# On the Probable Origin of Some Milk Fat Acids in Rumen Microbial Lipids[1]

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## Abstract

The quantity and character of the microbial lipid isolated from rumen digesta are interpreted as indicating that significant quantities of milk fat acids originate from rumen microbial synthesis of long chain acids from volatile fatty acids. Component fatty acid patterns are presented of rumen bacterial lipid, crude rumen protozoal lipid, blood serum lipid, and milk lipid isolated from samples taken from a lactating Holstein. Certain rumen bacterial lipid fractions are shown to be very rich sources of odd carbon acids and branched acids, and it is suggested that the major source of these acids in ruminant fats is from bacterial synthesis rather than animal synthesis.

THE DISCOVERY of small quantities of odd-numbered carbon and branched-chain fatty acids in ruminant milk and body fats by Hansen *et al.* (1,2,3), poses the question of their biological origin. El-Shazly (4) suggested that the odd and branched-chain volatile fatty acids, formed in the rumen by microbial degradation of amino acids, might provide the building blocks for the synthesis of the longer chain acids in the ruminant. It was visualized that successive addition of acetate units to propionic, *n*-valeric and isovaleric acids, for example, could yield the $C_{15}$ and $C_{17}$ odd and branched-chain acids found in ruminant fats. There has been an apparent inclination to postulate an animal synthesis of these long chain acids as evidenced by the approach used in some recent research (5,6,7). Gerson *et al.* (5) studied the distribution of radio-activity in the milk fat acids of a cow after an intra-jugular injection of carboxyl-labeled *n*-valeric acid. Verbeke *et al.* (6) made a similar study of milk fat acids and udder fat acids from a cow's udder perfused with carboxyl-labeled isovaleric acid. Both groups (5,6) concluded that the $C_5$ acids were not the direct precursors of the $C_{15}$ and $C_{17}$ acids of milk fat. Their results were interpreted as indicating a cleavage of the $C_5$ acids to yield $C_2$ and $C_3$ units, with the $C_2$ units being used for fatty acid synthesis. Horning *et al.* (7) have demonstrated, *in vitro,* the synthesis of $C_{13}$, $C_{16}$, and $C_{17}$ fatty acids from substrates such as propionyl-coenzyme A, isobutyryl-CoA, isovaleryl-CoA, etc., by addition of malonyl-CoA units catalyzed by a rat adipose tissue enzyme system. They suggested that the occurrence of only small amounts of long odd and branched-chain fatty acids in mammalian tissue probably reflects the low concentration of the appropriate acyl-CoA esters available for fatty acid synthesis compared to acetyl-CoA. The recent report by Bhalerao *et al.* (8), on the absence of $C_{13}$, $C_{15}$, and $C_{17}$ normal and branched-chain acids in the body fats of rats raised on corn oil or lard and the occurrence of these acids in body fats of rats raised on milk fat, suggests a dietary origin for these acids in the rat. It remains to be demonstrated, *in vivo,* that the appropriate short chain odd and branched-chain CoA esters become available to the Horning enzyme system. The above reports (5,6,8) can be interpreted as casting doubt on major origin of $C_{15}$ and $C_{17}$ normal and branched-chain acids through animal biogenesis. The observations of Gerson *et al.* (5) and Verbeke *et al.* (6), that milk fat acids of similar molecular weight containing odd and even numbers of carbon atoms had similar specific activities, may merely reflect addition of $C_2$ units to existing fatty acids of intermediate chain length by the mitochondrial system. As pointed out in the review by Wakil (9), the main pathway of fatty acid biosynthesis from short chain acyl-CoA precursors is conducted by the soluble enzyme system in the cytoplasm of the cell.

The expressed doubt of an animal origin for the odd and branched-chain acids requires one to look elsewhere for their biosynthesis. In light of the fact that these acids were first discovered in ruminant fats and that their occurrence in other animals, such as man and rat, might be traced to the eating of ruminant fats (8,10), it would appear that the rumen microbes should be examined as possible synthesizers. It is recalled that Reiser and Choudhury (11) observed normal stearate-rich body fat from a steer raised on a fat free diet. Among several possible explanations for this phenomenon, they listed rumen microbial synthesis of stearic acid from nonfatty precursors such as acetate. Saito (12) found 50% iso-$C_{15}$ acid and 15% iso-$C_{17}$ acid in the fatty acids isolated from *Bacillus subtilis* lipids. Akachi and Saito (13) found several other microbial lipids to be rich sources of branched-chain $C_{15}$ fatty acids and suggested that when these acids are found in animal lipids they may have originated from microbial synthesis in the host's gut. Evidence of the net synthesis of lipid in the intestinal tract of a human has recently been presented by James *et al.* (14), who found 18 g of fat per day in the feces of a subject consuming a diet containing only 12 g of fat per day. The intestinal origin of the fat was indicated by its high content of unusual oleic acid isomers.

