SHORT COMMUNICATIONS

Nitrogen Fixation in Rumen Contents Indicated by the Acetylene Reduction Test

By U. GRANHALL AND P. CISZUK

*Department of Microbiology and Department of Animal Nutrition,
Agricultural College, Uppsala, Sweden*

(Accepted for publication 16 November 1970)

Bergersen & Hipsley (1970) showed that N_2 -fixing bacteria could be isolated at high dilutions from the intestinal contents of humans, pigs and guinea pigs given a low-N diet. Acetylene reduction tests indicated that N_2 -fixation might occur in the intestines.

The possibility of rumen microbial assimilation of molecular nitrogen into usable protein has been considered for many years. Tóth (1948) reported N_2 -fixation in a nitrogen-free culture medium inoculated with bacteria from the rumen or the intestine. Apart from the presence of ammonia, the rumen might provide suitable conditions for N_2 -fixation because of its carbohydrate content, anaerobic milieu, near neutral pH and presence of N_2 . Potential N_2 -fixing bacteria present in the rumen are *Methanobacterium* and *Clostridium* and sometimes *Azotobacter* (Hungate, 1966).

Moisio, Kreula & Virtanen (1969) could not demonstrate N_2 -fixation in the cow's rumen. In their experiments $^{15}N_2$ was passed directly into the rumen, but the high price of $^{15}N_2$ prevented a more thorough investigation. On the other hand, Hardy, Holsten, Jackson & Burns (1968) applied the highly sensitive acetylene reduction technique to rumen samples from a steer and found acetylene reduction. The potential N_2 -fixing activity of a rumen calculated on the basis of the anaerobic results was 10 mg. N_2 fixed/rumen/day. We have used the same method more extensively in respect to animal species and kind of feed.

Samples were obtained from sheep, goat, reindeer and rabbit given rather different diets for a minimum of 2 weeks (Table 1). Rumen and intestinal contents were taken through permanent fistulas and kept, at 37°, for less than 30 min. Some exposure to air during sampling did not seem to influence the tests. N_2 -fixation was measured by the acetylene-reduction technique (Hardy *et al.* 1968). Subsamples of about 1 g. (fresh weight) were gassed with argon for 5 min. in rubber-capped bottles of 7 ml. capacity and then incubated anaerobically for 1 h. at 37° under an argon acetylene mixture containing 10 % (v/v) acetylene. One ml. gas samples were analysed for ethylene formation in a Varian 600 gas chromatograph equipped with a 9 ft stainless steel column of Porapak T run at 90°. N_2 served as carrier gas at a flow-rate of 25 ml./min. Control tests with TCA-treated samples did not give any detectable ethylene production.

Most of the rumen samples showed a small but significant production of ethylene (Table 1). The highest values were obtained some hours after feeding. The amount of ethylene produced did not seem to be greatly influenced by the protein content

of the feed. The sheep were given diets which were high, normal or low in crude protein, while the reindeer and the goat were fed on low-N diets based respectively on 50 or 85 % of lichens (*Cladonia*) which contain only 2 to 3 % crude protein in the dry matter. In a final experiment, conditions were made as suitable as possible for N_2 -fixation. One sheep (L) that had previously shown nitrogenase activity was chosen and given a diet containing only 3.8 % crude protein (Table 1). In spite of low rumen NH_3 content (diurnal variation 12.3 to 0.4 mg. NH_3 -N/100 ml.), relatively high pH (6.7 to 6.2), high carbon/nitrogen value and the addition of molybdenum, N_2 -fixation was not stimulated.

One sample of reindeer faeces and one from the colon content of a sheep showed no ethylene formation, while the caecum contents of a rabbit produced 0.38 nmoles/g./h. This animal was fed on lucerne pellets.

Table 1. *Acetylene reduction tests of rumen contents*

Animal	Feed	C ₂ H ₄ formation* (nmoles/g./h.)				
		Hours after feeding				
		0	0.5	1	2.5	5.5
Sheep L	Grass-hay, grain and artificially dried white clover	0.09	—	—	(1.42)	0.49
	Grass-hay, grain and soya meal	—	—	—	1.12	—
	Grass-hay, straw, starch, minerals + Mo	0.09	0.03	—	0.06	0.11
Sheep U	Grass-hay, grain and artificially dried clover	0.24	—	—	0.70	0.16
Sheep X	Grass-hay, grain and soya meal	0.00	—	—	0.00	0.00
Sheep H	Artificially dried clover	0.00	—	—	1.28	0.83
Goat	Lichens (<i>Cladonia</i>) and clover-grass-hay	—	—	—	0.60	—
Reindeer	Lichens and clover-grass-hay	—	—	0.77	—	—

* Mean value of triplicate tests.

Bergersen & Hipsley (1970) reported an acetylene reduction of 0.75 nmoles/g./h. for human faeces and 75 nmoles/22 h. for a whole guinea pig. Our values for rumen and rabbit caecum activity are of the same order (0.0 to 1.4 nmoles/g./h.). If a rumen which contains 5 kg. material shows an ethylene production of 1 nmole/g./h. throughout the day, it will correspond to a N_2 fixation of 1.1 mg./day. The sheep used here have a nitrogen requirement of the order 10 g./day. Even the N_2 -fixation indicated in the rabbit caecum is much too low to contribute substantially to the nutrition of the animal. Thus, although nitrogenase activity could easily be demonstrated in the rumen and intestines of widely different animals, the very low values, even on low-N diets makes it hardly reasonable to believe that the animals could benefit from it for nutritional purpose.

We wish to thank Professor Stig Hydén and Dr Göran Björnhag for placing the reindeer, goat and rabbit at our disposal and also for their help with the sampling from caecum and colon. A grant from the Swedish Natural Research Council made this investigation possible.

REFERENCES

- BERGERSEN, F. J. & HIPSLEY, E. H. (1970). The presence of N_2 -fixing bacteria in the intestines of man and animals. *Journal of General Microbiology* **60**, 61–65.
- HARDY, R. W. F., HOLSTEN, R. D., JACKSON, E. K. & BURNS, R. C. (1968). The acetylene–ethylene assay for nitrogen fixation: laboratory and field evaluation. *Plant Physiology* **43**, 1185–1207.
- HUNGATE, R. E. (1966). *The Rumen and its Microbes*. London: Academic Press.
- MOISIO, R., KREULA, M. & VIRTANEN, A. I. (1969). Experiments on nitrogen fixation in cow's rumen. *Suomen Kemistilehti B* **42**, 432–433.
- TÓTH, L. 1948. Nitrogen-fixing micro-organisms in the alimentary canal of herbivorous farm animals. *Experientia* **4**, 395–396.