A patch of vivid yellow water, seen south of Useppa Island on January 28, consisted almost exclusively of *Gymnodinium* sp. with a mixture of numerous larval invertebrates. The water was viscid and slimy, having the consistency of diluted syrup. A fish was seen dying in this water.

Chemical determinations disclosed no unusual salinities. Water temperatures ranged from 22.5° C. to 26° C. Samples showed a pH close to 8.2. The dissolved oxygen content was not low in brownish-red water near Fort Myers Beach except in an inshore area where large numbers of dead fish littered the water. In the yellow patch of water described above the oxygen content was low, being less than 33 per cent saturated. Hydrogen sulfide was reported earlier at Naples, and the hulls of several fishing vessels were seen with the white topside paint definitely blackened. During the period of investigation no H_2S could be detected.

An odorless but acrid gas causing stinging of the nostrils and hard coughing made life miserable for the residents of Captiva Island when a northwest wind caused a heavy surf on the Gulf Beach from January 22 to 26. This gas, which could not be identified, was not present during calm weather on January 28, but could be detected by boiling samples of Gulf and bay water. It was particularly strong in a sample of the yellow water. Lund (*1*) has previously called attention to presence of this gas during a similar heavy mortality of fishes on the Texas Coast.

A report giving full details of observations on this phenomenon will be published later.

Reference

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The Glyceride Structure of Natural Fats

K. F. Mattil and F. A. Norris

Swift & Company, Chicago, Illinois

Naturally occurring fats and oils consist predominantly of esters of glycerol and fatty acids, each hydroxyl group of the glycerol being esterified with a mole of fatty acid. A number of different fatty acids (usually at least four) make up any given fat. Because of the number of fatty acids and the fact that there are three positions on each glycerol molecule to which the fatty acids may be attached, a large number of isomeric glycerides is possible in a single natural fat. Thus, if n represents the number of component acids, the theoretical number of isomers possible from a maximum distribution of the acids over the three positions of glycerol is n^3. The actual number of glycerides chemically distinguishable from one another is $\frac{1}{2}(n^3 + n^2)$ (*4*).

In general, the glycerides of natural fats are highly mixed, simple triglycerides being the exception rather than the rule and occurring for the most part only in the relatively few fats in which a single fatty acid predominates. Since natural fats consist largely of isomeric glycerides, many of which differ from each other only slightly with respect to physical and chemical properties, the analysis of fats for their component glycerides has been an extremely difficult problem. For many years various investigators, especially Prof. Hilditch and his co-workers, have attempted to enlarge upon our rather meager knowledge of the glyceride structure of natural fats. The chief result of the investigations at the University of Liverpool has been the pronouncement of the so-called "rule of even distribution" (*3*). According to this hypothesis, the individual fatty acids of a fat tend to be apportioned evenly among the different glyceride molecules. In seed fats, for example, it is pointed out that trisaturated glycerides do not usually appear until the proportion of fatty acids reaches approximately 60 per cent. Numerous exceptions to this rule have been discussed by Hilditch, and a rough classification of fats has been made on the basis of their conformity to this rule.

An analysis of the "rule of even distribution," however, reveals the fact that truly "even" distribution is not proposed. Rather, it is maintained that, at fatty acid molar concentrations of approximately 60 per cent, homogeneous triglycerides of that fatty acid first appear—something less than "even" distribution. Frequently an association ratio (molar ratio of saturated to unsaturated fatty acids) of 1.4:1.0 is used, which is equivalent to a 58 per cent molar concentration of saturated fatty acids. On the basis of a truly "even" distribution, however, no homogeneous triglyceride should be formed until the molar concentration of the fatty acid in question is 66.7 per cent—that is, until more than two-thirds of the fatty acid molecules are the same. This is illustrated in Fig. 1, in which 10 glyceride molecules are shown graphically. It is apparent that 20 saturated fatty acid radicals could be attached to the glycerol molecules without any glyceride containing more than two saturated fatty acids. The 21st saturated fatty acid, however, must unite with a glyceride already containing two molecules of the same fatty acid, consequently producing a molecule of trisaturated glyceride. An extension of this reasoning to a greater number of glycerol molecules leads to the obvious conclusion that only when the molar concentration of the saturated fatty acids exceeds two-thirds would saturated triglycerides be formed under a truly "even" distribution. Thus, the molar concentration of trisaturated glycerides, $[S_3]$, can be related to the molar concentration of saturated fatty acids, [S], as follows:

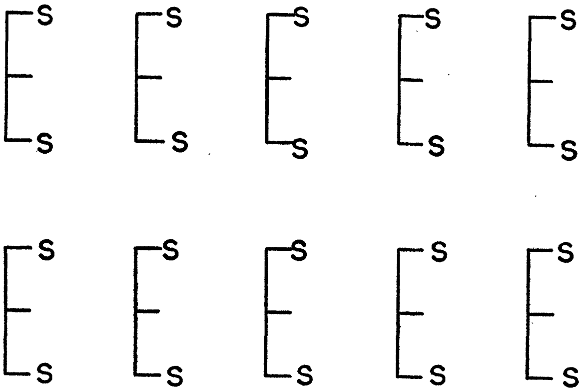

Fig. 1

$$[S_3] = 3\ ([S] - 66.7).$$

In general terms, if S_3 represents the triglycerides of any fatty acid, S, then the above equation defines "even" distribution as the term is employed in this paper.

Prof. Hilditch (*3*) has noted that fruit coat fats do not adhere well to the "even distribution hypothesis" and that land animal fats in general constitute notable exceptions. He has further pointed out that the glyceride structure of natural animal fats corresponds to that of synthetic mixed glycerides, in which the relationship of trisaturated glycerides, $[S_3]$, to mole per cent saturated acids, $[S]$, is expressed by the equation

$$[S_3] = k[S]^3,$$

where S_3 and S have the same significance as in the previous equation and k is a proportionality constant. Thus, the mole per cent of trisaturated glycerides is proportional to the cube of the mole per cent of saturated fatty acids. It should be noted that this equation is a specific example of the general equation $Y = k\ (X)^3$, which expresses the probability of any given arrangement of three independent variables. This, of course, is quite different from "even" distribution and, as will be shown later, really describes so-called "random" distribution.

In the chemical synthesis of glycerides from mixed fatty acids and glycerol, the amounts of the various glycerides formed would be expected to depend upon random collision and reaction, which is amenable to mathematical treatment on the basis of the laws of probability. Thus, the probability of oleic, palmitic, and stearic acids (equal reactivity assumed for each) uniting with glycerol depends upon their respective concentration; that is, the probability of oleo-palmito-stearin being formed is expressed by the equation:

$$[(OPS)] = k\ [O]\ [P]\ [S].$$

Increasing the concentration of any one of these acids increases the probability of that triglyceride being formed. It follows that if all the saturated acids were lumped together as S, the equation for the per cent of trisaturated glycerides would be:

$$[(SSS)] = k\ [S]\ [S]\ [S] = k\ [S]^3.$$

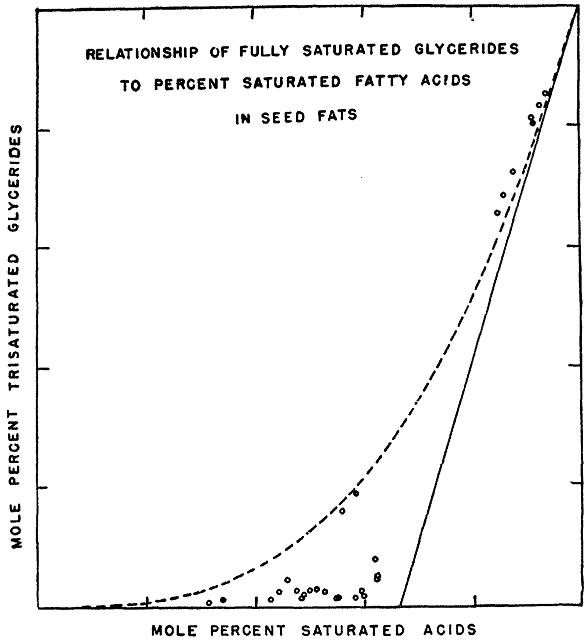

FIG. 2

Experimentally, the only component of a glyceride mixture which can be determined with relative ease and accuracy is the trisaturated glyceride fraction. In the past it has been tacitly assumed that the total glyceride structure may be deduced if the relationship between saturated fatty acids and trisaturated glycerides is known. As a result, this relationship has been rather extensively investigated (*3*). In Figs. 2, 3, and 4 representative data from the literature are compared with curves representing the above equations for "even" and "random" distribution.

From Fig. 2 it is apparent that the experimental data for seed fats do not coincide well with either the straight line representing "even" distribution or with the curve representing "random" distribution. However, it must be noted that, according to Hilditch's hypothesis, the intercept of the straight line with the axis would be at approximately 58 mole per cent saturated acid rather than at 66.7 per cent, which represents truly "even" distribution. This transposition of the curve to the 58 per cent intercept would, of course, result in a better agreement of some of the points with the so-called "even distribution hypothesis." It should also be noted that the points in the upper part of the curve fit either "even" or "random" distribution about equally well. To illustrate this point, in Table 1 we have compared the amount of trisaturated glycerides anticipated on the bases of "even" and of "random" distribution in fats containing large amounts of saturated fatty acids. It is evident that, at high saturated fatty acid concentrations, calculations based upon the two theories give so nearly identical results that data obtained for fats in this range (85 per cent or more saturated acids) cannot be interpreted as favoring either hypothesis.

TABLE 1

MOLAR CONCENTRATIONS OF TRISATURATED GLYCERIDES CALCULATED FOR FATS CONTAINING HIGH MOLAR CONCENTRATIONS OF SATURATED ACIDS

Molar concentration of saturated acids	Molar concentration of trisaturated glyceride	
	Calculated for "even" distribution	Calculated for "random" distribution
94	81.9	83.0
93	78.9	80.3
92	75.9	77.8
91	72.9	75.3
90	69.9	72.9
85	54.9	61.3

In Fig. 3 the molar concentration of saturated acids versus that of trisaturated glycerides is plotted for fruit coat fats. In this case it is seen that the data definitely do not agree with those expected on the basis of either hypothesis.

Similar data for animal fats, as shown in Fig. 4, reveal that the molar concentrations of trisaturated glycerides in animal fats closely approximate the values anticipated from the equation $[Y] = k\ [X]^3$. From this observation it may be generalized that the over-all glyceride structures of animal fats probably approximate those which would be anticipated from a "random" distribution of the fatty acids over the available glycerol molecules. The same generalization has been proposed by Dean, who used the terms "indiscriminate" and "haphazard" to define the distribution of fatty acids in animal fats and synthetic glycerides (*2*). The term "random distribution" was later used by Longenecker (*5*), who elaborated the general equation $[Y] = k [X]^3$ to include specific glycerides of all possible configurations. Similar equations have recently been published by Bailey (*1*).

