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QUANTITATIVE LEVELS, FATTY ACID COMPOSITION AND POSITIONAL DISTRIBUTION OF ACYL GROUPS IN ENCEPHALIC PHOSPHOGLYCERIDES DURING EMBRYONIC DEVELOPMENT OF THE CHICK AND DUCK

JOSE M. GONZALEZ-ROS* and A. RIBERA†
Department of Biochemistry, Faculty of Biology, Universidad Complutense de Madrid, Madrid-3, Spain

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Abstract—1. Phosphatidylcholine (PC) was the major phospholipid fraction in the embryonic encephalon, palmitic and oleic acids being the main acyl constituents.

2. These fatty acids were poorly represented in phosphatidylethanolamine (PE), whereas stearic, arachidonic and docosahexenoic acids had very high relative abundances.

3. The fatty acid composition of choline or ethanolamine phosphoglycerides from the embryonic encephalon did not show appreciable differences either during the developmental stages considered or between the two avian species.

4. The positional distribution of acyl groups in 1,2-diacyl-sn-glycero-3-phosphocholines showed a clear predominance of saturated fatty acids bound to sn-1 position of the glycerol moiety, whereas the unsaturated fatty acids were predominantly located in the sn-2 position.

INTRODUCTION

Lipids, primarily phospholipids, play an essential role in the structural organization, as well as functional and other properties of membranes (Van Deenen, 1965; Rouser *et al.*, 1968). Specifically regarding these functional aspects, an interdependence between the presence of chemically well-defined phospholipids and the activity of membrane enzyme and/or transport systems have been demonstrated (Fourcans & Jain, 1974; Hidalgo *et al.*, 1976; Sandermann, 1978). Embryonic development providing appearance of new functional activities, has been thought an appropiate model to follow possible lipid compositional changes. In the rat brain, morphological and physiological changes during postnatal development have been correlated with variations in the levels of different lipid classes (Wells & Dittmer, 1967).

In this paper, the variations in the level and fatty acid composition of the major phospholipid fractions of developing chick and duck embryo encephalons are described. In addition, the positional distribution of acyl groups in the glycerol moiety of phosphatidylcholine (PC), the major phosphoglyceride found during the embryonic stages of the brain of superior animals (Wells & Dittmer, 1967; Wood, 1974; Miyamoto *et al.*, 1966; Marshall *et al.*, 1966; Dalal & Einstein, 1969; DeKaban *et al.*, 1971; Kamazawa *et al.*, 1972; Dorman *et al.*, 1977; Shaikh & Palmer, 1976) is reported. The choline phosphoglycerides either in the encephalon (Wells & Dittmer, 1967; Dorman *et al.*, 1977) or in embryologically derived structures from the Central Nervous System (Dreyfus *et al.*, 1975; Johnston & Hudson, 1974) are comprised principally of phosphatidylcholine.

MATERIALS AND METHODS

Fertile eggs from *Gallus gallus* and *Anas boschas* obtained from a commercially available source were incubated under conditions of controlled temperature (37 C) and relative humidity (84%). Embryos were obtained at different incubation periods with the equivalency between the developmental stages of both avian species taken according to Romanoff (1967). The encephalons were excised and rinsed in a cold isotonic saline solution. Several encephalons were pooled according to age (30–200 embryos) and homogenized (1:1, w/v) in distilled water at 0 C (under nitrogen) in an Omni-Mixer Sorvall homogenizer (16,000 rev/min for three periods of 1 min each). The total lipids were extracted by the procedure of Bligh & Dyer (1959). The lipid extracts were washed with 0.73% NaCl solution and the organic phase was separated, dried over anhydrous sodium sulphate and evaporated to dryness. Total lipid content of encephalons was determined gravimetrically.

Phosphoglyceride classes were obtained directly from aliquots of the total lipid extracts by thin layer chromatography (TLC) on 0.5 mm layers of kieselgel G (E. Merck A. G., Darmstad, G.F.R.) using chloroform/methanol/water (65:25:4, v/v/v) as the developing solvent. The choline and ethanolamine phosphoglycerides were detected by spraying the lateral ends of the plates with the Dittmer reagent (Dittmer & Lester, 1964). The lipid containing-zones were scraped off the plates and extracted by successive treatments with 10 vol of chloroform/methanol (2:1, 1:1, 1:2 and 1:9, v/v) and finally methanol. Phosphoglycerides isolated by this method yielded single spots in two

* Present address: Department of Biochemistry, Medical College of Virginia, Richmond, Virginia 23298, U.S.A.

