Thiobarbituric Acid Reactive Substance Formation As Affected by Distribution of Polyenoic Fatty Acids in Individual Phospholipids

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Fatty acid profiles and distribution of individual phospholipids (PL) in the total PL of chicken liver, heart, plasma, and egg yolk were determined, and the contribution of each phospholipid to the thiobarbituric acid reactive substance (TBARS) formation was determined. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) produced from approximately 77% to over 90% of the total phospholipid TBARS, while 17–21% of the TBARS was formed by phosphatidylinositol (PI) and phosphatidylserine (PS). The PC, PE, PI, and PS fractions accounted for 86–97% of the total PL. When equal masses of each individual PL were analyzed and compared, PI was found to produce the highest and sphingomyelin (SP) the lowest amount of TBARS. In all analyzed tissues, both the TBARS concentration and the percentage of polyenoic fatty acid, especially arachidonic acid, were highest in PI followed by PE, PS, PC, CL, LyPE, LyPC, and SP. It was concluded that PL fractions with relatively higher concentrations of polyenoic fatty acids characteristically had greater potentials for TBARS formation and were more susceptible to oxidation.

INTRODUCTION

Many investigators have identified the phospholipid fraction of fat as being of primary importance in the development of oxidative deterioration in tissue lipids (Love and Pearson, 1971; Igene et al., 1980; Melton, 1983). Lee and Dawson (1973) and Igene et al. (1980) found that phospholipids in chicken muscle had a greater effect on lipid deterioration than the neutral lipids. A previous study in our laboratory (Pikul et al., 1984) showed that even when the same initial amounts of phospholipids, triacylglycerols, and cholesterol esters were used in TBARS assays, approximately 70% of the total TBARS present was generated by the phospholipids. The phospholipid fraction is characterized by its relatively high amount of polyunsaturated fatty acids (PUFA) compared to the other lipid fractions (Igene et al., 1980, 1981; Melton, 1983; Pikul et al., 1984).

The distribution of individual phospholipids in chicken liver has been published by Sgoutas (1966) and that in chicken liver and heart by Marion and Miller (1968). Information about the percentage of individual phospholipids in total egg yolk lipid is also available (Noble and Moore, 1965; Evans et al., 1967; Gornall and Kuksis, 1971; Tsiagbe et al., 1988). However, most authors separated the total egg yolk phospholipid into only four fractions. Phosphatidylcholine (PC) phosphatidylethanolamine (PE), and sphingomyelin (SP) were analyzed separately, while the fourth fraction pooled other phospholipids such as phosphatidylinositol (PI), lysophosphatidylethanolamine (LyPE), lysophosphatidylcholine (LyPC), and phosphatidic acid (PA). A few authors reported small amounts of PI, phosphatidylserine (PS), or cardiolipin (CL). The fatty acid composition of individual phospholipids from chicken liver was published by Sgoutas (1966) and that for PC and PE in chicken liver and plasma by Chung et al. (1967). The fatty acid profile of PC, PE, and SP from egg yolk phospholipid has already been thoroughly examined (Privett et al., 1962; Noble and Moore, 1965; Marion and Woodroof, 1968; Gornall and Kuksis, 1971). The results of these investigations indicate there are significant differences in the fatty acid composition among individual phospholipid classes. However, the amount of polyenoic fatty acids in the PL fractions reported by these authors sometimes differed.

The structural and metabolic functions of phospholipids indicate that they are actively involved in oxidation during processing and storage. Results published by Igene et al. (1980) show that during frozen storage of model meat systems the polyenoic fatty acids of PC and PE are not stable and undergo oxidation. The PUFA are extremely reactive, and through oxidation give rise to a number of carbonyl compounds. Marion et al. (1967) reported that the 22-carbon fatty acids with four or five double bonds in chicken muscle phospholipids were positively correlated with the TBARS values. Earlier, Lea (1957) studied thin-layer oxidation of egg PE and PC and found that PE oxidized much faster than PC. However, work done by Acosta et al. (1966) showed that lecithin from turkey muscle was more active in the early stages of autoxidation than cephalin; probably, as suggested by these authors, the lecithin fraction was not pure. Most of the PS was eluted with the lecithin; the SP fraction was relatively nonreactive with oxygen.

Although the role of phospholipids in TBARS formation has been extensively studied (Igene et al., 1980, 1981; Pikul et al., 1984), an exact estimation of the amount of TBARS generated by individual phospholipids has not been established. The purpose of this study was to identify and quantitate the individual phospholipids, to determine the fatty acid profile of each individual phospholipid fraction in chicken liver, heart, plasma, and egg yolk, and to study the roles of individual phospholipids in thiobarbituric acid reactive substance (TBARS) formation in edible poultry products.

