	First day			Second day			
	I	II	Mean	I	II	Mean	Calculated[a]
Methyl pelargonate	42.3	43.2	42.8	43.8	42.0	42.9	43.3
Methyl laurate	57.7	56.7	57.2	56.2	58.0	57.1	56.7

[a] Calculated from proportions of seed oils in the mixture and the fatty acid compositions of each.

A sample of body fat from mice fed on the mixed oil was analyzed for petroselinic acid content according to the two procedures and duplicate analyses gave the following values:

Method	Petroselinic %	
	I	II
A	15.3	15.7
B	15.4	15.6

The results show excellent agreement between the two methods for calculating the proportions of methyl oleate and petroselinate and a very good precision in the analysis. The addition of sodium bisulfite at the acidification step is necessary to reduce the free iodine which is liberated. If this is neglected byproducts are produced which interfere in the subsequent GLC analysis. The combination of extraction solvents was effective for both the monocarboxylic and dicarboxylic methyl esters. Petroleum ether in the solvent mixture reduced the water content and eliminated the necessity for using a drying agent, such as sodium sulfate.

The method requires about 3 hr for the preparation of the sample and has the advantage that no transfers are involved, which eliminates the possibility of losses and subsequent errors.

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The Simultaneous Determination of Both the Quantity and the Fatty Acid Composition of the Triglycerides in Three to Ten Microliters of Plasma[1]

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Abstract

An integrated system using chromatography on silica gel-impregnated glass paper, an internal standard and gas-liquid chromatography (GLC) makes possible the isolation of triglycerides, the determination of triglyceride, fatty acid composition, and the quantitative determination of triglyceride.

Recoveries are good and values for total triglyceride obtained by this system compare favorably with values obtained by application of the hydroxamate colorimetric method to the triglyceride eluate from silicic acid columns. The fatty acid composition of the cholesteryl ester (CE) fraction is obtainable at the same time by GLC of methyl esters prepared from the CE fractions which also appears as a distinct spot on these chromatograms.

Also, a densitometric method is described for triglyceride determination by photometry of the charred spots obtained after chromatography on the gel-paper.

THE LIMITED NUMBER of reports on serum triglyceride levels of animals and humans under various conditions of health and disease has been due at least in part to difficulties in measuring this lipid. Silicic acid column chromatography has been one of the more valuable methods of isolating triglycerides from serum extracts. Since this method requires constant monitoring of the physical and chemical conditions to effect a separation of the triglycerides from other components of the serum, it has gradually been replaced by methods using thin-layer and paper chromatography. An additional advance in the analysis of triglycerides has been the determination of their fatty acid composition by gas-liquid chromatography.

A detailed review of the glass paper chromatography of lipids has been published by Hamilton and Muldrey. These authors reported their experience and the advantages in separating various serum lipids by this method (1,2). Recently, a method has been described by Muldrey, Bowers, Miller and Hamilton (3), for the microassay of serum cholesteryl ester fatty acid (CEFA) patterns by densitometry of the charred spots produced after chromatography on silica-gel impregnated glass paper. Since the individual CEFA can be determined in 1–3 μl of plasma, it has been possible to perform serial measurements in single rats for several months (4).

In this study, a method was developed for measurement of serum triglycerides in 3 to 10 μl of plasma by use of a system consisting of chromatography on glass paper impregnated with silica gel (gel-paper) combined with an internal standard and gas liquid chromatography (the integrated system). By using

[1] Presented at the AOCS Meeting, Houston, April, 1965.

