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Incorporation of radioactive carbon from [^{14}C] alanine in the rumen of a cow given feed containing urea as the sole source of nitrogen

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Abstract. The utilisation of exogenic amino acid in a cow given feed containing urea and ammonium salts as the sole sources of nitrogen was studied by means of intraruminal administration of [^{14}C]alanine. The labelling of the trichloracetic acid-precipitated bacterial cell mass, the main volatile fatty acids (acetic, propionic and butyric acid) and a number of isolated amino acids after 1, 3, 8 and 26 h was determined. The rumen micro-organisms rapidly incorporated the [^{14}C]alanine into their cellular constituents. After the above-mentioned times the microbial cell mass was found to contain 47.4, 49.7, 70.0 and 80.0 % of the total activity of the rumen contents. Although the carbon skeleton of alanine can be used for the formation of many amino acids the rumen bacteria studied were shown to utilise only small amounts of this amino acid in their synthesis of Asp, Glu, Tyr and Phe. The combined label present in these 4 amino acids was 1.5, 1.9, 2.9 and 5.5 % of the total activity of the rumen fluid at the stated times. A considerable proportion of the [^{14}C]alanine was degraded to volatile fatty acids: label present in acetic, propionic and butyric acid totalled 40.8, 32.3, 23.0 and 5.0 % of that in the rumen fluid. Of these, acetic acid had the strongest labelling after 1 and 3 h, and propionic acid the weakest at all stages.

Introduction

The concentration of extracellular amino acids in the rumen is usually relatively low (Wright and Hungate 1967). Feed protein is degraded very rapidly in the rumen to peptides and free amino acids, which undergo extensive metabolism before passing further down the digestive tract (McDONALD 1948, 1952; EL-SHAZLY 1952, LEWIS 1955 and ANNISON 1956). LEWIS and EMERY (1962) studied the degradation of amino acids in rumen fluid *in vitro* and found that they form three groups according to their rate of deamination. Ser, Cys, Asp, Thr and Arg are deaminated almost completely, followed by Phe, Glu, Lys and Cys-Cys. Those with the lowest deamination rate are Try, Met, Ala, Val, Ile, Orn, His, Gly, Pro, Pro(OH) and σ-aminovaleric acid. MANGAN (1972) found, in *in vivo* studies, that the half-life of casein in the cow's rumen fell in the range 5.6–21.5 minutes, in degradation to peptides, free amino acids and ammonia. He further observed that there was a complete loss of amino acids from casein hydrolysate, Cys and Arg in the rumen in 15

minutes, and of Asp, Ser, Thr, Gly, Ala, Met, Tyr, Phe and His in 30 minutes. Also Val, Leu, Ile and Lys disappeared quickly, but their reappearance was regarded as evidence of the synthesis of these four amino acids in the rumen.

Ammonia is the principal source of nitrogen in microbial protein synthesis (BRYANT and ROBINSON 1962). Not all rumen bacteria can use exogenic amino acids, and those which can will grow better when ammonia is available in addition. The most probable explanation for the apparent failure of amino acids to compete with or inhibit the utilisation of ammonia is a low activity, or even absence, of mechanisms for the transport of amino acids through the membrane of the bacterial cell (ALLISON 1969).

The significance of these biosynthetic reactions can readily be demonstrated in cows which produce milk and meat from protein-free feed, in which urea and ammonium salts form the sole source of nitrogen and purified carbohydrates the sole energy source (VIRTANEN 1963, 1966, 1971). The level of free amino acids in the rumina of these cows is very low (MÄKINEN 1972), but the rate of synthesis of microbial protein is greater than that in cows given normal, proteinaceous feed (VIRTANEN 1966). In the work described below the utilisation of carbon from a single exogenous amino acid, namely [^{14}C]alanine, by the rumen bacteria of a cow on protein-free feed was examined. The work is one of a series of studies on the metabolism by such cows of many ^{14}C-labelled compounds (KREULA and RAURAMAA 1979).

Materials and Methods

A single dose of 237 μCi L-[U-^{14}C]alanine (Radiochemical Centre, Amersham) was given in 360 g of 0.1 % unlabelled alanine to the cow, *via* a rumen fistula and mixing thoroughly with the rumen contents. The feed of the cow was composed mainly of purified carbohydrates, urea and ammonium salts being the only source of nitrogen (VIRTANEN 1963, 1966, 1971).

Rumen samples were taken just before the administration of the labelled alanine and 1, 3, 8 and 26 h afterwards. No drinking water was given for 3 h after the alanine dose. The rumen liquor was strained immediately through glass-fibre cloth. The bacteria were isolated by centrifugation, washed several times with phosphate buffer containing inactive alanine and finally twice with distilled water; they were then suspended homogenously in water and lyophilised. Protein was precipitated from rumen liquor and lyophilised bacteria with 10 % trichloracetic acid (TCA). The precipitates were washed 4 times with the same reagent and the TCA was then removed with ether. Asp, Glu, Tyr and Phe were isolated by means of Dowex IX8 anion exchange resin from the bacterial protein, following our published method (SYVÄOJA and KREULA 1979).