MATERIALS AND METHODS

Materials. Nine-month-old New Hampshire × Columbian pullets were used in this study. They had been individually caged

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and fed ad libitum a high-protein, low-fat, corn–soybean meal based starter ration, described in detail in a previous study (Pikul et al., 1985). Egg yolk samples were prepared from 24 eggs collected from 50 birds within 24 h of oviposition. The eggs were divided into seven lots of four each and broken, the yolk were separated by hand from the albumin, and the yolk composite was throughly mixed and immediately weighed in preparation for total lipid extraction.

Blood was drawn, from the wing veins of six individual birds, into heparinized syringes and transferred into tubes containing 0.1% tetrasodium ethylenediaminetetraacetate (EDTA) at final concentration. The plasma was obtained by centrifugation at 1500*g* for 15 min. Individual plasma samples were kept at 4 °C for 2 h before total lipid extraction. After the six birds were sacrificed, the entire liver and heart were removed from each carcass, individually wrapped in aluminum foil, and kept on ice for 2 h prior to lipid extraction.

Total Lipid Extraction from Various Tissues. Lipid from the liver and heart was extracted with a chloroform–methanol solution (1:2) according to the basic procedure of Kates (1972) with modifications described in previous papers (Pikul et al., 1983, 1985). Total lipid in the organic extract was determined by gravimetric measurement using a Perkin-Elmer Model AD-2 autobalance (Oakbrook, IL). The samples were appropriately diluted with chloroform to a final concentration of 25 mg of total lipid/mL of chloroform.

Plasma lipid was extracted with a chloroform–methanol solution (2:1) according to the basic procedure of Folch et al. (1957) using conditions described in a previous study (Pikul et al., 1985). The chloroform extract was stored under nitrogen at −20 °C until further assay.

A two-step modified Folch extraction based upon work done by Smith et al. (1964) and described by Fletcher et al. (1984) was used for total yolk lipid extraction. Two grams of egg yolk was added to 60 mL of a chloroform–methanol solution (2:1), mixed with a Polytron ST-10 system (Brinkmann, Switzerland) for 1 min, transferred to Erlenmeyer flasks, and stored under nitrogen at 4 °C overnight. The homogenate was then filtered into a separatory funnel. The residue from the filter paper was mixed again and extracted a second time with an additional 40 mL of the chloroform–methanol solution (2:1) for 1 min. This mixture was also filtered and added to the original extraction in the separatory funnel. The flask and filter paper were rinsed twice with aliquots of the chloroform–methanol solvent. A potassium chloride solution (0.88%), equal to 20% of the amount of total solvent, was added to the separatory funnel, and the funnel was then shaken gently. This solution was kept under nitrogen for 4 h, after which time the chloroform phase had completely separated and was transferred to a 100-mL graduated cylinder. The chloroform extract was dried over anhydrous sodium sulfate, filtered, and evaporated under nitrogen to a volume of approximately 10 mL. The concentration of total lipid from this extract was determined by gravimetric measurement, and the volume of extract was adjusted with chloroform to give a final lipid concentration of 25 mg/mL of chloroform.

Separation and Quantitation of Phospholipid. Thin-layer chromatography plates were activated for 30 min at 120 °C and then transferred to a nitrogen chamber, where they were allowed to cool to room temperature. Chicken liver, heart, plasma, and egg yolk phospholipids were separated from their respective total lipids by using two-dimensional thin-layer chromatography (TLC) on silica gel H precoated plates (20 × 20 cm, 250 μm thick) impregnated with 7.5% magnesium acetate (Analtech, Inc.). A 50–100-μL sample of total lipid, dissolved in chloroform, was applied to the TLC plate corner with a Hamilton syringe under a stream of nitrogen gas. Simultaneously, the same volume of sample was spotted in the opposite corner of the plate. This spot was not chromatographed but was recovered and used to check the percent of recovery of the sample. The plate was allowed to stand in a nitrogen atmosphere until the chloroform evaporated from the chromatographed samples.

The chromatography jars were prepared 1 h in advance to allow for equilibration of the solvent system with the atmospheric conditions inside the jars. Separation of individual phospholipids was accomplished by using a solution of chloroform–methanol–ammonium hydroxide (65:25:5 v/v/v) in the first dimension and a solution of chloroform–acetone–methanol–glacial acetic acid–water (6:8:2:2:1 v/v/v/v/v) in the second dimension according to the procedure of Nelson (1972). The phospholipid spots were visualized by exposure to iodine vapor and identified with a standard mixture of individual phospholipids. The phospholipid standard mixture was phosphatidylcholine (egg), phosphatidylethanolamine (egg), phosphatidylinositol (bovine liver), phosphatidylserine (bovine brain), sphingomyelin (egg), cardiolipin (bovine heart), lysophosphatidylcholine (egg), and lysophosphatidylethanolamine (egg) obtained from Avanti Polar Lipids, Inc. (Pelham, AL). The standard was spotted on another TLC plate and simultaneously run with the samples. The individual phospholipid spots and silica gel were quantitatively scraped from the plates with a razor blade into individual Pyrex tubes and then prepared for the lipid phosphorus analysis by using the perchloric acid digestion method according to the procedure of Eng and Noble (1968).

