Crude protein ranged from 1.4 to 4.7% (Table I) of the dry matter, which is in accordance with the literature (2). Again, no significant correlation was found between protein and individual fatty acid contents.

The starchy characteristic of the cassava tubers is substantiated (Table II). The acid digestible carbohydrates in the green matter ranged from 11.8 to 40.7%. The total soluble carbohydrates ranged from 7.35 to 27.7 mg/g of green matter. More information about the oligosaccharides is needed for a better understanding of the carbohydrate metabolism of this euphorbiaceous plant.

ACKNOWLEDGMENTS

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A Quantitative Comparison of the Yields of Radiolysis Products in Various Meats and their Relationship to Precursors

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ABSTRACT

A detailed analysis has been made of the composition of radiolysis products formed in beef, pork, ham, and chicken. The yields of the various compounds are related linearly to irradiation dose, and the fat, fatty acid and triglyceride composition of the meats.

INTRODUCTION

The fact that various meats yield similar compounds in comparable amounts upon irradiation has long been established (1). A series of studies with meats (1-6) and model compounds (2-4, 7-12) has shown that the origin of the radiolytically induced components can be attributed to precursors in the meat such as the fats and proteins. Moreover, mechanisms for the reaction pathways have been adduced or proposed (7-9, 13), but the evidence in support of the hypotheses has been mainly qualitative. Recently, a quantitative study of the reaction pathways leading to radiolysis products in ethyl palmitate has been completed (14). In this study, a quantitative relationship is shown for the yields of the various radiolysis products in meat and the amount of their putative precursors.

EXPERIMENTAL

Preparation of Meats for Irradiation

Preparation of Chicken Samples. Broiler carcasses were separated into white meat and dark meat and skins with attached fat, and then frozen. Chicken rolls were formed by mixing 82% light meat and dark meat and 18% skins. To this mixture was added 0.75% salt (NaCl) and 0.3% sodium tripolyphosphate. The meat and additives were thoroughly mixed for ca. 20 min in a vacuum mixer and then stuffed into regenerated cellulose casings of appropriate size. The chicken rolls were enzyme inactivated by heating to an internal temperature of at least 68 C and not more than 74 C. The rolls were then spray washed, chilled and stored under refrigeration until packaged. Before packaging the casings were stripped off and the rolls ground twice and packed into 404 x 309 tin cans. After vacuum sealing, the cans were frozen pending irradiation.

Preparation of Beef Samples. Fresh, raw beef was deboned, trimmed and cut into chunks. The beef chunks were then mixed with 0.75% salt and 0.3% sodium tripolyphosphate in a vacuum mixer for 20 min. After mixing the beef was stuffed into casings, enzyme inactivated, ground, canned and stored in the same manner as chicken (vide supra).

Preparation of Ham Samples. Fresh, raw pork hams were mechanically pumped with curing brine to a 12% level, then skinned, deboned, trimmed and cut into 100 to 500 g chunks. The meat chunks and an additional 3% brine were mixed for 15 min to a tacky consistency followed by vacuum mixing for an additional 20 min.

Brine Composition

Water	25.5 Kg
Sodium tripolyphosphate	600.0 g
Salt (sodium chloride)	4.8 Kg
Sodium ascorbate	55.0 g
Sodium erythrobate	55.0 g
Sodium nitrate	10.0 g
Sodium nitrite	5.0 g

The mixed ham was then stuffed into casings, enzyme inactivated, processed, canned and stored as was the chicken (vide supra).

