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NITROGEN METABOLISM IN THE RUMEN

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THE MAJOR FUNCTION of ruminants as domestic animals is to act as converters of plant proteins to animal proteins: meat, wool, milk proteins and hides. By contrast, the major function of the rumen* is to provide acceptable sources of energy from otherwise indigestible polysaccharides; all other reactions in the rumen can be looked upon as secondary, and sometimes these prove to be a gain, sometimes a loss, in the animal's nutritional economy. Some of these complexities form the subject of this brief review.

The reactions undergone by nitrogenous substances in the rumen are essentially the consequence of microbial activity. The ruminal milieu is so favourable and so well controlled that the microbial population is one of the most dense found in nature. Nevertheless, the indications are that much of the chemical activity in the rumen is associated with the metabolism of the microbes rather than with their growth; the consequence of this is discussed below.

SOURCES OF RUMEN NITROGEN

The bulk of the nitrogen entering the rumen under ordinary conditions of feeding is in the form of protein. Some fodders—*e.g.*, young, rapidly growing plant leaves—have important fractions of non-protein-nitrogen (N.P.N.) comprising amino acids, amides and a wide variety of metabolites in small concentrations (Steward and Pollard, 1957). In some special instances, the fodder contains significant amounts of nitrates—occasionally, in toxic amounts. The saliva, which is secreted in such large volumes, contains both proteins and N.P.N.; the largest fraction of the N.P.N. is urea (McDonald, 1948). Urea also enters the rumen by diffusion from the blood (Simmonet *et al.*, 1957).

*In this paper the word "rumen" usually implies the complex of "rumen and reticulum" which function together as a unit.

DEGRADATION OF NITROGENOUS SUBSTANCES IN THE RUMEN

The microbial mass provides a strongly proteolytic system, the activity of which seems to be largely independent of the feed being consumed (Annison, 1956; Warner, 1956; Blackburn and Hobson, 1960). Consequently, degradation of feed proteins starts immediately after ingestion. The rate of protein breakdown varies greatly with the nature of the feed; in general, soluble proteins are rapidly attacked, while insoluble proteins are much more resistant (McDonald, 1952; Annison, 1956; Lewis, 1960). This difference is of interest especially in regard to plant leaf proteins which are readily soluble in dilute salt solutions, comparable to rumen liquor, while the leaf is fresh and green, but become highly insoluble when denatured by drying—as in hay-making or after the plant matures. The proteins of fodder concentrates form a very varied group; for example, much of the protein of peanut meal is readily soluble and quickly degraded in the rumen, while the alcohol-soluble protein, zein, of maize is quite insoluble in water and is very slowly attacked in the rumen. The susceptibility to ruminal digestion and the nutritive value of a protein can be affected by the influence of processing on the solubility of the protein of the feed stuff (Chalmers *et al.*, 1954).

The microbial proteolytic enzymes appear to be conventional in their properties, but amino acids and peptides do not accumulate in the rumen fluid. The concentrations are quite small in the rumen fluid but are increased after feeding. Most of the amino acid nitrogen is associated with the micro-organisms. It is interesting that, although absorption of ammonia and volatile fatty acids occurs in the rumen, amino acids are apparently not absorbed even when the concentration in the rumen is much higher than in the blood (Annison, 1956).

Amino acids are further subjected to degradation by microbial deaminases with the formation of ammonia. This enzymatic activity is dependent on the nature of the ration being consumed (el-Shazly, 1952) and can lead to the accumulation of high concentrations of ammonia.

Rumen contents also have high urease activity (Lenkeit and Becker, 1938), but the organisms primarily responsible for this activity have not yet been identified. Thus urea from saliva, the blood, or feedstuffs is very rapidly hydrolysed with the formation of ammonia and CO_2. Enzymes capable of attacking other nitrogenous substances have received little attention, though a very active desamidase has been demonstrated (McDonald, unpublished). It is probable that many minor dietary components are degraded with ammonia as the chief end product.

