THE POLYMORPHIC FORMS OR PHASES OF TRIGLYCERIDE FATS[1]

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Received May 14, 1941

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I. INTRODUCTION

The remarkable way in which fats melt, resolidify, and then melt again on further heating has been the subject of experimentation and speculation since the time of Chevreul. The double melting of tristearin was one of the first examples to receive extensive study, and Guth (17), among others, found that when the molten triglyceride was quickly chilled, it thereupon melted at 55°C., but shortly resolidified, to melt again at 71°C. as the heating proceeded. The cause for this behavior was usually assigned, mainly on the basis of analogy and assumption, to the existence of two

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[1] Presented at the Symposium on the Molecular Structure of Fats and Oils, which was held under the auspices of the Division of Biological Chemistry and the Division of Agricultural and Food Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Missouri, April 7-11, 1941.

different isomeric modifications, one melting at the lower and one at the higher temperature.

The study of the polymorphism of the triglycerides originated in such melting-point phenomena, but over the years, the description of the melting behavior of tristearin (or of other glycerides) has seldom been clearly given, and indeed, as will appear below, not until the advent of Malkin's (6, 7, 29, 30, 31) work in the last decade has any definite and clear basis for an understanding of the melting behavior of fats been available in the literature. To be sure, the predecessors of Malkin in the study of pure triglycerides had laid the foundation, and the many researches on simpler long-chain compounds, such as the fatty acids, had indicated the plan of attack, but it is notable that much of the more stimulating earlier work was performed, not upon single pure chemical compounds, but upon the complex mixtures of glycerides in the natural fats. Many of the concepts of Van Roon (48) and others on the fats of commerce, while not clear as to the nature of the crystalline structures involved, may be applied with profit to polymorphic and phase studies of triglycerides.

The point of view of the present paper is the consideration of triglycerides in terms of the solid physical forms or phases in which they exist. Emphasis has been and will be placed on melting and solidification phenomena, because historically the study of the polymorphic forms has been based almost exclusively on observations intimately associated with the heating and cooling of fats. Not until recently have other phenomena and other methods been brought to bear on the problems concerned with the phase relations of the triglycerides. Even these later investigations have invariably been supplemented and substantiated by studies of melting and crystallization behavior.

II. THE MEANING OF THE MELTING POINTS OF FATS

In the realm of commercial fats it has been appreciated for many years that the melting point of a sample depended on (*1*) the particular melting-point method used, and (*2*) the previous history of the sample. The typical condition of affairs has been indicated in the admirable statement of Van Roon (48) with regard to cacao butter: "Many methods have been proposed for determining the melting point of cacao butter, and in each the melting point is defined differently. None of these can be called generally useful. They simply lead to confusion, which is increased by the fact that the treatment of the fat in preparation for the melting point determination has a great effect on the result."

Unfortunately, such recognition of the difficulties has not always prevailed in the field of glyceride chemistry, and all too often melting-point data for glycerides are reported, which are not susceptible to exact comparison, even assuming the glycerides in question are of identical composition. For example, the melting point for β-laurodistearin has been given by various workers as 36°, 52°, 56.5°, 58°, 59.8°, 60.5°, and 68.5°C.

Until the stability and equilibrium relations between the various forms or phases in which a given glyceride may occur are clearly understood, such a profusion of melting-point results makes it practically impossible to establish anything precise in the way of characterizing properties. Each glyceride must be considered as a one-component system capable of changing form and properties without change in composition.

Since the work of Othmer (38), Loskit (27), Joglekar and Watson (22), and especially the later contributions of Malkin (6, 29, 30, 31) have made it clear that triglycerides can exist in at least four different modifications,—the γ-, the α-, the β'-, and the β-phases,—it is necessary to consider the actual melting-point determination in terms of these phases. Each phase has its own melting point, and in considering the melting point of any triglyceride it is necessary to realize which particular form of the glyceride is involved.

Perhaps the most significant method of ascertaining the melting point of an already solidified or processed fat is the so-called "quick method", essentially that described by Timmermans (47). The sample, without previous liquefaction, is placed in a small, thin-walled capillary, and this is thrust into a bath of known temperature. If the sample melts, similar samples are then thrust into the bath at lower temperatures, until a temperature is discovered at which the sample barely melts.

Suitably applied, this method also serves to distinguish the various phases mentioned above (30) from one another. Thus a very small quantity of a molten fat may be drawn into a thin-walled capillary, then quickly chilled. Under proper chilling conditions the γ-phase of the triglyceride is produced, but it is unstable and its melting point therefore must be obtained, not by slowly raising the temperature of the bath, which would allow it to be converted into a more stable phase, but by thrusting the capillary quickly into a bath of known temperature. At the gamma melting point liquefaction ordinarily occurs, followed by resolidification into the α-, β'-, or β-modifications, depending on conditions. The melting points of these phases then must be determined by similar methods, in which, of course, the production of the correct phase prior to the determination is imperative.

Even the special melting-point methods may fail to yield a reliable figure with some compositions, and cooling-curve and other techniques are required to obtain the temperature of melting (7).

From what has been said, it is apparent that the legendary conception of "double melting" of fats (see, especially, 42) must be replaced by "multiple melting", and the melting points must be given in terms of the different physical forms in which the solid fat may occur.

III. The Development of the Concept of Polymorphic Forms in Fats

The initial development of the concept of polymorphic forms in triglyceride fats goes back to the obscure beginnings of organic chemistry. It is remarkable that, as early as 1853, Duffy (11), building on the experience of earlier observers, actually discovered three melting points for "stearin" from mutton fat many times recrystallized. These three melting points agree rather well with the three melting points obtained for tristearin by the most reliable modern observers. Duffy noted that, with considerable impurity present, it was difficult to obtain the highest melting points, and that, as impurity was eliminated, it was less and less easy to find indications of the intermediate melting point. He stated clearly, apparently for the first time, (*1*) that the solidification temperature and the lowest melting point were practically identical and (*2*) that the melting point of crystals obtained from solvents was identical with the highest melting point obtainable with the glyceride itself. The assumption was made that the melting behavior arose from some form of isomerism.

It appears that many later observers, in the course of years of quoting and requoting, lost sight of the best of Duffy's work. They still, for the most part, kept to the idea of isomerism as the cause of double melting. The intermediate melting point was missed, probably because of the increased purity of the tristearin employed in experimentation.

In 1902, Guth (18) determined the melting point of tristearin as 71.5°C. Moreover, this was described as the sole melting point, provided the sample was held in the capillary sufficiently long before the determination was made. If examined soon after placing in the capillary the fat appeared to undergo two distinct meltings, the first occurring at a much lower temperature than the other. Guth also made the very illuminating disclosure that, at the lowest observed melting point, complete melting did not occur unless a very narrow capillary and a very small quantity of fat were used. This is still good technique today and is necessary because of the rapidity with which the low-melting unstable γ-form is transformed, or the melt crystallizes to give the alpha or more stable phases.

As a result of his investigations, Guth concluded that the rapidly cooled sample, when it resolidified, had in reality not passed into the crystalline state, but was in a condition analogous to that of undercooled water or a supersaturated solution, which upon slight disturbance thereupon passed into a crystallized condition. These ideas are not so far removed from Malkin's (7) modern conception of the lowest melting triglyceride as a "glass".

