SULPHUR METABOLISM AND EXCRETION STUDIES IN RUMINANTS

XII.* NITROGEN AND SULPHUR COMPOSITION OF RUMINAL BACTERIA

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Abstract

The total sulphur content of mixed ruminal bacteria from sheep fed two different diets was 0·41 and 0·45% of the dry matter, and 97% of this sulphur was in the organic form. The total nitrogen content was 8·9 and 9·0% of the dry matter.

The ratio of total nitrogen : total sulphur and of protein nitrogen : organic sulphur in the bacterial cells was 20·2–21·8 and 18·1–18·9 respectively. The latter values are a little higher than those found by others for plant protein.

These data are discussed in relation to the ruminant's requirement for and utilization of sulphur, and the application of nitrogen : sulphur ratios in techniques used for determining microbial protein synthesis.

I. INTRODUCTION

Information on the total nitrogen content of mixed ruminal bacteria is available (Reed *et al.* 1949; Weller 1957; Leibholz 1972; Smith and McAllan 1973) but there are no data on the total sulphur and reducible sulphur contents of ruminal bacteria.

Species of ruminal bacteria differ in their content of methionine and cystine (Purser and Buechler 1966) and the methionine or cystine content of mixed ruminal bacteria may vary amongst diets (Leibholz 1972). The nitrogen : sulphur ratio of protein from mixed ruminal bacteria might therefore alter with change in diet, since the composition of bacterial species change with diet changes (e.g. Warner 1962). Walker and Nader (1968) used the rate of [^{35}S]sulphide incorporation into microbial protein *in vitro* and the ratio of nitrogen : sulphur in the microbial cells to estimate the rate of synthesis of ruminal microbial protein. Walker and Nader (1970) appear to have applied the nitrogen : sulphur ratio obtained in the earlier study (Walker and Nader 1968) to their later data, but it may be that the ratio is not constant. Furthermore, recalculation of the data of Purser and Buechler (1966) indicate that the nitrogen : sulphur ratio in bacterial protein was approximately 19. Walker and Nader (1968) used a value of 11, which may be too low. If so, their estimates of the absolute rate of microbial synthesis and of the energetic efficiency of growth must also be low.

In the present work the total sulphur, reducible sulphur, total nitrogen, and α-amino nitrogen content of mixed ruminal bacteria was determined and the ratio of

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total nitrogen : total sulphur and of protein nitrogen : organic sulphur was calculated. The bacteria were obtained from sheep fed two diets of different composition, and differing methods of preparing the bacterial samples were used.

II. Materials and Methods

Three groups, each of two sheep, were fed rations 1 (groups 1 and 2) or 2 (group 3) once daily for at least 14 days prior to removing *c.* 1·5 litres of rumen liquor from each animal before feeding. The collected liquor was combined within groups for analysis.

Ration 1 consisted of 775 g of a mixture of 57% oaten chaff, 13% lucerne chaff, 1·3% minerals, and 29% of a proprietary concentrate (Wesfeeds Studbreeders Sheep Cubes, containing *c.* 39% mixed barley and wheat grain, 25% brewers grain residue, 15% linseed, 10% cotton seed, and 9% of molasses residue plus limestone, urea, minerals, and vitamins). Ration 2 was similar to ration 1 except that crushed wheat replaced the sheep cubes.

Collected rumen fluid was strained through bolting silk and centrifuged at 100 *g* for 7 min to remove feed particles and protozoa. The supernatants were decanted and subjected to this treatment twice more. The bacteria in suspension were then collected by centrifugation at 25,000 *g* for 20 min. The cells were dispersed and washed with isotonic saline prior to re-centrifugation. This process was repeated twice more. The cells were then dispersed evenly in a thick suspension. Half of the first preparation (A1) from group 1 (ration 1) was washed with 0·4M perchloric acid (Walker and Nader 1968), resulting in a preparation containing lysed cells (A2). Both preparations A1 and A2 were frozen and dried under reduced pressure. Analyses of preparations B from group 2 (ration 1) and C from group 3 (ration 2) were made on the wet cell suspensions immediately after preparation.