Since ammonia is the chief nitrogenous end-product of bacterial intermediary metabolism, it can be anticipated that this is another source of rumen ammonia. The nitrogenous end-products of protozoal metabolism have not been determined; urea could be a major excretory product and would be rapidly degraded to ammonia.

Further, the inorganic nitrate found in some fodders is rapidly reduced to nitrate and thence, via intermediates not yet identified, to ammonia. The conversion of nitrite to ammonia is very rapid so that high concentrations of nitrite are not produced; however, the nitrite is so readily absorbed and is so toxic that it can cause fatal intoxication (Lewis, 1951; Jamieson, 1959; Wang *et al.*, 1961).

It is clear, then, that ammonia is the key nitrogenous metabolite in the rumen and there is now a good deal of evidence to suggest that the nutritive value of the dietary nitrogen is in large part determined by the production and subsequent fate of ammonia.

THE FATE OF RUMEN AMMONIA

Three aspects need to be considered: (a) Passage out of the rumen with the fluids flowing through the rticulo-omasal orifice to the omasum and abomasum; (b) Absorption from the rumen; (c) Uptake for synthetic reactions by rumen microbes.

The output is determined simply by the ammonia concentration and the rate of flow of fluid to the omasum. Fairly satisfactory methods are now available for measuring this flow rate, based on the change in concentration of soluble, but non-absorbable, markers, the most effective of which have been polyethylene glycol (Sperber *et al.*, 1953) and ^{51}Cr-EDTA (Downes and McDonald, unpublished). ^{51}Cr-EDTA is the highly stable complex of chromium with ethylenediaminetetra-acetic acid; by using radioactive Cr, the analysis can be made accurately, simply and quickly. These methods are described as only "fairly" satisfactory because the calculations require an assumption that total rumen volume remains constant—that is, the output to the omasum is equal to the input from saliva and drinking water; over periods of about 24 hr this is probably close to the truth, but over short periods may be erroneous. The ammonia leaving the rumen by this pathway is readily absorbed by the gut into the portal blood stream.

Ammonia is also absorbed directly through the walls of the rumen and reticulum and flows via the ruminal veins into the portal vein near its entry into the liver. No really accurate procedures have been devised to measure the amount of ammonia thus absorbed, but there is no doubt that the quantities are important (McDonald, 1948; Head and Rook, 1955; Lewis *et al.*, 1957).

The ammonia absorbed by these two pathways passes into the liver, is taken up by the liver cells and converted to urea. In normal circumstances, the blood of the general circulation contains only traces of ammonia. However, when the rate of absorption of ammonia is increased by high concentrations in the rumen, the uptake capacity of the liver is exceeded, ammonia passes into the general circulation and signs of toxicity appear (see below).

It was noted above that urea passes into the rumen via the saliva and by direct diffusion. Thus a nitrogen-cycle is established: urea enters the rumen, is converted to ammonia, which is absorbed into the portal blood and converted to urea in the liver, urea passes into the general bloodstream and thus becomes available again for entry into the rumen. There are as yet no satisfactory means for measuring the quantities of nitrogen involved in this cycle and the quantities directly excreted as urea in the urine.

There is abundant evidence that ammonia can be used as a source of nitrogen for microbial growth—indeed three rumen organisms are known to require ammonia for growth (Bryant *et al.*, 1959; Bryant and Robinson, 1961). The micro-organisms use both amino acids and ammonia for growth; the relative contributions of the two are not known.

Growth implies the synthesis of the specific microbial proteins, which subsequently become available for digestion and absorption by the ruminant. Other compounds, notably the nucleic acid bases, are also formed (McDonald, 1954a); possibly as much as 10% of the microbial "protein nitrogen" consists of nucleic acid nitrogen.

