Alterations in lipid composition of plasma lipoproteins during deposition of egg yolk

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Abstract The profiles of total lipids and of the molecular species of individual lipid classes were compared among corresponding lipoproteins of plasma and yolk of the laying hen. A close qualitative correspondence was found in the makeup of the molecular species of glycerophospholipids and triglycerides of the very low density lipoproteins and the high density lipoproteins of plasma and yolk. There was a lower proportion of the trienoic triglycerides and of the dienoic glycerophospholipids in the egg yolk than in the plasma lipoproteins, and the greatest differences (20–30%) were noted between the high density lipoproteins. It was also observed that the plasma high density lipoproteins lost their cholesteryl esters upon entering the yolk. On the basis of these and comparable analyses of the plasma lipoproteins of the nonlaying hen, it is concluded that the laying hen synthesizes specific lipoproteins for deposition in the yolk, and these are carried in plasma and selectively transferred to the developing ovum without significant equilibration with the other plasma lipoproteins.

Supplementary key words total lipid profiles · direct gas–liquid chromatography · phospholipase C digestion · cholesteryl esters · molecular species · triglycerides · glycerophospholipids

THE LIPOPROTEINS found in the yolk of the egg are synthesized in the liver of the laying hen and are transported in the blood stream, from which they are believed to be passed more or less intact into the egg yolk (1–3). This belief is based largely on immunochemical comparisons of the apoproteins of corresponding density classes of lipoproteins of plasma and yolk (2, 4). Previous comparisons of lipid composition of plasma and yolk lipoproteins have been limited to analyses of total lipid extracts by silicic acid column chromatography and to gas–liquid chromatographic analysis of total fatty acids, which have given comparable results for corresponding fractions (4). From electron microscopic studies it has been concluded that the plasma lipoproteins enter the developing ovum by pinocytosis (5).

More recent analyses of the egg yolk triglycerides have demonstrated that they differ significantly from those of the liver and adipose tissue of the hen (3). The egg yolk also has been shown to contain little or no cholesteryl ester (6–8), while the plasma is rich in this lipid (5). Furthermore, the yolk lipoproteins are believed to be specifically synthesized by the liver in response to hormonal stimulation (1, 2), and it would be anticipated that they are selectively cleared from plasma during yolk formation. In the present study we have made a detailed quantitative comparison of the masses of the molecular species of lipids of the major plasma and yolk lipoproteins. The results support the hypothesis of a discriminate transfer of plasma lipoproteins to the developing ovum, and they serve as a basis for a subsequent study of egg yolk deposition by following the distribution of radioactive isotopes.

MATERIALS AND METHODS

Diet and sample collection

Experiments were carried out on 1.8–2.3-kg white leghorn hens fed a commercial laying mixture (18 Complete Layer Crumbs, Maple Leaf Mills Ltd., Toronto, Canada). Hens were kept on this diet and were laying regularly for at least 1 wk prior to being killed. Immature white leghorn pullets ranging from 0.5 to 1.1 kg and fed a

Abbreviations: VLDL, very low density lipoprotein, d < 1.006 g/ml; HDL, high density lipoprotein, d = 1.006–1.21 g/ml; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TG, triglyceride; PL, phospholipid.

commercial starter diet (Complete Starter Crumbs) were used as plasma donors for analyses of lipids from nonlaying hens. Blood was collected by heart puncture into a final dilution of 0.3% sodium citrate, and plasma was separated by centrifugation. Developing egg yolks in phase 3 (9, 10) were collected surgically from the laying hen ovaries and were immediately classified according to weight and stage of development. Studies on 59 yolks confirmed an earlier report (11) that the ovum of approximately 5 g in weight took up yolk most actively. Accordingly, such ova, designated E5, were selected for further analysis and comparison with plasma.

Preparation of lipoproteins

Undiluted plasma and yolk diluted five times with saline (d = 1.006) were immediately placed in centrifuge tubes and the lipoproteins were separated into low and high density (LDL + HDL, d > 1.006) and very low density (VLDL, d < 1.006) components by ultracentrifugation for 16 hr at 105,000 *g* in a Spinco model L ultracentrifuge. The tubes were sliced and VLDL samples were removed from the top. Additional saline was added and the samples were redispersed and both VLDL and LDL + HDL were recentrifuged for 16 hr. The tubes were again sliced and VLDL was collected. Plasma LDL + HDL was redispersed and yolk LDL + HDL was redissolved in 0.5 M sodium chloride prior to extraction of lipids. Lipoproteins of densities < 1.006 (VLDL), 1.006–1.063 (LDL), and 1.063–1.21 (HDL) were also prepared (12).

Lipid analyses

Total lipids were prepared (8) by repeatedly extracting (4 times) aliquots of the lipoproteins with 20 vol of chloroform–methanol 2:1 (v/v). The extracts were pooled and concentrated to small volume in a rotary evaporator; the concentrated solution was taken up in chloroform–methanol 2:1 (v/v) and diluted to volume. Neutral lipids were resolved by TLC in heptane–isopropyl ether–glacial acetic acid 60:40:3 (v/v/v) and phospholipids were prepared using a chloroform–methanol–glacial acetic acid–water 25:15:4:2 (v/v/v/v) solvent system (13). Lipid phosphorus was measured according to Haines (14).

