INTRODUCTION

Milk fat is one of the most palatable food components known to man. In the form of milk and various dairy products, and as an ingredient in cookery, it is widely consumed by all age groups. The demand for milk fat has been such as to establish selective breeding of cows for high fat production as a standard practice. It can be stated with the greatest confidence that the number of milk samples tested for fat content has been astronomical, but until recently milk fat composition and properties was a consideration of interest to a very few research workers. This inclination to let the cow do what she will regarding qualitative aspects of milk fat production is open to question for two reasons. First, it seems doubtful that further improvement can be made in various dairy products, on the assumption that composition and properties of milk fat are of little importance. Secondly, it seems unwise to ignore the current concern that many have for their weight and the state of their arteries. This concern has provoked a tremendous research effort in the field of lipid metabolism. Although

The dairy industry is not oblivious to these considerations, its research program on milk lipids seems somewhat inadequate. A mastery of milk fat synthesis, composition, and properties would at least be good insurance for the future; conceivably, it could be the basis of better and more healthful dairy products. The milk fat that makes for ideal texture in butter may not necessarily yield optimum dispersibility and flavor in dry whole milk. With respect to nutrition, the milk fat consumed by infants, normal adults, and elderly heart-patients may well represent three entirely different requirements from the standpoint of fatty acid composition.

Thus, as a Symposium topic, Milk Lipids seems appropriate and timely. The objective of the Symposium, stated simply, is to examine how milk fat is made, how its composition can be analyzed, how it may be metabolized in the body, and how it may deteriorate prior to consumption. In the limited time available the treatment of these subjects can hardly be exhaustive. However, it is hoped that the papers of this Symposium, together with other papers on milk lipids to be presented at this meeting, will indicate the frontiers of knowledge on the subject.

MILK FAT SYNTHESIS

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The origin of milk fat in general, and of the short chain fatty acids in particular, has resisted the investigations of scientists for more than half a century—even though their labors have been fruitful. Nevertheless, the theory of milk fat synthesis most widely accepted today differs only in degree of refinement from that proposed by Carlo Foà in 1912 (8), despite the development of chromatography, radioactive isotopes, the Warburg apparatus, and the like. This is not to imply that Foà's experiments and techniques left little to be desired nor that his work was overlooked by his successors. On the contrary, nearly everyone has credited Foà with being the first to use perfused udders for the study of synthesis of milk. However, it seems Foà's contributions were more significant than that. He insisted that the udder plays an active role in the synthesis of milk fat, absorbing certain triglycerides from blood and rejecting others. More important, he stated that the short chain fatty acids originate in the udder and that they are synthesized from noncarbohydrate sources. Further, Foà was apparently the first to state that lactose is synthesized in the udder from plasma glucose.

Foà apparently failed to convince his colleagues and successors of the validity of his observation, however, for a number of papers between 1934 and 1940 supported the idea that milk fat is synthesized entirely from plasma lipids (13, 25, 30, 46). This idea fitted nicely with Hilditch's observations on the fatty acid composition of ox blood and milk fat triglycerides, and in 1937 he proposed that the short chain fatty acids of milk fat resulted from a stepwise omega degradation of the long chain fatty acids, especially oleic, which were a part of the absorbed triglycerides (14). Hilditch's theory was well founded, and undoubtedly would be widely accepted today had not the Atomic Age made radioactive tracers available.

The first experimental evidence to indicate that the short chain fatty acids might arise by
an entirely different pathway came from the Russian workers Asimoff and Kashevaroff [cited by Nikitin, (34)] who, in 1940, reported that the mammary gland absorbs significant quantities of beta-hydroxybutyric acid from the plasma. Shaw and Knodt (44) confirmed this finding in 1941 and calculated that the beta-hydroxybutyrate absorbed was more than enough to account for the synthesis of all the milk fatty acids from C₆ through C₁₄.

Experimental work in ruminant metabolism was scant during the war years. In 1943, however, a paper by Barcroft and coworkers (2) suggested that the volatile fatty acids in milk fat might arise from volatile acids produced in the rumen. That suggestion apparently stimulated subsequent research. Immediately after the war, a large number of relevant articles appeared in the English journals. The volatile fatty acids found in the rumen and peripheral blood of cattle and sheep were identified as acetate, propionate, and butyrate (3, 6, 7, 19, 29, 32, 40, 41), and in 1949 G. L. McClymont (31) demonstrated that large amounts of acetate are absorbed by the mammary gland of the lactating cow.

