

## FATTY ACID COMPOSITION OF RED BLOOD CELL MEMBRANE PHOSPHATIDYLETHANOLAMINE AND PHOSPHATIDYLCHOLINE IN RAT, RABBIT, HUMAN AND DOG

SIÇAN, TAVŞAN, İNSAN VE KÖPEK ERİTROSİT MEMBRANI  
FOSFATİDİLETANOLAMİN VE FOSFATİDİLKOLİNİN YAĞ ASİDİ  
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### ABSTRACT

*RBC (red blood cell) membrane fatty acid percentages in PE (phosphatidylethanolamine) and PC (phosphatidylcholine) in different species like rats, rabbits, humans and dogs were measured and compared. The major fatty acids of membrane PE and PC in the species were same as  $C_{16:0}$ ,  $C_{18:1}$ ,  $C_{18:2}$ ,  $C_{18:3}$  and  $C_{20:4}$ . SFAs (Saturated fatty acids) ( $C_{16:0}$ ,  $C_{18:0}$  and  $C_{20:0}$ ) were lower in PE but higher in PC in all species in RBC membrane. Arachidonate ( $C_{20:4}$ ) and PUFAs (polyunsaturated fatty acid) ( $C_{18:2}$ ,  $C_{18:3}$  and  $C_{20:4}$ ) were higher in PE than PC. PC also had relatively high PUFA content.  $C_{20:4}$  was a prominent fatty acid of PE in rat, human and dog except rabbit.  $C_{18:2}$  was the prominent fatty acid in the rabbit. MUFA (monounsaturated fatty acid) ( $C_{16:1}$ ,  $C_{18:1}$ ,  $C_{18:1}$ ,  $C_{20:1}$  and  $C_{22:1}$ ) percentage was also higher in PE than PC.  $C_{18:1}$  was found considerably higher than  $C_{16:1}$  in all species. LCPUFAs (long chain polyunsaturated fatty acids) ( $C_{22:4}$  and  $C_{22:6}$ ) were significantly lower in the species PE and PC. Among LCPUFA,  $C_{22:6}$  was high in human RBC membrane PE and was not detected in dog.*

**Key Words:** fatty acids, RBC membrane, PE, PC, rat, rabbit, human, dog.

### ÖZET

*Rat, tavşan, insan ve köpek gibi farklı türlerin kırmızı kan hücreleri fosfatidiletanolamin (PE) ve fosfatidilkolini (PC) yağ asitleri tayin edilmiş ve karşılaştırılmıştır. Türlerin membran PE ve PC yağ asitleri başlıca  $C_{16:0}$ ,  $C_{18:0}$ ,  $C_{18:1}$ ,  $C_{18:2}$  ve  $C_{20:4}$  dir. Doymuş yağ asitleri (SFA) ( $C_{18:0}$ ,  $C_{18:0}$  ve  $C_{20:0}$ ) bütün türlerde PE de düşük ancak PC de yüksek düzeydedir. Arakidonat ( $C_{20:4}$ ) ve çoklu doymamış yağ asitleri (PUFAs) ( $C_{18:2}$ ,  $C_{18:3}$  and  $C_{20:4}$ ) PE de PC den daha fazladır. PC nin PUFA içeriği de nispeten yüksektir.  $C_{20:4}$  tavşan dışında rat, insan ve köpek PE nin de göze çarpan bir yağ asididir. Tavşanda ise bunun yerini  $C_{18:2}$  almıştır. MUFA (doymuş yağ asitleri) ( $C_{16:1}$ ,  $C_{18:1}$ ,  $C_{20:1}$  and  $C_{22:1}$ ) yüzdesi de yine PE nde PC den daha fazladır. Bütün türlerde  $C_{18:1}$ ,  $C_{16:1}$  den daha fazla miktarda bulunmuştur. LCPUFAs (uzun zincirli doymamış yağ asitleri) ( $C_{22:4}$  and  $C_{22:6}$ ) PE ve PC nde önemli derecede düşüktür. LCPUFA içinde  $C_{22:6}$  yüzdesi insan eritrosit membranındaki PE de yüksek bulunmuş, köpekte ise bulunmamıştır.*