Volatile fatty acid contents in rumen liquor were determined as described by COTTYN and BOUCQUE (1968). The main components, namely acetic, propionic and butyric acid, were isolated by the method of HARPER (1953) and the purity of the isolates checked by gas chromatography.

^{14}C was determined either with a liquid scintillation counter or in an ionisation chamber after oxidation of the sample to $^{14}CO_2$ using CuO catalyst.

Results and Discussion

The distribution of radioactivity in the rumen liquor is shown in Figure 1. 1, 3, 8 and 26 h after the alanine dose the proportion of the activity present in one litre of rumen fluid was 1.05, 0.85, 0.52 and 0.25 % of the total given. Most of the activity was found in the fraction precipitated with 10 % TCA, which comprised mainly rumen bacteria. The protein content of this fraction was 40, 45, 35 and 50 % after the above-mentioned intervals. The proportion of total rumen liquor activity precipitated with TCA increased throughout the 1-day period: 47.4 % after 1 h, 49.7 after 3, 70.0 after 8 and 80.0 % after 26 h. Though the incorporation of [^{14}C]alanine into TCA-precipited material and into microbial cells was very vigorous most of this activity was not present in the microbial protein. Thus while the carbon skeleton of alanine can be used for the biosynthesis of many amino acids it is evident that the rumen bacteria utilised only small amounts of it in the formation of cellular protein. The labelling of the isolated amino acids, namely Tyr, Phe, Asp and Glu was slight: 3.2, 3.9, 4.1 and 6.6 % respectively of the activity of the TCA precipitate and 1.5, 1.9, 2.9 and 5.5 % of the total activity of the rumen liquor. These amino acids together accounted for about 36 % of the total amino acid.

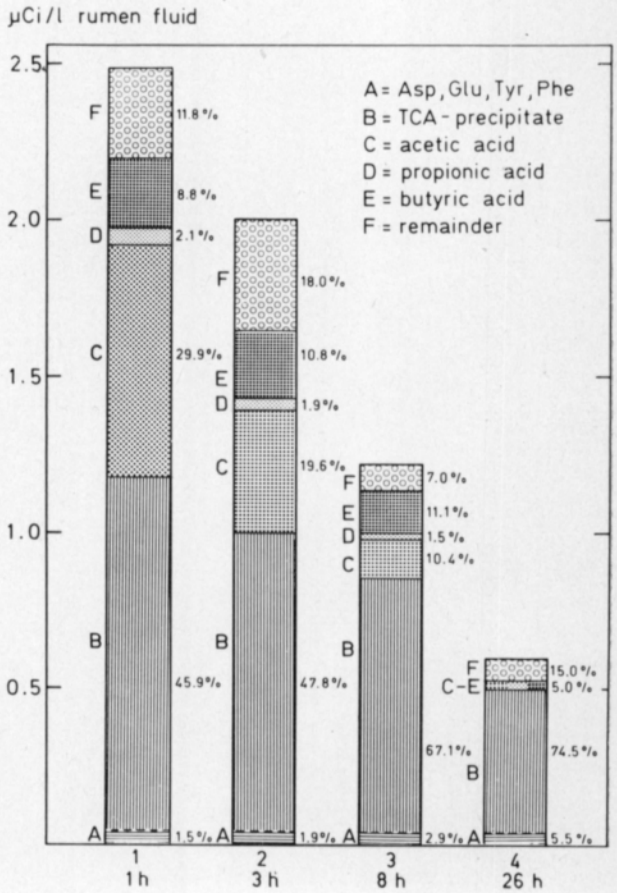

Figure 1. Labelling of volatile fatty acids and trichloracetic acid precipitate from rumen fluid 1—26 h after the administration of [^{14}C]alanine.

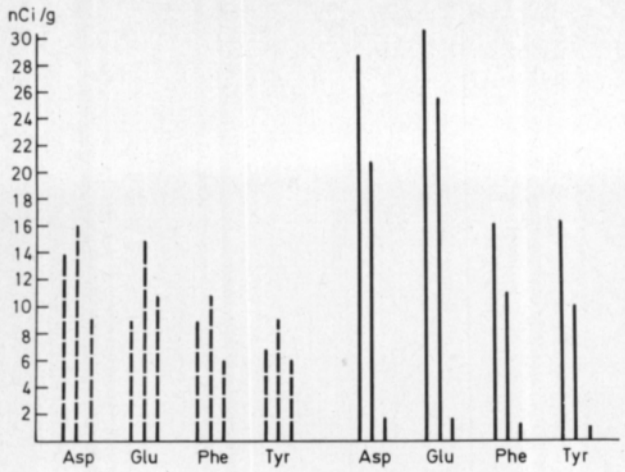

Figure 2. Specific activity of amino acids isolated from rumen bacterial protein after intraruminal administration of 237 μCi [U-^{14}C] alanine to a cow on protein-free feed (- - - -) and 220 μCi [U-^{14}C] sucrose (SYVÄOJA and KREULA 1979) to a cow on urea-rich low-protein feed (—). In the alanine study the rumen fluid samples were taken 1, 3 and 26 h after giving the label, in the sucrose study 1, 5 and 24 h.

