IX. AMMONIA CONTENT OF THE BLOOD IN NEPHRITIS.

BY DOROTHY STUART RUSSELL.

From the Hale Clinical Laboratory, London Hospital.

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IN October, 1921, Nash and Benedict [1921] published a paper in which they gave an historical review of previous work concerning the ammonia-content of blood, together with work which they themselves had done on this subject, using dogs under various conditions. They showed that low figures, ranging from 0.03 to 0.14 mg. per 100 cc., were obtained for the blood-ammonia in normal cases, and that in nephrectomised dogs the values were markedly reduced. They also found a higher percentage of ammonia in blood from the renal vein than elsewhere in the circulation. On these data they put forward the view that the kidneys are the site of ammonia-formation in the body, the small quantities present in the blood being regarded as an overflow from these organs into the systemic circulation.

In view of this hypothesis it was considered that a series of estimations of the blood-ammonia in cases of severe nephritis would be of interest. In selecting the cases, preference was shown for those with a marked nitrogen-retention, as it was thought that these would be the most likely to give abnormal figures. In the series quoted other details relating to the renal efficiency of the patients are given wherever available.

Method.

The Folin-Denis aeration method was employed, as described by Nash and Benedict. With the exception of Case 3, the time elapsing between the drawing of the blood and the beginning of aeration was not greater than 10 minutes. In the case mentioned the interval was five minutes longer, and the sample was placed on ice during this time. An electric suction pump was used for the aeration, and in all cases this step in the analysis lasted 15 minutes.

Controls.

Controls for the method, as employed, were carried out as follows:

- (1) 5 cc. of a standard ammonium chloride solution, containing 0.014 mg. N (or 0.28 mg. N %) were used for an analysis in place of 5 cc. of blood. The resulting solution in the acid-holding tube, when Nesslerised, gave an exact match with 5 cc. of the untreated standard simultaneously Nesslerised.
- (2) A sample of blood from a patient without known renal disease (Case 1, Table II) was analysed, and the ammonia estimated immediately. Without further delay 0.5 cc. of diluted ammonium chloride solution, containing

0.014 mg. N was added to the aerated blood, and a second aeration immediately performed, adding another 1.0 cc. of carbonate-oxalate solution. The resulting solution in the acid-holding tube gave an exact match with the same quantity of diluted standard in 5 cc. of ammonia-free water, simultaneously Nesslerised. This experiment was repeated on Case 11, Table II, adding 0.5 cc. containing 0.0047 mg. N, with the same result.

(3) Since it was suggested that this result might have been obtained as the result of incomplete removal of the blood-ammonia during the first aeration, the following investigation was made:

5 cc. of freshly drawn blood (Case 2, Table II) were aerated, and the blood-ammonia immediately estimated. With the least possible delay, a second aeration was performed on the same blood for another 15 minutes. The fluid in the acid-holding tube showed no trace of colour on Nesslerisation.

This experiment demonstrated not only the adequacy of the method as a means of analysis, but the purity of our reagents with respect to ammonia.

It should be added that, where the concentration of blood-ammonia was under 0.04 mg. N %, the values given are only approximate, since the depth of colour on Nesslerisation was too faint to admit of accurate estimation.

Table I gives figures for a series of 17 cases examined in the wards of the hospital. Of these Cases 1, 5, 12 and 17 died, but in only the last two mentioned was a post-mortem obtained. The diagnosis of chronic interstitial nephritis was confirmed, but the picture was complicated in Case 12 by the additional finding of infective endocarditis, which probably superimposed an acute infection on the already damaged kidneys.

These same four cases presented symptoms clinically recognised as uraemia, *i.e.* pronounced drowsiness with irregular muscular twitchings, uraemic smell and irregularities in respiration. In Case 12 this irregularity was Cheyne-Stokes in type. Case 1 showed a urea frost during 48 hours before death, a rare phenomenon in which urea crystallises on the surface of the skin giving it a rough, whitish appearance. Case 13 is of considerable interest, since the patient was not confined to bed, but actively engaged in domestic service and not apparently inconvenienced by her renal inefficiency. In other respects the table is self-explanatory. In estimating p_H of blood and plasma bicarbonate the method of Van Slyke and Cullen was employed [1922]. Table II gives a series of 11 cases in which the blood-ammonia was estimated in cases with no evidence of renal disease. Cases 5, 6, 7 and 8 were normal people, the rest were patients suffering from the diseases indicated. The number of cases investigated in this second series is not sufficient to enable one to draw conclusions, but one is led to think that the ammonia-content of the blood in normal people is almost negligible, and that the blood of ill people contains amounts of ammonia which, though small, will admit of estimation. In this class the value obtained for the blood-ammonia seems to be independent of

the nature of the illness, and the range of figures for the non-renal cases coincides with the range obtained for renal cases. More work along this line would be useful. It is possible that such factors as toxæmia might be found to play a part.

