Letter to the Editor

A. R. S. KARTHA, in several recently published communications, has attempted to show a) that the "even distribution rule" suggested by Hilditch as the governing principle in the structure of natural glycerides is erroneous (1, 2); b) that the chemical determination of trisaturated glycerides as practiced by Hilditch and others is inaccurate (1, 3), and c) that the preliminary crystallization procedure more recently used by Hilditch and co-workers, and by others, fails to give correct estimates of the different types of mixed glycerides present in a natural fat (1, 2).

Some of my former colleagues and myself (4) showed a) that Kartha's failure to effect adequate separation by crystallization of a very simple mixture of glycerides was caused by lack of appreciation of the technique to be followed in applying the crystallization procedure, b) that the observed contents of trisaturated glycerides in 14 natural fats were substantially the same when determined either by the chemical (oxidation) or the physical (crystallization) procedures, and c) that the contents of simple triglycerides (trisaturated, triolein, trilinolein, trilinlenin, tri-elaeostearin, triricinolein) as determined by the crystallization method in 25 vegetable fats of widely different fatty acid composition formed an entirely regular sequence. The relationship of simple triacyl glycerides to the proportion of the acid under consideration is the same whether the acid be saturated or one of the unsaturated acids mentioned.

It is desired in this note to refer to some further considerations raised by Kartha's recent communications to this Journal (2, 3):

1. The addition of acetic acid to the acetone solution of a fat during oxidation with permanganate (which was recommended by Boekenoogen *et al.* (5) prior to Dr. Kartha's publications) is undoubtedly useful in restricting secondary oxidation as well as, possibly, slight partial hydrolysis of glycerides during oxidation. But the chief source of hydrolysis of the azelao-glycerides produced in the oxidation lies in the emulsified mixture of neutral fat and acidic products of oxidation which is readily and invariably produced during removal of the latter from the neutral (trisaturated) glycerides by mild alkaline solutions (6); and it is evident that the presence of acetic acid during the primary oxidation in acetone solution cannot help at the later stage of removal of acidic scission-products in an alkaline medium.

2. Kartha (2) has devised an empirical computation which gives values for trisaturated, disaturated-mono-unsaturated, monosaturated-diunsaturated, and tri-unsaturated glycerides in good agreement with those determined by his modified oxidation method in the case of 27 natural fats.

Application of his formula to 46 natural fats which had been studied by the crystallization procedure showed "poor correlation" between the calculated and observed values: "throughout almost the entire series values for GS_2U and GU_3 are higher" when calculated by the formula "than those calculated from crystallization data, or according to the rule of even distribution." When a discrepancy between calculated and observed values exists, two explanations are possible: either all the observed experimental values are wrong, or there is something wrong with the mode of computation. Dr. Kartha has no hesitation in jumping to the conclusion that all the experimentally observed data must be erroneous.

3. Attention is invited to a feature of Kartha's experimental figures (*2*, Table I) which seems to merit notice although, curiously, he does not discuss it. If the proportions of mono-unsaturated-disaturated glycerides ("GS_2U") and of di-unsaturated-mono-saturated glycerides ("GSU_2") are plotted against the proportions of saturated acids in the total fatty acids of 23 natural fats studied by Kartha (ranging from 8 to 61% of saturated in the total acids), the "GS_2U" glycerides from a smooth curve rising to 80% or so of mono-unsaturated-disaturated glycerides at about 60% saturated acids in the total acids. On the other hand, the "GSU_2" glycerides reach a maximum of only 45-50% at about 22-33% of saturated acids and thereafter fall away again.

In other words, disaturated glycerides may form over 80% of a fat whereas diunsaturated glycerides never exceed 45-50%; conversely, monounsaturated glycerides may form over 80%, but monounsaturated glycerides never more than 45-50% of a natural fat. This means that, according to Kartha's data, *saturated* and *unsaturated* acids behave *entirely differently* as regards their distribution in mixed glycerides.

4. Such differential behavior between different fatty acid groups has not been observed before—indeed all other results have failed to indicate any selective difference between one acid and another as regards glyceride structure. Many other fats (in addition to the 46 quoted by Kartha) have now been studied, by my associates and by other workers, by the crystallization procedure, and it has become possible to plot graphs of the content of glycerides containing 1, 2, or 3 groups of a variety of acids (saturated, oleic, linoleic, linolenic, and others). A full analysis of these results is precluded in the present brief note but will be published elsewhere in the near future. Whatever acid be considered, the experimentally found points for glycerides containing 1, 2, or 3 of its groups are distributed about curves which are of the same shape for each individual acid and can indeed be superimposed. The content of glycerides containing *one* group of a particular acid reaches a maximum when that acid forms exactly one-third of the total acids and similarly the content of glycerides containing *two* groups of the acid is a maximum when the acid forms exactly two-thirds of the total acids. At these maxima about 85-90% of the whole fat consists of glycerides with respectively *one* or *two* groups of the particular fatty acid; but glycerides containing *one* group of a particular acid persist in small and diminishing amounts until that acid forms about 75% of the total acids while those containing *two* groups of the acid commence to appear in small quantities when the acid forms about 20-25% of the total acids. Similarly, simple triglycerides containing 3 groups of the same acid commence to appear when the acid forms from about 60% upwards of the total acids.

This is a more complete description than was possible, prior to the advent of the crystallization technique, of what has been called the "rule of even distribution." This term has been applied consistently by myself and my co-workers as an expression of the major "tendency" in glyceride structure. It has