AN ABSTRACT OF THE THESIS OF

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in General Science (Physical Science) presented on _February 26, 1981_

Title: URINARY METABOLITES OF BROMINATED FATTY

ACIDS-IDENTIFICATION AND KINETICS IN RATS.

Abstract approved: _____ Redacted for Privacy _____

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The rate of bromide excretion and identity of metabolites in
urine has been studied in rats fed corn oil (CO), brominated corn oil
(BCO), and the monoglycerides of dibromo-(DBS) and tetrabromostearate
(TBS). Inorganic bromine in the urine was determined by treating
with dimethyl sulfate in an acidic medium to form volatile methyl
bromide, which was then isolated from the sample via a head-space
technique, separated by gas chromatography and detected by flame
ionization. A linear relationship between detector response of the
head-space methyl bromide and bromide concentration in the liquid
phase was obtained with the concentration range studied. Prior to
treatment the bromide concentration in urine averaged 0.31 ± 0.11 mM
with an average daily excretion of 2.33 ± 0.79 μmoles of bromide.
Cumulative data indicated that 57 to 77% of the ingested bromine
was excreted as inorganic bromide in 144 hours. This suggested
the presence of an active debromination system in the rat. After 144
hours the urinary bromide concentrations had essentially returned to
the control levels. Maximum concentrations of bromide were observed
in urine 72 hours after treatment.

The urinary metabolites were identified using gas-liquid chromatography and mass spectrometry. Seven short-chain dicarboxylic acids and five brominated dibasic acids were found in the urine. 2,3-Dibromo-suberic acid, 3,4-dibromo-azelaic acid and 2,3-dibromo sebacic acid were detected in the urine of rats from all three treatment groups while 2-bromo-dodecanodioic acid and 2,3-dibromo-5-dodecenedioic acid were detected only in the urine of rats ingesting BCO. Formation of these metabolites from brominated fatty acids must involve ω-oxidation along with a mechanism for losing single carbons such as α-oxidation which played a minor role in the formation of these metabolites.

Urinary Metabolites of Brominated
Fatty Acids - Identification and Kinetics
in Rats

by

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Typed by Mary K. Paul for   Vidal Eliezer Sotillo

To my mother Bruna, my wife Delcy, parents, sisters and brother

ACKNOWLEDGEMENTS

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INTRODUCTION

Use and Distribution of Brominated Oils

Brominated vegetable oils are used in several foods to adjust
the density of flavoring oils and to enhance cloud stability in
beverages (Jacobs, 1959). Being of higher density than normal
oils a stable cloudy emulsion is achieved and the entrapped flavoring
components are also protected from oxidative degradation. In 1973
permission to use brominated vegetable oil in an amount not to exceed
15 ppm was extended pending the outcome of long term toxicity studies.
In 1974 on the basis of preliminary information permission to continue
using brominated vegetable oil was extended (Fine, 1974). Brominated
oils are listed as flame retardants (Kuryla, 1973) although the extent
of use for this purpose is not documented. There is also circumstantial
evidence that brominated fatty acids may occur as natural constituents
of oils from marine organisms (Lunde, 1972, 1973a; Tinsley and Lowry,
1980). The origin of these compounds in the marine environment is
an important question.

Toxicity of Brominated Oils

Young male rats showed reduced growth rate and enlargement
of liver and heart when they were fed 2.5% brominated cottonseed oil
for 80 days (Munro et al., 1969, 1972a). The increase in liver size

was associated with an accumulation of lipid. The increase in heart

size was also observed when the level of brominated cottonseed oil

was reduced to 0.5% of the diet. Microscopic examination of the heart

showed fatty degeneration involving the entire myocardium.

Gaunt et al. (1971a) feeding brominated maize oil at 0.05, 0.020, and

0.80% of the diet for 90 days produced comparable lesions in liver and

heart at the 0.80% level but growth rate was not affected. There

was some indication that the incidence of the heart lesions was influenced

by the composition of the brominated oil.

Munro et al. (1972b) observed a lower incidence of heart lesions

with brominated olive oil compared to brominated corn, sesame, or

cottonseed oils. Since the linoleate content is lowest in olive oil, the

data would suggest that a tetrabromostearate, derived from linoleate,

may be more active than a dibromostearate derived from oleate.

Gaunt et al., (1971a, 1971b) found lipid bound bromine in most

tissues analyzed, accumulating to a maximum concentration in about

13 weeks in adipose tissue, after feeding brominated oil. The nature

of the bromine containing compounds was not established.

It is clear that brominated oils produce significant pathological

changes in heart and other tissues, it is not known what components

of the treated oil are responsible.

Toxicity of Brominated Fatty Acids

Morrinson (1946) demonstrated that the oral toxicity of halogenated

fatty acids to mice increased with increasing atomic weight of the

of the halogen but decreased with increasing length of the carbon chain.

This means that the toxicity of α-bromo acids decreased with increasing chain length; the α-substituted acids were more toxic than their β-substituted counterparts. Le Poindevin (1965), working with shorter-chain fatty acids brominated on the 2-carbon, found that toxicity decreased as the chain length increased until minimum toxicity was reached at bromovaleric acid (C_5), further increases in chain length to carbon ten resulted in increased toxicity. On a molar basis the toxicity of 2-bromo acetate and 2-bromo decanoate were equivalent although a different mechanism of action was suggested.

Little if any information is available on the toxicological effects of brominated acids present in brominated oil, the dibromostearate and the tetrabromostearate. If the toxic effect of the oil is really greater than that observed with the bromostearates, one would assume that the oil contained other active constituents. These questions need to be resolved before the toxic hazard of these compounds to humans can be defined.

Metabolism of Substituted Fatty Acids

Deuel (1957) reported that in branched chain fatty acid metabolism in mammals, whenever there is a hindrance of β-oxidation, that ω-oxidation plays a much larger role in metabolizing these fatty acids. Therefore, a dicarboxylic acid of shorter chain length is formed and then eliminated through the urine as a conjugated or a free dicarboxylic acid.

Cyclopropene fatty acids (CPFA) are naturally occurring fatty acids found in cottonseed oil, kapok oil, and okra which contain a highly strained and reactive unsaturated three membered ring in the center of an 18 carbon (malvalic) or 19 carbon (sterculic) chain. CPFA are important because they exert a marked synergistic effect on Aflatoxin B_1 carcinogenesis (Lee et al., 1968, Lee et al., 1971). CPFA inhibit the liver fatty acid desaturase system, decrease the hepatic mixed function oxidase activity and cause cytological damage in liver parenchymal cells (Lee et al., 1968).

The metabolism of cis and trans 9, 10-methylene octadecanoic acid in rat adipose tissue was studied by Wood and Reiser, (1965). The main metabolites were cis-and trans-3,4-methylene dodecanoic acid. These metabolites result from the β-oxidation system continuing down the fatty acid chain with large amounts of the material accumulating in the adipose tissue. Nixon et al., (1977) studied the metabolism of [9-10-methylene-^{14}C] sterculic acid in corn oil and sterculia foetida oil fed rats. The majority of the label was excreted in the urine as short-chain dicarboxylic acids with an intact cyclopropane ring. The main metabolites were cis-3,4-methylene adipic acid and cis-3,4-methylene suberic acid. Formation of these metabolites requires β-and ω-oxidation plus reduction of the cyclopropene ring to a cyclopropane ring.

Possible routes for the endogenous formation for some short chains fatty acids are either ω-oxidation of long chain fatty acids (Preiss and Bloch, 1964) followed by β-oxidation or ω-oxidation of medium chain fatty acids (Mitz and Heinrikson, 1961).

Metabolism of Brominated Fatty Acids

The retention of lipid bound bromine in storage lipids of rats
(Gaunt et al., 1971a) suggests that mobilization of brominated fatty
acids may be slower than that of non-brominated fatty acids and that
general lipid mobilization is not necessarily impeded by brominated
fatty acids . The ratio of brominated fatty acids to total free fatty
acids should decrease through the period of high plasma free fatty
acid levels if bromination retards the progress of release. The studies
are too general in nature to confirm this conclusion.

The retention of lipid bound bromine after feeding of brominated
fatty acids may result from impaired metabolism of the substituted
fatty acids. Such a perturbation disarrangement could displace the
equilibrium among storage, mobilization, transport, and oxidation to
result in a net decrease in removal from storage.

Mohamed et al., (1980) studied the metabolism of brominated acids
by isolated mitochondria, the major site of fatty acid oxidation. The
product of bromination of palmitoleic acid (9, 10-dibromo palmitic
acid) could not undergo normal β-oxidation when incubated with
mitochondria isolated from hamster brown adipose tissue. It was
established that the conditions used for bromination of palmitoleic
acid did not introduce bromine at other than the 9, 10-positions.

Bromooctanoate inhibits oxidation of long and medium chain fatty
acids in perfused rat liver and in mitochondria isolated from rat liver.
Raaka and Lowenstein (1979a) presented evidence obtained in Vitro that

mitochondria convert bromooctanoate to bromo-3-ketooctanoyl-CoA, an α-haloketone which is probably the active form of the inhibitor. They also presented evidence that various enzymes of the β-oxidation pathway can use 2-bromooctanoyl-CoA and its β-oxidation products as substrates. Binding of mitochondrial CoA in the form of bromooctanoyl-CoA did not appear to be the cause of the inhibition (Raaka and Lowenstein, 1979b). Bromooctanoate did inactivate acyl-CoA: Acetyl-CoA-acyl transferase (Middleton, 1973). This enzyme catalyzes cleavage of substrates with 3-ketoacyl groups of four or more carbons (Seubert et al., 1968).

The potential for human exposure to brominated oils has been reviewed and the fact that these compounds can produce cellular changes in rats has been established. However, the toxicologically active components of the oils have not been defined. In view of the fact that the majority of the bromine is associated with di and tetrabromostearates (derived from oleic and linoleic acids) it is logical to expect that these would be the active components. To date this has not been established and studies are in progress in this laboratory to correct this deficiency. The following study was designed to:

1. Establish the presence of bromide ion in the urine of animals fed brominated oils or monoglycerides, thus providing evidence of a active debromination mechanism in the rat.

2. Identify the brominated organic metabolites of the brominated fatty acids in the urine and postulate a mechanism as to their formation.

A part of this analysis explored in this thesis research has been concerned with definition of the metabolism of single dose of brominated fatty acids in the rat in vivo. Rats were fed a single dose of brominated corn oil, the monoglyceride of dibromostearate or the monoglyceride of tetrabromostearate and the rate of excretion of inorganic bromide monitored. In addition, the identity of organic metabolites of the brominated metabolites was established. Such studies indicate the involvement of different metabolic pathways and provide a basis for more comprehensive in vitro studies.

MATERIAL AND METHODS

Materials

Brominated Compounds

Mazola corn oil was used to prepare brominated corn oil. Olive oil was used to prepare the monoglyceride of dibromostearate. Safflower oil was used to make the monoglyceride of tetrabromostearate.

Reagents and Standards

Sodium bromide   (J.T. Baker Analyzed Reagent Grade).

Dimethyl sulfate (Aldrich Chemical Co.).

Sulfuric acid (American Scientific & Chemical Reagent Grade).

Methanol (Analytical Reagent, Mallinckrodt Chemical Works).

Ether (Analytical Reagent, Mallinckrodt Chemical Works).

Hexane (Phillips Co.).

Dimethyl succinate (Eastman).

Dimethyl adipate (Eastman).

2,3-dibromo succinic acid (Pfaltz & Bauer, Inc.).

Brominated trans-3-hexenedioic acid (made in lab. 3/9/79).

Screw cap microreaction vials (one ml) with PTFE lined silicone septums were obtained from Kontes Scientific Glassware/instrument; and gas tight, push-button valve syringe (five ml) was obtained from Precision Sampling Corporation.

Preparation of Brominated Derivatives

Oleic and linoleic acids were isolated from olive and safflower oils (no preservatives) respectively by urea adduction. Saponified olive and safflower oils were previously washed with 50/50 ether: hexane.

The monoglycerides were prepared in a glycerol esterification vessel (10 in. deep, 7 3/8 in. inside diameter), mixer blade 2 5/8 in. above bottom with maxima capacity of 3500g (fatty acid and glycerol). Nitrogen bleed was added for unsaturated acids (Feuge et al., 1945; McCutcheon, 1955).

Prior to initiating the preparation of the brominated derivative of monoglyceride, a sample was methylated and analyzed on the GC to determine the relative fatty acid composition of the oil. The amount of Br_2 required from the composition of the oil was 51.244 ml Br_2/mole.

Three hundred grams of oil were weighed out into a 5,000 ml. round bottom flask and dissolved in approximately one liter of diethyl ether.

The entire reaction mixture was cooled to 0°C with a salt-ice bath.

Bromine was added, dropwise with stirring maintaining the temperature between 0-10°C. The tip of the dropping funnel was just above the surface of the oil. The oil was stirred for an additional 45-60 min. at 0°C until a red-yellow color persisted, indicating a slight excess of bromine.

The oil was washed with acidic sodium sulfite (2% + HCL) until the solution was clear, to remove excess bromine and then washed with three portions of water.

After drying over sodium sulfate, the solvent was removed as rapidly as possible on a ratatory evaporator to avoid loss of bromine from the oil.

