Isolation and Identification of Monoglycerides in the Intestinal Contents of Humans[1]

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SECTION A

General Procedures, Results, and Discussion

UNEQUIVOCAL evidence of the *in vivo* formation of monoglycerides is essential to the understanding of the mechanism of fat absorption. Many investigators have postulated that triglycerides are hydrolyzed by pancreatic lipase to partial esters, and numerous studies *in vitro* and *in vivo* have tended to confirm such a mechanism (1, 3, 6-9, 17). Frazer (10) stated that triglyceride absorption through the intestine requires an emulsion involving monoglycerides. Frazer and Sammons (11) concluded from their work on rats that a product of pancreatic lipolysis was monoglycerides. Their conclusions were based upon such properties of the intestinal lipids as acetyl values, neutralization values, and phthalic acid ester studies. Reiser and associates (16) fed rats synthetic triglycerides in which the glycerol was labeled with C^{14} and the fatty acids with conjugated double bonds and studied the resultant lymph lipids. They concluded that 55 to 75% of the glycerides was hydrolyzed to, and absorbed as, monoglycerides.

This report describes work establishing that monoglycerides are produced in the human intestinal tract through digestion of triglycerides. The monoglycerides were extracted from the contents of the small intestine, concentrated, and identified by several different methods, using techniques similar to those described by Kuhrt and associates (14).

Experimental

W. A. Clay and E. S. Nasset from the Department of Physiology and Vital Economics of the University of Rochester, School of Medicine and Dentistry, acted as subjects for this study. Each subject swallowed a Harris tube, and the position of the tubes was followed by fluoroscopic examination. After several hours each tube was at or near the ligament of Treitz. The test meal, consisting of a sandwich and fruit, as described in Section B, was then eaten. Samples of the intestinal contents were removed through the tube at periodic intervals. It was not possible with this arrangement to remove the partially digested food quantitatively.

Extraction and Purification of Monoglycerides. After dehydration of the samples the lipids were removed by stirring with large volumes of a warm ethyl ether:ethanol mixture. The ethyl ether:ethanol mixture extracted non-lipid materials; therefore the fat recovered from the ether-alcohol mixture after removal of the solvents was redissolved in ethyl ether and the insoluble non-lipid material was rejected. Monoglycerides were then isolated from the ether-soluble lipid material and purified by the solvent procedures outlined in Section D. The basic techniques are fully discussed in the previous paper (14).

Identification. The periodic acid oxidation method (12) for monoglyceride analysis was used to follow the progress of all the purification procedures. Free glycerol was determined by the method of Bradford and associates (4). The purity of the monoglyceride fractions was established by periodic acid analysis and the fractions were further identified as monoglycerides by saponification, by infrared absorption, and by countercurrent distribution (Table I).

Results and Discussion

Isolation of Monoglycerides from the Test Meal. Food which duplicated the experimental diet (Section B) was used to determine the amount of fat in the diet and its content of monoglycerides. The quantity of lipid from 200 grams of food was found to be 22.2 grams. From this lipid only 0.033 gram of monoglycerides was obtained (Section C). The monoglyceride fraction thus represented 0.15% of the total lipid present in the diet.

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TABLE I

Properties[a] of Crystalline Monoglycerides of Saturated Fatty Acids and Concentrates of Monoglycerides of Unsaturated Fatty Acids

Property	Monoglyceride Extract from					
	Intestinal Contents of				Reaction Product of Milk Fat and Glycerol	
	Dr. Nasset		Dr. Clay			
	Saturated acids NK-52B	Unsaturated acids NK-56C	Saturated acids NK-52A	Unsaturated acids NK-56A	Saturated acids NK-66	Unsaturated acids NK-65A
Periodic acid assay for monoglycerides—% theory	97	90	96	86	93	83
Fatty acids recovered—% theory	97.8	102.3	....	....	98	102.5
Neutralization value of recovered acids—% theory	98.8	97	....	....	*100*	99.5
Glycerol recovery—% theory	82.8	71.3	....	....	79.5	70.5
Infrared analysis	Practically pure	Practically pure	Practically pure	Practically pure	Practically pure	Practically pure
Countercurrent extraction						
Recovered in main fractions,%	94	89.6	93.3	69.5	92.4	60.0
Recovered in early fractions, %	....	....	....	18.8	....	17.7
Total monoglycerides, %	94	89.6	93.3	88.3	92.4	77.7
Free fatty acids—%	0	0.3	0	1.0	0	4.0
Free glycerol—%	0	0	0	0	0	0
Melting point—°C.	68.5-70.0	....	67.5-69.5	....	67.5-69.0	....

[a] "Theory" is the calculated properties of the monoglycerides of the saturated or of the unsaturated acids of milk fat.

Isolation of Monoglycerides from Intestinal Contents. Subject, Dr. Clay. The method for collecting the intestinal contents is described in Section B. The 180 ml. of intestinal contents yielded 13.3 grams of dried material. The extracted lipid fraction amounted to 3.83 g., or 28.8% of the dried intestinal contents. From this lipid 1.12 grams of essentially pure monoglycerides were obtained by the solvent extraction procedures reported in Section D and identified as monoglycerides in Sections F, G, and H. When the 0.32 gram of monoglycerides rejected in the purification procedures and used for analyses are considered in the total yield, the lipid fraction of the intestinal contents of subject, Dr. Clay, contained 37.6% of monoglycerides as compared to 0.15% in the lipid fraction of the original diet.