C₁³-labeled metabolites became available for biological research during this period, and almost immediately thereafter Popjík, Polley, French, and their coworkers in England [see Polley (9)], and Kleiber and coworkers at Davis, California (20, 21, 22), proved beyond reasonable doubt that the circulating volatile acids in the blood are indeed important precursors of milk fat.

LIPID PRECURSORS OF MILK FAT

Comparison of dietary fat with milk fat. Figure 1 compares the fatty acid composition of pasture clover lipids with milk fat and emphasizes the point that large changes must occur in the portion of dietary fat that eventually becomes a part of milk fat. This is not to imply that milk fat may be derived entirely from dietary fat; one can readily calculate that the average milk cow secretes about twice as much fat in milk as she eats in her feed. In other words, the cow must synthesize at least half her daily production of milk fat from nonlipid sources. The figure also shows that clover lipids contain large amounts of unsaturated fatty acids—oleic, linoleic, and especially linolenic, the last being almost completely absent in milk fat. In contrast, milk fat contains relatively large amounts of short chain fatty acids—acids which are not found in appreciable quantities in any other lipid fraction of the cow's body.

Effect of rumen microflora on dietary lipids. Figure 2 shows the fatty acid composition of lipids taken from rumen contents of sheep that were grazing on clover-rich pastures (Figure 1). Comparison of these two figures indicates that the dietary lipids undergo extensive change even in the first step of digestion. Shorland et al. (47) were attempting to explain why prolonged feeding of highly unsaturated oils failed to decrease the degree of saturation of body depot fat. Dietary lipids contain large proportions of C₁₀ unsaturated fatty acids, particularly linoleic acid, but very little stearic acid. The lipids isolated from rumen

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**Fig. 1.** Fatty acid composition of clover-rich lipids [data of Shorland et al., Reference (47)] compared with that of butterfat [Hilditch, Reference (47)].

**Fig. 2.** Fatty acid composition of rumen-content lipids [data of Shorland, Reference (47)].
contents, in contrast, contained very little linolenic acid but large amounts of oleic and stearic acids. These observations led to the conclusion that the highly unsaturated dietary acids are extensively hydrogenated in the rumen. Ruminal hydrogenation also accounts for the presence of trans isomers of fatty acids in depot and milk fat.

Comparison of rumen content lipids with blood lipids. The fatty acid composition of the plasma triglycerides is shown in Figure 3, where it may be seen that except for minor differences, there is a rather close similarity in molar percentage of fatty acids in this fraction of the plasma lipids and the lipids in the rumen. In contrast, the molar percentage of fatty acids in the plasma sterol esters (Figure 4) differs considerably from that of rumen content lipids. Since cholesterol esters of bovine plasma make up nearly 80% of the total lipids, they must be considered as possible precursors of milk fat. Cholesterol esters typically contain large amounts of C18 unsaturated fatty acids, and in this respect resemble dietary lipids more closely than depot or milk fat lipids. This observation, plus the little cholesterol in the diet of dairy cows (most of it is synthesized endogenously), led Lough and Garton (26) to propose that cholesterol is esterified with long chain fatty acids of dietary origin, either during or after their absorption from the gut. In other words, this may be viewed as a transport mechanism for the dietary unsaturated fatty acids.

Composition of blood lipids. Figure 5 was taken from the work of Garton and Duncan (11), who used silicic acid column chromatography to separate the various blood lipids of Ayrshire cows. This figure shows that approximately 80% of the circulating lipids are cholesterol esters, and that there are 7.5% triglycerides, 6.5% sterols, 3.8% phospholipids, 2.5% unidentified lipids, and only traces of free fatty acids. Milk fat, considerably different from plasma lipids, contains about 98% triglycerides, 1% phospholipids, and less than 1% cholesterol esters (17). Studies of the uptake of various plasma lipids by the mammary gland have been conducted over the past 50 yr. by determining the difference in concentration of a particular lipid fraction in the arterial blood supply to the udder and in the venous drainage from the udder. There

Fig. 3. Molar per cent fatty acids in plasma triglycerides of Ayrshire cattle. [Data of Lough and Garton, Reference (26)].

Fig. 4. Molar per cent fatty acids in cholesterol esters of Ayrshire cattle [data of Lough and Garton, Reference (26)].