GLC analyses of total lipid extracts, after a preliminary digestion with phospholipase C, and of fatty acid methyl esters were performed as reported earlier (8). Cholesterol, intact diglycerides, cholesteryl esters, and TG were analyzed using a Beckman GC-4 gas chromatograph with a special on-column injector heater. Runs were temperature programmed from 190 to 350°C at 10°C/min. The columns were stainless steel tubes 60 cm by 0.3 cm O.D. and were packed with 3% (w/w) JXR (a methyl silicone) on silanized Gas-Chrom Q, 100–120 mesh (Applied Science Laboratories, State College, Pa.). Peak areas were measured by an electronic integrator (Infotronics digital readout system, model CRS 104, Infotronics Ltd., Shannon Airport, Ireland). Quantitative estimates of the various lipids were obtained by comparison of the peak areas with that of tridecanoin added as an internal standard. Recoveries were determined using an equal weight mixture of cholesterol, tridecanoin, 1-stearoyl 2-palmitoyl-*sn*-glycerol, cholesteryl palmitate, and stripped lard as well as a standard mixture of simple triglycerides containing equal weights of trilaurin, trimyristin, tripalmitin, and tristearin. Recoveries were determined each day, and appropriate factors were used to correct for discrepancies due to differential detector response and loss on the column. The agreement with the composition of standards showed a relative error of less than 2% for major components (over 10% of total) and less than 5% for minor components (less than 10% of total). Fatty acid methyl esters were analyzed using a Varian Aerograph model 204 B gas chromatograph equipped with dual columns, dual hydrogen flame detectors, and on-column injectors. The columns were stainless steel tubes (180 cm by 0.3 cm O.D.) packed with 10% EGSS-X, 100–120 mesh (an ethylene glycol succinate–methylsiloxane copolymer) on Gas-Chrom P (Applied Science Laboratories). Runs were made isothermally at 180°C with a carrier gas (N_2) flow rate of 60 ml/min. Quantitation was performed by comparison of peak areas with the area of a known weight of methyl heptadecanoate added as internal standard. Peak identity and quantitative validity of the estimates of fatty acid proportions were established by comparison with the National Heart Institute fatty acid standards (mixtures D and F). The agreement with the standard composition showed a relative error of less than 1% for major components (over 10% of total mixture) and less than 5% for minor components (less than 10% of total mixture).

The composition of the various molecular species of glycerophospholipids was determined by combined TLC and GLC of the diglyceride moieties liberated by hydrolysis with phospholipase C, as previously described (15). The diglycerides were separated on the basis of unsaturation by TLC on silica gel G (Merck) containing 20% silver nitrate. The plates (20 × 20 cm) were developed in 1% methanol in chloroform. For this purpose the diglycerides were converted into the acetates by treatment with an excess of acetic anhydride–pyridine 10:1 (v/v) at room temperature. The carbon numbers of the diglycerides in the various fractions were determined by GLC under the conditions described for the direct examination of total lipid extracts.