Further contribution to the relationship of the materials melting at the two temperatures was made by Grün and Schacht (15). They prepared three mixed triglycerides, each of which exhibited an unstable (low-melting) and a stable (high-melting) form. It was then determined that the unstable form was converted into the stable modification when inoculated with the latter. The conclusion was reached, valid today, that the change was monotropic, no reversions occurring under any conditions. These investigators, however, still labored under the misconception that isomerism was involved and, indeed, Custodis (8) found later, probably erroneously, that the unstable form gave only half the theoretical value in the freezing-point determination of molecular weight.

Le Chatelier and Cavaignac (26) now introduced evidence to show that the melting of a triglyceride was a reversible process, the peculiar behavior reported by earlier workers being due to the slow velocity of the melting or crystallization. If the melting or solidification was carried out with an extreme degree of slowness, the reverse change occurred within 0.1 to 0.2°C. Rapid determinations were accordingly designated as the cause of divergent melting points. Up to this point they were perfectly correct as to the physical evidence, but no fact was found necessitating the assumption of the existence of different polymorphic forms, although such forms were not excluded. The issue was confused by minimizing the long-substantiated fact that solidified tristearin could be made to melt and resolidify at as low a temperature as 55°C., although the very same sample would yield a reproducible, reversible high melting point of about 71°C. However, of great importance was the establishment of the reversibility of the upper melting point.

In 1907 Bömer (5), substantiating Duffy (11), showed definitely that a high-melting modification always resulted when triglycerides were crystallized from solvents, and felt therefore that the melting point of such a preparation was to be regarded as the true melting point. He also indicated more definitely than most the exact conditions for obtaining the low melting point.

The unstable, glass-like nature of the low-melting modification was from time to time emphasized, other workers looking upon it as an anisotropic liquid phase (21). Malkin (7), the latest large contributor to the study of the polymorphism of triglycerides, regards the γ-form as a "glass".

The first definite intimation of a rediscovery of still other modifications than the so-called stable and unstable forms came in 1915, when Othmer (38) listed not two, but three, melting points for trimyristin and tristearin. Othmer's conceptions seemed to involve simple crystalline polymorphism and not isomerism, and thus in some respects a clarification of the problem resulted.

Loskit (27) a little later contributed strong confirmatory evidence for at least three different forms in which tristearin and other triglycerides could exist under proper conditions. Moreover, the microscopic structure of the three forms was specified,—the low-melting phase being of a "colloidal" glassy nature, the intermediate form consisting of spherulitic crystals, and the stable form separating in crystalline needles.

Efremov (12), in 1927, for all the obvious lack of purity of his materials and inaccuracies in his numerical values, obtained definite evidence of the three polymorphic forms in tristearin and tripalmitin and, as related below, made confirmatory experiments in binary glyceride systems.

Apparently ignoring the purport of Othmer's, Loskit's, and Efremov's experiments, and reviving ideas connected with isomerism, Joglekar and Watson (22), as late as 1930, concluded that only two different solid modifications of triglycerides existed. The stable form, which they call alpha (this is the beta form of Malkin, whose nomenclature is employed throughout this paper) separates in crystalline condition from solvents, and the unstable variety occurs when the molten fat is chilled, thus far confirming many previous investigations. In spite of its limitations, the paper of Joglekar and Watson contains much that is fundamental, such as an unusually clear exposition of the methods and conditions required to obtain the stable and unstable forms, and what appear to be highly accurate melting points. These investigators seem to have been the first also to note clearly in triglycerides the phenomenon of transformation in the solid state, so often seen in commercial fats. Thus, after chilling a sample of molten tristearin it was found that "the solid melted at 55°, the melting point of the β form (Malkin's γ), but resolidified in a few moments, showing that numerous nuclei of the α form (Malkin's β) had been produced by chilling. This formation of nuclei is, no doubt, responsible for the change of the β (Malkin's γ) into the α (Malkin's β) form in the solid state at room temperature frequently observed during the experiments. When it takes place there is a remarkable swelling into sponge-like formations, and the containing vessel is often broken".

While Joglekar and Watson (22) and later Rao and Jatkar (39) recognized but two modifications in simple glycerides, it will be recalled that Duffy, Othmer, Loskit, and Efremov believed that three forms were possible. Furthermore, in 1932, on the basis of melting-point and microscopic experiments, Weygand and Grüntzig (49, 50) came to the conclusion that as many as seven different polymorphic forms were able to exist. Their work seems to have been carried out with considerable accuracy, although it would appear that their original results in reality indicate with certainty only three forms for tristearin.

It is clear, therefore, that a very considerable body of evidence had been built up pointing to the occurrence of several distinct glyceride phases, yet until recently there had been no adequate description or characterization of these forms and no clear and fully convincing evidence as to their number for any particular glyceride.

IV. THE POLYMORPHIC FORMS OF SIMPLE TRIGLYCERIDES

A. INTRODUCTION

Before proceeding to the elucidation of the polymorphism of the simple triglycerides, there should be a brief consideration of the background of information on the polymorphism of the simpler long-chain compounds related to the triglycerides. There is a store of information here to draw upon, from which the following points are pertinent and significant in relating the larger field of long-chain compounds to that of the triglyceride fats.

(1) Polymorphism is almost universal in long-chain compounds.

(2) Long-chain compounds crystallize generally with two, four, or eight molecules in the unit cell.

(3) There are two main types of polymorphic forms, for which a conventional nomenclature has been fairly widely accepted: (*a*) Alpha form: In this form the long chains crystallize in positions perpendicular to the planes determined by the end groups. This form usually exhibits no alternation[2] in physical properties for odd and even number of carbon atoms. Only one alpha form exists for a given compound. (*b*) Beta forms: In these, the long chain crystallizes at an angle, which is not a right angle, to the plane of the end groups.[3] These forms generally exhibit alternation in physical properties between odd and even series. More than one such form may exist for a given compound.

(4) Transformations from one form to another are slowed up when impurity is present. Impurities may be other members of the same homologous series.

(5) Compound formation may occur between near members of a homologous series (44).

(6) Not only does the second component of a binary system tend to slow up transformation from one form to another, but sufficient amount of the second component may cause a form which is unstable in the single-component system to become the truly stable form in the binary system (45).

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[2] For a discussion of the geometrical basis of alternation, see Malkin (28). For a critical discussion of this geometrical explanation, see Eyring (13).

[3] For a presentation of the possible interrelation of the alpha and the various beta forms, see Schoon (43) and Fuller (14).

B. SIMPLE TRIGLYCERIDES

The long-anticipated phase basis for systematizing and explaining the behavior of triglycerides became a definite reality in 1934, when Clarkson and Malkin (7) presented convincing x-ray and other evidence on the polymorphism of triglycerides of chain length from ten to eighteen carbon atoms.

It now appears certain that the simple triglycerides occur in at least three phases,—the gamma, the alpha, and the beta. (A fourth form intermediate in stability between the alpha and beta forms occurs for the triglycerides with an odd number of carbon atoms.) The melting, solidification, and other properties of the triglycerides must therefore be interpreted and studied with respect to the phase or phases present.

Insofar as they can be applied generally, the main identifying characteristics of the three polymorphic forms are listed in table 1.

1. Phase melting points

When the melting points of the three modifications are determined for the simple glycerides, it is found that the beta phase exhibits the familiar property of alternation, whereas the alpha and gamma forms do not (7).