The phospholipid samples were digested with 0.9 mL of 70% perchloric acid and heated for 1 h at 190 °C in a tube heater. Each sample and standard tube was covered with a marble to prevent any loss of the mixture. Potassium phosphate, monobasic (Sigma Chemical Co., St. Louis, MO), was used as a phosphorus standard. When digestion was complete, the samples and standards were cooled to room temperature, and then 7.7 mL of mixed reagent was added to each tube. The mixed reagent contained 7 parts of deionized water, 0.5 part of 2.5% ammonium molybdate, and 0.2 part of Fiske-Subbarow reagent. The Fiske-Subbarow reagent was prepared by throughly mixing 30 g of $NaHSO_3$ in 200 mL of water and then adding 0.5 g of 1-amino-2-hydroxynaphthalene-4-sulfonic acid and 1 g of Na_2SO_3. The resultant solution was filtered and stored in a brown glass bottle no longer than 1 week. The contents of each tube were then mixed well, and the tubes were covered with a marble and placed in a boiling water bath for 10 min, which allowed color development. The tubes were again cooled and the contents mixed well. The samples were cleared by centrifugation at 1000*g* for 10 min at 4 °C. The absorbance of the samples and standards was read at 820 nm in a Beckman Model 25 spectrophotometer. Sample absorbance values were converted to micrograms of phosphorus from standard curves which had a concentration range of 0.25–10.00 μg of phosphorus.

Fatty Acid Analysis. Following the above thin-layer chromatography procedure, sample phospholipid spots were scraped from the TLC plates and placed into individual, capped test tubes for transmethylation, which was accomplished by using 5 mL of 14% boron trifluoride in methanol (Sigma) and 1 mL of dry benzene according to the procedure of Morrison and Smith (1964). Each tube was flushed with nitrogen, tightly capped, and heated in a steam bath for 1 h. After transmethylation, all tubes were cooled to room temperature, and then 10 mL of 4 °C distilled water was added. The methyl esters were extracted twice with 3-mL aliquots of hexane. The hexane was dried over anhydrous sodium sulfate. The fatty acid methyl esters present in the hexane extract were concentrated and stored under nitrogen gas in a −80 °C deep freezer.

Analysis of fatty acid methyl esters was accomplished with a Hewlett-Packard Model 5790A gas chromatograph equipped with a 30 m × 0.25 mm i.d. capillary column coated with SP-2340 with film thickness of 0.2 μm fused silica (Supelco Inc.). The oven was programmed for the temperatue range 190–210 °C with a heating rate of 1.3 °C/min. The injection and detector temperatures were both 250 °C. The hydrogen flow rate was 0.7 mL/min with a split ratio of 110:1. The detector signals were recorded with a Hewlett-Packared 3390A integrator. Eluting peaks were identified by comparison with retention times of known standards (Supelco Inc.; Nu Chek Prep., Inc.).

Thiobarbituric Acid Assay. Thiobarbituric acid reactive substances were quantitated by using a modification of the Buege and Aust (1978) procedure. On the day of use, a trichloroacetic acid (TCA)–thiobarbituric acid (TBA) stock solution consisting of 15% w/v TCA and 0.375% w/v TBA in 0.25 M HCl was prepared. This solution was mildly heated to assist in the dissolution of the thiobarbituric acid.

After the samples were chromatographed via thin-layer chromatography, the resulting phospholipid spots were scraped from the TLC plates into capped test tubes and immediately

Table I. Distribution of Individual Phospholipids in Chicken Liver, Heart, Plasma, and Egg Yolk (%)[a]

phospholipid fractions[b]	liver[c]	heart[c]	plasma[c]	egg yolk[c]
PC	48.91 ± 2.87[e]	45.76 ± 2.25[g]	71.15 ± 3.71[d]	69.25 ± 3.22[d]
PE	31.32 ± 1.95[d]	30.22 ± 1.76[f]	22.28 ● 0.95[c]	21.76 ± 1.18[c]
PI	8.40 ± 1.02[c]	6.65 ± 0.75[e]		2.59 ± 0.33[b]
PS	3.46 ± 0.42[b]	3.33 ± 0.28[c]	3.82 ± 0.41[b]	
SP	3.64 ± 0.31[b]	5.52 ± 0.51[de]	1.43 ± 0.17[a]	2.74 ± 0.28[b]
CL	3.04 ± 0.26[b]	5.20 ± 0.38[d]		
LyPC	1.23 ± 0.15[a]	2.20 ± 0.19[b]	1.32 ± 0.10[a]	2.58 ± 0.21[b]
LyPE		1.12 ● 0.17[a]		1.08 ± 0.15[a]