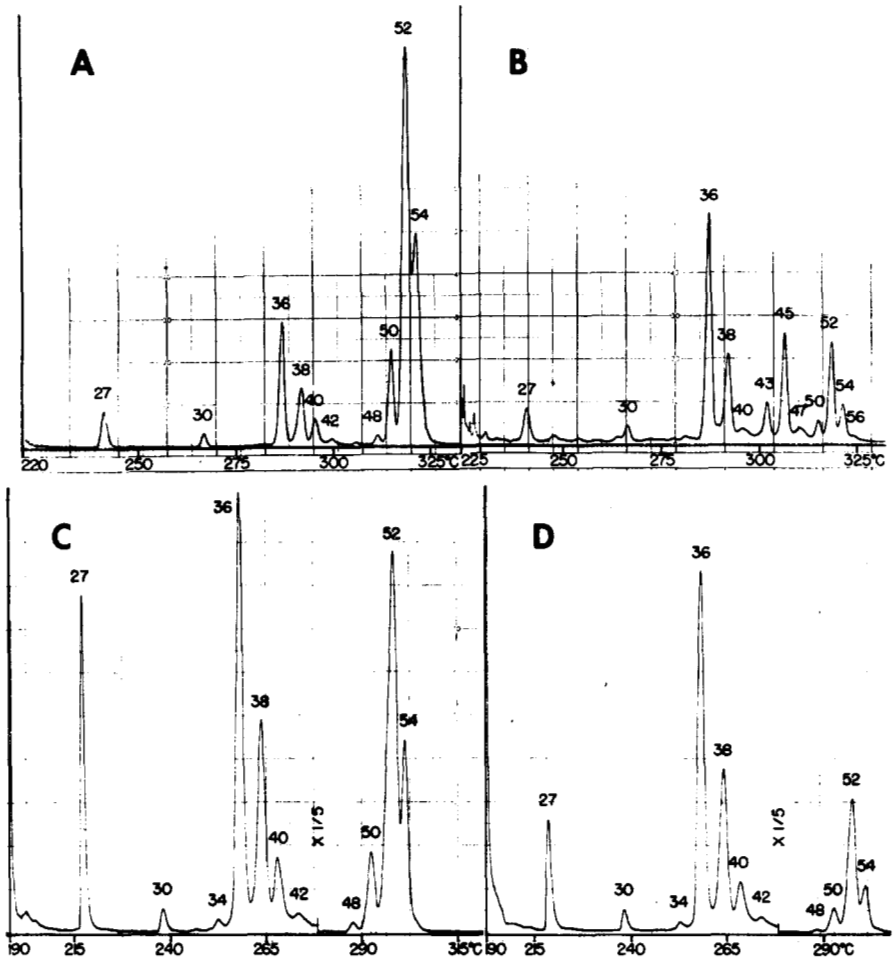

FIG. 1. Total lipid profiles of plasma and yolk lipoproteins. (*A*) VLDL of plasma, (*B*) LDL + HDL of plasma, (*C*) VLDL of yolk, (*D*) LDL + HDL of yolk. Conditions of chromatography: Beckman GC-4 gas chromatograph with 1-mV recorder and an electronic peak area integrator; dual stainless steel columns, 60 cm × 3 mm O.D., filled with 3% methyl silicone polymer on 100–120 mesh Gas-Chrom Q; carrier gas flow rate, 120 ml of nitrogen/min; injector, 300°C; detector, 340°C; column temperature, 190 to 350°C in 16 min. Peaks identified by total number of acyl carbon atoms in glycerides and total number of carbons in cholesterol and cholesteryl esters: 27, cholesterol; 30, tridecanoin internal standard; 34–42, diglyceride acetates derived from phospholipids; 43–47, cholesteryl esters; 48–56, triglycerides.

RESULTS

Resolution of lipoproteins

Neither the plasma nor the yolk contained significant amounts of lipoprotein that could be isolated by a 30-min centrifugation in NaCl of d = 1.006. Centrifugation for 16 hr was required to isolate large amounts of VLDL (d < 1.006) from both sources. The VLDL was purified by recentrifugation; there was no significant loss of lipid. Successive centrifugations of the subnatant solution in NaCl of d = 1.063 and 1.21 gave LDL and HDL, respectively. Since the LDL content was less than 6% of the total lipoproteins and the cholesteryl ester content was similar to that of the HDL, LDL and HDL were routinely collected and examined as a single density class. The d > 1.21 subnatant solutions contained small amounts of lipoprotein characterized by extremely low proportions of lipids. The comparisons of the lipid compositions were limited to the VLDL and combined LDL + HDL fractions; these are the major components of both plasma and yolk (8, 16).

Previous work (17) has shown that the lipoproteins of the chicken can be effectively separated using the usual densities of NaCl solutions provided the time of centrifugation is extended to 42 hr. In the present investigation, characteristic lipid profiles were obtained for both VLDL and LDL + HDL lipoprotein classes after recentrifugation for 16 hr. Further resolution of egg yolk

TABLE 1. Lipid class composition of lipoproteins from the domestic fowl

Lipid Classes	Laying Hen						Nonlaying Hen		
	Egg Yolk			Plasma			Plasma		
	Total[a]	VLDL[b]	HDL + LDL[b]	Total[a]	VLDL[c]	HDL + LDL[c]	Total[d]	VLDL[d]	HDL + LDL[d]
	mole %								
Cholesterol	6 ± 1	6 ± 1	8 ± 3	7 ± 1	6 ± 1	5 ± 3	15 ± 1	5 ± 1	11 ± 3
Phospholipid	23 ± 5	16 ± 5	32 ± 3	29 ± 7	16 ± 5	61 ± 13	42 ± 4	11 ± 4	44 ± 11
Cholesteryl esters	0 ± 0	0 ± 0	0 ± 0	3 ± 2	0 ± 0	20 ± 12	27 ± 2	3 ± 1	39 ± 10
Triglycerides	71 ± 8	78 ± 6	61 ± 6	62 ± 9	78 ± 7	15 ± 4	17 ± 2	80 ± 4	5 ± 2
Phosphatidylcholine	69	67	69	69	66	72			
Phosphatidylethanolamine	24	29	27	28	30	26			
Others	6	4	4	3	4	2			

[a] Averages of five hens ± SD.
[b] Averages of four hens ± SD.
[c] Averages of three hens ± SD.
[d] Averages of two pullets ± range.

VLDL and HDL lipoproteins by chromatography on hydroxyapatite (16) failed to yield subfractions of different lipid composition (8).