It is evident from figure 1 that tristearin, for example, may give a melting point of 71.5°, 65°, or 54.5°C., depending on which phase is present. As a practical matter, a given sample of tristearin may yield almost any melting point over this range, depending on the history of the sample before the determination is made. Accordingly, the special meaning of melting point, outlined earlier, must be recalled in dealing with the melting of the three triglyceride phases. The determination of melting point includes the processing necessary to induce the formation of the particular phase in question. Thus, to obtain the gamma melting points shown in the lower curve of figure 1, the triglyceride in molten condition should be rapidly chilled in a narrow, thin-walled capillary. Immediately the chilled sample is thrust into a bath of known temperature to see whether or not melting occurs. In either case a well-melted and newly chilled sample is thrust into a bath at higher or lower temperature, and so on until the true melting temperature is obtained.

By this method the melting point of the γ-form of the triglyceride is obtained. Properly determined, it is a definite, characterizing property.

A major concern in determining the true gamma melting point is obtaining the correct chilling rate to induce the formation of the gamma form and it alone. While the glycerides of C_{13}, C_{14}, and C_{15} acids solidify in the gamma form when chilled in an ice–salt mixture, members higher in the series solidify readily in ice. Tristearin, in fact, solidifies in the gamma form if merely cooled to room temperature.

TABLE 1

The polymorphic forms of simple triglycerides (tristearin)

	FORM		
	Gamma	**Alpha**	**Beta**
Relative stability	Least	Intermediate	Most; the only thermodynamically stable form
Melting point	Lowest; non-alternating for odd and even number of carbon atoms	Intermediate; non-alternating	Highest; alternating
Gross appearance	Vitreous, translucent	Intermediate	Generally opaque, even powdery
Microscopic appearance	Tiny black and white uniaxial crosses; a mosaic between crossed nicols	Much larger uniaxial crosses, not as brilliant	Small parallelograms; best, no nicols (can form spherulites)
X-ray evidence:			
Short spacings*	One, diffuse, 4.2 Å.	One, fairly sharp, 4.2 Å.	Multiple, sharp; 3.7, 3.9, 4.6 Å. strong; 5.3 Å. weak
Long spacings†	None found	Longer; two chain lengths (perpendicular chains)	Shorter; more than one, less than two, chain lengths (tilted chains)
Crystal structure	Possibly glass or liquid crystalline	Possibly orthorhombic (mesomorphic?)	Monoclinic
Density	Least	Intermediate	Greatest
Heat of crystallization	44 cal. per gram	Undetermined; probably intermediate	62 cal. per gram
Dielectric constant	Near liquid value	Undetermined	Lowest
How obtained:			
From liquid	Rapid cooling	Slow cooling	Very slow cooling
From other solid	Not possible	From γ-form near gamma melting point	From α-form near alpha melting point
Existence of type form in other long-chain compounds	Apparently *uniquely* in triglycerides	Quite general in mono- and diglycerides, paraffins, and esters; *not in saturated fatty acids*	Quite general in mono- and diglycerides, paraffins, esters, *and fatty acids*

* Short spacings refer to distances of separation of hydrocarbon chains.

† Long spacings refer to lengths of hydrocarbon chain.

It must be emphasized that the size of the sample is important. A large sample, cooling at unequal rates from surface to center, will, in most examples, exhibit a mixture of phases. Hence for melting-point determinations the necessity of the restriction to small quantities in thin-walled capillaries is apparent.

Usually the melting point of the alpha or intermediate phase is not so readily ascertained, since this form is probably never obtained in an absolutely pure state. This is because the melt may crystallize in a mixture of forms, or the gamma phase may transform to both alpha and beta, and the alpha, especially in some glycerides, may transform rapidly to beta.

In general, however, the melting point of the alpha phase is detected (7) by first obtaining the melting point of the gamma phase in a bath at a temperature where it just melts and resolidifies: gamma → liquid → alpha.

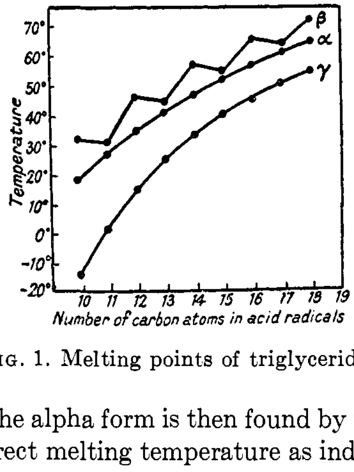

FIG. 1. Melting points of triglycerides

The melting point of the alpha form is then found by thrusting the capillary into a bath at the correct melting temperature as indicated by trial. Here again large samples obscure the result, since the α-phase changes to the β-phase in the solid state without melting, i.e., below the true alpha melting point. Hence it is that the usual melting-point procedure, wherein the temperature of the sample is raised gradually, never discloses the alpha melting point, although it may sometimes give an indication of the gamma melting point.

The maximum melting point, that of the stable β-phase, may be determined readily in many fats by ordinary procedure. With others, it is necessary that the β-phase be present initially. This condition is not always easily attained, since impurity or special composition may lead to an extremely slow formation of the β-phase in the stabilized fat. In such cases a very slow rise in the temperature of the bath, with periods of holding at temperatures below the melting point (tempering), are required to develop the true maximum melting temperature of the sample. For such samples the dilatometric curves illustrated by Hofgaard (20) yield illuminating results, and in some instances cooling and heating curves enable the true melting point to be obtained (30).

For some of the simple triglycerides table 2 lists the melting points of the three phases as determined by different workers. Considering possible differences in purity of samples and in technique, the values for a given phase are, in most instances, remarkably close, and amply support the existence of three distinct modifications.

While Clarkson and Malkin regard their values for the gamma melting point as being ranges of temperature, rather than points, it is probable that more careful technique would define these with considerable sharpness.

The whole discussion has been based on the assumption that but three polymorphic forms exist in the simple triglycerides. It will be recalled that Weygand and Grüntzig (*loc. cit.*) have reported seven forms for the even-carbon chain and four for the odd. Their results were based on melting-point and meticulous microscopic studies. Malkin in his first paper on triglycerides has seemed to dispose fairly of their case with regard to the triglycerides with an even number of carbon atoms and, indeed, inspection of the results as reported in table 2 will show how closely the melting-point figures fall in three well-defined groups.

However, Grüntzig (16) still maintains his earlier stand and reports a private communication from Malkin accepting the existence of four forms in the odd triglycerides, in confirmation of Weygand and Grüntzig's findings. It will be noted later that Malkin has found four forms for practically all mixed triglycerides that he has studied.

On the basis of the best available evidence it may be assumed for the present that in the simpler triglycerides the more important even members exhibit but three forms, while the odd members exhibit four.

Melting-point information on the simple unsaturated triglycerides is necessarily meager, considering how recently pure materials have become available and how low melting the compounds are. The data of Wheeler, Riemenschneider, and Sando (51) on triolein, combined with those of Carter and Malkin (33) for trierucin, show that the unsaturated homologous series of which these are members follows the saturated series in polymorphic behavior, each member exhibiting three melting points. Trilinolein, on the other hand, apparently exhibits but two polymorphic forms. The unsaturated glycerides show more rapid transformation of forms than do the corresponding saturated compounds.

The isomeric trielaidin and tribrassidin also fit into the general scheme with three polymorphic forms, according to Carter and Malkin (6).