[a] Percent individual phospholipid classes was calculated from phosphorus distribution without a correction factor. Data are presented as means ± standard deviations of 6 individual chickens and 24 eggs (4 eggs per group). [b] PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SP, sphingomyelin; CL, cardiolipin; LyPC, lysophosphatidylcholine; LyPE, lysophosphatidylethanolamine. [c] Mean values within the same column followed by different letters are significantly different ($P < 0.05$).

assayed for TBA content. Five milliliters of the TCA–TBA stock solution was added to each tube. On the basis of previous calculations all tubes then received 0.5% butylated hydroxytoluene (BHT) dissolved in absolute ethanol such that the final concentration was 250 μg BHT/mg of individual phospholipid. The contents of the tubes were mixed throughly. The samples were next heated in a boiling water bath for 15 min and cooled to room temperature before centrifugation at 1500*g* for 10 min. The absorbance of the supernate was measured at 532 nm against the blank, which contained all reagents added to sample tubes, including silica gel. The results were expressed as nanomoles of TBARS by using a molar extinction coefficient of 1.56×10^{-5} M^{-1} cm^{-1}.

Statistical Analysis. Analysis of variance and Duncan's multiple range test (Steel and Torrie, 1980) were used to determine the significance of differences among the means of the percentage of phospholipid fractions distribution and thiobarbituric acid reactive substances produced by individual phospholipids in chicken liver, heart, plasma, and egg yolk.

RESULTS AND DISCUSSION

Distribution of Individual Phospholipids in Various Tissues. A relatively high percentage of phospholipid in the total lipid was found in all the analyzed tissues. Chicken liver, heart, plasma, and egg yolk were found to contain 33.2%, 42.5%, 38.4%, and 31.9% phospholipid in the total lipid, respectively. These percentages of phospholipid in liver, heart, and plasma are about 7% lower than those found in our previous study of 4-month-old New Hampshire × Columbian pullets (Pikul et al., 1985) and are in agreement with data presented for liver and heart tissue of 20-week-old chickens by Marion and Miller (1968). Similar amounts of phospholipid were found in total liver lipid by Sgoutas (1966) and in plasma of layer chickens by Leszczynski et al. (1987). The percentage of egg yolk phospholipid in the total lipid was very similar to that in previous papers (Lea, 1962; Privett et al., 1962; Christie and Moore, 1972; Marion and Woodroof, 1968).

The distributions of individual phospholipids in chicken liver, heart, plasma, and egg yolk are presented in Table I. These results indicated that the PC and PE were present in relatively greater amounts than the other phospholipid fractions (from 76% in chicken heart to above 90% in chicken plasma and egg yolk). The percentage ratio of PC to PE ranged from 1.5 in chicken liver and heart to 3.2 in chicken plasma and egg yolk. Choline, ethanolamine, and serine plasmalogens were included in the PC, PE, and PS fractions from liver and heart and in the PE fraction of plasma and egg yolk. They were not estimated separately. PI and PS together comprised above 10% of the total phospholipid in chicken liver and heart; the percentage of PI was 2 times greater than the percentage of PS. The percentage of SP in the analyzed tissues exhibited the greatest range (from 1.4% in chicken plasma to 5.5% in chicken heart). CL was found only in chicken liver and heart tissues at concentrations of 3.0% and 5.2%, respectively. Small amounts of LyPC were present in all analyzed tissues (from 1.2% in chicken liver to 2.6% in egg yolk). The other phospholipid, which was found only in chicken heart and egg yolk at a level of about 1%, was LyPE.

Marion and Miller (1968) showed that when chicken liver and heart phospholipids were separated, the PC, PE, PS, CL, and SP were easily identified. Upon further analysis, they found that the PE and PS fractions also contained small quantities of PI, while the PC and SP fractions contained small amounts of LyPC. Sgoutas (1966), who separated total phospholipid from chicken liver, identified PC, PE, PS, PI, CL, SP, and LyPC. We found PC was present in the greatest quantity followed by PE, PI, PS, SP, CL, LyPC, and LyPE in chicken tissue. However, Marion and Miller (1968) reported higher amounts of SP and CL and a much lower percentage of PI in chicken liver and heart tissues. We found the distributions of PC, PE, PI, PS, and SP were similar to those reported by Yeo and Horrocks (1988) for beef liver and heart tissues.

The percentage of individual phospholipids in egg yolk in the present study was generally in agreement with data published by others (Noble and Moore, 1965; Parkinson, 1966; Evans et al., 1967; Momma et al., 1970; Gornall and Kuksis, 1971; Christie and Moore, 1972; Tsiagbe et al., 1988). However, most authors separated the total egg yolk phospholipids into only four fractions. PC, PE, and SP were analyzed separately, while the other phospholipids, such as PI, LyPC, LyPE, and PA, were analyzed as one fraction. These four fractions constituted 68.6–78.0%, 15.0–24.0%, 0.9–9.1%, and 2.2–3.4% of the total phospholipid, respectively.

