CHAPTER IV

DEVELOPMENTAL CHANGES IN LIPID COMPOSITION AND FLUIDITY CHARACTERISTICS OF RAT LIVER AND BRAIN MITOCHONDRIA

INTRODUCTION

The lipids are an important component of the membrane structure [1] and like the enzymes of mitochondrial membranes, the lipids are also seen to alter with age [1].

Studies on the changes in the PL content and composition of rat liver mitochondria have been reported [1-4]. However most of these studies are restricted either to the neonates [5,6] or 0 - 10 days postnatal rats [2]. Besides, these reports are contradictory. In the brain, the studies are mostly on the brain specific lipids such as cerebrosides, gangliosides and myelin (Chapter I). However, no studies on the developmental profiles of brain mitochondria heve been reported.

As described earlier (Chapters II and III of this thesis), the enzymes of mitochondrial energy metabolism in the liver and the kidneys followed a specific developmental pattern. Hence it was of interest to see if the lipids also change during development and also if the different stages of maturation of the two organs is mirrored in the lipid profiles of their mitochondria.

With a view to illustrating this point,studies were carried out to examine in the liver and brain mitochondria the following parameters: a) Total phospholipid (TPL) and Cholesterol (CHL) content and TPL/CHL molar ratios., b) the

% contribution and content of individual PL classes and c) fluidity parameters of the mitochondrial membranes.

MATERIALS AND METHODS

Chemicals

Kieselgel 60 H was purchased from E. Merck, Germany. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was purchased from Sigma Chemical Co., St.Louis, Missouri, U.S.A. Tetrahydrofuran (THF) was obtained from Thomas and Baker Co., London. Cholesterol was purchased from J.T.Baker Chemicals, N.J., U.S.A. All other chemicals and reagents were as described in Chapter II. All the solvents were of analytical grade purchased locally.

For all the experimental purposes double glass distilled water (D/W) was used.

Animals :

The details are as described in Chapter II of this thesis.

Isolation of mitochondria :

The procedure followed is primarily same as described in Chapter I, except that, instead of only one washing given to the mitochondria for enzyme work, both liver and brain mitochondria were washed thrice.

Lipid extraction :

This was essentialy as described by Folch et.al.[7]. The thrice washed mitochondria were resuspended in the isolation medium and appropriate aliquots of mitochondrial protein (8 to 10 mg) were taken in a final aqueous volume of 1.5 ml made up with either isolation medium or distilled water. To this 4.0 ml of freshly prepared 2 : 1 (v/v) chloroform : methanol mixture was added and the tube was vortexed vigorously and was centrifuged in a table top centrifuge at 2000 rpm for 10 minutes. The lower phase was transferred to another tube using a graduated syringe, the volume was noted. To the first tube once again 2.0 ml 2:1 chloroform : methanol was added and after re-extraction, the lower phase was once again measured and transferred to the tube already containing the first extract. The total volume of the pooled extract was noted and to this 0.2 volumes of 0.017 % $MgCl_2$ was added and after vortexing, the tube was centrifuged at 2000 rpm for 10 minutes. Once again the lower phase was transferred to another tube and the volume was made up to 4.0 ml with the 2:1 chloroform : methanol mixture.

From this final extract, aliquots were taken in separate tubes for estimating the total phospholipid (TPL), cholesterol (CHL) as well as separation of the PL into subclasses by thin layer chromatography (TLC).

Separation of phospholipids by TLC :

Preparation of plates :

Glass plates (20 x 20 cm) soaked overnight in chromic acid and washed repeatedly with tap water. The plates were final rinsed with D/W and dried. Before coating the plates with the silica gel slurry they were thoroughly rinsed with acetone.

Kieselgel 60 H was used to coat the plates. The slurry contained 7.0 gm of the silica gel powder mixed in 15 ml D/W per plate. This slurry was transferred to De Saga applicator with an apperture adjusted for 0.25 mm coat thickness, and spread evenly on the plates. These were allowed to dry overnight and those plates with an even and uniform coating were selected for further work.

Spotting of the samples :

Before spotting the samples the plates were activated by placing them in an oven set at 110^{o}C for 30 minutes.

