EXPERIMENTAL STUDIES ON THE AMMONIA POISONING OF THE GOAT I. ON THE CAPACITY TO DETOXICATE AMMONIA ENTERING THROUGH THE PORTAL BLOOD

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I. ON THE CAPACITY TO DETOXICATE AMMONIA ENTERING THROUGH THE PORTAL BLOOD

By

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The reactions of breakdown and synthesis of nitrogen materials by micro-organisms in the rumen is of the significance on the ruminant nutrition as well as the digestion of coarse fibrous materials.

Protein that enters the rumen is fermented by normally living micro-organisms giving rise to peptides, amino acids and ammonia. The non-protein nitrogen consumed can also produce ammonia. Simultaneously with those breakdown reactions there is a synthesis of microbial protein using the non-protein compounds formed in the rumen. It is not only amino acids and peptides that can be used in these synthetic reactions but ammonia nitrogen can also be readily incorporated into microbial protein. Because of such chracteristics of conversion of nitrogen materials in the rumen, many efforts had been made to spare protein food stuffs by supplying part of the nitrogen reqirement of ruminants in the form of more simple non-protein materials such as urea.

However, there are limits to the amounts of nitrogen materials which an organism can assimilate. The balance of the breakdown and synthetic reaction of the nitrogen materials is dependent upon the nature of the ingested protein and the amount and type of carbohydrate present (1).

It is well recognized that when the feed containing a high level of soluble

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protein and a low level of carbohydrate are fed, the concentration of non-protein, especially ammonia, of the rumen contents increases remarkably and a significant part of total nitrogen ingested are absorbed through the rumen wall as ammonia. (2,3) Ammonia absorbed into the ruminal vein is lead via the portal vein to the liver and usually converted to urea. Although a portion of blood urea returns to the rumen as the urea of the saliva, a major part of it is excreted into urine without utilization by the tissues. Therefore rapid and excessive production of ammonia in the rumen not only results in a wastage of nitrogen to the animal but may also give rise to toxic symptoms. In general, ammonia poisoning occures during a decrease of urea formation in the liver failure and during administration of large amounts of urea to the ruminant. However, it is considered in practice that high yielding dairy cows may always be exposed to danger, when a lot of ammonia enters the liver, by the very high intake of a protein diet. It is also possible that metabolic activities may be impaired through excessive ammonia production without any clinical disorder.

Several groups of workers (4–9) have studied the relationship between the ammonia concentration in the rumen and in the peripheral blood under various feeding conditions and on the metabolic consequences of artificially produced ammonia poisoning in the ruminant. However, little attention has been given to the extent of the detoxicating capacity of ammonia.

The present study was carried out to estimate the extent of the capacity of the liver to detoxicate ammonia entering through the portal blood and to know the correlation of ammonia concentration in the peripheral blood and in the rumen contents.

Experiment A: On the extent of the capacity to detoxicate ammonia infused into the portal vein of the goat

Experimental Procedure

1) Animal and infusion procedure

Nine adult goats of Saanen breed were used for the experiment. Each animal was fed with orchard hay and concentrates. Prior to the experiment, the polyethylen catheter was introduced into the portal vein to infuse an ammonia solution. The animal was anesthetized locally with the procain hydrochloride and the abdomen was opened. The polyethylen catheter was inserted into the mesenteric vein and its tip was reached at the porta hepatis. The other end of the tube was passed through the body wall and the abdomen was suitured. The catheter was filled with heparin-saline to prevent blockage from blood coagulation. An ammonium carbonate solution having different concentrations were infused constantly into the portal vein through the catheter. Before infusion an ammonium carbonate solution to be infused was neutralized with diluted acetic acid at pH 7.4. In these

experiments, although the amounts of ammonia nitrogen infused varied from 11.8 to 36.0 mg per hour per kg of body weight, the volume of infused solution was regulated at a rate of 100 ml per hour by employing a speed controlled injector.

2) Chemical method

Blood samplings from the jugular vein were carried out several times during the experimental period to determine the concentrations of ammonia and urea in the peripheral blood. Blood ammonia was estimated by the diffusion method of Conway & Cooke (10). Blood samples for the ammonia determination were delivered into the tube containing small amounts of potassium oxalate and mixed

Table 1. The amounts of ammonia-N infused into the portal blood of the experimental animals.