**Anahtar kelimeler:** yağ asitleri, RBC membranı, PE, PC, rat, tavşan, insan, köpek.

## INTRODUCTION

PUFAs play an important role in regulation of cellular functions. This could be either due to the fatty acids themselves or to their oxygenated derivatives called eicosanoids(1). It is also known that saturated fatty acids increase atherosclerotic tendency whilst polyunsaturated fatty acid (PUFA) mainly linoleic acid ( $C_{18:2}$  n-6) have been recommended for treatment and prevention of atherosclerosis and heart disease (2-4).

Different kinds of fatty acids are distributed in phospholipids in various animal tissues (5-13). In red blood cell (RBC) membrane, phospholipids have a structural role whilst in plasma phospholipids play a role in lipoproteins. High density lipoproteins and low density lipoproteins are particularly rich in phospholipids which surround the inner core of hydrophobic lipids. Thus there may be different phospholipids and fatty acids involved in each fraction. Great majority of the studies on fatty acids have been performed in the total fatty acids of RBC membrane and plasma (7-9, 14-21). However the data concerning the fatty acid of specific phospholipids is less. Comparisons can be made between the fatty acids of different phospholipids of RBC membrane to establish whether particular phospholipids are rich in particular fatty acids. One can also compare the fatty acids of different species.

The purpose of this study was therefore to compare the relative amounts of fatty acids of PC and PE in RBC membrane in different species like, rats, rabbits, humans and dogs.

## MATERIALS AND METHODS

### Diet

Dogs were fed Friskies Go-Dog pellets (Friskies Pet Care, U. K.) containing meat and animal derivatives, cereals, derivatives of vegetable origin, fats and oils, vegetable protein extracts and minerals as ingredients (oil 9 %, protein 24 %, , ash 9 %, fibre 5 %, vitamin E 60 mg/kg, vitamin A 5400 iu/kg, vitamin D 450 iu/kg, , copper 11 mg/kg) were fed to the dogs.

A high fibre diet (Beekay, Bantin and Kingman Ltd, U.K.) were fed to rabbits which had a fixed formula containing, grass meal, wheatfeed, barley meal, , ground oats, linseed meal, fats and oils, fish meal, minerals, vitamins and trace elements (crude protein 18 %, crude oil 4 %, crude fibre 9 %, nitrogen free extract 50 %, ash 7 %, dry matter 88 %). Also present were saturated fatty acids 0.75 %, unsaturated fatty acids 1.84 % and linoleic acid as 1.24 %. Added vitamins were vitamin A 36000 iu/kg, vitamin D3 2000 iu/kg, vitamin E 130 mg/kg.

Rat pellets were made up of crude protein 14.6 %, crude oil 2.6 %, crude fibre 4.3 %, ash 5.8 % and nitrogen free extract 62.7 %. (Fatty acids were palmitoleic acid ( $C_{16:1}$ ) 0.07 %, oleic acid ( $C_{18:1}$ ) 0.74 %, linoleic acid ( $C_{18:2}$ ) 0.56 %, linolenic acid ( $C_{18:3}$ ) 0.05 %, arachidonic acid ( $C_{20:4}$ ) 0.13 %, palmitic acid ( $C_{16:0}$ ) 0.31 % and stearic acid ( $C_{18:0}$ ) 0.04 %. The vitamins added were retinol 1922  $\mu\text{g}/\text{kg}$  (1  $\mu\text{g}$  retinol : 3.3 iu vitamin A activity),  $\alpha$ -tocopherol 68.3 mg/kg, cholecalciferol 15.1  $\mu\text{g}/\text{kg}$  (1  $\mu\text{g}$  cholecalciferol : 40 iu vitamin D3 activity). Rats had a low protein, high quality diet (Rat and Mouse No. 1 Modified, SDS Ltd., Witham, Essex) designed to maintain rats in good health over long periods.

