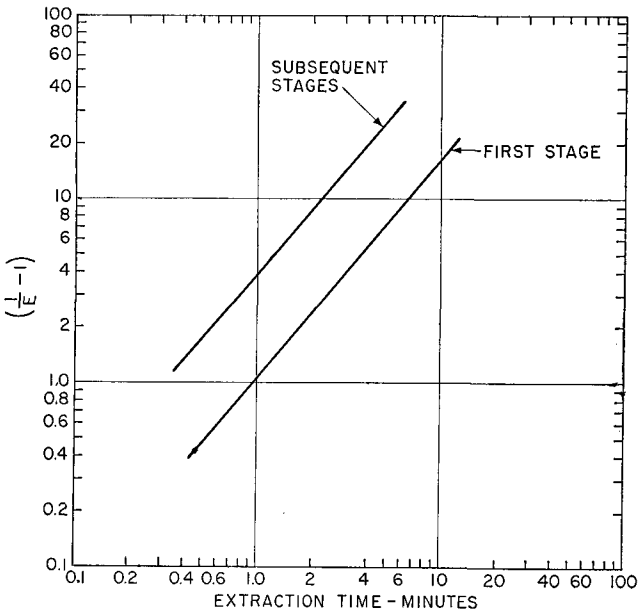

FIG. 5. Effect of design parameters upon solution hold-up of 15-mil. soybean flakes at 60°C.(140°F.).

Thus 4.0 equilibrium stages are required if a draining time of 4 min. is used between stages. For 2 min. of drainage approximately 4.5 stages would be required; for 1 min. of drainage approximately 5 stages; and for 0-min. drainage, approximately 10 stages.

Acknowledgment

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Summary

Hold-up of solution by the solids undergoing extraction has been shown to be an important factor in the design of percolation extraction systems. By means of a laboratory technique, hold-up was measured for a wide variety of prepared oilseeds. Empirical correlations of the data were obtained, and their significance was illustrated in a sample design calculation.

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The Metabolism of Triglycerides Containing cis and trans Octadecenoic Fatty Acids

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THE GEOMETRIC STRUCTURE of the unsaturated fatty acids in edible oils can be shifted from a *cis* to a *trans* configuration during hydrogenation (1). The amount of high-melting *trans* or "iso-oleic" acids formed is in direct proportion to the selectivity of the reaction, and, as recently pointed out (2), greater geometrical and positional isomerization than heretofore suspected occurs under conditions of high selectivity. It remains to be determined however whether the *trans* isomers are metabolized as efficiently as the *cis*. Kohl (3) reported in 1938 that it took over 30 days to clear the elaidic-acid reserves from the rat body after the acid had been fed for three days. Sinclair (4) made use of elaidic acid as a "label" to demonstrate the incorporation of fatty acids into the phospholipide fractions of the intestinal mucosa.

Both of these studies were made before the more accurate determination of *trans* acids by infrared analysis had been perfected. Phatak and Patwardhan (5) fed rats iso-oleic acids from hydrogenated peanut oil and reported that 94% was metabolized and 6% was found in carcass lipides. That elaidic acid is selectively deposited and that phospholipides contain less elaidic acid than neutral fat were reported by Collet and Favarger (6). Paul and McCay (7) have shown that guinea pigs do not utilize elaidic acid as efficiently as rats. Furthermore Aaes-Jorgensen and Dam (8) found a decrease in the growth rate of rats fed hydrogenated peanut oil. More recently Melnick and Deuel (9) demonstrated that iso-oleic acids are not antimetabolites for oleic acid in microorganisms.

In all studies reported to date the *trans* acids were mixtures obtained from hydrogenated-oil sources or were pure elaidic acid. In the present study known amounts of synthetic triglycerides containing unsaturated fatty acids with the *trans* bond in the 9 or 8 position were fed to rats, and the results were compared with those from hydrogenated oil. The total amount of fat excreted was determined, and various tissues were analyzed for the total amount of *trans* acids deposited.

Experimental

Weanling female rats were fed a synthetic basal diet composed of 31% casein, 63.8% cerelose, 5% Wesson salts (10), and 0.4% of a water-soluble vitamin mix of the following composition: choline 93.5%, thiamine 1.24%, riboflavin 1.24%, pyridoxine 1.24%, Ca pantothenate 2.48%, and folic acid 0.3%. In addition, each animal received approximately 100 mg. per day of linoleic acid (as trilinolein) and approximately twice the minimum daily requirements