Goat	A Body weight (kg)	B Amounts of infused ammonia-N (g/hr)	$\frac{B}{A}$ (mg/hr/kg)
1	36.0	1.3	36.1
2	27.0	0.9	34.3
3	28.0	0.81	29.0
4	31.5	0.78	25.0
5	36.0	0.60	16.6
6	23.0	0.30	12.6
7	32.0	0.40	11.8
8	36.0	water	—
9	45.0	saline	—

quickly and 1 ml of blood was put into a diffusion vessel within 20 seconds after blood sampling.

Urea concentration in blood was determined by the diffusion method of Conway & O'Mally (11) using urease.

In addition to the blood analyses, the physiological conditions of the animals following the infusion of an ammonium carbonate solution were observed.

Results and Discussion

1) Blood ammonia concentration

The ammonia concentration in the jugular blood before the ammonia infusion in nine goats was around 50 to 100γ per dl as seen in Fig. 1. Refering to the ammonia level in the peripheral venous blood, Head & Rook (5) reported 0.25 m mole per 1 in the cow and Lewis (4) found 0.04 to 0.1 m mole per 1 in the sheep under normal feeding conditions.

When goats were infused with water or physiological saline, ammonia concentrations of the blood did not change at all. On the other hand, when different concentrations of ammonium carbonate solution were infused into the portal vein, the curves of the blood ammonia concentration were clearly distributed