TABLE I

Determinations of Human Plasma, Triglyceride Levels by Three Methods: Densitometry, the Integrated System,[a] and Hydroxamate-Colorimetry of Triglyceride from Silicic Acid Column Chromatography

	Method				
	Densitometry mg/100 ml Plasma	Deviation from colorimetry	Integrated system mg/100 ml plasma	Deviation from colorimetry	Colorimetry mg/100 ml plasma
1	192	8	209	25	184
2	126	22	132	16	148
3	225	16	224	15	209
4	260	17	252	9	243
5	100	10	88	2	90
6	76	1	71	6	77
7	118	2	109	11	120
8	156	29	124	3	127
9	88	8	94	2	96
10	88	13	84	17	101
11	72	17	76	13	89
12	153	25	152	26	178
13	178	11	164	25	189
14	114	9	116	7	123
15	146	14	137	23	160
16	156	19	141	34	175
17	121	14	96	11	107
18	124	14	140	2	138
19	94	16	112	2	110
20	156	4	134	26	160
21	140	38	168	10	178
Mean Deviation from Colorimetry		14.6		13.6	

[a] Gel-paper, gas liquid chromatography with an internal standard.

the integrated system method it is possible to determine levels of plasma triglycerides and their fatty acid composition. Also, a densitometric method to determine triglyceride levels is described which is based on photometry of the charred spots obtained by treating the gel-paper chromatogram with sulfuric acid and heat.

Materials and Methods

In order to clean papers of organic materials, glass fiber papers (20 × 20 cm, paper)[1] are suspended on a stainless steel rod through a hole punched in the top center edge of the paper and heated in a furnace at 538C for 30 min. Cleaned papers are transferred with forceps to a manila folder which is wrapped in aluminum foil for storage. The coating solution is prepared by thoroughly mixing 100 ml of an aqueous solution of potassium silicate (15:1)[2] and 30 ml of an aqueous solution of ammonium chloride (5% w/v) in a glass tray 14 × 12 × 2 in. Single sheets of paper held by plastic clips are immersed in the coating solution for about 10 sec, a clean glass rod is passed over the surface of the paper to remove the excess coating solution and the papers are hung to dry at room temperature. When dry, the papers are heated at 538C for 20 min to sublimate the ammonium chloride. The small residue of nonvolatile salts is removed by an ascending chromatographic wash with distilled water. Washed papers are dried over a hot plate for about one hour, then stored as described above.[3] They remain usable for several months. More details in the technique of preparing the papers are given by Muldrey et al. (3).

The amount of 3–10 μg of triglyceride from plasma of mice, rats or humans is necessary for determining the amount and fatty acid composition of the triglycerides. Blood was collected after an overnight fast into 55 μl heparinized capillary tubes from the orbital venous sinus of CBA female mice or from the tail vein of Holtzman Sprague-Dawley female rats. The mice were fed Purina mouse chow and the rats Rockland rat chow ad lib. After centrifugation, the end of the capillary with the red cells is broken off and discarded. The end containing plasma is sealed and stored at −4C until analyzed.

TABLE II

The "Between-Day" Variation in the Values of Triglyceride-Fatty Acid Composition and Triglyceride Level of a Single Sample of Human Plasma Determined by the Integrated System Method[a]

Days	Methyl esters, milligrams percent					
	16:0	16:1	18:0	18:1	18:2	Total
1	20	1.0	2.8	33.1	7.6	65
2	19	1.1	2.8	30	5.5	59
3	21	1.1	3.1	29.1	5.4	59
4	19	0.8	2.2	29	7.0	58
5	22	1.8	2.8	29	8.4	64
6	20	0.7	4.4	30	7.8	63
7	20	0.7	1.7	28	5.3	56
8	19	0.5	4.3	36	8.4	68
9	22	0.9	2.6	36	6.1	68
10	20	0.7	2.2	32	5.4	60
Mean ±SD	20±1.2	0.93±0.36	2.9±0.87	31±2.9	6.7±1.3	62±4.2

[a] Gel-paper, gas liquid chromatography with an internal standard.