Fig. 5. Weight per cent of various lipids in Ayrshire blood [data of Garton and Duncan, Reference (11)].
seems to be unanimous agreement that the mammary gland absorbs large quantities of triglycerides from the blood. Indeed, several workers have estimated that the uptake of these glycerides is sufficient to account for the complete production of milk fat (13, 30, 46).

There is some disagreement on whether phospholipids and cholesterol esters are used for fat synthesis. Nikitin (34) claims work in Russia during the war demonstrated that up to 40% of milk fat is derived from phospholipids of the blood. This idea seems to be supported by two recent reports by Riis, Luick, and Kleiber at Davis (42), and by Glascock and his group in Reading (12). Even so, arterio-venous difference studies have consistently failed to show a significant uptake of either cholesterol esters or phospholipids by the mammary gland of dairy cattle, and there seems to be no experimental proof that either of these two lipids plays an important role in the net transport of fatty acids in other species (10).

Comparison of milk fat with depot and blood fat. The fatty acid composition of depot fat is shown in Figure 6. Depot fat contains large amounts of palmitic, stearic, and oleic acids and relatively few short chain fatty acids. In contrast, milk fat contains large amounts of short chain fatty acids and relatively little stearic and oleic acid. It may be of some interest to recall that changes in animal diet and health (18, 43) result in large changes in the fatty acid composition of milk fat but have little effect on the composition of depot fat. The principal difference in fatty acid composition between plasma triglycerides and milk fat (Figures 1 and 3) is due to the presence of C6 through C14 fatty acids in milk fat; the plasma glycerides contain relatively more palmitic, stearic, and C16 and C18 unsaturated fatty acids. Comparison of Figures 1 and 4 shows that the difference in fatty acid composition between plasma sterol esters and milk fat is even more striking—particularly in the large amounts of unsaturated fatty acids in the sterol esters. As mentioned earlier, there is little evidence that the udder absorbs cholesterol esters from the plasma; comparison of the fatty acid moieties of these two lipids shows that even if sterol ester fatty acids were available for milk fat synthesis, they would have to undergo extensive chemical change before they could become a part of the milk fat molecule.

These comparisons have shown that lipids that might be considered obligatory intermediates in the synthesis of milk fat differ considerably from one another in fatty acid composition. Blood lipids have been fractionated into four or five components, and each of these fractions has, at one time or another, been considered a possible precursor of milk fat. It now seems positive, however, that only the blood triglycerides are absorbed in quantities large enough to contribute significantly to milk fat synthesis. They have also shown that milk fat differs considerably in fatty acid composition from all other lipids in the ruminant body, particularly in regard to the presence of short chain fatty acids. Intensive metabolic activity by ruminal microorganisms results in the production of trans isomers of the dietary fatty acids. Unsaturated fatty acids undergo hydrogenation and, more important, large quantities of short chain fatty acids, especially acetate, propionate, and butyrate are synthesized from nonlipid dietary precursors.

NONLIPID PRECURSORS OF MILK FAT

The bulk of the acetate produced in the rumen seems to pass unchanged through the liver and into the general circulation (29). On the other hand, under normal dietary regimes, most workers now hold that little propionate or butyrate, as such, finds its way into the peripheral blood (3, 19, 29, 41). Propionate is thought to contribute most of its C to the synthesis of glucose (22, 32, 40). Butyrate is oxidized to beta-hydroxybutyrate by the ruminal epithelium and perhaps by the liver (1, 35, 48, 49). Some butyrate may escape into the general circulation, but one may readily calculate from arterial blood butyrate concentrations and blood flow through the udder that butyrate as such—even if completely absorbed and used exclusively for fat synthesis—can not contribute more than 13% of the C necessary for the synthesis of short chain fatty acids.

SYNTHESIS OF MILK FAT IN THE MAMMARY GLAND

The synthesis of milk fat in the mammary gland is not completely understood. The long chain fatty acids presumably come directly from the triglycerides which are absorbed from
the blood, and there is little evidence to suggest that other plasma lipids are important precursors of milk fat. It is now almost certain that the short chain fatty acids are synthesized from acetate and beta-hydroxybutyrate (5, 21, 24, 37, 38) according to the following scheme.

\[ \text{Beta-hydroxybutyrate} \rightarrow \text{butyrate} \]

Beta-hydroxybutyrate is absorbed into the udder (36, 44) and follows one of two major pathways: reduction to butyrate or cleavage between the second and third C atoms to yield methyl and carboxyl 2 C fragments. The carboxyl 2 C fragment seems to be used preferentially for fatty acid synthesis, and presumably follows the same pathway as acetate (24, 45). Head-to-tail condensation of these 2 C fragments is thought to account for 40% of the newly synthesized butyrate; the remaining 60% comes from direct reduction of the newly synthesized beta-hydroxybutyrate (38). Subsequent chain elongation, brought about by the stepwise addition of 2 C units on to the carboxyl end of the fatty acid, ultimately leads to the synthesis of all acids up to C₁₀ and perhaps even to C₁₈ (24, 38).