Distribution of lipid classes

Fig. 1 shows the total lipid profiles of the plasma and yolk lipoproteins as obtained by direct GLC (18). The peaks for cholesterol and cholesteryl esters are identified by the total carbon number and those for the diglyceride acetates and triglycerides by the total number of acyl carbons including the carbon atoms of the acetyl residues. The elution pattern of the yolk VLDL appears to be identical with that of the plasma VLDL, both of which are peculiar in that there are no cholesteryl esters. In contrast, the plasma LDL + HDL contains significant amounts of cholesteryl esters, which may reach the pro-

TABLE 2. Distribution of molecular species of triglycerides from different sources in the laying hen[a]

Saturation Class[b]	Carbon Number	Source			
		VLDL		LDL + HDL	
		Plasma	Egg	Plasma	Egg
		mole %			
Total	48	0.5	0.4	0.4	0.3
	50	8.4	7.6	8.0	6.9
	52	73.2	70.0	76.3	73.4
	54	18.0	22.0	15.3	19.5
Saturates	48	8.6			tr
	50	53.0 (<1)[c]	49.1 (<1)	50.0 (<1)	52.9 (<1)
	52	38.4	50.9	50.0	47.1
Monoenes	48	3.3	3.0	2.5	2.2
	50	32.8	32.4	35.9	32.1
	52	61.2 (6.2 ± 0.1)	61.7 (7.0 ± 0.1)	61.2 (7.4 ± 0.3)	61.3 (7.8 ± 0.7)
	54	2.7	2.9	0.4	4.4
Dienes	48	0.4	0.4	0.3	0.3
	50	8.6	8.5	8.0	7.4
	52	80.9 (37.0 ± 3.3)	81.9 (46.1 ± 2.3)	87.7 (39.3 ± 3.5)	82.8 (48.8 ± 3.7)
	54	10.1	9.3	4.1	9.5
Trienes	50	2.8	2.9	2.6	2.5
	52	73.4 (47.6 ± 4.0)	72.7 (37.3 ± 1.6)	77.1 (42.6 ± 4.7)	72.0 (31.4 ± 6.4)
	54	23.9	24.4	20.4	25.5
Polyenes	50	1.4	1.5	0.6	0.7
	52	31.7 (9.3 ± 0.5)	34.1 (9.6 ± 0.7)	35.4 (10.8 ± 1.5)	31.1 (12.0 ± 2.0)
	54	66.9	64.4	64.0	68.2

[a] The results in this table and in Tables 3–6 are averages of analyses of plasma and one E5 yolk (see text) from each of two hens; the range of values for individual species was within ± 2.6% of the mean value for major peaks (> 30% of a saturation class) and within ± 13.4% of the mean value for minor peaks (2–30% of a saturation class); relative error of analysis ≤ 5%.
[b] Total number of double bonds per glyceride molecule.
[c] The values in parentheses represent the percentage of each saturation class in the total triglycerides ± the range of two independent experiments. Five additional egg yolks from five other hens were also analyzed. The results agreed with the total values within the ranges indicated. The distribution of the triglycerides according to carbon number was not carried out.

TABLE 3. Fatty acid composition of triglycerides of different lipoproteins in the laying hen[a]

Saturation Class[b]	Lipoprotein Class	Source	Fatty Acid 14:0[c]	16:0	16:1	18:0	18:1	18:2	20:2
						mole %			
Total	VLDL	Plasma	0.6	26.3	4.1	7.4	46.7	14.9	
		Egg	0.9	25.0	3.6	7.6	47.6	15.3	
	LDL + HDL	Plasma	2.0	25.4	3.5	5.7	47.1	16.3	
		Egg	0.8	24.3	3.4	8.2	47.4	15.9	
Saturates	VLDL	Plasma	17.2	48.6		34.2			
		Egg	10.7	48.3		41.0			
	LDL + HDL	Plasma	37.1	34.3		28.6			
		Egg	15.9	46.6		37.5			
Monoenes	VLDL	Plasma	1.4	40.5	2.2	24.2	31.7		
		Egg	1.5	41.3	0.7	22.5	33.9		
	LDL + HDL	Plasma	5.6	38.0	3.6	23.8	29.0		
		Egg	2.0	39.2	0.7	24.6	33.5		
Dienes	VLDL	Plasma	0.7	30.7	1.3	5.1	58.8	3.3	tr
		Egg	0.4	30.4	2.0	7.6	55.9	3.5	0.3
	LDL + HDL	Plasma	1.9	29.5	3.4	6.5	55.7	2.3	0.7
		Egg	0.6	29.7	2.7	7.4	56.2	3.5	tr
Trienes	VLDL	Plasma	0.7	21.2	4.7	2.5	45.8	24.6	0.5
		Egg	0.6	21.2	3.8	2.7	47.7	23.5	0.5
	LDL + HDL	Plasma	5.3	24.5	2.8	1.3	44.8	21.4	tr
		Egg	0.5	21.6	3.9	3.1	46.4	24.2	0.4
Polyenes	VLDL	Plasma	1.5	10.7	6.4	3.1	42.3	35.5	0.5
		Egg	2.0	6.7	6.3	1.4	51.9	31.3	0.4
	LDL + HDL	Plasma	5.2	12.6	7.3	4.8	40.2	29.3	0.6
		Egg	7.0	20.8	2.1	9.8	43.1	16.7	0.4

[a] Averages of two hens; the range of values for individual acids was within ± 4.0% of the mean value for major bands (> 15% of a saturation class) and within ± 13.2% of the mean value for minor bands (2–15% of a saturation class); relative error of analysis ≤ 3%. Egg LDL + HDL polyene results are from a single experiment.
[b] Total number of double bonds per glyceride molecule.
[c] Number of carbon atoms: number of double bonds.

portion of the triglycerides. The neutral lipids are present in considerably smaller proportion than the phospholipids, which account for the bulk of the fraction.

