The Glyceride Structure of Swine Depot Fat[1, 2, 3]

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THE ORDER of the arrangement of fatty acids in animal glycerides has, for many years, been the subject of research, speculation, and controversy (1, 2, 3, 4). Investigations to elucidate the facts have been handicapped by the lack of suitable experimental techniques.

The most useful technique, until recently, has been that of fractional crystallization (5, 6), by which glycerides of differing species, based on their relative number of saturated or unsaturated fatty acids in the molecule, were isolated relatively pure, and weighed. The fat was said to be "randomly" or "evenly" distributed, depending upon whether the relative amounts of each glyceride type compared more closely to the amounts expected if the saturated and unsaturated fatty acids were distributed among them "randomly" or were partitioned so that each glyceride got its "even" share of each kind of acid. Later Kartha (7) determined the amount of trisaturated glycerides by a modified permanganate oxidation and calculated divergence from "random" or "even" distribution according to the divergence of only the trisaturated glyceride from the theoretical.

Results of the crystallization technique indicated that vegetable fats tended to follow an even distribution pattern while animal glycerides were mixed, though somewhat more "random" than "even" (1, 8, 9). The oxidation technique led Kartha to believe that all fats follow a "random" pattern unless the amount and nature of the saturated fatty acids would result in more solid trisaturated glycerides than could be tolerated by protoplasm under the environmental conditions (7).

More recently countercurrent distribution techniques (10, 11) on highly unsaturated vegetable oils confirmed the random hypothesis for these oils, but x-ray diffraction studies (12) resulted in the opposite conclusion.

It has been pointed out that the depot fat of animals may have both exogenous and endogenous origins (9).

Reiser and Dieckert (13) demonstrated a definite influence of exogenous fat on glyceride structure by feeding fat extracted from chickens or rats grown on a fat-free diet to other chickens or rats on an otherwise fat-free diet. They also found a class difference.

Recently it was found that pancreatic lipase attacks the glyceride ester linkages in the 1 and 3 positions preferentially and that the action can be made to stop at the monoglyceride stage under controlled conditions (14, 15, 16, 17). Using this technique, Mattson and Beck (14, 17) and Savary, Flanzy, and Desnuelle (18) have reported that the unsaturated fatty acids predominate in the 1 position in all glycerides studied except that of swine. In the last case the unsaturated acids predominate in the 1 and 3 positions. They will be discussed later in more detail.

Ruminant fat must be considered as a special case since Reiser and Reddy (19) have shown that rumen microorganisms hydrogenate unsaturated fatty acids, thus resulting in an inordinately high level of stearic acid.

The pig was chosen as the experimental animal for the present study. As far back as 1940 Hilditch and Pedelty (20) examined the back fat of a pig reared on low-fat diet for its glyceride structure. In view of the rapid strides made in such experimental techniques as low-temperature crystallization, disruptive oxidation, and the new enzymatic hydrolysis, it was thought that the examination of the glyceride structure and, more specifically, the positional linkage of the fatty acids in the various glyceride species of a depot fat of a pig reared on low-fat diet would throw new light on the mechanism of the biosynthesis of natural glycerides and the structure of animal fat.

Experimental

A male pig was reared for three months on a diet of solvent-extracted meat and bone scrap, soybean oil meal, and Brewer's rice supplemented with vitamins and salt. The total ether-soluble extract was 1%. It was considered that the small amount of this dietary fat would not measurably influence the depot fat structure but was necessary to prevent essential fatty acid deficiency.

Back fat was ground and heated on the water bath with mechanical stirring until the fat was melted. The filtered, dried, rendered fat was preserved under nitrogen at −22°C.

The fatty acid compositions of depot fat, monoglycerides, and free fatty acids were all determined spectrophotometrically (21). Glyceride species were determined by low temperature crystallization (5, 6) and disruptive oxidation (7) techniques.

Both the unfractionated fat and the isolated glyceride species were subjected to enzymatic hydrolysis according to the procedure of Mattson and Beck (14), by which the fatty acids in the 1 and 3 positions only are removed, although to a limited degree. The resultant fatty acids and the monoglycerides were separated by the use of ion exchange Amberlite IRA 400 (22) and countercurrent distribution (23). The fatty acid composition of each was determined spectrophotometrically.

Observations and Discussion

The fatty acid composition of the ration fat, determined spectrophotometrically (21), was: saturated acids 38.1%, monoethenoid 40.5%, diethenoid 20.8%, and triethenoid 0.6%. This constituted less than 1% of the ration. The fatty acid composition of the depot fat is shown in Table II.

Glyceride structure of the back fat was determined by low-temperature crystallization (5, 6) and disruptive oxidation (7) techniques. The results obtained by these two experimental techniques and those calculated from the fatty acid composition on the basis of "random" and "restricted random" distribution

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hypotheses are shown in Table I. It is readily seen that the values obtained by oxidation and crystallization agree very well. These values however differ significantly from the calculated values for both "random" type distributions and would differ even more from the "even" type distribution. The results closely resemble those of Luddy *et al.* (6) from a lard of unknown dietary background.

