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Fate of Cyst(e)ine Synthesized by Microbial Activity in the Ruminant Caecum

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Abstract

Cyst(e)ine synthesized by microorganisms in the caecum of sheep was labelled following the infusion of $Na_2{}^{35}SO_4$ into the terminal ileum. [^{35}S]Cyst(e)ine activity was detected in the faeces, but not in plasma or wool. Appreciable absorption of ^{35}S, presumably in the form of sulphide, into the circulation occurred, and its presence in saliva was demonstrated. It was concluded that nutritionally negligible quantities of cyst(e)ine are likely to be absorbed from the ovine large intestine.

Introduction

Microbial fermentation occurs in the ruminant caecum, and is similar in nature to that in the rumen. It is known that ammonia (McDonald 1948) and volatile fatty acids (Barcroft *et al.* 1944) are absorbed from the caecum. The small intestine is the site of absorption of amino acids, and there has been speculation as to whether microbial protein formed during caecal fermentation may be useful to the animal. This is apparently so in the horse (Slade *et al.* 1971) and some evidence exists suggesting that it may be the case for sheep (Demaux *et al.* 1961; Judson *et al.* 1975).

We have approached this problem by causing an isotopic label to be incorporated into cyst(e)ine synthesized by microbial activity in the caecum of sheep, so that the fate of the amino acid might be traced. We were unable to demonstrate absorption of the amino acid from the large intestine.

Methods

Three mature Scottish Blackface wethers were used in the present study, each fitted with a rumen cannula and re-entrant duodenal and ileal cannulae (Brown *et al.* 1968). The sheep were fed twice daily 500 g of a dried and pelleted grass containing 14% crude protein in the dry matter; digestibility of the dry matter was 73%. A solution that contained 1 mg (600 μCi) $Na_2{}^{35}SO_4$ per litre was continuously infused into the terminal ileum at a rate of 150 μCi/day to two of the sheep (*A* and *B*) for 2 and 4 days respectively. The third sheep (*C*) received a dose of 300 μCi $Na_2{}^{35}SO_4$ in 500 ml daily for 4 days, and the infusion solution in this case also contained 8 g glucose/100 ml as suggested by Thornton *et al.* (1970) to stimulate microbial activity in the caecum.

During the experiment all of the digesta flowing through the pylorus was diverted, and non-labelled donor material previously collected from the same sheep was infused into the duodenum instead, at a rate equal to that at which the digesta were removed. This was designed to prevent the absorption in the small intestine of any [^{35}S]cyst(e)ine that might be synthesized by bacteria in the rumen from [^{35}S]sulphate absorbed from the large intestine and reaching the rumen via saliva or the rumen wall.

Jugular blood, faeces and duodenal extrusa were sampled daily; after separation of the plasma, these samples were immediately frozen. Faeces and duodenal extrusa were freeze-dried before analysis. Wool samples were obtained from sheep *C* before and after infusion; on each side of the animal, a patch approximately 50 by 8 cm was clipped before infusion. Five weeks later, during which a total of some 200 g wool would have been grown (Doney 1964), 60 g of wool were clipped from these patches, and 16 separate 1-g samples were analysed. Measurements of [^{35}S]cyst(e)ine radioactivity in these samples were made using a method specific for cyst(e)ine. This method (see Herrick *et al.* 1972) involved reaction of the sample with hydrazine hydrate while sealed under nitrogen at 120°C for 18 h to convert the cyst(e)ine-sulphur to sulphide; H_2S was subsequently released by the addition of 3 M H_2SO_4 and carried in a stream of high-purity nitrogen into the sulphide-collecting solution of Johnson and Nishita (1952). Then 2·5% aliquots were taken for scintillation counting. The technique was described in detail by Elliott (1975).

Plasma activities of total [^{35}S]sulpate (Bird and Fountain 1970) were also measured. In one experiment (sheep *A*), saliva samples were obtained by persuading the animal to chew on balls of cotton wool which were then extracted with a small quantity of distilled water; counts of ^{35}S were made immediately on this material on a purely qualitative basis. The isotope was counted from each sample in duplicate using a Beckman model LS-133 liquid scintillation machine (with which approximately 0·05 nCi of activity could be detected). The results are expressed as nCi ^{35}S activity per ml (plasma) or per 100 mg (faeces, duodenal extrusa and wool).