Isolation of Monoglycerides from Intestinal Contents. Subject, Dr. Nasset. The 135 ml. of intestinal contents yielded 14.0 grams of dried material. The extracted lipid fraction weighed 5.7 g. or 40.6% of the dried intestinal contents. From this lipid fraction 2.46 grams of essentially pure monoglycerides were obtained by the solvent extraction procedures reported in Section D and identified as monoglycerides in Sections F, G, and H. When the 0.38 gram of monoglycerides rejected in the purification procedures and used for analyses are considered in the total yield, the lipid fraction of the intestinal contents of subject, Dr. Nasset, contained 50% of monoglycerides as compared to 0.15% in the lipid fraction of the original diet.

Identification of Monoglycerides from a Reaction Product of Milk Fat and Glycerol. Monoglycerides were prepared by chemical means to check the accuracy of the extraction procedures for the monoglycerides of milk fat; to characterize the monoglycerides of milk fat by chemical means, by infrared analysis, and by countercurrent extraction; and to compare the monoglyceride prepared by reacting milk fat and glycerol with the monoglycerides occurring in the intestinal contents after ingestion of a diet rich in milk fat. Milk fat and glycerol were reacted as reported in Section E, and the monoglycerides were separated and purified by the solvent procedures used to determine the monoglyceride content of the intestinal contents.

The extracted and purified fraction of the monoglycerides of the saturated acids of milk fat proved to be practically identical to the saturated acid monoglycerides recovered from the intestinal contents after ingestion of a diet containing milk fat products (Table I).

The extracted and purified fraction of the monoglycerides of the unsaturated acids of milk fat gave about the correct neutralization value (204) that was calculated (205) from the published data on the unsaturated acids of milk fat. This value was higher than the value (199) for the neutralization factor for the corresponding fraction from the intestinal contents. Low molecular weight monoglycerides are found in appreciable amounts in the first three tubes of the countercurrent extraction apparatus in the case of the milk fat but are practically absent from the extractions of the intestinal contents of one of the subjects, perhaps because they have already been absorbed from the intestines. Holt and associates (13) concluded that, in a mixed fat, absorption is favored by the presence of a) fatty acids containing one or more unsaturated linkages, and b) fatty acids with relatively short carbon chains.

Summary

The experiments described in this report show that the human intestinal tract contains a relatively large quantity of monoglycerides after the ingestion of a meal containing fat but essentially no monoglycerides. The results are as follows:

Two human subjects each ate a meal containing 22 grams of lipid, of which 0.15% was monoglycerides;
A sample of the intestinal contents of one of the human subjects contained 3.83 grams of lipid, of which 37.6% was monoglycerides; and
A sample of the intestinal contents of the other subject contained 5.70 grams of lipid, of which approximately 50% was monoglycerides.

The monoglyceride assay was confirmed by the isolation of crystalline saturated monoglycerides and the preparation of concentrates of the unsaturated monoglycerides. The monoglycerides were further identified by infrared spectrophotometry, by countercurrent extraction, by monoglyceride analysis (periodic acid oxidation), by fatty acid analysis, and by isolation of glycerol.

Thus, as predicted from experiments with animals, monoglycerides are normal and important components of the human intestinal contents during the digestion of a meal containing fats.

SECTION B

Experimental Diet and Collection of Samples of Intestinal Contents

Contents of Test Meal and Schedule of the Collection of Samples from the Small Intestine. Dr. Clay and Dr. Nasset swallowed Harris tubes for the purpose of collecting contents from the upper small intestine after eating the usual Wednesday department cold lunch on January 16, 1952. The composition of this meal, calculated from Tables of Nutritional Data (15) is shown in Table II. Both subjects made it a point to eat identical lunches. Also a third identical lunch was set aside for chemical analysis. The amounts of "dry matter" and "fat" actually recovered from this test meal are also shown in Table II. The Harris tube has a single lumen, and an elongated rubber balloon, containing some mercury, is attached at one end. This device facilitates passage of the tube into the

TABLE II

Test Meal Employed in Fat Digestion Experiment Values from Tables of Nutritional Data

Food items	Portion eaten	Weight	Dry matter	Fat	Fat	Calories
		g.	g.	%	g.	
Bread, white	2 slices	46	29.5	2.0	0.9	120
Butter	1 pat	7	5.9	81.0	5.7	50
Cheese, yellow cheddar	1 slice	24	14.6	32.3	7.7	150
Liver sausage	2 slices	60	24.6	20.6	12.3	154
Mayonnaise	....	5	4.2	78.0	3.9	36
Mustard	....	....	....	....	....	....
Jelly	1 tbsp.	15	9.8	....	....	....
Cream cheese	1 tbsp.	15	7.0	36.9	5.5	55
Grapes	6 grapes	25	4.6	0.4	0.1	18
Totals		197	100.2	....	36.1	583
Actual amount present in control meal (analysis by Kuhrt and Welch)		200	77.0	....	22.2	....

duodenum from the stomach. Despite this assistance it often requires considerable time to get the tube an appreciable distance into the small bowel. The position of the end of the tube was determined by several fluoroscopic examinations in the Department of Radiology.

