Occurrence and Biochemical Origin of Aliphatic Lactones in Milk Fat—A Review

Paul S. Dimick, Neil J. Walker,[1] and Stuart Patton

Freshly secreted milk fat contains small amounts of δ-hydroxy alkanoic acids esterified to glycerol which, upon hydrolysis during heating or storage, form a homologous series of saturated aliphatic delta-lactones. Aliphatic delta-lactones are not unique to lactating tissue, since they have been isolated in depot fat of both ruminants and monogastrics, although at lower concentrations. Correlative data on the lactone potential in ruminant milk fat under common variables such as feed, season, breed, stage of lactation, and metabolic disturbances provide a better definition of the origin and the factors which enhance or suppress their levels. Radiochemical studies have demonstrated that these trace flavor compounds are products from the endogenous biosynthesis of saturated fatty acids via delta-oxidation in the mammary gland. This mode of formation of aliphatic lactones and their relationship to lipid metabolism has challenging practical implications.

One rather remarkable area of flavor research which illustrates many of the evolving aspects and interrelationships in flavor science is that of the aliphatic (hydroxy acid) lactones of milk and milk products. This research was initiated from two very practical premises. One concerned the fact that stored forms of milk (dried or heat-sterilized fluids) have objectionable flavor that readily distinguishes them from the fresh product. The other involved the attractive flavor properties of butter as a cooking and baking additive and as a bread spread. As the ensuing review shows, the same homologous series of aliphatic lactones is involved in both phenomena. More recent work shows that these compounds, which are important in the palatability of man's food supply, are subject to quantitative variations in milk due to metabolic variables within the animal. Finally, and most significantly, a study of the origin of these lactones as δ-hydroxy acid precursors within the animal has shown that they are intermediates in lipid metabolism and evidence of a completely new and apparently ubiquitous pathway of fatty acid metabolism.

A principal impetus for research on the aliphatic lactones of milk arose from the unsuitable flavor properties of beverage-grade dry whole milk. Initially, it was assumed that some combination of processing, packaging, and storage variables could be found that would produce a dried milk which, when reconstituted, would have a flavor indistinguishable from the fresh fluid product. It became obvious in time that the problem was not that simple, for no measures seemed to prevent a "coconut-like" flavor which developed early in the storage life of the best dried whole milks. Thus, it became evident that a much more fundamental approach to the problem would be required, and that essential information on the basic nature of milk was lacking.

The particular flavor defect of dried whole milk was described by Lea *et al.* (1943) as "butter-toffee." Hetrick and Tracy (1945) noted characteristic flavor deterioration in the product which was not like that of fat oxidation. The findings of West (1948) and Musset *et al.* (1950) confirmed the existence of the flavor, demonstrated that it arises from the fat phase of the milk, and revealed it to be unaffected by antioxidants and oxygen levels. The fact that dried nonfat milk does not develop the defect provided further evidence of the off-flavor's lipid origin.

A study of volatile decomposition products of milk fat by Keeney and Doan (1951) indicated that a fraction having the coconut-like aroma also had the chemical properties of a lactone. It was subsequently postulated by Patton *et al.* (1954) that the lactone responsible for the off-flavor was δ-decalactone. This lactone does have the characteristic coconut-like or butter toffee aroma of heated milk fat. Thus, its identification in the medium by Keeney and Patton (1956) confirmed the postulation. Their evidence, together with that of Tharp and Patton (1960), indicated that related lactones, among them δ-dodecalactone, also are produced and involved to some extent in the flavor defect.

At about the same time, Boldingh and Taylor (1958, 1962) also detected these lactones in milk fat and viewed them as desirable contributors to butter flavor and aroma. There are no significant inconsistencies in the findings from these two lines of investigation. In addition to providing evidence as to what lactones are involved, both have indicated that the mechanism of lactone formation is nonoxidative and that the precursors are δ-hydroxy fatty acids esterified in the milk fat. The demonstration of optical activity in the δ-hydroxy fatty acid precursors by Boldingh and Taylor (1962) was particularly withering evidence against an autoxidative origin of the lactones. Rather, these findings have provided a sound basis for research on the technological application and control of lactones in food

Flavor Laboratory, Division of Food Science and Industry, The Pennsylvania State University, University Park, Pa. 16802

[1] Present address, New Zealand Dairy Research Institute, P. O. Box 1204, Palmerston North, New Zealand

products and on their metabolic origin and significance in the lipids of milk and tissue.

