I. ON THE PRESENCE OF CYANATE IN THE BLOOD.

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(Received November 7th, 1925.)

It has recently been asserted that considerable quantities of cyanate are found in the blood, namely 0.8–1.0 mg. HOCN per 100 cc. blood of the rabbit and cat [Montgomery, 1925].

These findings are of very great interest, since cyanates and cyanic acid, on account of their chemical nature, might be considered as precursors of urea in the liver [Bollman, Mann and Magath, 1924] as well as of ammonia in the kidneys [Nash and Benedict, 1921]. It might be presumed that, in neutral or in alkaline reaction, 2 mols. HOCN as well as 1 mol. $\text{NH}_4\text{.OCN}$ could give rise to urea, whilst cyanic acid, in acid reaction, is hydrolysed into ammonia and carbonic acid [cf. Werner, 1923]. Nowadays, such findings arouse more than ordinary interest, since, in the course of the last years, the conclusion has been reached that blood does not contain such quantities of ammonia and carbamate as were previously assumed¹, and also since the theory that cyanic acid gives rise to urea is considered by some to be one of the most probable [cf. Werner, 1923, and Montgomery, 1925].

However, in studying the ammonia formation in blood, I came to the conclusion that it was the same whether the blood, after collection, had stood either at acid or slightly alkaline reaction, so that it appeared unlikely that blood could contain such quantities of cyanate as recorded by Montgomery; for one would expect that the ammonia formation produced at acid reaction would be appreciably greater than that produced at alkaline reaction.

I therefore tried at first to determine the velocity of decomposition of cyanic acid. 1 cc. $N/5$ KOCN was mixed with 99 cc. "buffer mixture" of known reaction, and samples of 10 cc. were taken from time to time in order to determine the amount of ammonia formed, Folin's method being employed for this determination.

¹ Concerning the ammonium concentration in the blood, cf. Barnett [1917], Nash and Benedict [1921], Henriques and Gottlieb [1924], Parnas and Heller [1924], Parnas [1925]. According to Fauerholt [1924], the carbamate concentration in the blood must be very much smaller than the ammonium concentration. If, therefore, the ammonium-N concentration, according to the investigators mentioned above, is estimated at 0.03 mg. per 100 cc. blood at most, the carbamate-N concentration in a bicarbonate solution like blood can, according to Fauerholt, only be 0.2 % of this, or 0.00006 mg. per 100 cc. of blood.

Exp. 1. At 18° there were found the following values of $\text{NH}_3\text{-N}$, expressed in per cent. of the theoretically possible value formed at different reactions.

p_{H}	2.0	5.0	7.3	9.0
After 1 hour	98	78	1	1
„ 27 hours	100	99	3	2
„ 74 „	—	—	12	2
„ 17 days	—	—	91	7

Exp. 2. At p_{H} 2.0 the following $\text{NH}_3\text{-N}$ values were found—in per cent. of the theoretically possible amount—formed at different temperatures.

	0°		18°
After 1 minute	6	After 1 minute	14
„ 5 minutes	9	„ 5 minutes	80
„ 10 „	21	„ 25 „	100
„ 30 „	34	„ 62 „	100

Exps. 1 and 2 show the dependence of the formation of ammonia on the p_{H} and temperature indicating that practically all the cyanate in a sufficiently acid reaction (p_{H} 2.0) is decomposed into ammonia in the course of less than half an hour at room temperature and in the dilution employed, namely $N/500$.

Afterwards an investigation was made as to the possibility of detecting the presence of a certain amount of cyanate added to blood by allowing samples of such blood to stand at acid and slightly alkaline reactions (p_{H} 2.0 and p_{H} 9.0 respectively), the difference in the amount of ammonia formed in acid and alkaline reactions being used as a standard for the amount of cyanate; for, from the experiments described above, we may conclude that all the cyanate present may be considered as being hydrolysed by standing 1 hour at p_{H} 2.0, whereas the amount of cyanate hydrolysed at p_{H} 9.0 is so small that it need not be taken into account. After the blood had stood for a certain time at acid and alkaline reactions, the amount of ammonia formed was determined by Folin's method, the samples being cooled at 0° previous to the addition of excess of potassium carbonate, and, subsequently, the ammonia formed was removed by aerating for 3 hours at a low temperature (0°–4°). The ammonia determinations themselves were carried out by titration with $N/280$ NaOH in a recipient of 1 cc. $N/280$ H_2SO_4 [Henriques and Gottlieb, 1924].

Four such experiments were carried out, namely two with blood and two with plasma. In the experiments with plasma 90–95 % of the amount of cyanate added could be estimated, whereas in the experiments with blood only 60–70 % could be recovered owing to the technical difficulties occasioned by the great quantity of haemoglobin in the blood.

Simultaneously with each of these experiments control experiments were carried out both with samples of normal plasma and with samples of blood, though without an addition of cyanate, the procedure in the controls being exactly the same; however, as it was impossible to detect any difference between the ammonia formation at acid reaction and that at alkaline reaction, it appears quite improbable that the small quantities of ammonia found were due to decomposition of cyanate.

One of these experiments described in detail may serve as an example. About 200 cc. blood collected from a dog were cooled rapidly, and mixed with pure NH_3 -free Na oxalate. After 5 minutes' centrifugation the plasma was poured off and divided into two portions, one of which may be termed " P_0 "; the other portion of 50 cc. was mixed with 0.5 cc. of a freshly prepared solution of KOCN (0.220 g. in 100 cc.). This mixture, which may be termed " P_{NCO} ," should then contain 1.13 mg. of NCO' per 100 cc.