Feeding Procedures and Urine Collection

Twelve male OSU Wistar rats weighing 75-90 grams, were used throughout this study. All animals were fed a semi-synthetic powered diet (Tinsley and Lowry, 1972; Appendix I), containing 10% corn oil for 24 hours prior to transfer to metabolism cages, and collection of a 24 hour control urine sample. The animals were returned to hanging cages and fed ad lib. the control diet and water for three days. After starving for 24 hours the rats were divided into three experimental groups of four each being fed for one hour diets containing:

1. 10% corn oil.
2. 8% corn oil + 2% brominated corn oil.
3. 8% corn oil + 2% dibromostearyl glycerol.
4. 8% corn oil + 2% tetrabromostearyl glycerol.

Food intakes were recorded. The animals were placed in metabolism cages given control diet and water, and the urine was collected after 6, 24, 48, 72, and 144 hours.

To increase the level of brominated derivatives in urine, all animals were returned to their respective experimental diets for an additional two weeks, after which time 48-hour, urine samples were collected.

Analytical Procedures

Inorganic Bromide Analysis

A variety of gas chromatographic methods have been used for the determination of bromide (Archer, 1972; Corina et al., 1979) in biological fluids with limited success due to the complicated procedures used to form derivatives. Archer's method involved the oxidation of the bromide to bromine followed by addition to an unsaturated compound and subsequent assay of the bromo derivative by GC. Maiorino et al., (1980) have developed a simpler, more sensitive procedure for analysis of bromide for use in in vivo pharmacological studies. Bromide is converted to methyl bromide which is analyzed by a headspace gas chromatographic technique using a flame ionization detector (FID). A simpler but less sensitive method than Maiorino's for the determination of trace amounts (down to 0.05ppm) of bromide in water is reported (Nota et al., 1979). Bromide is converted to cyanogen bromide (Schülek et al., 1951).

$$Br^- + 2\ Cl_2 + 2\ CN^- \longrightarrow BrCN + ClCN + 3Cl^-$$
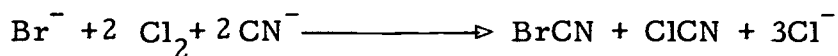
which is separated by gas chromatography and selectively detected with an electron capture detector.

The procedure used has been described by Maiorino et al., (1980). Vials, sulfuric acid, and methylating agent (dimethyl sulfate) were placed in a freezer for 45 minutes. 500 microliters of cold concentrated sulfuric acid were placed into one milliliter screw cap microreaction vials with PTFE lined silicone septums. 100 microliter aliquants of urine were pipetted into the vials followed by 100 microliters of dimethyl sulfate. The vials were sealed, mixed and heated at 60°C for 20 minutes. After completion of the reaction the vials were cooled at room temperature for 30 minutes, and one milliliter of head-space vapor withdrawn slowly (30 sec.) with a gas-tight syringe and injected onto the gas chromatograph for methyl bromide detection.

Known concentrations (0.1-5mM) of sodium bromide were prepared with distilled water. 100 microliter aliquants of sodium bromide were pipetted into vials containing 500 microliters of cold concentrated sulfuric acid and then treated as above. Peak areas were measured by 3352 B-Operator Commands Data System. Bromide concentrations were determined from a standard calibration curve.

For the head-space technique (methyl bromide analysis) a Varian Aerograph series 1400 gas chromatograph interfaced to a 3352 B-Operator Commands Data System was used. The instrument was fitted with a flame ionization detector, and a six-foot x 1/8 inch (i.d.) nickel column containing Porapak Q (100-120 mesh). The column was maintained at 110°C, the injection port at 165°C, and the detector at 170°C. Flow rates of nitrogen (carrier), hydrogen, and air were, 40, 30, and 300 ml/min respectively.

<u>Methyl Ester Derivatives</u>:

Gas-liquid chromatography

The urine sample was acidified to pH 1.0 by addition of 1M perchloric acid and held frozen at - 20°C. The samples were thawed, extracted with three volumes of diethyl ether and the ether extract methylated for gas-liquid chromatography analysis according to Lowry, 1977 (Appendix II).

The compounds were separated in a HP Model 700 gas chromatograph fitted with spoon injection system and using a 200 ft. capillary column coated with Apiezon L. The instrument had a hydrogen flame ionization detector and helium was the carrier gas. The column temperature was maintained at 170°C, and a sample size of five microliters was used.

Verification of the identity of the different compounds was done by gas - liquid chromatography followed by mass spectrometry. An instrument consisting of a gas chromatograph (four-foot Pyrex column filled with non-polar 7% OV-101 on Gas-Chrom 750) combined with a low resolution mass spectrometer (Varian 787 magnetic instrument) and operated with an ionization energy of 70 eV was used. The temperature was programmed from 50° to 300° at a rate of 10°C per minute.

RESULTS

Inorganic Bromide Excretion

Bromide Analysis Calibration Curve

A typical calibration curve and a gas chromatogram of the methyl bromide are shown in Figure 1. A linear relationship between detector response of the head-space methyl bromide and bromide concentration in the liquid phase exists within the concentration range studied. Chromatograms derived from urine of treated and untreated animals (Figure 2) illustrate the effectiveness of the procedure in discriminating between chloride and bromide.

Bromide Excretion in Urine from Treated Rat

Background bromide concentrations were determined in 24 hour urine samples taken before treatment with brominated compounds In the twelve rats the bromide concentration in urine averaged 0.30 ± 0.11 mM   with an average daily excretion of 2.33 ± 0.79 μ moles of bromide (Table 1).

Bromide levels in the urine of rats treated with brominated corn oil, and the monoglycerides of dibromo-and tetrabromostearate are summarized in Table 2. It can be seen from Figure 3. That after 144 hours the urinary bromide concentrations had essentially returned to the control levels. Maximum concentrations of bromide were observed in urine 72 hours after treatment.
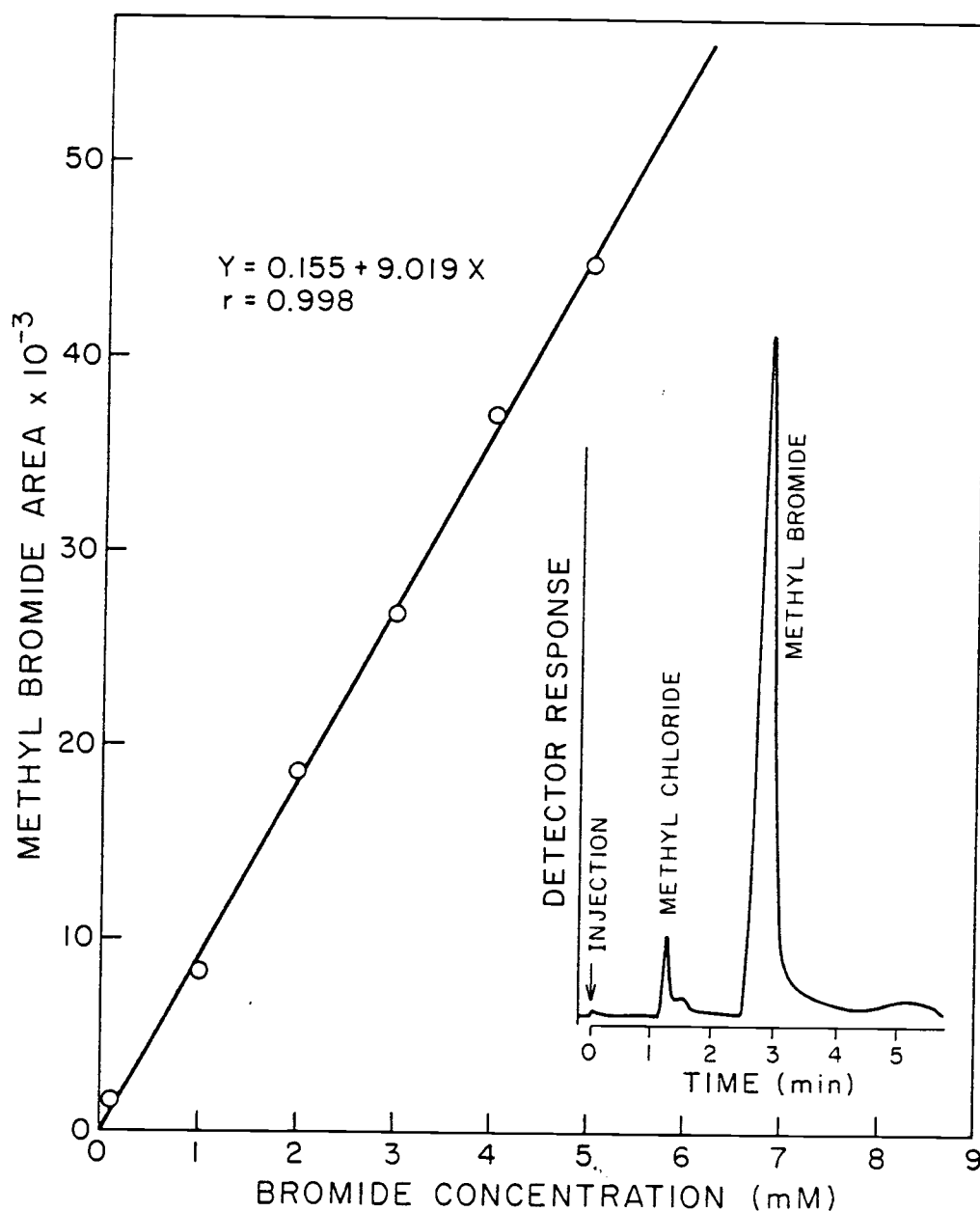
Figure 1. Gas chromatographic calibration curve for the determination of bromide in urine obtained by plotting peak area of methyl bromide per one ml. head-space gas against bromide concentration and typical gas chromatogram of the head-space gas obtained by injecting one ml. of standard sodium bromide 1mM.
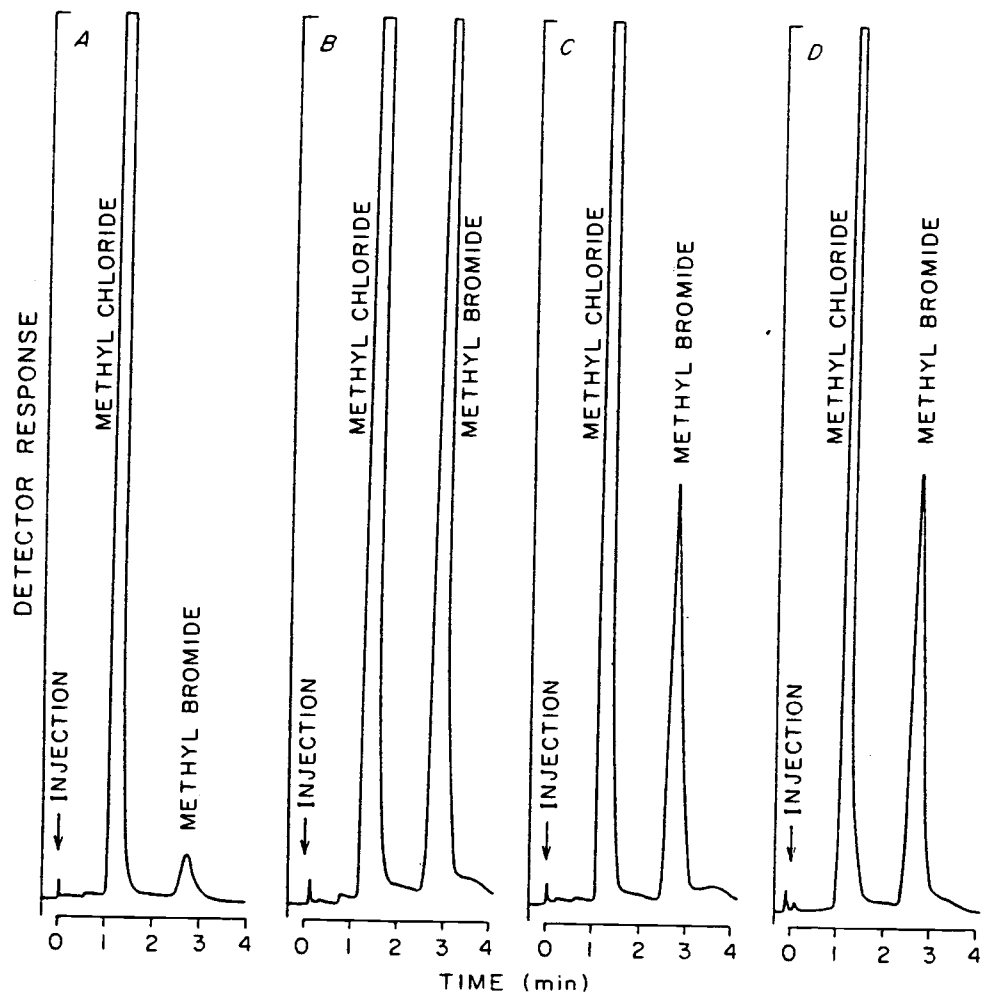
Figure 2. Typical gas chromatgrams derived from untreated 24 hour urine sample 10% corn oil (A) and treated animals with brominated corn oil (B), monoglyceride of dibromostearate (C) and monoglyceride of tetrabromostearate (D).
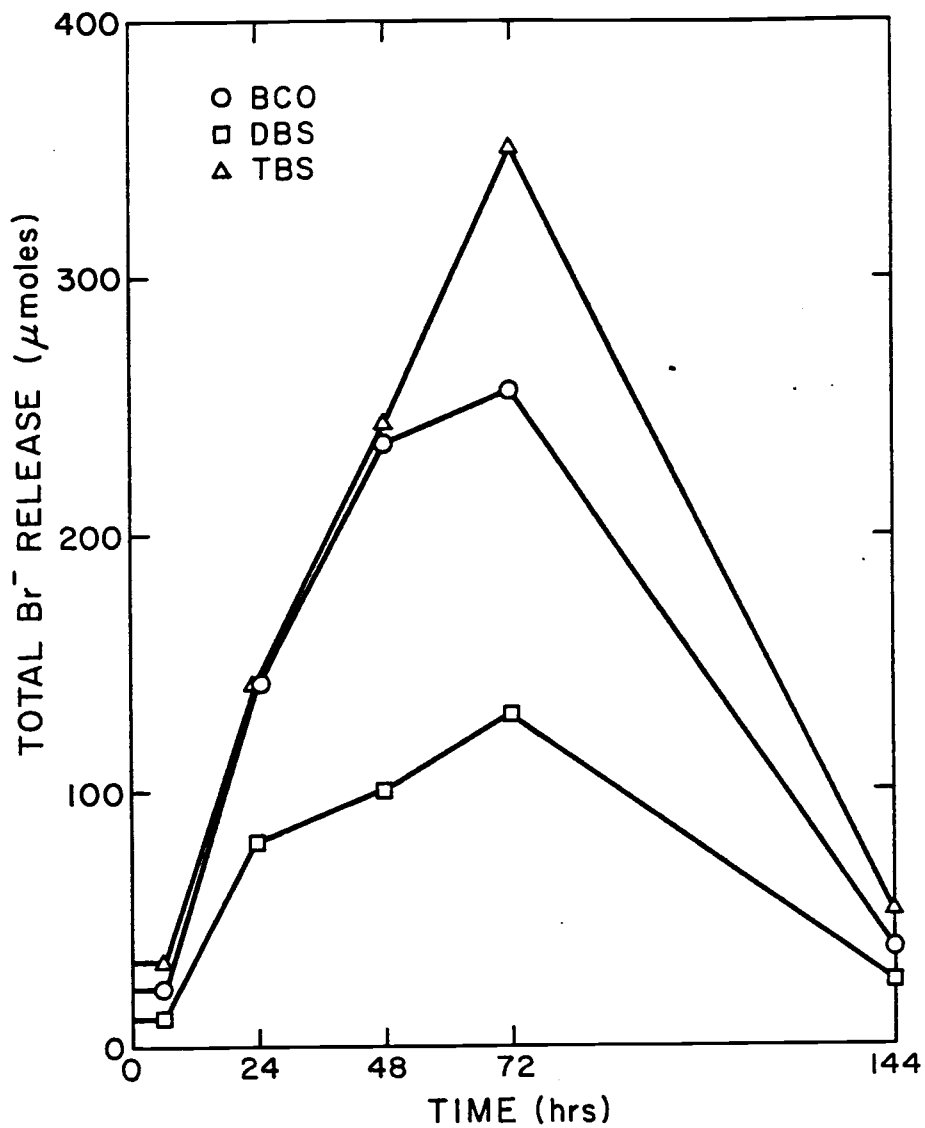
Figure 3. Gas chromatographic determination of the total bromide released from urine of rats treated with brominated corn oil, and the monoglycerides of dibromo and tetrabromostearate at different periods of times.