TECHNIQUES FOR ANALYSIS OF THE ALIPHATIC LACTONES

Isolation and Characterization. Keeney and Patton (1956) utilized methanol extraction and adsorption chromatography on neutral alumina to isolate the lactone fraction from heated butter oil. In later studies by Mattick *et al.* (1959), low-temperature crystallization of butter oil from acetone was employed to concentrate the lactone fraction prior to alumina adsorption chromatography. Subsequently, Tharp (1959) and Tharp and Patton (1960) reported on the use of vacuum steam distillation to remove the majority of the more flavorful lactones from milk fat.

Boldingh and Taylor (1962) employed a variety of techniques for the isolation of the lactones from butter oil. These methods included methanol extraction, molecular distillation, steam distillation, alkali extraction, and vacuum degassing to obtain a crude lactone fraction. Further purification was achieved by saponification, followed by extraction of unsaponifiable material, lactonization, urea extraction, and sodium carbonate extraction. These procedures are described in more detail in a subsequent publication by Boldingh *et al.* (1966). Forss *et al.* (1966) also reported on the use of molecular distillation at 50° C. and 0.001 torr for 8 hours to remove a portion of the lactones from butter oil.

Silicic acid adsorption chromatography has also been employed successfully to isolate crude lactone fractions from lipid extracts (Dimick *et al.*, 1966b; Jurriens and Oele, 1965a). Dimick and Walker (1967) compared the efficiency of recovery of the δ-lactones by this method to that by steam distillation. The results indicated that during isolation by silicic acid adsorption chromatography the shorter-chain lactones were not quantitatively recovered, whereas steam deodorization for five hours at 190° C. and 0.01 to 0.5 mm. of Hg with a steam flow of 100 ml. per minute (measured as condensate) failed to remove all of the longer-chain lactones. However, these two procedures still appear to be the most commonly used at present for isolation of the crude lactone fraction from a lipid extract.

Keeney and Patton (1956) employed paper chromatography of the hydroxamate derivatives (Keeney, 1957) to separate and aid in the identification of δ-decalactone and δ-dodecalactone in milk fat. De Jonge and Van der Ven (1965) prepared the hydroxyalkanilide derivatives of the γ- and δ-lactones. These derivatives were separated by paper chromatography (Boldingh *et al.*, 1966) and silicic acid column chromatography (Van Beers and Van der Zijden, 1966). Forss *et al.* (1966) and Urbach (1965) described the conditions for two-dimensional thin-layer chromatographic separation of the individual lactones.

Infrared spectroscopy (Boldingh and Taylor, 1962; Keeney and Patton, 1956), mass spectrometry (Day and Libbey, 1964; Khatri *et al.*, 1966), and nuclear magnetic resonance spectroscopy (Fioriti *et al.*, 1967) have been employed for the identification of lactones isolated from lipid systems.

Gas-liquid chromatography has also been universally employed to separate and identify naturally-occurring lactones. Dimick *et al.* (1966a) successfully employed both polar (10% diethylene glycol adipate + 2% phosphoric acid) and nonpolar (20% Apiezon L) stationary phases to identify the lactones in a variety of animal fats (Figure 1).

