CHAPTER II

DEVELOPMENTAL CHANGES IN THE ENZYMES OF RAT LIVER MITOCHONDRIAL ELECTRON TRANSPORT CHAIN

INTRODUCTION

The acquisition of fully developed mitochondria after birth is an important homeostatic mechanism that enables newborn mammals to successfully adapt to extrauterine life. During fetal life, the liver meets its energy requirement by anaerobic glycolysis, and the respiratory activity is low [1]. After birth many physiological and biochemical changes take place in a newborn animal, enabling it to adapt to its new environment. Many of the regulatory functions previously carried out by the placenta and other maternal organs need to be quickly assumed by the lungs, liver, kidneys and other organs of the neonate [2]. The proliferation of mitochondria is a continuous process during the entire period of neonatal rat liver development [3]. In contrast, maturation or differentiation of pre-existing mitochondria i.e. acquisition of ultrastructural, molecular and functional characteristics that define organelle function occurs very rapidly after birth, in the first postnatal hour and the enzymes that show a burst of activity and register much higher activities than in the fetal stage, in this period are known as the `neonatal cluster of enzymes' [1,2].

As outlined in Chapter I, some studies have been carried out to monitor the changes occuring in the hepatocyte enzymes and, to some extent, in the mitochondrial enzymes, during embryogenesis and in the perinatal period after birth. It has

been shown that during fetal development, the contribution by cellular organelles to the overall metabolism of the cell varies considerably. This may be due to the changing environment within the differentiating cell as well as to the fact that cellular organelles undergo a maturation process [4]. It has been demonstrated [5] that during the perinatal period rat liver mitochondria may undergo many significant changes in the morphology and metabolism. Investigations on the energy conservation system of fetal and neonatal rat liver mitochondria [4,5], point to the fact that the energy conservation system becomes truly operative only in the immediate postnatal period.

The postnatal studies have shown [4,5] an increase in the mitochondrial respiratory capacity and this is an important and necessary part of the adaptation [5]. Jani et.al. [6] have reported an age-dependent increase in the respiratory activities in rat liver mitochondria from 20-day-old to adult rats.

However, no detailed studies are available on the developmental pattern of the mitochondrial electron transport chain (ETC) and related enzymes over the complete range of age between postnatal to adult stage. Hence, it is of interest to study developmental pattern of liver mitochondrial enzymes and check for the changes taking place after the initial burst of activity. Studies were therefore carried out to monitor changes in the levels of: a) Primary dehydrogenases viz.

glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate - DCIP reductase (SDR), b) Mitochondrial ATPase activity under basal and Mg^{2+} and/or DNP- stimulated conditions, c) Substrate kinetics of cytochrome oxidase and Arrhenius kinetics of ATPase in sonic mitochondrial particles (SMP).

MATERIALS AND METHODS:

Chemicals:

Sodium salts of succinic acid and L-glutamic acid were purchased from British Drug Houses, UK. Sodium salt of L-ascorbic acid was purchased from Sarabhai Chemicals.

N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD), rotenone, 2,6-dichlorophenol-indophenol (DCIP), nicotinamide adenine dinucleotide (NAD^+), nicotinamide adenine dinucleotide reduced form (NADH), 2,4,-dinitrophenol (DNP) were purchased from Sigma Chemical Co. USA.

Adenosine-5'-triphosphate (ATP), bovine serum albumin (BSA), Tris(hydroxymethyl)aminomethane (tris) and sodium salt of oxaloacetic acid were purchased from SRL, India.

Sucrose (ExcelaR) was purchased from Qualigens, India.

All other chemicals were of analytical-grade, purchased locally.

For all experimental purposes double glass distilled water was used.

Animals:

Male albino rats of Charles-Foster strain of different ages i.e. 2 weeks, 3 weeks, 4 weeks, 5 weeks and adult (8-10 weeks) were used. Food and water were provided ad libitum to the mother (before weaning) or to the pups after weaning.