Allison *et al.* (15) found that *Ruminococcus flavefaciens,* a representative rumen cellulolytic bacterium which has an obligate growth requirement for isovalerate or isobutyrate, incorporated radio-activity from isovalerate-1-$C^{14}$ approximately equally into cellular protein (all as leucine) and cellular lipid. Analysis of the lipid indicated that 74% of the $C^{14}$ was in a $C_{15}$ branched-chain acid and 13% in the $C_{17}$ acid fractions. The $C_{15}$ branched-chain acid was a major fatty acid in this organism (Table II, last line). Similar recent studies have shown that $C^{14}$ from labled branched-chain volatile acids is incorporated mainly into branched chain higher fatty acids by strains of *R. albus* (16), *Bacteroides succinogenes,* and a *Borrelia* species (17). These observations, considered in light of the above discussion, have led us to give serious consideration to the possibility that significant quantities of ruminant milk fat acids may arise from microbial synthesis in the rumen. The

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analyses reported in this paper indicate that the rumen contains significant quantities of long chain fatty acid moieties of microbial origin which could be used for milk fat production. Certain rumen microbial lipid fractions were observed to be very rich sources of long odd and branched-chain acids.

## Experimental and Results

An investigation was made of the fatty acid composition of rumen microbial lipids, blood serum lipids, and milk lipids from a lactating Holstein cow maintained on a normal hay and grain ration.

*Isolation of Bacterial and Crude Protozoal Lipids.* Rumen digesta (2.5 l) was collected by stomach tube after the cow had been isolated from feed for 5 hr. The digesta was filtered through cheese cloth and the filter cake of coarse plant material was resuspended in a liter of water and filtered again. The combined filtrates were centrifuged in bottles (250 ml) for 2 min at 2,000 rpm in an International No. 1 centrifuge. The supernatant was decanted from the heavier packed fraction of protozoa (contaminated with feed particles), and passed through a Sharples supercentrifuge clarifier 3 times with the bowl revolving at 50,000 rpm. The packed bacterial fraction was scraped from the dismantled bowl. Each fraction was overlaid with methanol, and then ground in a mortar for 15 min with enough Celite 545 (previously acid and alkali washed and redried) to yield a slightly damp cake. The cake was transferred to a beaker and stirred for 12 hr with 1 l of methanol-chloroform (1–2). After filtration the cake was again extracted with 1 l of methanol-chloroform (1–2) for 4 hr and filtered. The combined filtrates were evaporated and the fatty residue purified on a cellulose column (19). The rumen bacterial fraction yielded 482 mg lipid, and the rumen protozoal fraction yielded 1,880 mg lipid. The protozoal fraction was contaminated with feed particles and bacteria. The name given to this crude fraction is one of convenience and reflects the belief, based upon the fatty acid composition of the lipids (Table II), that the major portion of the lipid isolated from this fraction was of protozoal origin. A small quantity of packed *Ruminoccoccus flavefaciens* cells (16) was similarly worked up on a smaller scale to yield 12 mg lipid from the cellulose column.

*Isolation of Blood Serum Lipid.* A blood sample (800 ml) was taken from the cow the day following the rumen sampling. Serum (350 ml) was obtained by the usual clotting and centrifuging procedures. The serum was mixed with 700 ml methanol and stirred for 1 hr, after which 700 ml chloroform was added and the stirring continued for 2 more hr. The mixture was filtered and 200 ml chloroform were added to the filtrate. After shaking, the chloroform layer was recovered. The filter cake from above was re-extracted with 500 ml methanol-chloroform (1–1) and the extract added to the first chloroform extract. Evaporation of the solvent and purification on a cellulose column (18) yielded 900 mg lipid.

*Isolation of Milk Lipid.* The 2 milkings following the rumen sampling were combined, and the cream obtained by centrifugation. A sample of the cream was extracted with methanol-chloroform (1–2). After evaporation of the solvent the milk lipid was passed through a cellulose column (18) to remove non-lipid contaminants. This cow was producing approximately 30 lb of 3.7% fat milk per day at this time.