Schedule for Each Subject. Dr. Nasset (January 16, 1952)—Harris tube was inserted through nose, 10:51 a.m.; he swallowed tube without difficulty and assumed reclining position on right side; end of the tube entered duodenum at 12:10 p.m.; at 1 p.m. the tube had progressed past the duodenal cap and was beginning to descend; he attended committee meeting at 1:05 to 2:15 p.m.; lunch was eaten 2:20 to 2:35; tube was approximately in the midline and approaching the ligament of Treitz at 2:45 p.m.; aspiration of contents was begun 3:05 p.m. with syringe attached to outer end of Harris tube; aspiration continued periodically until 4:30 p.m.

It is impossible with this arrangement to get a quantitative removal of the partially digested food; the experiment was designed to yield a representative sample over the period of most rapid evacuation of contents from the stomach. Total volume obtained was 135 ml. It was interesting to note that the contents as removed were not stained with bile until near the end of the collection, and they were always strongly acid, as determined by indicator paper.

Dr. Clay (January 16, 1952)—Several attempts to pass the tube through the nose were unsuccessful, and it had to be taken by mouth, with some discomfort and retching (approximately 11:45 a.m.); meal was eaten at approximately 1:15 p.m.; beginning time of aspiration of contents not noted; last fluoroscopic examination showed the tube to be at the ligament of Treitz. Total volume obtained was 180 ml. In this instance, also, bile did not appear until late in the collection, and the contents were quite acid.

SECTION C

Extraction of Monoglycerides from Experimental Diet

A 200-gram sample of food which duplicated the diets of Dr. Clay and Dr. Nasset was immediately frozen at —28°C. The frozen solids were dried under vacuum. The dried material, which weighed 77 grams, was extracted three times with 125 ml. of warm 4:1 ethyl ether:ethanol mixture. The dried food was then ground to pass a 20-mesh screen and extracted six times with 125 ml. of warm 4:1 ethyl ether:ethanol. The solvent was removed from the combined extracts under vacuum. The residue was redissolved in ether and filtered. The solvent was removed from the filtrate under nitrogen. The ethyl ether-soluble lipid fraction recovered weighed 22.2 grams.

The ethyl ether-soluble fraction of 22.2 grams was dissolved in 600 ml. of acetone, and the precipitated phospholipids were removed by filtration. The acetone was removed under vacuum and the recovered fat was dissolved in 800 ml. of ethyl ether. The ether solution was washed repeatedly with water, dried over sodium sulphate, and evaporated under nitrogen. Water-soluble materials, which were oxidizable with periodic acid, were removed in this step.

The acetone soluble fraction of 13.9 grams was extracted twice with 200 ml. of methanol. The methanol extracts were combined and the solvent removed under vacuum.

The very small amount of monoglycerides represented in the methanol-soluble fraction prevented the continuation of the usual extraction and purification procedure. The periodic acid analysis of the methanol-soluble fraction probably represents true monoglyceride, judging from the practically quantitative recovery of pure monoglycerides obtained in the subsequent steps when working up lipids from other sources. Therefore not more than 0.033 gram or 0.15% of the lipid portion of the experimental diet was monoglycerides.

TABLE III

Extraction of Monoglycerides from the Test Meal

Solvent	Monoglyceride Distribution			
	Soluble fraction		Insoluble fraction	
	Wt.	Purity	Wt.	Purity
	g.	%	g.	%
Ethyl ether	22.2	0.16	....	....
Acetone	13.9	0.25	6	0
Methanol	1.5	2.2	12	0

SECTION D

Extraction of Monoglycerides from Samples of Intestinal Contents

Subject—Dr. Clay. The 180-ml. sample of intestinal contents obtained from Dr. Clay was immediately frozen at —28°C. The frozen solids were dried under vacuum. The dried material, which weighed 13.3 grams, was extracted with six portions of 125 ml. of warm 4:1 ethyl ether:ethanol mixture. The solvent was removed from the combined extracts under vacuum. The residue was redissolved in ether and filtered. The solvent was removed from the filtrate under nitrogen. The ethyl ether-soluble lipid fraction recovered amounted to 28.8% of the dried intestinal contents.

The ethyl ether-soluble fraction of 3.83 grams was dissolved in 100 ml. of acetone, and the precipitated phospholipids were removed by filtration. The acetone was removed under vacuum, and the fat was dissolved in 400 ml. of ethyl ether. The ether solution was washed repeatedly with water, dried over sodium sulphate, and the ether solution evaporated under nitrogen.

The acetone soluble fraction of 3.38 grams was extracted twice with 300 ml. of methanol. The methanol extracts were combined and the solvent removed under vacuum.

The methanol soluble fraction of 2.68 grams was dissolved in 200 ml. of Skellysolve F and cooled to +5°C. The Skellysolve F insoluble fraction was removed by filtration and recrystallized from Skellysolve F. The Skellysolve F solutions were combined and the solvent removed under nitrogen.