Humans had a healthy balanced diet.

### **Collection and preparation of tissues:**

The blood used was from 3 months old male rats (n: 4), 10 months old male rabbits (n: 4), 30 year old male humans (n: 4) and 6 years old male dogs (n: 4). All the species were given the nutrients and energy for a balanced diet to ensure a healthy and active life.

RBCs and plasma were separated immediately by centrifugation at 1,000 g for 10 min after collecting the blood into a heparinized plastic beaker.

RBC membranes were obtained by the method of Burton *et al*, (22) and Steck and Kant (23)] by little modifications. The packed cells were washed three times with 3 volumes of 0.89 % NaCl, pH 7.4 solution and haemolyzed by rapid and thorough mixing with chilled 5 mM pH 8.0 phosphate buffer. 5 mM Ascorbic acid was added as an antioxidant. The supernatant was removed after centrifugation of the mixture at 22,000 g for 20 min. The RBC membrane pellet was washed by resuspending in 2.5 mM phosphate buffer, pH 8.0 and centrifuged as before. The RBC membrane became colourless after washing second time using 1.25 mM phosphate buffer, pH 8.0. The RBC membranes were colourless showing that no haemoglobin remained and extracted immediately.

### **Lipid extraction and analysis**

Lipids in RBC membrane were extracted twice by the method of Verdon and Blumberg's (24) by modifying the concentrations of SDS (sodium dodecyl sulphate), BHT (butylated hydroxy toluene), solvents and avoiding the day light.

RBC membrane was mixed with an equal volume of 0.02 % aqueous SDS in a large volumetric tube. Two volumes of 0.002 % BHT in ethanol containing pentamethyl-6-chromanol

(oc-tocopherol with no side chain, internal standard) synthesized (25) was added and mixed. All procedure was done on ice. 5 volumes of 0.00025 % BHT in 50 % diethylether / hexane was added, mixed and centrifuged for 3 min at 10,000 x g. The upper layer was removed and washed with glass-distilled water; dried by passage through a filter containing  $\text{Na}_2\text{S}_4\text{O}_4$  and evaporated to dryness on a rotary evaporator. The lipid was then dissolved in ether/hexane, transferred to a vial and evaporated to dryness under nitrogen. It was dissolved in 50 % ether/hexane and kept in deep freeze. One portion of the extract was analysed by HPLC for the determination of the recovery. The extraction recovery was 95-100 %. Another portion of the extract was used for separation of phospholipids and transmethylation of their fatty acids.

### **Separation of phospholipids by TLC**

Silicagel H plates 0.5 mm were prepared by mixing 40 g in 100 ml distilled water. The plates were allowed to dry in air and were activated in an oven at 120°C for an hour. Up to 2 mg of lipid extracts were applied in a thin line approximately 10 cm along the origin of 20 x 20 cm TLC plates as soon as they cooled to room temperature. Phospholipid standards were also applied as spots along the origin. The plates were developed with chloroform: methanol: acetic acid: water (25: 15: 4: 2 by vol) for 45 min in a tank which was lined with filter paper and saturated for an hour. Only the phospholipid standard spots were sprayed with 0.2 % anilinonaphtalene sulphonic acid in methanol (HPLC grade) and visualised under a UV lamp and marked. Blood phospholipids were then chromatographed with markers and were removed and eluted with petroleum ether/diethyl ether (30 %) (26).

### **Transmethylation and separation of fatty acids**

The lipids were transmethylated by the method of Christie, (27) as follows. 2.5%  $\text{H}_2\text{SO}_4$  (v/v), (2 vol) in anhydrous methanol was mixed with lipid sample (1-2 mg) and incubated for 2 hours at 70°C. After adding 5 vol of 5% NaCl saturated with  $\text{NaHCO}_3$ , the mixture was extracted 3 times with 3 vol of petroleum ether. It was evaporated to dryness and dissolved in a small volume of HPLC grade hexane.

