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Short Communications

Food Particles as a Site for Biohydrogenation of Unsaturated Fatty Acids in the Rumen

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On incubation of linoleic acid with strained rumen contents from sheep, it was observed that conversion of linoleic acid into $C_{18:1}$ *trans*-11 monoenoic acid and subsequently into stearic acid was largely associated with the food-particle fraction. The bacteria, protozoa and cell-free supernatant together contributed less than 30% to the overall change in the added $C_{18:2}$ fatty acid.

The intermediates produced during the conversion of C_{18} polyunsaturated fatty acids into stearic acid in the rumens of cattle and sheep have been much investigated (Viviani, 1970). However, little is known about the relative importance of the major components of the rumen contents in this process. Incubation of polyunsaturated fatty acids with suspensions of either rumen bacteria or rumen protozoa separately have led to the general assumption that it is the bacteria that are largely responsible for biohydrogenation and that the protozoa are of only secondary importance (Viviani, 1970). Recent work in our laboratory has been concerned with the role of the four major components of rumen liquor, i.e. bacteria, protozoa, the cell-free supernatant and the fine food particles, in the biohydrogenation of linoleic acid. The preliminary results of these experiments are now reported.

In these experiments approx. 1 litre of strained rumen contents was obtained from each of four fistulated Suffolk-cross sheep about 3 years old. Two of the sheep were ewes and two were castrated males. Each sheep received 0.5kg of hay and 0.5kg of concentrate mixture/day. The strained rumen contents were pooled and of the pooled rumen contents 3.2 litres were diluted to 8 litres with 3.2 litres of buffer solution (McDougall, 1948) and 1.6 litres of distilled water. All incubations were done at 39°C in a Microferm MF-114 fermentor equipped with automatic pH control (New Brunswick Scientific Co., New Brunswick, N.J., U.S.A.). The fermentor vessel containing the buffer solution was gassed for 10min with H_2+N_2 (5:95) to displace oxygen before addition of the rumen contents. Sucrose was used as a carbon+energy source for the rumen microorganisms and was added to the incubation vessel by continuous infusion at a rate of 3.1g of sucrose/100ml of strained rumen contents throughout the 6h period of incubation. Simultaneously $(NH_4)_2CO_3$ was added as a nitrogen source at one-fifth of the rate at which the sucrose was added. Linoleic acid [99% pure; Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K.] was added to the incubation vessel as an emulsion in 50ml of an aqueous 1% (v/v) solution of Tween 80 (polyoxyethylene sorbitan mono-oleate; Honeywill and Stein Ltd., London W.1, U.K.). A 500ml sample of the incubation mixture was taken before the addition of linoleic acid, and further 500ml samples were taken after the addition of linoleic acid at 1min, 20min, 40min, 1h, 2h, 4h and 6h.

Each 500ml sample was centrifuged at 100*g* for 5min to sediment the protozoa and fine food particles, which were subsequently obtained as uncontaminated preparations by repeated centrifugation and sedimentation under gravity in aq. 0.85% NaCl solution. The efficiency of this separation was checked by microscopic examination. The bacterial suspension comprising the supernatant of the initial 100*g* centrifugation was centrifuged at 35000*g* for 20min to yield a cell-free supernatant fraction. The pellet of bacteria was washed by centrifugation at 35000*g* for 20min in aq. 0.85% NaCl solution. Each fraction was freeze-dried to determine its dry weight. The subsequent lipid extractions and analyses were done as described previously (Moore *et al.*, 1969).

The products of hydrogenation during one experiment in which linoleic acid was incubated with strained rumen contents are shown in Table 1. About 90% of the $C_{18:2}$ acid added as substrate was utilized in the first hour of incubation, during which time there was a transient production of the $C_{18:2}$ *cis*-9,-*trans*-11 conjugated dienoic acid followed by a large accumulation of the $C_{18:1}$ *trans*-11 acid. This in turn was followed by rapid conversion into stearic ($C_{18:0}$) acid. These observations conform to the sequence of events observed by Polan *et al.* (1964). In the present work, the $C_{18:0}$ acid accounted for about 65–70% of the $C_{18:2}$ acid added by the end of incubation.

Table 1. *Biohydrogenation of linoleic acid during incubation with rumen contents*

Strained rumen contents (3.2 litres) from sheep were diluted to a final volume of 8 litres with buffer solution and water and incubated at 39°C with 0.3mg of linoleic acid/ml of rumen contents. Sucrose was infused as a carbon+energy source at a rate of 3.1g/6h per 100ml of rumen contents throughout the incubation. Samples of the incubation mixture were analysed for total fatty acid composition and not separated into plant material, bacterial, protozoal or supernatant fractions. All values shown are corrected for the endogenous sample taken before addition of linoleic acid to the incubation vessel and are expressed as percentages of the $C_{18:2}$ acid added to the incubation vessel.

