The glycerides of the heptane phase were separated into tri- and diglycerides on a column of silicic acid ⁴.

To prove the configuration of the diglyceride a sample of this fraction was hydrogenated (PtO₂, acetic acid) and oxidized with chromic acid in 97.5 per cent acetic acid. Using this method a 1,3-diglyceride consumes one atom of oxygen per mole, while a 1,2-diglyceride takes two. The results of such oxidations on synthetic 1,3-diglyceride and on the hydrogenated diglyceride obtained in the *in vitro* hydrolysis are seen in Figure 1 and proves that the configuration of the diglyceride formed during the pancreatic lipolysis was the 1,2-isomer.

Thus, by the action of pancreatic lipase *in vitro* long-chain triglycerides are degraded *via* the 1,2-diglyceride, mainly to the 2-monoglyceride. The monoglycerides, however, also contain about 20 per cent 1-monoglyceride. It remains to be elucidated whether this 1-monoglyceride is formed directly from the 1,2-diglyceride or is formed by isomerisation of 2-monoglyceride.

A method for distinguishing 1,2-diglycerides from 1,3-diglycerides is given.

With the methods outlined above 1,2-diglycerides can be prepared with lipase. This might prove valuable for preparation of 1,2-diglycerides containing unsaturated fatty acids as hitherto described chemical methods only can be used for the preparation of saturated 1,2-diglycerides.

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On the Protein Character of a Slime Produced by *Streptococcus cremoris* in Finnish Ropy Sour Milk

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In a previous paper ¹ the author proved the earlier statement that the extreme viscosity of Finnish ropy sour milk is due to the intense production of capsular mucus by the milk streptococci in question. Since some other non-haemolytic streptococci, *viz.* *Str. salivarius* and *Str. bovis*, are known to produce viscous polysaccharides ² it seemed feasible to assume that the mucus in ropy sour milk should be a polysaccharide too. The capsular material, however, failed to give the Hotchkiss reaction for polysaccharides ³ when studied microscopically in stained smears. In order to settle whether the slime produced by *Str. cremoris* strains is of polysaccharide character the mucous substance of ropy sour milk was investigated in greater detail.

Culture medium. Whey has proved to be the most favourable medium for slime production. Whey was prepared by clotting skimmed milk with rennet at 37° C for 1 hour, followed by filtration through cloth and sterilisation at 110° C for 15 min. No attention was paid to the clearness of the whey.

Preparation of the slime. Portions of sterile whey were inoculated with 2 % of a ropy sour milk and incubated at 25° C for 48 hours. It was necessary to use a natural ropy sour milk as inoculum in order to obtain sufficient amounts of the slime. In pure cultures of the streptococci in question the slime production is less vigorous and the slime formed does not always precipitate with ethanol as well as the slime in a mixed population. Apart from slime-producing and normal milk streptococci, the ropy sour milk contained *Oospora lactis* and some unidentified yeasts. During incubation the turbid whey turned clear and a flocculent sediment was formed. The sediment was centrifuged off and discarded. To the viscous clear supernatant were slowly added 1.25

volumes of ethanol under vigorous stirring. A sticky, filamentous precipitate settled immediately. The thin filaments were often more than 15 mm in length. No precipitate is formed in whey incubated with non-ropy streptococci. The precipitate was washed twice in 60 % ethanol and redissolved in water by dialysis against running tap water for 48 hours. This viscous dialysed solution was centrifuged to remove possibly undissolved particles and used for further analysis.

Analytical methods. After hydrolysis the composition of the slime was investigated by paper partition chromatography. For sugar analyses 1–2 ml of the dialysed slime solution was hydrolysed in $N H_2SO_4$ for 2 hours on a boiling water bath. The hydrolysate was neutralised to pH 4 with baryta using bromocresol green test paper as an indicator. The $BaSO_4$ precipitate was centrifuged off and the solution evaporated to a small volume ($< 100 \mu l$). The solution was chromatographed according to Sundman⁴. *n*-Butanol saturated with 16.7 % acetic acid and containing 2.5 % phthalic acid was used as solvent. The standards contained galactose, glucose, fructose, mannose, arabinose, xylose and ribose. The amount of analysis and standards applied on the same paper were always equal, mostly 10–15 μl , but the same standard was used in different concentrations. Thus the spots on the developed chromatogram are comparable. The use of different standard concentrations makes it possible to estimate the quantities of the sugars in the analysis from the chromatogram.

The protein component of the slime solution was investigated after hydrolysis at 100° C in 25 % H_2SO_4 for 15 hours. Neutralisation was performed as described above. The clear solution was evaporated to 1 ml, and examined by a modification of Woiwod's one-dimensional

technique⁵. Whatman's filter paper No. 1 was used instead of No. 4 and the eluant contained 0.1 % ninhydrin to avoid spray development. A casein hydrolysate prepared in the same way as the sample to be analysed, and known amounts of 14 amino acids were run as standards. The key chromatogram given by Proom and Woiwod⁶ for casein hydrolysates was also used to identify the spots in the casein-hydrolysate standard.

The filamentous precipitate formed by 1.25 volumes of ethanol in ropy whey is extremely sticky. When washed and centrifuged off it forms a gummy substance which can be handled without changing shape. Some results of the quantity and composition of the washed undialysed precipitate are listed in Table 1.

From the results it seemed evident that the slime could not be a polysaccharide. The low sugar content was probably due to lactose retained by the precipitate from the whey.

When dialysed the slime lost about one fourth of its dry weight. The sugar content was reduced to less than 1 %. Chromatograms of the dialysed slime showed that galactose is the main carbohydrate. Traces of glucose are always present. The results are given in Table 2.

No other carbohydrates could be demonstrated. Chromatograms where galacturonic acid was run as a standard showed that the galactose spot at R_F 0.16 did not contain galacturonic acid (R_F = 0.14).

Table 1. Properties of the slime precipitated by 1.25 vol. ethanol from whey.

Percentage of the precipitate in the whey. Calculated from the weight of washed, centrifuged precipitate		Composition of the dried precipitate		
Before drying	After drying	Ash	Nitrogen ¹	Carbohydrates ²
1.36 %	0.08 %	15 %	8.9 %	4 %

¹ Micro Kjeldahl determinations.

² Estimated as equal parts of galactose and glucose from paper chromatograms of the hydrolysed precipitate.

Table 2. The sugar content of the dialysed slime, estimated from paper partition chromatograms.

Experiment No.	Sugar content, calculated as % of dried dialysed slime		
	Galactose	Glucose	Total
1	0.40	< 0.10	< 0.50
2	0.72	< 0.35	< 1.07
3	0.54	< 0.21	< 0.75
Average	0.55	< 0.22	< 0.77

The presence of amino sugars was investigated according to Morgan and Elson⁷, but with a negative result.

The dialysed slime gave a strong biuret reaction and paper chromatographical protein analyses showed that the major part of the slime is a protein. The amino acid composition does not differ remarkably from that of casein. The spot pattern in the analysis run and in the casein hydrolysate run were nearly equal. Of the 11 spots and spot-groups respectively, visible in the one-dimensional casein run, 10 were present in the slime run. The spot at R_F 0.30 (20° C), in the position of prolin, was lacking. Some differences in spot intensities were also evident. No significant difference could be noticed with this

technique in the amino acid composition of the slime precipitate before and after dialysis.

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