Isolation of the liver mitochondria:

The rats of required age groups were killed by decapitation and the livers were excised, blotted on a filter paper, weighed on a triple beam balance and transferred to a glass beaker containing chilled (0-4^{o}C) isolation medium, as quickly as possible. Depending on the age group the number of livers pooled for a single experimental set preparation varied; 4 livers were pooled in the 14- and 21-day group, 3 in the case of 28- and 35-day groups and 2 livers for the adult group. The isolation medium contained 0.25 M sucrose, 10 mM tris-HCl, 1.0 mM EDTA, all at pH 7.4 and 250 µg per ml of BSA. The excised livers were thoroughly minced and then washed repeatedly in the chilled isolation medium for removing adhering blood. These were then transferred to a Potter-Elvehjem glass-teflon homogenizer with a wall clearance of 0.18 mm and homogenized to obtain 10% (w/v) homogenates.

The isolation of rat liver mitochondria was carried out as described by Katyare and Rajan [7], with some modifications [8]. The 10% (w/v) homogenates were transferred to centrifuge cups and centrifuged at 650 g for 10 minutes in a Sorvall RC 5 centrifuge to sediment the cell debries and nuclei. The supernatant obtained (post-nuclear supernatant) had a thin layer of lipid on top, which was removed using filter paper strips. Supernatant was then transferred to another centrifuge cup which was centrifuged at 6500 g for 10 minutes to pellet the mitochondria. The supernatant was discarded and the pellet was resuspended in the isolation medium and washed once by resedimentation at 6500 g for 10 minutes. The pelleted mitochondria were resuspended in chilled isolation medium to give a final mitochondrial protein content of about 10-15 mg/ml. These once washed mitochondria were used for enzyme analysis. All operations were carried out at 0-4^{o}C.

The mitochondria obtained by the above procedure have been tested for contamination by other subcellular organelles [8] and found to be free from contaminants such as microsomes (activity of the marker enzyme glucose-6-phosphatase only 5% of that obtained with pure microsomes).

Cytochrome oxidase assay : substrates kinetics

This was done polarographically using a Clark-type oxygen electrode.

The respiration medium (1.5 ml) consisted of 225 mM sucrose, 5.0 mM potassium phosphate buffer, 10 mM tris-HCl buffer, 0.2 mM EDTA, 20 mM KCl,all at pH 7.4 and 100 µg/ml BSA [9].

To the above isolation medium ascorbate + TMPD was added as the electron donor system. The concentrations of TMPD were varied from 10 µM to 100 mM while the ascorbate concentration remained constant. In addition 1.0 µM rotenone (prepared in absolute alcohol) was also added. Depending on the concentration of TMPD 2-8mg of mitochondrial protein was added to the assay system as the source of enzyme. The respiration rates were measured and the data obtained were then analyzed by the Lineweaver - Burk and the Eadie - Hofstee plots [10]. The values for Km and Vmax obtained from the two analyses were in agreement with each other; they were pooled and the average values reported.

Assay of mitochondrial dehydrogenases :

Glutamate dehydrogenase :

An assay system of final volume 1.0 ml contained 70 mM potassium phosphate buffer, 5.0 mM glutamate (both at pH 7.8), 15 mM NAD^+ and 0.5 - 1.0 mg mitochondrial protein. After a brief pre-incubation period of about 2 minutes at 37^oC the reaction was initiated by the addition of NAD^+. The increase

in absorbance at 340 nm was recorded in a Shimadzu UV 160 A spectrophotometer [11]. The specific activity was calculated using the E/mM/cm for NAD^+ of 6.22.

Malate dehydrogenase :

The assay system in a final volume of 1.0 ml contained, 10 mM potassium phosphate buffer, 2.5 mM oxalacetate, (both at pH 7.4), Triton X-100, 1.0 %, 1.5 mM NADH and about 10 µg mitochondrial protein. Similar to GDH the assay system was pre-incubated for 2 minutes at 37°C before starting the reaction by adding NADH [12]. The decrease in absorbance was followed at 340 nm in a Shimadzu UV 160 A spectrophotometer. Calculations for specific activity were similar to those for GDH.