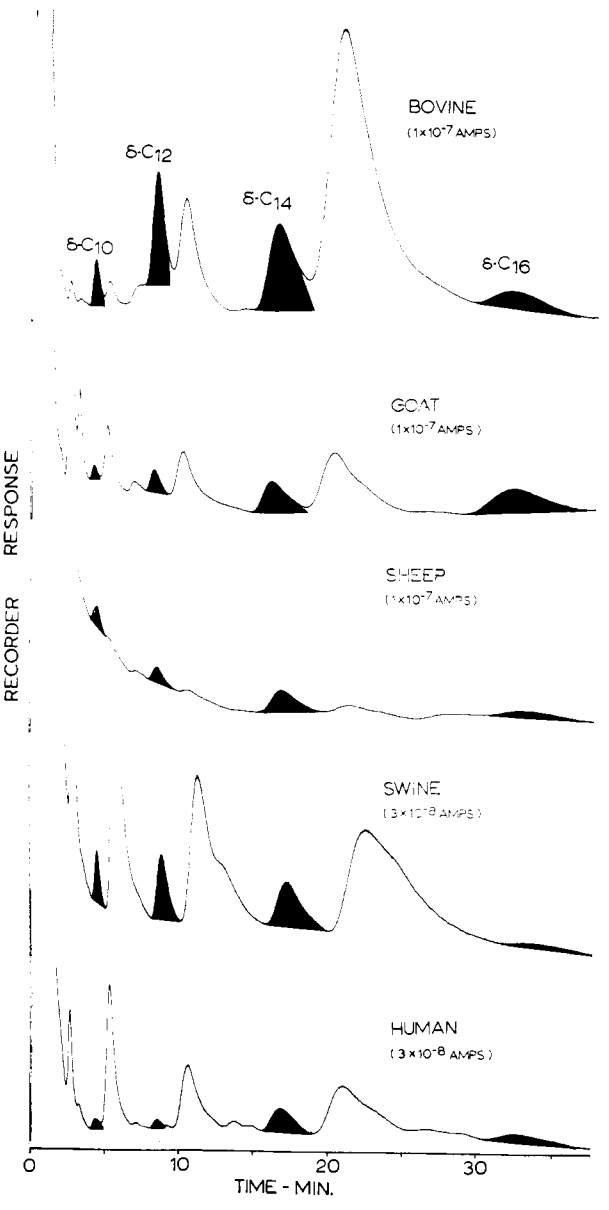

Figure 1. Gas chromatograms of lactones isolated from various milk fats (Dimick *et al.*, 1966a)

Quantitative Analysis. Gas-liquid chromatography has been extensively employed for quantitative estimation of the individual δ-lactones isolated from a lipid sample (Dimick *et al.*, 1966b). Lactones, used as standards in the quantitation, readily polymerize and cause excess tailing of the GC peaks. It was therefore necessary to depolymerize the authentic lactones by refluxing in alcoholic KOH, acidifying with 1*N* HCl, and lastly, extracting the lactone monomers with ethyl ether. The freshly depolymerized lactone was then made up to known concentration and injected onto the GC column to determine the peak areas. One calibration curve (concentration *vs.* GC peak area) does not accurately describe the relationship for the δ-lactones

of differing carbon chain length. This observation tended to rule out the use of one internal standard for quantitation of all lactones. Analyses were carried out isothermally at 185° C., using a column packed with 10% (w./w.) diethyleneglycol adipate + 2% (w./w.) phosphoric acid on Gas Chrom A. The gas chromatographic detector was of the argon ionization type.

Jurriens and Oele (1965a) have employed the isotope dilution technique for quantitative analysis of lactones in milk fat. Synthetic ^{14}C-labeled lactones were prepared and added in known quantity to the lipid sample. Specific activities of the isolated lactones were determined by gas-liquid radiochromatography. Van Beers and Van der Zijden (1966) have described a variation of this technique whereby the lactones are determined as their hydroxy acid anilide derivatives. Specific activities of these derivatives were determined by Geiger-Mueller assay for ^{14}C activity and with a spectrophotometer at 243 nm. for concentration measurement.

Control of Lactones in Processed Milks. The problem of controlling and preventing lactone-associated off-flavors in processed milks has suggested the merits of analyzing the lactones in such milks. A principal difficulty in this type of analysis is that of determining only existing lactones without causing erroneous results through conversion of the hydroxy acid glyceride precursors to additional lactone. The fact that lactones are usually recovered for analysis as trace constituents in a large amount of fat also tends to complicate the problem, because the fat should be eliminated without inducing any lactones. Patton (1961) devised a technique whereby liquid homogenized milks (pasteurized, sterilized, or dried) could be extracted with purified hexane to recover lactones present with the essential exclusion of milk fat. Analysis of the lactones was accomplished by gas chromatography of the hexane extracts. While this procedure is somewhat empirical, it appears to give results that relate well to the actual levels of lactones. Muck *et al.* (1963) employed this method in a study of evaporated milk which confirmed the presence of specific lactones and methyl ketones, and their importance to the flavor of the product.

A satisfactory procedure for the removal of lactones from milk fat has been described by Patton (1964). This involves steam deodorization of milk fat, by which process the lactones are formed from their precursors and removed by steam distillation under vacuum. The process also forms and eliminates methyl ketones from the fat at the same time. This produces a bland-flavored fat which, when properly fortified with antioxidants, is very stable toward flavor deterioration during storage (Nelson *et al.*, 1966; Wyatt and Day, 1965).

OCCURRENCE OF ALIPHATIC γ- AND δ-LACTONES

Table I represents an attempt to summarize the results in which the lactone concentrations in bovine milk fat have been determined. Wyatt *et al.* (1967) recently reported the presence of relatively large amounts of δ-octadecalactone in milk fat, in the region of 18.7 mole % of the total δ-lactone concentration. The occurrence of this compound has been observed at our laboratory, but at a concentration not exceeding 5 mole % of the total δ-lactones. One should consider the values from a relative rather than an absolute standpoint, based on variability due to environmental and physiological conditions of the animal, as reported by Dimick and Harner (1968).