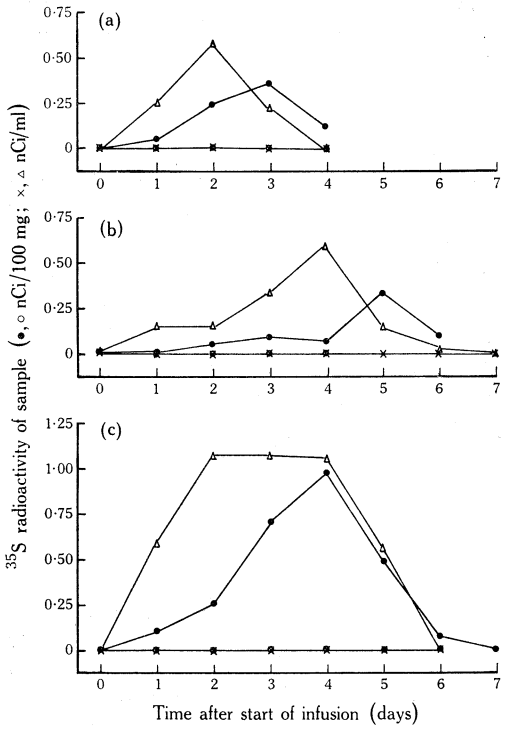

Fig. 1. ^{35}S radioactivity in various samples from three sheep (*A*, *B* and *C* shown in *a*, *b* and *c* respectively) following the infusion of $Na_2{}^{35}SO_4$ into the terminal ileum for (*a*) two or (*b*, *c*) four days. [^{35}S]Cyst(e)ine in faeces (●) and duodenal digesta (○) measured as nCi/100 mg dry matter; plasma [^{35}S]cyst(e)ine (×) and total [^{35}S]sulphate (△) measured as nCi/ml.

Results

The appearance of ^{35}S activity in the various fractions for each animal is shown in Fig. 1. The isotope was incorporated into microbial cyst(e)ine which appeared in the faeces, but no labelled cyst(e)ine was detected in the plasma at any time. Appreciable levels of [^{35}S]sulphate activity were obtained in plasma, consequent upon the absorption of isotope presumably in the form of sulphide. The secretion of this into

saliva was detected, in that the pattern of activity observed in saliva from animal *A* closely paralleled that of plasma; this was not quantitated, but was estimated to have reached almost 1 nCi/ml by the second day of infusion. No [^{35}S]cyst(e)ine was detected in duodenal extrusa at any stage.

Discussion

Earlier studies in this laboratory (Elliott 1975), in which infusions of $Na_2{}^{35}SO_4$ were made into the rumen, demonstrated rapid appearance of labelled cyst(e)ine in plasma and wool following its absorption from the small intestine; similar conclusions may be reached from the work of Bird (1972), who infused ^{35}S-labelled rumen bacteria into the abomasum. The present experiment was designed so that any [^{35}S]cyst(e)ine that appeared in faeces, plasma or wool must have been synthesized by microorganisms in the caecum. The presence of [^{35}S]cyst(e)ine in the faeces (Fig. 1) confirmed such synthesis, but any absorption that occurred was at an undetectable level.

Coelho da Silva *et al.* (1972) observed the disappearance of some 27% of total nitrogen and 22% of amino acid nitrogen between the ileum and anus of sheep. Although rapid proteolysis and deamination occurs in ovine caecal contents (Hecker 1971), it remains conjectural whether any amino nitrogen may have been absorbed, since most of the nitrogen that disappeared may have been absorbed as ammonia and excreted in the urine. Demaux *et al.* (1961) observed amino nitrogen to be absorbed from the caecum of sheep. Their technique, however, was unphysiological in that it involved isolation of part of the intestine and its vascular supply; doubt must therefore attach to the extrapolation of their results to the normal situation.

Recently, Judson *et al.* (1975) infused into the caecum of sheep ^{35}S-labelled rumen bacteria grown *in vitro*; while more than 99% of the infused activity was in the form of organically bound ^{35}S, over 90% of the plasma ^{35}S activity was in the reducible form, indicating that substantial breakdown of sulphur amino acids occurred in the caecum. They found that *c.* 60% of the organically combined S introduced into the large intestine appeared as such in the faeces, and that only 3% was absorbed in the organic form, although this was not conclusively shown to be in the form of sulphur acids; after 6 weeks, a proportion was detected in wool. By applying Judson's value of 60% to the results obtained from sheep *C*, the animal from which wool samples were obtained, it may be calculated that the cyst(e)ine synthesized in the caecum during the infusion contained a total 11·83 μCi of ^{35}S activity. Some 3·4% of the [^{35}S]cyst(e)ine synthesized could have been absorbed and stored in wool undetected by the analytical procedures adopted. Thus the present results, obtained using caecal bacteria, agree with those of Judson *et al.* (1975) from rumen bacteria, that nutritionally negligible amounts of cyst(e)ine are likely to be absorbed from the large intestine of sheep.

