Effects of dietary fat on the amounts and proportions of the individual lipids in turkey muscle

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1. Lipid extracted from breast and leg muscle of 10-week-old turkeys was fractionated by preparative thin-layer chromatography and five individual 'neutral' and eight individual phospholipid fractions, representing 95% by weight of the extractable lipid, were recovered from the plates for analysis.

2. The 'neutral' lipids from breast and leg muscle consisted mainly of triglyceride (202–497 and 1644–2333 mg/100 g), together with cholesterol (74 and 103 mg/100 g), free fatty acid (27 and 123 mg/100 g), diglyceride (17 and 66 mg/100 g) and cholesterol ester (9 and 12 mg/100 g).

3. The phospholipids contained phosphatidylcholine (367 and 500 mg/100 g), phosphatidylethanolamine (157 and 279 mg/100 g), phosphatidylinositol (60 and 109 mg/100 g), sphingomyelin (43 and 62 mg/100 g), phosphatidylserine (31 and 61 mg/100 g), 'cardiolipin' (23 and 35 mg/100 g), lysophosphatidylcholine (9 and 11 mg/100 g) and 'origin fraction' (6 and 7 mg/100 g), accounting together (700 mg and 1070 mg/100 g for breast and leg muscle respectively) for 98% of the lipid phosphorus extracted.

4. Partial replacement of carbohydrate in the cereal-based diet by beef fat (2·5%) or anchovy oil (2·5 or 5·0%) had no effect on the amount of any of the lipid fractions, except for triglyceride, which varied considerably and was lowest in tissue from groups receiving 2·5% anchovy oil.

In previous papers the effects of giving beef fat (2·5%) or anchovy oil (2·5%; 2·5% plus 0·02% ethoxyquin; 5%) in isocaloric cereal-based diets (2·3% lipid) to turkeys from 2 to 10 weeks of age were investigated, both in relation to the composition and stability of the triglyceride depot fat (Neudoerffer & Lea, 1966) and to the compositions of five neutral and six phospholipid fractions isolated from the muscle (Neudoerffer & Lea, 1967). It was shown that individual lipid fractions differed widely in fatty acid composition and in the degree to which they could be influenced by dietary fat supplements.

But quantitative determination of the actual amounts of each lipid fraction present in the muscle required a knowledge of the mean molecular weight of each fraction, and therefore could not be presented until the fatty acid analyses had been completed. This information, together with necessary preliminary investigations of the quantitative extraction of lipid from muscle and of the quantitative recovery of separated lipid fractions from preparative thin-layer chromatographic plates, are reported in the present paper.

EXPERIMENTAL

Material

The diets and performance of the birds have already been described (Neudoerffer & Lea, 1966, 1967). In brief, composite samples of breast and leg muscle were obtained from groups of young turkeys receiving the following diets to 10 weeks of age.

Expt 1

Diet A: 92 parts basal, 2·5 parts beef fat, 5·5 parts maize starch.
Diet B: 92 parts basal, 2·5 anchovy oil, 5·5 parts maize starch.
Diet C: same as B, plus 0·02 % ethoxyquin.
Diet D: 92 parts basal, 5·0 parts anchovy oil, 3·0 parts cellulose powder.

Expt 2

Diet A_1: 92 parts basal, 2·5 parts beef fat, 2·5 parts maize starch and 3 parts cellulose powder.
Diet E: 92 parts basal, 8 parts maize starch.

Extraction of the lipids

Folch, Lees & Stanley (1957) showed that lipids can be rapidly and completely extracted from many tissues and freed from non-lipid contaminants by a 'cold' extraction procedure, with partition between 'lipid' and 'aqueous' phases of controlled composition. Subsequently, other workers have modified the Folch procedure to suit their particular requirements. Bligh & Dyer (1959) changed the ratio of chloroform:methanol:water from 8:4:3 to 2:2:1·8 for extraction of the lipids from fish muscle, and Rhodes & Lea (1961) simplified the Folch method for routine extraction of the lipids of lamb's liver. Winter (1963) compared several of the cold extraction methods in current use and found that there was little difference between their efficiencies: he concluded that, if one was to be preferred, it should be the original procedure of Folch.