The aliquots for TLC were first made to zero volume after which a fixed small volume was made up using the 2 : 1 chloroform : methanol mixture. The sample was then spotted on to the plates. After spotting, the plates are once again placed in the oven for 2 minutes and then were placed in the solvent system for PL separation.

Single dimensional TLC for PL separation :

This was essentially according to the method described by Mangold [8]. The solvent system used was as follows:

Chloroform : Methanol : Glacial acetic acid : Water (v/v) (25:15:4:2).

The solvent jar was saturated with this solvent system before the plates are kept in it. After the run was over the plate was removed and air-dried to remove the solvent present on the plates. The plate was then placed in a jar containing iodine for visualizing the spots. Once all the spots develop the yellowish brown color, the plate was removed and the individual spots were marked and identified as per the pattern reported by Mangold [8].

Estimation of separated phospholipids :

The gel in the marked and identified spots was scraped off into numbered tubes. These tubes along with the aliquots for TPL measurement taken from the lipid extract were then subjected to digestion.

To all the tubes 0.5 ml 10 N H_2SO_4 was added and these were then placed in a sand-bath and heated for 2 - 3 h. At the end of this digestion period the tubes were cooled and a drop of 70 % perchloric acid was added. The tubes were once again heated in the sand-bath till the odour of chlorine was

no longer detectable. The tubes were then allowed to cool down then the phosphorus content was estimated essentially according to the method described by Bartlett [9].

Estimation of phospholipid phosphorous by Bartlett's method:

The reagents required for this assay are :

Standard phosphorous solution - 35.1 mg KH_2PO_4 in 100 ml D/W to which 1.0 ml concentrated sulfuric acid is added to give a final concentration of 80 µg Pi /ml. The working standard is obtained by diluting the above 1:40 times. The concentration is 2 µg Pi/ml.

b) Ammonium molybdate - 5% solution prepared fresh in D/W.

c) 10 N sulfuric acid - 28 ml concentrated sulfuric acid (36 N) added to 72 ml D/W.

d) 1-Amino-2-naphthol-4-sulfonic acid (ANSA)- a triturate similar to that prepared for Fiske and Subba Row method is prepared details as given in Chapter II of this thesis. For the assay 40 mg ANSA / ml is prepared fresh in D/W.

For the standard curve aliquots containing 0.4 to 4.0 µg of Pi were taken from the working standard and 0.5 ml 10 N sulfuric acid was added to each tube. The final volume was

made up to 3.7 ml with D/W, followed by 0.2 ml aqueous 5% ammonium molybdate and 0.1 ml freshly prepared ANSA. The tubes were then vortexed and placed in a boiling water bath for 10 minutes for color development afterwhich the tubes were removed and allowed to cool. The color developed was read against the reagent blanks in Carl-Zeiss spectrophotometer at 830 nm. A standard graph of O.D. versus phosphorous concentration was plotted from the data obtained.

The digested samples were assumed to have a volume of 0.2 ml and final volume was made up to 3.7 ml with D/W. The further protocol was similar to that for the standards. After the color development the samples were subjected to centrifugation at 2000 rpm for 10 minutes to sediment the silica gel and the O.D. of the supernatants were read at 830 nm.

Cholesterol estimation:

This was as described by Zlatkis et.al. [10] with some modifications. The following reagents were used:

a) Cholesterol standard - 1.0 mg/ml prepared in isopropanol.

b) Ferric chloride ($FeCl_3$) reagent - 10% $FeCl_3$ solution prepared in glacial acetic acid . The working reagent was prepared fresh by adding 1.0 ml this reagent to 100 ml of concentrated sulfuric acid with constant stirring.

For the standard curve aliquots containing 20 to 200 µg cholesterol were taken in a final volume of 0.5 ml made up with isopropanol. To this 1.5 ml of glacial acetic acid and 1.5 ml of ferric chloride reagent were added followed by vigorous vortexing. The tubes were kept at room temperature for 20 minutes and the color was read in an ERMA colorimeter using a 530 nm filter.