TABLE 2

Melting points of the polymorphic forms of simple triglycerides

TRIGLYCERIDE	DUFFY			OTHMER			EFREMOV			LOSKIT			JOGLEKAR AND WATSON			WEYGAND AND GRÜNTZIG							CLARKSON AND MALKIN			WHEELER ET AL.			CARTER AND MALKIN		
	III	II	I	I	II	III	(γ)	(β)	(α)	I	II	III	(α)	(−)	(β)	I			II			III	β	α	γ	β	α	γ	β	α	γ
																A	B	C	A	B	C	C									
Tristearin	69.7	64.2	52.0†	70.8	64.5	54.5	69.3	64.9	53.2	71.8	65.4	55	71.8	—	46.2	71.0	70.5	69.5	65.5	65.0	64.0	55.0	71.5	65.0	54.5						
Trimargarin																			64.5	63.0	60.0	50.5	63.5*	61.0	50						
Tripalmitin	62.8	61.7	46.0‡				67.5	60.0	48.4	64.8	55.0	45.4	65.6	—	46.2	65.5	64.5	63.5	58.0	57.0	55.5	45.5	65.5	56.0	45						
Tripentadecylin																			54.8	53.5	51.0	39.5	54.0*	51.5	40						
Trimyristin				55	41.8	32.1				56.5	48.5	47.0	56.5	—	33.0	56.5	56.0	54.5	48.5	48.0	47.0	32	57.0	46.5	33						
Tritridecylin																			44.4	43.5	41.0	24	44.0*	41.0	25						
Trilaurin				44.3	34					46.4		36.4	46.2	—	18.0	46.5	46.0	45.0	37.5	37.0	36.0	15	46.4	35.0	15						
Triundecylin																							30.5*	26.5	1.0						
Tricaprin										31.0		25.0	31.6	—	—								31.5	18.0	−15						
Trierucin																													32.5	25	6
Triolein																										4.9	−12	−32		Oil	
Tribrassidin																													57	43	36
Trielaidin																													41.5	37	15.5
Trilinolein																										−12.9	−43				

* A fourth polymorphic form reported by Malkin in private communication to Grüntzig.

† From mutton fat.

‡ From palm oil.

2. *Cooling and heating curves*

With the aid of cooling and heating curves, judiciously performed, a valuable insight has been obtained into the phase transformations which take place during the crystallization and melting of fats. This insight is deeper and more complete than can be obtained with melting points alone.

Malkin (7) was not the first to employ cooling and heating curves, although his curves are related more definitely to the phase changes which occur. Nicolet (37) revealed valuable indications as to the polymorphic behavior of tristearin and closely related commercial fats, as a replotting of his data will show (see figure 2).

While not explicitly stated in his paper, it is clear from the curves, especially in the light of other more recent information on the polymorphism of triglycerides, that his sample of tristearin supercooled to a minimum approximating the gamma melting point. Solidification in the gamma form then occurred, followed by transformation and then crystallization in the alpha form with a rise in temperature, a flat in the curve roughly approximating the alpha melting point. Further transformation and crystallization in the beta form then took place with another temperature rise to a maximum, which is approximately the temperature of melting of the beta phase. This behavior refers specifically to tristearin, but an inspection of the curve for hydrogenated linseed oil (iodine value = 2.7; 7.5 per cent non-stearic acids) indicates a very similar sequence of events during the cooling of this fat. It is important to observe also that the corresponding curve for hydrogenated soybean oil, which contains 8.1

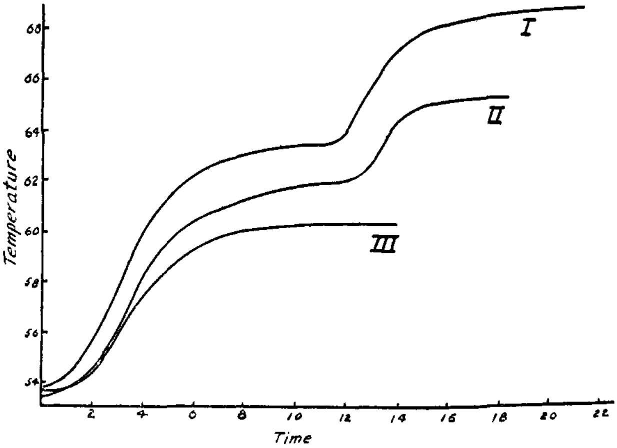

FIG. 2. Nicolet's cooling curves for triglycerides. Curve I, tristearin; curve II, hydrogenated linseed oil; curve III, hydrogenated soybean oil.

per cent of non-stearic acids and is thus slightly further removed from tristearin in composition, shows no second rise, although (according to Nicolet) such a rise did occur when the sample was seeded. A hydrogenated cottonseed oil (28 per cent palmitic acid), departing considerably in composition from tristearin, exhibited no second rise even with seeding. This behavior emphasizes the marked retarding influence of admixture on phase transformation, an influence the potency of which must be constantly kept in mind when dealing with commercial fats.

Still more pertinent and revealing interpretations are derived from Malkin's cooling and heating curves (see figure 3) for various triglycerides. His work was performed with small amounts (1 g.) of material in a jacketed system; temperatures were followed by means of thermocouples. The dotted cooling curve (for tristearin) shows the results of rather rapid cooling. It has one nick corresponding to the gamma melting point, again showing the gamma melting point as the approximate limit of supercooling.

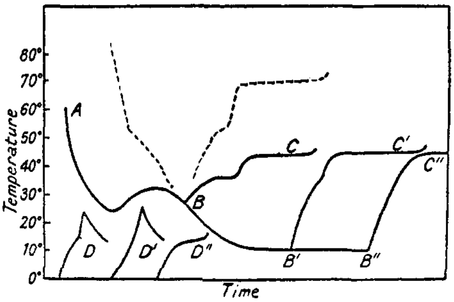

FIG. 3. Malkin's cooling and heating curves for various triglycerides

The dotted heating curve for tristearin shows by the first deflection a partial melting at the gamma melting point, then a sharp rise indicating alpha and beta crystallization, probably accompanied by alpha-to-beta transformation. There is no evidence of the alpha melting point, owing to the relatively slow heating gradient. Finally the long flat indicates beta melting.

Consideration of the solid curve (for trilaurin), marked *ABB′B″*, shows that, under the particular experimental conditions, the trilaurin did not supercool to the gamma melting point (15°C.) as it would with a faster cooling rate. The fat supercooled to about 25°C. to crystallize in the alpha form with a rise, due to heat of crystallization, almost to the alpha melting point.

The heating curve *BC* shows two distinct arrests. The curve starts above the gamma melting point, so the first arrest indicates melting of alpha to liquid, probably accompanied by transformation alpha → beta. Upon the disappearance of the alpha form, the crystallization of the beta form raised the temperature sharply to the second arrest, corresponding to the melting point of the beta phase.

The conversion of one form to another with elimination of an arrest in the curve is indicated in $B'C'$ and $B''C''$. The samples for these latter curves were held at room temperature (above the gamma melting point) for 1 and 3 hr., respectively. (Note that again no indication of the gamma form appears, although the curve starts below the gamma melting point. The trilaurin had crystallized entirely in other forms above the gamma melting point.) The result of storage at room temperature was that the alpha arrest disappeared, showing that, by tempering, the alpha form had been converted to the stable beta form.

The curves D, D', and D'' for trilaurin are examples of Malkin's heating-curve method of determining the melting point of the gamma form. The molten sample was rapidly chilled in a mixture of dry ice and ether. It was then set in a bath which had been found by trial to be near the gamma melting point. For the case when the temperature was too high, the curve rose rapidly past the temperature of the bath without exhibiting any pronounced break (D'). If the temperature was too low, there occurred a pronounced break in the curve before it began to rise sharply (D''). The gamma melting point was taken as the intermediate temperature at which the arrest was just sharply noticeable (D).