The fatty acid composition of the predominantly GS_2U and GSU_2 fractions obtained by low-temperature fractionation and of the monoglycerides and free fatty acids obtained from them by pancreatic lipolysis are shown in Table II. Even in the unfractionated depot fat the iodine value of the monoglycerides is much lower than that of the free fatty acids. This difference is much more pronounced in the GSU_2 fraction and is explained by the fatty acid composition of the fractions.

TABLE I

Glyceride Types of Swine Depot Fat

	Experimental		Calculated[a]	
	Oxidation	Crystallization	Random	Restricted random
	%	%	%	%
GS_3	2.2	2.2	7.2	2.2
GS_2U	31.0	30.3	30.3	38.2
GSU_2	55.5	57.3	42.5	41.7
GU_3[b]	11.3	10.2	19.9	17.9

[a] Calculated from the fatty acid composition of the depot fat.
[b] Determined by difference.

There are two possible GS_2U glycerides: GSSU and GSUS. Assuming the "U" to be oleic, the theoretical iodine values of the mixed fatty acids obtained from the 1 and 3 positions by pancreatic lipolysis would be 45 and 0, respectively. The iodine values of the resultant 2-monoglycerides would be 0 and 71, respectively. The experimental iodine values were 43 for the fatty acids and 18 for the monoglycerides. These results more nearly fit the GSSU structure.

Similarly there are two possible GSU_2 glycerides: GSUU and GUSU. Again assuming "U" to be oleic acid, the theoretical iodine value of the 1 and 3 position fatty acids would be 45 and of the 2-monoglyceride 71 for GSUU. In GUSU the respective theoretical values would be 90 and 0. The experimental values were 81 for the free acids and 30 for the monoglycerides. These values are a much closer fit to the GUSU structure than the GSUU. It must be concluded therefore that endogenous swine fat resembles mixed exogenous and endogenous swine fat in that the unsaturated acids are predominantly in the 1 and 3 positions.

TABLE II

Fatty Acid Composition of Swine Back Fat, or Its Constituent Disaturated and Diunsaturated Glycerides, and of the Monoglycerides and Free Fatty Acids Released from Them by Pancreatic Lipase

	Iodine[a] value	Satd.	Monoeth.[b]	Dieth.	Trieth.
		%	%	%	%
Depot fat	57.1	40.1	55.8	3.99	0.09
Monoglycerides	27.6	69.4	30.6	.....	.....
Free fatty acids	65.6	27.2	72.8	.....	.....
GS_2U fraction	28.9	67.5	32.5	.....	.....
Monoglycerides	18.3	79.6	20.4	.....	.....
Free fatty acids	42.5	52.7	47.3	.....	.....
GSU_2	65.1	31.3	64.5	3.99	0.23
Monoglycerides	30.1	67.8	30.6	1.56	.....
Free fatty acids	81.1	9.9	90.1	.....	.....

[a] Iodine value of fatty acids.
[b] Calculated as oleic.

It may also be seen from Table II that the depot fat contained about 4% linoleic acid, that the GS_2U fraction contained none, and that the GSU_2 fraction, 55% of the fat, contained 3.9%. It must be concluded therefore that the fatty acids of the SU_3 fraction, which was only 11% of the total fat, contained almost 20% linoleic acid. From Table II it is apparent that no linoleic was found in the fatty acids freed by lipolysis, even from the unfractionated fat. Therefore essentially all linoleic acid must have been in the 2 position.

Mattson and Beck (17) however reported 13% dienoic acid in lard of unknown dietary background. They found 17% of this in the fatty acids freed by pancreatic hydrolysis and only 6% in the resultant monoglycerides.

The presence of all the linoleic in the 2 position, and of all the oleic in the 1 and 3 positions points up the fallacy of discussing saturated and unsaturated acids collectively. Each fatty acid must have its own directed esterification. This factor might well explain much of the difficulty in drawing conclusions about glyceride structure from data that use only the "saturated" and "unsaturated" designations.

The glyceride composition of the back fat of this animal, reared on an essentially fat-free diet, was GS_3 2.2%, GS_2U 31.0%, GSU_2 55.5%, and SU_3 11.3% (Table I). The fatty acid composition was 40.5% saturated, 55.4% oleic, 4.0% linoleic, and 0.1% linolenic (Table II). The linoleic acid appears to be all in the 2 position and the oleic predominantly in the 1 and 3 positions.

One may consider these data as indicating that the glyceride structure tends toward an ideal in which there is an oleic acid in the 1 position on each glyceride molecule. The residual oleic acids are then placed in the 3 position and the linoleic acid in the 2 position of the dioleins. The saturated acids then complete the glycerides. With this scheme the present fat would contain 12% GOLO, 56% GOSO, 32% GOSS, and no GS_3, in which O represents oleic, L linoleic, and S saturated acids. The values found: 11.3, 55.5, 31.0, and 2.2%, respectively, are remarkably close to these values.