Standard curve of O.D. versus cholesterol concentration is plotted .

The sample tubes were made to zero volume and then 0.5 ml of isopropanol was added. The further protocol was similar to the one described above.

Measurement of membrane fluidity:

This was carried out essentially as described by Mehta et.al. [11], modified for mitochondrial membrane fluidity measurements [12]. Freshly isolated mitochondria were used for the measurements.

The fluorescence polarization measurements were carried out in buffered sucrose solution containing 0.32 M sucrose and 10 mM tris-HCl at a final pH 7.4. DPH (2 mM) used as the probe was dissolved in THF and stored in amber colored bottle in the refrigerator.

For the actual measurement, mitochondrial protein 0.2 mg/ml, final concentration, was taken in buffered sucrose and to this 5 µl of the stock probe (DPH) solution was added and the tubes were vigorously vortexed and left in dark for 30 minutes to permit equilibration of the probe into the membranes. The probe to lipid ratio was maintained around 1:200 to 1:300 [11].

The fluorescence polarization measurement was carried out in a Shimadzu RF-5000 spectrophotofluorimeter with a polarizer attachment and containing a resident programme for calculating and printing fluorescence polarization (P) values. The excitation and emission wavelengths used were 360 nm and 430 nm respectively and the bandwidth of 5 and 10 nm respectively for the two wavelengths. Data were accumulated for 5 seconds each for both vertical (parallel $P0^{o}$) and horizontal (perpendicular $P90^{o}$) setting of the polarizer.

From the P values other parameters i.e. fluorescence anisotropy (r), limited hindered anisotropy (r_∞) and order parameter (S) were calculated from the following formulae:

$$r = 2P/(3-P)$$

$$r_\infty = (4r/3)-0.1 \text{ and}$$

$$S = \sqrt{r_\infty/r} \qquad [13,14].$$

RESULTS

Tables 1 to 4 present the data for developmental changes in the liver mitochondrial lipids while the data for brain mitochondria are shown in Tables 5 to 8.

The TPL content did not change significantly in any of the age groups (Table 1) and was comparable to the adults. The CHL content on day 14 was 21% higher than the adult group, although the increase was not statistically significant. The 21- and 35-day groups had 2.1 and 2.6 fold higher CHL content. Consequently the TPL/CHL molar ratios in the 14 day groups was not significantly different from the adult but in the 21- and 35-day groups the ratios were reduced by about 50% indicating decreased fluidity.

Percent contribution by individual phospholipids is shown in Table 2. Lysophosphatidic acid in the 14-day group was about 30% of the adults while in the 21-day-group it was 18%. In 35-day group it was 6.5 fold higher. Percent contribution by SM in 14- and 21-days is lower (-30 and -26% respectively) while on day 35 it was higher (+40%) than the adults. The PC levels did not differ significantly in any of the age groups except on day 21 (+16%) while in case of PE all except on day 35 (30%) had similar values as the adult. PI, on the other hand, was highest on day 35 (about 40% higher). The 14- and 21- day group, however, had lower values than the

Table 1

Developmental changes in total phospholipid (TPL), cholesterol (CHL) content and TPL / CHL molar ratio in rat liver milochondria.

Age	TPL content (µg/mg mitochondrial protein)	CHL content (µg/mg mitochondrial protein)	TPL : CHL molar ratio
14 Days	210.0 ± 18.89	19.2 ± 0.83	5.72 ± 0.49
21 Days	217.5 ± 10.03	33.8 ± 3.80[a]	3.21 ± 0.20[b]
35 Days	222.8 ± 4.20	40.8 ± 2.90[b]	2.81 ± 0.24[b]
Adult	207.3 ± 17.62	15.9 ± 2.10	6.34 ± 0.46

Results are expressed as mean + S.E.M of 8 independent observations in individual group.

[a]$P < 0.002$; [b]$P < 0.001$ compared to the adults.