Since the quantity of fat, the cooling and heating gradient, and the special nature of the particular glyceride all affect the cooling and heating curves, it is not to be expected that the arrests should correspond under all conditions to the exact melting points or that arrests corresponding to every polymorphic form of the particular glyceride should be obtainable under conditions which can be prescribed as general for all glycerides.

In obtaining cooling curves, if the cooling rate is too fast, manifestations of the higher melting forms are apt to be lacking. If the rate is too slow, supercooling may be limited and manifestations of the unstable forms may not be obtained. Similarly for heating curves, too fast a rate may fail to reveal the more stable, higher melting forms. Too slow a rate may obscure the unstable forms. In any case, but more especially in the heating curve, the course of the physical changes depends on the previous history of the starting material, i.e., in the case of solid material for a heating curve the course of the curve depends in large part on the phases already present, as determined by previous crystallization history.

The same, or what seems to be corresponding, treatment for different triglycerides will not always yield the same manifestations of polymorphism. The greater spread of melting point and the greater transformation rates of lower members of the homologous series make each material practically a special case. A further complication is the slower transformation rate of glycerides with chains of odd carbon number as compared with the rate for even-number glycerides.

As a general rule, modified by the above considerations, it appears that extremely slow cooling from initial temperatures at a little above the beta melting point causes beta crystallization. From higher temperatures and with somewhat quicker cooling the alpha phase is obtained. Rapid chilling to a temperature below the gamma melting point induces the formation of the gamma modification. Of the two forms, alpha and beta, the former separates first, provided the liquid is held below the alpha melting point. Upon raising the temperature of a cooled sample, transformation to the more stable forms may occur, and variations in cooling and tempering conditions lead to mixtures of phases, which may sometimes be taken for additional phases (7).

3. Microscopic evidence

Information on the microscopic structure of fats is widely scattered, but for the most part does not take into account the different modifications. The most pertinent and valuable contributions with respect to triglyceride polymorphism are found in the work of Loskit (27). Additional material is given by Weygand and Grüntzig (49, 50) and by Malkin (30). No completely satisfactory characterization of the various phases by microscopic methods has been brought forward, but the following descriptions represent the best available information:

Gamma phase: Between crossed nicols there usually appear very tiny uniaxial crosses, indicating spherulite formation. The whole field may appear as a mosaic of tiny points of light so small as to make the detection of the uniaxial crosses difficult (27, 40, 49, 50).

Alpha phase: Much larger uniaxial crosses are formed, distinguished from those of the gamma phase by size and by the lesser contrast between the dark and light portions of the field. Conversion to the beta form is readily observed by destruction of the pattern (Malkin).

Beta phase: Spherulites are formed but not with such a striking pattern of uniaxial crosses. The form appears as a multitude of usually perfect little parallelograms of various sizes and shapes, some almost needle-like, others broader. Such shapes are also obtained by crystallization from organic solvents. The crystals are small for the higher members of the homologous series and larger for the lower members.

4. X-ray diffraction patterns

Perhaps the most conclusive differentiation of triglyceride phases comes from x-ray diffraction patterns. Not only does the pattern designate the phase present, but, of equal value, it enables changes and transformations to be followed. Thus, for example, the diffraction pattern indicates the conversion of the gamma form to alpha and beta as a sample containing the unstable modification is aged at a given temperature. If a characteristic ring pattern can be recognized as pertaining to one definite phase, then nothing need be known, as to calculated spacings or unit cells, in order to follow the subsequent career of the phase.

As pointed out by Davey (9), the quantitative x-ray study of mixtures of phases can be carried on accurately only if mixtures of known phase composition are used as a basis. For the fats no published x-ray evidence is based on such technique, and accordingly the study of phase transformation has at best been qualitative. Another limitation in the use of the x-ray for fats has been the rapidity with which change in phase occurs under some conditions. Nevertheless, as applied by Malkin and his coworkers, the x-ray pattern provides a highly valuable means of attacking many of the problems connected with the polymorphism of the triglycerides.

TABLE 3

X-ray spacings for phases of triglycerides

GLYCERIDE	LONG SPACINGS		BETA SHORT SPACINGS	
	Beta	Alpha	Even	Odd
Tristearin	45	50.6	3.7, 3.9, 4.6, 5.3	
Trimargarin	43.5	48.5		3.65, 4.0, 4.6, 5.3
Tripalmitin	40.6	45.6	3.7, 3.9, 4.6, 5.3	
Tripentadecylin	38.9	42.9		3.65, 4.0, 4.6, 5.3
Trimyristin	35.8	41.2	3.7, 3.9, 4.6, 5.3	
Tritridecylin	34.1	37.7		3.65, 4.0, 4.6, 5.3
Trilaurin	31.2	35.6	3.7, 3.9, 4.6, 5.3	
Triunidecylin	29.6	33.0		
Tricaprin	26.8		3.7, 3.9, 4.6, 5.3 4.2* 4.2 diffuse†	
Trierucin	51.1	55.0	3.70, 3.84, 4.03, 4.60, 5.24	
Triolein			Oil	
Tribrassidin	53.6	60.9	3.75, 3.95, 4.95, 5.35	
Trielaidin	44.1		3.75, 3.95, 4.95, 5.35	

* Alpha short spacings.

† Gamma short spacings.

For example, Clarkson and Malkin (7) found that a characteristic beta pattern is obtained when purified triglycerides are crystallized from solvents. If, however, the beta crystals are melted and then allowed to flow onto a thin glass strip, either the alpha or the gamma phase is obtained, depending on the rate of cooling, the pattern changing accordingly.

In the foregoing discussion the ring *pattern* characteristic of a phase has been emphasized, but in table 3 and figure 4 the actual measured long and side spacings for the various phases of triglycerides from tricaprin to tristearin are compared.

As has been said, it is not necessary to know the crystal spacings or the crystal structures derivable from them to study polymorphic changes, but to understand the nature of the polymorphic forms and their changes it is well to glean from x-ray information all that is there.

As Malkin makes clear, a full solution of the triglyceride structure must await such single-crystal studies as have been made by Müller (34, 35) on fatty acids and paraffins. However, from Malkin's x-ray data the following inferences may be drawn:

Gamma form: The hydrocarbon chains are probably in fairly general alinement in the micro-structure. However, according to Clarkson and Malkin (7), the single diffuse band in the x-ray spectrum indicates that the chains are "frozen" in random degrees of rotation about the chain axis. The diffuseness of the band indicates that distances between chains are not very precisely determined. The lack of long spacings, if it is not due to difficulty of observation, indicates that the ends of the hydrocarbon chains, i.e., the methyl groups and the glyceryl groups, do not determine planes.

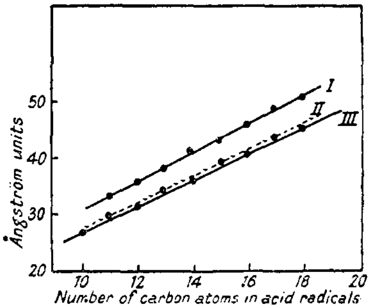

FIG. 4. Long x-ray spacings of triglycerides

Even liquids and glasses will give a diffuse band (for instance, near the melting point liquid paraffins give a spacing of 4.6 Å. (36), as compared with 4.2 for the gamma spacing), though possibly not so well defined as the gamma diffuse band. Clarkson and Malkin conclude that the gamma form is a glass. (It must be noted, however, that the gamma form is anisotropic, as previously mentioned; besides, it shows very considerable volume and heat changes on melting. The possibility that the gamma form is in a mesomorphic or liquid crystalline state does not seem to be ruled out.)