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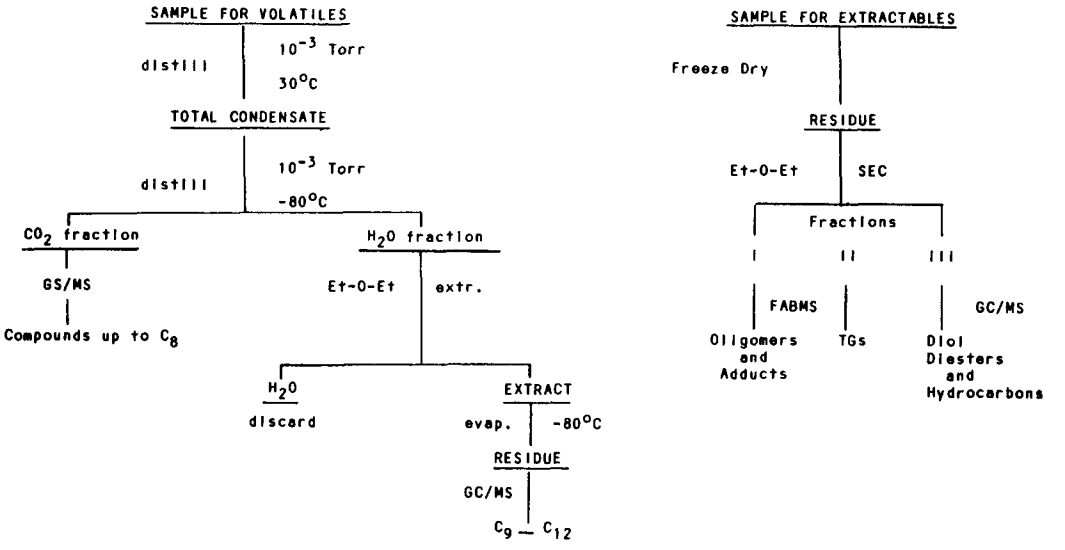


FIG. 1. Analytical Scheme for Separation and Identification of Radiolysis Products. Vacuum distillable products are separated into two fractions and further analyzed by GC/MS. The residue after freeze drying is separated by size exclusion chromatography and further analyzed by GC/MS.

TABLE II

Fat and Fatty Acid Composition of Meats

			Pork	Ham	Chicken	Beef	
Fat content[a]			4.3	7.3	11.7	15.4	Derivative
Fatty acid composition[b]				% of total fat			hydrocarbon (C_n−1)
16:0	Palmitic	(P)	23.1	23.2	21.7	25.4	Pentadecane
16:1	Palmitoleic	(P_o)	3.2	3.2	5.2	5.1	Pentadecene
18:0	Stearic	(S)	11.3	10.9	6.3	12.5	Heptadecane
18:1	Oleic	(O)	47.5	47.4	35.3	44.4	Heptadecene
18:2	Linoleic	(L)	10.8	11.0	26.1	3.8	Heptadecadiene

[a]Section 24.005 AOAC Methods, 13th ed., 1980, p. 376.
[b]AOAC-IUPAC Gas Chromatographic Method No. 28.057, AOAC Methods, 13th ed., 1980, p. 447.

Preparation of Pork Samples. Fresh, raw pork was deboned, trimmed and cut into chunks. The pork chunks were then mixed with 0.75% salt and 0.3% sodium tripolyphosphate in a vacuum mixer for 20 min; 0.01% ground black pepper was added to the pork chunks prior to vacuum mixing for seasoning. After mixing the pork was stuffed into casings, enzyme inactivated, ground, canned and irradiated in the same manner as the other meats (vide supra).

Before irradiation, the meat samples were chilled to −40 C±5 C and held at that temperature during irradiation. All the meats were irradiated under identical conditions, in the cans, at nominal doses of 3, 6 and 9 Mrads.

The accuracy of the radiation doses given was determined from dose-distribution measurements in the Co^{60} source used for irradiation to be ≤ ±0.05% of the nominal dose.

Analytical Methods

The amounts of the radiolysis products in the meat samples were determined according to the scheme depicted in Figure 1. Since details of the methods used have been described in several prior publications (1, 3, 5, 6, 15, 16), only a brief summary of the methods is given here.

The fat content of the meats and the fatty acid composition of the fats were determined by AOAC methods cited in Table II.