Table I. Concentrations of γ- and δ-Lactones Isolated from Bovine Milk Fat

Carbon Number	δ-Lactones, P.P.M.	γ-Lactones, P.P.M.
C_6	2.0[a]	trace
C_7	trace	trace
C_8	2.6[b]	0.5[c]
C_9	trace	0.2[c]
C_{10}	15.0[d], 18.0[e]	1.2[d]
C_{11}	0.7[d]	0.5[d]
C_{12}	34.5[d], 33.0[e]	1.6[d]
C_{13}	1.5[d]	0.5[d]
C_{14}	34.0[d], 39.0[e]	1.4[d]
C_{15}	6.4[d]	1.3[d]
C_{16}	23.2[d], 32.0[b]	1.3[d]

[a] Parliment *et al.* (1966).
[b] Dimick and Walker (1967).
[c] Kinsella *et al.* (1967).
[d] Jurriens and Oele (1965a).
[e] Dimick and Harner (1968).

Van der Zijden *et al.* (1966) reported the presence of trace levels of unsaturated aliphatic γ- and δ-lactones —namely, δ-tetradec-9-ene-lactone, δ-dodec-9-ene-lactone, and γ-dodec-6-ene-lactone in butterfat. The principal contributors to butter flavor have been the δ-deca- and δ-dodecalactones, as evidenced by their addition to margarine to enhance the flavor quality. The relative flavor potencies of the γ- and δ-decalactones are roughly equivalent and have a flavor threshold in the order of 1 to 2 p.p.m. in milk (Patton *et al.*, 1954). As pointed out by Forss *et al.* (1966) in a study of the lactones in Australian butter oil, the role of the γ-lactones and trace levels of δ-lactones of chain length shorter than 10 carbons, as well as the unsaturated lactones, is not clear. These should be evaluated as to their role in butter flavor. The presence of the δ-lactones in lipid sources other than milk was first realized by Boldingh and Taylor (1962) when they reported their occurrence in beef tallow, however, in lower concentrations than that of milk fat. Subsequently, Dimick *et al.* (1966a) reported the presence of δ-lactones in steer, sheep, and swine depot fats. This study also provided evidence of their occurrence in numerous ruminant and nonruminant milk fats—namely, from goat, sheep, swine, and human. A quantitative comparison between the δ-lactone concentrations—that is, δ-C_{10}, C_{12}, and C_{14} lactones—reveals that the milk fats from the monogastric species were substantially lower than the ruminants, being approximately 5 p.p.m. for human and 14 p.p.m. for swine, as compared to 20 p.p.m. for sheep, 53 p.p.m. for goat, and 90 p.p.m. for cow. Lactone precursors occur commonly in animal fats, and therefore ultimately may play a role in establishing the flavor of these products and/or products in which they are utilized. In studies on the changes in meat fats by various processing, Watanabe and Sato (1968) have identified the δ-C_6, δ-C_{10} to C_{16} even-carbon, δ-C_{11} to C_{15} odd-carbon, and γ-C_7, C_8, C_9, and γ-C_{10} to C_{16} lactones in beef depot fat. These authors point out that these compounds may help extend the use of meat fats in shortening, margarine, and other oil products.

Both aliphatic γ- and δ-lactones have also been found in plant materials. Extensive studies by Jennings and colleagues (Jennings and Sevenants, 1964; Sevenants and Jennings, 1966; Tang and Jennings, 1968) have demonstrated the presence of γ- and δ-lactones in the volatile essence of peaches, pears, and apricots, and have shown that the γ-series predominate. Similarly, Silverstein *et al.* (1965) isolated caprolactone in pineapples, and Allen (1965) found even-numbered δ-lactones in coconut oil. Hydrogenated (Kawada *et al.*, 1966) and highly oxidized (Fioriti *et al.*, 1967) soybean oil have yielded γ- and δ-lactones upon analysis, the majority being γ-lactones. These are postulated to arise from the high levels of hydroperoxides from the unsaturated fatty acids. Under these treatment conditions, subsequent oxidation and hydrogenation will yield lactones.

PARAMETERS EFFECTING LACTONE POTENTIAL OF MILK FAT

Throughout the years of study on the aliphatic γ- and δ-lactones in bovine milk fat, identification and origin of these trace compounds has been emphasized. Of practical importance, further improvements in flavor quality of the various forms of beverage milk require answers to such questions as why some milks produce a greater degree of coconut-like defect than others. Patton (1965) noted that two milks processed under identical conditions, but one from California and the other from Wisconsin, had extremely differing degrees of lactone flavor. The influence of such common variables as feed, season, breed, and stage of lactation could have influenced the lactone potential of these samples, based on the observations recently reported by Dimick and Harner (1968). Their studies show that a pronounced seasonal trend occurred in the lactone potential of bovine milk fat from 276 animals tested (Figure 2). When the animals were on pasture, the lactone output in the fat was lower than when the animals were on a barn-feeding regimen, averaging 67 and 96 p.p.m., respectively. The sine-like curve for the seasonal data appeared to be influenced by the type of feed the animals were consuming. When animals were shifted from normal ration to a complex mix containing chopped alfalfa hay and heat-treated corn, a diet which depresses the fat content of milk, a marked decrease