Succinate - DCIP reductase :

The assay system consisted of 120 mM potassium phosphate buffer, pH 7.4, 20 mM sodium succinate, 1.5 mM KCN (prepared fresh every time before use), 0.05 mM DCIP and 0.5-1.0 mg mitochondrial protein in a final volume of 1.0 ml. Following a breif pre-incubation period of 2 minutes at 37°C the reaction was initiated by adding DCIP. The decrease in absorbance at 600 nm was followed in a Shimadzu UV 160 A spectrophotometer [13]. For calculating the specific activity the E/mM/cm of 21 for DCIP was used.

Assay of mitochondrial ATPase :

Liver mitochondrial ATPase was assayed as per the method described by Katyare and Satav [14].

In an assay medium containing 50 mM tris-HCl pH 7.4, 75 mM KCl and 0.4 mM EDTA, 6.0 mM $MgCl_2$ and/or 0.1 mM DNP were added wherever indicated. After a 2 minute pre-incubation of the reaction medium at 37°C, 200 - 250 µg mitochondrial protein was added. The reaction was initiated by adding 6.0 mM ATP and allowed to continue for 10 minutes at 37°C. The final volume after adding ATP was 0.5 ml. At the end of the incubation period, the reaction was terminated by adding 0.1 ml of 5.0% SDS (w/v) [14]. Estimation of the inorganic phosphate liberated was by the method of Fiske and Subba Row [15].

Preparation of sonic mitochondrial particles (SMP) :

The mitochondria suspended in the isolation medium were subjected to sonication in a Branson sonifier. The sonication was carried out for a total period of 2 minutes in short bursts of 10 secs. followed by a 10 secs pause inbetween and this process was alternated. This precaution is necessary for preventing heat accumulation and for allowing heat dissipation [14].

The sonicate was centrifuged at 10,000g for 10 minutes in a Sorvall RC 5 centrifuge, to sediment unbroken mitochondria. The supernatant was further centrifuged at 100,000g for 1 h.

in an OTD Combi Ultracentrifuge to pellet the SMP which were then resuspended in the isolation medium to obtain a final protein concentration of about 1 mg/ml and stored frozen. All the operations were carried out at 0-4°C. The SMP were used within 1 week of preparation for the enzyme assays.

SMP ATPase asays : Arrhenius kinetics :

The reaction mixture for this assay contained 50 mM tris-HCl,pH 7.4, 75 mM KCl, 0.4 mM EDTA and 6.0 mM $MgCl_2$. 50 µg of SMP protein was pre-incubated in this assay medium for 2 minutes and the reaction was initiated by adding 6.0 mM ATP, this made up the final volume to 0.5 ml. For the pre-incubation and the actual assay the temperature was varied between 5 - 53°C with a difference of 4°C between the consecutive temperature points. The time of incubation depended on the temperature of assay; longer periods for lower temperatures and shorter periods for higher temperatures.

The liberated inorganic phosphate was estimated by the method of Fiske and Subba Row [15].

The value of energy of activation in the high (E_1) and low (E_2) temperature range and the phase transition temperature (T_t°C) were determined from the Arrhenius plots [16].

Protein estimation :

This was essentially according to the method of Lowry et.al. [17] with some modifications as described in Kumthekar and Katyare [18].

The standard stock was 100 µg/ml BSA containing a few drops of 0.1 N NaOH.

The other reagents used were :

a). Reagent A : 5 times concentrated stock solution containing 20 gm.anhydrous Na_2CO_3, 4.0 gm. NaOH and 200 mg Na K tartarate dissolved in 200 ml. this was diluted 1:5 before use.

b). Reagent B : 0.5 gm $CuSO_4,7H_2O$ in 100 ml.

c). Reagent C : Reagent A (1:5 diluted) 50 ml + 1 ml Reagent B, freshly prepared.

d). Folin - Ciocalteau reagent (FC reagent): This was prepared fresh before use by diluting the stock 1:1.