Analysis of Volatiles

The samples were distilled under high vacuum at room temperature, and the distillate was collected for 6 hrs in a gas bottle held at −196 C (15). The condensed distillate was then fractionated by placing the gas bottle containing it in a dry ice-ethanol bath (ca. −80 C) and collecting the distillate in another gas bottle held at −196 C. This second distillate (CO_2 fraction) was analyzed by combined wide range temperature programmed gas chromatography–fast scanning mass spectrometry employing a TRIS/SCOT gas chromatography column programmed from −100 C to 125 C at 5 C/min and a Bendix Model MA-2 TOF mass spectrometer (2). The sample was transferred onto the chromatographic column from a U-shaped trap fitted with a two-way valve by sweeping with helium carrier gas. A typical chromatogram showing the separation of volatile radiolysis products has been given in a prior publication (3).

The residue of the second distillation (water fraction) was extracted with diethyl ether. The extract was then placed in a dry ice ethanol bath (ca. −80 C) and the ether distilled off under high vacuum. The extract from the water fraction was analyzed by GC/MS using a CW 20M SCOT column temperature programmed from 0 C to 200 C at 5 C per min.

Duplicate determinations were made for each sample of meat irradiated at the respective doses.

Analysis of Organic Extractable (Non-Volatile) Compounds

Residues of the meat samples after freeze drying were extracted overnight in a Soxhlet with diethyl ether. The

TABLE I

Yield of Major Radiolysis Products in Various Meats as a Function of Dose

Compound	Dose (Mrads)											
	Beef						Pork					
	0[b]	3	6	9	G	r	0	3	6	9	G	r
Group 1[c]												
Pentane	5	103	288	306	5.1×10^{-4}	0.98	135	207	386	348	3.8×10^{-4}	0.93
Hexane	2	129	239	354	4.6×10^{-4}	0.99	8	97	183	288	3.5×10^{-4}	0.99
Heptane	3	186	343	537	5.8×10^{-4}	0.99	38	128	266	367	3.6×10^{-4}	0.99
Octane	8	126	298	412	3.9×10^{-4}	0.99	24	81	193	273	2.3×10^{-4}	0.98
Pentene	0	28	62	87	1.4×10^{-4}	0.99	26	61	82	98	1.2×10^{-4}	0.99
Hexene	0	38	86	122	1.7×10^{-4}	0.99	2	37	61	101	1.3×10^{-4}	0.99
Heptene	0	51	110	163	1.9×10^{-4}	0.99	7	56	158	206	2.2×10^{-4}	0.98
Octene	0	42	96	158	1.7×10^{-4}	0.99	3	46	102	143	1.4×10^{-4}	0.99
Group 2[d]												
Pentadecane	0	23	27	58	2.76×10^{-2}	0.91	0	27	77	150	6.04×10^{-2}	0.99
Heptadecene	0	35	42	93	4.05×10^{-2}	0.91	0	40	90	180	6.76×10^{-2}	0.98
Heptadecadiene	0	5	7	13	0.60×10^{-2}	0.96	0	15	26	33	1.83×10^{-2}	0.99
Hexadecanal	0	70	110	240	10.06×10^{-2}	0.95	0	50	100	80	6.22×10^{-2}	0.60[e]
Dihexadecanoyl propanedioldiester (1,2)	0	40	90	170	2.85×10^{-2}	0.99	0	30	50	180	1.78×10^{-2}	0.97
Hexadecanoyl octadecenoyl propanedioldiester (1,2)	0	40	90	150	2.60×10^{-2}	0.99	0	120	220	280	6.51×10^{-2}	0.99
	Ham						Chicken[a]					
Group 1[c]												
Pentane	14	40	92	137	1.8×10^{-4}	0.98	110	176	246	356	3.5×10^{-4}	0.99
Hexane	2	27	65	97	1.2×10^{-4}	0.99	11	67	159	221	2.7×10^{-4}	0.99
Heptane	6	79	158	227	2.5×10^{-4}	0.99	21	86	268	428	3.9×10^{-4}	9.96
Octane	7	56	127	182	1.7×10^{-4}	0.99	25	49	182	225	1.8×10^{-4}	0.93
Pentene	4	14	32	44	0.6×10^{-4}	0.98	35	52	81	119	1.2×10^{-4}	0.98
Hexene	1	10	25	33	0.4×10^{-4}	0.99	5	28	53	77	0.9×10^{-4}	0.99
Heptene	1	43	78	136	1.4×10^{-4}	0.99	6	52	161	240	2.4×10^{-4}	0.98
Octene	1	20	43	61	0.6×10^{-4}	0.99	5	45	85	119	1.1×10^{-4}	0.99
Group 2[d]												
Pentadecane	0	20	30	100	3.57×10^{-2}	0.91	0	6	26	35	1.60×10^{-2}	0.97
Heptadecene	0	36	49	150	5.14×10^{-2}	0.91	0	26	52	65	3.43×10^{-2}	0.98
Heptadecadiene	0	15	25	40	1.92×10^{-2}	0.99	0	25	41	55	2.99×10^{-2}	0.99
Hexadecanal	0	80	100	150	8.83×10^{-2}	0.97	0	50	60	110	5.73×10^{-2}	0.93
Dihexadecanoyl propanedioldiester (1,2)	0	50	60	75	2.11×10^{-2}	0.93	0	10	30	55	0.87×10^{-2}	0.99
Hexadecanoyl octadecenoyl propanedioldiester (1,2)	0	130	190	320	6.38×10^{-2}	0.97	0	20	65	80	1.52×10^{-2}	0.96