Fatty Acid Profile of Individual Phospholipids. The fatty acid compositions of individual phospholipids from chicken liver and heart tissues are presented in Tables II and III. Palmitic acid, which was the most abundant fatty acid, constituted about 30% of the liver and heart PC, while stearic acid accounted for only about 10% of the PC. Dimethyl acetal (DMA) derivatives of hexadecanal [16:A] and octadecanal [18:A] occurred at concentrations of 4.4% in liver and 3.0% in heart PC. Arachidonic acid was present in concentrations of 11.9% and 14.7% in chicken liver and heart PC, respectively. Stearic acid was the largest constituent of PE. The concentration of this saturated fatty acid ranged from 25.7% in liver to 30.4% in heart. The liver and heart PE contained about twice as much polyenoic fatty acids as the PC, especially arachidonic acid, which occurred at concentrations of 14.8% and 25.4%, respectively. These data are in agreement with data presented by Sgoutas (1966) and Chung et al. (1967) for chicken liver PC and PE fractions. Stearic acid reached an unusually high concentration in PI, ranging from 44% in heart to 47% in liver, while the palmitic acid concentration was only 3.2% and 8.0%, respectively. PI was found to be exceptionally rich in arachidonic acid, which occurred at concentrations of 20.9% in liver and 28.1% in heart. The PI fatty acid composition in the present study was in agreement with data published by Sgoutas (1966) for chicken liver except that Sgoutas found a greater concentration of arachidonic acid. The liver and heart CL fraction was characterized by relatively low

Table II. Fatty Acid Profile of Individual Phospholipid Classes from Chicken Liver[a]

fatty acids[b]	phospholipid fractions[c]						
	PC	PE	PI	PS	SP	CL	LyPC
12:0	0.21	0.33	0.17	0.28	0.38	0.21	0.10
14:0	0.33	0.38	0.06	0.25	0.85	0.26	1.65
14:1*n*-5	0.05	0.10	0.10	0.18	0.15	0.15	0.21
15:0	0.56	0.14	0.04	0.41	0.21	0.41	0.46
15:1	0.07	0.27		0.71	0.05	0.65	
16:A	1.72	1.74		0.68			2.06
16:0	30.31	15.48	8.02	9.37	31.25	1.74	33.02
16:1*n*-7	0.87	1.86	0.45	0.78		0.95	1.16
17:0	2.40	2.14	1.94	2.96	3.71	0.59	0.41
17:1	0.10						0.36
18:A	2.71	2.91		1.75			4.17
18:0	9.59	25.67	47.18	39.08	18.51	1.36	24.06
18:1*n*-9	24.74	15.68	7.47	13.28	3.20	12.53	12.83
18:2*n*-6	10.16	8.43	4.03	8.48	2.72	60.36	9.06
18:3*n*-6	0.08		0.18				
18:3*n*-3	0.04	0.62				0.38	0.13
20:0	0.12			0.84	3.68		0.36
20:1*n*-9	0.20	0.20				0.75	0.97
20:2*n*-6	3.91	1.86	2.11	1.54	1.63	9.57	5.26
20:3*n*-6	0.15	0.43	3.21	0.74		0.48	1.27
20:4*n*-6	7.18	14.81	20.91	11.13	1.07	2.08	2.46
20:3*n*-3	0.28						
20:5*n*-3	1.44	1.04	2.17				
22:0				2.46	13.15	0.16	
22:2*n*-6					1.32	5.28	
23:0					2.50		
22:4*n*-6	0.36	0.53	1.15	1.13		0.78	
22:5*n*-6	1.14	1.68	0.81	2.17		0.44	
24:0					5.48		
22:6*n*-3	1.28	3.70		1.78		0.46	
24:1*n*-9					10.14	0.41	
saturated	47.95	48.79	57.41	58.08	79.72	4.73	66.29
monoenoic	26.03	18.11	8.02	14.95	13.54	15.44	15.53
dienoic	14.07	10.29	6.14	10.02	5.67	75.21	14.32
polyenoic	11.95	22.81	28.43	16.95	1.07	4.62	3.86

[a] Data are presented as means of two separate determinations of pooled sample from six individual chickens. [b] As area percent of total fatty acids. [c] See Table I.