Table 1 gives the numerical values calculated for the various lipid classes in the individual lipoproteins and in the total yolk and plasma. In addition, the proportions of the two major glycerophospholipids, phosphatidylcholine and phosphatidylethanolamine, found in the various lipoproteins have been recorded separately. Analyses of these phospholipids were carried out after TLC of the total lipid extracts. There is very little variation in the ratio of the major phosphatides and in the amount of the other phospholipids, mainly sphingomyelin and lysolecithin (8). The table also includes values for the plasma lipids of a nonlaying hen, whose VLDL and LDL + HDL characteristically contain cholesteryl esters. Other samples of plasma lipoproteins from laying hens showed a similar relationship among their lipid moieties and to the lipid moieties of the corresponding yolk lipoproteins. There were other, lesser differences in the lipid profiles of the laying and nonlaying hen plasma lipoproteins, when examined by direct GLC.

Molecular species of triglycerides

In Table 2 it is seen that the relative amounts of the total triglyceride classes as well as of the various types of triglycerides in each saturation class were similar in all of the lipoproteins. Thus, the dienes of plasma VLDL (37.0%) and LDL + HDL (39.3%) were present in comparable proportions as were the trienes of plasma VLDL (47.6%) and LDL + HDL (42.6%). Upon transfer to egg yolk, the dienoic triglycerides increased by 25 and 24% in the VLDL and LDL + HDL fractions, respectively, while the trienoic triglycerides fell by 22 and 26%. These changes are especially important because the dienes and trienes make up the bulk of the triglycerides in both plasma and yolk lipoproteins.

The above relationships among the triglycerides of the different lipoproteins were derived on the basis of the examination of the intact triglycerides. The results are confirmed by the fatty acid analyses presented in Table 3. The overall fatty acid compositions of the VLDL and LDL + HDL from plasma and yolk were characterized by high contents of oleic (47–48%), palmitic (24–27%), and linoleic (13–16%) acids. These amounts were essentially the same as those reported previously (1, 8, 19) for the total egg yolk triglycerides.

Molecular species of glycerophospholipids

As seen from Table 1, phospholipids are the major class of lipids in plasma HDL + LDL and the second most abundant class in all other lipoprotein fractions.

TABLE 4. Distribution of molecular species of phosphatidylcholine from different sources in the laying hen[a]

Saturation Class[b]	Carbon Number	Source: VLDL Plasma	Source: VLDL Egg	Source: LDL + HDL Plasma	Source: LDL + HDL Egg
		mole %			
Total	34	0.5	0.5	0.4	0.4
	36	83.9	82.8	81.7	81.6
	38	13.8	14.4	15.4	15.4
	40	1.6	2.1	2.2	2.3
	42	0.2	0.2	0.2	0.3
Saturates	34	68.8	83.3	80.4	80.8
	36	31.3 (<1)[c]	16.7 (<1)	19.6 (<1)	19.2 (<1)
Monoenes	34	0.8	0.7	0.8	0.6
	36	91.6 (47.5 ± 1.2)	91.6 (51.9 ± 1.7)	91.6 (44.8 ± 5.6)	92.0 (54.8 ± 1.4)
	38	7.5	7.8	7.6	7.4
Dienes	36	77.5	77.2	78.8	76.9
	38	22.5 (41.0 ± 1.0)	22.8 (38.2 ± 1.6)	21.2 (43.1 ± 6.2)	23.1 (35.3 ± 1.0)
Trienes	36	37.8	36.4	32.8	40.9
	38	62.2 (2.0 ± 1.0)	63.1 (1.6 ± 0.8)	66.9 (2.2 ± 1.1)	58.8 (1.8 ± 0.9)
	40		0.5	0.3	0.3
Polyenes	36	4.9	3.9	4.2	4.2
	38	32.4	31.6	33.6	34.0
	40	55.9 (9.4 ± 2.8)	58.5 (8.2 ± 3.8)	54.4 (9.8 ± 2.4)	58.2 (8.1 ± 4.2)
	42	6.9	6.0	7.8	3.6

[a] Averages of two hens; the range of values for individual species was within ± 3.7% of the mean value for major peaks (> 10% of a saturation class) and within ± 6.8% of the mean value for minor peaks (2–10% of a saturation class); relative error of analysis ≤ 5%.

[b] Total number of double bonds per molecule.

[c] The values in parentheses represent the percentage of each saturation class in the total phosphatidylcholine ± the range of two independent experiments (see also footnote *c* of Table 2).

More than 94% of the phospholipids in all lipoproteins contained glycerophospholipids, which were made up of about 70% phosphatidylcholine and 30% phosphatidylethanolamine.