[a]Average of duplicate determinations.

[b]Amounts found at zero dose subtracted from radiation yields.

[c]Concentrations given in μg/Kg of meat.

[d]Concentrations given in mg/kg of fat (uncorrected for water).

[e]Value for yield at 9 Mr presumed in error but included in least squares calculation. If omitted, r=0.99.

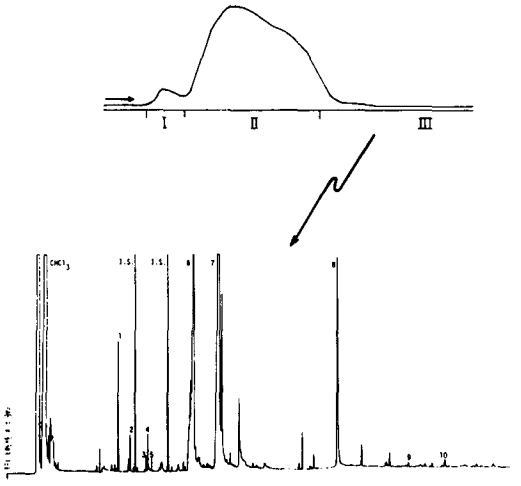


FIG. 2. Upper Trace: Size exclusion chromatogram (SEC) of ether extract of irradiated chicken (Co^{60}), 4.5 Mrads, −30 C. Column: 64 ft × 3/8 in. 60 Å Styragel; Eluent: $CHCl_3$ at 2 ml/min; Refractive index detector. Sample size (4 ml). Fractions: I, Triglyceride adducts; II, Triglycerides; III, Extractable radiolysis products. Lower Trace: Gas chromatogram of radiolysis compounds found in SEC/Fraction III. Fused silica bonded phase (DB-1) open tubular column, 60 m × 0.32 mm; He, 2 ml/min; temp. 100-320 C @ 5 C/min; Peak Nos. (1) pentadecane; (2) hexadecadiene; (3) heptadecadiene; (4) heptadecene; (5) heptadecane; (6) palmitic acid; (7) oleic acid; (8) cholestadiene; (9) 1,2:dihexadecanoyl propanedioldiester; (10) 1,2:hexadecanoyl octadecenoyl propanedioldiester. Internal Standards (I.S.) in elution order: $C_{16}H_{34}$ and $C_{18}H_{38}$.

extracts were separated by size exclusion HPLC (4, 6). The compounds smaller than triglycerides were collected. This fraction (III, Figs. 1 and 2) was analyzed by combined GC/MS for qualitative identification of the components and by gas chromatography using internal standards and retention volumes for quantitative determinations (6, 10, 12). An example of the separation and analysis of the extractable compounds is shown in Figure 2.

As with the analysis of volatile compounds, duplicate determinations were made for each sample.