Table 1. Bromide concentration determined in 24 hour urine samples
before treatment with brominated compounds.

Urine Sample	ml Urine	Bromide conc. mM	Bromide daily excret. μ moles
1	10.7	0.30	3.20
2	9.4	0.40	3.76
3	9.8	0.33	3.23
4	12.2	0.24	2.98
5	4.0	0.57	2.26
6	6.0	0.23	1.35
7	8.1	0.20	1.63
8	9.7	0.16	1.53
9	6.2	0.40	2.48
10	7.0	0.25	1.72
11	5.2	0.38	1.99
12	7.2	0.25	1.83
Mean		0.31	2.33
SD		±0.11	±0.79

Table 2. Bromide levels in the urine of rats treated with brominated corn oil, and the monoglycerides of dibromo and tetrabromostearate.

Urine Sample	Total Br Intake μmoles	6 hours ml urine	Br⁻ conc. μmoles/ml	Total Br⁻ μmoles	24 hours ml urine	Br⁻ conc μmoles/ml	Total Br⁻ μmoles	cumulative Br⁻ excreted μmoles	48 hours ml urine	Br⁻ conc μmoles/ml	Total Br⁻ μmoles	cumulative Br⁻ excreted μmoles
BCO	830	1.3	22.30	29.0	8.1	16.63	135.0	164.0	11.0	26.88	296.0	460.0
	1200	0.6	19.50	11.7	11.0	16.14	178.0	189.7	6.0	41.79	251.0	440.7
	1070	1.5	20.00	30.0	2.9	33.49	97.0	127.0	2.6	56.62	147.0	274.0
	950				11.1	14.92	166.0	166.0	10.9	23.08	252.0	418.0
Mean	1010		20.6	24.0		20.30	144.0	161.7		37.09	237.0	398.2
SD				10.3				25.9				84.5
DBS	810	2.0	6.80	13.6	3.0	28.57	85.7	99.3	3.9	31.00	121.0	220.3
	650	10.5	1.20	12.6	12.3	7.80	95.7	108.3	12.0	10.26	123.0	231.3
	570	1.5	6.20	9.3	2.6	19.90	51.8	61.1	3.0	21.50	65.0	126.1
	490	1.5	6.00	9.0	11.8	7.49	88.4	97.4	11.0	8.20	90.0	187.4
Mean	630		5.05	11.0		15.94	80.0	91.5		17.74	100.0	191.3
SD				2.3				20.8				47.3
TBS	1080	4.1	6.80	27.9	5.9	27.73	164.0	191.9	8.5	32.33	275.0	466.9
	1210	1.3	27.80	36.1	4.4	28.64	126.0	162.1	2.5	36.33	303.0	465.0
	1080	2.0	18.40	36.8	6.0	24.52	147.0	183.8	10.5	29.80	313.0	496.8
	1210	0.3			6.1	24.25	148.0	148.0	7.0	34.78	243.0	391.0
Mean	1145		17.70	34.0		26.29	146.3	171.5		33.31	283.5	454.9
SD				5.0				20.1				45.0

Table 2. Bromide levels in the urine of rats treated with brominated corn oil, and the monoglycerides of dibromo and tetrabromostearate. (Continued)

| | 72 hours | | cumulative | 144 hours | | | cumulative | |
ml urine	Br conc μmoles/ml	Total Br μmoles	Br excreted μmoles	ml urine	Br conc μmoles/ml	Total Br μmoles	Br excreted μmoles	Br excreted μmoles
18.5	11.91	220.0	680.0	28.1	1.80	50.5	730.5	
13.5	22.03	297.0	737.7	26.0	2.08	50.4	788.1	
6.6	40.74	269.0	543.0	18.1	1.43	25.8	568.8	
11.0	21.80	240.0	658.0	43.7	0.37	16.0	674.0	
	24.12	257.0	654.7		1.42	36.0	690.4	
			80.4				93.5	690.4
9.9	16.45	163.0	383.0	22.0	1.37	30.0	413.3	
12.8	12.81	164.0	395.3	31.0	0.73	22.7	418.0	
4.5	22.09	99.0	225.1	14.2	1.92	27.3	252.4	
12.5	8.24	103.0	290.4	31.3	0.62	19.5	309.0	
	14.90	132.0	323.5		1.16	25.0	348.4	
			80.6				81.1	
								348.4
11.3	30.24	342.0	808.9	20.0	3.60	72.0	880.9	
15.0	24.85	373.0	838.0	24.8	1.56	38.6	876.7	
23.5	17.35	408.0	904.8	31.0	1.17	35.1	939.9	
13.5	29.04	392.0	783.0	18.8	3.53	66.3	849.3	
	25.37	378.8	833.7		2.47	53.0	886.7	
			52.5				38.1	886.7

Cumulative plots of bromide excretion (Figure 4) indicate that 57 to 77% of the ingested bromine is excreted as inorganic bromide in 144 hours. This suggests the presence of an active debromination system in the rat.

Steady State Bromide Concentraiton in Urine

It is assumed that concentrations of bromide and organobromides approach steady - state concentrations in urine after a longer feeding period. Concentrations of bromide in urine from animals fed the brominated derivatives for two weeks are sumarized in Table 3. Daily excretion rates are substantially higher than those observed in animals receiving a single dose (Table 2).

Identification of Dibasic Acids in Urine

The non-brominated and brominated dibasic acids found in the urine of rats treated with brominated corn oil, and the monoglycerides of dibromo and tetrabromostearate are shown in Table 4 and 5.

Non-brominated Compounds

A typical gas chromatogram of methylated urine extract from a rat fed brominated corn oil (Figure 5), presented excellent resolution for the components eluted for the first half of the chromatogram. However, short chain fatty acids below C_4 are not distinguished from the solvent peak and the last five components of interest have
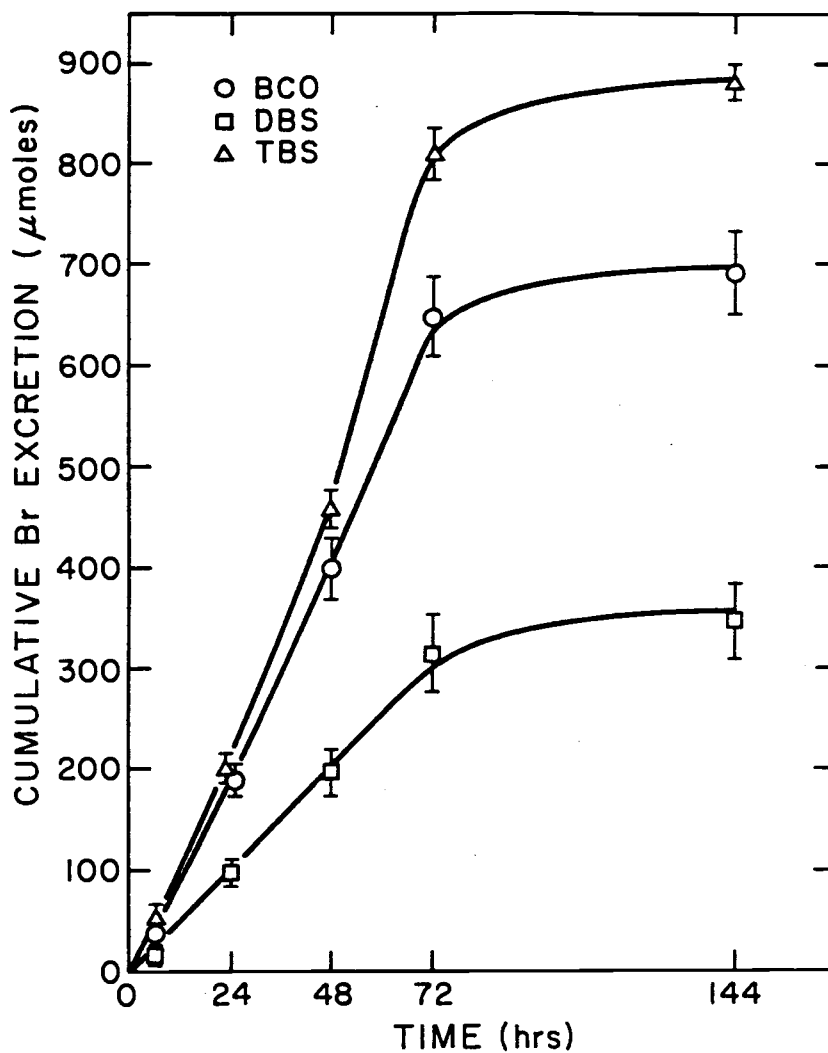
Figure 4. Cumulative bromide excretions against time after treatment with brominated corn oil, and the monoglycerides of dibromo and tetrabromostearate.

Table 3. Steady state bromide concentration in the urine of rats
         treated with brominated corn oil, and the monoglycerides
         of dibromo and tetrabromostearate for two weeks.

Urine Sample	Total Br Intake μmoles/day	ml Urine/48 hrs.	Bromide conc mM	Bromide daily excret. μ moles
BCO	1773.0	30.0	225.6	3385.0
	1510.0	22.0	374.8	4125.0
	1704.5	24.0	323.4	3880.0
	1711.0	30.0	255.4	3830.0
Mean	1674.6		294.8	3800.0
SD			±67.23	±308.3
DBS	1131.0	29.5	196.4	2895.0
	1047.0	30.0	198.7	2980.0
	798.5	22.0	272.2	2990.0
	853.5	30.5	135.7	2070.0
Mean	957.5		200.8	2730.0
SD			±55.8	±445.0
TBS	1730.5	28.0	366.1	5125.0
	1808.5	31.0	324.5	5030.0
	1672.5	29.5	340.4	5020.0
	1692.0	47.0	215.3	5060.0
Mean	1725.9		311.6	5061.0
SD			±66.4	±47.4

BCO: Brominated corn oil

DBS: Monoglyceride of dibromostearate

TBS: Monoglyceride of tetrabromostearate

Table 4. Urinary metabolites of non-brominated compounds in brominated corn oil, and the monoglycerides of dibromo-tetrabromostearate fed rats.