*Fractionation of Lipids.* The blood serum lipid was separated into the fractions indicated in Table I by the silica gel chromatographic procedure of Bottcher *et al.* (19). The rumen bacterial and protozoal lipids were each separated into 2 fractions by chromatography of 200 mg on columns composed of 10 g dry silicic acid and 5 g of Celite 545 (purified as before). The relatively nonpolar lipids plus free fatty acids were eluted, as one fraction, with chloroform methanol (99–1). The polar lipids were eluted with methanol. The first fraction (99–1) was separated into neutral and free fatty acid fractions on carbonate columns. Ten g of analytical grade Celite was ground in a mortar with 8 ml of 5% $Na_2CO_3$. This mixture was slurried with either hexane or chloroform and packed into a chromatographic column. The lipid in either hexane or chloroform was applied to the column and the neutral lipid passed rapidly through the column. After no more lipid could be eluted with hexane or chloroform the column was extruded into 50 ml of 5% $H_2SO_4$. The acidified mixture was extracted 3 times with 50 ml quantities of ethyl ether to recover free fatty acids. The carbonate column method had previously been tested by submitting 100 mg stearic acid dissolved in 5 g corn oil to the procedure. Quantitative recovery of neutral corn oil and 95 mg of stearic acid was obtained. However, when applied to the bacterial and protozoal lipids considerable material was lost. This lost material is listed as unclassified lipid in Table I, along with the other results of the above described fractionation. More detailed current work on the fractionation of rumen microbial lipids has revealed the presence of anthrone positive material, and it may be tentatively concluded that the unclassified lipid was, in part, glycolipid. Also, current work on the polar lipid fractions involving detailed silica gel chromatography, monitored by phosphorous analysis, has indicated that the material classified as polar lipid was mainly phospholipid. Garton and Oxford (20) found 39% acetone insoluble material in rumen bacterial lipids from sheep, which they classified as phospholipids.

TABLE I
Class Composition of Lipids

| Lipid | % |
|---|---|
| Bovine blood serum | |
| Pigment plus hydrocarbon | 2.5 |
| Glyceride plus free fatty acid | 12.5 |
| Cholesterol ester | 46.5 |
| Phospholipid | 38.5 |
| Rumen bacteria | |
| Neutral lipid | 41.0 |
| Free fatty acid | 13.0 |
| Polar lipid | 30.0 |
| Unclassified lipid | 16.0 |
| Rumen protozoa[a] | |
| Neutral lipid | 23.0 |
| Free fatty acid | 24.0 |
| Polar lipid | 35.0 |
| Unclassified lipid | 18.0 |

[a] Contaminated with feed particles and bacteria.

*Preparation of Methyl Esters.* The various lipid fractions were methylated or transmethylated by dissolving them in 30 volumes of methylene chloride or chloroform plus 20 volumes of dry 1 *N* HCl-methanol. After standing overnight at room temperature, most of the solvent was evaporated in a stream of nitrogen, and the methyl esters extracted from the residue with hexane. The hexane extracted was dried with $Na_2SO_4$ and then evaporated to an appropriate volume for application to the gas chromatograph. This method for preparing methyl esters was developed after studying the procedures of Bottcher *et al.* (19), who transmethylated phospholipids by refluxing them for 2 hr with 6 *N* HCl-methanol. The chlorinated hydrocarbon has the important function of dissolving lipids in the reagent. Many lipids are only slightly soluble in methanol, even at reflux tempera-