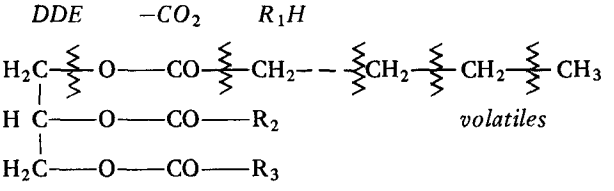
Data Processing

The analytical data were acquired and stored in a data bank initially on a Digital Equipment Corporation (DEC) PDP 15/76 computer and transferred subsequently to a DEC PDP 11/34 computer. The computation of the precision of the yield determinations was based on the entire population of values, because it was observed that absolute deviations were nearly constant. No deviations were found outside the limits of $\pm 2\sigma$ and the yield values recorded in Table I are the averages of the duplicate determinations. Yield-dose dependency and yield-precursor dependency relationships were determined by performing linear least squares regression analyses (17) of the data (Tables I and II). Duplicate analyses of a second set of irradiated chicken samples were made to establish reproducibility of the radiation procedure. The computation of triglyceride composition of the meat fats from fatty acid analyses was performed as described in a prior publication (18). The computer also was used to compute the weighted triglyceride precursor abundance for formation of the dioldiesters. Graphic plots of the data were generated by the computer as required.

RESULTS AND DISCUSSION

The analytical data showing the amount of the several radiolysis compounds formed in the various meats at various doses is given in Table I. The linear dependence of yield as a function of dose (5) was verified by computing the least squares linear regression correlation coefficient, r, for each compound. The slopes of the yield/dose equations provide an evaluation of the G-values (G = μmole/Kg/Mrad) for the several compounds. Values of r and G also are given in Table I.

The mechanism for formation of certain radiolysis products from lipid precursors has been postulated previously (2, 3,7-9, 13, 14). For example,



Thus, short chain hydrocarbons are perceived to arise from homolytic cleavage of the aliphatic portion of the fatty acids in a triglyceride, the long chain hydrocarbons by loss of the entire alkyl radical, and dioldiesters (DDE) by loss of acyloxy moieties. The quantitative dependence of each class of radiolysis product on its precursor is established below.

Dependence on Fat Content

The origin of volatile hydrocarbons and other related compounds observed among the components formed in irradiated meats and model lipid components, viz., triglycerides and fatty acid methyl esters, has been postulated to be due to homolytic cleavages of the aliphatic side chains of fatty acid moieties (1, 2). The dependence of yield of several of the volatile radiolysis products, mainly hydrocarbons, C_5–C_9, on the fat content, was demonstrated in a prior study of the effect of various parameters on the formation of radiolytic compounds in beef (5). In this study the quantitative relationship of the yield of volatile hydrocarbons to the fat content of several meats shows the linear dependence of such compounds on their fat precursor.

The fat content of the various meats is given in Table II. A plot of the G-values for the volatile short chain hydrocarbons as a function of the fat contents of the four meats studied has shown a linear relationship. The relationship for all the relevant compounds is summarized in Table III by citing the correlation coefficients for the linear least square regression equations. All of the aliphatic hydrocarbons, C_5–C_9, both alkanes and alkenes, when derived from four different meats, are seen to have a highly correlated linear dependence on fat content.

The fat content of the four principal meats studied, viz., beef, pork, ham and chicken, ranged from ca. 7-15%. In order to test the validity of the concept for a meat with a high fat content, data for bacon ($\sim$50% fat) were compared with the other meats (Fig. 3). Since it has been established previously that the yield of radiolysis product as a function of dose may be treated colligatively as a group (5), the data presented in Figure 3 are shown as the sum of the yields of the four principal alkanes.