To test the efficiency of the extraction of lipids from turkey meat, breast muscle from three sources was treated by the Folch procedure, as modified by Rhodes & Lea (1961), but using the solvent to sample ratio suggested by Winter (1963) and working at 1° to minimize autoxidative or hydrolytic decomposition. Other portions of the same muscle were ground, freeze-dried and extracted for 1 h in darkness under nitrogen by a continuous hot extraction procedure, with chloroform:methanol (2:1) containing 0·5 mg/100 ml quinol. Sufficient 0·9 % (w/v) sodium chloride solution was then added to extracts prepared by both methods to give an 8:4:3 ratio of chloroform:methanol:water and, after stirring for 1 min, the phases were separated by centrifugation at 0°. Cooling to −20° followed by recentrifugation was sometimes necessary to clarify the lower, lipid-containing phase. The yield of lipid was ascertained by drying samples of the extract to constant weight at 40° under reduced pressure. Washing the hot extract in this way was found to give consistently higher yield than evaporating the unwashed extract followed by re-extraction of the residue with chloroform, a discrepancy attributable to incomplete extraction of the lipid by the chloroform in the latter pro-

cedure. The results (Table 1) showed that the Folch extraction procedure gave 12–14% less lipid than the hot extraction method.

A number of variations of the proposed cold extraction procedure were therefore carried out using tissue no. 1, in an attempt to improve its efficiency. These were (*a*) applying the Folch extraction without the Rhodes–Lea modifications; (*b*) varying the periods of maceration; (*c*) varying the ratio of solvent to weight of tissue, within the limits suggested by Winter; (*d*) changing the order of addition of the solvents during maceration; (*e*) carrying out the extraction at room temperature; (*f*) carrying out the extraction according to the method of Bligh & Dyer. The yields of lipid were: procedures *a–e*, 1·10 ± 0·02 g lipid/100 g tissue; procedure *f*, 1·05 ± 0·04 g lipid/100 g tissue; which showed that a single cold extraction could not be made to yield as much lipid as hot extraction. The method of Bligh & Dyer appeared to be slightly inferior to that of Folch.

Table 1. *Yields of lipid from three samples of turkey muscle obtained by hot and cold extraction procedures*

Extraction procedure	Lipid obtained (g/100 g tissue)		
	1*	2†	3‡
Hot	1·28	1·19	1·13
Cold	1·10	1·04	1·00

* Breast muscle from 6½ lb commercial bird.
† Breast muscle from birds described by Lea *et al.* (1966), diet 4.
‡ Breast muscle from birds described by Lea *et al.* (1966), diet 1.

Table 2. *Composition of the lipids extracted from turkey muscle by three procedures*

Lipid fraction	Content per 100 g/tissue		
	Hot extraction	Method of Folch *et al.* (1957)	Method of Bligh & Dyer (1959)
Total lipid*	1·19 ± 0·02 g	1·04 ± 0·02 g	0·99 ± 0·04 g
Phosphorus*	25·6 ± 0·3 mg	24·3 ± 0·6 mg	23·2 ± 0·7 mg
Nitrogen*	11·1 ± 0·2 mg	11·3 ± 0·4 mg	—
Free fatty acids*	2·2 ± 0·3 mg	2·0 ± 0·2 mg	2·0 ± 0·2 mg
Carboxylic ester	3·45 m-equiv.	2·80 m-equiv.	2·68 m-equiv.
Re-extraction of tissue by hot method			
Total lipid	Trace	0·17 g	—
Phosphorus	Trace	1·5 mg	—

* Means and standard deviations.

The compositions of lipid extracted from tissue 2 by three procedures are given in Table 2. Bligh & Dyer extracts gave high and variable nitrogen contents which could be reduced by drying the lower phase, as suggested by Marion & Woodroof (1965), but only at the expense of a slight loss of lipid. The phosphorus contents, total fatty acid contents (as recovered after saponification) and carboxylic ester values (as determined by the hydroxylamine method of Snyder & Stephens (1959)) of the lipid

extracts confirmed the conclusion that the hot extraction method yielded more lipid than the cold, but, unexpectedly, indicated that the difficultly extractable lipid was mainly non-phospholipid. Since determination of the free fatty acid content of the extracts, by the method of Duncombe (1963) as adapted by Hanson & Olley (1963), showed no evidence of any hydrolytic decomposition during hot extraction, this procedure was standardized in further work.

Although acidification of the solvents used in the cold extraction process might well have increased the yield of lipid, particularly of phosphatidylinositol (Dawson, 1965), this method could not be employed because it would certainly destroy the plasmalogens.

Quantitative analysis of the lipids

Only very limited information relating to the content of the individual lipids in poultry muscle has been published to date. Marion & Woodroof (1965) reported values for the neutral lipids of chicken, and Acosta, Marion & Forsythe (1966) for turkey muscle, both by gravimetric procedures. Total phospholipid content, based on phosphorus determination, has also been given, but no detailed analyses of the individual phospholipids of turkey muscle appear to be available.

