The Absorption of Ammonia from the Rumen of the Sheep

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The peculiar anatomical arrangement of the digestive tract of the ruminant presents special problems in the elucidation of the processes of digestion in these species. The food is received, after swallowing, into the capacious rumen and reticulum where extensive fermentative changes are effected by the abundant micro-organisms which inhabit these organs. This unique arrangement confers on the ruminants an efficient mechanism for the digestion of cellulose and other polysaccharides of their exclusively herbaceous diet. The favourable conditions maintained in the rumen and reticulum for the growth of micro-organisms result in extensive conversion of the nitrogen of the animal's food to the use of the micro-organisms for their own growth. That ammonia should appear in the ruminal contents is not surprising in view of the fact that ammonia is the chief nitrogenous end product in the breakdown of proteins by bacteria, while at the same time ammonia is the chief source of nitrogen in the nutrition of many bacteria (Stephenson, 1939).

Schwarz & Steinlechner (1925) were unable to detect ammonia in the rumen contents of cattle, but their observation is at variance with that of all subsequent workers, e.g. Mangold & Schmitt-Kramer (1927); Harris, Work & Henke (1943); Wegner, Booth, Bohstedt & Hart (1941); Lenkeit & Becker (1938). Interest in the non-protein nitrogen fractions in the rumen contents has chiefly been directed to the phenomenon of the utilization by ruminants of urea and amides as a source of protein through the mediation of the micro-organisms of the fore stomachs; recent reviews of the extensive literature on this subject have been given by Smith (1945) and McClymont (1946). That urea is rapidly converted to ammonia by microbial activity in the rumen was demonstrated by Lenkeit & Becker (1938) and confirmed by later workers. However, it appears to have been overlooked that the saliva in those species studied contains urea in concentrations proportional to that in the blood (Hench & Aldrich, 1922, 1923; Schmitz, 1923; Komarov & Stavratsky, 1940). Observations recorded in this paper show that urea occurs in the saliva of the sheep, and in view of the fact that the sheep secretes very large volumes of saliva (Scheunert & Trautmann, 1921; Scheunert & Krzywanek, 1930) this source of urea, and hence of ammonia, in the rumen contents assumes some significance.

Little attention has previously been paid to the role of ammonia in the digestion of natural food-stuffs in the rumen; some representative data on the changes in the concentration of ammonia in the rumen contents are presented in this paper. Ammonia in the ruminal contents appears to derive chiefly from the deamination of food proteins and from urea secreted in the saliva. Ammonia could disappear from the rumen liquor by its utilization by bacteria for their growth, by passage in the ruminal ingesta from the rumen to the abomasum, or by direct absorption.

Barcroft, McAnally & Phillipson (1944) demonstrated that volatile acids (acetic, propionic and butyric) are absorbed from the rumen. Further, it has long been known that ammonia is absorbed from the intestinal tract of monogastric animals (Folin & Denis, 1912; Parnas & Klisiecki, 1926; Koprowski & Uninski, 1939). These facts suggested that ammonia might be absorbed from the rumen of the sheep; the experiments reported in this paper demonstrate that this is indeed the case.

METHODS

Ammonia in blood was estimated by the method of Conway & Cooke (1939). For the collection of blood from the visceral veins, the technique developed by Barcroft *et al.* (1944) was used. The experimental animal was anaesthetized with nembutal (pentobarbital), the abdomen was opened and the visceral veins exposed. Blood was withdrawn into a syringe, with as little interference to blood flow as possible. No technique is as yet available for withdrawing blood from the ruminal veins in the normal, unanaesthetized animal.

Ammonia in rumen liquor was estimated by the method of Conway (1947) using potassium metaborate as the alkaline reagent. Corrections for the slight deamination caused by the alkali were applied. The rumen liquor was prepared by passing through muslin ruminal contents collected through a permanent rumen fistula.

Urea in saliva was estimated by the method of Conway & O'Malley (1942).

RESULTS

Ammonia in blood

Preliminary analyses of the jugular blood of normal sheep confirmed the general conclusion of Conway (1947) that in the blood of the general circulation there is almost no ammonia present. Conway has shown that ammonia is formed in

human blood within a few minutes after collection, and that during absorption of ammonia in the Conway unit gradual deamination occurs. In the experiments quoted below the technique adopted necessitated the passage of some 2-4 min. from the time of insertion of the hypodermic needle into the vein until the blood sample had been delivered into the Conway units (analyses were performed in triplicate or quadruplicate). No data are available for applying corrections for the rate of ammonia formation or rate of deamination by alkali in the blood of sheep. For this reason Conway's corrections for deamination as applied to human blood have been used, but no correction has been applied for ammonia formation during the interval between collection of the blood and its mixture with the potassium carbonate in the Conway unit. Thus no special accuracy can be claimed for the values given for ammonia in blood drawn from the jugular vein or the carotid artery; it can only be concluded that these values are extremely small. In analyses of blood from the ruminal veins, the errors involved are of comparatively little significance.