† Present address: Tecnicas Instrumentales Biologicas, Facultad de Ciencias, Universidad de Palma de Mallorca, Spain.

dimensional TLC using chloroform/methanol/water (65:25:4, v/v/v) and *n*-butanol/acetic acid/water (60:20:20, v/v/v) as developing systems. The same solvents were used in the two-dimensional TLC (0.3 mm layers of kiesselgel G) for phosphorus determinations on separated phosphoglycerides. The plates were dried at room temperature for 12 hr, spots were detected with iodine vapors and marked with a needle. After iodine sublimation, the relevant zones were scraped off the plate and digested in 0.9 ml of 70% perchloric acid at 250° C for 25 min. The color development was carried out according to Rouser *et al.* (1966). Blank values were determined by ashing comparable amounts of the silica-gel from a lipid-free area of the plate. Analytical data represents an average of duplicate determinations on two different samples.

The positional distribution of the acyl groups in the 1,2-diacyl-sn-glycero-3-phosphocholine was determined by hydrolysis with phospholipase A_2 (E.C. 3.1.1.4) present in *Crotalus adamenteus* venom (Sigma Chemical Co., St Louis, MO, U.S.A.), as described by Van Golde & Van Deenen (1967). The lysoderivatives and the corresponding free fatty acids were processed as described by Abad *et al.* (1976).

Preparation of the methyl esters and analysis of the fatty acids by gas liquid chromatography (GLC) were performed as described by Metcalfe & Schmitz (1961) and Abad *et al.* (1976), respectively. The presence of kiesselgel G in the methanolysis mixture did not interfere with the final analysis. For the identification of the peaks, known mixtures of standard saturated and unsaturated fatty acid methyl esters from Applied Science Lab. (State College, PA, U.S.A.) and Sigma Chemical Co. (St Louis, MO, U.S.A.) were used. GLC results represent an average of duplicate determinations from at least three different samples and are expressed as molar percentages.

RESULTS AND DISCUSSION

Figure 1 shows the total lipid content variations during the developmental stages considered in the two avian species. According to the data, the increasing amount of total lipids does not merely reflect the increase in encephalon size, but a successive lipid enrichment in the developing tissue. This enrichment is also detected in the phospholipid classes which are present (Fig. 2) and may be related to the intensive phospholipid and general lipid accumulation associated with the initiation of the active myelination phase, beginning in the chick embryo from 18th–19th incubation days and continuing into postnatal periods (Skaikh & Palmer, 1976; El-Eischi, 1967; Kurihara & Tsukada, 1968; Mezei & Palmer, 1974).

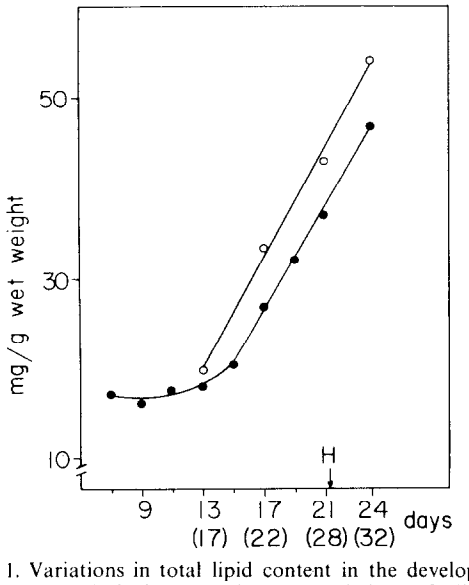

Fig. 1. Variations in total lipid content in the developing chick (●) and duck (○) embryo encephalons. Ordinate numbers in brackets indicate equivalent developmental stages in duck encephalons compared to the chick embryo (numbers above). H indicates the time of hatching.

