Lipolysis and Hydrogenation in the Rumen

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Two important activities of the rumen micro-organisms are (1) lipolysis of the lipids containing esterified fatty acids that are ingested by the ruminant and (2) hydrogenation of unsaturated fatty acids (Garton, 1964). Comparisons of the fatty acid composition of the free fatty acids and the esterified lipids of rumen contents suggest that hydrogenation of the fatty acids does not occur until they are hydrolysed from glycerolipid (Garton, Lough & Vioque, 1961; Hawke & Robertson, 1964; Patton & Kesler, 1967). For example, Patton & Kesler (1967) found that the fatty acids of neutral lipids, polar lipids and free fatty acids isolated from the rumen contained 6.6, 7.1 and 70.0% of stearic acid respectively, whereas the same fractions contained 3.6, 1.3 and 0.0% of linolenic acid. This communication reports the use of $2[1-^{14}\text{C}]$ -linolenoyl-3-oleoyl-1-palmitoylglycerol as substrate in incubations with rumen fluid to investigate the relationship between lipolysis and hydrogenation. The synthetic triglyceride was prepared by the methods discussed by Mattson & Volpenhein (1962), with 1-palmitoylglycerol as starting material (Hartman, 1960).

Rumen fluid was obtained from a cow with a rumen fistula that had been grazing on fresh pasture. After the rumen fluid had been strained through two layers of cheesecloth to remove large food particles, 20 ml. was incubated for 3 hr. with the ^{14}C -labelled triglyceride (0.01 g., $2.5\mu\text{C}$) under an atmosphere of N_2 at 38° . The reaction mixture was evaporated to dryness *in vacuo* on a rotary evaporator, the residue adjusted to approx. pH 2.0 by the addition of m-HCl and then immediately extracted four times with 20 ml. of diethyl ether by shaking at room temperature. The combined extracts were dried with anhydrous Na_2SO_4 , evaporated to dryness at 40° and dissolved in dry hexane.

The lipid products of the reaction were separated by t.l.c. on silica gel H (E. Merck A.-G., Darmstadt, Germany) with hexane-diethyl ether-acetic acid (70:30:1, by vol.). A radiochromatogram scan showed that 70% of the radioactivity was present in fatty acids and the remaining 30% in triglyceride. T.l.c. on a preparative scale was then used to separate the remainder of the lipid into free fatty

acids and triglycerides. Pure fractions of these components were obtained by rechromatographing the fractions obtained from the first preparative thin-layer chromatogram. The triglycerides were hydrolysed with 5% (w/v) KOH in methanol, and the constituent fatty acids of the triglycerides and the free fatty acids were methylated with diazomethane. Simultaneous analysis of the methyl esters and radiochemical analysis of the radioactive components were carried out by connecting a gas-liquid chromatograph (Aerograph 1520) to a proportional radioactive detector (Nuclear-Chicago Biospan model 4998). The g.l.c. separations were carried out on a 5 ft. $\times$ $\frac{1}{4}$ in. stainless-steel column, packed with 12% (w/w) diethylene glycol succinate on Celite (60–80 mesh), at 165° with He as carrier gas. One-fifth of the effluent from the gas-liquid chromatograph was passed into the hydrogen flame-ionization detector and the remaining four-fifths, mixed with an approximately equal volume of butane, were passed into the proportional detector.

The radiochromatograms obtained from the methyl esters prepared from the triglyceride and free fatty acid fractions of the incubation mixture (Fig. 1) showed that, whereas linolenic acid was the only radioactive component of the constituent fatty acids of the triglyceride fraction after incubation with rumen fluid, appreciable hydrogenation of the linolenic acid had occurred in the free fatty acid fraction. Comparisons of retention volumes of the unknown radioactive components relative to methyl palmitate with the values obtained for standard radioactive compounds established that the hydrogenated components were methyl stearate and the methyl esters of monoene and diene fatty acids. Only a small proportion of the original linolenic acid remained. The distribution of the radioactivity in the methyl esters, obtained by digital readout and by measurement of the peak areas by planimetry, was: $\text{C}_{18:0}$, 39.5%; $\text{C}_{18:1}$, 40.7%; $\text{C}_{18:2}$, 17.4%; $\text{C}_{18:3}$, 2.4%. The $\text{C}_{18:2}$ component consisted of two incompletely resolved components, the minor and faster-moving component probably being a conjugated isomer of methyl linoleate (Kepler, Hirons, McNeill & Tove, 1966).

The chromatogram of the mass response (Fig. 1)

Fig. 1. G.I.c. separation of the methyl esters of the fatty acids prepared from 20 ml. of rumen liquor incubated with 2[1- 14 C]-linolenyl-3-oleoyl-1-palmitoylglycerol (0.01 g., 2.5 μ c) for 3 hr. at 38°. (I) Radioactive methyl esters prepared from (a) free fatty acids and (b) triglycerides; (II) mass-detector record of methyl esters prepared from (a) free fatty acids and (b) triglycerides.

showed that the free fatty acids were much more saturated than the fatty acid components of the triglyceride, which is consistent with published observations (Hawke & Robertson, 1964). Measurements of peak areas gave 50.5 and 6.3% of methyl stearate in the respective lipid fractions. However, the comparison of the distribution of 14 C in the free fatty acids and the triglycerides shows unequivocally that hydrogenation only proceeds after the fatty acids have been removed from ester combination. Further, the absence of products of hydrogenation from the small amounts of 14 C-labelled diglyceride and monoglyceride isolated from rumen liquor that had been incubated with the synthetic [14 C]triglyceride for shorter periods of time gives further confirmation of a requirement for a free fatty acid substrate in biohydrogenation in the rumen.

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