Figure 2 compares the labelling of the 4 isolated amino acids in the present work with that found when [U-^{14}C]sucrose was fed to a cow on urea-rich low-protein feed (SYVÄOJA and KREULA 1979). It is evident that the bacteria synthesised these amino acids from sucrose at about double the rate from alanine. The retention time of sucrose in the rumen was shorter than that of alanine.

In studies with sheep fed a diet of pelleted, dried grass meal, PORTUGAL and SUTHERLAND (1966) found very little incorporation of labelled aspartic and glutamic acid into rumen microbial protein; both amino acids were metabolised rapidly, with incorporation of much of the ^{14}C into short chain fatty acids and CO_2. Similar results were obtained by BORCHERS (1967): he used [U-^{14}C]glucose and ^{14}C-labelled amino acids from an acid hydrolysate of *Chlorella* and found that rumen microorganisms utilised glucose more readily than exogenic amino acid carbons for the synthesis of cellular protein.

Measurements of the labelling of the main volatile fatty acids of rumen fluid 1, 3, 8 and 26 h after feeding [^{14}C]alanine showed that acetic, propionic and butyric acid, which together accounted for 92—94 % of the total rumen volatile fatty acids, contained 40.8, 32.3, 23.0 and 5.0 % of the total activity of the rumen fluid. Acetic acid carried the strongest labelling in the first two samples (29.9 and 19.6 % of the total activity of the rumen fluid), butyric acid in the last two samples (11.1 and 2.6 %), and propionic acid had the weakest labelling in all samples (2.1—0.8 %), as shown in Figure 1.

Lactose is synthesised in the udder from blood glucose. Propionate is the most important source of glucose in fed ruminants not absorbing glucose from the small intestine. LINDSAY (1979) estimated that 40—60 % of the glucose entry rate derives from propionate. That the lactose was labelled 7 and 21 h after feeding the [^{14}C]alanine (11 and 15 nCi/g respectively, KREULA and RAURAMAA, unpublished results) showed that the blood glucose had carried label. The above-mentioned weak labelling of propionic acid after feeding [^{14}C]alanine does not by itself account for the incorporation of ^{14}C into the lactose — glucose must have been formed *via* pyruvate and oxaloacetate directly from alanine. Further study is needed before the contribution of amino

acids to gluconeogenesis can be assessed. BERGMAN and HEITMANN (1978) estimated that 15—25 % of the glucose is derived from amino acids in amply-fed sheep, glutamic acid and alanine being the most important substrates. In starved sheep 35 % of the glucose entry rate derives from amino acids (LINDSAY 1979). BLACK (1968) and BLACK et al. (1968) reported that amino acids can provide 33—50 % of the carbon required for glucose synthesis in the lactating COW.

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SELOSTUS

Radioaktiivisen hiilen inkorporoituminen ureaa ainoana typenlähteenään saaneen lehmän pötsissä ^{14}C-alaniinista

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Eksogeenisen aminohapon hyväksikäyttöä tutkittiin ureaa ja ammoniumsuoloja ainoana typen lähteenään saaneella 0-lehmällä antamalla pötsiin ^{14}C-alaniinia. Leimaantuminen määritettiin 1, 3, 8 ja 26 h kuluttua trikloretikkahapolla saostuvasta bakteerimassasta, haihtuvien rasvahappojen pääkomponenteista (etikka-, propioni- ja voihaposta) sekä muutamista eristetyistä aminohapoista. Pötsin mikro-organismit inkorporoivat ^{14}C-alaniinia nopeasti solumateriaaliinsa. Yhden pötsinestelitran sisältämästä kokonaisaktiiviisuudesta todettiin mikrobimassan sisältävän yllä mainittujen aikojen kuluttua 47.4, 49.7, 70.0 ja 80.0 %. Vaikka alaniinin hiilirunkoa voidaan käyttää monien aminohappojen muodostumisessa bakteerit käyttivät tätä aminohappoa vain vähän Asp, Glu, Tyr ja Phe muodostumiseen. Näiden eristettyjen aminohappojen leimaantuminen oli 1.5, 1.9, 2.9 ja 5.5 % pötsinestelitran totaaliaktiivisuudesta mainittuina näytteenottoaikoina. Huomattava osa ^{14}C-alaniinista hajosi haihtuviksi rasvahapoiksi. Etikka-, propioni- ja voihapossa todettiin 40.8, 32.3, 23.0 ja 5.0 % annetusta aktiivisuudesta samoina näytteenottoaikoina. Haihtuvista rasvahapoista etikkahapon leimaantuminen oli voimakkain ja propionihapon heikoin.