Phospholipids. Individual phospholipid fractions, separated on silica gel thin-layer plates by the procedures already described (Neudoerffer & Lea, 1967), were determined by estimation of their phosphorus content, using the method of Parker & Peterson (1965) in which the determination is carried out without elution from the silica. Care, however, was necessary for quantitative results.

Preliminary investigation showed that although all six phospholipid fractions, phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylcholine (PC), sphingomyelin (SP) and lysophosphatidylcholine (LPC), could be quantitatively determined in the presence of silica, losses were liable to occur during chromatographic separation and subsequent manipulation. (1) Application of lipid too close to the lane marker line resulted in losses due to part of it reaching the channel free of adsorbent: these losses were prevented by applying lipid only to the central part of each lane. (2) Although satisfactory separations of phospholipids were readily obtainable when the plates could be handled under dry atmospheric conditions, even brief exposure to a moist atmosphere caused troublesome 'band spreading'. (3) Losses during transference of the lipid-containing silicic acid from the plate to the digestion vials were avoided by suspending the plate face downwards and scraping the the lipid-containing area into a funnel carried in the neck of the digestion flask. Lipids were made visible on the plate with iodine vapour or with a solution of fluoresceine (0·02 %): neither reagent interfered with the phosphorus determination.

Phosphorus was determined by the method of Bartlett (1959), as modified by Boettcher, van Gent & Pries (1961), the volume of 70 % perchloric acid used, however, being increased from 0·2 to 0·4 ml in order to wet the silica gel completely. Subsequently reduction of the volume of water added from 4·4 to 4·2 ml still left the acidity of the final solution within the range for maximum colour development (Bartlett, 1959).

Recoveries of six phospholipids obtained after chromatography of the pure fractions

are given in Table 3, and these factors, ranging from 87 to 100%, were used to correct the experimental values obtained from thin-layer chromatographic analyses of the turkey muscle phospholipids.

Neutral lipids. Determination of the amounts of di- and tri-glycerides present in the total lipid extracts was based on the glycerol contents of the fractions of these two lipids, after recovery from the thin-layer plates. Cholesterol and cholesterol esters were determined by assay of free cholesterol before and after saponification. The amount of free fatty acid in the lipid extract was determined colorimetrically, as copper complex (p. 118). Lipid 'X', present only in very small amounts, was not determined.

Table 3. *Recovery of phospholipid reference standards from plates after thin-layer chromatography*

Phospholipid standard	Amount of P applied to plate (μg)	Amount of P recovered from plate (μg)*	Recovered (%)
Phosphatidylethanolamine	5·36	4·94±0·09	92
Phosphatidylserine	5·44	5·01±0·08	92
Phosphatidylinositol	5·61	4·88±0·15	87
Phosphatidylcholine	4·99	4·79±0·11	96
Sphingomyelin	5·05	4·95±0·06	98
Lysophosphatidylcholine	5·10	5·10±0·05	100

* Means and standard deviations for twelve determinations.

Lipid glycerol can be determined by colorimetric (Lambert & Neish, 1950; Jover, 1963) or enzymic (Boltralik & Noll, 1960; Spinella & Mager, 1966) methods, but these are multi-step procedures, usually involving saponification of the lipid and recovery of the freed glycerol in an aqueous phase. Losses of glycerol easily occur during evaporation of the water (Le Baron, Folch & Rothleder, 1957). More recently, a gas chromatographic method for the determination of glycerol as acetate has been described (Horrocks & Cornwell, 1962), but doubt remains (Holla, Horrocks & Cornwell, 1964; Jellum & Björnstad, 1964) about the quantitative recovery of glycerol triacetate during the isolation of the derivatives. It was therefore decided to investigate the feasibility of determining glycerol gas chromatographically, as its trimethylsilyl (TMS) ether derivative.

Langer, Connell & Wender (1958) have shown that alcohols react with silylating reagents (trimethylchlorosilane and hexamethyldisilazane) to give volatile trimethylsilyl ether derivatives, and the use of these reagents has been routinely adapted for the synthesis of volatile derivatives of carbohydrates, including glycerol, for gas chromatography (Sweeley, Bentley, Makita & Wells, 1963; Lee & Ballou 1965; Brower, Jeffery & Folsom, 1966). The method of Sweeley *et al.* (1963) was followed.