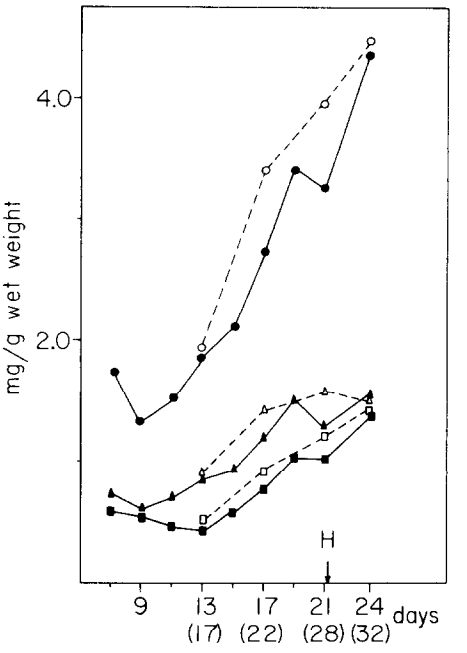

Fig. 2. Changes in the amount of inorganic phosphorus contained in lipid classes from the developing chick (closed figures and solid line) and duck (open figures and dashed line) embryo encephalons. H and numbers in brackets as in Fig. 1. Circles indicate total lipid phosphorus contents, whereas triangles and squares indicate PC and PE, respectively.

As can be seen in Fig. 2, PC is the major phospholipid fraction in the developmental stages considered in both avian species (~40%). This is in good agreement with data reported by different groups for higher animals encephalons (Wells & Dittmer, 1967; Wood, 1974; Miyamoto *et al.*, 1966; Marshall *et al.*, 1966; Dalal & Einstein, 1969; DeKaban *et al.*, 1971; Kanazawa *et al.*, 1972; Dorman *et al.*, 1967; Shaikh & Palmer, 1976). In Table 1 is shown the fatty acid composition of PC in the developing encephalon, with palmitic and oleic comprising approx 80% of the total. This relative abundance in saturated and monounsaturated fatty acids in encephalic PC is in agreement with previously described data from higher animals (Wood, 1974; Miyamoto *et al.*, 1966; Marshall *et al.*, 1966; Baker & Thompson, 1972; Svennerholm *et al.*, 1972) and contrasts strongly with the fatty acid composition of PE (Table 2) in which the presence of polyunsaturated fatty acids is fairly considerable (Wood, 1974; Miyamoto *et al.*, 1966; Baker & Thompson, 1972; Svennerholm *et al.*, 1972; Dhopeshwarkar & Subramanian, 1975). These structural differences between both major phospholipid fractions could be interpreted on the basis of a possible meta-

Table 1. Fatty acid composition of PC from the developing avian embryo encephalon

Age (incubation days)	Fatty acids 14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:4	22:5	22:6
Chick embryo										
7	1.38	52.78	4.36	6.41	20.78	1.06	5.95	0.98	0.74	5.53
9	1.43	51.93	4.94	5.46	21.95	1.26	6.47	1.25	0.67	4.63
11	1.41	53.06	4.83	5.00	22.78	1.58	4.73	0.82	0.70	4.99
13	1.51	54.07	5.11	5.31	24.66	2.03	3.34	0.52	TR*	3.44
15	0.55	55.25	4.58	4.90	24.87	1.80	3.90	0.61	TR	3.52
17	1.43	57.42	3.03	4.85	23.33	2.27	3.71	0.65	TR	3.31
19	0.99	55.85	3.00	7.00	23.46	1.00	4.71	0.52	TR	3.46
21 (hatched)	0.78	54.66	2.72	6.70	25.22	1.91	3.80	TR	TR	4.20
24	1.35	55.07	2.19	8.14	26.06	0.57	3.71	TR	TR	2.90
Duck embryo										
17	0.90	51.43	3.65	6.10	27.83	0.76	5.45	TR	1.09	2.77
22	1.03	59.41	1.79	5.86	23.00	0.51	4.53	TR	0.92	2.99
28 (hatched)	TR	53.39	1.68	8.69	26.21	TR	5.14	0.74	1.02	3.12
32	TR	53.75	1.69	8.82	27.66	TR	4.14	TR	0.74	3.20

* TR = traces; fatty acid abundance less than 0.5%.

bolic specificity in the biosynthetic pathways in charge of acyl groups binding to the glycerol moieties.

On the other hand, the absence of variation in the fatty acid composition of PC or PE during the development is nearly absolute and suggests, in conjunction with the almost total absence of interspecific changes, a remarkable organ specifity regarding the fine chemical structure of the phospholipids in the developing avian encephalon.