The results of six experiments are given in Table 1; it was consistently found that the blood of the ruminal veins contained significant amounts of ammonia; the values averaged 1.7 mg. ammonia N/100 ml. blood.

Barcroft *et al.* (1944) had previously observed that the concentration of volatile fatty acids was higher in the anterior ruminal vein than in the posterior ruminal vein; they ascribed this to the fact that the posterior ruminal vein drained an area of the rumen which covered a gas space, which thus, in part, removed the ruminal mucosa from contact with the ingesta. In Exps. 5 and 6 (Table 1),

analyses were made of blood from both ruminal veins and in each case the level of ammonia was slightly higher in the anterior ruminal vein.

The observations made in Exp. 2 confirm the findings of Folin & Denis (1912) and of Parnas & Klisiecki (1926) that ammonia is also absorbed from other regions of the gastro-intestinal tract.

In order to establish that the ammonia found in the ruminal veins is in fact derived from the ruminal ingesta, two experiments were performed. Two sheep were fasted for 24 hr. and then the contents of the rumen and reticulum were removed through a rumen fistula, and the rumen was washed out as thoroughly as possible with warm water. Under nembutal anaesthesia the oesophagus was ligated, the fluid again removed from the rumen and replaced with warm water, and the posterior ruminal vein exposed by laparotomy. Samples of blood from the ruminal vein were taken for estimation of ammonia. The fluid was then removed from the rumen and replaced with 4 l. of a warm solution of ammonium acetate. After an interval of 30 min., blood samples were again taken from the posterior ruminal vein and from the carotid artery. The results of the two experiments are set out in Table 2.

It has been found difficult to wash the rumen quite free of ingesta, and this explains why some ammonia remains in the rumen fluid after washing, and why the blood draining the washed rumen still contains more ammonia than the blood in the general circulation. The marked rise in the level of ammonia in the ruminal blood, after the rumen has been filled with a dilute solution of ammonium acetate, leaves no doubt that ammonia can be absorbed from the rumen.

Table 1. *Concentration of ammonia in the blood from different vessels in sheep*

Vein	Exps.	Ammonia N (mg./100 ml. blood)					
		1	2	3	4	5	6
Jugular	—	0.0	0.0	0.1	—	—	—
Carotid (artery)	—	—	—	—	0.07	0.02	0.06
Posterior ruminal	—	1.9	1.9	1.5	1.4	2.2	1.3
Anterior ruminal	—	—	—	—	—	2.4	1.6
Abomasal	—	—	0.1	—	—	—	—
Intestinal	—	—	0.6	—	—	—	—
Caecal	—	—	0.8	—	—	—	—

Table 2. *Absorption of ammonia from the rumen of sheep*

Origin of samples	Ammonia N			
	Experiment 7		Experiment 8	
	(mg./100 ml. blood)	(mg./100 ml. ruminal fluid)	(mg./100 ml. blood)	(mg./100 ml. ruminal fluid)
Ruminal fluid, beginning of experiment	—	0.2	—	0.4
Posterior ruminal vein, 1st sample	0.13	—	0.36	—
Ruminal fluid containing ammonium acetate	—	15.4	—	22.6
Posterior ruminal vein, 2nd sample	0.93	—	1.64	—
Carotid artery	0.07	—	0.07	—

Ammonia in the rumen contents

In the course of studies on the digestion of protein in the sheep, numerous analyses of the content of ammonia in the rumen liquor were made; it has been repeatedly observed that ammonia N may constitute a significant fraction of the total N in the rumen liquor. A typical curve showing changes in the concentration of ammonia in the rumen liquor after feeding on pasture are given in Fig. 1;

Fig. 1. Change in concentration of ammonia in the ruminal contents of a sheep after grazing at pasture.

the animal was fasted overnight and returned to the pasture after two preliminary samples of rumen liquor had been collected. The figure shows clearly that the concentration of ammonia rose steadily for some hours after feeding on pasture, and attained a maximum value of about 35 mg. ammonia N/100 ml. rumen liquor.

Urea in saliva

Samples of mixed saliva were collected from three sheep on 2 days just prior to feeding. The values obtained for the concentration of urea in the saliva are given in Table 3.