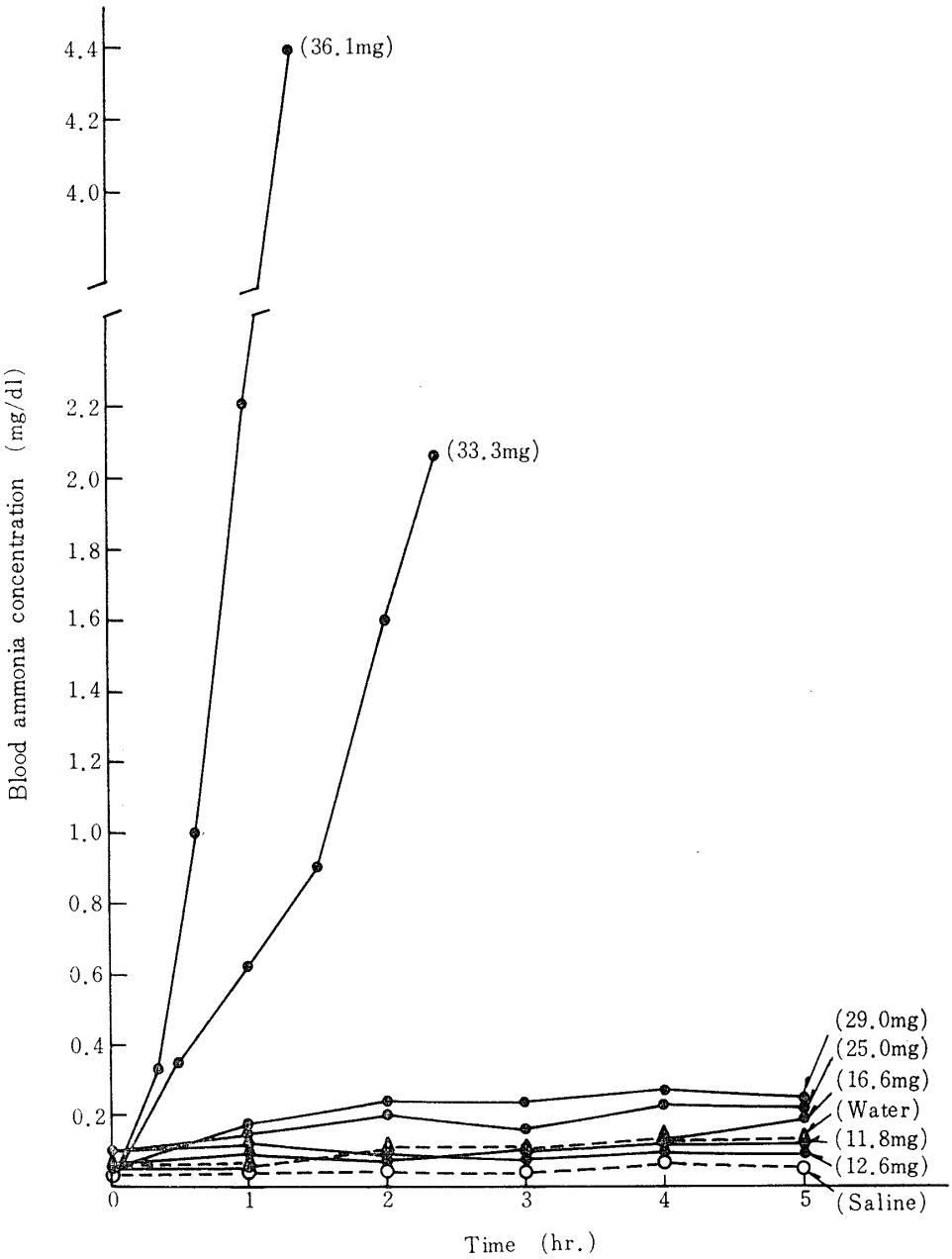

Fig. 1. Changes in the ammonia concentration in the jugular blood during infusion of ammonium carbonate solution into the portal blood of the goat.
Figures in parentheses represent the infusion rate of ammonia-N (mg per hour per kg of body weight)

into two zones. As given in Fig. 1, in the experiments with infusion rates of 11.8, 12.6 and 16. 6mg per hour per kg of body weight the curves of blood ammonia concentrations were almost the same pattern as in water and saline infusions. In the experiments with infusion rates of 25 and 29 mg per hour per kg, although the concentration of blood ammonia increased sligthly during infusions, its concentration did not rise beyond level of 300γ per dl. However, when ammonia solution was infused in the rates of 33 and 36 mg per hour per kg, the blood ammonia con-

centration sharply increased in a short period. Both animals revealed toxic symtoms such as somnolence, drowsiness and tetany, and died within one and half hours and two and half hours of infusion period.

It has been recognized that ammonia absorbed from the rumen and reaching the liver via the portal vein, is usually removed and is not therefore normally found other than in trace amounts in the peripheral blood. The result in the present experiments indicates that the liver has a certain extent of the capacity to detoxicate ammonia entering through the portal vein. Lewis (4), who used sheep in which the ammonia content of the rumen was increased stepwise by repeated additions, found that although the ammonia concentration of the portal blood increased with that of the rumen contents, no significant change in the ammonia concentration of the arterial blood occured until the portal blood contained about 0.8 m mole per liter and that above this level the ammonia concentration in the arterial blood increased at almost the same rate as the concentration in the portal blood. We did not determine the ammonia concentrations in the portal and arterial blood. It is possible that the ammonia concentration in the jugular venous blood may be lower than that in the arterial blood because ammonia is combined with glutamic acid to form glutamine in the brain.

Lewis (4) found that toxic symptoms were developed when the ammonia concentration of the arterial blood reached 0.4 to 0.5 m mole per 1. In the present experiments it was observed that the animals revealed light symptoms such as restlessness and somnolence when the ammonia concentration of jugular blood exceeds level of 300γ per dl and that such concentration of the jugular blood ammonia were estimated only in the infusion rates of 33 and 39 mg per hour but not in the rates under 29 mg per hour. Therefore it is considered that the extent of the capacity to detoxicate ammonia entering through the portal blood may be around 30 mg per hour per kg of body weight and that when the amount of ammonia entering exceeds its capacity the ammonia concentration in the peripheral blood remarkably increases and toxic symptoms are encountered.

2) Blood urea concentration

Fig. 2 showed the changes of urea concentration in the jugular blood during the infusion with different concentrations of ammonium carbonate solution.

No significant change in the blood urea concentration was detected in the infusion of water or physiological saline. In each of the experiments in which ammonia solution was infused, blood urea concentration remarkably increased during the infusion period. The urea formation in the liver increases proportionally with an increase in quantity of ammonia infused as indicated by gradually increasing the urea concentrations in the jugular blood. However, the quantitative information on the capacity of urea formation in the liver could not be obtained since there was no indication of any significant difference in the urea concentration between the died and the torelated animals with ammonia infusion.

In the present experiment the maximum urea concentration in the jugular blood was around 60 mg per dl. Lewis (4) reported that the urea concentration in the arterial blood reached 60 mg per dl when the ammonia concentration in the arterial blood increased 0.5 m mole per 1 by the repeated addition of an ammonium acetate solution into the rumen.

It has been established that a part of the urea in the blood returns into the rumen as urea of the saliva and most of it is excreted into the urine without the utilization by the tissues. Since the urea concentration in the peripheral blood and the quantity of the ammonia infused through the portal vein were roughly parallel as shown in Fig. 2, the urea concentration of the peripheral blood may reflect the degree of nitrogen wastage, as suggested by Annison (12).

