On the Structure of the Liquid State of Triglycerides

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Neutron diffraction data on trilaurin were recently presented by Cebula *et al.* [Cebula, D.J., D.J. McClements, M.J.W. Povey and P.R. Smith, *J. Am. Oil Chem. Soc. 69*:130 (1992)] with an interpretation of a liquid structure related to the nematic state of liquid crystals. Arguments for an alternative interpretation, consistent with curved stacks of bilayer units proposed earlier, are presented in this paper, based mainly on the analogy in diffraction behavior with polar lipid liquids/L2-phases.

KEY WORDS: Bilayer organization, liquid structure, triglyceride melt.

Cebula *et al.* (1) have recently reported an interesting neutron diffraction study of trilaurin in the solid and liquid state. Their following two main conclusions are related to earlier work by me (2) and a co-worker (3): i) "The classical picture of structure proposed for liquid triglycerides is given by Larsson's paracrystal concept, in which liquid crystalline smectic structure is postulated. Such a structure cannot be sustained by the neutron diffraction evidence here." ii) Hernqvist's model (3) for the temperature dependence could not be confirmed; no such temperature effects were seen.

The second conclusion is clearly justified by Cebula *et al.* (1), and this is important new information on the liquid state. The first conclusion, however, is a matter of interpretation of the diffraction data, which is the subject of the present paper.

It is obvious that any long-range periodicity or structure in a liquid state must be highly dynamic, occurring in domains of colloidal size at the most. The interpretation of diffraction data from the liquid state is certainly not simple, and the lack of straightforward analysis means that indirect comparative methods must be applied. In my original proposal of the bilayer-type of structure, a main argument was the analogy between the low-angle diffraction band exhibited by triglycerides and by L2-phases of polar lipids. Since then additional work on the structure of L2-phases (4,5) has confirmed the proposed structure consisting of "differently oriented stacks of small smooth lamellae" (5). The same structure model, with a continuum of curved bilayer units in an overall isotropic arrangement of the L2-phase, came out of this additional work as that originally proposed for liquid triglycerides. It is necessary to summarize this work before relating it to the triglyceride melt and the interpretation by Cebula *et al.* (1).

A long-chain paraffin or alcohol in the liquid state does not exhibit a band in the low-angle region of the diffraction pattern. Polar lipids, and a triglyceride melt, however, give such a diffraction band, with a position corresponding to the thickness of the lipid bilayer (in the liquid crystalline type of conformation). When the melt of a polar lipid, for example a monoglyceride, takes up water and forms an L2-phase, there is no discontinuity in the diffraction pattern; only a successive shift in the position of the band with water content. L2-phases are true liquids with stacks of curved lipid bilayers in a dynamic organization, as evident from X-ray work (4) and freeze-fracture electron microscopy (5). The difference between this type of structure and the amorphous type of structure in a long-chain alcohol melt is demonstrated in Figure 1. Even if a triglyceride is not expected to form lamellae as well-defined as polar lipid melts do, the similarity in diffraction behavior is a strong indication of the occurrence of the same type of structure. Any structure analysis of the triglyceride melt should benefit from relating it to the two known extremes; the amorphous paraffin melt and the layered structure of polar lipid melts.

A basic assumption in the work by Cebula *et al.* (1) is "If there was any layer-like structure at all, then glycerol moieties of adjacent molecules should reside in the same plane parallel to the plane of the layers. Such partition of the glycerol from the fatty acid chains (normally observed in crystalline triglycerides) would produce some form of (00L) series. A layer system might also be expected to show some high-angle (hk) reflections due to glycerol-glycerol organization within the layer." This is not correct, as is demonstrated clearly by diffraction characteristics of L2-phases discussed above [which never have been observed to show "(00L) series" but only one band], as well as by all liquid-crystalline lipid phases. All liquid-crystalline lipid phases contain a lipid bilayer with molecules in a liquid-like disorder. Therefore, they show no "high-angle (hk) reflection."

The main argument by Cebula *et al.* (1) against lamellae in the liquid state is the difference in diffraction pattern of liquid trilaurin with fully deuterated acyl chains and that of trilaurin with only the glycerol region deuterated. The wide-angle band with 2θ about 60°, corresponding to chain-

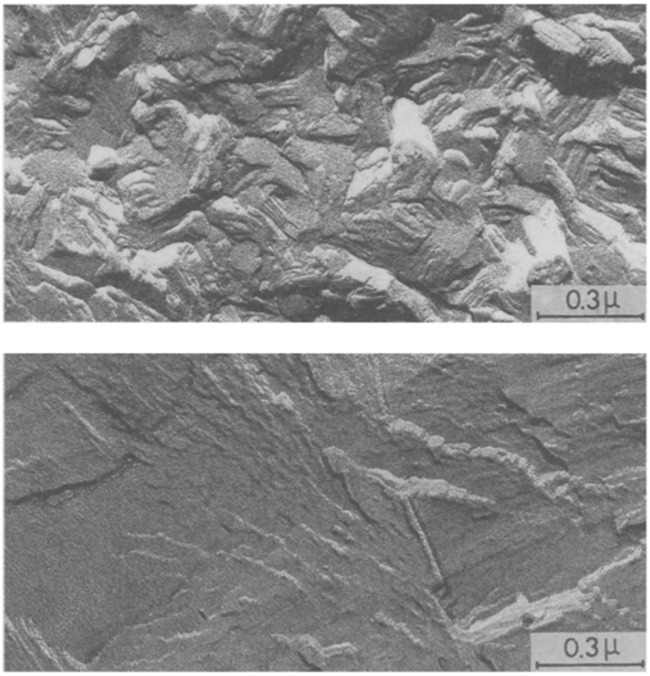

FIG. 1. Freeze-fracture electron micrographs of a liquid monoglyceride (mainly of linoleic acid) (shown above) and of liquid dodecanol (shown below) after (5).

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chain correlations in the case of perdeuterated chains, is strongly suppressed when only the glycerol group is deuterated. This is, however, not surprising. It must be kept in mind that the frequency of the X-ray or neutron beam used in diffraction experiments is so high in relation to molecular movements in the sample that the beam will only "see" atoms in a static position. The high degree of molecular mobility, characteristic of the liquid state, must of course be reflected in the dynamics of the proposed lamellae, and the glycerol groups of each dimer must be expected to move considerably in relation to the center of the lamellae. If we assume a relative mobility of ±3Å of adjacent dimers, which seems reasonable, it is obvious that no band at about 60° in 2θ can be expected but rather a broad and weak hump, like that observed in the "LLL-DH" diffraction pattern.

A final comment on the nematic type of structure proposed by Cebula *et al.* (1): The general requirement for nematic phases is the occurrence of stiff, rod-like molecules. The characteristic property of hydrocarbon chains in the liquid-type of conformation is the lack of rigidity and, therefore, of direction properties. In the wide diversity of liquid-crystalline phases found in lipid-water systems, a nematic type of structure has never been observed.

The paper by Cebula *et al.* (1) has clearly brought our knowledge on the liquid triglyceride structure to a higher level by providing new valuable experimental data, although the last word on interpretation certainly has not been said. A challenging feature of their "LLL-DH" diffraction pattern, for example, is an indication of a weak feature near $2\theta = 40°$ [which might correspond to a third order of a bilayer spacing, as reported in the case of the trimyristin melt (2)].

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[Received April 20, 1992; accepted May 8, 1992]