For the standard plot, BSA in a range between 4 - 40 µg was taken in a final volume of 0.5 ml. To this 2.5 ml Reagent C was added and this was vigorously vortexed and incubated at room temperature for 20 minutes. Next, 0.25 ml FC reagent (diluted 1:1) was added. the mixture was once again vigorously vortexed and incubated for 30 minutes. After this the OD

readings were taken in an ERMA colorimeter using a 660 nm filter. A standard graph of OD versus BSA concentration was plotted.

To estimate the unknown protein, aliquots from the samples were diluted and suitable aliquots were taken from these diluted samples. The final volume was made up to 0.5 ml and the protocol described above was followed and from the standard graph the protein content was calculated.

Inorganic phosphate estimation :

This was essentially according to the method described by Fiske and Subba Row [15].

The reagents used were :

a). Inorganic phosphorus standard (80 µg/ml): 35.1 mg of KH_2PO_4 dissolved in 100 ml of distilled water. The working standard was obtained by diluting this 1:10.

b). Molybdate II reagent : 25 gm of ammonium molybdate was disssolved in 200 ml and to this 300 ml 10 N H_2SO_4 was added. the final volume was made up to 1000 ml.

c). 1-Amino-2-naphthol-4-sulfonic acid (ANSA) : A triturate of 0.2 gm ANSA, 1.2 gm Na-sulfate and 1.2 gm Na-bisulfite was prepared and stored in amber bottle. The ANSA solution is prepared fresh before use by dissolving 40 mg ANSA/ml D/W.

For the standard graph the working standard (8µg/ml) was used to obtain a range between 1 - 16 µg of Pi in a final volume of 3.5 ml. To this 0.4 ml molybdate II reagent followed by 0.1 ml ANSA was added and readings were taken between 8 - 12 minutes of adding ANSA reagent in an ERMA colorimeter using a 660 nm filter. A standard graph of OD versus Pi concentration was plotted.

For the assay of liberated Pi from ATPase assays after terminating the reaction the final volume was made up to 3.5 ml and then a protocol similar to the one described above was followed. The OD values were used to calculate the Pi content from the standard graphs.

RESULTS:

The results of the developmental profile for the primary dehydrogenases (Table 1) shows the activity to be gradually increasing with the age. The 14-day-group has the lowest activity for all the dehydrogenases. However, the extent of increase from the 14-day to the adults varied for the individual enzyme.

GDH activity in the 14-day-group was only 25% of the activity in the adult group. The activities in 21- and 35-day- groups were about 43% and 53% of the adults. The MDH activities in the 14- and 21-day-groups were almost the same,

Table 1

Developmental changes in rat liver mitochondrial dehydrogenases.

Age	Glutamate dehydrogenase	Malate dehydrogenase	Succinate - DCIP reductase
14 Days	21.4 ± 1.41[a]	7249.5 ± 183.5[a]	10.1 ± 0.46[a]
21 Days	46.2 ± 2.03[a]	7682.6 ± 638.4[a]	29.4 ± 1.89[a]
35 Days	36.6 ± 5.48[a]	9311.6 ± 181.5[a]	36.8 ± 3.78[a]
Adult	86.1 ± 3.95	15002.1 ± 686.2	68.2 ± 5.81

Results are expressed as mean ± S.E.M of 6 independent observations for individual groups

Activity - nmoles/ min / mg mitochondrial protein

[a]$P < 0.005$ compared to the adults.

Table 2

Developmental changes in rat liver mitochondrial ATPase activity.

Age	ATPase activity			
	Basal	+ Mg^{2+}	+ DNP	+ Mg^{2+} + DNP
14 Days	4.6 ± 0.22[b]	5.8 ± 0.31[b]	6.0 ± 0.28	7.8 ± 0.53[b]
21 Days	10.2 ± 0.71[a]	25.7 ± 1.05	33.1 ± 1.40[b]	43.8 ± 2.51[b]
35 Days	6.7 ± 0.22[b]	14.3 ± 0.87[b]	30.5 ± 2.01[b]	28.0 ± 1.92[b]
Adult	14.1 ± 0.93	23.7 ± 1.30	62.4 ± 4.64	69.9 ± 3.44

Results are expressed as mean ± S.E.M of 6 independent observations in individual group.