The Skellysolve F insoluble fraction NK-52A has been further identified as the monoglyceride of saturated fatty acids by melting point (Table I), by infrared analysis (Figure 1), and by countercurrent distribution (Figure 3).

The Skellysolve F soluble fraction of 1.7 grams was extracted twice with 100 ml. of 70% methanol. The methanol extracts were combined; the alcohol removed under vacuum; and the fat dissolved in 100 ml.

TABLE IV

Extraction and Purification of Monoglycerides from Samples of Intestinal Contents

Solvent	Monoglyceride Distribution of Samples from							
	Dr. Clay				Dr. Nasset			
	Soluble fraction		Insoluble fraction		Soluble fraction		Insoluble fraction	
	Wt.	Purity	Wt.	Purity	Wt.	Purity	Wt.	Purity
	g.	%	g.	%	g.	%	g.	%
Ethyl ether	3.83	38	....	....	5.7	49	....	....
Acetone	3.38	40	0.35	0	5.26	53.2	0.4	0
Methanol	2.68	49	0.6	0	4.86	56.5	0.3	0
Skellysolve F (+5°C.)	1.70	36	0.72	96	3.2	40	1.42	97
70% Methanol	0.50	86	1.0	18	1.2	90	1.9	10.5

of ethyl ether. The ether solution was water-washed and dried over sodium sulphate and the ether removed under nitrogen. The 70% methanol insoluble fraction had an acid value of 1.40.

The 70% methanol soluble fraction NK-56A has been further identified as the monoglycerides of unsaturated fatty acids by infrared analysis (Figure 2) and by countercurrent distribution (Figure 5).

The extraction and purification data are shown in Table IV.

The total weight of purified monoglycerides obtained from the sample of intestinal contents was 1.12 grams. A total of 0.19 gram of impure monoglycerides was rejected in the purification procedure as well as 0.13 gram of impure monoglycerides contained in the various samples used for analyses. Therefore 1.44 grams, or 37.6% of the lipid fraction of the intestinal contents was monoglycerides.

Subject—Dr. Nasset. The 135-ml. sample of intestinal contents obtained from Dr. Nasset was immediately frozen at —28°C. The frozen solids were dried under vacuum. The dried material weighed 14.0 grams. The lipid fraction recovered amounted to 40.6% of the dried intestinal contents.

The extraction and purification were similar to those of the Dr. Clay sample, and the data are reported in Table IV. The 70% methanol-insoluble fraction had an acid value of 0.84.

The Skellysolve F insoluble fraction NK-52B has been further identified as the monoglyceride of saturated fatty acids by its melting point and by analyses of the fatty acids and glycerol (Table VII), by infrared analysis (Figure 1), and by countercurrent distribution (Figure 3).

The 70% methanol-soluble fraction NK-56C has been further identified as the monoglycerides of the unsaturated fatty acids by analyses of the fatty acids and glycerol (Table VII), by infrared analysis (Figure 2), and by countercurrent distribution (Figure 5).

The total weight of purified monoglycerides obtained from the sample of intestinal contents was 2.46 grams. A total of 0.20 gram of impure monoglycerides was rejected in the purification procedures as well as 0.18 gram of impure monoglycerides contained in the various samples used for analyses. Therefore 2.84 grams, or 50% of the lipid fraction of the intestinal contents, was monoglycerides.

TABLE V

Extraction and Purification of Monoglycerides from a Reaction Product of Butterfat and Glycerol

Solvent	Monoglyceride Distribution			
	Soluble fraction		Insoluble fraction	
	Wt.	Purity	Wt.	Purity
	g.	%	g.	%
Acetone	57	55	2	0
Skellysolve F (+5°C.)	35	37.7	19.3	93
70% Methanol	10.9	83	23.1	14.7

SECTION E

Extraction of Monoglycerides from a Reaction Product of Butter Fat and Glycerol

A monoglyceride reaction product was made by heating and stirring 85 grams of water-washed butterfat, 30 grams of C.P. glycerol, and 0.1 gram of calcium hydroxide at 230°C. for one hour. The reaction mixture was dissolved in 1,000 ml. of ethyl ether and the ether solution washed four times with water; dried over sodium sulphate; and the ether solution evaporated under nitrogen. Fifty-nine grams of the ether-soluble fraction was dissolved in 800 ml. of acetone and the insoluble material removed by filtration. The acetone was removed under vacuum and the fat dissolved in 600 ml. of ethyl ether. The ether solution was washed repeatedly with water, dried over sodium sulphate, and the ether solution evaporated under nitrogen.

The water-soluble, water-washed fraction of 59 grams was dissolved in 600 ml. of warm Skellysolve F and cooled to +5°C. The Skellysolve F insoluble fraction was removed by filtration and recrystallized from Skellysolve F. The Skellysolve F solutions were combined and the solvent removed under nitrogen.

The Skellysolve F insoluble fraction NK-66 has been identified as the monoglycerides of saturated fatty acids of milk fat by its melting point (Table I), by analyses of the fatty acids and glycerol (Table VII), by infrared analysis (Figure 1), and by countercurrent distribution (Figure 4).