Several methods have been used in an attempt to estimate the degree of conversion of fodder nitrogen to microbial protein: the specific chemical properties of pure proteins (zein and casein) in artificial diets were used by McDonald (1954b) and McDonald and Hall (1957); Gray *et al.* (1953) and Weller *et al.* (1958) used physical fractionation together with analysis of lignin/nitrogen ratios for experiments with sheep fed wheaten chaff; Williams *et al.* (1953) used estimations of numbers of rumen microbes with varying protein content of the ration. These varied methods of approach all indicate that a large proportion of the protein available to the ruminant may, in fact, be derived from the bodies of rumen microbes. The methods are still not satisfactory for the study of ruminants on ordinary rations; the ideal procedure would differentiate between microbial and fodder proteins and measure the rate of movement of total protein from the reticulum to the omasum and abomasum.

NON-PROTEIN NITROGEN AS A PROTEIN SUBSTITUTE

From the foregoing comments, it is evident that ammonia is a key intermediate in the transition between fodder proteins and microbial proteins. Long before this was known, N.P.N. in the form of urea had been exploited as a protein substitute; an enormous literature has appeared on this subject, but it is much to be regretted that the emphasis in research has been primarily dietetic rather than physiological. This has resulted in the curious situation that it is still not clear just what factors determine the usefulness of urea in ruminant rations. This uncertainty is reflected in the remarkable fact that urea is widely used in cattle rations in the U.S.A. and very little used in Europe—and this in spite of the similarities in cattle husbandry in the two regions.

Most of the experimental work in Europe and U.S.A. has been directed toward production rations for dairy cattle or fattening beef cattle. In South Africa and Australia, on the other hand, the centre of interest has been on the improvement of low quality rations for maintenance or "survival" of sheep and cattle.

Only three general principles governing the utilization of urea have been firmly established:

(1) Urea contributes useful nitrogen to the ruminant by virtue of being converted to ammonia in the rumen and then being used for the synthesis of protein during microbial growth. It is obvious that, when the basal diet supplies sufficient protein (or other nitrogen) for maximum growth rate of the microbes, an addition of urea can have no useful effect.

(2) This synthesis of protein requires a source of energy, in the form of carbohydrate, for microbial growth. Starch is the most useful carbohydrate, sugars somewhat less useful, and cellulose seems to be quite unsatisfactory. The work of

Lewis and McDonald (1958) suggests that levans and xylans would probably be as useful as starch. The various substances contributing to the "hemicellulose" fraction of roughages have not been adequately tested.

(3) The mode of feeding and the quantity of urea fed must be selected to avoid fatal toxic reactions.

These principles provide much too inadequate a basis for prediction of the usefulness of urea in any given situation; at present, therefore, the practical application must be closely related to experimental conditions or else developed on a trial-and-error basis.

It does not seem to be generally appreciated that there are strict limitations on the growth of organisms (and hence protein synthesis) in the rumen. Under favourable conditions, the microbes can occupy about 20% of the volume of rumen fluid; if we imagine every organism had a volume of 1 μ^3, the rumen contents would contain 2×10^{11} organisms per millilitre. Growth in numbers then can occur only as fast as digesta leave the rumen and are replaced with saliva and ingesta. A consideration of the available data suggests that all rumen organisms can experience, on average, only about three generations per day. If the growth rate is much faster than this, it must be balanced by an equivalent death-rate with lysis; and, since soluble protein does not accumulate in the rumen fluid, the products of lysis must be metabolized by other organisms; the net effect then, in regard to protein synthesis, would simply be equivalent to the smaller number of generations per day. It is noteworthy that, although three generations per day is a high growth rate for the large protozoa, it is very slow growth for small bacteria. Warner (1962) has observed protozoal growth rates up to seven generations per day in the rumen of sheep recuperating from a fast. Most cultures have achieved rates of only one generation per day. The growth rates of rumen bacteria have not been studied. Fast-growing bacteria can have generation intervals less than half an hour.

The conclusion that ruminal bacteria experience only about three generations per day seems much at variance with the high metabolic activity of the rumen microbes as evidenced by the bursts of gaseous output, voltaile fatty acid production and ammonia formation in the first few hours after feeding. Nevertheless, this conclusion seems inescapable, and it is tempting to speculate that strenuous competition for living space and nutrients is a factor in the preservation of the highly characteristic biota of the rumen.