TABLE II

Component Fatty Acids of Various Lipid Fractions

| Lipid | % Fatty acid[a] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12:0 | 13:0br | 13:0 | 14:0br | 14:0 | 15:0br | 15:0 | 16:0br | 16:0 | 16:1 | 17:0br | 17:0 | 18:0 | 18:1 | 18:2 | 18:3 | 18:4 or 20:1 |
| Milk | | | | | | | | | | | | | | | | | |
| Total lipid | 4.7 | .... | 0.3 | .... | 14.1 | 1.1 | 1.4 | 0.2 | 35.3 | 1.3 | 0.8 | 0.8 | 8.9 | 19.5 | 1.5 | tr | |
| Bovine blood serum | | | | | | | | | | 5.3 | | | | | | | |
| Glyceride plus FFA[b] | tr | tr | tr | tr | 1.4 | 1.3 | 1.7 | 0.6 | 23.5 | | | 1.7 | 17.4 | 27.3 | 18.1 | | 1.7 |
| Cholesterol ester | ... | ... | ... | .... | 0.6 | 0.9 | 0.7 | 0.2 | 4.8 | 2.3 | 0.3 | 0.2 | 0.5 | 3.3 | 80.5 | 2.0 | 3.7 |
| Phospholipid | 0.3 | 0.2 | 0.9 | .... | 1.8 | tr | 0.6 | 0.4 | 20.9 | 2.2 | 3.7 | 3.0 | 21.1 | 15.6 | 29.1 | | |
| Rumen bacteria | | | | | | | | | | | | | | | | | |
| Neutral lipid | 0.6 | tr | tr | 0.7 | 2.0 | 6.7 | 4.5 | 2.9 | 26.1 | 1.5 | 1.5 | 0.4 | 10.7 | 16.2 | 18.1 | | 7.9 |
| Free fatty acid | tr | tr | tr | tr | 0.9 | 1.9 | 1.2 | .... | 16.9 | 0.8 | 0.5 | 1.9 | 58.9 | 12.5 | 4.3 | | |
| Polar lipid | 1.5 | 1.2 | 0.7 | 1.3 | 3.8 | 20.3 | 8.2 | 1.6 | 30.6 | 1.3 | 1.2 | 0.8 | 6.5 | 10.2 | 10.7 | | |
| Rumen protozoa | | | | | | | | | | | | | | | | | |
| Neutral lipid | .... | .... | .... | .... | 1.0 | 0.5 | 1.1 | 2.2 | 26.5 | 0.7 | 1.2 | tr | 12.3 | 17.1 | 27.5 | | 9.7 |
| Free fatty acid | .... | .... | .... | .... | tr | .... | 0.6 | tr | 14.2 | .... | 0.6 | 1.3 | 68.3 | 4.9 | 4.9 | | |
| Polar lipid | tr | tr | 0.7 | tr | 1.6 | 3.7 | 2.0 | 1.1 | 37.5 | 1.2 | 2.8 | 0.8 | 10.3 | 20.3 | 14.6 | | 3.2 |
| *R. flavefaciens* | | | | | | | | | | 3.4 | | | | | | | |
| Total lipid[b] | 0.7 | 1.5 | tr | 2.0 | 2.5 | 43.7 | 4.3 | 9.3 | 19.0 | | | 1.8 | 4.1 | 6.9 | 0.8 | | |

[a] Identified by shorthand designation where number to left of colon indicates carbon chain length—to right, number of double bonds. Branched chain acids indicated by br. Less than 0.2% indicated by tr.

[b] Chromatographed only on diethyleneglycol succinate.

ture. The quantitative nature of our transmethylation has been verified by isolation of the theoretical amounts of free glycerol in applying the method to butterfat and corn oil.

*Gas Chromatographic Determination of Methyl Esters.* The gas chromatograph contained a 6.5 ft, 4 mm ID column packed with 38% diethyleneglycol succinate on 60–80 mesh Celite; a katherometer detector; and helium carrier gas. Operating condtions were: injector preheater, 350C; column and detector 193C; 18 lb psi inlet pressure; and 2–4 mg sample size. Under these conditions stearate and oleate separated in 30 min, with oleate having a relative retention time of 1.18 compared to stearate. Samples were chromatographed both before and after removal of unsaturated esters by a modification of the mercuric acetate adduct procedure of Kishimoto and Radin (21). This modification involved elution of the saturated esters from a 5 g column of grade III alumina with 40 ml of hexane-benzene (1–1) instead of the Skellysolve elution from Florisil. Except in the two cases noted in Table II, the esters were also chromatographed on an Apiezon L column in order to confirm the tentative identity from the polyester column. The esters were identified by reference to authentic compounds and to the properties published by Farquhar *et al.* (22). Quantities of each ester as weight % of total chromatogram were calculated by triangulation. In calculating the milk fat data allowance was made for the occurrence of 10% $C_4$–$C_{10}$ acids in the fat. Results are presented in Table II.

## Discussion

Examination of Table II reveals the high level of odd and branched-chain acids in the polar lipid fractiton from the rumen bacteria. These acids are also easily detectable in the blood serum lipids. Thus, they are available to be taken up by the mammary gland for incorporation into milk fat.