Peak #	Relative Ret.time	Molecular Weight	Formula	Name (dimethyl ester)
1	0.54	146	$CH_3-O-\underset{\underset{O}{\|\|}}{C}-CH_2-CH_2-\underset{\underset{O}{\|\|}}{C}-O-CH_3$	Succinate
2	0.82	160	$CH_3-O-\underset{\underset{O}{\|\|}}{C}-CH_2-CH_2-CH_2-\underset{\underset{O}{\|\|}}{C}-O-CH_3$	Glutarate
3	1.00	174	$CH_3-O-\underset{\underset{O}{\|\|}}{C}-CH_2-(CH_2)_2-CH_2-\underset{\underset{O}{\|\|}}{C}-O-CH_3$	Adipate
4.	182	188	$CH_3-O-\underset{\underset{O}{\|\|}}{C}-CH_2-(CH_2)_3-CH_2-\underset{\underset{O}{\|\|}}{C}-O-CH_3$	Pimelate
5	282	202	$CH_3-O-\underset{\underset{O}{\|\|}}{C}-CH_2-(CH_2)_4-CH_2-\underset{\underset{O}{\|\|}}{C}-O-CH_3$	Suberate
6	498	216	$CH_3-O-\underset{\underset{O}{\|\|}}{C}-CH_2-(CH_2)_5-CH_2-\underset{\underset{O}{\|\|}}{C}-O-CH_3$	Azelate
7	600	230	$CH_3-O-\underset{\underset{O}{\|\|}}{C}-CH_2-(CH_2)_6-CH_2-\underset{\underset{O}{\|\|}}{C}-O-CH_3$	Sebacate

Table 5. Urinary metabolites of brominated compounds in brominated corn oil, and the monoglycerides of dibromo-tetrabromostearate fed rats.

Peak	Molecular Weight	Formula	Compound Name (dimethyl ester)	Diet		
				BCO	DBS	TBS
A	338	$CH_3-O-\underset{O}{\overset{Br}{C}}-CH-(CH_2)_8-CH_2-\underset{O}{C}-O-CH_3$	2-bromo-dodecanodioic acid	X		
B	362	$CH_3-O-\underset{O\ Br}{C}-\overset{Br}{CH}-CH-CH_2-(CH_2-)_3\underset{O}{C}-O-CH_3$	2,3-dibromo-suberic acid	X	X	X
C	376	$CH_3-O-\underset{O}{C}-CH_2-\overset{Br}{CH}-\underset{Br}{CH}-CH_2-(CH_2-)_3\underset{O}{C}-0-CH_3$	3,4-dibromo-azelaic acid	X	X	X
D	390	$CH_3-O-\underset{O}{C}-\overset{Br}{CH}-\underset{Br}{CH}-(CH_2)_6-\underset{O}{C}-O-CH_3$	2,3-dibromo sebacic acid	X	X	X
E	416	$CH_3-O-\underset{O}{C}-\overset{Br}{CH}-\underset{Br}{CH}-CH_2-CH=CH-(CH_2)_5-\underset{O}{C}-O-CH_3$	2,3-dibromo-5-dodecenedioic acid	X		

BCO: Brominated corn oil

DBS: Monoglyceride of dibromostearate

TBS: Monoglyceride of tetrabromostearate

25.
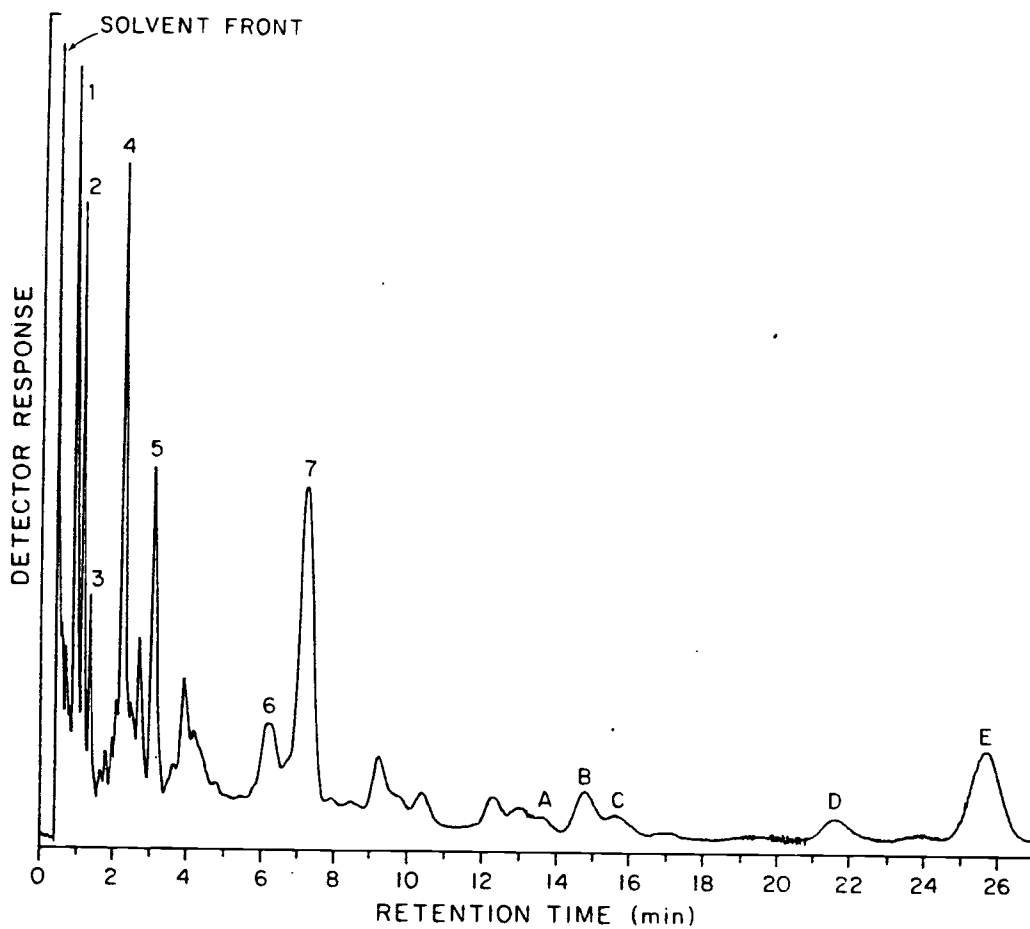
Figure 5. Typical gas chromatogram of methylated urine extract from a rat fed brominated corn oil.

longer retention times so that their peaks are very broad. In the chromatogram mentioned above were identified seven non-brominated short chain fatty acids (Table 4) by comparison of retention times of standards as well as by mass spectra.

The mass spectrometry investigation of the methyl esters of short chain fatty acids and their major ion fragmentation are summarized in the following steps:

a. Cleavage of butoxy radicals from the molecular ions appears to produce the base peaks at m/e M-73

b. Ions at m/e M-101 are probably produced by the α-cleavage of the acid alkyl group (R) from the molecular ions. Ions at m/e M-55 logically result from a similar cleavage of a butyl group followed by a double hydrogen rearrangement (McLafferty, 1963)

An example of a proposed fragmentation mechanism for a non-brominated compound is shown in Appendix III.

Brominated Compounds

Peaks labelled with letters A through E (Figure 5) were analyzed by MS. Table 5 shows the distribution of these five compounds in the urine of rats fed the BCO, DBS, and TBS diets. Peak B, C and D were found to have identical mass spectra as those of the corresponding peaks of DBS and TBS fed rats.

The mass spectra and their interpretation for the brominated fatty acid metabolites found in the urine are shown in Figures 6, 7, 8, 9 and 10. The most intense peaks were the same for the five compounds, m/e 55(I), 59(II), and 74(III).

$$CH_2 = CH - C \equiv \overset{+}{O}$$

I (m/e 55)

$$CH_3 - O - \overset{+}{\underset{\underset{O}{\parallel}}{C}} -$$

II (m/e 59)

$$CH_3 - O - \overset{+}{\underset{\underset{OH}{\mid}}{C}} = CH_2$$

III (m/e 74)
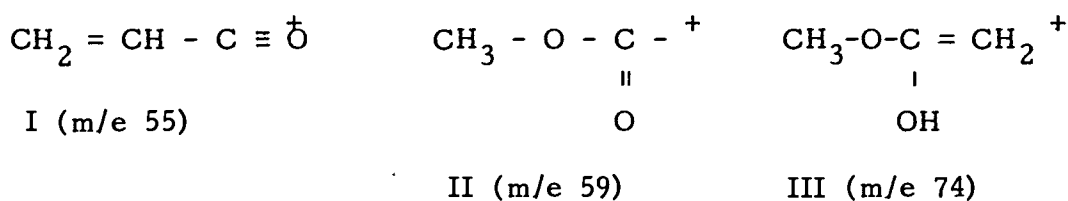
In addition, the fragmentation patterns and the very characteristic isotope distribution of bromine, confirmed the presence of these five brominated fatty acids in the urine. The fragment ion at m/e 79 corresponded to $\overset{+}{Br}$ and the presence of the fragment ion at m/e 81; m/e 95 - 97, m/e 96 - 98 etc. were indicative of isotopic pattern of bromine - 81 (Table 6).

Table 6. Mass fragment characteristic of methyl ester of dibasic acids and brominaned compound (Ryhage and Stenhagen, 1959; McLafferty, 1963; Beynon et al. 1968).

m/e	Fragment lost	Remarks
M-31	$-OCH_3$	Characteristic of all methyl esters of dibasic acid.
M-32	$-OCH_3+H$	
M-60	$-COOCH_3+H$	This peak is prominent only for short chain esters.
M-63	$(-OCH_3)_2+H$	Prominent for all esters except methly adipate.
M-64	$(-OCH_3)_2+2H$	
M-74	$-CH_2-COOCH_3$	Characteristic of all methyl esters.
M-91	$-COOCH_3+-OCH_3+H$	M-92 is higher than M-91 from methyl azelate upwards.
M-92	$-COOCH_3+-OCH_3+2H$	
M-105	$-CH_2-COOCH_3+-OCH_3+H$	Low for methyl adipate, high for higher esters.
M-106	$-CH_2-COOCH_3+-OCH_3+2H$	Marked only for very long chain esters.
M-79/81	^{79}Br and ^{81}Br	Characteristic of all methyl ester of brominated dibasic acids.
M-$^{80/82}_{81/83}$	$-HBr$, H_2Br	
M-93/95	$[-CH_2-Br]+$	
M-105/107	$[-CH=CH-Br]^+$	
M-165/167	$[-CH_2-\underset{Br}{CH}-COOCH_3]$	

Peak A

The mass spectrum (Figure 6) of peak A (Figure 5) shows
fragments characteristic of methyl esters dibasic acids (Table 6).
The most prominent series of peaks, however, are from the ions of
masses 84, 98, 112, 126 etc. The peak due to the rearranged
ion of m/e 74 (McLafferty, 1963), the series of peak 59, 87, 101,
115 .... due to methylester fragment ions and the loss of methoxy, and
two hydrogen atoms are also found. However, the peak heights of
the methoxy carbonyl ions decrease in a regular manner with increasing
mass of the ion. The parent peak is not present in the MS of
compound A. Based on these observations, the molecular weight of
compound A would be m/e 310. The proposed fragmentation ions
are shown in Scheme 1.