Table 4 shows the molecular weight distributions of the diglyceride moieties of phosphatidylcholine after a preliminary resolution of their diglyceride moieties by argentation TLC. The data indicate that within each saturation class the relative amounts of a given molecular species were similar or were the same (within experimental error) in the VLDL and LDL + HDL of plasma and yolk. However, the proportions of the saturation classes in the corresponding plasma and yolk lipoproteins, especially in the LDL + HDL, appear to be significantly different. Table 5 shows similar data for the phosphatidylethanolamines. The proportions of the various molecular species in a given saturation class appear to be identical. Also, the proportion of the various molecular species in the total phosphatidylethanolamines in the VLDL and LDL + HDL fractions ot plasma and yolk appear to be essentially identical.

The overall composition of the fatty acids of the glycerophospholipids of the egg yolk lipoproteins (Table 6) is similar to that reported previously for whole egg yolk (14, 20). Assuming that the positional distribution of the saturated and unsaturated fatty acids in the phosphatides was comparable, it may be concluded that they possess similar molecular species, since the corresponding classes of phosphatidylcholine and phosphatidylethanolamine gave identical molecular weight distributions for both VLDL and LDL + HDL of yolk. Similarly, it may be concluded that the phosphatidylcholines and phosphatidylethanolamines of the plasma lipoproteins possess comparable compositions of molecular species.

DISCUSSION

There is evidence that the liver of the laying hen secretes specific lipoproteins in greatly increased amounts during the onset of laying (2). On the basis of the present study, these lipoproteins must possess lipid compositions similar to those of the yolk and significantly different from those of the plasma lipoproteins of the nonlaying hen. It may be pointed out, however, that the comparison between the lipid profiles of laying and nonlaying hens involved animals of different weights. The nonlaying hens weighed only 0.5–1.1 kg since hens mature and start laying before they reach 1.8 kg, which was the minimum weight of the laying hens. Hens that are mature and past their prime laying time have decreased amounts of specific lipoproteins, especially VLDL and phosvitin, and their plasma lipoprotein patterns are similar to those of immature hens. However, the cessation of egg production is not abrupt, and healthy hens

TABLE 5. Distribution of molecular species of phosphatidylethanolamine from different sources in the laying hen[a]

Saturation Class[b]	Carbon Number	Source: VLDL Plasma		Source: VLDL Egg		Source: LDL + HDL Plasma		Source: LDL + HDL Egg	
		mole %							
Total	36	28.4		27.6		28.4		27.5	
	38	41.3		38.1		39.8		38.6	
	40	27.7		30.9		29.1		30.6	
	42	2.6		3.4		2.8		3.4	
Saturates		n.d.[c]		n.d.		n.d.		n.d.	
Monoenes	36	63.4	(20.5 ± 0.2)[d]	61.1	(21.7 ± 1.0)	62.0	(20.7 ± 0.6)	63.2	(17.4 ± 2.0)
	38	36.6		38.9		38.0		36.8	
Dienes	36	35.3	(29.6 ± 4.2)	34.7	(17.1 ± 1.6)	38.9	(30.0 ± 1.6)	35.1	(19.8 ± 1.6)
	38	64.7		65.3		65.1		64.9	
Trienes	36	7.6		5.2		5.0		21.9	
	38	91.2	(1.9 ± 1.0)	93.4	(1.5 ± 0.8)	93.3	(1.9 ± 1.1)	78.1	(1.7 ± 0.9)
	40	1.2		1.3		1.7		trace	
Polyenes	36	0.1		0.9		0.2		0.2	
	38	9.3	(48.0 ± 5.8)	8.4	(59.7 ± 1.2)	9.1	(47.4 ± 3.0)	7.5	(61.2 ± 1.8)
	40	73.8		73.6		73.0		74.8	
	42	16.8		17.1		17.7		17.6	

[a] Averages of two hens; the range of values for individual species was within ± 3.2% of the mean value for major peaks (> 15% of a saturation class) and within ± 4.1% of the mean value for minor peaks (2–15% of a saturation class); relative error of analysis ≤ 5%.

[b] Total number of double bonds per glyceride molecule.

[c] n.d., not detected.

[d] The values in parentheses represent the percentages of each saturation class in the total phosphatidylethanolamine ± the range of two independent experiments (see also footnote *c* of Table 2).

in this condition are hard to obtain, since they are not commercially useful. The immature hens are more reliable and readily available.

From Table 1 it can be seen that upon transfer to yolk, the lipid moieties of the VLDL and LDL + HDL lipoproteins are handled differently. While the plasma VLDL (82%) retains its TG/PL ratio of 5/1, the plasma LDL + HDL (18%) undergoes a change in TG/PL from 1/4 to 2/1. Summation of lipoproteins from yolk (87% VLDL and 13% LDL + HDL) and from nonlaying pullet (24% VLDL and 76% LDL + HDL) failed to support the notion that the lipoproteins of the laying hen consist of yolk lipoproteins plus the plasma lipoproteins of the nonlaying pullet.

Triglycerides provide the bulk of the lipid in all the lipoproteins except the plasma LDL + HDL. A comparison of the mass distribution of the molecular species of triglycerides between the VLDL and HDL fractions ought to indicate any similarities among the plasma and yolk lipoproteins, as well as the possible existence of a common glyceride pool for both plasma lipoproteins and both yolk lipoproteins.