The Skellysolve F soluble fraction of 35 grams was extracted twice with 100 ml. of 70% methanol. The methanol extracts were combined and the alcohol removed under vacuum and the fat dissolved in 100 ml. of ethyl ether. The ether solution was water-washed and dried over sodium sulphate and the ether removed under nitrogen.

The 70% methanol soluble fraction NK-65A has been identified as the monoglycerides of the unsaturated acids of milk fat by analyses of the fatty acids and glycerol (Table VII), by infrared analysis (Figure 2), and by countercurrent distribution (Figure 4).

The extraction and purification data are given in Table V.

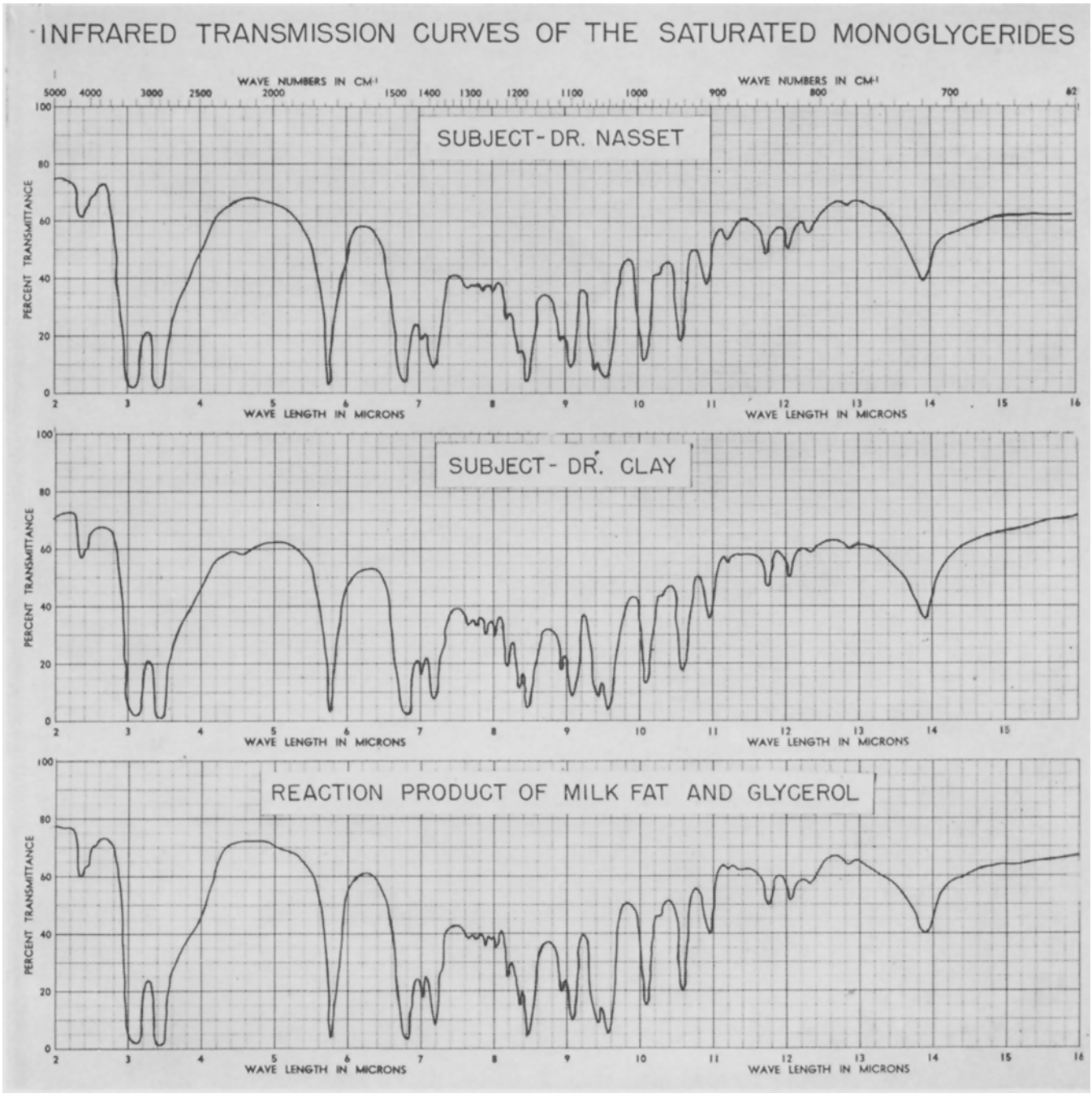

FIG. 1. Infrared spectra of saturated monoglycerides isolated from intestinal contents and from reaction product of milk fat and glycerol.

SECTION F

Identification of Monoglycerides by Chemical Methods

Identification by Saponification. The monoglycerides of the saturated fatty acids and of the unsaturated fatty acids were expected to be predominantly those of the saturated and unsaturated fatty acids which occur in milk fat. The average composition of the saturated and unsaturated acids of milk fat reported by Bailey (2) is presented in Table VI.

To identify further the extracted and purified samples as monoglycerides of these particular acids, several samples were split into fatty acids and glycerol. The identification of fraction NK-52B serves as an example of the analytical methods.