This methylated sample extracts and the standards (methyl esters of fatty acids) were then applied to Silicagel 60 G F<sub>254</sub> TLC plates (0.5 mm thick) using petroleum ether : diethyl ether : acetic acid (90 : 10 : 1 by vol) as the developing solvent (28) for the purification of methyl esters. Short- and long-chain methylated fatty acid esters were widely separated from cholesterol and more polar compounds which remain at or near the origin and also from hydrocarbons and any BHT added as an antioxidant, which migrated ahead. Only the standards were visualised by spraying with dodecamolybdophosphoric acid in ethanol and developing a

blue colour by heating. The methyl esters of fatty acids, were eluted from the silicagel with petroleum ether / hexane (50 %) containing 0.01 % BHT. The solvent was then evaporated in a rotary evaporator and dissolved in a small volume of HPLC grade hexane and stored in the deep freeze until analysis by GC.

### Estimation of methyl esters of fatty acids by GC

Methyl esters of fatty acids were separated and determined by a Hewlett Packard 5890 A gas chromatography with an x - meter Carbowax 20 m capillary column. The detector temperature was 230 °C. Helium was used as a carrier gas (50 ml / min). The oven temperature was programmed to rise from 50°C to 230° C at 12° C / min.. Retention times and peak areas were measured with a reporting integrator Hewlett Packard model, SP 4270. Fatty acids were identified by comparing their retention time with those of standard methyl fatty acid esters from Sigma Ltd. Recovery of fatty acids was estimated by adding a known amount of PC with two C15 saturated fatty acids attached to the mixture to be transmethylated that are rarely found in nature (27).

## RESULTS

The fatty acid composition of RBC membrane of the four species are shown in tables 1-4. The main fatty acids of RBC membrane PE and PC were C<sub>16:0</sub>, C<sub>18:0</sub>, C<sub>18:2</sub>, C<sub>18:2</sub> and C<sub>20:4</sub> and followed by the others. These fatty acids comprised nearly 93 % of total RBC membrane PC and PE phospholipid fatty acids. Other fatty acids were present in minimal concentrations. Saturated fatty acids such as C<sub>16:0</sub>, C<sub>18:0</sub> and C<sub>20:0</sub> averaged 43 % in PE of rabbit and dog, 24 % in rat PE and 34 % in human PE whereas it averaged 55 % in PC among all the species. PUFA such as C<sub>18:2</sub>, C<sub>18:3</sub> and C<sub>20:4</sub> were found in large amounts in RBC membrane of species. PUFA and arachidonate (C<sub>20:4</sub>) levels were higher in PE than PC (Table 5). Arachidonate was the major PUFA in RBC membrane of rat, human and dog whereas linoleate (C<sub>18:2</sub>) was the major one in the rabbit. MUFA was also lowest in rabbit than the other species. Arachidonate was the main PUFA in PE whereas C<sub>18:2</sub> was the main one in PC in the rat (Table 1) and human (Table 3). C<sub>18:2</sub> was higher in both PE and PC phospholipids of rabbit (Table 2). There was not much difference between the percentage levels of fatty acids between PE and PC of the rabbit unlike the other species. In RBC membrane, the LCPUFA (C<sub>22:2</sub> and C<sub>22:6</sub>) was significantly lower in the species phospholipids. Among the LCPUFA, C<sub>22:6</sub> was found in the highest concentration in human RBC membrane PE (Table 3) and was not detected in dog.

RBC membrane contained 11-26 % MUFA with high levels of C<sub>18:1</sub> both in PE and PC of the species.

