1. Førland, T. and Østvold, T. *Acta Chem. Scand.* **20** (1966) 2086.
2. Østvold, T. *Acta Chem. Scand.* **20** (1966) 2187.
3. Kleppa, O. J. and McCarty, F. G. *J. Phys. Chem. In press.*
4. Neil, D. E., Clark, H. M. and Wiswall, R. H. *J. Chem. Eng. Data* **10** (1965) 21.

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Cholesterol in the Milk of Cows on Normal and Protein-free Feeds

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The presence of cholesterol in milk has not attracted a great deal of attention; there have been a number of estimations of the concentration of cholesterol in milk and most of the figures fall in the range 11—14 mg per 100 ml milk, equivalent to about 0.35 % of milk fat. A small proportion only is esterified.[1] Cholesterol is known to account for the major part of the sterol fraction of butterfat: the identity of the other components, except for lanosterol,[2] has not been established.

The concentration of cholesterol in the lipid of isolated fat-globule membrane material [3] and in the lipid found associated with the milk proteins [4] is high, and cholesterol other than that found in the fat globules amounts to some 14 % of the total milk cholesterol,[1] so that its concentration in the bulk fat phase is relatively low: butter or butterfat figures average about 250 mg cholesterol per 100 g fat.

Only few investigations on the dependence of the milk cholesterol level on feeding, season, stage of lactation, and breed have been reported. Nataf *et al.* found no seasonal differences,[5] and others have found neither breed nor seasonal differences, though some lactation trends have been noted.

Recently a significant role in milk fat synthesis has been indicated for cholesterol, and it has been proposed that cholesterol ester is an agent in the transfer of fatty acids to form the triglycerides of milk fat.[6] Later the same authors found an active system in freshly-secreted milk capable of synthesising glyceride from fatty acid.[7] Thus it appears that cholesterol is secreted as part of milk just as are the other factors responsible for the synthesis of milk fat.

Virtanen has described a series of experiments which have shown that it is possible to maintain the milk production of dairy cows at a relatively high level over a period of several years with a feed in which the protein has been completely replaced by urea and ammonium salts.[8] The feed consists basically of purified carbohydrates (starch, cellulose and sucrose). Small quantities of vegetable oil (35—130 g per day) have been included in the feed; thus the fat intake of the test cows is low. As part of these experiments milk cholesterol values were determined over a period of three years and compared with those of samples taken from cows of the same breed (Ayrshire) on normal feed.

Experimental. Milk fat was determined by the Röse-Gottlieb or Gerber methods and Röse-Gottlieb fat was used for cholesterol determination. Total cholesterol was found by saponification of the fat, precipitation of the cholesterol with digitonin and ferric chloride-sulphuric acid colorimetry. The method, free of interference from lanosterol, was found to give reliable results. The same colorimetric procedure was applied directly to the cholesterol ester fraction separated by column chromatography on silica gel.

Results and discussion. Milk samples were collected from eight farms with herds on normal feed. Silage feed gave a mean value of 300 mg cholesterol per 100 g milk fat, hay feed 320, hay-roots 340, and pasturage 330. There were no significant differences among the various winter feeds: the overall means (93 samples) were 317 ± 4 (standard error) mg cholesterol per 100 g fat, 137 ± 2 mg per kg milk, and cholesterol ester 5 % of the total cholesterol.

The mean figures for the five cows receiving the protein-free feed were 400, 370, 480, 435, and 435 mg cholesterol per 100 g milk fat; the overall means (85 samples) were 413 ± 6 mg per 100 g fat, 231 ± 5 mg per kg milk, and cholesterol ester 6 % of the total.

Such elevated milk cholesterol values as the result of feeding have not previously been reported. They are consistent with evidence found by other investigators that milk fat is derived directly from blood lipid as well as by synthesis within the mammary gland [9] and that the proportions of the milk fat contributed by these two mechanisms are not always the same.[10] With the protein-free feed, thus, it appears that the proportion of the milk fat derived from blood lipid is relatively small: the low concentration of lipid in the blood plasma, normal blood volatile fatty acid levels, and the low concentration of the C_{18}-fatty acids in the milk fat too suggest that this is so. With mammary gland synthesised triglyceride making the greater contribution to the production of the milk fat, the secretion of greater than normal amounts of cholesterol in relation to other milk components would seem to follow as a consequence.

Though the cows on the protein-free feed showed no marked trends in milk cholesterol figures as the lactation period progressed, there was a steady increase throughout the lactation of the normally-fed cow.

A more detailed report will be published elsewhere.[11]

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1. Mulder, H. and Zuidhof, T. A. *Neth. Milk Dairy J.* **12** (1958) 173.
2. Morice, I. M. *J. Chem. Soc.* **1951** 1200.
3. Thompson, M. P., Brunner, J. R., Stine, C. M. and Lindqvist, K. *J. Dairy Sci.* **44** (1961) 1589.
4. Ansbacher, S. and Supplee, G. C. *J. Biol. Chem.* **105** (1934) 391.
5. Nataf, B., Mickelsen, O., Keys, A. and Petersen, W. E. *J. Nutr.* **36** (1948) 495.
6. Patton, S. and McCarthy, R. D. *J. Dairy Sci.* **46** (1963) 396.
7. McCarthy, R. D. and Patton, S. *Nature* **202** (1964) 347.
8. Virtanen, A. I. *Science* **153** (1966) 1603.
9. Riis, P. M. and Moustgaard, J. *Use of Radioisotopes in Anim. Biol. and the Med. Sciences* **2** (1962) 167; Ref. *Dairy Sci. Abstr.* **25** (1963) 2295.
10. Riis, P. M. *Aarsberetn. Inst. Sterilitetsforskn. K. Vet.-Landbohøjsk., Kbh.* **1961** 159; Ref. *Dairy Sci. Abstr.* **24** (1962) 2279.
11. Homer, D. R. and Virtanen, A. I. *Milchwissenschaft. To be published.*

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Carotenoids of Flexibacteria

IV.* The Carotenoids of two Further Pigment Types

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According to Lewin and co-workers [1,2] almost all flexibacteria produce carotenoids, and may be divided into four pigmented types. We have previously published the structural elucidation of carotenoids synthesized by selected representatives of two of these types,[3,4] and now report on investigations of the third and fourth pigment type.

1. *Flexithrix* sp. The yellow strain QQ-3, provisionally designated by Lewin as *Flexithrix* sp., produced in high yield (0.08 % of the dry weight) a single carotenoid, directly compared with authentic synthetic [5] and natural zeaxanthin (I) from Hoffmann-La Roche; see Table 1.

A mixed-melting-point determination gave no depression. Partition ratios,[6] co-chromatography tests,[7] and the qualitative and quantitative composition of their iodine-catalyzed isomerization mixtures,[8] strongly supported identity. The infrared spectra, measured in KBr pellet, were nearly identical, and differed from that of isozeaxanthin (β-carotene-4,4′-diol) in the low-frequency absorptions associated with the hydroxyl groups.[9] Moreover, both the *Flexithrix* pigment and authentic zeaxanthin samples were more strongly adsorbed than isozeaxanthin by prolonged chromatography on kieselguhr paper [7] (5 % acetone in petroleum ether). On acetyla-

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