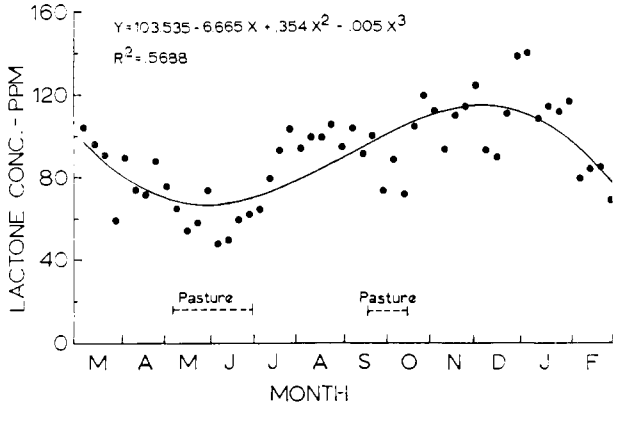

Figure 2. Seasonal variation in aliphatic delta-lactone potential (C_{10}, C_{12}, C_{14}) of butter oil (Dimick and Harner, 1968)

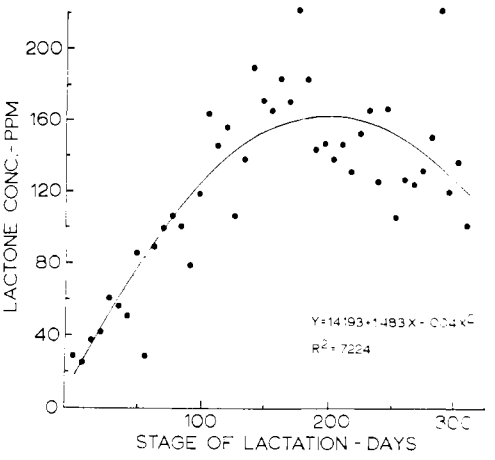

Figure 3. Variation in aliphatic delta-lactone potential (C_{10}, C_{12}, C_{14}) of butter oil with stage of lactation (Dimick and Harner, 1968)

in the δ-C_{10} and δ-C_{12} lactone resulted (Dimick *et al.*, 1966b). The diet of the bovine does influence the lactone potential of the resulting milk fat. Primary to to practical challenge, one may possibly foresee the advantage of selecting milks from animals on differing diets, depending on the desire for this flavor attribute. Similarly, the positive correlation between the lactone potential and that for methyl ketones (Dimick and Walker, 1968) over season suggests a combined contribution of these compounds in the susceptibility of a dairy product to develop a stale flavor.

The composition of bovine milk changes considerably throughout lactation. Similarly, it has been shown that the lactone potential also varies (Figure 3). Immediately following parturition, the lactone potential ranges from 25 to 30 p.p.m., increases to 170 to 180 p.p.m. at 150 days, and decreases during the remainder of the lactation period. The computed correlation coefficients between the lactone potential and some of the variables tested during lactation are presented in Table II. These data reveal two important points; first, there is significant correlation between the amounts of δ-lactones and methyl ketones, both classes of nonoxidative compounds formed in fresh milk fat, and secondly, there is similarity in the degree of significance and direction (positive and negative) of the correlation coefficients

Table II. Correlation Coefficients Between Lactone, Monocarbonyl, and Methyl Ketone Potentials and Tested Variables [a]

Variables	Aliphatic Delta-Lactones, r	Mono-carbonyls, r	Methyl Ketones, r
State of lactation	0.636[b]	0.555[b]	0.543[b]
Fat test	−0.611[b]	−0.386[b]	−0.401[b]
Milk yield	−0.021	−0.226	−0.218
Fat yield	−0.443[b]	−0.406[b]	−0.435[b]
Short-chain fatty acids	0.829[b]	0.388[b]	0.443[b]
Aliphatic delta-lactones	—	0.308[c]	0.441[b]

[a] Dimick and Walker (1968).
[b] 1% level of significance $r = 0.376$.
[c] 5% level of significance $r = 0.291$.