Isolation of Saturated Acids—NK-52B. A 0.2153-gram sample of the Skellysolve F insoluble fraction, NK-52B, prepared from the intestinal contents of subject, Dr. Nasset, was saponified, using 0.3 gram of potassium hydroxide dissolved in 1 ml. of water and 1 ml. of ethanol. The mixture was heated under reflux for one hour. Three ml. of water were added, and the aqueous residue was acidified with dilute sulfuric acid. The precipitated acids were taken up in ethyl ether and washed repeatedly until free of mineral acid. The ether solution was dried with sodium sulphate and the ether removed under nitrogen. The

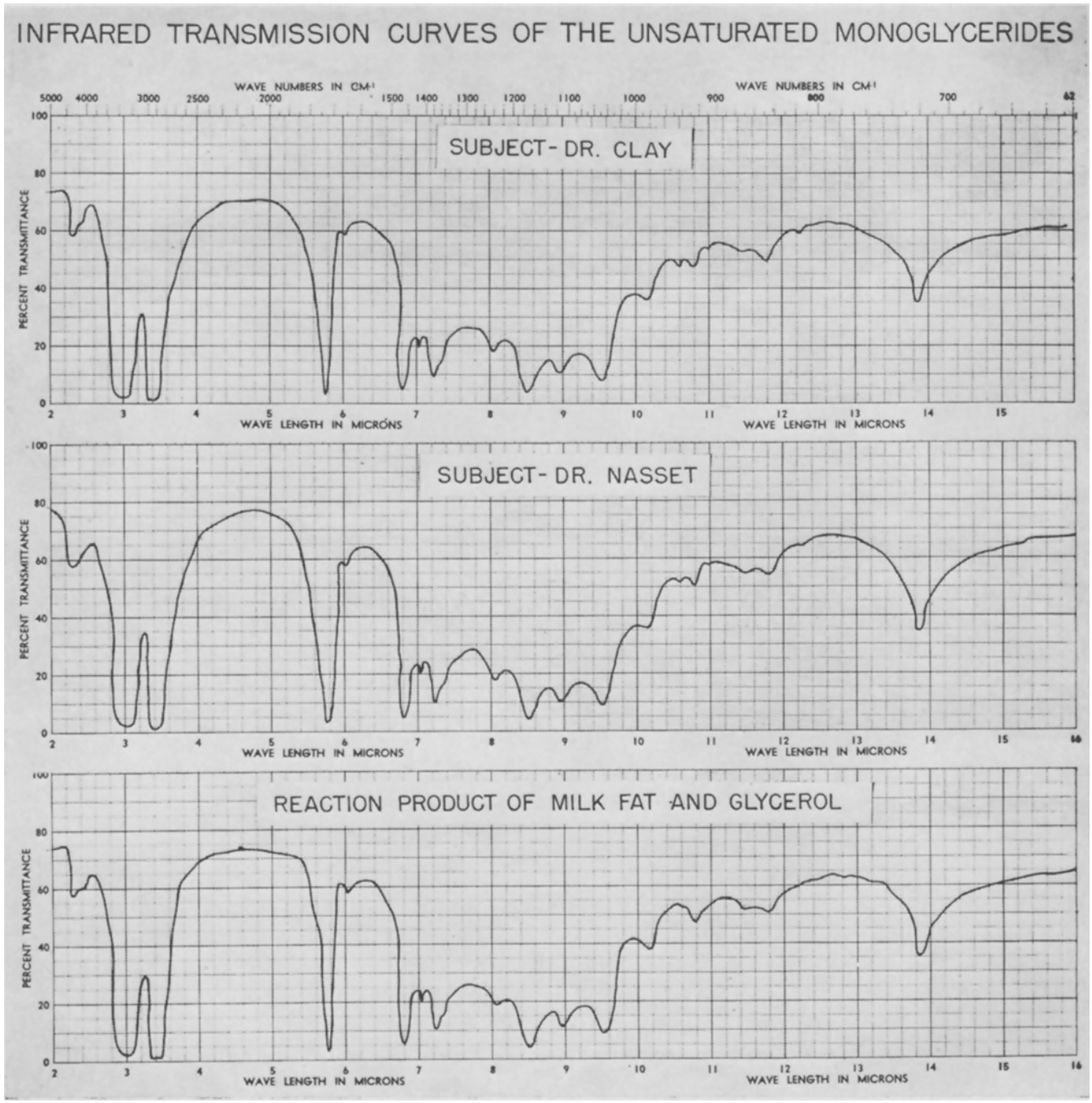

FIG. 2. Infrared spectra of unsaturated monoglycerides isolated from the intestinal contents and from reaction product of milk fat and glycerol.

dried weight was 0.159 gram or 74% (theory for saturated acids in the monoglycerides of milk fat, 75.7%). The neutralization value of the acids was 240 (theory for the saturated acids of milk fat, 243).

Isolation of Glycerol—NK-52B. As an added check, an attempt was made to recover most of the small quantity of glycerol from the aqueous phase.

The water-soluble materials and all the washings from the saturated acids were dried under vacuum. The salt residue was extracted with ethanol. The ethanol extracts were evaporated, and the resulting syrupy residue accounted for 25.9% of the original material (theory for glycerol in the saturated monoglycerides of milk fat, 30.2%). By periodic acid oxidation (4) this material was found to be 96.5% glycerol. It is assumed that some of the glycerol was lost in the various collection and evaporation steps.