Bacterial cells were analysed for total sulphur and reducible (inorganic plus ester) sulphur by the methods of Bird and Fountain (1970). The mean of six determinations per sample was used. In order to check the results two other methods of total sulphur analyses were employed: the alkaline oxidation method of Tabatabai and Bremner (1972) and the acid oxidation method of Johnson and Nishita (1952).

Bacterial cells were analysed for total nitrogen in triplicate by the macro-Kjeldahl technique. Free ammonia nitrogen in the bacterial sample was determined by the method of Conway (1957).

Analysis of total α-amino nitrogen was performed on hydrolysates of bacteria using the method of Lee and Takahashi (1966). Triplicate samples (0·5 g, 7·5% dry matter) of the cell suspensions were hydrolysed with 10 ml 6N HCl in sealed tubes after sparging with N_2. An autoclave set at 5 kg/cm^2 and 125°C was used and the optimum time for hydrolysis was determined from the results of samples hydrolysed over periods of 15, 20, and 25 hr. The hydrolysates were diluted to 250 ml with 0·5M citrate buffer before analysis. The α-amino nitrogen values for the hydrolysed cells were adjusted for the small amount of ammonia nitrogen in the bacterial samples prior to hydrolysis. Any ammonia nitrogen released during hydrolysis was assumed to be derived from amino nitrogen, although amide nitrogen (if present) would also contribute some ammonia nitrogen.

The dry matter content of the bacterial cells or cell suspensions was determined by drying triplicate samples at 105°C for 20 hr, and organic matter was subsequently determined by ignition at 550°C for 6 hr.

III. Results and Discussion

The organic matter, nitrogen, and sulphur contents and the composition of the nitrogen and sulphur of bacterial cells is shown in Table 1.

The total sulphur data are based on the method of Bird and Fountain (1970). Of the other methods used the acid oxidation technique gave a mean which was 100% and the alkaline oxidation technique a mean which was 101·7% of the values in Table 1.

Washing the bacterial cells with perchloric acid to release intracellular fluids and soluble nitrogen and sulphur compounds did not substantially alter either the

nitrogen or the sulphur content of the cells (preparation A2 *v.* A1; Table 1). There was 6·9% less nitrogen and 3·0% less sulphur in the lysed cells (A2). Approximately 80% of the sulphur loss was due to the removal of reducible sulphur compounds from the cells.

TABLE 1

NITROGEN, SULPHUR, AND ORGANIC MATTER CONTENT OF MIXED RUMINAL BACTERIA

The origin of preparations A1, A2, B, and C is described in Section II

Quantity measured	Composition of preparation (dry matter basis):			
	A1	A2	B	C
Organic matter (%)	77·13	82·66	83·59	84·21
Ash (%)	22·87	17·34	16·41	15·79
Nitrogen:				
Total nitrogen (%) (N_1)	7·89	7·35	9·02	8·92
Crude protein (%) (= $N_1 \times 6{\cdot}25$)	49·3	45·9	56·4	55·8
Ammonia nitrogen (%)	—	—	0·06	0·06
α-amino nitrogen (%) (N_2)	—	—	7·82	7·51
(% of total nitrogen)	—	—	86·7	84·2
True protein (%) (= $N_2 \times 6{\cdot}25$)	—	—	48·9	46·9
Sulphur:				
Total sulphur (%)	0·322	0·312	0·446	0·410
Reducible sulphur (%)	0·013	0·005	0·014	0·012
(% of total sulphur)	3·9	1·6	3·1	2·9
Organic sulphur (%)	0·309	0·307	0·432	0·398
(% of total sulphur)	96·1	98·4	96·9	97·3
Nitrogen : sulphur ratio				
Total nitrogen : total sulphur	24·50	23·52	20·24	21·78
Protein nitrogen : organic sulphur	—	—	18·11	18·87

The true protein content of bacterial crude protein was approximately 85%, a value similar to that reported by Purser and Buechler (1966) for 22 strains of ruminal bacteria (mean 86%) and by Demeyer *et al.* (1972) (83·5%). In the present experiment the contribution of amino nitrogen to total nitrogen may be overestimated due to the possible inclusion of amide nitrogen in the amino nitrogen fraction. However, Smith and McAllan (1970) concluded that amino nitrogen together with nucleic acid nitrogen could account for almost all of the bacterial nitrogen, so the amide nitrogen fraction is probably a minor component.