Free glycerol was obtained from the triglyceride and diglyceride fractions by transmethylation (in presence of the silica gel) with 2 and 1 ml respectively of 5% dry methanolic hydrochloric acid, in sealed ampoules at 80° for 1 h. The methylating solvent was then decanted from the silica gel into small test tubes (5 ml), and the residual silica washed with methanol. The combined solvents were then removed under nitrogen at 50° and the last traces of methanol and hydrochloric acid removed

by drying briefly at 30° under reduced pressure. To the residue, consisting of glycerol and methyl esters, 0·1 ml of silylating reagent (dry pyridine: hexamethyldisilazane: trimethylchlorosilane, 8:2:1) was added. The solution was thoroughly mixed (Whirlimix; Fisons Ltd), allowed to stand at room temperature for 5 min and centrifuged to settle the flocculent precipitate.

For gas chromatography a 5 ft glass column packed with SE-30 silicone gum on celite was used, the top 3–4 in. of the column packing being replaced after twenty-five injections. At a column temperature of 100° the glycerol peak eluted in about 8 min; thereafter, the temperature was increased at 8°/min to expedite removal of the methyl esters. The gas flow was 40 ml/min and the chart speed 30 in./h.

Experiments with glycerol (redistilled 'Analar', chromatographically pure) showed that conversion into the TMS derivative was quantitative, that the product was stable for at least 8 h and that a linear weight–peak area relationship existed from 0 up to at least 40 μg glycerol/15 μl injection sample. Glycerol TMS derivative was also prepared from triolein that had been chromatographed on and recovered from TLC plates in the normal way: recoveries approximating to 100 % were obtained (Table 4).

Table 4. *Recovery of glycerol trimethylsilyl (TMS) derivative from triglyceride after chromatography*

Glycerol (μg) applied to plate as triolein	Glycerol (μg) found as TMS derivative*	Recovered (%)
1·17	1·15 ± 0·05	98
2·34	2·37 ± 0·07	101
4·68	4·70 ± 0·08	101

* Means and standard deviations for twelve determinations.

TMS derivatives of inositol were prepared by the method of Flint, Lee & Huggins (1965), which is essentially similar to the procedure used for glycerol except for the use of dimethyl sulphoxide in addition to pyridine as solvent. The method was used for identification, but was not standardized for quantitative analysis.

Cholesterol was determined on the total lipid extracts, before and after saponification, by the method of Galanos, Aïvazis & Kapoulas (1964).

RESULTS

Total lipids. The amounts of lipid extracted from the breast and leg tissue of turkeys raised on the six diets are given in Table 5. Groups A and A_1 are shown separately, although the differences between them were slight. The amount of extractable lipid varied with diet in both leg and breast muscle, the variation being consistent with the differences already observed in the yield of lipid from the skin tissues (Neudoerffer & Lea, 1966). These differences, however, were due to fluctuations in 'neutral' lipid deposition only; the phospholipid content in both tissues remained virtually unaffected by diet.

Leg muscle contained about three times as much total lipid as breast muscle, the

differences being due to slightly (about 50%) higher phospholipid and much higher (four to seven times) neutral lipid levels in the leg muscle (Table 5). This resulted in reversal of the neutral lipid to phospholipid ratio from approximately 1:2 in the 'leanest' breast muscle to approximately 2:1 in the leg muscle. Although small quantitative differences were observed between breast and leg muscle lipids, there were no qualitative differences between the types and numbers of the individual lipid present in the two tissues (Neudoerffer & Lea, 1967).

Table 5. *Lipid content (mg/100 g tissue) of muscular tissue from turkeys on diets E–D**

Lipid ...	E	A	A_1	B	C	D
			Breast muscle			
Total	1191	1385	1349	1112	1055	1320
Neutral lipid	456	643	545	362	311	559
Phospholipid	707	690	705	707	677	714
% accounted for	97·5	95·6	92·7	95·8	93·7	97·5
			Leg muscle			
Total	3512	3930	3790	3105	3210	3881
Neutral lipid	2359	2644	2438	1893	2082	2657
Phospholipid	1079	1079	1091	1048	1056	1043
% accounted for	97·1	94·7	93·1	94·6	98·0	95·4
Ratio, leg: breast						
Neutral	5·17	4·11	4·48	5·24	6·70	4·75
Phospholipid	1·52	1·56	1·55	1·48	1·56	1·46

* See p. 116.

'Neutral' lipids. Detailed analyses of the composition of the neutral lipids from both breast and leg muscle are given in Table 6. Triglyceride was by far the largest constituent of the neutral lipid fraction, representing 27·8 and 57·0% of the total lipid from breast and leg muscle respectively: it was also the only individual lipid analysed to show fluctuations in amount with diet greater than the experimental error of the determination. These pronounced differences in triglyceride storage occurred despite the fact that the birds received isocaloric diets and remained healthy throughout the experiments.