The unsaturated monoglyceride fraction extracted from the intestinal contents of Dr. Nasset and the saturated and unsaturated monoglyceride fractions extracted from the reaction product of milk fat and glycerol also were examined by saponification with the results reported in Table VII.

Other Chemical and Physical Properties. The melting points of the three saturated monoglyceride fractions obtained by the capillary method and reported in Table I are all similar. The purity of all the saturated and unsaturated monoglyceride fractions was

further established by determination of the percentage of free fatty acids and free glycerol as reported in Table I.

Identification of the Low Molecular Weight Unsaturated Monoglycerides. The countercurrent distribution of the unsaturated monoglycerides prepared from the reaction product of milk fat and glycerol resulted in an appreciable amount of material in the first three tubes.

A 0.014-gram of the first three tubes from the countercurrent distribution of the 70% methyl alcohol-soluble fraction prepared from the reaction product of milk fat and glycerol was saponified, using 0.1 gram of potassium hydroxide dissolved in 1 ml. of water and 1 ml. of ethyl alcohol. The mixture was heated under reflux for one-half hour. Two ml. of water were added, and the aqueous residue was acidified with dilute sulfuric acid. The supernatant acid layer was taken up in ether and washed repeatedly. The ether solution was dried and the ether removed under nitrogen. The residue weighed 0.0103 gram, or 73.5% of the original material. The neutralization value was 235.8, and the calculated average molecular weight was 239.

The monoglyceride content of the combined material in the first three tubes of the countercurrent extraction was 94%, calculated as the monoglycerides of the acids with average molecular weight of 239.

SECTION G

Examination of Monoglyceride Fractions by Infrared Spectrophotometry

The instrument, techniques, and lists of wavelengths are the same as those used in the preceding paper (14).

Crystalline Saturated Monoglycerides. The spectra (Figure 1) of both crystalline saturated monoglycerides isolated from the samples of intestinal contents compared very well with the chemically prepared product, NK-66. All of the peaks listed previously for saturated monoglycerides were found in the samples without any shift in wavelength. A few minor variations occurred in absorption intensities, but no peaks disappeared and no new peaks appeared. There were minor absorption differences of weak peaks between 7.60μ and 8.30μ. NK-52B has a stronger peak at 11.36μ which is very weak in NK-66. A weak shoulder peak at 10.35μ is weaker in NK-52B. Nujol mulls of these samples were also prepared and found to give good quality characteristic spectra.

Liquid Unsaturated Monoglycerides. The spectra (Figure 2) of the unsaturated monoglycerides checked very well with that of the chemically prepared product, NK-65A, and the previously described spectra of unsaturated monoglycerides. The main difference between the unsaturated monoglycerides was produced by the small amount of solid monoglycerides that was present in all samples including the standard. The low molecular weight monoglycerides constituting the first three tubes of the countercurrent extraction of NK-65A had a spectrum that was the most characteristic for unsaturated monoglycerides since it had only a small amount of solids.

TABLE VI

The Saturated and Unsaturated Fatty Acid Composition of Milk Fat

Acid	Composition of fatty acids	Contribution to average molecular weight
	%	
Saturated acids		
Butyric	4.9	4.4
Caproic	2.0	2.3
Caprylic	2.4	3.5
Capric	3.7	6.3
Lauric	6.4	12.7
Myristic	20.7	42.7
Palmitic	42.7	109.0
Stearic	14.8	42.2
C_{20} saturated	2.2	6.9
Average molecular weight	....	230.0
Unsaturated acids		
Decenoic	1.0	1.7
Dodecenoic	0.7	1.4
Tetradecenoic	5.1	11.5
Hexadecenoic	19.4	49.3
Octadecenoic	63.6	180.0
Octadecedienoic	7.1	20.0
C_{20} and C_{22} unsaturated	3.1	9.7
Average molecular weight	....	273.6

SECTION H

Countercurrent Extraction Experiments

A countercurrent distribution was made on each of the purified monoglyceride fractions on a 50-tube apparatus described by Craig and Post (5). The solvent system used in the distribution was Skellysolve B and aqueous methanol solution containing 15% water by volume; the procedure is described in detail in the preceding paper (14).

The curve for NK-52A in Figure 3 was developed from 0.1029 g.; the peak in the curve, including the extrapolation of the sides to zero ordinate, contains 0.096 grams or 93.3% of the fraction. The curve for NK-52B was developed from 0.1002 g.; the peak in the curve including the extrapolation of the sides to zero ordinate contains 0.0942 grams or 94% of the fraction.

The curve for NK-66 in Figure 4 was developed from 0.0999 g.; the peak in the curve, including the extrapolation of the sides to zero ordinate, contains 0.0922 grams or 92.4% of the fraction. The curve for NK-65A was developed from 0.1033 g.; the peak at

TABLE VII

Identification of Monoglycerides by Saponification

Sample	Fatty acid recovery		Neutralization value		Glycerol recovery	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
	%	%			%	%
Saturated monoglycerides						
Intestinal contents—Dr. Nasset	74.0	75.7[a]	240	243[a]	25.9	30.2[a]
From the reaction product of milk fat and glycerol	74.2	75.7[a]	239	243[a]	25.3	30.2[a]
Unsaturated monoglycerides						
Intestinal contents—Dr. Nasset	80.5	78.7[b]	199	205[b]	19.2	26.4[b]
From the reaction product of milk fat and glycerol	80.6	78.7[b]	204	205[b]	19.0	26.4[b]

[a] Calculated as the monoglycerides of saturated acids of milk fat.
[b] Calculated as the monoglycerides of unsaturated acids of milk fat.