Table 2

Developmental changes in phospholipid composition of rat liver mitochondria. (% TPL)

Phospholipid class	Age			
	14 Days	21 Days	35 Days	Adult
Lysophosphatidic acid (lyso)	0.5 ± 0.08[d]	0.3 ± 0.06[d]	11.1 ± 0.65[d]	1.7 ± 0.14
Sphingomyelin (SM)	1.9 ± 0.30[d]	1.6 ± 0.30[d]	8.5 ± 0.54[b]	6.1 ± 0.47
Phosphatidyl-choline (PC)	45.8 ± 0.40	51.3 ± 2.60[a]	38.0 ± 0.73	41.2 ± 2.78
Phosphatidyl-inositol (PI)	2.4 ± 0.25[d]	1.8 ± 0.38[d]	9.3 ± 0.18[d]	6.8 ± 0.34
Phosphatidyl-serine (PS)	1.4 ± 0.09	1.1 ± 0.08[d]	7.7 ± 0.42[d]	2.4 ± 0.17
Phosphatidyl-ethanolamine (PE)	36.5 ± 0.83	36.6 ± 2.10	21.4 ± 1.07[d]	31.5 ± 2.32
Diphosphatidyl-glycerol (DPG)	11.6 ± 1.00	6.9 ± 0.32[d]	6.6 ± 0.23[d]	11.6 ± 0.54

Results are expressed as mean ± S.E.M of 8 independent observations for individual groups.

[a] $P < 0.02$; [b] $P < 0.005$; [c] $P < 0.002$; [d] $P < 0.001$ compared to the adults.

Table 3

Developmental changes in the content of individual phospholipid classes in rat liver mitochondrial. (μg/mg mitochondrial protein)

Phospholipid class	Age			
	14 Days	21 Days	35 Days	Adult
Lysophosphatidic acid (lyso)	0.9 ± 0.24^{e}	2.3 ± 0.21^{d}	23.5 ± 0.93^{e}	3.9 ± 0.31
Sphingomyelin (SM)	4.1 ± 0.60^{e}	2.5 ± 0.33^{e}	17.1 ± 0.84	17.6 ± 1.69
Phosphatidyl-choline (PC)	96.1 ± 2.70	104.6 ± 2.70	84.9 ± 1.48	92.2 ± 9.40
Phosphatidyl-inositol (PI)	5.1 ± 0.43^{e}	4.9 ± 0.47^{e}	20.5 ± 0.34^{e}	9.3 ± 0.80
Phosphatidyl-serine (PS)	3.0 ± 0.37^{e}	1.8 ± 0.49^{e}	17.4 ± 1.03^{b}	12.9 ± 0.94
Phosphatidyl-ethanolamine(PE)	76.5 ± 2.25	86.0 ± 9.5	47.5 ± 2.02^{d}	77.0 ± 6.50
Diphosphatidyl-glycerol (DPG)	24.9 ± 1.62	16.1 ± 1.54^{a}	15.5 ± 0.30^{c}	22.2 ± 1.80

Results are expressed as mean ± S.E.M. of 8 independent observations for individual group.

$^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.005$; $^{d}P < 0.002$; $^{e}P < 0.001$ compared to the adults.

Table 4

Developmental changes in the membrane fluidity parameters in rat liver mitochondria.

Age	Fluorescence polarization (P)	Fluorescence anisotropy (r)	Limited hindered anisotropy (r_{ec})	Order parameter (S)
14 Days	0.131 ± 0.003	0.089 ± 0.002[b]	0.017 ± 0.003[b]	0.466 ± 0.03
21 Days	0.161 ± 0.002[d]	0.113 ± 0.001[c]	0.053 ± 0.002[d]	0.664 ± 0.009[c]
35 Days	0.163 ± 0.003[d]	0.111 ± 0.003[a]	0.054 ± 0.004[c]	0.673 ± 0.015[c]
Adult	0.138 ± 0.004	0.101 ± 0.003	0.032 ± 0.004	0.551 ± 0.028

Results are expressd as mean ± S.E.M. of 8 independent observations for individual group.

[a]P < 0.05; [b]P < 0.01 ; [c] P < 0.002 ; [d]P < 0.001 compared to the adults.

adults (only about 20 - 25%). PS level was only 60 and 45% in the 14- and 21-day groups respectively and about 3.2 fold higher in 35-day group. DPG % contribution in 14-day group is similar to the adult while the 21- and 35-day groups both had about 40% lower values.

