X. THE DETERMINATION OF CYANATES IN BLOOD.

By EDWARD GEORGE MONTGOMERY.

From the Physiological Laboratory, Trinity College, Dublin.

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THE occurrence of cyanic acid during the oxidative deamination of glycine, alanine and sarcosine has been quoted recently in support of the hypothesis that this acid is an important intermediate product in the formation of urea in the animal body [Fearon and Montgomery, 1924]. Additional support was claimed from the work of Nicloux and Welter [1922], as reviewed by Riggs in *Chemical Abstracts* [1923].

At the time the original paper was not available, but a subsequent study of the work revealed the unexpected fact that Nicloux and Welter do not claim to have obtained any positive evidence for the occurrence of cyanic acid in the blood of the dog. Their data, however, are also capable of the interpretation given by Riggs. "Ten parts of plasma of dog blood mixed with 1 part of satd. NH_4Cl soln. and incubated 36 hrs at 35° showed an increase of about 5 % in the urea content over that of the initial plasma. By adding 0.1 mg. KCNO to 1 cc. of plasma- NH_4Cl mixture, the increase of urea in 36 hrs was 21 %. Cyanates therefore occur in blood" [Riggs, 1923].

With the object of removing this ambiguity, the blood of the rabbit and the cat was examined for cyanate by a method similar to that of Nicloux and Welter with slight modifications.

In several animals a positive result was obtained. For the rabbit the cyanate value was found to be 0.7 mg. per 100 cc. plasma; and for the cat a value of 1.0 mg. per 100 cc. plasma was obtained, expressed as HOCN .

THE METHOD OF DETERMINING CYANATE IN BLOOD.

This can most readily be accomplished by converting the cyanate into urea by incubation with a suitable ammonium salt solution in accordance with the equation:

This change is not quantitative as some of the cyanate undergoes hydrolysis to ammonia and carbon dioxide, depending on the time and temperature of the incubation, and the concentration of the added ammonium salt. Nicloux and Welter found that a 0.1 % solution of KCNO , incubated at 35° with a saturated solution of NH_4Cl , gave 36 % of the possible urea in 12 hours, and 77 % in 48 hours.

Recovery of cyanate as urea. 20 cc. of a fresh KOCN solution (0.101 %) were incubated with 0.2 g. $(\text{NH}_4)_2\text{SO}_4$ at 40° for 24 hours. The urea recovered represented 77.1 % of the total cyanate added.

For the determination of the urea the xanthydrol method was applied as in the previous paper [Fearon and Montgomery, 1924]. The more delicate urease methods of estimating urea are not so suitable, since Fearon [1923] and Mack and Villars [1923] have shown the formation of cyanic acid during the decomposition of urea by urease; consequently confusion might arise between any cyanic acid present in the plasma and cyanic acid formed by the influence of the enzyme.

Details of method. 30 to 40 cc. of plasma were obtained by centrifugalisation of the fresh blood and divided into two equal samples, A and B. A, the control, was treated with toluene and incubated in a sterile tube for 24 hours at 43° . 0.2 g. of ammonium sulphate was added to B, which was incubated for the same time under the same conditions. An equal volume of concentrated Tanret's reagent¹ was then added to each tube, and the contents filtered. The filtrates were treated with equal volumes of glacial acetic acid, and 1 cc. of a 10 % solution of xanthydrol in methyl alcohol.

At the end of 12 hours the precipitates of dixanthylurea were filtered off, washed with 3 cc. absolute alcohol saturated with dixanthylurea, dried and weighed, and the weight of the urea present determined by dividing the weight of the dixanthylurea by 7.

The solubility of the dixanthylurea in the acetic acid mixture under the conditions of the experiment was determined and found to be 1.6 mg. per 100 cc.

Table I. *Recovery of urea added to 5 cc. of blood plasma.*

Exp. 1.	Initial urea content of plasma ...	...	2.23 mg. per 5 cc.
	Final urea content of plasma ...	...	3.74 " " 5 "
	Estimated amount of urea added ...	...	1.51 " " 5 "
	Actual amount of urea added ...	...	1.56 " " 5 "
Exp. 2.	Initial urea content of plasma ...	...	1.53 mg. per 5 cc.
	Final urea content of plasma ...	...	3.68 " " 5 "
	Estimated amount of urea added ...	...	2.15 " " 5 "
	Actual amount of urea added ...	...	2.20 " " 5 "

Table II. *Cyanic acid concentration of rabbit plasma.*

Rabbit. Three hours after meal of 130 cc. of milk. Urea determined in 10 cc. of plasma.