ORIGIN OF MILK FAT GLYCEROL

The absorption of triglycerides from blood necessarily implies the absorption of equimolar quantities of glycerol. Since sufficient triglyceride is absorbed to account for the complete synthesis of milk fat, one might assume the same applies to glycerol. The fallacy of this logic is well known and applies to nearly all statements which are based on absorption studies. The absorption of a substance does not necessarily mean the synthesis of a chemically similar substance. Moreover, the absorbed substance might be catabolized or used for the maintenance of structure and function within this very active tissue. The origin of milk fat glycerol seems to be a case in point.

Figure 7 compares the radioactivity in milk fat glycerol with plasma glucose following the intramammary infusion of uniformly C¹⁴-labeled glucose (28). It also shows the summated specific activity of C¹⁴ in milk fat glycerol and plasma glucose. The ratio of these two terms is known as the transfer quotient (23) and expresses the importance of glucose as a precursor of glycerol. In this trial, it indicates that 68% of milk fat glycerol is derived from plasma glucose.

Although this calculation shows that a large fraction of milk fat glycerol comes from glucose, it tells nothing of the two pathways which might be involved: The synthesis of glycerol from glucose in the udder or the synthesis of plasma lipid glycerol from glucose, presumably in the liver (4), and its subsequent absorption into the udder. Recent work in our laboratory (27), shown in Figure 8, indicates that glycerol synthesis from glucose does, indeed, occur in the udder. This figure compares the radioactivity in glycerol taken from the infused and noninfused quarters of lactating cows following the intramammary infusion of C¹⁴-labeled glycerol, glucose, fructose, butyrate, propionate, and acetate. Only glucose and glycerol lead to high labeling in milk fat glycerol and in these two trials glycerol isolated from the infused quarter is much more radioactive than glycerol taken from the noninfused quarters. Glycerol per se can not be considered an important precursor of milk fat glycerol since there is little, if any, free glycerol in the blood of cattle. However, the high labeling after the infusion of C¹⁴-labeled glycerol is of unusual interest, since it implies that glycerol, if synthesized in the udder, becomes a part of the milk fat molecule.

In contrast, large quantities of glucose are absorbed in the udder (13, 33) and, therefore, the high labeling in milk fat glycerol after the infusion of C¹⁴-labeled glucose means that glycerol is synthesized in the udder. In this regard, our results confirm earlier findings with rabbits (39) and cows (50).

These results suggest a new concept of milk fat synthesis. Up to now we have generally
conceived of milk fat synthesis as a rearrangement or substitution of fatty acids on preformed triglyceride molecules which are absorbed from the blood. The synthesis of glycerol from glucose in mammary tissue and the subsequent incorporation of glycerol into milk fat means that milk fat, to an unknown extent, must be synthesized de novo within the mammary gland from glycerol and free fatty acids.

REFERENCES
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Gas chromatography is one of those remarkable developments in science with far-reaching effects. Primarily, it is a means of separating mixtures of volatile organic compounds in a highly efficient manner. This principle is of such general importance that it is difficult to predict the many ultimate uses that gas chromatography may have. Although broad exploration of the method began about 5 yr. ago, it is already evident that gas chromatography will be indispensable in lipid analysis. At the moment, it is most appropriately applied in determining fatty acid composition of lipids through analysis of the methyl esters. Its use in the analysis of steroids and intact triglycerides is being probed in a number of laboratories. Although vapor pressures of such classes of compounds are discouragingly low at reasonable temperatures, some members of these groups have been gas chromatographed satisfactorily.

To date, milk lipids have not received extensive appraisal with this new analytical tool. A recent report (6) from our laboratory reviewed the limited literature and explored qualitative and quantitative problems in gas chromatographic analysis of fatty acid methyl esters of milk fat. In brief, the study showed that such analysis is feasible and that gas chromatography holds much promise in application to


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