TABLE 6. Fatty acid composition of major glycerophospholipids in yolk and plasma lipoproteins of the laying hen[a]

Lipoprotein Class	Source	Fatty Acid 14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:4	22:6
		mole %								
Phosphatidylcholine										
VLDL	Plasma	0.9	38.7	0.5	11.0	24.5	17.0	4.3	1.4	1.7
	Egg	1.1	38.2	0.6	10.6	27.9	16.7	3.4	0.6	1.0
LDL + HDL	Plasma	0.3	37.0	1.2	9.5	27.8	18.4	3.5	0.7	1.5
	Egg	1.2	37.2	0.7	10.5	27.9	16.6	3.8	1.0	1.2
Phosphatidylethanolamine										
VLDL	Plasma	1.4	20.4	tr	26.6	16.4	13.9	10.8	5.1	5.4
	Egg	2.2	20.3	2.0	25.4	15.4	10.7	13.3	5.2	5.5
LDL + HDL	Plasma	1.2	25.1	3.0	26.1	17.5	11.5	8.6	2.7	4.2
	Egg	0.9	19.3	tr	28.9	18.3	12.3	12.1	4.1	4.3

[a] Averages of two hens; the range of values for individual acids was within ± 4.0% (phosphatidylcholine) and ± 5.8% (phosphatidylethanolamine) of the mean value for major acids (> 10% of the total) and within ± 11.5% (phosphatidylcholine) and ± 15.4% (phosphatidylethanolamine) of the mean value for minor acids (2–10% of the total); relative error of analysis ≤ 3%.

Earlier analyses of egg yolk triglycerides (8) have identified the major molecular species as the *sn*-glycerol-1-palmitate-2,3-dioleate and its racemate and *sn*-glycerol-1-palmitate-2-linoleate-3-oleate and its racemate in the dienoic and trienoic triglycerides, respectively. The present determinations of the yolk triglycerides correspond closely to the previously obtained distributions of the saturation classes and molecular weights. It is most likely that the triglycerides of the egg yolk VLDL and LDL + HDL possess similar stereochemical configurations of the fatty acids. Since the plasma triglycerides are synthesized in the liver, the suggestion that the molecular species of plasma and yolk triglycerides are qualitatively the same is in agreement with the findings of Husbands (3), who noted that the fatty acids of the 1-, 3-, and 2-positions in hen's liver and yolk triglycerides have a similar distribution.

On the basis of the similarities in the fatty acid composition and in their positional placement, it would appear that the VLDL and LDL + HDL triglycerides of the plasma and yolk were all derived from a single pool synthesized in the liver. Unaccounted for, however, remains the reciprocal difference in the proportions of dienoic and trienoic triglycerides in the VLDL and LDL + HDL fractions, as well as the further increase in the proportions of the dienoic triglycerides in both VLDL and LDL + HDL fractions upon their transfer to the egg yolk. Either the VLDL and LDL + HDL classes of plasma lipoproteins are therefore made up of heterogeneous subfractions of varying permeability into the ovum or the lipid moieties were specifically rearranged during a largely indiscriminate transfer of all lipoproteins to give the class proportions of the yolk triglycerides without upsetting the overall fatty acid composition, molecular association, and positional placement.

During transfer to yolk, there was a proportional increase in the monoenes and a decrease in the dienes of phosphatidylcholine of both VLDL and LDL + HDL. On transfer of lipoprotein phosphatidylethanolamines to the yolk, however, the proportion of dienoic species in VLDL and LDL + HDL was significantly reduced, and that of polyenoic species was considerably increased. These changes in the plasma glycerophospholipid composition would not affect the overall fatty acid composition (Table 6), although the slight changes were in the direction expected.

A more direct comparison of the lipids of the plasma and yolk lipoproteins would require a resolution of the yolk lipoproteins secreted by the liver and the lipoproteins of the nonlaying hen, which thus far has not proved practical by ultracentrifugation. McIndoe (2), however, has claimed that a characteristic phospholipoprotein can be precipitated from the plasma of a laying hen upon dilution with water. Repetition of this precipitation in our laboratory showed that the isolated material was contaminated with lipoproteins containing cholesteryl esters, which obviously were not transferred to yolk. Furthermore, we have been able to demonstrate in other studies that at least one VLDL and one HDL component of the yolk lipoproteins is immunochemically the same as lipoproteins in the plasma of the nonlaying hen (21).

The mechanisms bringing about the above indicated changes in the triglyceride and phospholipid ratios and in the proportions of the unsaturation classes of the lipids of the plasma lipoproteins upon transfer to egg yolk are incompatible with indiscriminate pinocytosis as suggested previously (5). Variance between the expected and actual PL/cholesterol ratio in plasma LDL + HDL and the decrease in proportion of certain species of glycerides and phosphoglycerides and the increase in others, along with a complete disappearance of the steryl ester fraction, points to a selective transfer of specific subfractions of plasma VLDL and LDL + HDL to the egg yolk. The alternative possibility that the ovary of the laying hen might intervene actively in the selection of the triglycerides and the glycerophospholipids which accompany the apoproteins of the plasma lipoproteins into the newly formed eggs has been proved unlikely in view of studies[1] of distribution of radioactivity in plasma and yolk lipids after administration of labeled glycerol and by assaying the lipid-metabolizing enzymes in the ovary (22). The extensive mass analyses reported here on a limited number of animals are therefore consistent with parallel studies of independent analytical parameters (21, 22) as well as with the results of previous more limited analyses in a larger number of animals (8, 14, 20). The above conclusions may therefore represent a valid interpretation of a physiological phenomenon which is not readily disturbed by simple experimental manipulation.

