts upon urea whether in urine or in solution in water. Krogh obtained the result by combustion of the carbon monoxide.

The author and Canti showed by the blood test and by combustion that carbon monoxide is also formed when sodium hypobromite acts upon the urea contained in cerebro-spinal fluid and also on pure urea. Its production admits of an easy class or lecture demonstration.

The amount of carbon monoxide contained in the nitrogen when sodium hypobromite of the usual strength is used to act upon a 2 % solution of urea is 0.7 % of the total gas.

Carbon monoxide is also produced along with nitrogen when sodium hypochlorite acts upon urea, when ammonia or caustic soda acts upon dichlorocarbamide, and when sodium hypobromite of suitable strength acts upon semicarbazide, hydrazodicarbonamide, azodicarbonamide, and acetylcarbamide.

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