Table III. Fatty Acid Profile of Individual Phospholipid Classes from Chicken Heart[a]

fatty acids	phospholipid fractions							
	PC	PE	PI	PS	SP	CL	LyPC	LyPE
12:0	0.08	0.05	0.17	0.27	0.51	0.08	0.81	0.70
14:0	0.52	1.32	0.21	0.25	0.41	0.10	0.95	0.89
14:1*n*-5	0.53	0.88	0.12	0.17	0.31	0.15	0.26	0.71
15:0	0.25	0.17			0.35	0.10	0.37	1.16
15:1	0.40	0.63		0.41	0.15		0.41	
16:A	2.21	3.05		0.63			2.17	1.25
16:0	29.53	6.26	3.16	6.33	26.58	1.68	36.83	25.32
16:1*n*-7	0.50	1.54	0.48	0.57		0.91	0.81	0.88
17:0	2.15	1.61	2.17	3.10	2.17	0.68	2.17	2.18
17:1	0.31	0.32						
18:A	0.82	2.87		1.28			0.59	1.85
18:0	9.26	30.43	44.01	35.67	24.82	1.41	24.75	35.18
18:1*n*-9	22.68	10.67	6.52	10.39	4.97	10.11	9.76	8.65
18:2*n*-6	14.31	8.41	6.22	15.71	4.45	73.81	11.16	8.95
18:3*n*-6	0.19	0.10	0.17			0.26		
18:3*n*-3	0.22							
20:0	0.20			2.13	4.38		0.17	
20:1*n*-9	0.35	0.38		0.71		0.76	0.38	
20:2*n*-6	1.22	1.51	1.90	1.60	3.68	1.21	3.76	5.19
20:3*n*-6	1.61	1.10	2.71	1.51		0.36	1.24	2.98
20:4*n*-6	9.87	25.42	28.08	12.71	1.51	2.36	3.41	4.11
20:3*n*-3		0.08	2.48					
20:5*n*-3	0.80							
22:0				1.07	7.91	0.36		
22:1*n*-9				0.56		0.92		
22:2*n*-6					2.53	1.64		
23:0					1.57			
22:4*n*-6	0.71	1.47	0.89	2.33		1.23		
22:5*n*-6	0.87	1.22	0.71	1.14		0.45		
24:0					5.75			
22:6*n*-3	0.41	0.51		1.46		0.75		
24:1*n*-9					7.95	0.67		
saturated	45.02	45.76	49.72	50.73	74.45	4.41	68.81	68.53
monoenoic	24.77	14.42	7.12	12.81	13.38	13.52	11.62	10.24
dienoic	15.53	9.92	8.12	17.31	10.66	76.66	14.92	14.14
polyenoic	14.68	29.90	35.04	19.15	1.51	5.41	4.65	7.09

[a] See Table II.

amounts of palmitic and stearic acids and very high quantities of linoleic acid, which ranged from 60.4% in liver to 73.8% in heart. Others (Sgoutas, 1966; Marion and Miller, 1968) have also reported very high concentrations of linoleic acid in the CL fatty acid profiles from chicken liver and thigh muscles. The liver and heart LyPC contained more saturated and less monounsaturated fatty acids than PC, and the amount of polyenoic fatty acids in LyPC was about 3.2 times less than that found in the PC fraction. The fatty acid profile of LyPE, which is found only in heart phospholipid, was generally similar to the fatty acid composition of LyPC. However, LyPE contained about 1.5 times more polyenoic fatty acids than LyPC. Also, LyPE had a lower percentage of palmitic acid and a higher percentage of stearic acid than LyPC, but the sum of these fatty acids represents about 60% of the total fatty acids found either in LyPC or in LyPE fractions.

The fatty acid composition of each phospholipid found in chicken plasma is shown in Table IV. The distribution of plasma fatty acids in PC, PE, PS, SP, and LyPC fractions was very similar to the fatty acid composition of the individual phospholipids from liver. However, the percentage of polyenoic fatty acids in each plasma phospholipid was slightly lower than in liver phospholipid. The highest amount of polyenoic fatty acids, especially arachidonic acid, in plasma phospholipids was found in PE, followed by PS and PC fractions. The fatty acid composition of plasma PC found in this paper was similar to that presented by Chung et al. (1967) for 10-month-old New Hampshire hens.

Palmitic acid and oleic acid were the major constituents of egg yolk PC, each at a level of above 30% (Table V). Palmitic acid and stearic [18:0] acid were the main saturated components found in PC, at concentrations of about 45% of the total fatty acids. Linoleic acid accounted for about 11% of the total fatty acids. Arachidonic acid, which was a major constituent of the polyenoic fatty acids, occurred in 8.5% of the total PC fatty acids. The egg yolk PC fatty acid composition was in agreement with results published by others (Privett et al., 1962; Noble and Moore, 1965; Marion and Woodroof, 1968; Momma et al., 1970; Gornall and Kuksis, 1971; Christie and Moore, 1972).