Urea content of plasma A	...	...	55.3 mg. per 100 cc.
" " " " B	...	...	56.4 " " 100 "
Increase represents 0.78 mg. cyanic acid per 100 cc.			

Table III. *Cyanic acid content of cat plasma.*

Urea determinations in 12.5 cc. of plasma before and after incubation with ammonium sulphate.

Cat I.	Urea content of plasma A	...	...	63.3 mg. in 100 cc.
	" " " " B	...	...	64.7 " " 100 "
	Urea increase represents 1.04 mg. cyanic acid in 100 cc.			
Cat II.	Urea content of plasma A	...	...	61.3 mg. in 100 cc.
	" " " " B	...	...	63.17 " " 100 "
	Urea increase represents 1.05 mg. cyanic acid in 100 cc.			

¹ Tanret's reagent: mercuric chloride (2.71 g.), potassium iodide (7.2 g.), in 66.6 cc. of glacial acetic acid, made up to 100 cc. with distilled water.

By means of control experiments the limits of error of the method were found to lie within ± 0.2 mg. per 100 cc.

Confirmatory test for cyanate in blood plasma. To determine if the increase in urea due to incubation with the ammonium salt arose from pre-existing cyanate or from some other source, such as the hydrolysis of some unidentified ureide, a determination was carried out on rabbit blood plasma after acidification by the addition of 3 cc. $N/10$ H_2SO_4 to 15 cc. plasma. This would destroy any cyanate present by converting it into free cyanic acid which is rapidly hydrolysed in acid solution. The urea content of this plasma is shown in the following table:

Table IV. *Urea content of acidified plasma after incubation for 24 hours.*

Urea content of plasma A	...	...	56.6 mg. per 100 cc.
" " " " B	...	...	56.7 " " 100 "

This confirms the belief that the increase in plasma urea after incubation with ammonium salts is due to the presence of cyanate, since previous acidification of the plasma checks the formation of urea.

SIGNIFICANCE OF CYANIC ACID IN THE BLOOD.

Since it is so closely related to urea, the occurrence of cyanic acid in the plasma suggests that it is either a precursor or a product of the urea formed in the organism. It may represent amino-nitrogen on its way to be converted into urea, or it may result from the enzyme dissociation of urea. The evidence available is strongly in support of the former view. There is at present no ground for the belief that the enzyme urease is present in the tissues of the human body, and considerable indirect evidence that it does not occur. Perfusion of surviving dog's liver with dog's blood, saline, and urea (up to 4 g. per litre) showed no increase in ammonia; consequently, Wakeman and Dakin [1911] concluded that the formation of urea appeared to be an irreversible reaction. Luck [1924] has recently demonstrated the presence of urease in the gastric mucosa of the Carnivora and Ruminantia, but this may be due to ferments taken in with the food or arising from micro-organisms in the food. The possibility of extraneous infection must always be borne in mind in working with urea as Werner [1918] has shown; the dissociation of urea in aqueous solution at low temperature is due, probably entirely, to infection. The number of micro-organisms capable of decomposing urea is very large.

The occurrence of a variable amount of cyanate in plasma will also explain the varying values for blood ammonia obtained by different observers. Solutions of cyanate in low concentration may be completely hydrolysed to ammonia and carbon dioxide by prolonged aspiration at room temperature; and the earlier and more drastic methods for estimating blood ammonia no doubt owe their high values to the unrecognised presence of cyanate.

CONCLUSIONS.

1. Cyanic acid is present in the blood plasma of the cat to the extent of 1.04 mg. %, and in the blood plasma of the rabbit to the extent of 0.78 mg. %, some hours after a protein meal.
2. Its presence is considered to be of significance in the formation of urea in the animal body.
3. The varying results found in the determination of the blood ammonia may be due to the presence of cyanic acid.

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