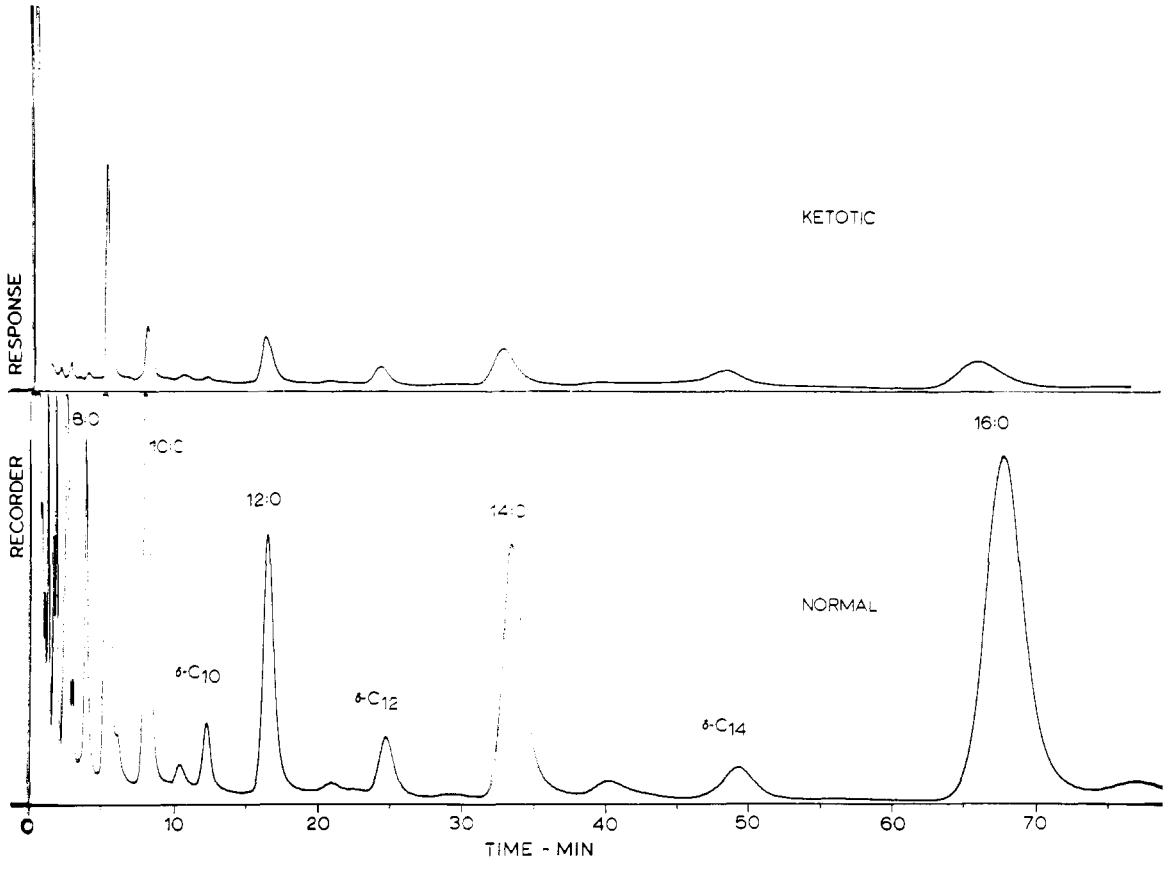

Figure 4. Gas chromatograms of total steam volatiles from butter oil of animal when in a ketotic and normal condition (Dimick and Harner, 1968)

between these compounds and the variables tested. The effects of a metabolic disorder such as ketosis in the bovine drastically depresses the lactone output (20 to 64%) in the resulting milk, as dramatically illustrated in Figure 4. These results, along with the dietary implications, strongly relate the level of lactone potential to the milk fat-synthesizing capability in the ruminant.

BIOCHEMICAL ORIGIN OF THE γ- AND δ-LACTONES IN MILK FAT

Freshly secreted milk fat contains essentially no free lactones, and the formation of these compounds is induced by a post-secretion treatment such as heating. This phenomenon implies that the lactone precursors are present in bound form. Mattick *et al.* (1959) provided initial evidence that the precursor of δ-decalactone in fresh milk fat was the δ-hydroxy decanoic acid existing as an ester. This observation has been confirmed and expanded by several groups of investigators. Boldingh and Taylor (1962) reported that infrared spectrophotometric analyses of certain fractions of unheated butterfat indicated presence of hydroxy acid glycerides, and that these fractions yielded lactones on hydrolysis. They concluded that the δ-hydroxy acids were built into a triglyceride in combination with two normal fatty acids. Jurriens and Oele (1965b), using column and thin-layer chromatography, isolated a butterfat fraction which yielded lactones on heating and hydrolysis. R_f values of this fraction compared well with those of synthetic monohydroxyacyl triglycerides. The presence of a free hydroxy group in the lactone precursor was demonstrated by acetylation of the precursor fraction and inhibition of lactone formation. Similar inhibition was recently observed by Parliment *et al.* (1966), who formed the trimethylsilyl derivatives of the lactone precursor fraction. Upon hydrolysis of the latter derivatives with phosphoric acid, however, the lactone potential was restored.