Scheme 1. Fragmentation ions for compound A.

$$CH_3-O-\overset{\overset{\displaystyle}{\underset{\displaystyle O}{\parallel}}}{C}\!-\!\underset{153}{\overset{Br}{CH}}\!-\!CH_2-CH_2-CH_2-(CH_2)_3\!-\!CH_2-CH_2-CH_2-\overset{\overset{\displaystyle}{\underset{\displaystyle O}{\parallel}}}{C}\!-\!O-CH_3$$

(cleavage labels: 59, 79/81, Br, 167, 181, 195, 201, 87, 73, 59, 31)

M=338

$[CH=CH-Br]^+$

$\{CH_3-O-\overset{\overset{\displaystyle Br}{|}}{\underset{\underset{\displaystyle O}{\parallel}}{C}}-CH-\}^+$

$\{CH_3-O-\overset{\overset{\displaystyle Br}{|}}{\underset{\underset{\displaystyle O}{\parallel}}{C}}-CH-CH_2\}^+$

m/e 105/107   m/e 151/153   m/e 165/167

$\{CH_3-O-\overset{\overset{\displaystyle Br}{|}}{\underset{\underset{\displaystyle O}{\parallel}}{C}}-CH-CH_2-CH_2\}^+$ and by adding $-CH_2-$ the fragments will
be m/e 195, m/e 209, m/e 223, m/e 237
and m/e 251

$\{CH_3-O-\overset{\overset{\displaystyle Br}{|}}{\underset{\underset{\displaystyle O}{\parallel}}{C}}-CH-CH_2-CH_2-CH_2-CH_2\}^+$ $+\{-\overset{\overset{\displaystyle}{\underset{\displaystyle OH}{|}}}{C}-O-CH_3\}^+ = $ m/e 268/270

$M-\{[CH_3-OH]+CH_3\}^+$
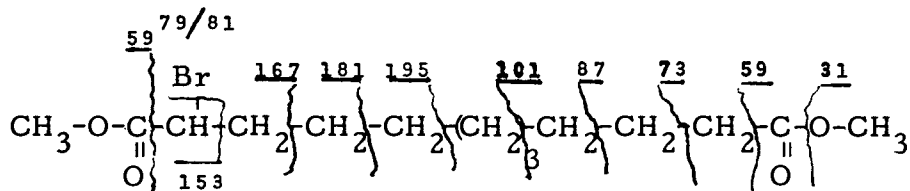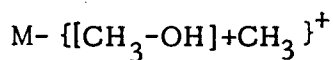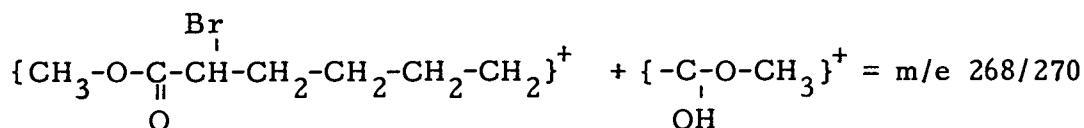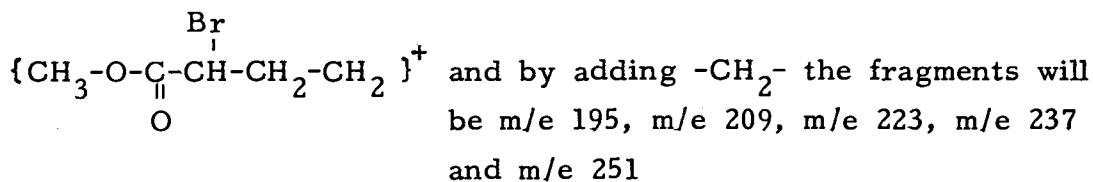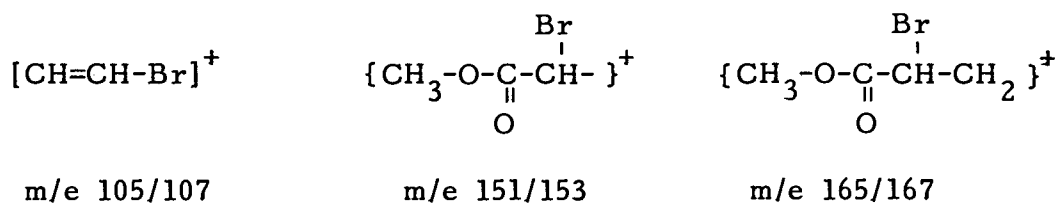
m/e 291/289

Peak B

The mass spectrum (Figure 7) also shows fragments characteristic of methyl esters of dibasic acids (Table 6): $[M-31]^+$ (m/e 327/329/331), $[M-Br]^+$ (m/e 279/281), $[M-HBr]^+$ (m/e 278/280), $\{M-[Br+32]\}^+$ m/e 247/249), and $\{M-[Br+64]\}^+$ (m/e 215/217) are present. The series m/e 53, 67, 81, and 95 corresponding to the empirical formula $[C_n H_{2n-5}O]^+$ and the series 71, 85, 99, and 113 corresponding to $[C_n H_{2n-3}O_2]$ are also present. The m/e 74 representative of the McLafferty rearrangement of typical metheyl esters is moderately intense (40%), this would suggest that at least three saturated carbons exist adjacent to one of the ester ends. Scheme 2 shows the fragmenation ions for compound B.

Figure 7. Mass spectrum, molecular weight and formula of compound B found in two percent BCO, DBS, and TBS diets.

Scheme 2. Shows the proposed fragmentation ions for compound B..

$$CH_3-O-C-CH-CH-(CH_2)_4-C-O-CH_3$$

with labels: 79/81 Br above; 31, 59, 153, 247, 85 along the chain; O and Br below.

M=362

$[CH_2-Br]^+$ $[CH_3-Br]$ $[Br-CH-CH-CH_2]^+$ $[Br-CH-CH_2-Br]^+$

m/e 93/95   m/e 94/96   m/e 119/121   m/e 187/189

$[M-Br]^+$ $\{M-[HBr+CH_3O]\}^+$ $[CH_3-O-\overset{\text{O}}{\underset{\|}{C}}-CH-Br]^+$

m/e 279/281   m/e 247/249   m/e 151/153

$\{CH_3-O-\underset{O}{\overset{Br}{C}}-CH-CH_2\}^+$ Loss of one molecule of methoxy carbonyl

m/e 165/167   and two molecules of bromine show

m/e 219/221

$\{M-[Br+[CH_3-OH] + [-COOCH_3+H]\}^+$

m/e 187/189
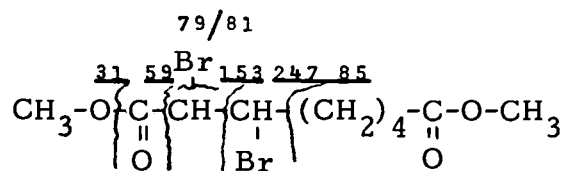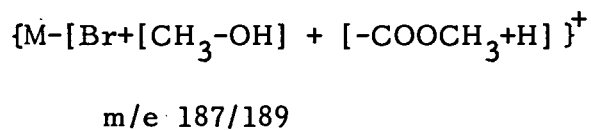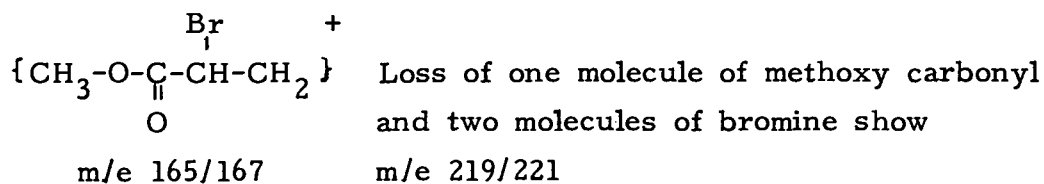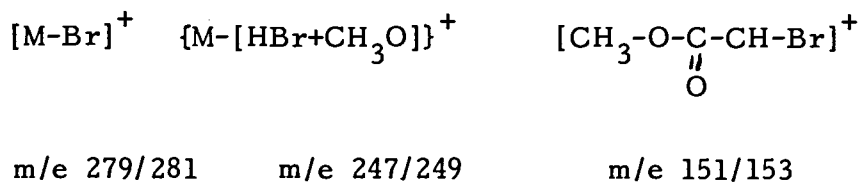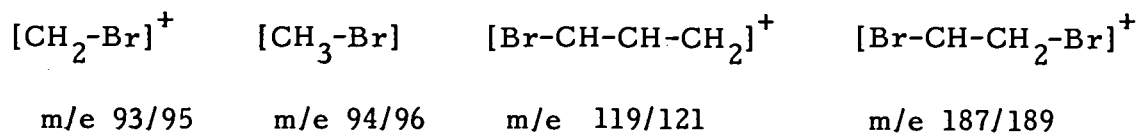
Peak C

Compound C shows the typical mass spectrum (Figure 8) fragmentation pattern of methylated dibasic acids (Table 6): [M-31] m/e 341/343/345, [M-Br] m/e 293/295, [M-Br-32] m/e 261/263, [M-Br-64] m/e 229/231, [M-Br-32-60] m/e 201/203, and [M-2Br-H] m/e 213) are present. The series m/e 53,67, 81, 95, and 109 corresponding to the empirical formula $[C_nH_{2n-5}O]^+$ and the series 71, 85, 99, 113, and 127 corresponding to $[C_nH_{2n-3}O_2]^+$ are also present. Scheme 3 shows the fragmentation ions for compound C.
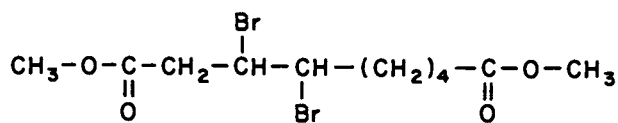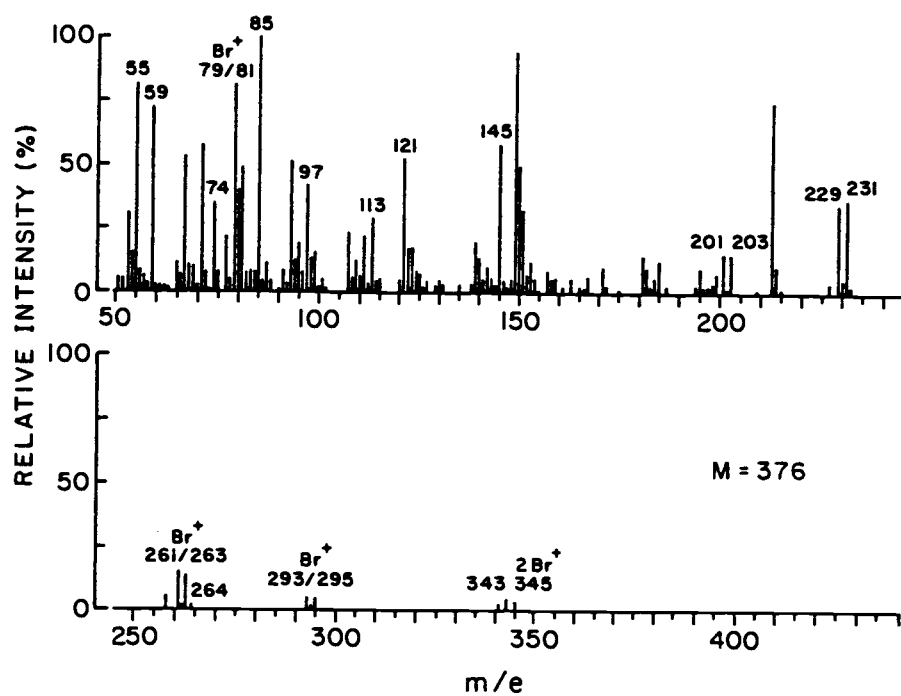
Figure 8. Mass spectrum, molecular weight, and formula of compound C found in the urine of rats treated with BCO, DBS, and TBS.

Scheme 3.   Fragmentation ions for compound C.

$$\overset{79/81}{}$$

$$CH_3-O\overset{31}{|}C\overset{59}{|}CH_2\overset{74}{|}CH\overset{Br}{|}CH\overset{168}{|}\overset{181}{|}(CH_2)_4\overset{85}{|}C-O-CH_3$$

(structure with Br at top, carbonyl O groups, Br/263 below)

$[CH_2-Br]^+$ $[CH_2-CH_2-Br]^+$ $[CH_3-CH_2-Br]^+$

m/e 93/95   m/e 107/109   m/e 108/110

$[M-CH_3-O]^+$ $[C_2H_6Br]^+$ $M-\{Br+[CH_3-OH]+[COOCH_3+H]\}$

m/e 341/343/345   m/e 109/111   m/e 201/203

$M-\{Br+2[CH_3-OH]\}$ $M-\{Br+[CH_3-OH]\}$ $M-[2Br+H]$

m/e 229/231   m/e 261/263   m/e 213

$$M-\;\{Br-CH-\overset{Br}{CH}-CH\}$$ $[CH_3-O-\overset{}{\underset{O}{C}}-CH_2-CH_2-CH_2-CH_2]$