C_{18} acid	Time after addition of $C_{18:2}$ acid ...	Composition (% of added $C_{18:2}$ acid)						
		1min	20min	40min	1h	2h	4h	6h
$C_{18:2}$ *cis*-9,*cis*-12 acid		24.1	12.3	11.0	9.0	5.0	3.9	5.5
$C_{18:2}$ *cis*-9,*trans*-11 acid		12.5	11.4	4.1	2.3	2.8	2.1	2.9
$C_{18:1}$ *trans*-11 acid		50.3	62.8	76.5	38.3	28.5	20.2	25.0
$C_{18:0}$ acid		13.1	13.4	8.4	50.3	63.7	73.7	66.5

Fractionation of the incubation mixture showed that, immediately after addition of linoleic acid to the incubation mixture, only about 6% of the added $C_{18:2}$ acid was associated with the protozoa, the remainder being more or less equally distributed between the food particles, the bacteria and the cell-free supernatant fractions. Table 2 shows that the major conversion of $C_{18:2}$ acid into $C_{18:1}$ acid and subsequently into $C_{18:0}$ acid was associated with the food-particle fraction, and it was calculated that the changes that occurred in the bacterial, protozoal and cell-free supernatant fractions together accounted for less than one-third of the overall change in the C_{18} acid added to the incubation mixture. Table 2 also shows the similar results of a second experiment done under identical conditions. About 85% of the linoleic acid added as substrate was hydrogenated to stearic acid by the end of incubation, and the major conversion of linoleic acid into $C_{18:1}$ acid and subsequently into stearic acid was associated with the food-particle fraction. A third experiment gave similar results.

That the food particles provide an important site for the adsorption of lipids in the rumen of sheep has been shown by previous workers (Ward *et al.*, 1964). However, the significance of this in terms of the biohydrogenation process in the rumen has been largely neglected; most studies *in vitro* have involved microbial suspensions from which the food particles have been deliberately removed (Wilde & Dawson, 1966). Viviani & Borgatti (1967, cited by Viviani, 1970) observed that complete hydrogenation of polyunsaturated fatty acids present in linseed oil took place *in vitro* only in the presence of complete rumen liquor; removal of the particulate fraction precipitated by centrifugation at 800*g* greatly diminished the biohydrogenating capacity of the rumen liquor. Hawke & Silcock (1970) observed a similar decrease in biohydrogenation on centrifuging strained rumen liquor from cattle at only 50*g*, but neither of these groups of workers analysed the particulate fractions for fatty acid composition.

The present experiments involving the incubation of free linoleic acid with strained rumen contents provide unequivocal evidence that there is considerable hydrogenation of the fatty acids associated with the food particles. One explanation for this observation is that the hydrogenation is effected by extracellular hydrogenases produced by the bacteria in suspension. Hawke & Silcock (1970) attributed the decrease in biohydrogenation on removal of the 50*g* sediment to the removal of hydrogenating bacteria adhering to the food particles. We consider that the possibility in our experiments that the hydrogenation could be associated entirely with the bacteria adhering to the food particles is unlikely on the following grounds. Using total rumen digesta from sheep, Weller *et al.* (1958) estimated that the numbers of bacteria associated with the rumen solids were approximately the same as the numbers present in the liquid phase. The rumen contents used by us were strained before use; this straining effectively removed all particles whose longest dimension was about 0.5mm, i.e. by far the greater proportion of food particles in the rumen. Also, the preparations of food particles analysed by us for fatty acid composition were washed free of bacteria by repeated agitation and centrifugation. Although washing does not remove all the bacteria adhering to the food particles, it seems unlikely that the remaining bacteria could contribute more to the hydrogenation process than do the bacteria in free suspension in the rumen liquor. It is accepted by many workers that lipolysis of esterified lipids in the rumen takes place extra-

Table 2. *Percentage of the added C_{18} acid accounted for by the C_{18} acids recovered from each fraction from rumen liquor*

Strained rumen contents (3.2 litres) from sheep were diluted to a final volume of 8 litres with buffer solution and water and incubated at 39°C with 0.3mg of linoleic acid/ml of strained rumen contents. Sucrose was infused as a carbon+energy source at a rate of 3.1g/6h per 100ml of rumen contents throughout the incubation. Each 500ml portion taken at the times shown was fractionated by centrifugation into plant material, protozoa, bacteria and cell-free supernatant, and each fraction was analysed for fatty acid composition. All values shown have been corrected for endogenous fatty acid composition. —, Not determined.