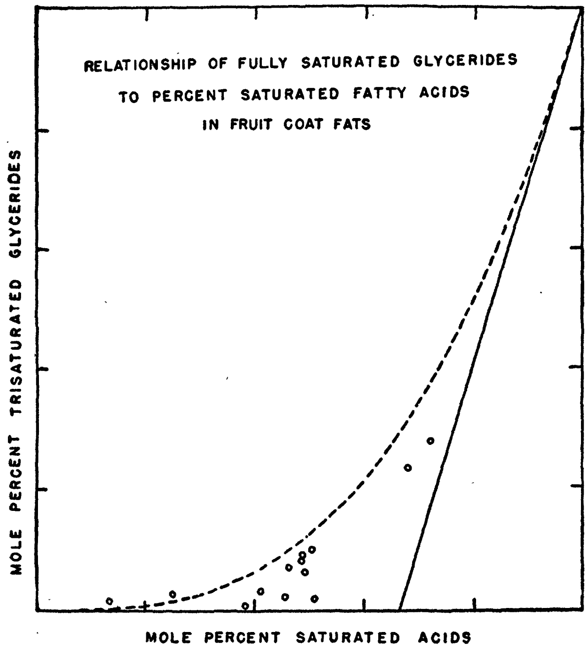

FIG. 3

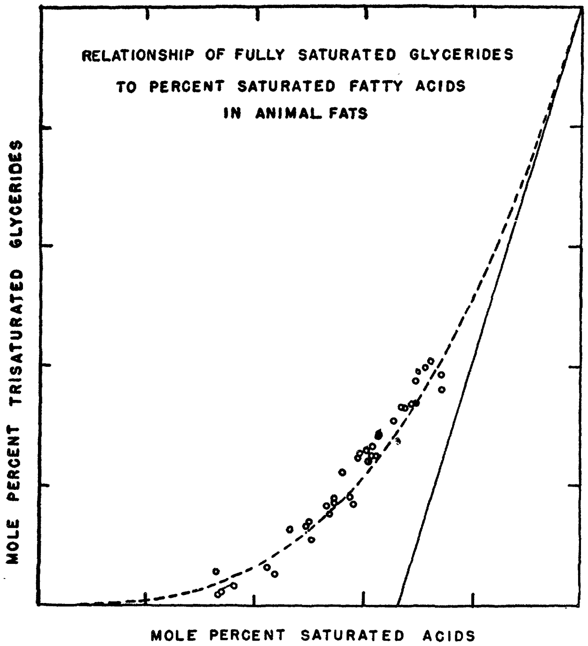

FIG. 4

In summary, an analysis of representative data showing the relationship of the molar concentrations of trisaturated glycerides to those of saturated acids in natural fats reveals that in the case of animal fats the glycerides are formed by a "random" or nearly "random" distribution of fatty acid radicals among the glycerol molecules. It has been shown that the "even distribution hypothesis" does not represent truly "even" distribution, but rather is an approach to the latter, being more or less arbitrarily fitted to experimental data. No general class of fats adheres to truly "even" distribution. Seed fats appear to approach the trisaturated glyceride contents anticipated by the "even distribution hypothesis," but even here the data are inconclusive.

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Effect of Vitamin B Complex on Inactivation of Estrone *in Vivo* and *in Vitro*

BERNHARD ZONDEK and MICHAEL FINKELSTEIN[1]

Hormone Research Laboratory,
Hebrew University, Jerusalem

In 1934 Zondek (*6*) found that estrone is inactivated by animals and plants. In experiments carried out *in vivo,* rats were injected with estrone and killed a few hours later. The bodies were finely minced, extracted, and the extract assayed as to estrogenic activity. Only about 2–4 per cent of the estrogenic activity of the injected estrone solution was recovered. Subsequent hydrolysis improved the recovery to 6–20 per cent. The major part of the inactivation was therefore not due to esterification. In numerous experiments inactivation of estrone by rats *in vivo* was consistently observed. Over a period of many years we have not chanced upon a single case in which such inactivation failed to occur.

In experiments carried out *in vitro,* pulps of various organs were incubated for some hours with estrone at 37°C., the mixture then being tested for estrogen activity. It was found that liver brei inactivates estrone to an extent of about 90 per cent, while of other organs tested only spleen inactivates estrone to a small degree. Hydrolysis of the inactive mixture does not liberate any estrogenic activity. It should be mentioned that not all livers tested inactivate estrone: for some unexplained reason, liver brei of normal rats occasionally fails to mediate this reaction.

Since heating inactivates liver pulp estrone, it may be concluded that the inactivation is enzymatic. This enzyme, which has been designated by one of us (B. Z.) as estrinase, has been obtained in cell-free extracts of liver and of various plants (*6, 7*). The plant enzyme is closely associated with tyrosinase and inactivates estrone presumably by oxidation (*5*).

Recently Biskind and Biskind (*1*) have pointed to the existence of a relationship between the amount of vitamin B com-

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