The values of 20% and 30% oleic acid found in the monoglycerides of the GS_2U and GSU_2 fractions, respectively, after lipolytic action (Table II) may result from the sum of errors in the lipolytic technique, the separation of the glyceride types, and the separation of monoglycerides from di- and triglycerides.

Hanahan has shown in beef, rabbit, rat, guinea pig and dog liver, and egg lecithin that the unsaturated acids are in the 1 position only and the saturated in the 2 position only (24). In a series of studies on the biosynthesis of triglycerides and phospholipides it has been shown that both follow the same routes from glycerol to phosphatidic acid (25, 26, 27). It should be expected therefore that any triglycerides thus formed should have unsaturated acids in the 1 and saturated in the 2 position. Whatever acids are available to complete the synthesis of triglycerides would occupy the 3 position. This concept can only account for glycerides of the types GUSU and GUSS, which, if they represent all of the GSU_2, and GS_2U, together make up about 86% of fat in the present

study. The 2 to 1 ratio of the two may be dictated by the relatively greater amounts of unsaturated acid present as well as by a possibly higher degree of specificity of the acylating enzyme for the unsaturated acid and 3 position. The presence of 10% GU_3 and 2% GS_3 is evidence that the specificity of the acylating enzyme is not perfect or results, as is strongly suggested by the present data, from the linoleic acid being oriented to the 2 position.

Evidence contrary to the above hypothesis is the data by Savary, Flanzy, and Desnuelle (18) that other animal fat, such as beef, mutton, horse, and goose, as well as the vegetable fats, such as palm oil, sunflower oil, and cocoa butter, appear to have the GSUS structure. More needs to be known about the structure of lecithin and the relationships of lecithin and triglyceride biosynthesis.

In goose fat Savary *et al.* (18) found that the iodine values of the total acids, the acids released by pancreatic lipolysis, and those remaining esterified after such lipolysis, were 71, 68, and 72, respectively. These values show no distinction between the 1, 2, and 3 positions.

Ruminant tallow must be considered as a special case because of its much higher degree of saturation and stearic acid content than nonruminant. Rumen microorganisms appear to hydrogenate dietary unsaturated acids (19).

Not only may the mechanism of resynthesis of absorbed fatty acids into glycerides be different from *de novo* synthesis, but the nature and proportions of the fatty acids available for synthesis also will be different. Any evidence for glyceride structure therefore based on animal fats in which the influence of exogenous fatty acids is not excluded is difficult, if not impossible, to interpret. The GSUS structure of the exogenous fat of any animal is thus still an open question.

Summary and Conclusions

A male pig was reared for three months on a diet containing about 1% fat and 0.2% dienoic acid. The back fat was fractionated into glyceride types by crystallization methods, and the fatty acid composition of each was determined by the spectrophotometric procedure. The relative amounts of the glyceride types almost perfectly fit the hypothesis that there is an oleic acid in the 1 position of each glyceride molecule, that the residual oleic acids are in the 3 position, that the linoleic acid is in the 2 position of the resultant dioleins, and that the saturated acids complete the structure.

The GS_2U and GSU_2 fractions were hydrolyzed by pancreatic lipase. The iodine values of the resultant monoglycerides and free fatty acids indicated that the unsaturated acids are predominantly in the 1 and 3 positions and the saturated in the 2 position. From these data and from previous knowledge of the structure of liver lecithin and of the mechanism of lecithin and triglyceride synthesis, which are discussed, it is postulated that the structure of endogenous animal fat is a resultant of the specificity of the acylating enzyme and of the nature and relative proportions of available fatty acids. The possible influence on depot fat structure of exogenous fat, of the dynamic state of the glycerides, and of the homeostatic mechanism are discussed.

Linoleic acid, when fed at a low level, was found to be preferentially deposited and not utilized for energy purposes.

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Addendum

Since this paper was written, a number of important contributions to the problem of glyceride structure have appeared. Mattson and Lutton (1) reported a pancreatic lipolytic study of vegetable and animal glycerides. They confirmed the observation that pig fat differs from other natural glycerides in that the 1 and 3 positions are mainly occupied by unsaturated acids and the 2 position mainly by saturated. In a sample of presumably commercial lard, dienoic acid constituted 6.4% of the 2-monoglyceride fatty acids and 12.6% of the total fatty acids. On the other hand, in a sample of pig fat from an animal reared on 25% safflower seed oil, 47% of the 2-monoglyceride acids and 59% of the total acids were dienoic. This emphasizes the effect of relative amounts of fatty acids discussed in the present paper. From the distribution of the fatty acid in the 1, 2, and 3 positions Mattson and Lutton concluded that "random distribution does not occur in either vegetable or animal fat."

In an important communication to the editor R. J. Vander Wal (2) has resolved the apparent conflict between the proponents of the random distribution hypothesis and those who champion the hypothesis of specific orientation. He points out that it is quite possible for the proportions of glyceride types (GS_3, GS_2U, GSU_2, and GU_3) to conform to random distribution and at the same time for the unsaturated acids to be oriented to the 1, 2, or 3 positions within the mixed (GS_2U and GSU_2) glycerides. This important hypothesis has been experimentally confirmed by C. G. Youngs (3).

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