From each of these two portions of plasma were then taken 4 samples of 5 cc. each, two of which were allowed to stand with 10 cc. of N tartaric acid, whilst the two others stood with a mixture of 10 cc. of N tartaric acid + 3 cc. of 3.5 N K_2CO_3 , whereby the reaction of the mixture became approximately p_{H} 2.0 and p_{H} 9.0, respectively. After standing for two hours in a water-bath at 18° , the mixtures were cooled to 0° , and so much 3.5 N K_2CO_3 solution was added to them that each sample finally contained 10 cc. of 3.5 N K_2CO_3 altogether. (This was done on account of the possible amount of ammonia contained in the reagents employed, so that the blank value might be considered as being constant.) Subsequently the amount of ammonia contained in the eight samples was determined as described above.

(1)	5 cc. of P_0 on standing at p_{H} 2.0	gave 0.005 mg. $\text{NH}_3\text{-N}$
(2)	" " "	" " 0.005 "
(3)	" " "	" p_{H} 9.0 " 0.005 "
(4)	" " "	" " " 0.004 "
(5)	5 cc. P_{NCO} "	" p_{H} 2.0 " 0.022 "
(6)	" " "	" " " 0.022 "
(7)	" " "	" p_{H} 9.0 " 0.005 "
(8)	" " "	" " " 0.006 "

As is seen from the above experiment, there is no essential difference between the values of $\text{NH}_3\text{-N}$ found in normal plasma which had stood at acid and alkaline reactions respectively, and those found in cyanate plasma which had stood at alkaline reaction, the accuracy of the $\text{NH}_3\text{-N}$ determination being about ± 0.001 mg. Hence the quantities of NH_3 formed in these experiments cannot be due to the content of cyanate. In the samples (Nos. 5 and 6), however, which contained an addition of cyanate, and which had stood at acid reaction, an additional formation of $0.022 - 0.005 = 0.017$ mg. $\text{NH}_3\text{-N}$ was found, which corresponds to 1.02 mg. of NCO' per 100 cc., or 90 % of the amount calculated.

Since the accuracy of the $\text{NH}_3\text{-N}$ determination per 100 cc. of plasma is ± 0.001 mg., the possible error per 100 cc. is 0.04 mg. N, which corresponds to 0.12 mg. NCO' . This value is one-tenth of the values which Montgomery states that he has found in normal plasma.

In order to verify whether an organism is able to transform cyanate into urea, and since the literature contains nothing that throws light on the effect and the destiny of cyanate in the organism, experiments were subsequently carried out on three rabbits to which a cyanate solution was given

per os; these animals died however in the course of less than an hour, presenting characteristic symptoms of poisoning. The doses applied varied between 0.7 and 0.19 g. of NCO' per kg. rabbit. The KOCN solution employed was very nearly neutralised and gave no positive reaction for cyanide.

Rabbits, however, occupy a somewhat special position in regard to nitrogen metabolism, for, unlike human beings and dogs, they are unable to excrete an excess of inorganic acids in the form of ammonium salts [cf. Pohl and Münzer, 1900; Kettner, 1902; Lyman and Raymond, 1919; and Palladin, 1924], and consequently the following experiment was carried out.

A dog weighing 16 kg. received a continuous intravenous injection of a N/3 cyanate solution at the rate of 0.42 g. cyanate-N per hour. This rate of N-transmission corresponds only to the metabolism of about 300 g. of flesh per 24 hours. However, the animal rapidly exhibited symptoms of poisoning similar to those observed in the experiments with the rabbits and died after 66 minutes. The animal had then received altogether 1.38 g. of NCO', or 0.086 g. per kg.

During the experiment the amount of cyanate contained in the blood was determined by a method similar to that described above. At the beginning of the injection the value of cyanate found was 0.0 g. per 100 cc. of plasma; after 60 minutes' injection the value was 14.4 mg., and, after death, 15.1 mg.

It is possible that a certain amount of the injected cyanate may have been excreted, or transformed into other substances, but the figures obtained indicate that there has been no time for a significant excretion to take place. For, if one assumes a uniform distribution of the salt in the quantity of water contained in the organism, and estimates this to be two-thirds of the weight of the animal, the amount of cyanate per 100 g. of fluid should be 13 mg. In all these experiments the same pure preparation of potassium cyanate ("Kahlbaum") was used, but it cannot be denied that sodium cyanate would have been preferable if it had been possible to procure this in the same purity. However, it is out of the question that the animals may have been killed by the quantities of potassium ions applied simultaneously, as much larger doses of KCl and of KNO₃ may be given intravenously [Hald, 1904] as well as *per os* [Norn, 1925], without causing death.

It is possible that the method used by Montgomery for the detection of cyanate in the blood is not specific, or at any rate may possibly give rise to the formation of products of decomposition if the plasma be allowed to stand for 24 hours at 43° with toluene, which cannot be considered as a reliable antiseptic¹. It is also possible that the blood contains cyanate in another form or combination, whose qualities differ from those of cyanate dissolved in water or added to the blood from outside. Without such an assumption the experiments described above indicate that cyanate is not a precursor of urea, as assumed by Werner.

¹ Similar, but aseptic, experiments have shown that cyanate cannot be present in the blood in concentrations exceeding 10 mg. per 100 cc. of plasma [Nieloux and Welter, 1922].

SUMMARY.

The experiments described exclude the presence of cyanate in the blood in concentrations exceeding 0.1 mg. per 100 cc. of plasma.

Cyanate, given intravenously or *per os*, is toxic; hence cyanate, as present in ordinary solutions, cannot be considered as a precursor of urea.

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