The amount of 3–10 μl of plasma is pipetted with calibrated micropipettes directly onto the paper 1.5 cm above the bottom edge. (Pipettes are cleaned by successive aspiration of 0.1 M sodium citrate, water, and isopropyl ether-ethanol (1:2), and occasionally they are cleaned with dichromate cleaning solution.) Eight samples are placed on each paper. Immediately, before the plasma dries, 10 to 20 μl of isopropyl ether-ethanol (1:2) are pipetted over the serum to release the lipid from the lipoproteins. When dry the plasma is overspotted a second and third time with isopropyl ether-ethanol. Alternately, a lipid extract may be applied directly to the paper. Ten microliters of isooctane containing a known amount (2 to 4 μg) of glyceryl triheptadecanoate is applied to each spot of plasma or lipid extract on the paper. The glyceryl triheptadecanoate serves as an internal standard for determination of the concentration of triglycerides.

If the cholesteryl ester fatty acids are to be determined, a known amount (6 to 12 μg) of the internal standard, cholesteryl heptadecanoate is applied onto the paper over the triglyceride standard. The chromatogram is developed in 100 ml of isooctane[4] and 2 ml isopropyl acetate. In this system the triglyceride has an R_f of about 0.7 and the free cholesterol about 0.5. Cholesterol esters run as a tight spot at the solvent front. The lipids are located by spraying papers with Rhodamine-6G. Areas of paper containing the separated lipids are cut out, cut into small strips and with forceps placed into culture tubes (7 ml). Four milliliters of acetone-hexane (1:1) is added, the tubes are capped with Teflon-lined caps and shaken on a Vortex mixer for 30 seconds. The solvent is transferred to 7 ml tubes. The papers are washed a second time with 2 ml of the acetone-hexane mixture. The second wash is transferred and the combined extract is taken to dryness under nitrogen.

To methylate the fatty acids, 2 ml of methanol-sulfuric acid (9:1) is added, the tubes capped and incubated at 65C for one hour. When the samples have cooled, 1 ml of distilled water and 2 ml of hexane are added. The tubes are shaken on a Vortex mixer for 30 sec and centrifuged. The hexane layer is transferred with disposable capillary pipettes into 3 ml conical shaped tubes and taken to dryness with

[1] H. Reeve Angel and Company, No. 934-AH, Schleicher and Schuell Co., No. 29; or Gelman Instrument Company.

[2] Potassium Silicate-Electronics 200, Electrochemical Department, E. I. DuPont, Wilmington, Delaware.

[3] Silica-gel coated paper may be obtained from Applied Science Laboratories, State College Pennsylvania or Gelman Instrument Company, Ann Arbor, Michigan.

[4] 2,2,4-trimethylpentane, Phillips Petroleum Company, Bartlesville, Oklahoma.

TABLE III

Fatty Acid Composition of a Solution of Safflower Oil: Comparison of Results (A) Eluting Lipid from Gel-Paper, (B) Rinsing Lipid from Gel-Paper, (C) Adding Lipid Directly to Tube for Methylation Without Prior Gel-Paper Chromatography

	Methyl esters Percentage composition				
	16:0	18:0	18:1	18:2	Total μg
A	4.4	1.6	9.7	84	18
	5.8	2.0	11.4	81	17
	4.4	1.6	9.4	84	19
	4.7	1.5	9.9	84	17
	4.4	1.4	9.0	85	18
	4.5	1.6	9.0	85	21
	4.3	1.5	8.5	86	19
B	5.5	2.4	10.8	81	17
	5.4	2.0	10.6	82	17
	6.3	2.2	12.2	79	20
	6.3	2.2	10.7	81	19
C	5.6	2.1	11.6	80	21
	6.7	2.7	12.9	78	17
	5.4	2.0	10.7	82	17
	5.7	2.0	11.0	81	18
	5.3	2.1	10.6	82	17

nitrogen. Ten microliters of hexane is added to the residue and 3–4 μl of the solution is injected with a 10 μl Hamilton syringe into a gas-liquid chromatograph.[5] In this study, a Lovelock argon ionization detector with a radium foil source was used. The column, a 6 ft × 3 mm I.D. glass U-tube was packed with 15% ethylene glycol succinate on Gas-Chrom W.[6] Column temperature was kept at 176C.