It is noteworthy that no [^{35}S]cyst(e)ine was detected in duodenal extrusa, although the isotope was present in saliva, and presumably was available to the rumenal microflora. No attempt was made to quantitate this, but an estimate based on the probable secretion of some 3 litres of saliva per day by sheep eating ground feed (Wilson and Tribe 1963), 13% incorporation of rumen sulphate into microbial protein (Gawthorne and Nader 1976) and the daily duodenal flow of about 2·5 g cyst(e)ine in 500 g dry matter (Elliott 1975), lead to the conclusion that [^{35}S]cyst(e)ine would have been present in duodenal digesta in quantities insufficient to have been detected.

The results presented here are pertinent to cyst(e)ine, and may not necessarily apply to other amino acids. However, it seems reasonable to conclude that the availability to the host of microbial protein in the ruminant large intestine is, at best, slight.

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References

Barcroft, J., McAnally, R. A., and Phillipson, A. T. (1944). Absorption of volatile fatty acids from the alimentary tract of the sheep and other animals. *J. Exp. Biol.* **20**, 120.

Bird, P. R. (1972). Sulphur metabolism and excretion studies in ruminants. VI. The digestibility and utilization by sheep of ^{35}S from ^{35}S-labelled ruminal micro-organisms. *Aust. J. Biol. Sci.* **25**, 195.

Bird, P. R., and Fountain, R. D. (1970). A method for the determination of sulphur in some biological materials. *Analyst (London)* **95**, 98.

Brown, G. F., Armstrong, D. G., and MacRae, J. C. (1968). The establishment in one operation of a cannula into the rumen and re-entrant cannulae into the duodenum and ileum of the sheep. *Br. Vet. J.* **124**, 78.

Coelho da Silva, J. F., Seeley, R. C., Thomson, D. J., Beever, D. E., and Armstrong, D. G. (1972). The effect in sheep of physical form on the sites of digestion of a dried lucerne diet. 2. Sites of nitrogen digestion. *Br. J. Nutr.* **28**, 357.

Demaux, G., Le Bars, H., Molle, J., Rerat, A., and Simmonet, H. (1961). Absorption des acides aminés au niveau du rumen de l'intestin grêle et du coecum chez le mouton. *Bull. Acad. Vet. Fr.* **34**, 85.

Doney, J. M. (1964). The fleece of the Scottish Blackface sheep. 4. The effects of pregnancy, lactation and nutrition on seasonal wool production. *J. Agric. Sci.* **62**, 59.

Elliott, R. (1975). Ph. D. Thesis, University of Newcastle-upon-Tyne.

Gawthorne, J. M., and Nader, C. J. (1976). The effect of molybdenum on the conversion of sulphate to sulphide and microbial protein-sulphur in the rumen of sheep. *Br. J. Nutr.* **35**, 11.

Hecker, J. F. (1971). Metabolism of nitrogenous compounds in the large intestine of sheep. *Br. J. Nutr.* **25**, 85.

Herrick, H. E., Lawrence, J. M., and Coahran, D. R. (1972). Simple methods for determination of methionine and cystine in legume seeds. *Anal. Biochem.* **48**, 353.

Johnson, C. M., and Nishita, H. (1952). Microestimation of sulphur in plant materials, soils and irrigation waters. *Anal. Chem.* **24**, 736.

Judson, G. J., Abdelsamie, R., and Bird, R. G. (1975). Digestion and utilization of ^{35}S-labelled bacteria placed in the caecum of the sheep. *Aust. J. Agric. Res.* **26**, 743.

McDonald, I. W. (1948). The absorption of ammonia from the rumen of the sheep. *Biochem J.* **42**, 584.

Slade, L. M., Bishop, R., Morris, J. G., and Robinson, D. W. (1971). Digestion and absorption of ^{15}N-labelled microbial protein in the large intestine of the horse. *Br. Vet. J.* **127**, x1.

Thornton, R. F., Bird, P. R., Somers, M., and Moir, R. J. (1970). Urea excretion in ruminants. 3. The role of the hind gut (caecum and colon). *Aust. J. Agric. Res.* **21**, 345.

Wilson, A. D., and Tribe, D. E. (1963). The effect of diet on the secretion of parotid saliva by sheep. I. The daily secretion of saliva by caged sheep. *Aust. J. Agric. Res.* **14**, 670.

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