Short Communication

The Effect of Defaunation on the Phospholipids and on the Hydrogenation of Unsaturated Fatty Acids in the Rumen

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The mixed micro-organism population from the rumen of sheep can rapidly hydrogenate dietary unsaturated C_{18} fatty acids (linolenic acid, linoleic acid, oleic acid) to stearic acid. Although a number of bacterial species that can convert linolenic acid and linoleic acid into octadecenoic acids have been isolated (Polan, McNeill & Tove, 1964; Kemp & White, 1968; Mills, Scott, Russell & Smith, 1969; Sachan & Davis, 1969), the organism(s) that can convert the latter into stearic acid is unknown.

Wright (1959) found that washed mixed rumen protozoa would convert linseed oil into stearic acid, and washed preparations of the rumen holotrichs *Isotricha prostoma* (Gutierrez, Williams, Davis & Warwick, 1962) and *Isotricha intestinalis* (Williams, Gutierrez & Davis, 1963) were able to hydrogenate oleic acid to stearic acid. In contrast, Chalupa & Kutches (1968) found that, of washed rumen protozoa, only the oligotrichs and not the holotrichs could hydrogenate linoleic acid and oleic acid. The difficulty in deciding whether the protozoa themselves are responsible for the hydrogenations observed is that some viable bacteria would undoubtedly be present in the protozoal preparations (Gutierrez & Davis, 1959; White, 1969), and the influence of these may not be adequately controlled by the inclusion of antibiotics in the incubation medium.

The recent introduction of a comparatively simple method for defaunating ruminants (Abou Akkada *et al.* 1968) has allowed us to obtain further information on the role of rumen protozoa in the hydrogenation of fatty acids and in particular to determine whether they are responsible for the hydrogenation of octadecenoic acids to stearic acid. A sheep (60kg.) fed on a hay-chaff (1000g.)–oats (200g.) diet was treated with 5g. lots of dioctyl sodium sulphosuccinate (Monoxol OT; British Drug Houses Ltd., Poole Dorset) introduced through a rumen fistula on 4 successive days. The animal temporarily lost its appetite, but it was 'weaned' back to its original diet over a period of a week by feeding with fresh herbage and long hay. It was kept isolated from other ruminants or personnel handling these animals to avoid reinfection of the rumen. Microsopic examination of the rumen contents showed that the flourishing ciliate-protozoal population was completely eliminated by the detergent treatment. Occasionally what appeared to be a small flagellate protozoon could be seen, but the mass of this per ml. of rumen fluid was completely insignificant compared with the original protozoal complement. Rumen contents were removed and, after being strained to remove coarse food particles, 10ml. was incubated with [1-^{14}C]-linolenic acid or [1-^{14}C]oleic acid for 1hr. or 2hr. respectively (Wilde & Dawson, 1966; Kemp & Dawson, 1968). The fatty acids were isolated from the incubation medium and the extent of the hydrogenation was assessed by g.l.c., or by t.l.c. with radioactive scanning as described previously (Wilde & Dawson, 1966; Kemp & Dawson, 1968).

The normal rumen micro-organisms before defaunation were able to convert a substantial portion of the added labelled oleic acid into stearic acid. Some of the radioactive linolenic acid was fully hydrogenated to stearic acid and there was also a build-up of the octadecadienoic acid and octadecenoic acid intermediates involved in the process (Wilde & Dawson, 1966; Kemp & Dawson, 1969) (Table 1). The extent of the hydrogenation varied from day to day, presumably owing to factors such as the magnitude of unlabelled dietary substrate in the rumen or variations in the type and quantity of the micro-organisms present. The administration of detergent initially led to the complete suppression of the hydrogenation of both substrates, although isomerization of linolenic acid to *cis–trans–cis*-octadeca-9,11,15-trienoic acid (Kepler & Tove, 1967; Kemp & Dawson, 1968) still occurred. As the animal recovered from the effects of the detergent the ability to hydrogenate both substrates was almost completely recovered, even though the rumen remained devoid of ciliate protozoa (Table 1). The successive recovery of the ability to hydrogenate trienoic acid to dienoic acid and then the latter to monoenoic acid and eventually stearic acid was apparent (Table 1).

Table 1. *Biohydrogenation of linolenic acid and oleic acid before and after defaunation*

Dioctyl sodium sulphosuccinate (5g.) was administered on days 6–9 inclusive.

Day		0	2	5	8	12	14	16	18	23	25
Ciliated protozoa present ...		++++	++++	++++	+	0	0	0	0	0	0
	Fatty acid	Distribution of labelling after incubation (%)									
[1-^{14}C]Oleic acid	$C_{18:1}$	47	33	35	100	100	100	100	45	65	65
	$C_{18:0}$	53	67	65	0	0	0	0	55	35	35
[U-^{14}C]Linolenic acid	$C_{18:3}$	3	30	16	17	59	49	50	25	24	36
	$C_{18:3}$ (conj.)	0	7	2	83	41	45	5	9	6	9
	$C_{18:2}$	9	13	15	0	0	6	35	34	9	21
	$C_{18:1}$	41	30	49	0	0	0	10	27	19	26
	$C_{18:0}$	47	20	18	0	0	0	0	5	42	8

Further experiments were performed to test the inhibition by the detergent of the hydrogenation of linolenic acid by rumen contents from a normal sheep. Dioctyl sodium sulphosuccinate (0·3–0·5mg./ml.) almost completely suppressed hydrogenation, although isomerization into various conjugated trienoic acids still occurred. It would appear therefore that the initial suppression of hydrogenation during defaunation is due to a direct action on the micro-organisms involved, which grow again as the detergent disappears from the rumen. The reappearance in the rumen of the complete system for the hydrogenation of both linolenic acid and oleic acid to stearic acid in the absence of ciliate protozoa indicates that the latter are not essential for the processes involved. However, although a given volume of ciliate-free rumen fluid seemed to be only slightly less effective than the normal fluid in hydrogenating these substrates, it cannot be said with complete certainty that protozoa play no role in biohydrogenation in the normal rumen.

An examination of the phospholipid composition of the micro-organisms present in the rumen after defaunation was made to see whether this treatment led to any deficiency of the phospholipids known to be present in rumen protozoa (Dawson & Kemp, 1967). The most dramatic change was the almost complete absence of the choline-containing phospholipids, phosphatidylcholine and sphingomyelin. This is presumably due to the absence in bacteria of the enzyme CTP–choline phosphate cytidylyltransferase (EC 2.7.7.15) involved in the synthesis of choline-containing phospholipids (Borkenhagen & Kennedy, 1957). It is suggested that, provided sufficient time (2–3hr.) is given to allow lipolysis of the dietary phosphatidylcholine (Dawson, 1959), then the concentration of phosphatidylcholine in the rumen could be used as a satisfactory quantitative indicator of protozoal activity.

The rumen contents of the defaunated sheep were also devoid of phosphatidylhydroxyethylalanine, which confirms that this phospholipid is characteristic of the protozoal compliment (Kemp & Dawson, 1969). Ciliatine-containing phospholipids (diglyceride aminoethylphosphonate, ceramide aminoethylphosphonate) (Dawson & Kemp, 1967) were also absent, but, perhaps surprisingly, ceramide ethanolamine phosphate was present.

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