Calculation of the concentration of individual fatty acids is based on the internal standard, methyl heptadecanoate. Area measurements are determined from the chart recordings. The width of the triangle at one half its height is multiplied by the height of the triangle. The concentration of total serum triglyceride levels is determined by summation from values of the individually determined fatty acids.

Plasma for triglyceride and free cholesterol analysis by the *densitometric* method is pipetted directly onto the paper as previously described. Standards of free cholesterol and triolein are mixed so that 10 μl of isopropyl ether-ethanol (1:2) contains 0.3, 0.6 and 1.2 μg of each. The fourth standard solution contains only triolein (2.4 μg) since the regression for free cholesterol is not linear at this concentration. The standard solution and 1 to 3 μl of six plasma samples (or appropriate volumes of lipid extracts) are pipetted onto the paper 1.5 cm above the bottom edge. The amount of 10–20 μl of isopropyl ether-ethanol (1:2) is pipetted over the plasma, as previously described, to release the lipid from the lipoproteins. The chromatogram is developed as previously described. The solvent on the chromatogram evaporates in about 8 min at room temperature. A strip across the bottom of the chromatogram (2.0 cm) containing the origins is cut off. The paper is chromatographed in 5% aqueous H_2SO_4 to the level of the solvent front and then it is immediately placed on a glass rod in a vented oven (212C). Charred spots are developed in 10 min. The spots are measured by densitometry as described previously (3,5). The amount of free cholesterol and triglyceride is calculated by reading the unknowns from the curves of the respective standards. For determination of the triglyceride levels it is necessary to correct for the saturated fatty acids, palmitate and stearate, which do not char under these conditions. Palmitate and stearate constitute approximately one third of the total triglyceride fatty acids in the plasma of humans, rats and mice on diets which

[5] Barber-Colman Model 10.
[6] Applied Science Laboratories, State College, Pennsylvania.

TABLE IV

Determinations of the Triglyceride Fatty Acid Composition and Triglyceride Level in 3 to 20 μl of a Single Sample of Human Plasma but a Constant Amount of Internal Standard (A) and the Repeated Analysis of 10 μl of Plasma with Various Amounts of the Internal Standard Added (B) by the Integrated System Method [a]

	Volume plasma analyzed, μl	Amount of internal standard added,[b] μg	Methyl esters milligrams percent				
			16:0	16:1	18:0	18:1	18:2
A	3	2	24	2.3	4.0	32	9.4
	5	2	17	2.4	2.0	33	7.0
	10	2	19	1.3	2.1	32	7.0
	20	2	20	2.0	4.0	32	6.0
B	10	4	25	3.1	1.3	39	12.2
	10	2	23	2.5	1.3	36	9.6
	10	1	23	1.8	2.0	35	8.8
	10	0.6	24	1.8	2.9	39	9.6

[a] Gel-paper, gas liquid chromatography with an internal standard.
[b] Glyceryl triheptadecanoate.

are not abnormally high in unsaturated or saturated fats. For this reason the quantity of triglyceride is calculated by multiplying 1.5 times the value obtained by photometry of the charred spots.

The isolation of triglycerides from extracts of plasma by silicic acid column chromatography was performed by minor modification of the method described by Barron and Hanahan (6) and quantitated by the hydroxamate reaction (7).

Results

Triglyceride levels of human plasma were determined by the three different methods (Table I). The values ranged from 70 to 260 mg per 100 ml plasma. It is to be noted that the use of a factor to correct for palmitate and stearate, which do not char, is satisfactory.

In order to estimate the "between-day" variations in the determination of the fatty acid composition and the triglyceride level by the integrated system method, repeated analyses were performed on a single sample of human plasma (Table II). The degree of variation in the quantities of oleate, palmitate, and linoleate was the same order of magnitude. The greatest variation was in the estimation of palmitoleate and stearate, both of which were in low concentration. Since myristate and arachidonate were in trace amounts in the plasma sample, these fatty acids were not measured.

