XCV. AMMONIA CONTENT OF CANINE BLOOD AFTER ORAL ADMINISTRATION OF AMMONIUM SALTS AND AMMONIA

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VENULET [1934] was the first to observe that NH_3 administered orally renders the blood system actually more acid. The researches of Venulet *et al.* [1936] showed that there was a fall in the alkali reserve of the blood after oral and intravenous administration of NH_3 . These researches established a hitherto unexpected influence of added NH_3 on the chemical composition of the blood in the living subject.

Keith & Whelan [1926] administered 100 g. of NH_4Cl intravenously to dogs, and after 2 hr. could find no changes in the ammoniacal nitrogen ($\text{NH}_3\text{-N}$) in the blood. The amount of urea present simultaneously increased considerably. The removal of the liver was not followed by any increase in the quantity in the blood. In disagreement with these authors, Sasaki Takema [1936] observed an increase in the blood NH_3 of dogs after intravenous injections of NH_4Cl .

Pugsley & Anderson [1934] reported enhanced diuresis and a higher blood Ca content in rats after the oral administration of 250 mg. of NH_4Cl .

Alwall *et al.* [1936] noticed that the introduction of NH_3 into the stomach of rabbits evokes strong acidification but that introduction into the duodenum has absolutely no influence on the alkali reserve of the blood. As in both experiments marked symptoms of poisoning appeared, there can be no doubt that the NH_3 undergoes absorption. The acidifying action of NH_3 when introduced into the stomach is explained by the authors thus: the NH_3 is neutralized in the stomach and enters the blood in the form of NH_4Cl . These workers hence confirm the critical remarks of Parnas [1927] with regard to Venulet's paper that the effect of the acidifying action of NH_3 orally is really due to the working of the stomach.

Lazzaro [1934] reported enhanced blood NH_3 in some diseases, such as hepatic cirrhosis, acute yellow atrophy of the liver, asthma, and pulmonary tuberculosis.

Galloro [1932] noted an increase in blood NH_3 in a state of exhaustion, starvation and after intravenous injections of ovalbumin. Glucose administered orally to the extent of 1 g. per kg. live-weight prevents the increase in blood NH_3 caused by fatigue.

The object of the present work was to investigate the influence of oral administration of ammonium salts (NH_4Cl) and of ammonia on blood NH_3 and urea.

METHODS

The procedure of determining blood NH_3 has a long history behind it. After Nencki & Zaleski's first experiments [1901] many other increasingly precise methods were published. As this progress was achieved, it was found that the values of NH_3 found in the blood declined steadily. We have, in our experiments,

applied the method of determining blood ammonia elaborated by Conway [1935].

Urea was determined by the method of Conway [1933], and the normal blood urea content of canine blood was found to vary between 10 and 15 mg./100 ml.

EXPERIMENTAL

All the experiments were made on large dogs weighing from 18 to 22 kg. After determining the normal curve of NH_3 formation, the animals were given a solution of ammonium chloride containing 0.5 g. NH_4Cl per kg. live-weight. Samples of blood were taken 15 min. after the oral administration of the NH_4Cl , and then after 30, 45, 60, 75, 90 min. and so on. After each collection of blood, the curve of NH_3 formation was constructed by making a number of determinations within 10 min. after the blood was shed. The blood urea content was determined simultaneously. In this way experiments were carried out on four dogs (A, B, C, D), after administering NH_4Cl and also on three dogs (F, G, H), in which ammonium hydroxide was substituted for the chloride (4 ml. of 10 % NH_4OH in 40 ml. water). The blood was collected from a neck vein through a thick hypodermic needle direct into a paraffined Erlenmeyer flask containing potassium oxalate.

RESULTS

Ammonia content of normal canine blood

Fig. 1 shows that, as in man [Conway, 1935] and rabbit [Conway & Cooke, 1938], the NH_3 content of the circulating blood of the dog is either zero or below the analytical level. In canine blood we have the same characteristic rise

Fig. 1. Ammonia content of canine blood after shedding. — Dog A. --- Dog B.
.... Dog C. —·— Dog D.

immediately after shedding and ending within 5 min., the NH_3 being then formed very slowly. The curve of this NH_3 formation, extrapolated to zero time after shedding, passes through the origin. The only difference between the canine and human blood, with respect to NH_3 content and formation, is that the NH_3 content of the former is somewhat higher at the fifth minute, being 50–70 $\mu\text{g. NH}_3\text{-N}/100$ ml. instead of 40–50 $\mu\text{g.}/100$ ml. for the latter.

Ammonia content of canine blood after oral administration of NH_4Cl (0.5 g./kg.)

The results for the four dogs are summarized in Table I. The table gives the mean values of the blood NH_3 at regular 15 min. periods after administration. The blood NH_3 figures refer to the analyses conducted immediately after shedding, and after 5 and 10 min. respectively. For the 60 min. period after administration, observations for only 3 animals (B, C, D), were available.