The other four constituents of the neutral lipid fraction analysed accounted for, together, about 10% of the total extractable lipid. All four were present at higher concentration in leg muscle than in breast muscle, but their relative proportions were not appreciably affected by diet. Free cholesterol, representing 6·0 and 3·0% of the total breast and leg muscle lipid respectively, was a possible exception, with a slight depression of level in both legs and breast muscle from birds receiving 2·5% fish oil (diets B and C).

The free fatty acid fraction represented 2·3 and 3·4% of the total lipid of breast and leg muscle respectively. The consistency of this fraction would appear to rule out formation by post-mortem hydrolysis, and suggests that small proportions of free fatty acids are present in vivo.

The diglyceride content was low, representing 1·5 and 1·9% of the total lipid of breast muscle and leg muscle respectively. No distinction was made between the 1,2- and 1,3-isomers when this lipid was assayed.

Table 6. *Composition of the 'neutral' lipids (mg/100 g tissue) of muscle of turkeys on diets E–D**

Lipid...	E	A	A_1	B	C	D	Individual lipid as % of total lipid† (weight basis)
			Breast muscle				
Cholesterol ester	10	11	9	9	5	12	0·7±0·1
Triglyceride	327	497	414	251	202	409	27·8±6·0
Free fatty acid	25	29	26	28	30	27	2·3±0·4
Diglyceride	13	21	18	20	15	22	1·5±0·2
Cholesterol	81	85	78	56	59	89	6·0±0·7
Total wt‡	456	643	545	362	311	559	—
% of total lipid	—	—	—	—	—	—	38·2±6·1
			Leg muscle				
Cholesterol ester	10	15	13	12	9	14	0·4±0·1
Triglyceride	2033	2303	2116	1644	1811	2333	57·0±2·4
Free fatty acid	133	141	128	87	121	131	3·4±0·4
Diglyceride	57	66	71	61	69	73	1·9±0·1
Cholesterol	126	119	110	89	72	106	3·0±0·5
Total wt‡	2359	2644	2493	1893	2082	2657	—
% of total lipid	—	—	—	—	—	—	65·5±2·8

* See p. 166.
† Means and standard deviations, based on between-treatment differences, of the six analyses.
‡ Does not include traces of hydrocarbons, lipid 'X' and monoglycerides.

Cholesterol esters were present only in trace amounts, at a concentration of approximately 0·5% of the total lipid. Only about one-eighth of the total cholesterol was therefore present in the esterified form.

Phospholipids. The results of the phospholipid analyses are given in Table 7. The extractable phospholipid phosphorus remained constant for both the breast tissue and the leg tissue, despite variation in the experimental diets.

Phosphorus was detected in all eight fractions separated on thin-layer plates by the solvent system of Skipski, Peterson & Barclay (1962), six of these being identified as PE, PS, PI, PC, SP and LPC. The fraction running above PE and described as 'cardiolipin' probably contained phosphatidic acid, phosphatidylglycerol and polyglycerophosphate, but was not further fractionated. The phosphorus remaining at the origin could have been due to traces of ganglioside.

Quantitative analysis of the phospholipids on TLC using other solvent systems revealed no additional lipids and it was concluded that no other phospholipid was present as a major constituent. Rechromatography of the PS and PI fractions, which were the most difficult to separate from neighbouring lipids, showed that both were pure.

Variation in diet had no effect on the relative concentrations of the individual phospholipids. There were, however, small but consistent differences between the

Table 7. *Composition of phospholipid fraction (mg/100 g tissue)* of muscle of turkeys on diets E–D†*