The content of the individual phospholipid classes (Table 3) mirrors the pattern in Table 2. Lysophosphatidic acid content on day 14 and 21 (-77% and -42% respectively) was lower than the adults while in the 35-day group it was 6 fold higher. SM content was only 23% and 14% in the 14- and 21-day groups compared to the adults. PC content did not change significantly in any of the age groups. PI content on day 14 and 21 was 46% lower and in 35-day group 2.3 times higher than the adults. PS content was quite low in the younger age groups i.e. 14- and 21-days (4.3 fold and 7.2 fold respectively) while 35-day group had 35% higher content. PE is significantly different only in 35-day group (-38%). DPG is lower by about 30% in the 21- and 35-day groups.

The fluorescence polarization (P values) (Table 4) on day 21 and 35 were significantly higher than the adult values (+17%). The fluorescence anisotropy (r) on day 14 is 14% lower while on day 21 and 35 it is 12% higher than the adult. The differences in fluidity parameters though small in magnitude, are highly significant. The limited hindered anisotropy (ra)is 45% lower on day 14 while 21- and 35-day groups have

70% higher values. The order parameter (S) is about 87% of adult values in the 14-day group while on day 21 and 35 it is about 20% higher.

Development-induced changes in the brain mitochondrial TPL (Table 5) are reflected in lower content (43%) in the 14-day group and 20 to 25% lower content in the other two age groups. CHL content differed in the different age groups although these wre not statistically significantly. The TPL/CHL molar ratio was lower in all the age groups as compared to the adults; by 34% on day 14, by 20% on day 21 and by 30% on day 35.

The percent contribution by individual PL classes is shown in Table 6. Lysophosphatidic acid levels were about 12.5 times lower on day 21 and 2.2 times higher on day 35 compared to the adults. SM is about 65% lower in the 14-day group and has twice the adult level in 35-day group. PC levels did not altear much except in the 35-day group where it was 12% lower. The PI contribution was about half the adult level in the 14-day group while on day 21 it was 26% lower. PS was 60% and 30% lower on day 14 and 35 respectively. PE contribution only slightly changed. However these small changes were statistically significant when compared to the adults. Contribution by DPG in the 14-day group was the highest which decreased gradually by about 60 % till the adult stage.

Table 5

Developmental changes in total phospholipid (TPL), cholesterol (CHL) content and TPL/CHL molar ratio in rat brain mitochondria.

Age	TPL content (µg/mg mitochondrial protein)	CHL content (µg/mg mitochondrial protein)	TPL : CHL molar ratio
14 Days	383.5 ± 22.30[e]	73.3 ± 2.80	2.58 ± 0.24[d]
21 Days	507.6 ± 24.20[b]	79.0 ± 5.80	3.12 ± 0.18[b]
35 Days	538.3 ± 4.10[a]	93.9 ± 6.60	2.84 ± 0.19[c]
Adult	675.3 ± 56.40	85.5 ± 8.30	3.90 ± 0.19

Results are expressed as mean ± S.E.M of 8 independent observations for individual groups.

[a]P < 0.05 ; [b]P < 0.02 ; [c]P < 0.005 ; [d]P < 0.002;

[e]P < 0.001 compared to the adults.

Table 6

Developmental changes in phospholipid composition of rat brain mitochondria. (% TPL)

Phospholipid class	Age			
	14 Days	21 Days	35 Days	Adults
Lysophosphatidic acid (lyso)	2.0 ± 0.45	0.2 ± 0.01[c]	5.6 ± 0.40[c]	2.5 ± 0.24
Sphingomyelin (SM)	1.5 ± 0.25[c]	4.6 ± 0.06	8.5 ± 0.60[c]	4.3 ± 0.13
Phosphatidyl-choline (PC)	40.2 ± 1.08	41.2 ± 0.67	37.8 ± 0.81[c]	42.9 ± 0.32
Phosphatidyl-inositol (PI)	1.7 ± 0.13[c]	2.6 ± 0.35[a]	3.5 ± 0.54	3.5 ± 0.12
Phosphatidyl-serine (PS)	1.4 ± 0.39[b]	3.2 ± 0.43	2.3 ± 0.33[a]	3.3 ± 0.24
Phosphatidyl-ethanolamine (PE)	37.6 ± 0.66	41.1 ± 0.40[c]	34.7 ± 0.77[c]	39.0 ± 0.12
Diphosphatidyl-glycerol (DPG)	15.4 ± 1.03[c]	7.0 ± 0.21[a]	7.1 ± 0.66	5.8 ± 0.47