Table I

Time after adminis- tration of NH_4Cl , min.	Mean blood NH_3 content ($\mu\text{g. N/100 ml.}$) analysed after shedding			Observed formation of NH_3 in shed blood $\mu\text{g. N/100 ml.}$	
	Immediately after	5 min. after	10 min. after	After 5 min.	After 10 min.
0	11.9 (0.7)	55.1	59.8	43.2	47.9
15	1013.0 (0.6)	1132	1107	119	94
30	605.0 (0.9)	749	627	144	22
45	296.0 (1.0)	320	319	24	23
60	64.0 (1.0)	114	151	50	87
75	48.0 (0.9)	79	82	31	34
90	8.0 (0.8)	43	48	35	40

The bracketed figures give the mean time in minutes after shedding.

The peak of the rise in blood NH_3 must occur at some time less than 15 min., at which time there is a mean value of $1013 \mu\text{g. NH}_3\text{-N/100 ml.}$, as determined 0.6 min. after shedding. 90 min. after administration the blood NH_3 content has again returned to zero.

The "alpha" rise at high blood ammonia concentrations

The immediate rise in the blood ammonia content after shedding and terminating within 5 min. has been termed the "alpha" ammonia [Conway & Cooke, 1938], to distinguish it from other phases in the curve of NH_3 formation. The present experiments give an opportunity of finding how this formation is influenced by high initial blood NH_3 concentrations. Table I again summarizes the necessary data, the last two columns giving the increase at the 5 and 10 min. periods over the concentrations obtained immediately after shedding. It appears from the table that such an increase in the blood NH_3 occurs at all levels—as judged from the mean values—and that it comes to an end after 5 min. The amount of this formation is not influenced in any definite way by the initial height of the blood NH_3 . As seen from the last column of Table I it varies as the blood NH_3 changes but in no definite relation thereto. At the highest concentrations observed the experimental error alone will cause marked variations. At

Table II. *Blood collected 15 min. after oral administration of NH_4Cl*

Time range after shedding min.	Blood NH_3 content as $\mu\text{g. N/100 ml.}$			
	Dog A	Dog B	Dog C	Dog D
0.5	—	676.5	952.8	—
0.75	1498	—	—	923.8
1.1	—	550.2	—	—
1.5	—	—	—	826.0
2.0	1305	559.2	1206.9	—
2.5	—	—	—	859.2
3.0	1205	—	—	—
3.5	—	—	1302.0	1038.2
4.0	1246	523.2	—	—
5.0	1498	506.2	1427.7	1096.8
7.5	—	—	—	988.6
10.0	1426	676.5	1336.6	—

these highest levels also, there appears to be a greater fluctuation in the blood NH_3 in the 10 min. period after shedding than can be attributed to analytical errors. Table II illustrates this. In the blood from two animals there is actually a decrease over the first 5 min., and for one blood an unduly large increase. That such effects cannot be altogether attributed to analytical errors—which will here no doubt play some part—is shown by the close agreement or uniform change of the results in Table III, for blood samples taken 45 min. after NH_4Cl administration.

Table III. *Blood collected 45 min. after oral administration of NH_4Cl*

Time range after shedding min.	Blood NH_3 content ($\mu\text{g. N/100 ml.}$)			
	Dog A	Dog B	Dog C	Dog D
0.5	—	—	274.3	—
0.75	—	—	—	297.4
1.25	—	282.4	—	311.8
1.5	331.2	—	289.0	—
1.75	—	289.1	—	—
2.25	—	294.0	—	326.0
2.5	328.8	—	291.6	—
3.0	326.4	302.1	—	—
3.5	—	—	300.2	329.1
4.5	—	306.9	306.0	343.4
5.0	316.8	309.3	308.8	345.3
10.0	310.8	309.3	311.8	345.3

Ammonia content of blood after oral administration of NH_4OH

Here the data from three dogs (F, G, H) are available. These indicate a rather marked difference from the figures after NH_4Cl administration. The blood NH_3 falls with great rapidity, so that—as shown in Table IV (*vide* also Fig. 3)—it returns to normal level 45 min. afterwards. Within the interval of 15–45 min. it has fallen from about 300 $\mu\text{g. NH}_3\text{-N/100 ml.}$ to zero.

Table IV

Time after administration of NH_4OH min.	Mean blood NH_3 content ($\mu\text{g. N/100 ml.}$) analysed after shedding			Observed formation of NH_3 in shed blood $\mu\text{g. N/100 ml.}$	
	Immediately after	5 min. after	10 min. after	After 5 min.	After 10 min.
0	9.9 (0.8)	63.6	66.3	53.7	56.4
15	305.4 (0.6)	365.6	367.0	60.2	61.6
30	199.5 (0.8)	266.1	267.7	66.6	68.2
45	3.2 (1.0)	56.7	58.8	53.5	55.6

The bracketed figures give the mean time in minutes after shedding.