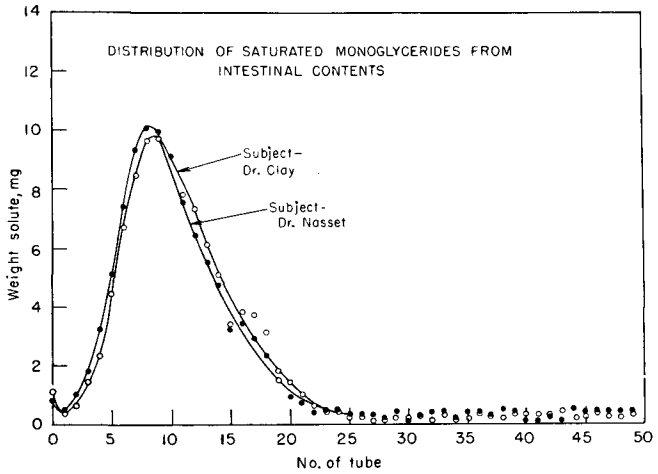

FIG. 3. Countercurrent extraction curves for the saturated monoglycerides isolated from intestinal contents.

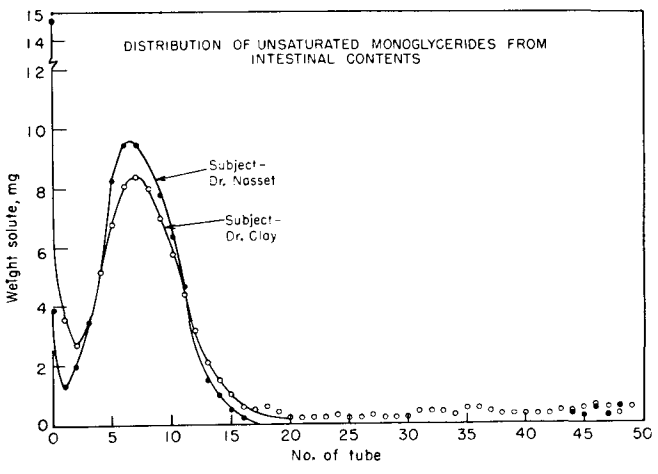

FIG. 5. Countercurrent extraction curves for the unsaturated monoglycerides isolated from intestinal contents.

tube 7 in the curve, including the extrapolation of the sides to zero ordinate, contains 0.062 grams or 60% of the fraction. That portion of the curve represented by the first three tubes was examined. A sample was saponified and the average molecular weight of the fatty acids determined (see Section F). The monoglyceride content by the periodic acid oxidation analysis was 94%. The infrared analysis of the contents of the first three tubes likewise indicated practically pure unsaturated monoglycerides. This quantity of low molecular weight, unsaturated acid monoglycerides amounts to 0.0186 grams, or 17.7% of the fraction. Thus the total monoglycerides as shown by the countercurrent distribution amounts to 77.7%.

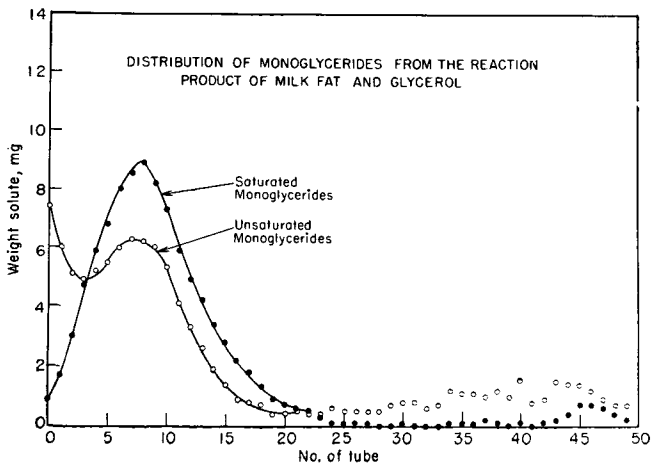

FIG. 4. Countercurrent extraction curves for saturated and unsaturated monoglycerides isolated from reaction product of milk fat and glycerol.

The curve for NK-56A in Figure 5 was developed from 0.0982 g.; the peak at tube 7, including the extrapolation of the sides to zero ordinate, contains 0.0682 grams or 69.5% of the fraction. That portion of the curve represented by the first tube and portion of the second tube, while not examined, can be assumed to be similar to the low molecular weight acid monoglycerides found in milk fat and would amount to 0.0184 gram or 18.8% of the fraction. The total monoglycerides would then be 88.3%. The curve for NK-56C was developed from 0.0795 g.; the peak at tube 7, including the extrapolation of the sides to zero ordinate, contains 0.0712 gram or 89.6% of the fraction.

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