ATPase activity - μmoles of Pi liberated/h/mg mitochondrial protein

[a] $P < 0.01$, [b] $P < 0.001$ compared to the adults.

Table 3

Developmental changes in substrate kinetics parameters of rat liver mitochondrial cytochrome oxidase.

Age	Km	Vmax
14 Days	0.56 ± 0.14	39.3 ± 8.36[c]
21 Days	0.39 ± 0.05[a]	117.0 ± 3.24[c]
28 Days	0.85 ± 0.02[c]	510.0 ± 35.30[c]
35 Days	0.53 ± 0.05	392.0 ± 26.56[b]
Adult	0.53 ± 0.02	217.3 ± 10.17[c]

Results are expressed as mean ± S.E.M. of 6 independent observations in individual group.

Km - mM

Vmax - μmoles of Pi liberated /min/mg mitochondrial protein

[a]$P < 0.05$; [b]$P < 0.005$; [c]$P < 0.001$ compared to the adults.

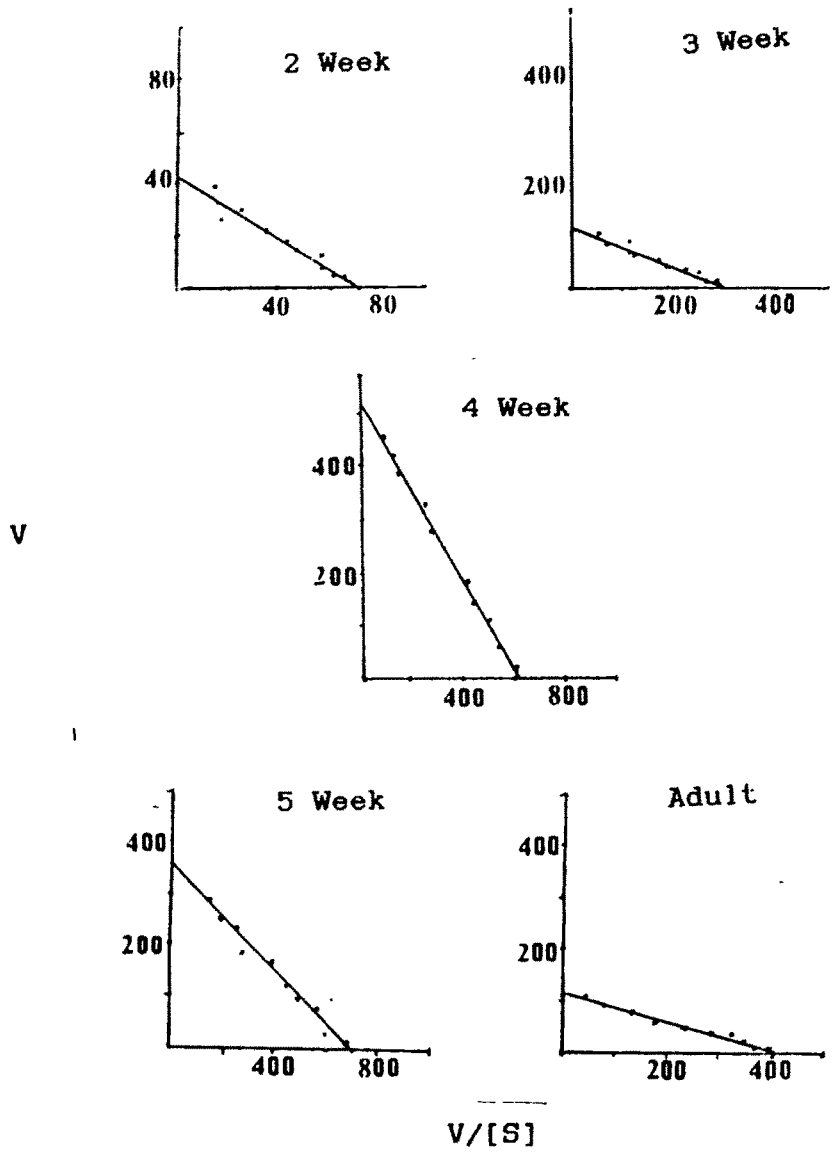

Figure 1

Typical Eadie - Hofstee plots depicting changes in rat liver mitochondrial cytochrome oxidase. Substrate kinetics.

Table 4

Developmental changes in the Arrhenius kinetics parameters of rat liver SMP ATPase.

Age	Phase transition temperature (Tt), ^{o}C	Energy of activation, (KJ/mole)	
		E_1	E_2
14 Days	------ [d]	-----[d]	37.9 ± 3.16
21 Days	19.2 ± 0.47	30.5 ± 0.85[b]	65.6 ± 1.38[c]
35 Days	------ [d]	-----[d]	28.6 ± 1.85[a]
Adult	23.7 ± 3.57	18.0 ± 3.35	33.7 ± 5.86

Results are expressed as mean ± S.E.M of 6 independent observatins in individual group.