Malondialdehyde Formation by Individual Phospholipids from Various Tissues. The percentage of TBARS obtained from individual phospholipids is presented in Table VI. PC was found to produce from about 35% (chicken liver and heart) to 59% (chicken plasma and egg yolk) of the total TBARS present in the total phospholipid. PE generated from 34% (egg yolk and chicken plasma) to 42% (chicken liver and heart) of the total phospholipid TBARS. These two phospholipid fractions contributed from 77% (chicken liver and heart) to 95% (chicken plasma) of all the TBARS produced by the total phospholipid in each analyzed tissue. Relatively high concentrations of TBARS were also produced by the liver and heart PI fraction, which ranged from 14% to 17%, while only a third as much TBARS was found in the egg yolk PI fraction, probably because egg yolk has a lower initial concentration of PI in the total phospholipid than

Table IV. Fatty Acid Profile of Individual Phospholipid Classes from Chicken Plasma[a]

fatty acids	phospholipid fractions				
	PC	PE	PS	SP	LyPC
12:0	0.03	0.08	0.17	0.96	0.62
14:0	0.21	0.21	0.36	0.84	0.95
14:1*n*-5	0.04	0.15		0.48	0.31
15:0	0.11	0.09	0.71	1.06	
15:1	0.05	0.12		0.30	
16:A		0.53	2.17		
16:0	34.01	15.90	10.52	33.36	38.18
16:1*n*-7	1.12	0.66	1.15	0.66	0.36
17:0	1.73	2.08	0.49	1.76	1.89
17:1	0.05	0.31			
18:A		0.28	3.17		
18:0	12.87	32.93	41.72	20.14	26.21
18:1*n*-9	29.37	16.82	9.13	5.75	12.52
18:2*n*-6	11.76	7.63	10.36	3.51	10.98
18:3*n*-6	0.10	0.08			
18:3*n*-3	0.05		0.98		
20:0	0.08		0.45	5.78	1.59
20:1*n*-9	0.09	0.26			0.17
20:2*n*-6	0.21	0.28	4.82	2.11	3.17
20:3*n*-6	0.33	0.43	1.68		0.98
20:4*n*-6	5.60	15.35	9.86	0.55	2.07
20:3*n*-3	0.57				
20:5*n*-3	0.20				
22:0			0.28	9.17	
22:2				1.45	
23:0				1.00	
22:4*n*-6	0.11	0.91	0.85		
22:5*n*-6	0.85	2.30			
24:0				4.67	
22:6*n*-3	0.46	2.60	1.13		
24:1*n*-9				6.45	
saturated	49.04	52.10	60.04	78.74	69.44
monoenoic	30.72	18.32	10.28	13.64	13.36
dienoic	11.97	7.91	15.18	7.07	14.15
polyenoic	8.27	21.67	14.50	0.55	3.05

[a] See Table II.

chicken liver and heart. The PS produced from 3.4% to 4.5% of the TBARS found in chicken liver and plasma, respectively. The CL fraction was responsible for only 1.7–3.1% of the total TBARS obtained in chicken liver and heart phospholipid. Only trace amounts of TBARS were found in SP and LyPC from all analyzed tissues. The same was true for LyPE from chicken heart and egg yolk. The following phospholipid fractions produced from 0.3% (liver LyPC) to 1.0% (heart SP) of the TBARS found in the total phospholipid (Table VI). These differences in the percentage of TBARS produced by individual phospholipid are the results of the potential for oxidation of each phospholipid fraction as well as the large differences in the percentage of individual phospholipids of the total phospholipid in the analyzed tissues. The potential formation of TBARS by individual phospholipid classes from various tissues indicates the ratio % TBARS/% PL (Table VI). For example, PC was a major constituent of all of the phospholipid fractions (45–71%) and contributed 34–59% of the total TBARS measured; the ratio % TBARS/% PL ranged from 0.73 to 0.86. PE constituted 22–31% of the total phospholipid and produced 33–43% of the TBARS found in the total phospholipid; the ratio ranged from 1.32 to 1.52. In contrast, SP accounted for approximately 1.4–5.5% of the total phospholipid but produced only 0.4–1.0% of the TBARS that occurred in the total phospholipid; the ratio % TBARS/% PL ranged from 0.18 to 0.25. The relative inactivity of the SP fraction to TBARS formation supports the earlier findings of Acosta et al. (1966). Lea (1957) has studied thin-layer oxidation of egg PE and PC. He found that PE oxidized much faster than the PC fraction.

Table V. Fatty Acid Profile of Individual Phospholipid Classes from Egg Yolk[a]

fatty acids	phospholipid fractions					
	PC	PE	PI	SP	LyPC	LyPE
12:0	0.03		0.31	1.15	0.35	0.75
14:0	0.21	0.21	0.58	0.69	0.41	0.96
14:1*n*-5			0.16	0.22		
15:0	0.07	0.87		1.48	1.21	1.87
15:1						
16:A		0.52				
16:0	33.16	17.31	8.67	40.85	45.79	39.61
16:1*n*-7	1.10	1.65	0.57		1.18	0.75
17:0	0.91	0.86	2.16	4.15	1.45	2.41
17:1	0.09					
18:A		0.51				
18:0	12.53	30.02	46.71	15.48	24.97	27.19
18:1*n*-9	31.59	19.49	8.58	4.16	13.31	10.45
18:2*n*-6	11.08	8.37	5.41	3.46	6.48	6.27
18:3*n*-6	0.13					
18:3*n*-3	0.06					
20:0	0.09			5.81	0.19	
20:1*n*-9	0.08					
20:2*n*-6	0.12	0.30	2.28	2.68	2.13	6.07
20:3*n*-6	0.28		2.04		0.56	1.16
20:4*n*-6	5.47	13.70	22.01	0.51	1.97	2.51
20:5*n*-3	0.10					
22:0	0.41			7.56		
22:4*n*-6	0.29	1.15	0.17			
22:5*n*-6	1.67	2.21	0.35			
24:0				4.86		
22:6*n*-3	0.45	2.83				
24:1*n*-9	0.08			6.94		
saturated	47.41	50.30	58.43	82.03	74.37	72.79
monoenoic	32.94	21.14	9.31	11.32	14.49	11.20
dienoic	11.20	8.67	7.69	6.14	8.61	12.34
polyenoic	8.45	19.89	24.57	0.51	2.53	3.67