Dependence on Fatty Acid Composition

The longer chain hydrocarbons, C_{15}–C_{17}, have been shown (vide supra) to be formed by decomposition of an acyloxy radical produced by irradiation of a triglyceride (7-9).

$$TG \xrightarrow{\sim} RCO_2\cdot \rightarrow R\cdot + CO_2$$

$$R\cdot + TG \rightarrow RH + TG\cdot$$

The amount of hydrocarbon, therefore, must depend on the fatty acid composition of the meat. The fatty acid com-

TABLE III

Correlation of G-Value of Radiolysis Product[a] with Amount of Precursor

Compound	r[b]
Volatile Hydrocarbons with Fat Content	
Pentane	0.95
Hexane	0.99
Heptane	0.81
Octane	0.82
Pentene	0.95
Hexene	0.98
Heptene	0.71[c]
Octene	0.99
Extractable Compounds with Fatty Acid Moieties[d]	
Pentadecane	0.87
Heptadecene	0.90
Heptadecadiene	0.97
Hexadecanal	0.93
Dioldiesters with Parent Triglycerides	
Dihexadecanoyl propanedioldiester (1,2)	0.90
Hexadecanoyl octadecenoyl propanedioldiester (1,2)	0.97

[a]Data from Tables I and II.

[b]Linear least squares correlation coefficients.

[c]In the beef samples, heptene is partly coeluted from the GC column with acetaldehyde which occurs in copious amounts and must be determined by deconvolution. A possible error may result in the poorer correlation for heptene.

[d]Data given only for compounds in major abundance.

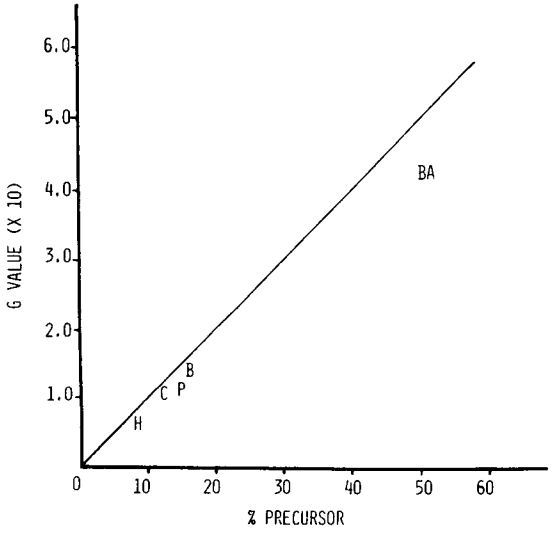


FIG. 3. Correlation graph showing linear correspondence of the yield of volatile hydrocarbons (sum of yields of pentane, hexane, heptane and octane) as a function of fat content in ham (H), chicken (C), pork (P), beef (B) and bacon (BA). The correlation coefficient of the linear least squares regression line for the data points is 0.998.

positions of the meats studied here are given in Table II. Also shown are the hydrocarbons expected as derivatives. Their linear dependence on dose is confirmed by the correlation coefficients given in Table I. The correlation of the yields (G-values) as a function of per cent precursor is given in Table III. An example displayed graphically in Figure 4 is given for the dependence of heptadecadiene ($C_{17:2}$) on the per cent of linoleic acid in the fat of beef, ham, pork and chicken respectively.

Another typical radiation product derived from the fat in meat is an aldehyde corresponding to a fatty acid moiety of the triglyceride.

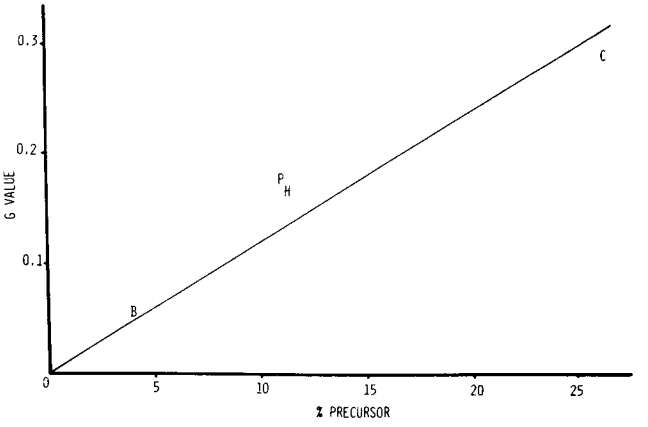


FIG. 4. Graph showing linear dependence of G value for heptadecadiene on per cent linoleic acid in various meat fats. (B, beef; P, pork; H, ham; C, chicken) Correlation coefficient, r, 0.97.