The total nitrogen content of ruminal bacteria varied from 7·35 to 9·02% of dry matter (Table 1). Differences in the total nitrogen content of bacteria have been shown by Reed *et al.* (1949) (7·6–8·1% of dry matter), Weller (1957) (9·3–12·4% of dry protein extract), Leibholz (1972) (8·5–10·5% of dry matter), and Smith and McAllan (1973) (3·8–7·6% of dry matter). In the present experiment it is possible that the drying of preparations A1 and A2 may have caused losses of nitrogen and sulphur. The total nitrogen content of preparation A1 was 7·89% compared with 9·02% for preparation B, which was obtained from sheep fed the same ration. However, since considerable variation in the concentration of individual organisms

in the rumen can occur amongst sheep, even when fed the same ration (Williams and Moir 1951; Warner 1962), differences found in the total nitrogen and sulphur content and nitrogen : sulphur ratio of mixed ruminal bacteria in the present experiment may be due partly to differences amongst sheep.

The small amount of reducible sulphur found (less than 4% of total sulphur) indicates that bacteria from the rumen are similar to *E. coli* in this regard. However, there is a marked disparity between the concentration of total sulphur in rumen bacteria (0·32–0·45% of dry matter) and that reported by Roberts *et al.* (1955) for *E. coli* (1·1% of dry matter). Cystine and methionine constitute almost all of the organic sulphur in *E. coli* (Roberts *et al.* 1955), and probably also in ruminal bacteria; therefore, since the reducible sulphur content of these cells is low the sulphur in cystine plus methionine should approximate the total sulphur content. Calculations from the data of Weller (1957), Purser and Buechler (1966), Williams and Dinusson (1973), and Leibholz (1972) result in mean total sulphur values of 0·92, 0·83, 0·64, and 0·58 g sulphur per 100 g of amino acids respectively. Allowing for a nucleic acid content of approximately 15% of total nitrogen (Smith 1969; Smith and McAllan 1973) and assuming that 56% of the bacterial dry matter is crude protein (as in the present work), the respective mean total sulphur concentrations (as percentage of dry matter) would be *c.* 0·44, 0·40, 0·33, and 0·28%. These values appear to substantiate those reported here. Despite reports that the amino acid composition of ruminal bacteria is relatively constant (Weller 1957; Purser and Buechler 1966), there seems to be considerable variation between strains of organisms (Purser and Buechler 1966) and between diets (Leibholz 1972) in the amounts of methionine and cystine in the protein of ruminal bacteria. Thus, the methionine and cystine contents (percentage of total amino acids) of bacterial protein reported by Purser and Buechler ranged from 1·3 to 3·3% and from 0·7 to 1·7% respectively. Leibholz's data for methionine and cystine are 0·8–2·1% and 0·6–1·0% respectively. As with reported variation in total nitrogen content it is not clear to what extent these differences are due to analytical variation. However, it may be anticipated that the total sulphur content of protein from mixed ruminal bacteria could vary, depending upon the diet fed and thus on the composition of the ruminal bacteria (Williams and Moir 1951).

The ratios of total nitrogen : total sulphur (20·2–21·8) and of protein nitrogen : organic sulphur (18·1–18·9) in preparations B and C do not differ greatly, and indicate that the ratio of total nitrogen : total sulphur, which is readily determined, should adequately represent protein nitrogen : protein sulphur values of ruminal bacteria. The present data are in the range of the mean values for protein nitrogen : sulphur calculated from the data of Weller (1957), Purser and Buechler (1966), Williams and Dinusson (1973), and Leibholz (1972), assuming that 100 g amino acids contain 16 g nitrogen. The respective data, 17·4, 19·3, 25·0, and 27·6, indicate a wide range of nitrogen : sulphur ratios. Also, the ratio of nitrogen : sulphur in plant protein has been regarded as reasonably constant (14–17·5—see Stewart and Porter 1969), but Jones *et al.* (1971) have shown that this ratio in tropical legume protein may vary widely from the value of 17·5 established for legumes by Dizkshoorn and van Wizk (1967). It is therefore probable that the nitrogen : sulphur ratio in the protein of mixed ruminal bacteria will in some instances depart from that found here (18·1–18·9). Certainly this ratio, or that of total nitrogen : total sulphur (20·2–21·8), differs substantially from that of Walker and Nader (1968).