Tables 3 and 4 show the positional distribution of acyl groups in the glycerol moiety of PC in encephalon of both avian species. It can be observed that the molar percentage of palmitic acid in sn-1 position is considerably higher than in sn-2, whereas the opposite occurs for oleic acid. Stearic acid is exclusively located at the sn-1 position, while the more unsaturated linolenic, arachidonic and docosahexenoic acids position on sn-2. Only minor fatty acids, such as palmitoleic acid, are randomly distributed between both sn-1 and sn-2 positions. The data described above is

Table 2. Fatty acid composition of PE from the developing avian embryo encephalon

Age (incubation days)	Fatty acids 14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:4	22:5	22:6
Chick embryo										
13	1.23	11.82	0.64	23.17	8.74	1.52	16.38	4.39	8.27	23.85
21 (hatched)	TR	13.45	TR	24.18	8.92	0.71	15.29	5.20	7.11	25.14
Duck embryo										
17	1.42	10.71	1.26	23.97	10.02	1.41	17.81	3.34	4.17	25.89
28 (hatched)	0.93	12.68	1.42	25.86	9.17	1.72	15.93	4.73	5.60	21.96

Table 3. Relative abundance of fatty acids bound at sn-1 position of PC

Age (incubation days)	Fatty acids 14:0	16:0	16:1	18:0	18:1	18:2
Chick embryo						
7	0.67	62.93	5.20	11.46	19.74	TR
9	1.01	64.40	5.48	10.72	18.39	TR
11	0.88	64.13	5.21	10.54	19.23	TR
13	0.88	64.80	4.11	11.46	17.61	1.13
15	0.75	62.90	4.62	8.47	21.83	1.42
17	1.33	67.09	2.54	9.33	18.10	1.60
19	0.63	77.62	3.79	6.78	11.16	TR
21 (hatched)	0.55	64.60	2.16	15.58	16.13	0.96
24	1.69	69.48	2.16	13.83	12.77	TR
Duck embryo						
17	0.63	59.20	3.11	10.72	26.02	0.53
22	1.76	68.75	0.93	13.43	15.11	TR
28 (hatched)	TR	67.30	1.47	16.28	14.94	TR
32	TR	68.26	1.38	17.14	13.22	TR

Table 4. Relative abundance of fatty acids bound at sn-2 position of PC

Age (incubation days)	Fatty acids 14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:4	22:5	22:6
Chick embryo										
7	1.04	27.81	4.74	TR	32.26	2.55	14.71	2.50	2.19	12.19
9	1.38	37.81	5.76	TR	29.16	2.28	12.25	1.85	1.04	8.46
11	1.02	45.30	4.72	TR	27.32	2.23	10.03	1.24	0.71	7.42
13	1.39	48.15	4.52	TR	29.75	2.75	6.72	1.03	TR	5.69
15	0.97	34.83	6.98	TR	38.46	3.06	8.43	1.32	TR	5.94
17	TR	36.75	4.09	TR	36.96	3.92	9.89	1.43	TR	6.95
19	0.81	35.52	4.97	TR	39.22	2.00	10.39	1.19	TR	6.09
21 (hatched)	0.94	42.94	3.55	TR	34.57	2.88	7.64	0.88	TR	6.60
24	2.05	36.55	3.93	TR	40.71	1.18	8.66	1.27	0.68	4.96
Duck embryo										
17	1.30	40.92	4.28	TR	36.26	1.08	9.38	0.94	1.57	4.26
22	3.67	45.28	2.67	TR	31.88	TR	9.06	0.66	1.32	5.44
28 (hatched)	1.72	43.74	2.80	TR	35.47	0.73	9.24	TR	1.79	4.51
32	1.15	44.99	2.03	TR	36.24	1.13	8.71	TR	1.53	4.22

in agreement with the general assumption that in animal phosphoglycerides the saturated fatty acids are mainly located at sn-1 position whereas the unsaturated ones are predominantly at sn-2 (see for instance Abad *et al.*, 1976; Hildebrand & Law, 1964; Haverkate & Van Deenen, 1965; Brockerhoff & Ackman, 1967; Fernandez-Sousa *et al.*, 1971).

The PC positional distribution of acyl groups also indicates a strong maintenance in the phospholipid structure during the encephalic development, as well as low interspecific differences between the avian species studied.

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