In order to determine if there were a loss of individual fatty acids during paper chromatography, the following study was performed. Ten microliters of a solution of safflower oil, a highly unsaturated triglyceride, and 10 μl of a solution of glyceryl triheptadecanoate were pipetted multiple times onto gel papers and chromatographed. In some trials the lipid was

TABLE V

Recovery of Triglyceride Fatty Acids Added to Plasma Extracts When Determined by the Integrated System Method [a]

	Triglyceride fatty acid (methyl ester)	Added mg per 100 ml	Recovered mg per 100 ml M±SD (N)	Percent recovered
A	Palmitate	13	13±2.4(10)	101
B	Palmitate	26	24±2.8(10)	95
A	Palmitoleate	1.4	1.6±1.2(10)	.....
B	Palmitoleate	2.8	2.5±0.8(10)	.....
A	Stearate	2.4	2.3±2.0(10)	.....
B	Stearate	5.1	4.2±1.3(10)	.....
A	Oleate	10	11.1±1.9(10)	108
B	Oleate	20	20±3.6(10)	98
A	Linoleate	31	29±3.1(10)	94
B	Linoleate	62	66±3.1(10)	107
A	Total	58	57±4.0(10)	99
B	Total	119	117±6.8(10)	103

[a] Gel-paper, gas liquid chromatography with an internal standard.

TABLE VI

Serial Determinations of the Triglyceride Fatty Acid Composition and Triglyceride Levels of Plasma from Individual Rats and Mice by the Densitometric Method and by the Integrated System Method [a]

		Method of analysis		Methyl esters milligrams percent					Percentage composition				
		Densitometry, total [b]	Integrated system total [c]	16:0	16:1	18:0	18:1	18:2	16:0	16:1	18:0	18:1	18:2
Rat 1—Day	1	55	50	15	4.2	4.5	16	17	29	8.4	8.8	32	22
	2	70	74	18	3.6	8.4	25	19	26	4.9	11.4	34	25
	3	63	72	20	7.1	6.3	22	17	28.0	9.9	8.7	30	23
Rat 2—Day	1	30	29	6.2	2.4	3.8	10	6.4	22.1	8.3	13.0	36	22
	2	59	60	22	4.6	5.6	18	10	36.0	7.7	9.3	31	16
	3	26	23	7.0	1.4	3.1	7.8	4.5	31.0	6.1	13.4	34	20
Rat 3—Day	1	53	41	12	2.2	3.1	13	10	30	5.4	7.6	32	25
	2	42	39	11	2.4	4.1	18	9	28	6.2	10.5	32	22
	3	50	48	10	2.2	4.9	18	16	21	4.6	10.7	35	33
Mouse 1—Day	1		43	5.8	2.0	2.1	21	12	14	4.7	5.1	51	26
	5		54	11	3.7	2.4	24	13	20	6.7	4.5	44	25
	9		58	15	1.6	3.4	25	13	25	2.8	5.8	43	23
	13		50	12	2.7	3.1	20	12	24	5.3	6.2	41	24
	17		47	11	3.4	2.5	19	11	24	7.1	5.3	41	23
	21		44	12	3.6	2.1	16	10	28	8.2	4.8	37	22
Mouse 2-Day	1		43	9	1.7	2.3	19	11	21	4.0	5.4	44	26
	5		36	8	1.5	2.2	14	9	24	4.2	6.1	40	26
	9		36	9	2.2	1.9	14	8	28	6.0	5.1	41	23
	13		34	11	2.2	1.7	12	7	31	6.3	5.0	36	21
	17		45	11	1.1	3.0	20	11	24	2.5	6.7	44	24
	21		45	10	1.8	3.1	19	11	23	4.0	7.0	42	24

[a] Gel-paper, gas liquid chromatography with an internal standard.
[b] Volume of plasma analysed—3 μl.
[c] Volume of plasma analysed—10 μl.

eluted from the paper and in others it was rinsed off. Fatty acid levels were determined following methylation and gas chromatography of the methyl esters (Table III). In the third part of this study the solution of safflower oil was pipetted directly into the tube for methylation; the paper chromatography step was not performed. Fatty acid levels were determined as described above. Rinsing the lipid from the gel-paper gave somewhat better results than the elution technique.