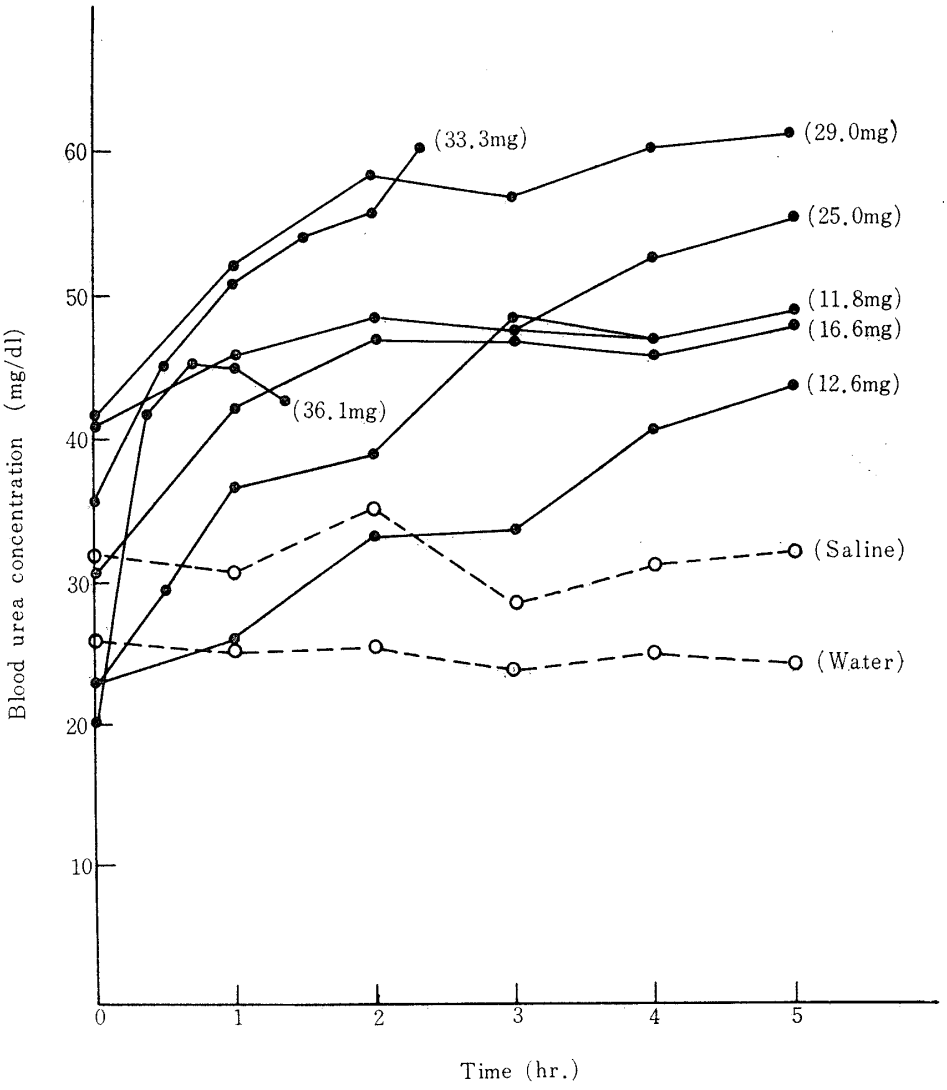

Fig. 2. Changes in the urea concentration in the jugular blood during infusion of ammonium carbonate solution into the portal blood of the goat.
Figures in parentheses represent the infusion rate of ammonia-N (mg per hour per kg of body weight)

Experiment B: On the correlation of the ammonia concentration in the portal and the rumen contents of the goat

Experimental Procedure

Three goats of Saanen breed with the rumen fistula were used in the experiments.

Just before the experiment most of the rumen contents were removed through the rumen fistula and the emptied rumen was washed out several times with the warm physiological saline. Then the ammonium carbonate solution having a different concentration was placed in the emptied rumen through the rumen fistula.

The samples of the jugular blood and of the rumen contents were removed at frequent intervals to determine the ammonia concentration. Blood ammonia was estimated by the diffusion method of Conway & Cooke (10). The ammonia of the rumen contents was determined by the diffusion method of Conway (13).

Results and Discussion

As described above, the goats revealed symptoms of ammonia poisoning when the ammonia concentration in the jugular blood exceeded around 300γ per dl. It is important to know the correlation of the ammonia concentration in the rumen contents and the peripheral blood.