Results are expressed as mean ± S.E.M of 8 independent observations for individual groups

[a]$P < 0.05$; [b]$P < 0.002$; [c]$P < 0.001$ compared to the adults.

Table 7

Developmental changes in content of individual phospholipid classes in rat brain mitochondria.
(μg/mg mitochondrial protein)

Phospholipid class	Age			
	14 Days	21 Days	35 Days	Adult
Lysophosphatidic acid (lyso)	4.5 ± 2.33^{c}	0.8 ± 0.26^{d}	2.7 ± 2.20^{d}	3.9 ± 0.31
Sphingomyelin (SM)	4.5 ± 1.16^{d}	2.7 ± 0.31^{d}	45.9 ± 3.02^{d}	31.3 ± 0.91
Phosphatidyl-choline (PC)	147.5 ± 8.45^{d}	217.7 ± 7.21^{d}	197.1 ± 10.90^{d}	277.6 ± 8.93
Phosphatidyl-inositol (PI)	6.2 ± 0.68^{d}	14.4 ± 2.19^{c}	22.1 ± 5.40	23.9 ± 0.83
Phosphatidyl-serine (PS)	5.5 ± 0.36^{d}	17.3 ± 2.64	14.3 ± 0.86^{b}	18.9 ± 0.91
Phosphatidyl-ethanolamine(PE)	136.5 ± 4.84^{d}	217.7 ± 6.40^{d}	193.8 ± 10.60^{d}	271.4 ± 1.15
Diphosphatidyl-glycerol (DPG)	55.8 ± 4.31^{d}	37.6 ± 2.16	30.8 ± 1.62^{b}	35.4 ± 1.20

Results are expressed as mean ± S.E.M of 8 independent observations for individual groups

$^{a}P < 0.05$; $^{b}P < 0.005$; $^{c}P < 0.002$; $^{d}P < 0.001$ compared to the adults.

Table 8.

Developmental changes in the membrane fluidity parameters in rat brain mitochondria.

Age	Fluorescence polarization (P)	Fluorescence anisotropy (r)	Limited hindered anisotropy (r_∞)	Order parameter (S)
14 Days	0.211 ± 0.002^{d}	0.154 ± 0.001^{d}	0.102 ± 0.002^{d}	0.811 ± 0.004^{d}
21 Days	0.164 ± 0.003	0.112 ± 0.002^{a}	0.053 ± 0.003	0.664 ± 0.013
35 Days	0.147 ± 0.003^{c}	0.101 ± 0.002^{d}	0.039 ± 0.003^{b}	0.567 ± 0.007^{d}
Adult	0.169 ± 0.006	0.123 ± 0.005	0.058 ± 0.006	0.700 ± 0.021

Results are expressd as mean ± S.E.M of 8 independent observations for individual group.

$^{a}P < 0.05$; $^{b}P < 0.02$; $^{c}P < 0.005$; $^{d}P < 0.001$ compared to the adults.

The lysophosphatidic acid content in the brain mitochondria (Table 7) was only 24% of adult levels on day 14 while on day 21 the content was still lower (only 5%) the 35-day group had 60% more content than the adults. SM content was also about 90% lower in the 14- and 21-day groups while the 35-day group had 47% higher values. PC content was the maximum in adults and was lower by 47% in the 14-day group, 22% in 21-day group and 30% in the 35 day group. PI also was lowest in the 14-day group (26% of adult content) while the 21-day group had 60% of the adult levels. PS content on day 14 was 70% lower and 24% lower on day 35 than the adults. PE profile was similar to that of PC. 14-day group had about 60% more DPG than the adults while the 35-day group had about 13% lower content.