Values for lipid concentrations were not significantly different when 3, 5, 10 or 20 μl of a single sample of human plasma were analysed by gel-paper, gas-liquid chromatography with an internal standard (Table IV). The results show that the precision of the method was good when various amounts of plasma were analysed. Another study was performed by pipetting 10 μl aliquots of a single plasma sample onto the paper and adding different amounts of the internal standard (0.6, 1.0, 2.0, 4.0 μg). There was no significant difference in the results.

Studies were conducted in which known amounts of cottonseed oil methyl esters were added to analysed plasma extracts. Recoveries were satisfactory except for palmitoleate and stearate (Table V). These were minor components of the cottonseed oil and could not be determined accurately under the conditions of the experiment. By adding smaller amounts of the internal standard and operating the gas chromatograph at a higher sensitivity it should be possible to accurately determine palmitoleate and stearate. No attempt was made to analyze them accurately.

Serial measurements of the triglyceride fatty acid composition and concentrations of triglyceride of plasma from rats and mice were performed by the integrated system method and the densitometric method (Table VI). There is fairly good agreement in the results of the two methods. Blood was taken from each of three rats on three consecutive days and from each of two mice every four days for 21 days. The triglyceride and the fatty acid levels were relatively constant for both the rats and mice.

Discussion

A microtechnique is described for determining simultaneously the fatty acid composition and the concentration of plasma triglyceride in 3 to 10 μl of plasma by means of chromatography with an internal standard on glass paper impregnated with silica gel (gel-paper) and gas-liquid chromatography. This enables one to perform serial measurements of these lipids in small animals. The triglycerides and cholesterol esters are easily and rapidly separated from plasma by gel-paper chromatography. If a factor is used to correct for saturated fatty acids which do not char, densitometry of the charred spots can be used to quantitate the triglycerides. The combination of the gel-paper chromatography and gas-liquid chromatography makes it possible to determine the fatty acid composition of the isolated triglycerides. The addition of an internal standard, glyceryl triheptadecanoate, makes possible the determination of serum concentrations of each fatty acid of the triglyceride. Ten microliters of plasma is usually used to determine the triglyceride fatty acid composition and level of triglycerides in plasma of humans, mice and rats. The least amount of plasma triglyceride necessary for quantitation of the individual fatty acids by this method is 2 μg and the optimal quantity is 10 μg. Two micrograms of the internal standard is satisfactory for serum triglyceride concentrations ranging from 20 to 150 mg per 100 ml plasma. For plasma levels of 150 to 400 mg per 100 ml plasma, it is necessary to use 4 μg of the internal standard.

An added advantage of the integrated system method is that the cholesteryl ester as well as triglyceride fatty acid levels can be measured in the same sample of plasma. In order to do this, a second internal standard, cholesteryl heptadecanoate, is used. The cholesteryl esters run at the solvent front as a distinct spot and can be rinsed from the paper, methylated, and then the methyl esters determined by gas-liquid chromatography.

The densitometric method is simple and rapid to perform and, therefore, offers advantages for serial triglyceride determinations, as in a triglyceride tolerance test or acute and chronic studies both in small animals and in humans.

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The Reaction of Ethylene Oxide with Oleic Acid

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Abstract

The base-catalyzed reaction of ethylene oxide with oleic acid can be divided into two stages. The first stage consists of a slow reaction of oleic acid with ethylene oxide to form principally ethylene glycol monooleate; other reactions such as esterification, transesterification and polyglycol formation lag behind. In the second stage, after the addition of approximately one mole of ethylene oxide, the reaction accelerates and transesterification equilibrium is rapidly attained. The composition of products containing several molecules or more of ethylene oxide can be calculated satisfactorily on the assumption of random addition of ethylene oxide and random esterification of the hydroxyl groups. The uncatalyzed reaction is much slower and transesterification equilibrium is attained slowly, if at all. A reaction mechanism based on the difference in basicities of the carboxylate and alkoxide ions (and the relative rates of the competitive ethylene oxide reactions) is presented for the base-catalyzed reaction.