Alpha form: Here also the hydrocarbon chains are in alinement and "frozen" in random degrees of rotation about the chain axis (Clarkson and Malkin (7)). However, the relative sharpness of the single line for side spacings indicates quite constant distances between hydrocarbon chains. The presence of long spacings proves that the ends of the hydrocarbon chains determine planes, and, further, the spacings are of such magnitude as to prove that the hydrocarbon chains are perpendicular to the end-group planes and that the structural unit of the crystal has a double chain length. This perpendicular relationship is the distinguishing feature of the "alpha" forms as observed for paraffins, fatty acid esters, etc.

Beta form: The multiplicity of short spacings probably indicates that the hydrocarbon chains are fixed in a definite position as to their relative degree of rotation about the chain axis. The end groups determine a plane and again the structural unit has a double chain length, but from the shorter long spacings that the alpha form exhibits, the hydrocarbon chains must be tilted at an angle of about 65° to the end-group planes (see figure 5). This tilt of the chains is the distinguishing feature of the beta forms and, according to Malkin, it gives the basis for alternation in the homologous series. (The nature of the fourth solid form, β', mentioned for triglycerides having an odd number of carbon atoms in the chain is presumably like that of the beta form in general features.)

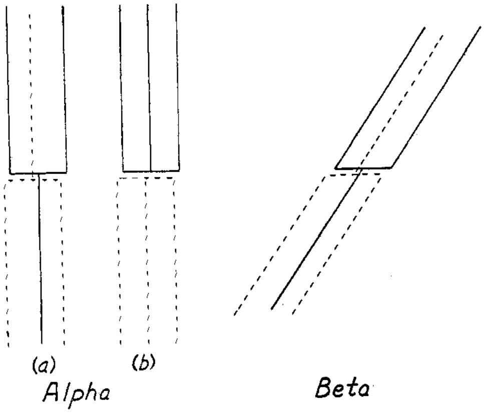

FIG. 5. Malkin's conception of molecular configuration

It is possible that mixtures of crystallites of the forms and variations in the degree of structural order in the gamma and alpha forms can give rise to small variations in melting point. Whether the formation of solid solutions, involving different forms, is actually possible has not been settled.

An alternative structure (b), with the three chains of a given molecule side by side, Malkin is inclined to rule out for mechanical reasons that appear in a three-dimensional model.

5. *Miscellaneous properties of the triglyceride phases*

The literature on volume changes with transformation and melting concerns chiefly the natural and commercial fats. Indeed, the literature on the densities of tristearin in various states has probably not been greatly improved upon since the work of Duffy (11) and Kopp (23) about 1850. Duffy's results are given in table 4.

TABLE 4

Specific volumes of tristearin

THIRD (HIGHEST) MELTING POINT OF SUBSTANCE	TEMPERATURE OF DETERMINATION	MODIFICATION			
		1 (gamma)	2 (alpha)	3 (beta)	Liquid*
°C.	°C.				
65	15	1.0129			
66.5	15	1.0124			
	15	1.0134	0.9900	0.9825	
69.7	15			0.9824	
	51.5	1.0416		0.9910	
	65.5			1.0069	1.0816
	68.2			1.0260	

* Joglekar and Watson (22) furnish data on the density of several liquid glycerides at several temperatures.

Duffy's conclusions, true or not, were that degree of impurity, as indicated by the first column, little affected the densities of the various forms. From the following brief table a better indication of volume changes can be obtained:

PHASE CHANGE	APPROXIMATE VOLUME CHANGE AT GIVEN TEMPERATURE	TOTAL CHANGE FROM LIQUID
	per cent	*per cent*
Liquid to gamma	4.4	4.4
Gamma to alpha	2.3	6.7
Alpha to beta	0.8	7.5

The greater density shown for the more stable forms is apparently contrary to the usual experience of tremendous swelling during crystallization and transformation of completely hydrogenated fats approaching tristearin in composition and of tristearin itself (22). Air pockets and vacuoles accompanying the crystallization may be the causes of the discrepancy.

Rao and Jatkar (39) obtained the following values for the heat of crystallization of the gamma and beta forms of tristearin and the beta form of tripalmitin:

GLYCERIDE	HEAT OF CRYSTALLIZATION		HEAT OF TRANSITION
	Gamma form	Beta form	
	cal. per gram	*cal. per gram*	*cal. per gram*
Tristearin	44.2	61.6	17.4
Tripalmitin		62.3	

The following heats of transition of the gamma form to the beta form were obtained from heat of solution data:

Tristearin	15.6 calories per gram
Trilaurin	15.4 calories per gram

Presumably the heat of crystallization for the alpha form would be intermediate between the values for the gamma and beta forms. It is interesting to note that the heat changes on melting of the two forms, gamma and beta, are roughly proportional to the corresponding volume changes.

Bhide and Bhide (4) found that the dielectric constant of tristearin varies with the previous history of the sample. They found that D of γ-tristearin is lower but near that of the liquid phase. The beta form gives the lowest value of all.

	D
Liquid at 56.8°C	2.82
at 90.5°C	2.76
Beta form at 31.0°C	2.42
at 54.0°C	2.45
at 67.0°C	2.51

Labrouste (25) and Dervichian (10) have introduced the following evidence to show that the highest melting form, in the case of monomolecular films of triglycerides without external pressure on the film, is the gamma form. It is mesomorphous, that is, it approaches in nature the liquid crystalline state. (*1*) When sufficient compression piles up visible particles of this form, the particles are observed to melt at the gamma melting point. (*2*) The gamma form spreads spontaneously on a water surface (unlike the stable form). (*3*) The "melting" point in the two-dimensional case has been found for several triglycerides to correspond very closely with the three-dimensional gamma melting point (see table 5).

At lower temperatures reversible transformation to another form is observed. The surface area of this form (63 Å.^{2} per molecule) corresponds to the x-ray dimensions of the tilted beta form in three dimensions.

With external pressures on the films, evidences of these forms and of one more "solid" form are obtained. This other form, that of minimum film area (55 Å.^{2} per molecule), corresponds naturally to the three-dimensional alpha form with its chain perpendicular to the end-group planes. The fact that the area of the gamma form (71 Å.^{2} per molecule) indicates a more tilted structure even than that of the beta form is at first startling, for this means, if Dervichian's argument holds, that in the three-dimensional case during the various transformations a tilted gamma form transforms to an erect alpha form and it in turn to a tilted beta form.

In considering the interrelation of the monomolecular film state to those in three dimensions, account must be taken of a change in chain alinement, for in the case of the monomolecular film, the chains are known to be aligned according to the alternative scheme of figure 5 (Adam (1)), ruled out by Malkin for the three-dimensional case.

TABLE 5

GLYCERIDE	MELTING IN FILMS		ORDINARY MELTING	
	Beginning of mesomorphous T_s	Real liquefaction	Vitreous	Stable
	°C.	°C.	°C.	°C.
Tristearin	46–7	55	54.5–55	70.8–71.5
Tripalmitin	35	45	45 –46.2	64.8–65.6
Trimyristin	21	29	32 – 3	55 – 7
Trilaurin	0	2–14	14 –16	44.3–46.4

V. The Phases of Mixed Triglycerides

The experimental investigation (6, 29, 30, 31) of the phase constitution of mixed triglycerides has been limited to glycerides containing only two kinds of fatty acid chains in the molecule, each chain having carbon atoms in even numbers ranging from ten to eighteen.