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REFERENCES

1. Flickinger, R. A., and D. E. Rounds. 1956. The maternal synthesis of egg yolk proteins as demonstrated by isotopic and serological means. *Biochim. Biophys. Acta.* **22:** 38–42.

[1] Gornall, D. A., and A. Kuksis. 1972. Distribution of [2-^{3}H]-glycerol among lipids of plasma and yolk lipoproteins in laying hens. Submitted for publication.

2. McIndoe, W. M. 1959. A lipophosphoprotein complex in hen plasma associated with yolk production. *Biochem. J.* **72:** 153–159.
3. Husbands, D. R. 1970. The composition of triglycerides from liver, egg yolk and adipose tissue of the laying hen. *Biochem. J.* **120:** 365–371.
4. Schjeide, O. A., M. Wilkens, R. G. McCandless, R. Munn, M. Peterson, and E. Carlsen. 1963. Liver synthesis, plasma transport, and structural alterations accompanying passage of yolk proteins. *Amer. Zool.* **3:** 167–184.
5. Schjeide, O. A., F. Galey, E. A. Grellert, R. I-S. Lin, J. de Vellis, and J. F. Mead. 1970. Macromolecules in oocyte maturation. *Biol. Reprod. Suppl.* **2:** 14–43.
6. Martin, W. G., N. H. Tattrie, and W. H. Cook. 1963. Lipid extraction and distribution studies of egg yolk lipoproteins. *Can. J. Biochem. Physiol.* **41:** 657–666.
7. Schneider, H., and N. H. Tattrie. 1968. Mutual solubility of the lipid components of egg yolk low-density lipoprotein. *Can. J. Biochem.* **46:** 979–982.
8. Gornall, D. A., and A. Kuksis. 1971. Molecular species of glycerophosphatides and triglycerides of egg yolk lipoproteins. *Can. J. Biochem.* **49:** 51–60.
9. Marza, V. D., and E. V. Marza. 1935. Formation of the hen's egg. *Quart. J. Microscop. Sci.* **78:** 134–189.
10. Van Durme, M. 1914. Nouvelle recherches sur la vitellogenèse des oeufs d'oiseaux aux stades d'accroissement, de maturation, de fécondation et début de la segmentation. *Arch. Biol.* **29:** 71–200.
11. Warren, D. C., and R. M. Conrad. 1939. Growth of the hen's ovum. *J. Agr. Res.* **58:** 857–893.
12. Mookerjea, S., D. Jeng, and J. Black. 1967. Studies on the synthesis of plasma glycolipoprotein and hepatic subcellular glycoprotein in early choline deficiency. *Can. J. Biochem.* **45:** 825–838.
13. Skipski, V. P., R. F. Peterson, and M. Barclay. 1964. Quantitative analysis of phospholipids by thin-layer chromatography. *Biochem. J.* **90:** 374–378.
14. Haines, D. S. M. 1966. The effects of choline deficiency and choline re-feeding upon the metabolism of plasma and liver lipids. *Can. J. Biochem.* **44:** 45–57.
15. Kuksis, A., and L. Marai. 1967. Determination of the complete structure of natural lecithins. *Lipids.* **2:** 217–224.
16. Gornall, D. A., and A. Kuksis. 1971. Resolution of egg yolk lipoproteins by chromatography on thin layers of hydroxylapatite. *Can. J. Biochem.* **49:** 44–50.
17. Hillyard, L. A., H. M. White, and S. A. Pangburn. 1972. Characterization of apolipoproteins in chicken serum and egg yolk. *Biochemistry.* **11:** 511–518.
18. Kuksis, A., L. Marai, and D. A. Gornall. 1967. Direct gas chromatographic examination of total lipid extracts. *J. Lipid Res.* **8:** 352–358.
19. Christie, W. W., and J. H. Moore. 1970. The structure of egg yolk triglycerides. *Biochim. Biophys. Acta.* **218:** 83–88.
20. Holub, B. J., and A. Kuksis. 1969. Molecular species of phosphatidyl ethanolamine from egg yolk. *Lipids.* **4:** 466–472.
21. Gornall, D. A., T. J. Delahunty, and A. Kuksis. 1971. Immunochemical relationships among plasma and egg-yolk lipoproteins of the laying hen. *Biochem. J.* **125:** 97P–98P.
22. Gornall, D. A., A. Kuksis, and N. Morley. 1972. Lipid-metabolizing enzymes in the ovary of the laying hen. *Biochim. Biophys. Acta.* **280:** 225–230.