Incase of the fluidity parameters the P value on day 14 was 25% higher while day 35 had 13 % lower value than the adults (Table 8). The r value on day 14 was 25% higher while on day 21 and 35 it was 10 and 20% lower respectively. The r_{α} value was also 76% higher in the 14-day group while on day 35 it was 30% lower than the adult. The S value on day 14 were 60% higher and on day 35 20% lower than the adults.

DISCUSSION:

The constant levels attained by TPL within the liver mitochondria from day 14 till the adult stage suggests that the TPL content of mitochondria, even in the neonatal life is critical. Previous work [8] has shown that maximum levels are attained on the 1st postnatal day itself while others [4-6] reported higher values in the adults than the fetus. The CHL content was also found to change and minimum amounts were seen in the adults. The changes in the CHL content when compared with the liver SMP ATPase Arrhenius kinetics, the absence of phase transition on day 35 and adults is seen to correspond with the maximum concentration of CHL in these groups.

The changes in CHL content influence the TPL/CHL molar ratio, which plays an important role in the control of membrane fluidity. This aspect is discussed in details later (vide infra).

The two major phospholipids i.e. PC and PE both together contribute about 70 to 90% of the TPL while the remaining i.e. DPG and other minor phospholipids contribute the rest. The maximum contribution by the minor phospholipids is on day 35. The contribution by the two major PL does not vary much except in the 35-day group and even on day 14 the content is similar to the adult. Thus the requisite amount gets accumulated within the early postnatal period itself, while the minor PLs

have the least content in the 14-day group. Thus the amounts necessary for the function are attained only later, around 35 days. The reports available also confirmed the high percentage of TPL being contributed by PC and PE [1-4,7,8]. Miyahara et.al. [8] have reported that in liver PC and PE content was similar to the adults even in the first postnatal day while DPG increases from an initial low amount to the high content in adults. However, as is evident from the data presented here, although, adult levels are much higher the content on day 21 and 35 is lower.

It has been shown that the gross distribution of main phospholipids of liver mitochondria does not appear to be different in the adult and young rats. Their fatty acid composition also either only slightly changed or remained similar between birth and first postnatal week [4,6].

As can be seen from the present data lysophosphatidic acid is maximum on day 35 and PC and PE content is the least. Since lysophosphatidic acid is a degradation product of PL metabolism this increased content would mean that the membrane lipid turnover is maximum in this age group and the major contribution comes from PC and PE. In their report Pollak and Harsas [6] have checked the alterations in the PL catabolism of mitochondria during rat liver development and have observed that in periods of maximum catabolism the levels of lysophosphatidic acid increased with the concomitant decrease in PC and PE content.

In Chapter II the kinetic properties of the liver mitochondrial cytochrome oxidase (C.O.) were studied and these showed developmental changes. DPG is an absolute requirement for the efficient functioning of this enzyme and plays a catalytic role [17-19]. DPG content is known to be high in isolated cytochrome oxidase [17]. However, it is also known even after lipid depletion i.e. extraction with 2:1 (v/v) chloroform : methanol, phospholipase A treatment, acetone-ethanol extraction or treatment with Triton X-100 can not fully remove the tightly bound DPG and a certain amount is left unextracted. Such results confirm the conclusion that DPG plays an important role, both functional and probably structural in the CO complex [18]. The difficulty in extraction suggests that one or two molecules of tightly bound DPG are either covalently linked to the enzyme or are deeply buried amongst the polypeptides of the C.O. complex [17]. Hence just as adult levels of DPG are attained by day 14 it is possible that the required and absolutely essential DPG might already been sequestered by the enzyme complex in the fetal or early neonatal stage and hence the changes in the DPG content seen in the present study do not reflect a concomitant change in cytochrome oxidase activity. Also as seen in Chapter II, the enzyme of liver mitochondria attained very high activities comparable to the adults within hours after birth and hence the acquired amount of DPG might also have been accumulated by the enzyme complex in the early postnatal

period. As can be observed from the results the C.O. kinetics properties do not correlate with DPG content in the rat liver mitochondria. The reports discussed above [17-19] could explain this absence of correlation, since if already the required amount of DPG is accumulated any change in the gross DPG content would not affect the C.O. characteristics. Thus the observed results could be explained either on the basis of alterations in the subunit structure or by alterations in the fatty acid composition of the lipids.