m/e 175   m/e 115
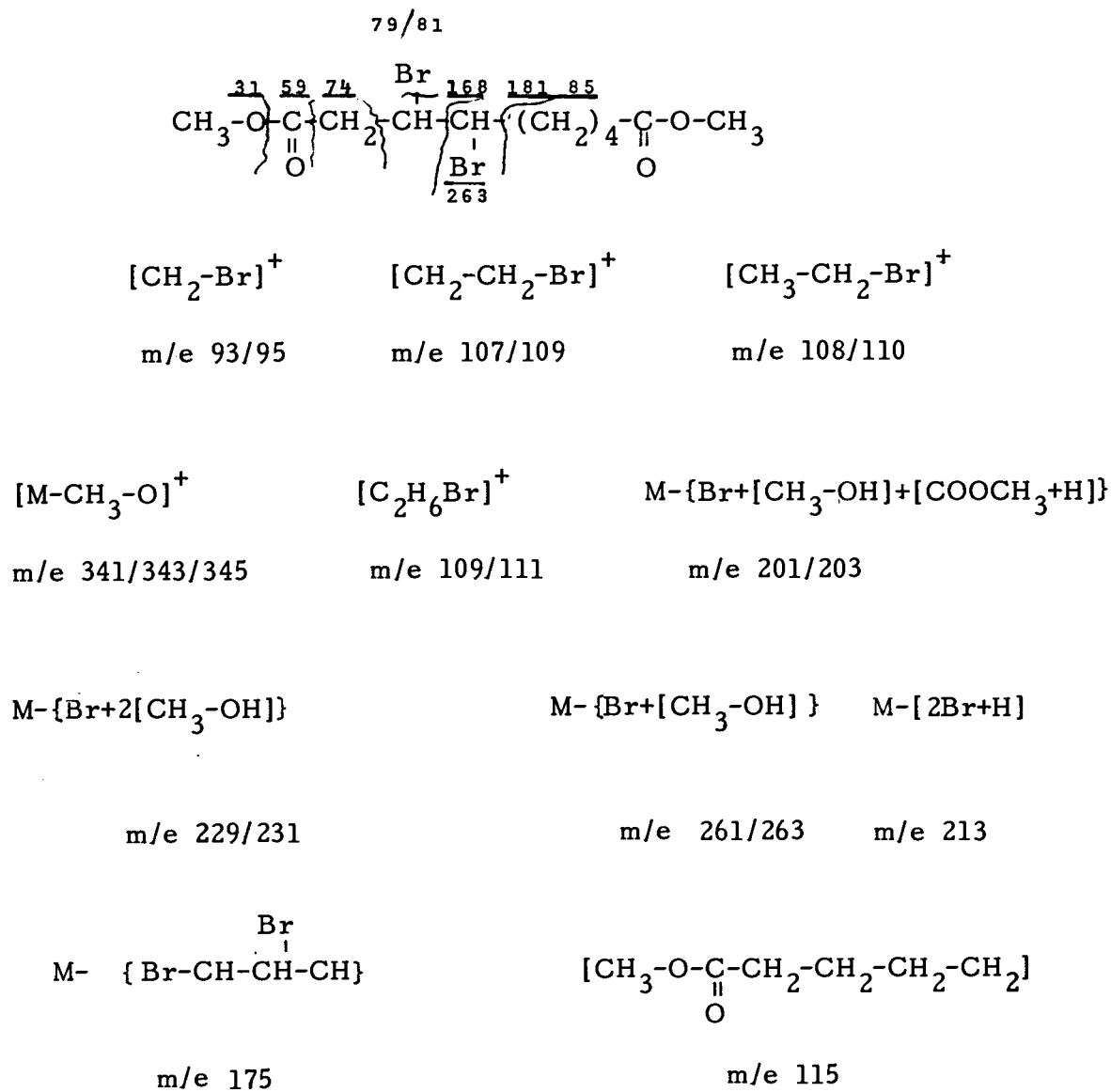
The parent peak is not present in the MS of compound C (Figure 8).

Peak D

The mass spectrum of compound D (Figure 9) shows the typical fragments of bromine and dibasic acid methyl esters: M-31 (m/e 355/357/359), $[M-Br]^+$ (307/309), $[M-HBr-Br]^+$ (m/e 227), and $[CH_2Br]^+$ (m/e 93/95). The parent peak is absent, but would correspond to a molecular weight of m/e 390. The same series $[C_nH_{2n-5}O]^+$ m/e 53, 67, 81, 95, 109, and 123, and $[C_nH_{2n-3}O_2]^+$, m/e 71, 85, 99, and 113 are present. Also, m/e 74 (75%) is very intense indicating a carbon length of three or more from the ester. A probable structure for compound D is shown in Figure 9. The proposed fragmentation ions of this compound are illustrated in Scheme 4.

Figure 9. Mass spectrum, molecular weight, and formula of compound D found in the urine of rats treated with BCO.

Scheme 4. Fragmentation ions for compound D.

$$CH_3-O-\underset{\underset{O}{\|}}{C}\{CH\}CH\{CH_2-CH_2\}CH_2\{CH_2\{CH_2\{CH_2-\underset{\underset{O}{\|}}{C}\{O-CH_3$$

(labels: 59, 79/81 Br, 246, 275, 101, 87, 74, 31; Br; 153)

m/e 390

$[CH_2-Br]^+$ $M-[2Br+H]^+$ $M-\{[CH_3-O]^++Br\}$ $M-\{[CH_3O]+HBr\}$
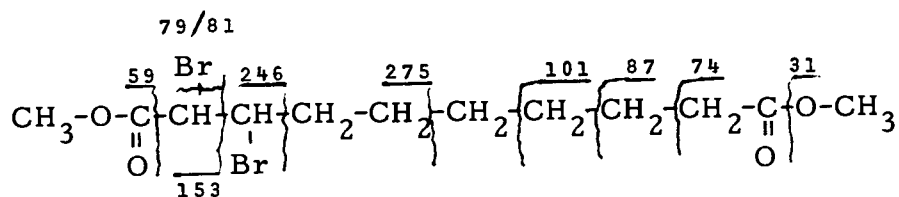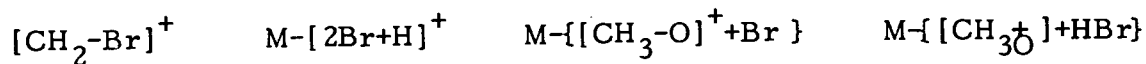
m/e 93/95   m/e 227   m/e 276/278   m/e 275/277

$M-\{Br+2[CH_3OH]^+\}$ $[M-Br]$ $[M-31]$
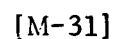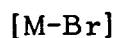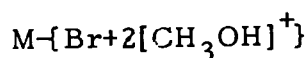
m/e 243/245   m/e 307/309   m/e 355/357/359

$M-\{Br+[CH_3-OH]+[COOCH_3+H]\}$ $M-[2Br+H+CH_3-OH]$

m/e 215/217   m/e 195

$M-\{2Br+H+2[CH_3OH]\}$ $M-\{2Br+H+CH_3OH+[-COOCH_3+H]\}$

m/e 163   m/e 135

$M-\{2Br+H+[CH_3-OH]+[-CH_2-COOCH_3+H]\}$

m/e 121

$M-\{2Br+H+[CH_3-OH]+[-CH_2-CH_2-COOCH_3+H]\}$
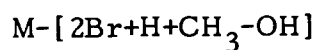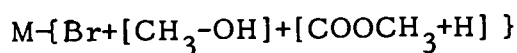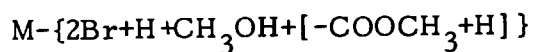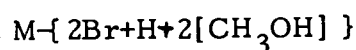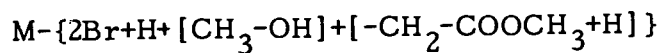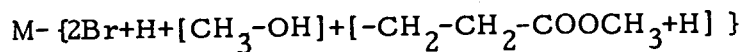
m/e 107

Peak E

The mass spectrum (Figure 10) of peak E (Figure 5) shows fragments characteristic of methyl esters dibasic acids and isotope bromine distribution (Table 6)., The [M-31] ion at m/e 385 was clearly evident as a result of the loss of a methyl group. The fragment ion at m/e 333/335 was the result of elimination of a bromine atom from the parent molecular ion. [M-HBr]$^+$(m/e 334), [M-2Br]$^+$)m/e 254), [M-2HBr] (m/e 252) are present. The fragment ions at m/e 55, 59, 74, 85, 87, 91, 105, and 119 were characteristic of methyl esters, along with the two series $[C_nH_{2n-5}O]^+$ and $[CnH_{2n-3}O_2]^+$. Based on these observations, the molecular weight of compound E would be m/e (385+31) = 416. The parent peak is not present in the mass spectrum of compound E (Figure 10). The proposed fragmentation ions are shown in scheme 5.
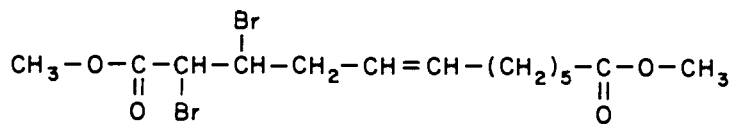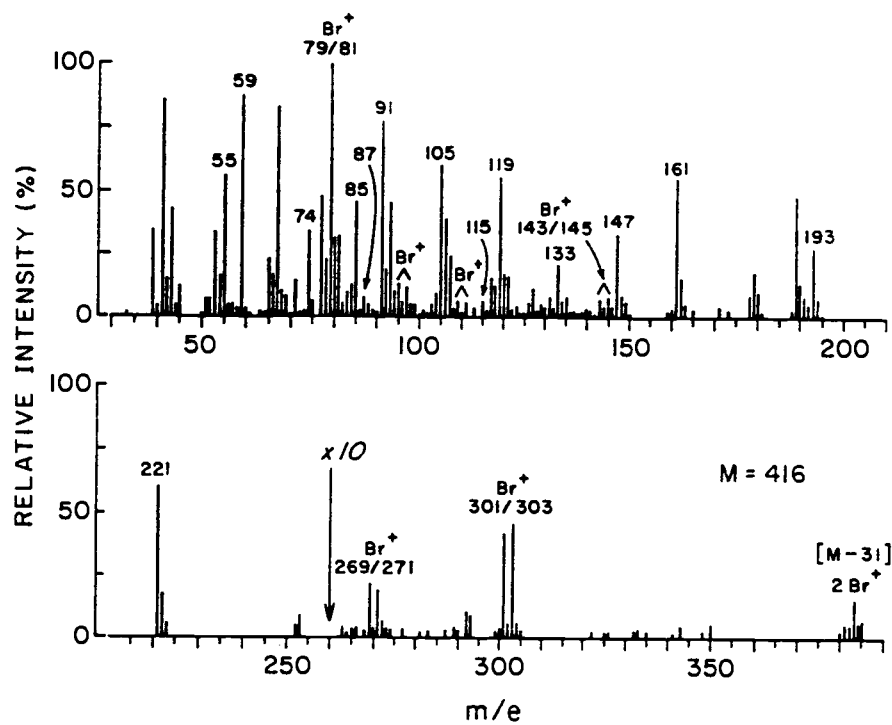
Figure 10. Mass spectrum, molecular weight, and formula of compound E found in the urine of rats treated with BCO.

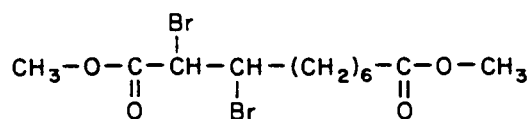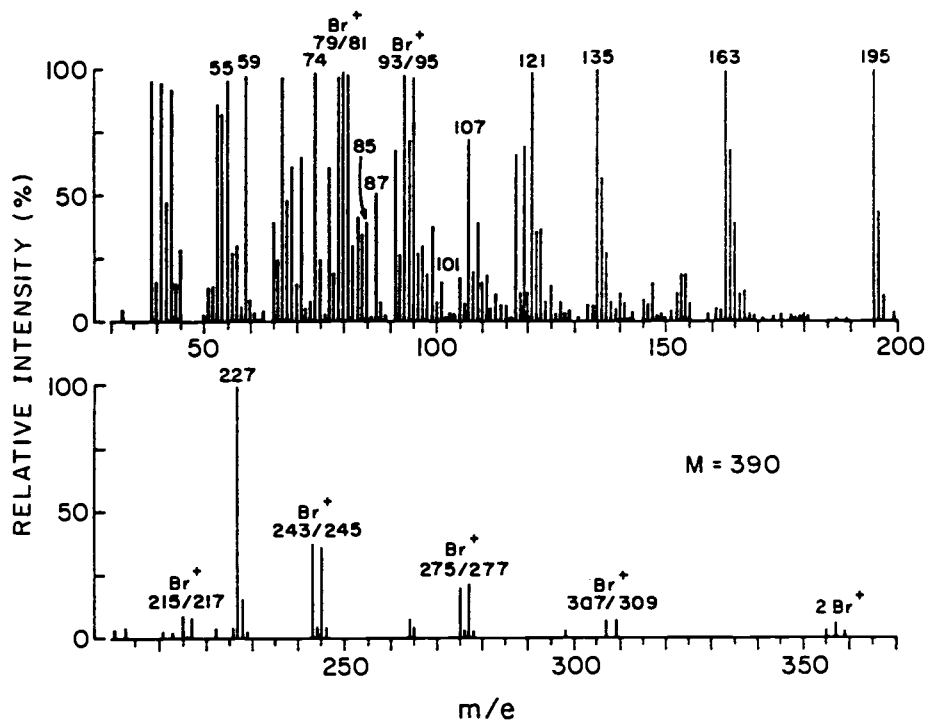## Scheme 5. Fragmentation ions for compound E.

$$\overset{\text{79 81}}{\underset{\text{91 59}}{\text{Br}}}\ \overset{\text{107 109}}{}\ \overset{\text{115 101 87 74}}{}$$

$$CH_3-O-\underset{O}{\overset{}{C}}-CH-\underset{Br}{\overset{}{CH}}-CH_2-CH=CH-CH_2-CH_2-CH_2-CH_2-CH_2-\underset{O}{\overset{}{C}}-O-CH_3$$

M=416

$$[CH_3-O-\underset{O}{\overset{}{C}}-CH_2-CH_2]^+$$
m/e 87

$$[CH_3-O-\underset{O}{\overset{}{C}}-CH_2-CH_2-CH_2]^+$$
m/e 101

$$[CH_3-O-\underset{O}{\overset{}{C}}-CH_2-CH_2-CH_2-CH_2]^+$$
m/e 115

$[CH_4-Br]^+$
m/e 95/97

$[CH_2-CH_2-Br]^+$
m/e 109/111

$[M-Br]^+$	[M-HBr]	$[M-H_2Br]$	[M-2Br]	[M-2HBr]
m/e 335	m/e 334	m/e 333	m/e 254	m/e 252

$M-\{2Br+H+[CH_3-OH]\}$
m/e 221

$M-\{Br+2[CH_3-OH]\}$
m/e 269/271

$M-\{Br-[CH_3OH]+[(-CH_2)_7-COOCH_3+H]\}$
m/e 143/145

[M-31]
m/e 383/385

[M-32]
m/e 382/384

$[M-31-Br]^+$
m/e 302/304

$[M-32-Br]^+$
m/e 301/303

$M-\{2Br+H+[CH_3-OH]+[-COOCH_3+H]\}$
m/e 161

$M-\{2Br+H-[-COOCH_3+H]\}$
m/e 193

$M-\{2Br+H-[CH_3-OH]+[-CH_2-COOCH_3+H]\}$
m/e 147

$M-\{2Br+H+[CH_3OH]+[-CH_2-CH_2-COOCH_3+H]\}$
m/e 133

$M=133+[-CH_2]\}$
m/e 119

$M=133+[-CH_2-CH_2]\}$
m/e 105

$M=133+[-CH_2-CH_2-CH_2]^+\}$
m/e 91

DISCUSSION

When rats are exposed to a single dose of a brominated fatty acid a major proportion of the ingested bromine is recovered as bromide ion in the urine. An effective debrominating process is thus in operation. This could involve the gut microflora since this capability has been demonstrated in bacterial systems (Goldman et al., 1966, 1968). Bromide ion produced in this manner would have to be absorbed and then excreted.

