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Short Communications

On the Mechanism of the Hydrolysis of Glycerides by Pancreatic Lipase

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Schönheyder and Volqvartz [1] have found that by the action of pancreatic lipase tripropionyl glycerol most probably is degraded to 1,2-dipropionyl glycerol. Further degradation was not observed in their experiments.

Quite recently Mattson, Benedict, Martin and Beck [2] have provided evidence that during lipolysis of long-chain glycerides in the lumen of the small intestine of the rat, the monoglycerides formed predominantly are of the 2-configuration. As a consequence of this they found it reasonable to assume that the diglyceride formed is the 1,2-isomer. In *in vitro* experiments they were, however, able to identify only 1-monoglycerides.

Studying the course of the *in vitro* hydrolysis of longchain triglycerides by rat pancreatic lipase we have found that the monoglycerides formed *in vitro* under our conditions predominantly are of the 2-configuration. Further we have obtained definite evidence that the diglyceride formed is the 1,2-isomer, by using the chromic acid oxidation method described by Bergström, Theorell and Davide [3].

One experiment is given as an example:

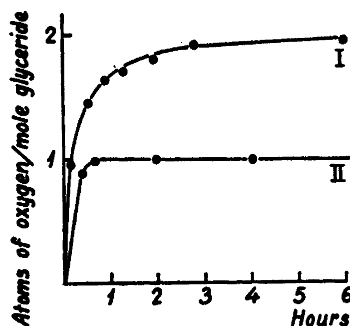

Fig. 1. Oxidation by chromic acid in 97.5 per cent acetic acid at 37° of synthetic 1,3-dipalmitin (II) and of hydrogenated diglyceride obtained from in vitro hydrolyses of olive oil (I).

2 ml olive oil was shaken for 1 hour at 40° with a mixture of 10 ml rat bile-pancreatic juice and 2 ml 0.1 *N* HCl (pH 6.4) [4]. After dilution with water the lipid mixture was extracted with ether, that was washed, dried and evaporated off. From the mixture of glycerides and fatty acids obtained the later were removed by passing the mixture through a column of Amberlite IRA-400. 1.215 g of glycerides was obtained. This was distributed in four separatory funnels between heptane and 80 per cent aqueous ethanol saturated with each other. The ethanol phase, in which the monoglycerides were to be expected contained after evaporation 57.2 mg substance. This was dissolved in 5 ml $CHCl_3$ and 1-monoglycerides assayed on two 1 ml portions by the periodic acid method. The values obtained corresponded to 2.2 mg 1-monoolein. After isomerisation of two other 1 ml portions by ferric chloride a total amount of 10.0 mg 1-monoglyceride was estimated. Thus 50.0 mg total monoglyceride was found in the 80 per cent ethanol phase, 22 per cent of which was 1-monoglycerides and 78 per cent 2-monoglycerides. About the same figures were obtained from several similar experiments.

The glycerides of the heptane phase were separated into tri- and diglycerides on a column of silicic acid [5].

To prove the configuration of the diglyceride a sample of this fraction was hydrogenated (PtO_2, 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