Kinsella *et al.* (1967) isolated a similar fraction from unheated butterfat by column and thin-layer chromatography, and confirmed that the lactone odor was absent until the fraction was heated. Lactones were released from this fraction by pancreatic lipase, which suggests that the hydroxy acids are esterified at the α-position of the triglyceride. The resulting monoglyceride did not yield lactones upon heating or hydrolysis. Recently, Wyatt *et al.* (1967) identified a series of γ- and δ-hydroxy acids in the polar glyceride fraction of fresh milk fat as their trimethylsilyl ether methyl esters by comparison of the mass spectra and gas chromatographic retention times with those of authentic compounds.

All evidence to date, therefore, establishes that the precursors of γ- and δ-lactones in freshly secreted milk fat are the corresponding γ- and δ-hydroxy fatty acids esterified to a triglyceride (Figure 5).

Logically, the next question pertains to the origin of these hydroxy fatty acids. Several recent studies have shed some light on this area.

The actual existence of a homologous series of lactones, hence of lactone precursors, and the discovery of Boldingh and Taylor (1962) that the series of even-

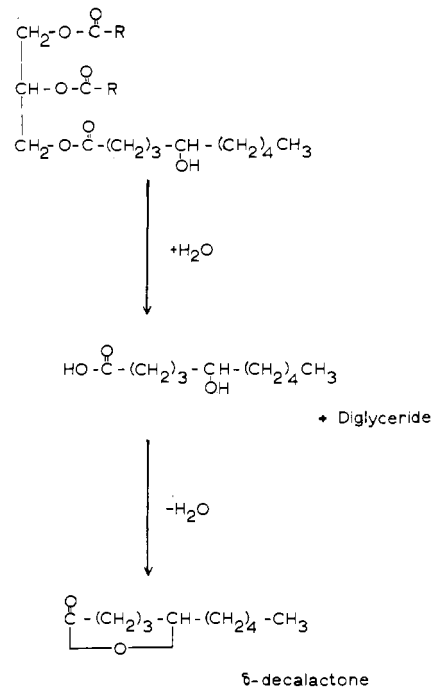

Figure 5. Structure and breakdown of a typical lactone precursor

numbered δ-hydroxy fatty acids exists in only one of two possible optically active forms, positively demonstrate the biological origin of these compounds. In addition, the lack of any evidence showing the presence of homologous series of γ- or δ-lactones in plant lipids, especially in any species used for animal feed, suggests that the γ- and δ-hydroxy fatty acids are products of endogenous animal metabolism. Results obtained in the classical studies by Virtanen (1966) supported this suggestion. Delta-lactones were found in the milk fat of cows fed highly purified diets containing measured amounts of pure vegetable oils to at least the same levels as in the milk of control cows fed normal diets.

The studies by Dimick and colleagues have been discussed previously concerning the widespread occurrence of the lactones and their positive correlation with the amount of short-chain (C_4 to C_{14}) saturated fatty acids in milk under various environmental conditions. These observations encouraged the authors to speculate on the possible involvement of acetate in the formation of δ-hydroxy acids. They implied that these acids are intermediates in or products derived from the process of fatty acid synthesis in the mammary gland. The biosynthesis of saturated C_4 to C_{16} acids from acetate, which occurs in the mammary gland, is well established and is the subject of several reviews (Folley and McNaught, 1961; Garton, 1963).

Van der Ven (1964) reported on the isolation of γ- and δ-keto fatty acids from milk fat in trace amounts. The possibility of a relationship between these acids and the γ- and δ-hydroxy fatty acids was suggested, even to the extent that the hydroxy acids may be formed directly from the keto acids by enzymatic reduction. The presence of enzymes in the mammary gland having this capability has not been demonstrated, however.

The presence of keto stearic and hydroxy stearic acids in rumen lipids and milk fat has been established by Katz and Keeney (1966) and Keeney *et al.* (1962). Theoretically, it is possible to derive series of γ- and δ-hydroxy fatty acids by oxidation of these acids, but no evidence has been forthcoming to show that this mechanism does, in fact, explain the origin of the δ-lactone precursors.

Walker *et al.* (1968) investigated the origin of δ-lactones (δ-hydroxy acids) in ruminant milk fat obtained from a goat following intravenous and intramammary administration of 1-^{14}C-acetate. Gas-liquid radiochromatography was employed to demonstrate that ^{14}C from 1-^{14}C-acetate was incorporated in vivo into the aliphatic δ-lactones of goat milk fat. The δ-C_{10}, C_{12}, C_{14}, and C_{16} lactones were isolated from the milk fat collected at 5, 10, 15, 20, and 25 hours following administration of the tracer to two goats. The specific radioactivities of the δ-lactones were of comparable magnitude to the specific activity of the saturated fatty acids of corresponding chain length. The data also revealed that the C_{18} acids, constituting 35 to 45% by weight of the milk fatty acids, were not isotopically labeled. This observation is consistent with the view that the major portion of the C_{18} fatty acids of milk are derived from blood plasma lipids, and precludes the possibility that the δ-hydroxy fatty acids are formed to any significant extent by oxidative degradation of the C_{18} saturated or unsaturated fatty acids.