The studies on fluidity parameters is important since maintenance of membrane fluidity is a pre-requisite for proper functioning of a cell. Lipids play an key role in determining the membrane fluidity. The concept of fluidity applied to PL bilayers refers to orientational and dynamic properties of the lipid hydrocarbon chains in the bilayer matrix [12]. Proteins in a biological membrane perturb the lipid environment and depending on their nature and concentration, influence the membrane fluidity [20]. However, the lipids are the major determinants of membrane fluidity [14]. Cholesterol plays a key role since it appears to maintain the bilayer matrix in an intermediate fluid state [21].

The membrane fluidity changes in the different age groups are paralleled by a change in the CHL content in these groups and this also leads to changes in the TPL / CHL molar ratio although there is no change in the TPL content. This ratio is high on day 21 and 35 and these also have the most rigid

membranes. An increase in fluorescence anisotropy and order parameter in biomembranes is generally reflected in a decrease in TPL : CHL ratio [15,22]. Although various PL classes and ratios are known to regulate the membrane fluidity [9,12,15,23], in the present study, none of the PL classes either alone or as ratios correlated with the changes in fluidity. Hence, it seems that it is either the change in the CHL content or the alterations in the fatty acid composition which regulates the membrane fluidity.

The lipid content of the brain is considerably higher than that of the other organs [24]. Experimental evidence in various animals and in humans demonstrates that modification of the lipid composition of brain occurs around birth and during early postnatal period [25] and the fatty acid composition of the PLs was also modified [25,26].

The studies on the brain mitochondrial composition and contents show differences from those obtained with the liver mitochondria. The TPL content gradually increased. These results substantiates the previous reports where gradual increas in the TPL content during development was noted [28-30]. Brain mitochondria are known to contain higher amounts of CHL [31] compared to the heart and liver mitochondria [32], which is known to increase during development [32,33]. In the present study a similar increase in the CHL content with age is observed.

The changes in the TPL and CHL content leads to the changes in TPL/CHL molar ratio. A low TPL/CHL ratio decreases fluidity in both biological and artificial membranes [25,34], the changes in the TPL/CHL vis a vis the membrane fluidity results in the present study, however, do not show any correlation.

PC and PE contents in all the age groups are similar to each other, as are the PS and PI contents. The PC and PE, within each age group have the maximum content and this is true for all the age groups, which is in agreement with the previous reports [1,28,29].

The changes in DPG content do not correlate with the changes in the C.O. substrate kinetics results similar to the liver enzyme.

The brain mitochondrial membrane fluidity is seen to be lowest in the young 14-day group as against the liver mitochondria, which is the most fluid. However, with age the brain mitochondrial membrane becomes more fluid. On the whole most of the lipid constituents known to regulate fluidity changes, in the individual age groups, do not show any correlation in the present studies. Only SM content and SM/PC and SM/PE ratios seem to exert some control. In addition the fatty acid composition and changes in the subunit characteristics also could regulate the kinetic properties of this enzyme.

SUMMARY

The changes in lipid content and membrane fluidity characterictics were studid in liver and brain mitchondria from rats of different age groups.

The TPL content in liver mitochondria did not show any change in any of the age groups while in brain ther was agradual increase with adults having the maximum content.

CHL content and TPL/CHL ratio also showed organ-specific and age-specific changes.

The % contribution of TPL and content of the individual PL classes also varied withage, PC and PE being the major contributors. The major PLs did not change much with age although the minor classes increased in content from the initial low amounts to reach adult values.

The fluidity parameters revealed that the liver mitochondria have amore fluid membrane as compared to the brain mitochondria, although with each tissue ther were developmental changes in the fluidity parameters.

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