The procedure used in this work for isolating the microbial lipids from the rumen was not quantitative. Microorganisms were undoubtedly clinging to the coarse feed particles which were removed in the first straining step. Weller *et al.* (23) found that about one-third of the rumen microbial nitrogen was retained on plant fibers even after these were washed twice. Further losses would be expected in the centrifuging steps and it would be surprising if our methods for extracting the lipids from the packed cells were quantitative. We have also neglected fatty acids of microbial origin which may be in cell free rumen fluid following lysis of microorganisms. The lipid quantities isolated from 2.5 l of rumen digesta, namely, 482 mg bacterial lipid and 1.88 g protozoal lipid, thus represent much less than the quantities expected to be present in this amount of rumen material under natural conditions.

The capacity of the rumen in a Holstein cow has been estimated to be in the range of 150–200 l (24). Sperber *et al.* (25) estimated the passage of 150–170 l of rumen fluid to the lower digestive tract during a 24 hr period in a 530 kg cow. As a first approximation, it would thus appear that there is a complete turnover of rumen material in 24 hr. Taking a conservative estimate of 150 l of rumen material passing to the lower digestive tract in 24 hr, it can be estimated that our experimental cow had at least 29 g of bacterial lipid and 113 g of protozoal lipid available to its digestive process during this period of time (e.g., $.482 \times 150/2.5 = 29$, $1.88 \times 150/2.5 = 113$). The cow was producing approximately 500 g

butterfat in 24 hr (e.g., $30 \times 454 \times .037 = 500$). It would appear from the above figures that serious consideration should be given to the possibility that rumen microbial lipids are significant contributors to the fatty acid composition of milk fat and other ruminant lipids.

Continuing the above line of thought for the case of the $C_{15}$-branched-chain acids, it is observed (Table II) that the milk fat contained 1.1% of these acids. This means that approximately 5.5 g of these acids were being secreted by the mammary gland in 24 hr. (e.g., $500 \times .011 = 5.5$). The rumen bacterial neutral lipid, free fatty acids, and polar lipid contained, respectively, 6.7, 1.9, and 20.3% of $C_{15}$-branched acids in their component fatty acids. Considering these values, together with the class composition in Table I, it is possible to make a rough calculation of the amount of $C_{15}$-branched acid of bacterial origin passing to the lower digestive tract in 24 hr. The assumptions in this calculation are that the neutral lipid is of the glyceride type and that the polar lipid is mainly phospholipid. A cephalin type phospholipid would contain approximately 65% fatty acid moieties of average $C_{16}$ chain length. Calculations are:

$$29 \times .41 \times .067 = .80 \text{ g}$$
$$29 \times .13 \times .019 = .07 \text{ g}$$
$$29 \times .30 \times .203 \times .65 = 1.15 \text{ g}$$
$$\text{Total } C_{15} \text{ branched acid from bacterial lipid } \overline{2.02 \text{ g}}$$

This 2 g quantity of $C_{15}$-branched acid plus the 1 g of $C_{15}$-branched acid from the polar protozoal lipid (e.g., $113 \times .35 \times .65 \times .037 = .95$), passing to the lower digestive tract from the rumen during a 24 hr period, could easily account for more than half of the 5.5 g of $C_{15}$-branched acid in the butterfat.

It is now generally accepted that the major source of protein nitrogen for milk and meat production in ruminants is rumen microbial protein. Dietary protein and nonprotein nitrogen are largely converted to microbial protein in the rumen (23). The digestive mechanisms which make this microbial protein available to the animal would be expected to go hand in hand with mechanisms which would make the microbial lipid available. The presence of both proteolytic and lipolytic enzymes in the pancreatic and intestinal systems is well known.

This work is interpreted as providing support for the suggestion of Akachi and Saito (13) that the branched-chain acids of animal fats are derived from bacterial lipids in the gut. It also supports the suggestion of Reiser and Choudhury (11) regarding the possible rumen microbial origin of part of the stearic acid in ruminant body fats. Hydrogenation of dietary unsaturated fatty acids by rumen organisms has been recognized as being responsible for the characteristic *trans* fatty acid isomers in ruminant fats (26). It is suggested that attention should also be given to the probability of microbial synthesis of long chain fatty acids in the rumen from the volatile faty acids, which are always present in relatively high levels (e.g., 80–120 mmole VFA/l rumen fluid).