The mixed triglycerides as a class may exist in at least four different solid modifications. They thus exhibit an even more complex melting and crystallization behavior than the simple triglycerides with an even number of carbon atoms in the chain. The character of the evidence with respect to the existence of and relations between the separate forms is similar to that already presented for the simple triglycerides, and is derived mainly from cooling curves and the x-ray record.

In addition to the gamma, alpha, and beta phases, the mixed triglycerides, as stated above, are able to exist in a fourth solid form. This is called the β'-form, and like all the other forms but beta, is thermodynamically unstable, with melting point intermediate between those of the alpha and the stable beta forms. With respect to the end-group planes the tilt of the chains is usually, although not always, intermediate between the tilts observed for the alpha and the beta forms. Ordinarily also the x-ray spectrum for the side spacings appears in a narrower band for the β'- than for the β-form.

A. THE SYMMETRICAL MIXED TRIGLYCERIDES

The transformations from one modification to another are more rapid than in the simple triglycerides. Accordingly it is more desirable, and in fact sometimes necessary, to determine the melting points from the heating and cooling curves, rather than in capillary tubes.

These glycerides, like the simple triglycerides, exhibit spherulite formation, but appear to be distinguished from them by a striking rippled effect as the crystallization field is viewed under the microscope.

The symmetrical triglycerides of the following classifications have been studied by Malkin and his coworkers.

1. Glycerides with fatty acids differing in chain length by two carbon atoms

(*a*) Glycerides of the type $CH(OCOR')(CH_2OCOR)_2$, where $R > R'$ by two carbon atoms: e.g., β-palmitodistearin.

(*b*) Glycerides of the type $CH(OCOR)(CH_2OCOR')_2$, where $R > R'$ by two carbon atoms: e.g., β-stearodipalmitin.

The following distinctions are observed: (*1*) glycerides of group a tend to solidify more readily in the gamma form; those of group b in the alpha form. (*2*) Glycerides of group b tend to give more evidence of the alpha form on melting; those of group a apparently tend to transform from the gamma form directly to the β'- or β-form. (*3*) Whereas the side spacings in the x-ray spectrum for the beta form of glycerides of group a are similar to those for the simple triglycerides, those for glycerides of b are in a narrower band.

There is a remarkable agreement in melting points between corresponding members of groups a and b, e.g., for β-palmitodistearin and β-stearodipalmitin.

2. Glycerides with fatty acids differing in chain length by four, six, or eight carbon atoms

(*c*) Glycerides of the type $CH(OCOR')(CH_2OCOR)_2$, where $R > R'$ by four carbon atoms: e.g., β-myristodistearin.

(*d*) Glycerides of type $CH(OCOR)(CH_2OCOR')_2$, where $R > R'$ by four carbon atoms: e.g., β-stearodimyristin.

(*e*) Glycerides of the type $CH(OCOR')(CH_2OCOR)_2$, where $R > R'$ by six carbon atoms: e. g., β-laurodistearin.

(*f*) Glycerides of the type $CH(OCOR)(CH_2OCOF')_2$, where $R > R'$ by six carbon atoms: e. g., β-stearodilaurin.

(*g*) The glycerides β-decodistearin and β-stearodidecoin.

From Malkin's results it appears that, with the exception of β-palmitodilaurin and β-stearodimyristin, the long spacings of these glycerides in the stable beta form correspond to twice the length of a single molecule or four chain lengths (instead of the normal two chain lengths for groups a and b and for the simple triglycerides). In some cases this is also true of the β′-forms. The significance of this behavior is obviously related in some way to the disparity in length between the fatty acid chains.

X-ray spacings and melting points arranged under the foregoing grouping are brought together in table 6.

TABLE 6

Melting points of symmetrical and unsymmetrical mixed triglycerides

GROUP	GLYCERIDE	ISOMER	MELTING POINTS OF SYMMETRICAL MIXED TRIGLYCERIDES				ISOMER	MELTING POINTS OF UNSYMMETRICAL MIXED TRIGLYCERIDES			
			β	β′	α	γ		β	β′	α	γ
			°C.	°C.	°C.	°C.		°C.	°C.	°C.	°C.
a...	Palmitodistearin....	β	68	64	56	50	α	65	61	57	50
	Myristodipalmitin..	β	60	55	46	37	α	57	52	47.5	36
	Laurodimyristin....	β	50	45	35	24	α	46.5	42	37	22
	Decodilaurin........	β	38.5	33	23	8	α	35.5	31	26	5
b...	Stearodipalmitin....	β	68	65	59	49	α	62.5	59.5	55	46.5
	Palmitodimyristin..	β	58.5	55	49	38	α	54	50.5	45.5	34
	Myristodilaurin.....	β	48	44	37	24	α	43.5	39	33.5	19
	Laurodidecoin......	β	37.5	34	25	6	α	30	26	17.5	0
c...	Myristodistearin....	β	62.5	59	56	47	α	62	57.5	54	44
	Laurodipalmitin....	β	53.5	50	47	34	α	54	49.5	45	32
	Decodimyristin.....	β	43.5	40	37	16	α	43.5	38	32	15
d...	Stearodimyristin....	β	55.5	53	47	33	α	56	52	46	36
	Palmitodilaurin.....	β	45.5	42.5	35	19	α	46.5	43	33	20
	Myristodidecoin....	β	34	30	21	3	α	34.5	31	20	3
e...	Laurodistearin......	β	60.5	58	52	36	α		52	47	36
	Decodipalmitin.....	β	51.5	48	42	20	α	45.5	41	37	23
f....	Stearodilaurin......	β	47	43	38	21	α	45	41.5	31	20
	Palmitodidecoin....	β	40	36	27	6	α	35	32	24	2
g...	Decodistearin.......	β	57	53	47	30	α	49	46	42.5	33
	Stearodidecoin......	β	44.5	40	34	5	α	41	38	32	13

B. THE UNSYMMETRICAL MIXED TRIGLYCERIDES

These glycerides have been classified after the manner of the classification for the symmetrical glycerides. Important characterizing features of the unsymmetrical triglycerides are their poor crystalline appearance and their slowness of transformation, especially from the β' to the stable β-phase. It is even difficult sometimes to obtain the beta form by crystallizing from solvent (6). Impurity as an explanation for this crystallization behavior can apparently be ruled out, but there is the possibility that these glycerides when synthetic are a racemic mixture of optical isomers.

It will be noted from table 6 that, unlike the case for symmetrical glycerides, no marked difference in side spacings exists between groups a and b. The side spacings of the stable beta forms are very similar to those of the simple triglycerides.

Carter and Malkin confirmed the observation of King and coworkers (2, 3, 32, 41) that the melting points of the unsymmetrical isomer were, in general, a few degrees below those of the symmetrical isomer.

The x-ray and melting point data for the unsymmetrical compounds appear in table 6. As in the case of the symmetrical glycerides where the chain lengths of the acids differ by more than two carbon atoms, the long spacings frequently correspond to four instead of two chain lengths, especially for the most stable beta forms.

New means of identification of glycerides would be of real aid in the study of fat composition. Melting points have been widely used but have usually been subject to some uncertainty, owing to the possible polymorphism. An interesting use of the multiple melting points obtained by Malkin *et al.* is reported in a paper by Hilditch and Maddison (19). A crystallization fraction of cottonseed oil was completely hydrogenated. Other evidence showed it to be a monopalmitodistearin. Careful recrystallizations yielded a substantial portion of a product with the following melting points:

GLYCERIDE	MELTING POINTS, °C.			
	γ	α	β'	β
Palmitodistearin from cottonseed oil	50	56	64	67.7
α-Palmitodistearin	50	57	61	65
β-Palmitodistearin	50	56	64	68

It could "thus be established that in the monopalmitoglycerides of cottonseed oil, the palmitic group is exclusively attached to the beta or central hydroxyl group of the glyceryl radical."