**Table 1.** Fatty acid composition of PE and PC in RBC membrane of rat

<u>Fatty acids (%)</u>			<u>Fatty acids (%)</u>		
	<u>Mean</u>	S.E.		Mean	S.E.
PE			PC		
C <sub>16:0</sub>	<b>13.0</b>	1.2	C <sub>16:0</sub>	39.5	1.9
C <sub>16:1</sub>	2.30	0.6	C <sub>16:1</sub>	0.50	0.5
C <sub>18:0</sub>	7.60	0.8	C <sub>18:0</sub>	16.0	0.9
C18:1	13.3	1.7	C <sub>18:1</sub>	8.50	0.5
C <sub>18:2</sub>	6.70	0.6	C <sub>18:2</sub>	16.5	0.4
C <sub>18:3</sub>	<b>0.14</b>	0.3	C <sub>18:3</sub>	0.30	0.3
C <sub>20:0</sub>	1.40	0.7	C <sub>20:0</sub>	0.40	0.4
C <sub>20:1</sub>	0.11	0.0	C <sub>20:1</sub>	0.80	0.4
C <sub>20:2</sub>	<0.1		C <sub>20:2</sub>	<0.1	
C <sub>20:3</sub>	N.D.		C <sub>20:3</sub>	0.58	0.2
C <sub>20:4</sub>	46.5	0.7	C <sub>20:4</sub>	9.80	1.6
C <sub>20:5</sub>	0.86	0.6	C <sub>20:5</sub>	N.D.	
C <sub>22:0</sub>	<0.1		C <sub>22:0</sub>	0.44	0.4
C <sub>22:1</sub>	<0.1		C22:1	<0.1	
C <sub>22:4</sub>	<0.1		C <sub>22:4</sub>	<0.1	

Values are percentages of total.

N.D.: Not detected.

Each figure represents the mean  $\pm$  S.E. of four individuals.

**Table 2.** Fatty acid composition of PE and PC in RBC membrane of rabbit

	<u>Fatty acids (%)</u>			<u>Fatty acids (%)</u>	
	<u>Mean</u>	<u>S.E.</u>		<u>Mean</u>	<u>S.E.</u>
PE			PC		
C <sub>16:0</sub>	22.5	14	C <sub>16:0</sub>	30.7	19
C <sub>16:1</sub>	1.06	0.4	C <sub>16:1</sub>	0.06	0.0
C <sub>18:0</sub>	15.5	0.6	C <sub>18:0</sub>	19.6	0.8
C <sub>18:1</sub>	8.56	1.5	C <sub>18:1</sub>	11.0	0.6
C <sub>18:2</sub>	28.9	0.9	C <sub>18:2</sub>	31.2	1.2
C <sub>18:3</sub>	1.03	0.3	C <sub>18:3</sub>	0.82	0.3
C <sub>20:0</sub>	0.39	0.6	C <sub>20:0</sub>	0.38	0.4
C <sub>20:1</sub>	N. D.		C <sub>20:1</sub>	0.22	0.2
C <sub>20:2</sub>	0.84	0.2	C <sub>20:2</sub>	0.98	0.3
C <sub>20:3</sub>	0.67	0.1	C <sub>20:3</sub>	0.84	0.2
C <sub>20:4</sub>	8.30	0.7	C <sub>20:4</sub>	2.55	0.5
C <sub>20:5</sub>	0.83	0.1	C <sub>20:5</sub>	0.19	0.2
C <sub>22:0</sub>	<0.1		C <sub>22:0</sub>	<0.1	
C <sub>22:1</sub>	N.D.		C <sub>22:1</sub>	<0.1	
C <sub>22:4</sub>	<0.1		C <sub>22:4</sub>	<0.1	
C <sub>22:6</sub>	1.01	0.2	C <sub>22:6</sub>	0.33	0.3
C <sub>23:0</sub>	0.78	0.1	C <sub>23:0</sub>	0.22	0.0

Values are percentages of total.

N.D.: Not detected.

Each figure represents the mean ± S.E. of four individuals.