Phospholipid fraction	E	A	A_1	B	C	D	Individual lipid as % of‡: Phospholipid (P basis)	Individual lipid as % of‡: Phospholipid (wt basis)	Individual lipid as % of‡: Total lipid (wt basis)
					Breast muscle				
Cardiolipin (see p. 122)	24·7	21·4	22·2	25·0	20·0	25·1	3·3 ± 0·3	3·2 ± 0·3§	1·9 ± 0·3
PE	156·2	160·1	162·5	157·4	148·9	154·2	22·5 ± 0·8	21·9 ± 0·7	12·8 ± 1·2
PS	30·3	28·0	30·6	33·4	30·8	33·7	4·2 ± 0·3	4·3 ± 0·3	2·6 ± 0·4
PI	61·8	59·1	64·8	60·0	57·1	57·2	7·3 ± 0·4	8·3 ± 0·4	4·9 ± 0·5
PC	378·5	365·6	363·2	374·3	360·9	358·4	51·7 ± 1·3	51·8 ± 1·1	30·4 ± 3·4
SP	42·3	42·3	44·8	42·3	42·3	42·3	6·1 ± 0·2	6·0 ± 0·2	3·5 ± 0·3
LPC	8·2	8·2	9·8	9·8	9·8	8·2	1·9 ± 0·2	1·3 ± 0·1	0·7 ± 0·1
Origin	5·0	5·0	7·4	5·0	7·5	7·5	0·8 ± 0·2	0·9 ± 0·2§	0·5 ± 0·1
% of total lipid	—	—	—	—	—	—	—	—	57·2 ± 6·0§
mg P/100 g tissue	29·3	28·8	29·0	28·2	28·4	28·9	28·7 ± 0·4	—	—
% P recovered from plate	97·7	96·8	98·4	100·0	95·4	98·2	97·7 ± 1·5	—	—
					Leg muscle				
Cardiolipin (see p. 122)	41·7	42·1	40·1	27·8	27·4	32·7	3·2 ± 0·6	3·5 ± 0·6§	0·9 ± 0·4
PE	276·4	285·1	282·9	276·1	276·9	274·9	26·5 ± 0·6	25·9 ± 0·4	7·9 ± 0·7
PS	54·6	63·2	65·8	61·6	61·5	61·9	5·5 ± 0·3	5·7 ± 0·4	1·7 ± 0·2
PI	98·1	112·6	115·4	109·2	114·7	102·7	8·8 ± 0·6	10·1 ± 0·7	3·1 ± 0·4
PC	514·6	495·9	501·8	500·0	496·1	493·7	46·5 ± 0·8	46·3 ± 0·9	14·2 ± 1·5
SP	67·3	62·5	65·4	59·8	62·5	62·3	5·9 ± 0·1	5·8 ± 0·2	1·8 ± 0·1
LPC	14·7	11·5	14·7	8·2	9·8	9·8	1·6 ± 0·3	1·0 ± 0·2	0·3 ± 0·1
Origin	9·9	5·0	5·0	5·0	7·5	5·0	0·6 ± 0·1	0·6 ± 0·1§	0·2 ± 0·1
% of total lipid	—	—	—	—	—	—	—	—	30·1 ± 2·9
mg P/100 g tissue	44·2	43·9	44·4	42·3	42·2	43·2	43·3 ± 0·9	—	—
% P recovered from plate	98·8	99·0	98·9	98·8	100·0	96·1	98·6 ± 1·3	—	—

* Calculated by multiplying P content by mean molecular weight of the individual lipid.
† See p. 116 for diets.
‡ Means and standard deviations, based on between-treatment differences of the six analyses.
§ Molecular weight used was averaged molecular weight of six known fractions.

proportions of the individual lipids in breast and leg muscle. The proportions of the 'acidic' phospholipids, PE, PS and PI, were higher and of PC lower in the physically more active leg muscle than in breast muscle. The proportions of sphingomyelin remained the same in both tissues.

Plasmalogen lipids were found in both muscles, being present primarily in the phosphatidalethanolamine and phosphatidalcholine forms, though traces of phosphatidalserine were also found. The concentrations of these substances varied with the tissue but were unaffected by diet. In breast muscle phosphatidalethanolamine constituted 42 % of the ethanolamine fraction; in leg muscle it was only 36 %. Phosphatidalcholine accounted for approximately 11 % of the total 'phosphatidylcholine' content of breast muscle and for 10 % of the corresponding fraction of leg muscle.

DISCUSSION

The values presented for the individual lipid constituents of muscle total 95 % of the total extractable lipid. Lipid 'X' and the hydrocarbon and monoglyceride fractions (the latter two constituting about 1·5 % of broiler tissue) (Marion & Woodroof, 1965) were not determined, and would be included in the missing 5 %. Calculated weights of cardiolipin and 'origin fraction' of the phospholipids are rough estimates only, since the precise molecular weights of these substances were not known. It would appear from the results presented that no major lipid class was overlooked in the analysis.

Total lipid. The levels of total lipid found in the breast and leg muscle of the 10-week-old turkeys were higher than those reported for 16-week-old turkeys by Acosta *et al.* (1966), probably owing, in part, to a lower efficiency of the cold extraction procedure employed by Acosta *et al.* These authors also reported only negligible differences between the lipid contents of leg and breast muscle, whereas the present results show that leg muscle contained approximately three times as much lipid as breast muscle, a finding in good agreement with the observations of Marion & Woodroof (1965), of Marion (1965) and of Mickelberry, Rogler & Stadelmann (1966) for the corresponding chicken tissues.