The results collected from the different experiments are given in Fig. 3. As shown in the figure, the ammonia concentrations in the jugular blood did not increase over 200γ per dl until the rumen contents contained about 50 mg per dl. When the rumen concentration exceeded this level, blood ammonia concentration gradually increased as the rumen concentration increased.

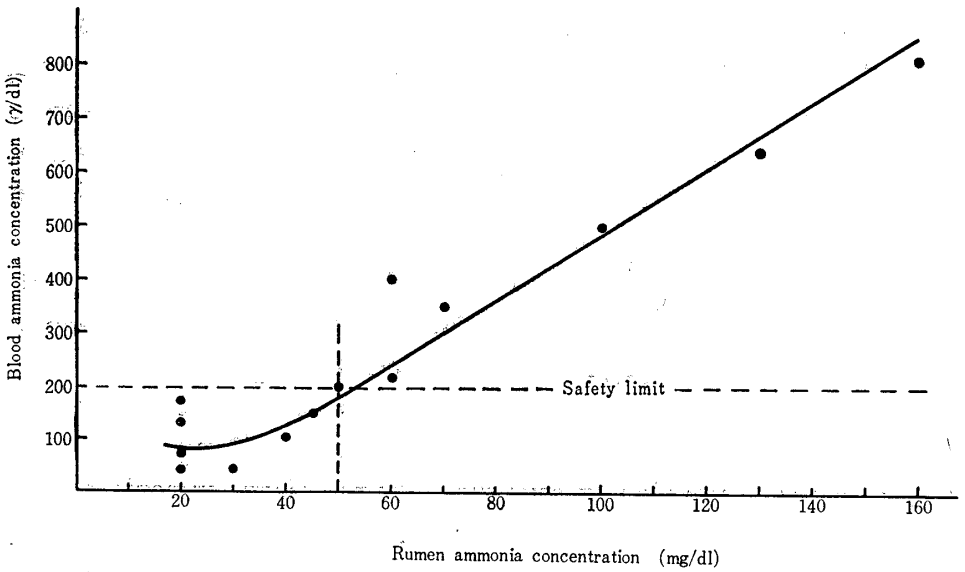

Fig. 3. The correlation of ammonia concentration in the jugular blood and in the rumen contents of the goat.

Lewis (4) determined the ammonia concentrations in the rumen contents and in the peripheral blood under various feeding conditions and after adding repeated doses of ammonium acetate to the rumen, found that ammonia appears in the peripheral blood when the rumen concentration exceeds 55 to 60 m moles per 1. The critical levels in the rumen ammonia are higher than that observed in the present experiment. It may be possible that this discordance of the critical level in the rumen ammonia may have been due to the experimental procedure in which the ammonia solution was placed in the emptied rumen in the present experiment.

When a gradual increase occured in the ammonia level in the jugular blood various toxic signs were revealed. It would appear there is a close interrelation between the ammonia concentrations in the jugular blood and the severity of a symptom of poisoning during the experiments of the portal infusion and the ruminal introduction of the ammonium carbonate solution. The following symptoms were observed; restlessness appeared whithin about 200 to 250γ per dl of blood ammonia, somnolence in 250 to 300γ per dl, atoxia in 300 to 400γ per dl, coma in 400 to 500γ per dl, labored breathing in 500 to 700γ per dl, tetany in 700 to 1000γ per dl and death in over 2000γ per dl.

Summary

(A) An ammonium carbonate solution having different concentrations were constantly infused into the portal vein by employing a portal vein catheter to estimate the extent of the capacity to detoxicate ammonia entering through the portal blood of the goat.

1) No significant increases in the ammonia concentrations of the jugular venous blood were detected until the infusion rate of ammonia was about 30 mg per hour per kg of body weight. However, when the infusion rate of ammonia exceeds this level the blood ammonia concentration greatly increased and the animals died in a short period after the appearance of the poisoning symptoms.

2) The increases of urea concentration in the jugular blood were roughly paralleled by the increasing of the infusion rate of ammonia. However, there was no indication of any significant difference in the urea concentration between the died and the tolerated animals with the ammonia infusion.

(B) An ammonium carbonate solution having different concentrations were placed in the emptied rumen to learn the correlation of the ammonia concentration in the rumen contents and the jugular venous blood of the goat.

1) The ammonia concentration in the jugular blood did not increase over 200γ per dl until the rumen contents contained about 50 mg per dl. However, when the rumen concentration exceeds this level the blood ammonia concentration gradually increased as the rumen concentration increased.

2) It would appear there is a close interrelation between the ammonia con-concentrations in the jugular blood and the severity of a sign of poisoning.

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