**Table 3.** Fatty acid composition of PE and PC in RBC membrane of human

	<u>Fattv acids (%)</u>		<u>Fattv acids (%)</u>		
	Mean	<i>SE.</i>	Mean	S.E	
PE			PC		
C16:0	18.8	0.4	C16:0	31.5	0.9
C16:1	1.35	0.4	C16:1	1.80	0.2
C18:0	11.2	0.8	C18:0	17.2	0.5
C18:1	21.7	1.0	C18:1	13.7	0.4
C18:2	9.18	0.7	C18:2	25.8	0.7
C18:3	0.23	0.1	C18:3	0.22	0.1
C20:0	0.36	0.1	C20:0	0.28	0.0
C20:1	<0.1		C20:1	0.29	0.0
C20:2	<0.1		C20:2	0.30	0.0
C20:3	0.47	0.1	C20:3	1.59	0.2
C20:4	20.4	0.7	C20:4	3.75	0.5
C20:5	1.25	0.3	C20:5	0.58	0.0
C22:0	N.D.		C22:0	N.D.	
C22:1	N.D.		C22:1	<0.1	
C22:4	<0.1		C22:4	<0.1	
C22:6	4.60	0.2	C22:6	<0.1	

Values are percentages of total.

N.D.: Not detected.

Each figure represents the mean  $\pm$  S.E. of four individuals.



**Table 4.** Fatty acid composition of PE and PC in RBC membrane of dog

	<u>Fatty acids (%)</u>		<u>Fatty acids (%)</u>		
	<u>Mean</u>	<u>S.E.</u>	<u>Mean</u>	<u>S.E.</u>	
PE			PC		
C16:0	15.4	0.8	C16:0	20.0	1.3
C16:1	0.47	0.1	C16:1	0.96	0.2
C18:0	26.9	0.5	C18:0	26.2	1.1
C18:1	11.6	1.0	C18:1	16.6	1.0
C18:2	2.89	0.5	C18:2	12.7	0.7
C18:3	N.D.		C18:3	0.45	0.1
C20:0	0.32	0.1	C20:0	0.43	0.0
C20:1	<0.1		C20:1	N.D.	
C20:2	0.45	0.1	C20:2	0.30	0.0
C20:3	N.D.		C20:3	3.30	1.8
C20:4	39.6	0.7	C20:4	11.6	1.5
C20:5	0.98	0.3	C20:5	0.18	0.0
C22:0	N.D.		C22:0	0.24	0.1
C22:4	0.63	0.3	C22:1	0.59	0.1
C22:6	N.D.		C22:4	N.D.	
C23:0	N.D.		C23:0	0.29	0.1

Values are percentages of total.

N.D.: Not detected.

Each figure represents the mean  $\pm$  S.E. of four individuals.

**Table 5. Percentage of SFA, MUFA, PUFA and arachidonate in RBC membrane PE and PC**

	<u>Species</u>			
	<u>Rat</u>	<u>Rabbit</u>	<u>Human</u>	<u>Dog</u>
-PE	24	43	34	43
SFA (%)				
PC	60	51	51	50
PE	17	11	26	12
MUFA (%)				
PC	11	11	16	19
PE	58	42	33	43
PUFA (%)				
PC	29	35	31	26
PE	51	9	23	40
Arachidonate				
(%) PC	11	3	4	12

SFA: Saturated fatty acids ( $C_{16:0}$ ,  $C_{18:0}$  and  $C_{20:0}$ )

MUFA: Monounsaturated fatty acids ( $C_{16:1}$ ,  $C_{18:1}$ ,  $C_{20:1}$  and  $C_{22:1}$ )

PUFA: Polyunsaturated fatty acids ( $C_{18:2}$ ,  $C_{18:3}$ ,  $C_{20:4}$ )

## DISCUSSION

Each species had the same major fatty acids in PE and PC of RBC membrane. Arachidonate and PUFA were highest in PE than PC. PC also had relatively high PUFA content. Arachidonate was a prominent fatty acid of PE in the species except rabbit.  $C_{18:2}$  was the prominent one in the rabbit. The finding that linoleic acid was in a lesser concentration than arachidonic acid in the RBC membrane of the species except rabbit, suggests that the conversion of linoleate to arachidonate could be more active in these species than the rabbit.