Complete elucidation of the actual pathway of δ-hydroxy fatty acid synthesis could not be realized from this study. It would seem feasible that in mammary gland tissue, where fatty acid biosynthesis from acetate is intense, δ-hydroxy fatty acids may be products of, or intermediates in, the normal biosynthetic pathway.

In further experiments, Dimick *et al.* (1969) administered the sodium salt of 1-^{14}C-dodecalactone via intramammary infusion to a lactating goat. The metabolic activity of the lactone was apparent by the labeling patterns in the C_4 through C_{16} fatty acids composing the milk fat triglycerides (Figure 6). The trend in specific

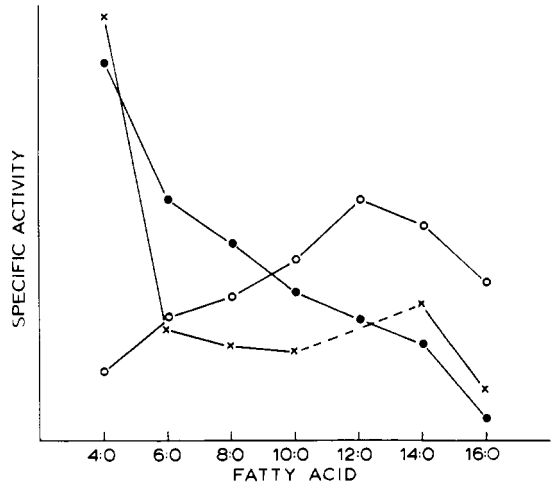

Figure 6. Specific activity patterns in milk triglyceride fatty acids following intramammary infusion into fed lactating goats (Dimick *et al.*, 1969)

(○) sodium 1-^{14}C-acetate, 5-hr. milk
(●) sodium 1-^{14}C-δ-hydroxy laurate, 3-hr. milk
(×) sodium 1-^{14}C-laurate, 3-hr. milk

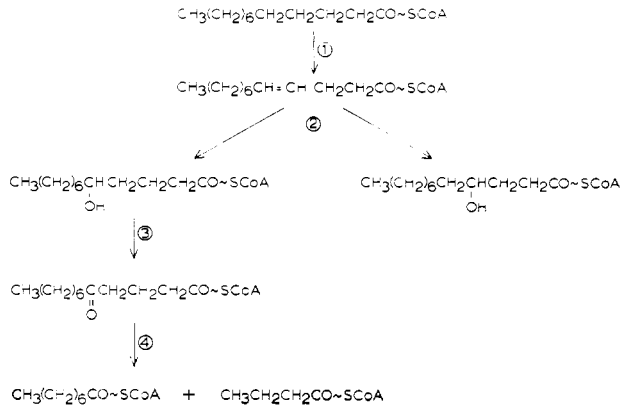

Figure 7. Proposed pathway for δ-oxidation of saturated fatty acids (Dimick *et al.*, 1969)

radioactivities of the fatty acids is characteristic of a four-carbon unit involvement in the synthesis of milk fatty acids, as elaborated on by Bines and Brown (1968) and Ahrens and Luick (1964). More particularly, the specific radioactivity is highest in the butyrate and decreases as chain length of the fatty acid increases, in contrast to the two-carbon involvement, where the C_{10} and C_{12} acids have the highest specific radioactivities. This four-carbon unit possibly results from cleavage between the number 4 and 5 carbons, as influenced by the hydroxyl on the number 5 carbon of the hydroxy acid administered to the goat.

To further elaborate this unique phenomenon, 1-^{14}C-dodecanoic acid was similarly infused into the lactating gland of a goat. Label in the resulting milk was present at high levels in both the γ- and δ-lactones. The glyceride fatty acids of this fat were also isotopically labeled with the same specific radioactivity pattern as when the sodium 1-^{14}C-dodecalactone was administered (Figure 6). These workers therefore proposed the existence of saturated fatty acid δ-oxidation in the ruminant mammary gland. This new endogenous oxidation within the gland is the pathway for the formation of both the γ- and δ-hydroxy acids, and therefore the resulting lactones (Figure 7). Establishing the quantitative significance of the δ-oxidation pathway for the four-carbon contribution to the synthesis of short chain fatty acids and defining the specific enzymes involved will require further research.

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