Short Communications

Non-Involvement of δ -Hydroxy Acid in the Oxidation of Milk Fatty Acids

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The oxidation of medium-chain aliphatic fatty acids at the γ - and δ -carbon atoms has been proposed (Dimick, Walker & Patton, 1969) as a pathway that yields C_4 units for the biosynthesis of butyrate and other short-chain fatty acids in ruminant milk lipids. Evidence for this mechanism, obtained by radioactive-tracer experiments with lactating goats, may be summarized as follows: (1) radioactive δ -hydroxy fatty acids were isolated after the intravenous and intramammary administration of $[1-^{14}C]$ acetate (Walker, Patton & Dimick, 1968); (2) intramammary infusion of δ -hydroxy $[1-^{14}C]$ dodecanoate labelled the short-chain fatty acids in milk triglycerides; (3) radioactive γ - and δ -hydroxy fatty acids (lactones) and glyceride fatty acids were isolated from milk after the infusion of $[1-^{14}C]$ dodecanoate.

The investigations now reported have failed to demonstrate radioactivity in the γ - and δ -hydroxy acids isolated from milk after the administration of $1-^{14}C$ -labelled decanoate and dodecanoate to lactating goats. This casts considerable doubt on the role of the hydroxy acids as hydrated intermediates derived from the $\gamma\delta$ -monoene in the previously proposed oxidation pathway.

The sodium salts of $[1-^{14}C]$ decanoate (1.0mCi/8.2mg) and $[1-^{14}C]$ dodecanoate (1.0mCi/9.5mg) were each infused via the teat canal into one side of the gland of two lactating goats. Milk samples were collected at specified times from both the infused and the non-infused side of the gland. The lipids were recovered and the γ - and δ -hydroxy fatty acids (lactones) and triglycerides isolated as previously described (Walker *et al.* 1968). Triglyceride fatty acids were converted into methyl esters (Christopherson & Glass, 1969); these were analysed by g.l.c. and were assayed for radioactivity (Dimick *et al.* 1969).

Samples of milk drawn at 3, 6 and 9 h after the administration of labelled decanoate were analysed for radioactivity and the incorporation of ^{14}C into the pooled total milk lipids was found to be 12% for the infused side and 0.9% for the non-infused side. Tracer incorporation from the dodecanoate was

11% for the infused side and 0.6% for the non-infused side. It was observed in the decanoate experiment that the radioactivity in the esterified short-chain fatty acids (C_4 to C_8) in the milk fat

Fig. 1. Gas-liquid radiochromatogram illustrating the presence of ^{14}C in the γ - C_{12} and δ - C_{12} lactones isolated from commercial $[1-^{14}C]$ dodecanoate by t.l.c. (a) Mass scan of authentic compounds; (b) radiochromatogram of tracer contaminants. The column was a U-shaped glass tube (6ft $\times$ 0.25in) packed with 5% (w/w) Apiezon L on 90-100-mesh Chromosorb W-AW. The operating conditions were: column temperature, 165°C; detector temperature, 240°C; flash heater, 230°C; carrier gas (helium) flow rate, 80ml/min; operating voltage for proportional counter, 2300 V; quench gas (propane) flow rate, 8ml/min; combustion tube (CuO) temperature, 700°C.

isolated from the infused side ranged from 4.5 to 6.8% of the total fatty acid label, whereas these same acids isolated in the milk fat from the non-infused side of the gland contained 19.1–20.0% of total radioactivity in the esterified fatty acids. Results from the dodecanoate experiment indicated less incorporation into the short-chain fatty acids, with values of 2 and 5% respectively. The patterns of specific radioactivities of the glyceride short-chain fatty acids confirmed the results previously reported (Dimick *et al.* 1969), i.e. the maximum specific radioactivity occurred in the C₄ acid and decreased as chain length of the fatty acid increased.

The γ - and δ -hydroxy fatty acids, analysed as their corresponding lactones, ranged from 40 to 50 p.p.m. in the milk lipids from the decanoate and the dodecanoate experiments. No difference in lactone concentration was evident between the infused and non-infused sides in both experiments. No ¹⁴C could be detected in these compounds by gas-liquid radiochromatography, trapping and counting the radioactivity of the effluent from g.l.c. separations and t.l.c. This lack of radioactivity in the hydroxy acids is contrary to the results previously reported (Dimick *et al.* 1969). The radioactive purity of the commercial [1-¹⁴C]dodecanoate used in the previously reported study was checked solely by gas-liquid radiochromatography, and the extent of possible contamination with the lactones may have been so small that it remained undetected. In the present study the commercial [1-¹⁴C]-dodecanoate was purified by t.l.c. on silica gel G with light petroleum (b.p. 30–60°C)–diethyl ether–acetic acid (45:5:1, by vol.) as solvent. Radioactivity was evident on the t.l.c. plates in the region below the labelled fatty acid, and it is known that lactones and hydroxy acids migrate to this region. Analysis of this contaminant fraction by t.l.c. and

g.l.c. demonstrated the presence of radioactive compounds that co-chromatographed with standard γ - and δ -lactones (Fig. 1); the contaminants were found to contain 0.27% of the total radioactivity. Several commercial samples of [1-¹⁴C]dodecanoic acid were found to be contaminated with lactone-like material, whereas samples of [1-¹⁴C]decanoic acid proved to be free of radioactivity associated with lactones.

The presence and role of the δ -hydroxy fatty acids in milk lipids and animal tissue (Dimick, Patton, Kinsella & Walker, 1966) remains an intriguing question, but there would now seem to be no reason for supposing that these compounds are primary intermediates in the oxidation of fatty acids in the mammary gland. Moreover, the lack of radioactive labelling of these intermediates places doubt on the existence of the δ -oxidation pathway as originally proposed. However, the present results have confirmed the previous finding (Dimick *et al.* 1969) that active oxidation of the medium-chain fatty acids in the mammary gland occurs with subsequent contribution of carbon for the synthesis of short-chain fatty acids.

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