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

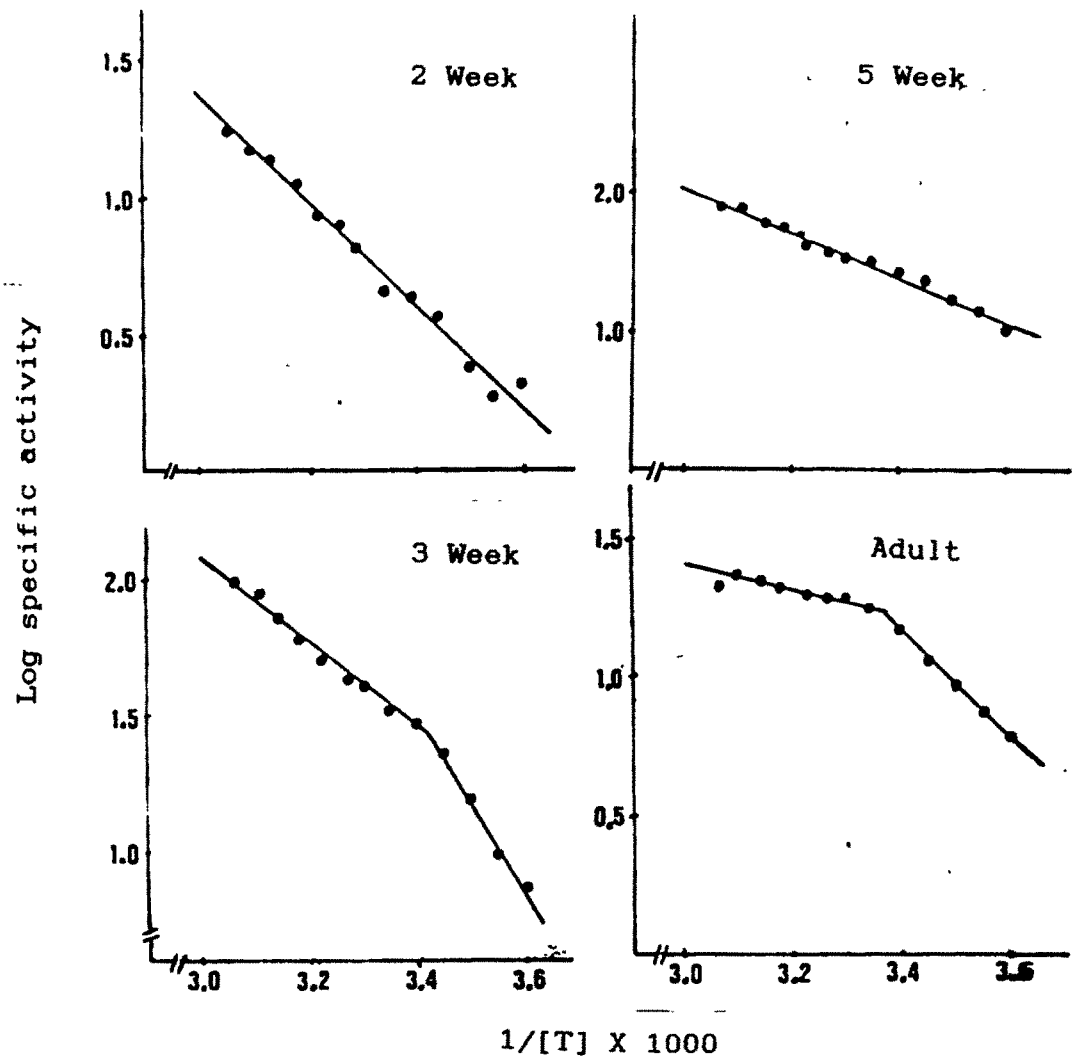

Figure 2

Rat liver SMP ATPase. Typical plots depicting developmental changes in Arrhenius kinetics

amounting to about half the adult value, while in the 35-day-group activity increased to 62% of the adult value. SDR activity was 65% and 57% lower in the 14- and 21-day-groups, in the 35-day-group it was about half the adult value.

The ATPase activities under basal and Mg^{2+} and/or DNP-stimulated conditions are shown in Table 2. Basal activity was the least in the 14-day-group, increased substantially on the 21st day and then decreased in the 35-day-group by 54%. The Mg^{2+} ATPase also displayed more-or-less similar pattern. The DNP-stimulated ATPase activity was only about 10% of the adults in the 14-day-group, while in the 21- and 35-day groups it was about half the activity in adult. The pattern of Mg^{2+} + DNP-ATPase paralleled that of basal and Mg^{2+} ATPase.

Figure 1 shows typical Eadie - Hofstee plots for cytochrome oxidase substrate kinetics in the five age groups. Measurement of Km and Vmax of cytochrome oxidase revealed that Km values decreased on day 21 but increased on day 28, while in other age groups the values were similar to the adults (Table 3). For the Vmax, the highest values were noted on day 28 which was about 2.5 fold higher compared to the adults and the lowest values were on day 14; about 82% lower than the adults.

Figure 2 represents the typical Arrhenius plots of the liver SMP ATPase for the different age groups. From the Arrhenius kinetics plots, the phase transition temperature and