The blood urea content after oral administration of NH_4Cl and NH_4OH

Blood urea analyses were carried out for the same dogs and in the same experiments summarized in Tables I and IV. The data are given in Tables V and VI. In Figs. 2 and 3 the mean blood urea changes are compared with those of the blood NH_3 after NH_4Cl and NH_4OH administration. To facilitate the graphical comparison, the blood urea-N in Fig. 2 is given in units 10 times greater than that of the blood NH_3 .

It is clear from Tables V and VI and Figs. 2 and 3 that a marked increase of the blood urea occurs when the blood NH_3 has returned to zero, the significance of which is discussed below. The great reactivity of the liver to blood NH_3 , resulting in the formation of urea is also manifest.

Fig. 2.

Fig. 2. Mean curves of blood NH_3 and blood urea for four dogs after oral administration of NH_4Cl . — Blood urea. - - - Blood ammonia.

Fig. 3.

Fig. 3. Mean curves of blood NH_3 and blood urea for three dogs after oral administration of NH_4OH . — Blood urea. - - - Blood ammonia.

Table V. *Urea determinations*

Time after oral administration of NH_4Cl min.	Blood urea content (mg. urea-N/100 ml.)			
	Dog A	Dog B	Dog C	Dog D
0				
15	10.1	—	15.4	13.6
25	—	26.0	—	—
30	12.1	—	18.1	15.6
45	12.2	21.7	24.2	—
60	—	23.6	24.6	15.8
75	15.4	27.9	28.0	23.5
90	18.4	27.0	33.1	21.4
105	—	27.0	33.3	20.5
120	14.4	—	27.2	15.8

Table VI. *Urea determinations*

Time after oral administration of NH_4OH min.	Blood urea content (mg. urea-N/100 ml.)		
	Dog F	Dog G	Dog H
0	14.2	18.6	13.3
15	14.2	41.0	18.6
30	48.4	43.0	18.0
45	51.3	50.0	53.1

DISCUSSION

When we consider the rapidity with which the blood NH_3 falls from a very rapidly attained peak value after administration of ammonium salts or NH_3 , it is not surprising that the normal content of NH_3 in circulating blood is either zero or below the analytical level. After administration of ammonium hydroxide the blood NH_3 drops from approximately $300 \mu\text{g. NH}_3\text{-N}/100 \text{ ml.}$ to zero within 30 min., and during this time also (15–45 min. after administration) considerable amounts of ammonia are doubtless still entering from the intestine.

Only a very small part of such decrease can be attributed to excretion by the kidneys, and probably the fall is mostly accounted for by the activity of the liver.

The blood urea rises to a considerable height when compared with the blood NH_3 , and it is noteworthy that the most rapid increase generally occurs when the blood ammonia has returned again almost to the zero level with a marked increase continuing when this level is reached. This indicates a considerable lag between the initial stage of the fixation of the NH_3 by the liver and its final appearance as urea. From what we know of the stages of urea formation [Krebs, 1932] the first stage would represent the formation of citrulline from ornithine, NH_3 and CO_2 , the succeeding stages—if we may judge from these experiments—proceeding comparatively slowly. It may be inferred that the ornithine content of blood or liver will be a determining factor in the rapid removal of traces of NH_3 from the blood stream, and will explain why in the fasting animal an increase in the blood NH_3 has been observed [e.g. Parnas & Wagner, 1924].

The immediate or “alpha” rise in the blood NH_3 after shedding is apparently independent of the absolute value of the free blood NH_3 , which is only to be expected if it derives from the deamination of some blood substance. At the same time the analyses at the highest blood NH_3 concentrations observed indicate an interplay between some fixation and some formation of the free NH_3 .

SUMMARY

1. The normal blood NH_3 content for resting dogs is either zero or below the analytical level, when determined by the method of Conway. There appears also the same characteristic rise immediately after shedding and ending within 5 min., as described for the human subject [Conway, 1935].

2. Oral administration of NH_4Cl (0.5 g./kg.) causes a marked increase in the blood NH_3 , the highest mean value of approximately 1.0 mg./100 ml. being observed 15 min. after administration and the peak probably occurring in less than this time. The return to normal occurred within 90 min.

3. When the blood NH_3 is so increased there is also an increase in the blood urea of 2–36 mg./100 ml. over 90 min. The steepest rise in the blood urea occurred from 60 to 90 min. after administration of NH_4Cl , and about 45 min. after administration of NH_4OH , when the blood NH_3 had returned almost to zero, indicating a marked lag in the urea formation after an initial NH_3 fixation.

4. Oral administration of NH_4OH (4 ml. of 10 % NH_4OH in 40 ml. water) is followed by a moderate rise in the blood NH_3 . In 15 min. it reached 0.3 mg./100 ml. but returned to normal in the next 30 min.

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