'Neutral' lipids. The proportions of the individual neutral lipids found in turkey are similar to those reported by Marion & Woodroof (1965) for chicken muscle, but the weights of lipids are higher in turkey than in chicken muscle. The same neutral lipids are also present in chicken liver (Sgoutas, 1966) although, as might be expected, the relative proportions and quantities differ considerably. Marion & Woodroof (1965) found no effect of several dietary fats, including menhaden oil, on the triglyceride concentration, calculated by difference from figures given by Marion (1965). Similarly, Mickelberry *et al.* (1966) showed that neither breast nor liver lipid (the latter mainly triglyceride) content was affected by diet but that lard or maize oil feeding raised the triglyceride content of chicken leg muscle significantly. In the present experiments the triglyceride content of turkey breast and leg tissue was lowest in the groups (B and C) receiving 2·5 % anchovy oil, but there were no consistent differences between the other dietary groups (Table 6).

The cholesterol contents of turkey muscle in the present experiments compare well with the cholesterol levels in chicken muscle found by Marion & Woodroof (1965), although these workers suggested that their own results might be too high because of interference in measurement, possibly by impurities, and that the lower values obtained by Mickelberry *et al.* (1966) might be more accurate for chicken muscle.

Fluctuations in muscle cholesterol content with diet were observed by Mickelberry *et al.* (1966), who found that dietary lard or carbohydrate lowered the cholesterol level, whereas maize oil (high in polyunsaturated fatty acids (PUFA)) raised it. This observation is in conflict with the present results, where PUFA from 2·5 % dietary anchovy oil slightly lowered the cholesterol plus cholesterol ester level, supported by Hill's (1966) findings that menhaden oil lowered chicken serum cholesterol level and that the effect was increased by ethoxyquin (EMQ). It is not clear, however, why 5 % dietary menhaden oil failed in our experiment to exert a greater hypocholesterolaemic effect than 2·5 %, although Lewis, Brown & Page (1966) have recently reported that the hypocholesterolaemic effect of PUFA is only temporary and that the serum cholesterol content, in time, returns to its original level. These authors, however, did not investigate whether the concentration of PUFA given affected the rate of return to the original level.

The free fatty acid content of the turkey breast muscle was similar to that found by Marion & Woodroof (1965) in chickens, but the leg muscle free fatty acid content was somewhat higher. All the values are much higher than those reported for chicken tissue by Gilpin, Dawson, Toepher & Warren (1952). It seems likely that improved extraction techniques, with more efficient rupture of lipoprotein complexes, increased the efficiency of extraction of the small amounts of free fatty acid present. Increasing the periods of storage at $-70°$ of some of the tissue before extraction failed to raise the free fatty acid content, as might have been expected if enzymic hydrolysis of glycerolipids was occurring during normal handling.

The cholesterol esters and diglyceride contents found in turkey leg and breast muscle are similar to those previously reported for chicken muscle.

Although cholesterol constituted 6 % of breast muscle lipid and only 3 % of leg muscle lipid (on a total lipid basis), it is interesting to note, in relation to a possible membrane function of this lipid, that the phospholipid:cholesterol ratio was approximately the same (10:1) in both tissues (Tables 5 and 6).

Phospholipids. The lipid phosphorus content of muscle from these 10-week-old turkeys was considerably higher than that found in 16-week-old birds by Acosta *et al.* (1966). The discrepancy could be partly due to age difference, since Marion (1965) has reported that the phospholipid content of chicken muscle changes with age, but is probably attributable in the main to a lower efficiency of the cold extraction procedure employed by Acosta. The total phospholipid content found in chicken muscle by Marion & Woodroof (1965) was also lower than in the corresponding tissues from turkeys in the present experiments. The difference could be partly due to an inherent difference between chickens and turkeys but it could also be due in part to the mode of calculation of phospholipid content.

The eight individual classes of phospholipid found in turkey muscle in the present

work are the same as those found in chicken liver by Sgoutas (1966) and in pigeon breast muscle by Gray & MacFarlane (1961). There is a considerable measure of agreement also between the proportions of the individual phospholipids in chicken liver and in turkey muscle lipid, the main differences being higher phosphatidylethanolamine and lower sphingomyelin levels in the liver lipid. But the differences between pigeon breast muscle and turkey breast and leg muscle are much more striking, possibly because pigeon breast muscle is more active than turkey leg muscle and much more active than turkey breast muscle. The total extractable lipid phosphorus in pigeon muscle was found to be three times greater than in turkey breast muscle and about twice as high as in turkey leg muscle. The main differences in proportions of individual phospholipids were a lower PI and a much higher cardiolipin content in the pigeon muscle, together with slightly higher PE and lower SP contents. The greater abundance of cardiolipin in the more active (pigeon) muscle probably reflects a localization of cardiolipin in the mitochondria and a higher density of mitochondria in the more active muscle (Bloor, 1943; Sheltawy & Dawson, 1966).