energy of activation, below (E_1) and above (E_2) the phase transition temperature were obtained (Table 4). The 14- and 35-day groups were strikingly different from the adults due to the absence of phase transition. Besides, the values of E_2 also changed. In the 21-day group the E_1 was about 70% higher and E_2 about double the adult values. The phase transition temperature increased by $4.5^{o}C$.

DISCUSSION:

From the data presented, it is clear that the activities of the primary dehydrogenases, i.e. GDH, MDH and SDR followed specific developmental pattern. Previous work by Hallman [4] on fetal rat liver mitochondria has shown that between the 19th gestational day and 8th postnatal day there is some increase in the GDH activity. Subramaniam and Katyare [19] found higher GDH activity in mouse liver mitochondria at weaning compared to the adults. Similarly, in the present studies the activity decreased after weaning i.e. around 21-days of age.

MDH, on the other hand, showed a gradual increase in activity from the second week onwards up to the adult stage. Hallman [4] has also shown a similar increase between the 8th postnatal day and the adults. Though, the increase was not to the same extent as obtained by us in the present studies.

SDR, a membrane-bound dehydrogenase also gradually increased in activity with age. The fetal SDH activity is reported to be very low which was found not to alter appreciably during gestation [20,21]. Hallman [4] had reported a gradual increase after 19th gestational day to the 8th postnatal day and adult stage. While there seemed to be no change in the activity of this enzyme in suckling, weaning and adult mice [19]. The succinate-cytochrome-c-reductase, an enzyme spanning the same expanse as SDR in the ETC also showed low activity in the 2-to 4-day prenatal rat liver [21]. Valcarce et.al. [1] have shown this enzyme to gradually increase in activity after birth, though the maximum increase was immediately after birth.