Debromination could also involve the hepatic mixed function oxidase complex. Oxidative dechlorination has been described for a number of short chain aliphatic compounds (Van Dyke and Wineman, 1971). It is assumed that the enzyme system would also be active with brominated analogues and the longer chain derivatives. More extensive in vitro studies are required to explore these variables. Enzymatic dehalogenation of chlorinated fatty acids has been reported by Kaufman (1961, 1962).

The debromination of the fatty acids could also involve the action of glutathione. Alkyl-S-transferase, a soluble enzyme, catalyzes the substitution of a halogen through the action of the glutathione sulfhydryl group (Barnsley, 1966).

The nature of the debrominated products is not known but either unsaturated or possibly hydroxylated derivatives could possible then be oxidized to carbon dioxide. Some preliminary observations with ^{14}C-labelled derivatives would substantiate this suggestion.

A smaller proportion of the ingested brominated acid is metabolized to dicarboxylic acids prior to excretion in the urine. Formation of these derivatives requires oxidation of the methyl end of the fatty acid chain ω-oxidation. This process was first reported by Verkade, (1938) and enzymatic system defined in vitro by Robbins, (1961). Subsequently the dicarboxylic acid could be degraded in the β-oxidation pathway from both ends of the molecule.

This sequence would result in derivatives with even numbered chains, the fact that a brominated dicarboxylic acid with an odd-numbered chain was detected indicates that α-oxidation removal of one carbon must also be contributing. To account for the location of bromine atoms it is also necessary to postulate α-oxidation. Consequently the following scheme (Figure 11) outlines metabolic sequences which must be involved in the metabolism of the brominated fatty acids.

Three of the brominated fatty acids were detected in urine from rats fed the dibromostearate and a possible metabolic sequence which might account for their formation is given in Figure 12. All five of the brominated derivatives have been detected in urine from rats fed the brominated corn oil. Since tetrabromostearate is the major component of BCO a metabolic scheme for the formation of the derivatives from this precursor is outlined (Figure 13). Why the five derivatives are not detected in urine from rats fed the tetrabromostearate is not apparent.

$$CH_3-(CH_2)_7-\overset{\overset{\displaystyle Br}{|}}{C}H-\overset{}{C}H-(CH_2)_7COOH$$
$$\underset{\underset{\displaystyle Br}{|}}{}$$

Mitochondria

Transport   β-oxidation

$$CH_3-(CH_2)_7-\overset{\overset{\displaystyle Br}{|}}{C}H-\overset{\underset{\displaystyle Br}{|}}{C}H-(CH_2)_3-COOH$$

Transport

$$HOOC-(CH_2)n-\overset{\overset{\displaystyle Br}{|}}{C}H-\overset{\underset{\displaystyle Br}{|}}{C}H-CH_2-COOH$$

$$HOOC-(CH_2)_{n-1}-\overset{\overset{\displaystyle Br}{|}}{C}H-\overset{\underset{\displaystyle Br}{|}}{C}H-CH_2-COOH$$

β-oxidation

Transport

$$CH_3-(CH_2)_7-\overset{\overset{\displaystyle Br}{|}}{C}H-\overset{\underset{\displaystyle Br}{|}}{C}H-CH_2-COOH$$

Microsomes

ω-oxidation

$$HOOC(CH_2)_7-\overset{\overset{\displaystyle Br}{|}}{C}H-\overset{\underset{\displaystyle Br}{|}}{C}H-CH_2-COOH$$ $\xrightarrow[\alpha\text{-oxidation}]{}$ $$HOOC-(CH_2)_6-\overset{\overset{\displaystyle Br}{|}}{C}H-\overset{\underset{\displaystyle Br}{|}}{C}H-CH_2-COOH$$
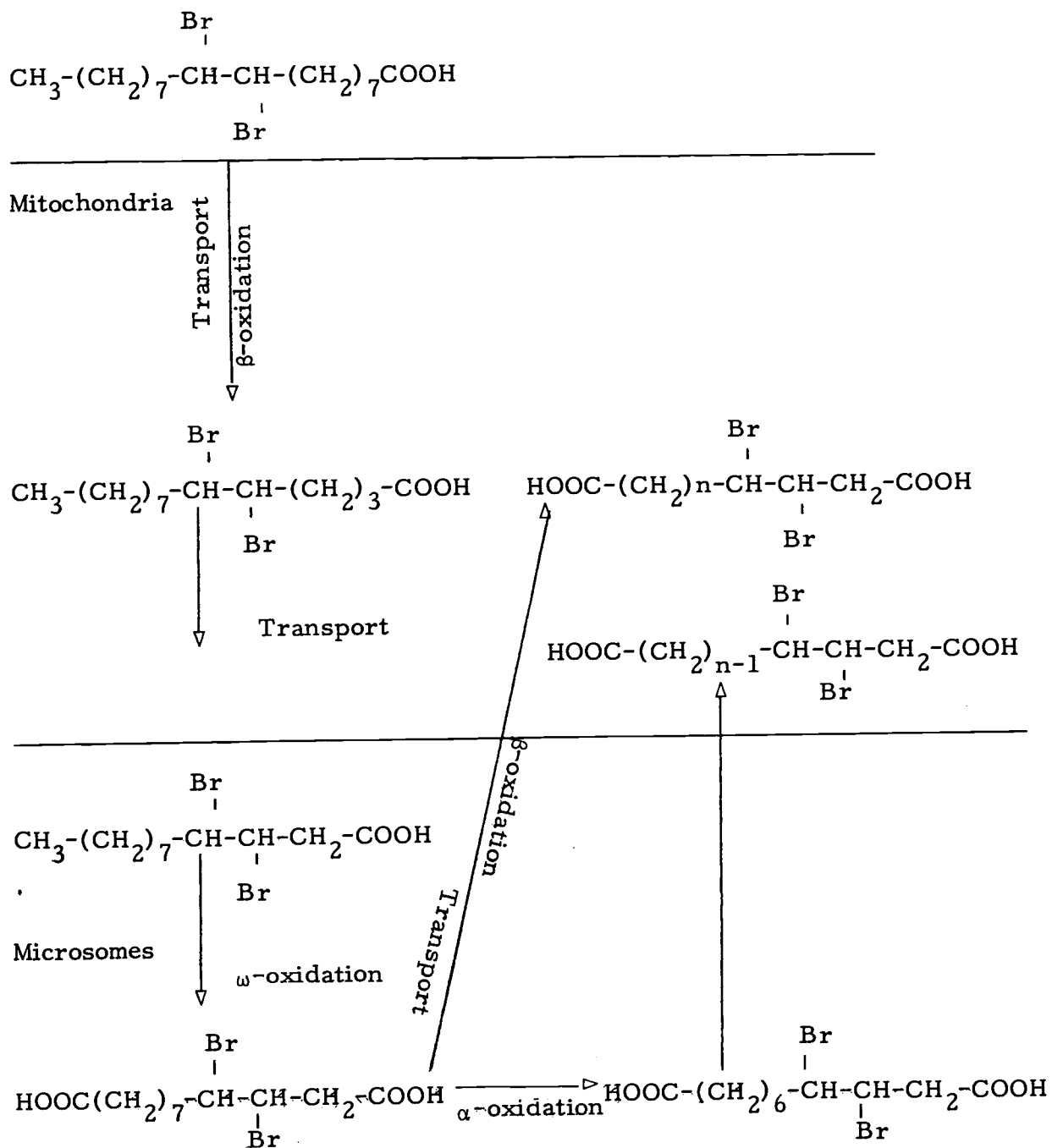
Figure 11. Proposed metabolic sequences involved in the metabolism of the brominated fatty acids.

$$CH_3-(CH_2)_7-CH-CH-(CH_2)_7-\overset{Br\;\;Br}{\underset{O}{C}}-OH$$

β-oxidation

$3xC_2$

$$CH_3-CH_2-CH-CH-(CH_2)_7-\overset{Br\;\;Br}{\underset{O}{C}}-OH$$

ω-oxidation

$$HO-\overset{Br\;\;Br}{\underset{O}{C}}-CH_2-CH-CH-(CH_2)_7-\overset{}{\underset{O}{C}}-OH$$

α-oxidation

$1xC_1$

$$HO-\overset{}{\underset{O}{C}}-CH_2-CH-CH-(CH_2)_6-\overset{Br\;\;Br}{\underset{O}{C}}-OH$$ α-oxidation $$HO-\overset{Br\;\;Br}{\underset{O}{C}}-CH-CH-(CH_2)_6-\overset{}{\underset{O}{C}}-OH$$

$1xC_1$

2,3-dibromo-sebacic acid

β-oxidation

$1xC_2$

β-oxidation

$1xC_2$

$$HO-\overset{Br\;\;Br}{\underset{O}{C}}-CH_2-CH-CH-CH_2-(CH_2)_3-\overset{}{\underset{O}{C}}-OH$$ $$HO-\overset{Br\;\;Br}{\underset{O}{C}}-CH-CH-(CH_2)_4-\overset{}{\underset{O}{C}}-OH$$

3,4-dibromo-azelaic acid   2,3-dibromo suberic acid
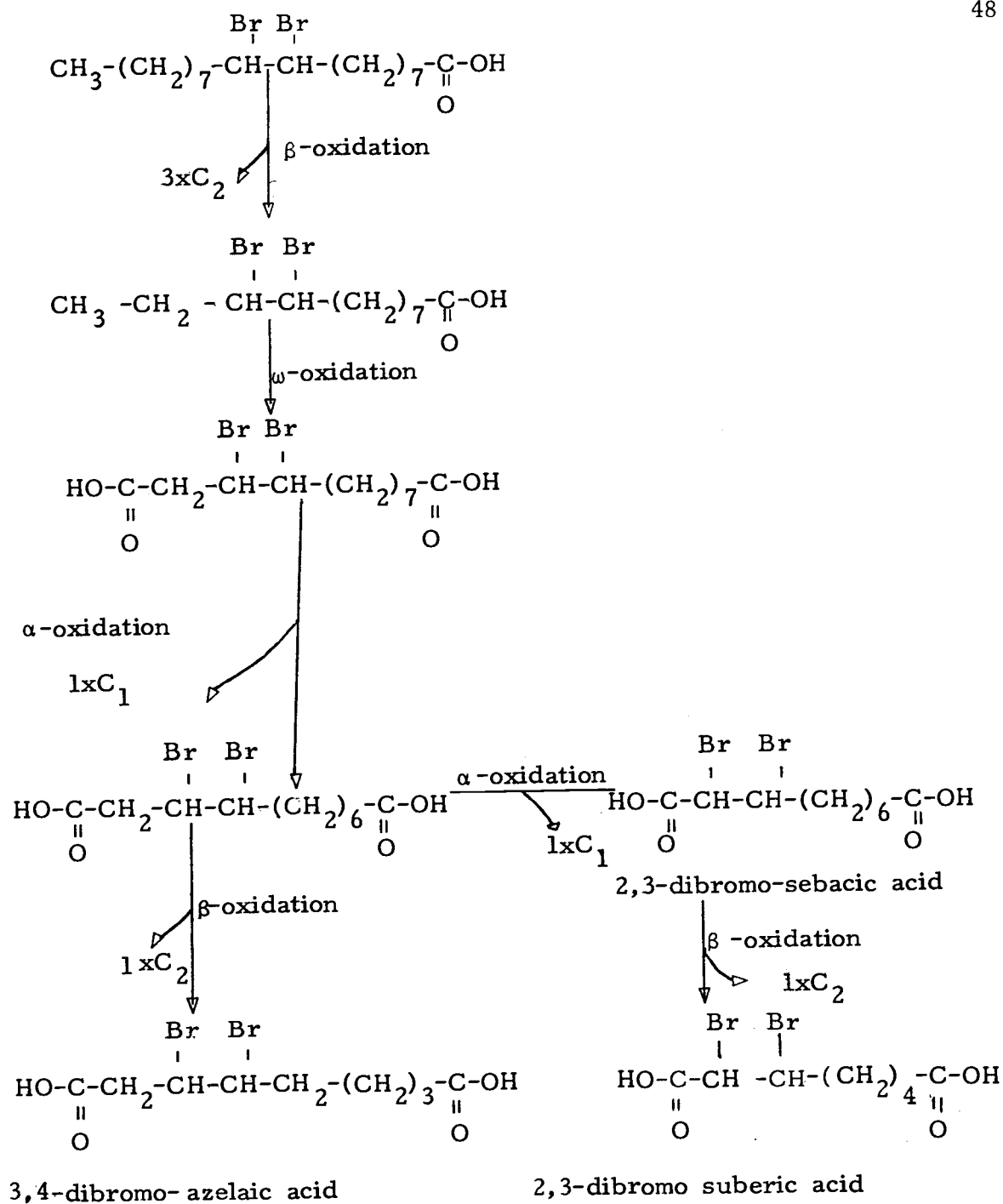
Figure 12. Proposed pathway for monoglyceride of dibromostearate in rats.