To use the x-ray spacings as a basis for identification would involve very careful discrimination at the present stage. Consider the four compounds given below:

GLYCERIDE	α LONG SPACINGS	β CHARACTERISTIC SHORT SPACINGS	β LONG SPACINGS	β' LONG SPACINGS
Symmetrical:				
β-Palmitodistearin	50.5	4.6, 5.3	44.2	47.5
β-Stearodipalmitin	50.2		43.2	44.7
Unsymmetrical:				
α-Palmitodistearin	48.8	4.6, 5.3	46.5	44.7
α-Stearodipalmitin	47.8	4.6, 5.3	42.5	43.9

β-Stearodipalmitin could be distinguished from the others by the *absence* of the two very characteristic short spacings of the most stable or beta form. α-Palmitodistearin could be distinguished from the others by the greater length of its long spacing for the beta form. The presence of the beta form would have to be definitely assured.

A distinction between β-palmitodistearin and α-stearodipalmitin could be made on the basis of the shorter long spacing of the alpha form of the latter glyceride.

To identify a *single* glyceride by x-ray alone without known materials for comparison would be difficult even if the number of possible glycerides could be limited to a few. However, the first steps have been taken to make such identification a real possibility, and, combined with multiple melting points, the x-ray method should serve in many instances to give far more certain results than have hitherto been possible.

VI. Multicomponent Systems of Triglycerides

When it is considered that each individual triglyceride may exist in three or four crystalline forms depending on the treatment of the sample, it is not surprising that so little is precisely established with regard to the phases present in known mixtures of triglycerides or, especially, in natural fats and oils. The fats of commerce are multiple-component systems indeed. The simplest of these chemically are completely hydrogenated products from such materials as soybean oil. These approach tristearin in composition, but by no means closely.

Added to the major complication of polymorphism are the other factors of possible formation of solid solutions (Reimer and Will (40)), uncertainty as to the composition of natural fats or of mixtures of supposedly pure compounds, and a general sluggishness of triglycerides in their approach to equilibrium.

Investigations previous to the work of Kremann (24) appear to have been of little significance. Indeed, the work of Kremann on the ternary system tristearin–tripalmitin–triolein and on the constituent binary systems, though extensive, cannot now be regarded as of real value. Coming before the elucidation of the polymorphic relationships involved, his results suffer from uncertainty as to the nature of the solid phases obtained. This uncertainty is increased by apparent impurity of materials.

In the opinion of Efremov (12), "all mixtures of tristearin and tripalmitin, independent of composition, repeat entirely the properties of the components", in the sense that all compositions manifested three polymorphic forms. He gave clear evidence that the gamma form, at least, exists through the whole range of possible compositions. His evidence for other forms, by cooling curves and melting points, is less clear. Despite some apparently sound conclusions as to the existence of three polymorphic forms, it is certain from the numerical values of the melting points given that Efremov's materials were not of the purest, at least in the case of tripalmitin.

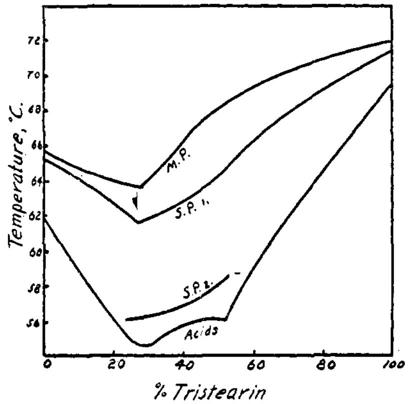

FIG. 6. Mixtures of tripalmitin and tristearin

Probably the best data for the binary system tristearin–tripalmitin are those of Joglekar and Watson (22). These concern, for the most part, the beta form.

In figure 6 their melting-point and main-solidification-point curves indicate pretty clearly that the system exhibits a eutectic in the alpha (Malkin's beta) form. Some degree of solid solution formation might be presumed by analogy with other long-chain compounds. Whether a single solid solution phase exists over the whole composition range cannot be decided.

Joglekar and Watson (22) found for a limited range of composition that two solidification points could be obtained, according to whether the cooling was slow or rapid. They concluded that the lower solidification points referred to the unstable beta (Malkin's gamma) modification. However, it would seem from the magnitude of the numerical values that these second solidification points could more readily refer to the alpha form of Malkin, which otherwise missed the attention of Joglekar and Watson.

That the cooling curve behavior of some of the hydrogenated glycerides of commerce show the same features of polymorphism as tristearin was shown by Nicolet (37).

In a calorimetric study of fat consistency, Straub and Malotaux (46) encountered the phenomenon of polymorphism in connection with a "palmitodistearin" and with coconut oil.

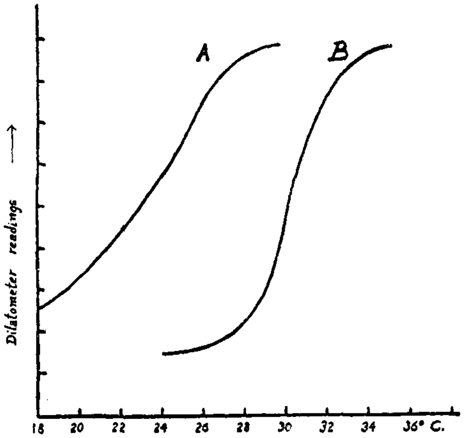

FIG. 7. Effect of the previous treatment of a fat on the course of melting. Curve A, dilatometer curve for cacao butter after chilling and warming immediately; curve B, dilatometer curve for cacao butter after chilling and holding at 24°C. for 15 hr. before warming.

Some very interesting manifestations of polymorphism are to be found in the dilatometric studies of Van Roon (48) and Hofgaard (20).

In figure 7 Van Roon showed how profoundly the previous treatment of a commercial fat, through its effect on polymorphic behavior, can affect the course of melting. The dilatometer curves for cacao butter (A) after chilling and warming immediately and (B) after chilling and holding at 24°C. for 15 hr. before warming (rate of warming, 4°C. every hour) are shown below. Curve A is a temperature–volume curve of cacao butter consisting largely of unstable low-melting modifications. Curve B shows the behavior of the same fat after transformation of much of the unstable to more stable forms.

Similar curves for many fats have been obtained by Hofgaard (20). He has pointed out that the state and physical behavior of a fat may depend in large measure on the ability of the triglyceride to crystallize in various modifications. By means of dilatometric technique the divergence of fats from the state of equilibrium at some given temperature can be observed, and thus the conversion of one phase to another may be followed. (If the fats are not 100 per cent solid, the density changes due to polymorphism may be confused with density changes resulting simply from readjustment of solid–liquid proportions.)

Hofgaard noted that the phenomenon of polymorphism is clearly traceable in beef tallow, cacao butter, shea butter, palm oil, and Illipe fat, but coconut and palm kernel oils show little or no signs of the appearance of unstable forms (perhaps an indication of the greater rate of transformation in fats of low molecular weight). With increased degree of hardening (hydrogenation), the tendency to solidify in unstable forms is more pronounced. (This may indicate merely that, as the melting point rises, so also does the temperature where the transformation rate becomes rapid increase.)

The clarification of the crystallization behavior of fats on a theoretical and practical basis is not yet far advanced, but it is definitely beyond the confused stage of less than a decade ago.

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