TABLE IV

Triglyceride Composition of Various Meats[a]

	Beef	Pork	Ham	Chicken
POO	19.8	17.7	17.8	9.6
POP	11.3	8.6	8.7	5.9
POS	11.2	8.4	8.2	3.4
SOO	9.7	8.7	8.4	2.8
PP_OO	4.5	2.4	2.4	2.8
P_OOO	4.0	2.5	2.5	2.3
POL	3.4	8.1	8.3	14.2
PPS	3.2	2.1	2.0	1.1
OOL	3.0	8.3	8.5	11.5
SOS	2.7	2.1	1.9	–
P_OOS	2.2	1.2	1.1	–
OLL	–	1.9	2.0	8.5
SOL	1.7	3.9	3.9	4.1
PLL	–	–	–	5.2
PPL	–	2.0	2.0	4.4
PLS	–	1.9	1.9	2.5
P_OOL	–	–	–	3.4

[a]Computed (see Ref. 18) from fatty acid composition in Table II.

$$TG \xrightarrow{\gamma} RCO\cdot + DG\cdot$$

(DG · is the diglyceride radial less a hydrogen atom)

$$RCO\cdot + TG \rightarrow RCHO + TG\cdot$$

The most abundant aldehyde thus formed is hexadecanal derived from the palmitic acid moiety in the fat. Yield data for this compound shows a linear dependence on the amount of palmitate in the triglycerides (Table III).

Dependence on Triglyceride Composition

Another class of compounds found in abundance among the radiolysis products is the propane dioldiesters. They are the compounds formed corresponding to a loss of the acyloxy moiety in a homolytic cleavage.

$$TG \xrightarrow{\gamma} PDDE\cdot + RCO_2\cdot$$

$$PDDE\cdot + TG \rightarrow PDDE + TG\cdot$$

As in the case of the simpler compounds, the propane dioldiesters are expected to show a linear dependence on their precursors, viz., the various triglycerides in the fat. Although the triglyceride content of meat fat may vary widely and be comprised of a large number of different tri-

TABLE V

Weighted Composition of Triglyceride Precursors for Certain Dioldiesters[1]

Dioldiester	Beef	Pork	Ham	Chicken
16:0/16:0	5.3	4.2	4.2	3.8
16:0/18:1	19.7	23.9	24.0	17.1

[1] By computer calculation from the fatty acid composition of the meat.

glycerides, it is now possible to elucidate the composition rather easily from the fatty acid composition by a computer prediction (18). Thus, the triglyceride compositions of the meats analyzed in this study have been determined and are given in Table IV. The yields of the two most abundant propanedioldiesters found by analysis in the various irradiated meats are given in Table I. The correlation coefficients for the yield of propanedioldiester with irradiation dose shows as with the other radiolysis products a typical linear dependence.

There are two principal dioldiesters found in the analysis of the radiolysis products, viz., the dipalmityl (i.e., dihexadecanoyl) and the mixed hexadecanoyl (C_{16}) and octadecenoyl ($C_{18:1}$) diesters. (Although a dioctadeceonyl also is found, its abundance is much less than expected. Since the oleate moiety is known to participate in reactions leading to adduct compounds (11, 19, 20), a low abundance of the C18:1/C18:1 dioldiester may be expected to be due to reactions which compete with the simple cleavage reaction.)

In order to demonstrate the dependence of dioldiesters on triglyceride composition, it is necessary to compute a weighted precursor composition based on the triglyceride composition of the meat. Taking into account the probable distribution (21) of the fatty acids in a meat triglyceride and the fact that the analytical procedure provides data only for the 1,2 diesters, the amount of precursor is computed by dividing by three the percentage composition of each triglyceride containing the requisite fatty acid and summing the amounts. The results of such a computation for all the meats is shown in Table V. The correlation of dioldiester yield with precursor may then be established from the data of Table I and Table V, and the results are given in Table III.

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