Walker and Nader (1968, 1970) determined the rate of incorporation of sulphide into microbial protein *in vitro* and, from the ratio of nitrogen : sulphur in microbial protein, calculated the rate of microbial protein synthesis. The nitrogen : sulphur ratio of *c.* 11 used by Walker and Nader appears to be too low. Walker and Nader (1970) calculated a daily yield of 14·4 g microbial crude protein per 100 g of organic matter digested in the rumen. Assuming that the microbial protein was largely bacterial protein, if their data for protein is adjusted by a factor of 1·9 (i.e. 21/11) the estimated yield of microbial crude protein per 100 g of organic matter digested in the rumen would be approximately 27 g. This is a little greater than the maximum estimates of microbial or bacterial protein yield (g/100 g of organic matter apparently digested in the rumen) of 23·3 (Hume 1970) or 25 (Hogan and Weston 1971; Lindsay and Hogan 1972). However, as previously discussed, it is probably not valid to apply data on nitrogen : sulphur ratios obtained under one set of conditions to other experiments which may have been carried out under different conditions. It is suggested that where data on nitrogen : sulphur ratios are required, as in techniques for measuring microbial protein synthesis (Walker and Nader 1968, 1970), such information be obtained for each experiment rather than relying on a fixed ratio for all circumstances.

In order to maximize the efficiency of utilization of dietary nitrogen by bacteria the ratio of nitrogen : sulphur supplied should not be greater than the ratio of those elements in the bacterial cells. The present data indicate that this ratio should be not greater than 21. Bird (1972*a*) found that the ratio of the increment of nitrogen : sulphur stored by sheep was 13·5 when sulphate was added to a sulphur-deficient diet. Therefore, in order to maximize the efficiency of utilization of dietary nitrogen by sheep the nitrogen : sulphur ratio in the feed should be not greater than 13·5. When comparing the nitrogen : sulphur requirement of the ruminant system with that of the ruminal bacteria it should be noted that the ruminant tissues have a definite requirement for sulphate *per se* (see Bird 1972*b*). Ruminants, particularly sheep, also produce substantial quantities of wool or hair keratin which has a high sulphur content and a nitrogen : sulphur ratio of 4–6 (Tristram 1953). The overall dietary nitrogen : sulphur ratio required by the ruminant system must therefore be less than that required by ruminal bacteria. The ratios proposed here are consistent with this.

The nitrogen : sulphur ratio of most body tissue protein is approximately 15, or less (Beach *et al.* 1942; Tristram 1953); thus it is evident that microbial protein is deficient in sulphur amino acids for body tissue synthesis, and that some deamination of non-sulphur amino acids must occur. The difference between the nitrogen : sulphur ratio of bacterial protein (*c.* 18·5) and wool (*c.* 5) must accentuate this loss, since half of the absorbed sulphur amino acids may be used for wool synthesis (Bird 1972*b*), thereby leaving an excess of non-sulphur amino acids. Nolan and Leng (1972) calculated that for sheep ingesting 23·4 g nitrogen per day, 16·4 g of urea nitrogen per day was synthesized daily from deaminated amino acids and from ammonia absorbed from the post-ruminal gut tract. Only a third of this urea was recycled to the gut and the remainder was excreted. Recycling of urea to the gut and re-utilization by ruminal or intestinal microbes therefore does not completely offset the excessive deamination losses of amino nitrogen occasioned apparently by the high nitrogen : sulphur ratio of bacterial protein in relation to the lower nitrogen : sulphur ratio required by the host tissues.

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