$CH_3-(CH_2)_4-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-CH_2-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_7-\overset{O}{\underset{||}{C}}-OH$

$2xC_2$ β-oxidation   β-oxidation

$2xC_2$

$CH_3-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-CH_2-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_7-\overset{O}{\underset{||}{C}}-OH$

ω-oxidation

$CH_3-(CH_2)_4-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-CH_2-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_3-\overset{O}{\underset{||}{C}}-OH$

2Br

$HO-\overset{O}{\underset{||}{C}}-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-CH_2-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_7-\overset{O}{\underset{||}{C}}-OH$

$CH_3-(CH_2)_4-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_6-\overset{O}{\underset{||}{C}}-OH$ —ω-oxidation— $HO-\overset{O}{\underset{||}{C}}-(CH_2-)_3CH_2-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_6-\overset{O}{\underset{||}{C}}-OH$

β-oxidation C_2 $3xC_2$ β-oxidation $1xC_1$ α-oxidation

$HO-\overset{O}{\underset{||}{C}}-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-CH_2-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_5-\overset{O}{\underset{||}{C}}-OH$

$CH_3-(CH_2)_4-\overset{Br}{\underset{|}{CH}}-CH-\overset{O}{\underset{||}{C}}-OH$

$HO-\overset{O}{\underset{||}{C}}-(CH_2-)_3CH_2-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_5-\overset{O}{\underset{||}{C}}-OH$

3Br   β-oxidation   2Br   ω-oxidation   β-oxidation

$HO-\overset{O}{\underset{||}{C}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_7-\overset{O}{\underset{||}{C}}-OH$

2-bromo-dodecanedioic acid

$HO-\overset{O}{\underset{||}{C}}-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-CH_2-CH=CH-(CH_2)_5-\overset{O}{\underset{||}{C}}-OH$

2,3-dibromo-5-dodecenedioic acid

$HO-\overset{O}{\underset{||}{C}}-(CH_2-)_3CH_2-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-\overset{O}{\underset{||}{C}}-OH$

2,3-dibromo-suberic acid

$2 xC_2$

C_2+2Br

$HO-\overset{O}{\underset{||}{C}}-\overset{Br}{\underset{|}{CH}}-\overset{Br}{\underset{|}{CH}}-(CH_2)_6-\overset{O}{\underset{||}{C}}-OH$

2,3-dibromo-sebacic acid

$HO-\overset{O}{\underset{||}{C}}-(CH_2-)_3CH_2-\overset{Br}{\underset{|}{CH}}-CH-CH_2-\overset{O}{\underset{||}{C}}-OH$
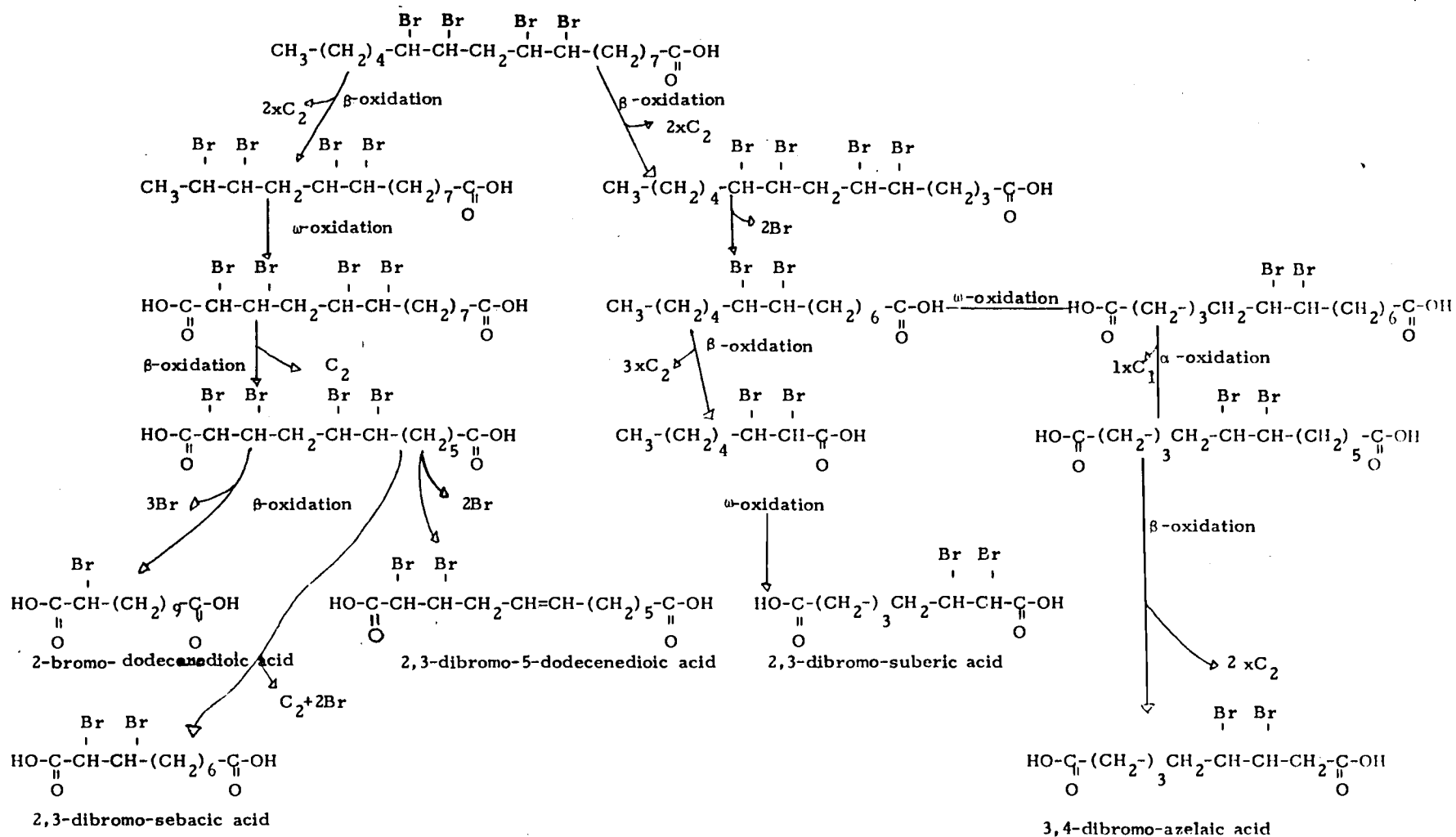
3,4-dibromo-azelaic acid

Figure 13.   Proposed pathway for monoglyceride of tetrabromostearate.

These data have demonstrated that brominated fatty acids can be extensively debrominated by the rat and in addition can be degraded to simpler dicarboxylic acid, by a combination of oxidative processes. These data contrast with observation made in vitro that dibromopalmitate was not oxidized by mitochondrial preparations from hamster brown adipose tissue (Mohamed et al., 1980). Further studies would be required to explore the exact metabolic sequences involved in the oxidation of these compounds.

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A P P E N D I X

APPENDIX I

Ration Composition of the Semi-Synthetic Diet

Ingredient	Percent by Weight
Casein[1]	22.0
Cerelose[2]	63.0
Fat[3]	10.0
H.M.W. salts[4,5]	4.0
Vitamin pre-mix[6,7]	1.0

[1]The Borden Chemical Co., Dominquez, Calif.

[2]Corn Products Co., New York, N.Y.

[3]Corn oil

[4]Nutritional Biochemicals Corp., Cleveland, Ohio

[5]Zinc was added at a level of 6mg/kilo as zinc acetate

[6]Provided as mg/100g of diet, the following: Thiaminehydrochloride, 0.4; riboflavin, 0.8; Pyridoxine hydrochloride, 0.50; D-calcium Pantothenate, 4.0; inositol, 20; menadione, 0.40; folic acid, 0.40; niacin, 4.0; choline dihydrogen citrate, 424; biotin, 0.03; B12, 0.02.

[7]Vitamins A, D and E were added in ethanol solution to provide per 100g of diet 0.875 IU of Vitamin A 125 units of Vitamin D and 3.64mg of D- α-tocopherol acetate.

APPENDIX II

Preparation of Methyl Esters (Lowry, R. 1977)

The sample is placed in a screw cap culture tube and any solvent is removed under nitrogen gas flow. 3.0 ml. volumes each of the catalyst (HCl/methanol) and diethyl ether are then added, mixed, the tube is capped, and heated in a block at 80°c for 90 minutes. At the end of the heating period the tube is cooled and extracted as follows: 3.0 ml. of water and 3.0 ml. of hexane are added, the tube is then capped, shaken vigorously and centrifuged briefly. Remove the upper phase and place in a clean tube. A second extraction with 3.0 ml. of hexane: ether (1:1) is made in like manner. Sample is ready for gas chromatography.

APPENDIX III

The Proposed Fragmentation Mechanism of

Succinic Acid-Dimethyl Ester

It is at once apparent that in this case methoxy carbonyl-methoxy carbonyl interaction does not dominate the fragmentation mechanisms. The ion [M-31] (m/e 115) is the intense peak, appears to be of greater importance.
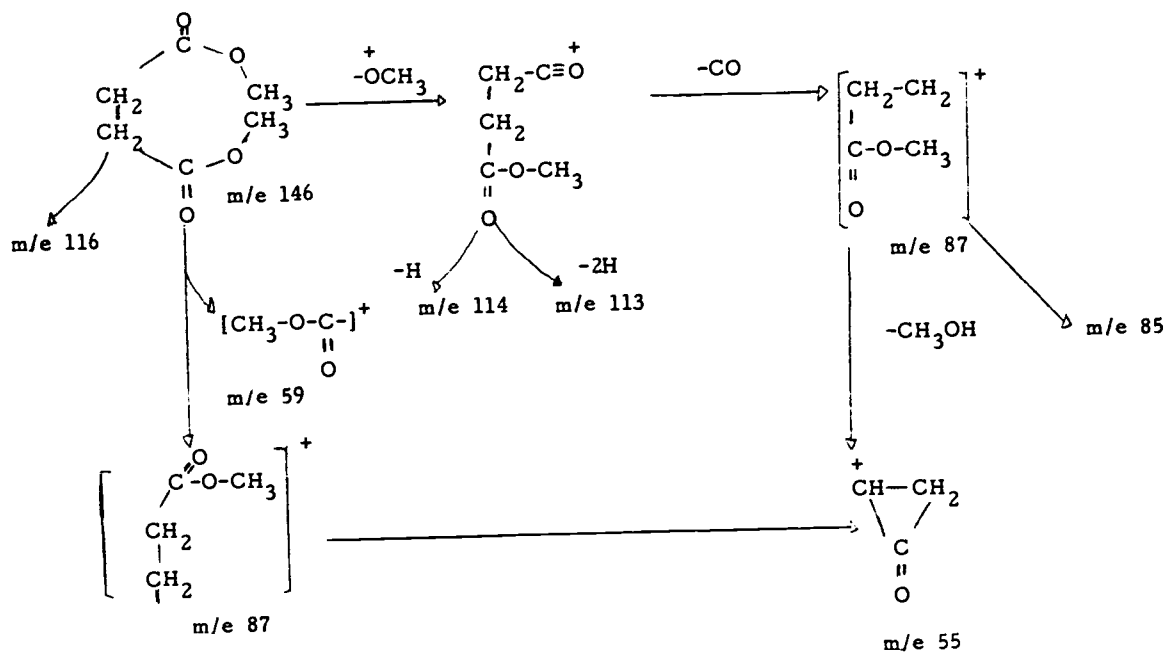
The methoxy carbonyl-methoxy carbonyl interaction leads to methoxy expulsion from the molecular ion as involving a five-membered ring transition state. Loss of $CH_3-O-\overset{+}{\underset{\underset{O}{\|}}{C}}-$ from the molecular ion is still an important process in this compound. The peak of m/e 45 is characteristic for methyl esters (McLafferty, 1963). Small peaks at this mass number due to ions formed by double cleavage and rearrangement are seen in the spectra of all methoxy esters.