THE TOXICITY OF AMMONIA

Ammonia absorbed from the rumen and other parts of the gastro-intestinal tract is carried in the portal blood and removed by the liver (McDonald, 1948; Lewis *et al.*, 1957). However, when ammonia concentration in the rumen fluid exceeds a level of about 50 mg N/100 ml, the rate of absorption is so great that the liver cannot completely remove the ammonia, which passes on to the general circulation (Lewis *et al.*, 1957). Toxicity may be evident when the ammonia concentration rises above 5 μg N/ml (Lewis, 1960), but insufficient information is available to state the probable fatal concentration. The balance of evidence favours the view that poisoning is due to a direct toxic effect of the ammonia on tissues.

The only major source of dietary intoxication is from the excessive consumption of urea. Coombe and Tribe (1958) have shown that the sheep can consume very large quantities of urea, provided the daily intake is spread over a sufficient number of hours. Undoubtedly, the principal precaution is to ensure that the feeding regime is such that the ammonia concentration in the rumen is not allowed to reach excessive levels.

It has been shown by Head and Rook (1955), Johns (1955) and Annison *et al.* (1959) that ruminants grazing high quality pasture may experience such high concentrations of rumen ammonia that a rise in peripheral blood concentration could be expected. There seems to be no proof yet that such a situation produces disease in sheep or cattle. However, studies in human medicine (Bessman, 1956) suggest that, in conditions with impaired liver function, ammonia intoxication may be important; since numerous diseases in sheep and cattle lead to acute or chronic hepatitis, this aspect merits more attention.

THE INFLUENCE OF RUMINAL DIGESTION ON BIOLOGICAL VALUE OF FODDERS

Very little work has been reported on the amino acid metabolism of ruminants. However, the experiments of Black et al. (1952) and Downes (1961) strongly suggest that ruminants have requirements for essential amino acids similar to those for man and rat. It may, therefore, be forecast that the usefulness of absorbed nitrogen will be determined chiefly or exclusively by the amino acid assemblage taken up by the gut; all other absorbed substances, especially ammonia and the nucleic acid bases, will contribute nothing to the nutritional economy of nitrogen, although, of course, these substances will find their way into the body's nitrogen pool. It seems probable that the only exception to this generalization will be the instance when the dietary regime provides conditions such that the potential rate of microbial synthesis exceeds the supply of nitrogen in the feed; this could occur with a low total nitrogen intake, a high starch intake, and a continuously low concentration of ammonia nitrogen in the rumen. The re-cycling of nitrogen, via urea, could then lead to a highly efficient use of nitrogen for maintenance.

The nutritional value of any fodder nitrogen will thus depend on the amount and composition of the mixture of proteins which leave the rumen; this mixture comprises undigested fodder protein and microbial proteins; the rather small amount of amino acids and peptides in rumen contents (Annison, 1956) cannot alter the picture appreciably. The analyses of Weller (1957) suggest that the proteins of plant leaves and of rumen microbes are not greatly different in amino acid assemblage and hence in nutritive value. Thus for grazing animals, the nutritional problem resolves itself into ascertaining the actual amount of protein that reaches the abomasum and intestines for digestion and absorption, the true digestibility of this protein, and its biological value.

There seems to be a general tendency to equate high quality pasture with high protein content. Yet it seems inevitable that from such pasture a considerable amount of the fodder nitrogen would be wasted owing to the processes of digestion. On other grounds, too, it seems apparent that rich pasture often has a protein content higher than cattle and sheep can use efficiently. The anomaly here cannot yet be resolved. It is possible that the excessive protein is efficiently used as a source of energy; that high protein is necessary to obtain the high feed intake required for high animal production; but it is also possible that the criteria for assessing pasture quality need revision. In any case, it is evident that a greater understanding of the physiological processes involved in digestion, absorption, metabolism and production (growth, milk secretion, wool growth) is essential for a rational approach to efficiency in the use of pastures for animal production.

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