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of the fat-soluble vitamins.[3] Thirty-four rats were kept on the basal diet for two weeks, and the feces were collected for analysis. Four of these rats were then sacrificed after a 24-hour fast to serve as a control group. The remaining 30 rats were divided into six equal groups and fed the basal diet plus 10% of the various triglycerides (Table I). The fat was added at the expense of cerelose. Olive oil served as the source of *cis* or natural fat. The glyceryl tri*(trans)*-8-octadecenoate was prepared from methyl *trans*-8-octadecenoate (11) and triacetin by the method described by Lundberg (12). The crude triglyceride was crystallized twice from acetone. The trielaidin, trilaurin, and triolein were prepared from the distilled

TABLE I
Characteristics of Fats in the Diet

Group No.	Fat in diet	*Trans*	I.V.
		%	
I	Olive oil	0	85.5
II	Glyceryl tri*(trans)*-8-octadecenoate	63.2	76.0
III	Trielaidin	88.5	74.0
IV	Margarine-base stock	33.6	73.5
V	Trilaurin	0	0
VI	Triolein	0	84.0

methyl esters and triacetin. The margarine-base stock for Group IV was from a commercial preparation of partially hydrogenated soybean oil. The trilinolein, which was given as the essential-fatty-acid source, was prepared by low-temperature crystallization of refined safflowerseed oil.

The rats were fed these diets for a total of 16 days, the daily feed consumption was noted, and feces samples were collected over the entire period. No significant differences in growth rate were observed among the groups during this short feeding period. After 24 hours of fasting the rats were anesthetized and sacrificed. The hearts, livers, and intestinal tracts were removed, weighed immediately, and saved for analysis. The lipides were recovered from the dried acidified feces by 24-hr. extraction in a Soxhlet apparatus with 25% acetone and 75% Skelly Solve F. The livers were dried, and the lipides were extracted in the same manner. The lipides from the intestinal tracts were obtained by grinding the entire tracts with an equal weight of anhydrous sodium sulfate, then subjecting the mixture to four extractions with acetone/Skelly Solve F. The heart lipides were obtained by grinding the hearts with sodium sulfate and extracting for 24 hrs. in a Soxhlet apparatus with the same solvent. The rest of the carcasses, including head, tail, and feet, were cut into small pieces and ground in a Waring Blendor.

[3] One drop per week of a fat-soluble vitamin mixture composed of 5.0 g. vitamin A concentrate (200,000 USP units per g., courtesy of Distillation Products Inc.), .0054 g. vitamin D_2, and 2.535 g. vitamin E concentrate in 100 ml. of olive oil.

The ground carcasses were extracted four times with acetone/Skelly Solve F. During the first two extractions the solvent was allowed to stand in contact with the tissue for 24 hrs. each, and during the third and fourth extractions for 8 hrs. each. All solvents were removed under vacuum after drying with anhydrous sodium sulfate, and all extractions were carried out quantitatively. In order to determine the amount of total fat which was extracted by this procedure the tissue residue was digested in 50% hydrochloric acid at 60°C., and the solution was extracted three times in a separatory funnel with Skelly Solve F. The solvent was removed from the combined extracts, and the amount of fat which had remained in the tissues was determined. The results showed that the extraction procedure had removed 96% of the total carcass lipides.

The *trans* determinations were made by infrared analysis, using the baseline method of Jackson and Callen (13). The fatty acid analysis of the carcass lipides was carried out spectrophotometrically.

Results and Discussion

The fatty acid composition of the carcass lipides is reported in Table II. These results are similar to those obtained by the many previous workers who have determined the fatty acid compositions of the carcass lipides of rats on various diets. Such results have been extensively discussed by Hilditch (14) and Deuel (15).

The presence of *trans* acids was noted in all the tissues and in the feces samples from the rats fed glyceryl tri*(trans)*-8-octadecenoate, trielaidin, and the margarine-base stock. No *trans* acids were found in the tissues or feces of the olive-oil, trilaurin, or triolein groups or in those of the rats sacrificed at the beginning of the experiment (Group VII, no fat). It appears then that there is no interconversion of *cis* to *trans* acids in the rat organism since no *trans* acid is found in the tissues unless it has been fed in the diet. As shown in Table III, the highest amount of *trans* deposited was in the carcass lipides. The glyceryl tri*(trans)*-8-octadecenoate group received the equivalent of 33 g. of the *trans* structure of the triglyceride, and 3.8 g., or 11.5% of the *trans* fed, were found in the carcass lipides. The trielaidin group received 55 5 g. of *trans*, and 6.0 g., or 10.8%, were found in the carcass lipides. The margarine-base stock group received 23.0 g., and 2.1 g., or 9.1%, were found in the carcass lipides. All these groups excreted between 0.7 and 0.9% of the *trans* acids fed. The intestinal-tract lipides contained the next highest amounts of *trans* acids: 0.15 g. (0.45%), 0.36 g. (0.65%), 0.11 g. (0.47%) in groups II, III, and IV, respectively. The heart and liver samples contained the least amount of *trans* by weight; the livers be-