[a] See Table II.

The percentage of polyenoic fatty acids, especially arachidonic acid, followed the same order of concentration in the phospholipid fraction as TBARS (Tables II–V). The highest amount of polyenoic fatty acids was found in PI followed by PE, PS, and PC. Much lower concentrations were measured in LyPE followed by LyPC and SP. This relationship between the amount of polyenoic fatty acids and TBARS formation supports earlier research in which autoxidation, specifically in fatty acids with three or more methylene-interrupted double bonds, was found to be responsible for TBARS formation (Dahle et al., 1962). Pryor et al (1976) proposed a mechanism that explained more adequately the reason autoxidized dienes produced very little malondialdehyde (MDA) compared to autoxidized trienes. Other researchers (Kanazawa et al., 1983; Pearson et al., 1983) have reported that MDA was not a main product nor a major thiobarbituric acid reactive substance during autoxidation of linoleic acid. The fact that each isomer of methyl arachidonate monohydroperoxide is capable of yielding TBARS (Terao and Matsushita, 1981) leaves open the possibility that each molecule of fatty acid with four or more methylene double bonds may produce two molecules of MDA. In addition to being more reactive to oxidation than linoleic acid, fatty acids with four or more double bonds may also produce higher yields of MDA. Therefore, the relatively high amounts of arachidonic acid as well as substantial amounts of dodecatetraenoic, dodecapentaenoic, and dodecahexaenoic acid found in PI, PE, PS, and PC may be sufficient to account for the TBARS found in these phospholipid fractions. Phospholipids with fatty acids containing high amounts of polyenoic fatty acids (PI, PE, PS, and PC) are therefore apparently more responsible for

Table VI. Percentage of Thiobarbituric Acid Reactive Substances Produced by Individual Phospholipids from Chicken Liver, Heart, Plasma, and Egg Yolk (A)[a] and TBARS Formation Potential by Individual Phospholipid Classes (B)[b]

phospholipid fractions[c]	liver		heart		plasma		egg yolk	
	A[d]	B[d]	A[d]	B[d]	A[d]	B[d]	A[d]	B[d]
PC	35.55 ± 1.87[g]	0.73[d]	34.83 ± 1.51[f]	0.76[e]	59.02 ± 2.05[d]	0.83[c]	59.26 ± 1.93[e]	0.86[d]
PE	41.22 ± 2.17[f]	1.32[f]	42.57 ± 2.03[e]	1.41[g]	35.63 ± 1.37[c]	1.60[e]	33.14 ± 0.98[d]	1.52[e]
PI	17.05 ± 1.22[e]	2.03[g]	13.94 ± 1.17[d]	2.10[h]			5.87 ± 0.37[c]	2.27[f]
PS	3.56 ± 0.41[d]	1.03[e]	3.36 ± 0.36[c]	1.01[f]	4.54 ± 0.52[b]	1.19[d]		
SP	0.64 ± 0.05[b]	0.18[a]	1.03 ± 0.09[b]	0.19[a]	0.36 ± 0.03[a]	1.25[a]	0.53 ± 0.04[ab]	0.19[a]
CL	1.67 ± 0.13[c]	0.55[c]	3.10 ± 0.39[c]	0.60[d]				
LyPC	0.31 ± 0.02[a]	0.25[b]	0.65 ± 0.05[a]	0.30[b]	0.45 ± 0.03[a]	0.34[b]	0.73 ± 0.05[b]	0.28[b]
LyPE			0.52 ± 0.04[a]	0.46[c]			0.47 ± 0.03[a]	0.44[c]

[a] Data are presented as means ± standard deviations of three individual samples pooled from six chickens and 24 eggs (8 eggs per group). [b] B, ratio = percentage of TBARS produced by individual phospholipid classes/percentage of individual phospholipid classes (Table I). [c] See Table I. For comparison of phospholipids composition see Table I. [d] Mean values within the same column followed by different letters are significantly different ($P < 0.05$).

the generation of TBARS than the CL, LyPE, LyPC, and SP fractions in chicken liver, heart, plasma, and egg yolk.

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