This probably indicates the lower activities of elongase and  $\Delta^5$ -desaturase for the formation of arachidonic acid from linoleic acid in the rabbit. Saturated fatty acids comprised about 24-43 % the fatty acids found in PE while PUFA comprised about 33-58 %. In contrast PC had higher percentage of SFA than PUFA. SFA comprised about 51-60 % whereas PUFA comprised about 26-35 % the fatty acids found in PC in the RBC membrane of the species. Among the main SFA, percentage of C<sub>16:0</sub> was higher compared to C<sub>18:0</sub> in PE and PC except dog. C<sub>18:0</sub> was higher than C<sub>16:0</sub> in dog RBC membrane PE and PC. MUFA % was also higher in PE than PC. C<sub>18:1</sub> was found considerably higher than C<sub>16:1</sub> in all species RBC membrane phospholipids. This could be due to the location difference of the two phospholipids; PC being located outside of the membrane bilayer whereas PE located towards the inside of the membrane bilayer. In the outer layer, PC is renewed by exchange between plasma lipoproteins (29). These unsaturated fatty acids are the major fatty acids contributing to the permeability, fluidity of the membrane and also to the functioning of the membrane via receptors and membrane bound enzymes. Unsaturated fatty acids may be more demanded in the inside rather than outside of the membrane. 22-Carbon fatty acids were present in small amounts in most species. Among the LCPUFA, C<sub>22:6</sub> was present in rabbit and human PE and very little was present (less than 0.1 %) in dog and rat, showing that the enzymes capable of synthesizing C<sub>22:6</sub> (elongase and  $\Delta^5$ -desaturase) were lower in dog and rat. C<sub>22:4</sub> was also present in very little amounts. This could also be due to the diet. The fact that 22 carbon fatty acids are present in some of the vegetable oils and in the fats of aquatic origin, C<sub>22:6</sub> is being high in fish oil; it is not surprising that dog and rat showed very little % of 22 fatty acids. These animals did not consume foods which contained appreciable amounts of 22 carbon fatty acids. The data presented by other workers demonstrated that RBC fatty acid composition reflects the type of fat in the diet. Fish oil-enriched diet causes profound changes in the fatty acid pattern of RBC membrane and serum phospholipids in rabbits. Fish oil results in high amounts of C<sub>20:5</sub>, C<sub>22:5</sub> and C<sub>22:6</sub> (6). These results are in agreement with other species's results like monkeys (30), rats (7) and humans (31).

The data obtained for rabbit was in accord with the findings of van den Boom *et al* (32). Their values for C<sub>18:0</sub> and C<sub>18:1</sub> were higher than the values reported here. The percentage of fatty acids in PC of dog RBC membrane were in agreement with those of Slappendel *et al* (33), rat RBC membrane values were in agreement with Tichelaar *et al* (19) and Ghosal *et al* (14) with little variations. Ghosal *et al* found higher levels of C<sub>16:0</sub> and C<sub>18:0</sub> and lower levels of C<sub>20:4</sub> compared to our results. They also found lower values of C<sub>18:0</sub>, C<sub>18:2</sub>, and C<sub>20:4</sub> compared

to our findings. Tichelaar *et al* (19) observed lower levels of  $C_{20:4}$  in PE and higher in PC compared to our results. Their  $C_{22:4}$  and  $C_{22:6}$  values were also a little higher than our values.

## CONCLUSION

The major fatty acids estimated in PE and PC of different species like a herbivore (rabbit), a carnivore (dog) and omnivores (human and rat) appeared to be same in RBC membrane. PUFA % was higher in PE than PC. Among PUFA  $C_{18:2}$  was the prominent one in rabbit while  $C_{20:4}$  was the prominent one in the others. This could be due to the lower activities of elongase and  $\Delta^5$ -desaturase synthesizing arachidonate from linoleate in the rabbit. SFA and MUFA were higher in PE than PC. Among MUFA,  $C_{18:1}$  was considerably higher difference of these two phospholipids.  $C_{22:6}$  and  $C_{22:4}$  (LCPUFA) were present in little amounts.  $C_{22:6}$  was present in human and rabbit PE whereas it was less than 0.1 % in rat and dog. This could be either due to the diet or to the enzymes synthesizing  $C_{22:6}$  (elongase and  $A^5$ -desaturase) being low in these species.

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