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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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-

* Part III. *Acta Chem. Scand.* **20** (1966) 1970.

Table 1. Direct comparison of zeaxanthin from *Flexithrix* strain QQ-3 with authentic zeaxanthin.

Property	Zeaxanthin			
	Authentic		*Flexithrix* strain QQ-3	
Melting point in °C	203–204	(205–206)[5]	205–206	
Abs.max. in mμ in				
petroleum ether (b.p. 40–60°C)	448.5	474.5	448.5	474.5
acetone	452	479	452	479
$E\ ^{1\%}_{1\,cm}$ at 452 mμ in acetone	2340		2310	
Partition ratio				
petroleum ether/95 % methanol	5:95		4:96	
petroleum ether/85 % methanol	27:73		26:74	
R_F-value, Schleicher & Schüll No. 287 paper; 10 % acetone-petroleum ether				
trans	0.59	(64 *)	0.59	(61 *)
neo U	0.35	(36 *)	0.35	(39 *)

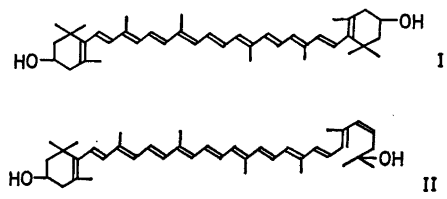
* % of total in iodine-catalyzed equilibrium mixture.

tion the *Flexithrix* pigment furnished a single monoacetate, which was subsequently converted into a diacetate. These derivatives were identified with the corresponding acetates of authentic zeaxanthin, judged from identity in R_F-values [7] and partition ratios.[6]

Neither the *Flexithrix* pigment, nor authentic zeaxanthin yielded ketonic products when treated with *p*-chloranil, a selective oxidant of allylic hydroxyl groups.[10] No smooth dehydration was observed in acidified chloroform.[11] Prolonged treatment of each sample resulted in low yields of the same reddish products (*cf.* Ref. 12).

From the evidence discussed above it may be concluded that the characteristic carotenoid of *Flexithrix* strain QQ-3 is zeaxanthin (I). This di-ol was not present here as an ester, since saponification did not alter the R_F-value. Zeaxanthin is a common carotenoid of many higher plants [13] and algae.[14] *Flexithrix* strain QQ-3 is a very convenient source of this carotenoid, because no other carotenoid was present and it was easily obtained in the pure state.

2. *Saprospira thermalis* Lewin was selected as representative of the fourth pigmented type. Due to the low carotenoid content (0.002 % of the dry weight) obtained under the cultural conditions used (darkness; *cf.* Ref. 15), only a preliminary investigation could be carried out. This organism produced a single carotenoid,



here referred to as S.t. 483, exhibiting absorption maxima at 483 and 510 mμ in acetone. The electronic spectrum was indistinguishable from that of flexixanthin (II), a carotenoid previously isolated from flexibacteria.[4,16] However, on kieselguhr paper [7] S.t. 483 was considerably more strongly adsorbed than flexixanthin (II). The result of hydride reduction supported the presence of a conjugated carbonyl group in S.t. 483. Alkali treatment converted the pigment to a more polar product, suggesting the presence of α-ketol or ester groups. On acetylation of alkali-treated S.t. 483, five intermediate acetates were detected by periodical paper-chromatographic analysis,[17] thus demonstrating the presence of at least three hydroxyl groups accessible for acetylation. Under conditions for silylation [18] the peracetate furnished no trimethylsilyl ether, indicating the absence of tertiary hydroxyl groups in S.t. 483.

Alkali-treated S.t. 483 was resistant towards allylic oxidation with *p*-chloranil,[10]

as well as towards allylic elimination with acidified chloroform.[11] Attempted acid hydrolysis of any glycosidic linkage [17] in 1'-position was also negative.

Some properties of S.t. 483 and its derivatives are summarized below. R_F-values refer to Schleicher & Schüll No. 287 paper (kieselguhr paper) [7] developed with 20 % acetone in petroleum ether. S.t. 483, $R_F = 0.20$; alkali-treated S.t. 483, $R_F = 0.10$, partition ratio in petroleum ether/75 % methanol 10:90; peracetate of alkali-treated S.t. 483, $R_F = 0.78$.

The available data for S.t. 483 are insufficient for a sound discussion of its structure. A direct comparison of alkali-treated S.t. 483 with a carotenoid (saponified "fraction IV") recently isolated from a *Micrococcus* sp. by Bamji and Krinsky [19] might be profitable.

A compilation of our studies on the carotenoids of flexibacteria has been presented elsewhere.[20,21] The distribution pattern of carotenoids in these organisms in relation to taxonomy will be treated by others.[2]

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Cultivations were carried out by Civ.ing. M. Fall through the courtesy of Dr. C. G. Hedén, Bakteriologisk Bioteknik, Karolinska Institutet, Stockholm 60, Sweden. Cells were harvested from 20 l of *Flexithrix* and 200 l of *Saprospira thermalis* culture. Materials and methods were similar to those previously described.[3,4] S.t. 483 was chromatographed on columns of cellulose powder; other experimental details are presented elsewhere.[21]

Gifts of natural and synthetic zeaxanthin and synthetic isozeaxanthin were obtained from Dr. O. Isler, Hoffmann-La Roche, Basel.

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