Rumen liquor fraction	C_{18} acid	Time after addition of $C_{18:2}$ acid ...	Composition (% of added $C_{18:2}$ acid)						
			1min	20min	40min	1h	2h	4h	6h
Expt. 1									
Plant material	$C_{18:0}$		6.8	—	2.4	44.0	55.8	60.0	55.7
	$C_{18:1}$ *trans*-11		31.7	43.9	47.2	9.2	8.8	8.2	8.9
	$C_{18:2}$ *cis*-9,*trans*-11		11.3	9.0	4.1	2.0	2.5	2.1	2.2
	$C_{18:2}$ *cis*-9,*cis*-12		6.7	5.2	2.4	—	—	—	—
Protozoa	$C_{18:0}$		4.5	2.4	3.3	3.0	4.5	12.9	10.8
	$C_{18:1}$ *trans*-11		0.8	2.8	3.2	3.1	1.9	3.0	2.8
	$C_{18:2}$ *cis*-9,*trans*-11		—	—	—	—	—	—	—
	$C_{18:2}$ *cis*-9,*cis*-12		0.8	0.1	—	—	—	—	—
Bacteria	$C_{18:0}$		1.9	11.0	2.3	2.3	2.1	—	—
	$C_{18:1}$ *trans*-11		13.5	12.5	24.3	23.6	13.0	6.6	7.3
	$C_{18:2}$ *cis*-9,*trans*-11		1.2	2.4	—	0.3	0.3	—	0.7
	$C_{18:2}$ *cis*-9,*cis*-12		7.2	6.6	1.0	2.7	2.7	3.3	4.1
Cell-free supernatant	$C_{18:0}$		—	—	0.3	1.0	1.3	0.8	—
	$C_{18:1}$ *trans*-11		4.3	3.6	1.7	2.4	4.8	2.5	6.0
	$C_{18:2}$ *cis*-9,*trans*-11		—	—	—	—	—	—	—
	$C_{18:2}$ *cis*-9,*cis*-12		9.3	0.5	7.6	6.3	2.3	0.6	1.4
Expt. 2									
Plant material	$C_{18:0}$		24.8	28.1	37.5	51.4	63.1	68.6	69.1
	$C_{18:1}$ *trans*-11		19.2	48.4	47.0	35.2	22.3	19.1	12.4
	$C_{18:2}$ *cis*-9,*trans*-11		12.4	2.8	—	0.1	—	0.8	0.1
	$C_{18:2}$ *cis*-9,*cis*-12		19.1	0.2	—	0.2	0.7	0.8	1.0
Protozoa	$C_{18:0}$		2.5	—	0.1	0.1	3.8	—	0.8
	$C_{18:1}$ *trans*-11		1.2	0.4	—	0.1	—	—	—
	$C_{18:2}$ *cis*-9,*trans*-11		0.4	0.2	—	0.2	—	—	—
	$C_{18:2}$ *cis*-9,*cis*-12		0.8	—	0.2	—	—	0.2	—
Bacteria	$C_{18:0}$		5.2	7.0	6.3	7.4	7.5	9.5	15.7
	$C_{18:1}$ *trans*-11		5.4	11.0	7.7	3.5	1.2	0.1	—
	$C_{18:2}$ *cis*-9,*trans*-11		2.0	0.2	—	—	—	—	—
	$C_{18:2}$ *cis*-9,*cis*-12		4.9	—	—	0.2	—	—	—
Cell-free supernatant	$C_{18:0}$		—	—	0.1	0.3	0.7	0.7	0.8
	$C_{18:1}$ *trans*-11		0.7	1.3	1.0	0.5	0.4	0.2	—
	$C_{18:2}$ *cis*-9,*trans*-11		0.4	0.2	0.1	0.3	0.1	0.1	0.1
	$C_{18:2}$ *cis*-9,*cis*-12		0.9	0.2	—	0.5	0.1	—	—

cellularly (Viviani, 1970) and, although it is widely acknowledged that lipolysis is rapidly followed by hydrogenation (Hawke & Silcock, 1970), the possibility that hydrogenation may also be an extracellular process appears to have been largely overlooked. The present work provides evidence that such a process may exist.

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