Introduction

THE ALKALI-CATALYZED reactions of ethylene oxide with long-chain fatty acids are used for the production of a number of commercially important emulsifiers and nonionic detergents (1,2). A simplified representation of the reaction is

$$RCOOH + n\ C_2H_4O \longrightarrow RCOO(C_2H_4O)_nH \quad (1)$$

The reaction is customarily conducted with an alkaline catalyst and other reactions such as transesterification,

$$2\ RCOO(C_2H_4O)_nH \rightleftarrows RCOO(C_2H_4O)_nOCR + HO(C_2H_4O)_nH \quad (2)$$

and direct esterification,

$$RCOOH + RCOO(C_2H_4O)_nH \rightarrow RCOO(C_2H_4O)_nOCR + H_2O \quad (3)$$

have been observed (2–4).

Polyoxyethylene (8) stearate (MYRJ 45—Atlas Chemical Industries, Inc.), which is the reaction product of 8 moles of ethylene oxide per mole of stearic acid, is approximately a 1:2:1 molar mixture of free polyol, monoester and diester (3). This distribution is the one expected (10) if ester interchange equilibrium (Equation 2) is reached rapidly. Larger mole ratios of mono- to diester have been reported for early stages of oxyethylation (4–7). In view of the interest in polyoxyethylene glycol esters in foods, pharmaceuticals, etc., it seemed desirable to make a thorough study of the effects of reaction conditions, including extent of oxyethylation on the composition of the products. Oleic acid was chosen for the study rather than stearic acid because the oleate esters are liquids and easy to handle and to observe for homogeneity.

Experimental

Starting Materials

Oleic acid was Emersol 233LL (Acid No., 202; average mole weight, 278), obtained from Emery Industries, Inc. Commercial ethylene oxide and sodium hydroxide were used without purification.

Procedure

Fatty acid-catalyst mixtures were prepared by dissolving powdered sodium hydroxide in oleic acid at elevated temperatures and decanting the mixture from the small quantity of aqueous phase which settled out upon standing. The ethylene oxide reactions were conducted in a one-liter stirred autoclave under a nitrogen atmosphere. Ethylene oxide was added incrementally maintaining autoclave pressure constant to ± 3 psig. Details of the equipment and procedure were given previously (11). In order to obtain samples representative of operating conditions, small quantities were removed rapidly from the autoclave into a flask cooled in a solid carbon dioxide-isopropanol bath.

Separation of Reaction Products and Analysis

The products are for the most part homogeneous. Those containing between 1.0 and 2.5 moles of ethylene oxide per mole acid yielded a small quantity of a second liquid phase, largely glycols, upon standing at room temperature. Before sampling for analysis, the products were mixed by shaking. The mole ratio of epoxide reacted with acid was estimated from the oxyethylene content of the product, determined by the method of Siggia et al. (8). The products were analyzed for acid value, hydroxyl value and saponification value by methods essentially those of the American Oil Chemists' Society. Free and total ethylene glycol were determined by periodate oxidation before and after saponification of the oleate ester. Water content was determined by Karl Fischer reagent or by gas-liquid chromatography.

Samples were processed to yield (a) free polyols, (b) combined mono- and diesters, and (c) total (free and combined) polyols. The procedure utilized to obtain the total polyols was similar to that described by Birkmeier and Brandner (3), except that samples were concentrated under controlled conditions to prevent loss of the lower glycols. The separation of free polyols from the combined esters was accomplished by extracting a benzene solution with aqueous 10% sodium sulfate solution. In some of the extractions, emulsions were obtained, which were broken by the addition of small quantities of isopropanol. The free polyols (and the total polyols obtained after saponification) were recovered as aqueous solutions, concentrated and the chain length of the glycols estimated from the hydroxyl number. Alternatively, GLC was used to estimate free polyols directly using a column