Acosta *et al.* (1966) and Peng & Dugan (1965) analysed the phospholipids of turkey and of chicken muscle respectively, but they only fractionated the extractable phospholipids into 'kephalin', lecithin and sphingomyelin groups. However, the overall pattern of proportions that emerges does show some agreement between their results and those presented here, provided that cardiolipin, PE, PS and PI are considered to be included in the 'kephalin' fraction. 'Kephalin' is shown to constitute a higher proportion of the phospholipids of leg than of breast muscle.

Peng & Dugan reported a sphingomyelin level for both tissues of 3·5 %, which is lower than the 6 % (on phosphorus basis, Table 8) found in turkey muscle. However, additional unseparated sphingomyelin was apparently present in at least one other of Peng & Dugan's lipid fractions. Neither Peng & Dugan's nor the present results afford any support for the very high level of 14 % sphingomyelin reported by Acosta *et al.* (1966), a result which would appear to be in error owing to contamination of the fraction isolated, with other classes of phospholipid.

If, as now appears possible, individual phospholipids may perhaps perform specific biological functions in tissues, variation of the diet would not be expected greatly to affect their proportions in the muscle of healthy animals. It has already been shown that fatty acid metabolism and the fatty acid composition of individual lipids can be profoundly affected by dietary fat (Neudoerffer & Lea, 1966, 1967), but it now appears that these changes occur without change in the proportions and concentrations of the individual lipids themselves, except for triglyceride.

Aside from considerations of metabolism, there is now evidence (Acosta *et al.* 1966) that lipids of muscle are selectively involved in autoxidation during the processing and storage of meat. Instability of this kind has previously been associated with the PUFA content of lipids, and deterioration in egg and milk products has been related to the breakdown of phospholipids containing the more highly unsaturated fatty acids (Lea, 1957). Mattsson & Swartling (1963) observed that the relative rates of oxygen absorption by individual phospholipids could be explained in part, though perhaps not entirely, by the degree of unsaturation of their constituent fatty acids.

The distribution of fatty acids between the different lipids of the tissue therefore becomes of considerable interest in relation to food preservation and storage.

For animals raised on a normal cereal-based diet the phospholipids are virtually the only constituents of the tissue which contain small amounts of highly unsaturated fatty acids, and on them the autoxidative stability of the meat must largely depend. However, when diets high in PUFA are fed, the stability of the meat can be depressed by deposition of these fatty acids in the glyceride constituents of muscle and adipose tissue also, and such deposition, on a weight basis, may considerably exceed deposition in the phospholipids (Tables 8 and 9).

Table 8. *Weight distribution (mg/100 g tissue) of 20:5 fatty acid among major muscle lipids of turkeys on diets E–D**

	Breast muscle					Leg muscle				
Lipid	E	A	B	C	D	E	A	B	C	D
Triglyceride	—	—	16·3	13·1	40·9	—	—	95·4	105·0	214·6
Phosphatidylcholine	11·3	14·6	32·2	33·2	38·7	tr	14·9	39·0	35·2	48·4
Phosphatidylethanolamine	2·2	2·9	14·0	13·1	17·7	3·6	6·6	27·6	27·1	34·9
Phosphatidylinositol	tr	0·8	5·0	4·5	5·7	tr	0·7	7·3	7·6	9·7
Phosphatidylserine	0·1	0·3	0·8	0·7	1·2	tr	tr	1·9	1·8	2·8

tr = trace. * See p. 116.

Table 9. *Weight distribution (mg/100 g tissue) of 22:6 fatty acid among major muscle lipids of turkeys on diets E–D**

	Breast muscle					Leg muscle				
Lipid	E	A	B	C	D	E	A	B	C	D
Triglyceride	—	—	5·0	4·0	15·5	—	—	36·2	39·8	88·7
Phosphatidylcholine	7·6	21·9	19·8	21·7	25·4	tr	14·9	22·5	22·8	29·6
Phosphatidylethanolamine	3·1	5·3	30·2	28·4	36·4	5·5	8·0	49·1	48·2	60·0
Phosphatidylinositol	tr	0·7	2·3	2·3	3·7	tr	0·6	4·4	4·7	6·8
Phosphatidylserine	tr	0·8	3·6	3·0	4·3	tr	1·1	5·5	5·5	7·1

tr = trace. * See p. 116.

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