Table II. *Values for blood-ammonia in cases without renal disease.*

Case	Diagnosis	Blood-NH ₃ in mg. N %	Remarks
1	Addison's disease	0.05	
2	Rheumatic fever	0.088	
3	Catarrhal jaundice	0.02	
4	Diabetes mellitus	0.02	No ketosis present
5	Normal	Very faint trace	Blood drawn in laboratory and transferred to pump immediately
6	"	Nil	
7	"	Faint trace	
	[repeated]	" "	Stood 10 mins. before aeration
8	Normal	" "	
9	Carcinoma of stomach	" "	
10	? Duodenal ulcer	" "	
11	Hepatic enlargement	0.05	

DISCUSSION.

If we adopt the view that the ammonia present in the urine has been preformed in the blood and simply excreted by the kidneys, it seems legitimate to expect that cases with marked urea-retention would also show ammonia-retention to a comparable degree, particularly if the formation of urea from ammonia is a reversible reaction. If, however, Nash and Benedict [1921] are correct in their hypothesis that the kidneys are the site of ammonia-formation, we might reasonably suppose that there would be no ammonia-retention corresponding to high values of the blood urea, since ammonia-formation would be suppressed in common with other renal functions.

A consideration of Table I at once shows that no ammonia-retention has been found in any of the cases. The values obtained vary from 0.09 mg. N % to zero and are of the same order as those of the cases in Table II.

Next, if the blood-ammonia is not renal in origin, but is derived from other organs it might be expected, on the filtration hypothesis, that the ratio between urea and ammonia in the urine would bear a close relation to the same ratio in the blood.

Taking as normal figures:

Urea in urine	2000 mg. %.
Ammonia	33 mg. %.
Urea in blood	25 mg. %.

$$\text{Then ammonia in blood} = \frac{33 \times 25}{2000} = 0.4 \text{ mg. \%}.$$

This figure is over four times as great as the maximum value observed.

Also, if ammonia is preformed in the blood, we ought to find a definite relation between the values for blood-ammonia and urinary ammonia. In case 16, the excretion of ammonia in a 24 hourly specimen was equivalent

to 57.4 mg. N %, whereas the blood-ammonia was only 0.02 mg. N %. Supposing her weight to be 50 kilos, and the volume of her blood 4,250 cc. (85 cc. per kilo) the total ammonia-content of her blood would be 0.85 mg. N. The whole volume of her blood must therefore circulate through the kidneys completely 384 times per 24 hours, and the kidneys must be 100 % efficient in removing all ammonia from the blood, in order to make up the figure for the urine ammonia. Or, in other words, the total blood flow through the kidneys must be 1632 litres per 24 hours. This is a possible rate of flow, according to Starling [1915], but it is highly improbable that a kidney with such impaired functions as are shown in this case could rise to a 100 % efficiency with respect to ammonia excretion.

Further evidence may be adduced from the consideration of the ammonia-content of urine and blood in acidosis. A large percentage of the total acidity of the urine is represented by ammonia as ammonium salts. This amounts, in normal persons, to about 30 mg. %. The ammonia in the blood, at a generous estimate, is present to the extent of 0.1 mg. %. But if ammonia is used as a neutralising agent for non-volatile acids in the blood it might be expected that, where increased acid is circulating, there would be a proportionate increase in the blood ammonia. Thus case 17 shows a marked acidosis with a decrease in the plasma bicarbonate (taking the normal as 60 vols. %) equal to 0.02 gram-equivalents per litre. If only a quarter of this were neutralised by ammonia (25 % being a low value for the percentage of acid neutralised by ammonia in urine) the figure for the blood-ammonia nitrogen would equal 0.005 gram-equivalents per litre = 0.005×14 g. N per litre = 0.007 g. N %. This is 70 times in excess of the maximum value obtained in any case in the series. Similarly, arguing on the same lines, the figure for Case 13 would be 0.004 g. ammonia N %, and in Case 5 it would be 0.0049 g. ammonia N %. Both of these are approximately 40 times in excess of the values found. It is perhaps noteworthy, in this connection, that Cases 17 and 13 are the only cases in Table I in which no trace of blood-ammonia could be found.

SUMMARY.

1. Twenty-three estimations of the blood-ammonia for a series of 17 cases are recorded, in patients showing advanced renal disease. In all cases the values reported are under 0.1 mg. % and are comparable to the values recorded for eleven cases with no known renal disease.
2. Controls for the technique employed are described.
3. It is argued that the evidence available lends support to Nash and Benedict's hypothesis that the kidneys are the site of ammonia formation in the body, and that the traces found in the systemic circulation represent an overflow from these organs.

REFERENCES.

- Nash and Benedict (1921). *J. Biol. Chem.* **48**, 463.
Starling (1915). *Principles of Human Physiology*, 1151.
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