Current work in this laboratory involves a more detailed characterization study of rumen microbial lipids. It is anticipated that identification and quantitative determination of unique components of microbial lipids will provide natural markers which may be used for quantitative studies of the role of these lipids in animal nutrition. If it is found that the quantity of one or more of these compounds is proportional to the amount of bacterial protoplasm present, they might be used as markers to estimate the quantity of bacterial protoplasm in material leaving the rumen to the lower digestive tract, or in that intimately associated with plant fibers, or in material from various locations within the rumen, etc. For instance, if one assumes that protozoa do not synthesize $C_{15}$-branched-chain acids it can be estimated, based upon the quantities of these acids in the polar lipids, that 15–20% of the fatty acids in the polar protozoal lipid isolated in this study were of bacterial origin. This approach would be analogous to that of Weller *et al.* (23), who used diaminopimelic acid as a natural indicator of the quantity of bacterial nitrogen in the rumen.

REFERENCES

1. Hansen, R. P., F. B. Shorland, and N. J. Cooke, Biochem. J., *61*, 547 (1955).
2. Hansen, R. P., F. B. Shorland, and N. J. Cooke, Chemistry and Industry 1149 (1956).
3. Shorland, F. B., T. Gerson, and R. P. Hansen, Biochem. J., *61*, 702 (1955).
4. El-Shazly, K., *Ibid.*, *51*, 647 (1952).
5. Gerson, T., J. C. Hawke, F. B. Shorland, and W. H. Melhuish, *Ibid.*, *74*, 366 (1960).
6. Verbeke, R., M. Lauryssens, G. Peeters, and A. T. James, *Ibid.*, *73*, 24 (1959).
7. Horning, M. G., D. B. Martin, A. Karmen, and P. R. Vagelos, J. Biol. Chem., *236*, 669 (1961).
8. Bhalerao, V. R., J. Endres, and F. A. Kummerow, J. Dairy Sci., *44*, 1283 (1961).
9. Wakil, S. J., J. Lipid Research, *2*, 1 (1961).
10. Insull, W., and E. H. Ahrens, Biochem. J., *72*, 27 (1959).
11. Reiser, R., and R. B. R. Choudhury, JAOCS, *36*, 129 (1959).
12. Saito, K., J. Biochem. Japan, *47*, 710 (1960).
13. Akashi, S., and K. Saito, *Ibid.*, *47*, 222 (1960).
13. James, A. T., J. P. W. Webb, and T. D. Kellock, Biochem. J., *78*, 333 (1961).
15. Allison, M. J., M. P. Bryant, M. Keeney, and I. Katz, J. Dairy Sci., *44*, 1203 (1961).
16. Allison, M. J., M. P. Bryant, I. Katz, and M. Keeney, Studies on the Metabolic Functions of Branched-Chain Volatile Fatty Acids, Growth Factors for Ruminococci. II. Biosynthesis of Higher Branched-Chain Fatty Acids and Aldehydes. Submitted to J. Bacteriol.
17. Wegner, G. H., University of Wisconsin, Personal communication (1961).
18. Lea, C. H., and D. N. Rhodes, Biochem. J., *54*, 467 (1953).
19. Bottcher, C. J. F., F. P. Woodford, E. B. Van Houte, and C. M. Van Gent, Rec. trav. chim., *78*, 794 (1959).
20. Garton, G. A., and A. E. Oxford, J. Sci. Food Agric., *6*, 142 (1955).
21. Kishimoto, Y., and N. S. Radin, J. Lipid Research, *1*, 72 (1959).
22. Farquhar, J. W., W. Insull P. Rosen, W. Stoffel, and E. H. Ahrens, Nutr. Rev., *17*, August Supplement (1959).
23. Weller, R. A., F. V. Gray, and A. F. Pilgrim, Brit. J. Nutr., *12*, 421 (1958).
24. Dukes, H. H., "The Physiology of Domestic Animals," 7th ed., Cornell University Press, Ithaca, New York, 1955, p. 352.
25. Sperber, I., F. Hyden, and J. Ekmn, Proc. VII Intern. Cong. Animal Husb., Madrid (1956).
26. Bartlet, J. C., and D. G. Chapman, Agr. Food Chem., *9*, 50 (1961).

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# New Methods of Analyzing Industrial Aliphatic Lipids[1]

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Methods are described for the rapid fractionation of classes of lipids containing the hetero elements N, P, and S, such as primary amines, secondary amines, tertiary amines, and quaternary ammonium salts containing one, two, or three long-chain moieties; amides, nitriles, and other nitrogenous lipids; alkyl sulfates and sulfonates; alkyl phosphates and phosphonates.

Thin-layer chromatography on silicic acid separates lipids according to classes of compounds. After isolation by thin-layer chromatography, each lipid class is amenable to further fractionation, according to chain length and de-

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