TABLE II
Fatty Acid Composition of Carcass Lipides

Group No.	Dietary fat	Saturated	Monoene	Diene		Triene	Tetraene
				Nonconjugated	Conjugated		
		%	%	%	%	%	%
I	Olive oil	20.7	67.0	4.4	0.3	0	2.9
II	Trans 8	31.1	55.9	2.5	1.7	0	4.3
III	Trans 9	20.5	70.2	2.3	0.9	0	1.7
IV	Marg. stock	23.5	67.2	3.5	1.1	0	0.7
V	Trilaurin	31.4	58.0	2.6	0.4	0	2.9
VI	Triolein	19.4	71.9	2.3	0.3	0	1.6
VII	Fat-free	27.0	61.5	4.6	0.3	0	2.2

TABLE III

Total Dietary *trans* Acids Found in Tissue

Group No.	Feces	Heart	Liver	Intestinal	Carcass
	%	%	%	%	%
II Glyceryl tri(*trans*)-8-octadecenoate	0.9	0.009	0.03	0.45	11.5
III Trielaidin	0.7	0.012	0.13	0.65	10.8
IV Margarine-base stock	0.8	....	....	0.47	9.1

tween 0.01 and 0.06 g. (0.03 to 0.13%) and the hearts between 0.003 g. and 0.007 g. (0.009 to 0.012%) of *trans* per group. The fate of the *trans* acids is summarized in Table IV, from which it can be seen that the rats being fed *trans* acids were capable of metabolizing them quite efficiently. The position of the *trans* double bond, that is, whether it was in the "natural" 9 position or in the 8 position, appears not to affect the efficiency of metabolism. The effect of the level of dietary fat on the deposition and metabolism of *trans* acids was considered in further studies and will be published elsewhere (16).

TABLE IV

Fate of *trans* Acids

Group	Percentage deposited in tissues	Percentage excreted	Percentage metabolized
II Glyceryl tri(*trans*)-8-octadecenoate	11.9	0.9	87.2
III Trielaidin	11.6	0.7	87.7
IV Margarine-base stock	9.7	0.8	89.5

Summary

Weanling rats were fed diets containing triglycerides composed of both *cis* and *trans* fatty acids for 16 days. The animals were sacrificed, and the lipides were extracted quantitatively from the heart, liver, feces, and the rest of the carcass. Infrared analyses were carried out to determine the fate of the *trans* acids. *Trans* acids with the double bond either in the 8 or 9 position are metabolized efficiently by the rat organism.

Acknowledgment

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Hydroxylation of Methyl Oleate. A New, Direct Method[1]

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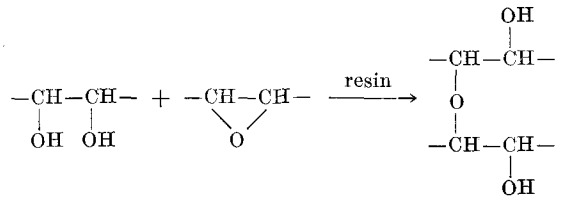
SOME TIME AGO (4) our laboratory began to develop a procedure to eliminate undesirable by-products in the epoxidation of fatty oils with hydrogen peroxide. An aim of this program was to investigate solids which might catalyze the reaction of hydrogen peroxide and acetic acid to form peracetic acid. Solid catalysts were expected to provide special surface effects in epoxidation for high yields not readily obtained with mineral acids. In the course of this investigation, polystyrene sulfonic-acid exchange resins of medium or low porosity were found to catalyze epoxidation with virtual exclusion of by-products under certain conditions (2). Additional work on these catalysts led to the discovery of a promising system for hydroxylation of methyl oleate.

Thus methyl oleate was converted directly to methyl 9,10-dihydroxystearate with hydrogen peroxide and acetic acid, catalyzed by polystyrene sulfonic-acid exchange resins of high porosity. Unlike other hydroxylation procedures (6, 7) resin catalysis allowed the ester linkage to remain substantially intact. The dihydroxy derivative was obtained in yields up to 74%. By-products of the reaction consisted of small amounts of epoxide and a polymeric hydroxy ether. The polyether (8) appears to be the product of the reaction of the glycol and epoxide formed transiently.



Polyether formation is catalyzed by porous exchange resins of the type specified, but not by the less porous resins suitable for epoxidation.

Data obtained in developing this new technique tend to support the interpretation that resin catalysis in hydroxylation involves absorption of the fatty oil. Successful epoxidation probably requires that sorption of fatty oils be limited to adsorption on the surface of the resin.

Experimental

Hydroxylation of Methyl Oleate. A mixture of 29.2 g. (0.10 mole) of technical methyl oleate (iodine number 87.1), 7.5 g. of resin (4% cross-linkage) containing 1.33 g. acetic acid, and 1.97 g. of glacial acetic

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