Table 3. Concentration of urea N in the saliva of sheep

Sheep no.	Urea N on 2 days (mg./100 ml.)
1	8.2, 13.6
2	6.5, 7.4
3	15.0, 10.4

DISCUSSION

The fact that ammonia is absorbed from the rumen raises several interesting points. Since urea is present in the saliva it appears that a N circulation occurs during the normal digestive process; the salivary urea is converted by microbial activity in the rumen into ammonia, which may be absorbed into the venous blood and passed via the portal vein

into the liver; here the ammonia will be converted into urea and again become available for secretion in the saliva. The significance of this cycle will clearly be dependent on the relation between the amount of urea N secreted daily in the saliva and the amount of ammonia N absorbed daily, for it seems probable that any nitrogen thus absorbed in excess of that secreted in the saliva would constitute a wastage in metabolism. Data are not yet available to allow accurate assessment of these quantities, but preliminary calculations suggest that ammonia N absorbed may well exceed by significant amounts the urea N secreted in the saliva. Barcroft *et al.* (1944) estimated the rate of flow of blood from the rumen and reticulum as 225 ± 65 ml./min. (This figure has been recalculated from the data of Barcroft *et al.* (1944) as the actual values for total blood flow from the rumen and reticulum are not quoted in their paper.) If then the efferent blood contains 1.5 mg. ammonia N/100 ml. and the rate of flow be taken as 200 ml./min., the absorption of ammonia amounts to 4.3 g. N/day. For comparison, values for saliva may be taken as 5 l./day with 10 mg. urea N/100 ml. representing a salivary secretion of 0.5 g. N/day. Further, it appears that nitrogen, derived from the food, may in part be lost to the animal by this means and will thus constitute a factor in determining the biological value of the food protein. It is evident, too, that when urea is fed to the animal as a source of nitrogen, part at least will be absorbed from the fore stomachs as ammonia and hence be valueless to the animal.

SUMMARY

1. Ammonia occurs only in traces (if at all) in the blood of the general circulation of the sheep while the venous blood draining the rumen contains about 1.5 mg. ammonia N/100 ml.; it is, therefore, concluded that ammonia is absorbed from the rumen. Addition of a solution of ammonium acetate to the rumen after removal of the ingesta produces a prompt rise in the level of ammonia in the blood draining the organ. It is calculated that the quantity of ammonia N absorbed from the rumen may be of the order of 4–5 g./day.

2. Urea occurs in significant quantities in the saliva of the sheep. Attention is drawn to the circulation of nitrogen in the normal digestive process, viz. urea in saliva is converted in the rumen into ammonia, which may be absorbed into the portal blood stream and converted by the liver to urea, which again becomes available for secretion in the saliva.

3. Ammonia derived from the deamination of proteins in the feed contributes an important fraction of the nitrogen of the ruminal ingesta.

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The Biochemistry of Bacterial Toxins

2. THE ENZYMIC SPECIFICITY OF *CLOSTRIDIUM WELCHII* LECITHINASEBy MARJORIE G. MACFARLANE, *Lister Institute of Preventive Medicine, London*

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The general properties of a lecithinase present in the toxic culture filtrates of *Clostridium welchii* were described by Macfarlane & Knight (1941), who concluded that this enzyme was probably identical with *Cl. welchii* alpha toxin, the main lethal component of these filtrates. Since none of the *Cl. welchii* toxins examined hydrolyzed diphenyl-, monophenyl- or β -glycero-phosphate, or nucleic acid, this enzyme appeared to be a true lecithinase, as distinct from a phosphodiesterase, although the linkage attacked is of the diester type leading to the formation of phosphorylcholine and a diglyceride. In a further study of the enzyme specificity, Macfarlane (1942) reported briefly that kephalin was not decomposed by *Cl. welchii* toxin, but sphingomyelin was slowly hydrolyzed; the products of this hydrolysis, however, were not isolated.

Recently Zamecnik, Brewster & Lipmann (1947), who were unaware of this finding, examined the specificity of the toxin by an elegant adaptation of the manometric technique, and stated that it was inactive towards sphingomyelin, as well as to the other phospholipins tested. It seemed possible that the negative result of these authors with sphingomyelin was due to the short reaction time and low toxin concentration employed, but it was desirable to confirm the earlier positive results in more detail, since the specificity of the lipolytic activity of the

toxin is a matter of some interest. *Cl. welchii* toxins in general appear to be free from phosphoesterases, lipase and nuclease, and the specificity of action might therefore be exploited for the differential estimation of phospholipins, for the elucidation of phospholipin structure or for the stepwise degradation of lipoprotein compounds.

The sample of sphingomyelin used in these experiments was kindly given to me by Dr O. Rosenheim, as one which, from its method of preparation as an ether-insoluble fraction, was free from lecithin; it contained, however, about 25 % cerebroside. Several hydrolyses of this crude sphingomyelin with *Cl. welchii* toxin have been carried out, in which from 70 to 90 % of the phosphorus present was converted into a water-soluble compound, with a satisfactory recovery of phosphorylcholine. It proved difficult, however, owing to the overlapping solubilities in the solvents tried, to separate the fatty product, which on the classical formula of sphingomyelin should be lignoceryl sphingosine, from the accompanying cerebroside in sufficient yield and purity to characterize the product, though evidence of the formation of a nitrogenous product free from phosphorus and carbohydrate was obtained. It is clear also, since the starting material may be a mixture of 'sphingomyelins', that the hydrolysis product may be a mixture containing a variety of fatty acid residues,