The glutamate stimulated respiration did not show any increase with age as reported by Jani et.al. [6]. Thus, the glutamate-dependent respiration does not match with the dehydrogenase activity. However, Roosevelt et.al. [22] have shown that the dehydrogenases are never limiting in their activity and always have much higher activity than the other ETC enzymes. Hence, although the respiration is low, glutamate dehydrogenase activity could still remain high so as to ensure constant supply of electrons into the ETC.

Mitochondrial ATPase activity, similar to GDH was seen to decrease after the weaning stage. A major change taking place at this stage is a shift from a liquid milk diet to solid food and this shift seems to exert some change in the liver

metabolism. Because of this the mitochondria seem to function at a lower rate as compared to the pre-weaning rats as well as the post-weaning and adult rats. However, the exact mechanism remains unclear.

Mg^{2+} and/or DNP - stimulated ATPase activities in the fetal liver mitochondria were found to be low while in the adult the activity and stimulation both were maximum [4]. Mackler et.al. [20,21] have reported that ATPase activity remains constant in fetuses from 10th to 14th gestational day. On the other hand, studies on mice [19] showed results similar to ours.

The increased ATPase activity could mean an in situ increase in the activity of the enzyme as ATP synthase. This increase could be necessary to ensure supply of ATP to the liver cells for carrying out the required metabolic functions. During gestation the functioning of the oxidative energy metabolism is very low and hence the ATP synthase function is also low. In addition the glycolytic system provides the major part of the energy requirements in the fetus and various workers have shown the adenine nucleotide pools to have lower levels in the fetal and neonatal livers [3,23,24]. Thus the lower activity of ATPase in the younger rats is in keeping with the trend of the other ETC enzymes. Valcarce et. al. [1] have shown that the content and the activity of F_1 ATPase increased within hours after birth and by 2 hours the activity was 30% higher and the content had increased 2 fold.

The alterations in mitochondrial ATPase could be due to the concomitant changes in the content and the concentration of various phospholipids (PL) classes in the mitochondrial membrane. Within the PL the fatty acid composition varies with the age [25] and since interactions between the PL polar head groups and F_1 ATPase are known, specific electrostatic or net ionic charge on the PL molecules could lead to activation of reconstituted mitochondrial ATPase [25]. The activity of ATPase has been shown to increase several fold by the addition of PL, especially acidic PL. This superior stimulatory activity by acidic PL might indicate that negatively charged ionic environment facilitates the interaction between the ATPase and the PL. The acidic PL may elicit higher activities from the ATPase by influencing its catalytic capacity. Thus the activity can also be controlled by changing the PL environment. As can be noted (Chapter IV) the PLs too show a specific developmental pattern. However, none of the phospholipids either alone or in combination seem to control the ATPase activity and the changes occuring in PL content and ATPase activity do not correlate with each other. Thus the fatty acids in the PL could also be a controlling factor.

Cytochrome oxidase is the terminal enzyme of mitochondrial respiratory chain, responsible for virtually all oxygen consumption in mammals [26].

The changes in the K_m and V_{max} seen in the present study show that 35-day age group has the maximum Km, i.e. the lowest

affinity and the highest Vmax. In the other age groups the two parameters seem to vary independent of each other.

As with ATPase, cytochrome oxidase also is influenced by the lipid environment of the membrane. Different workers [27-30] have shown that this enzyme has an absolute requirement for cardiolipin (DPG) and this enzyme has the maximum amount of DPG than any other PL tightly bound to it [31]. Depletion or variation in concentration of DPG has been shown to alter the enzyme activity [27]. Thus any alteration in the concentration of DPG with age could modify the enzyme activity. As can be seen from the data in Chapter IV, the PL of the mitochondria do follow a developmental pattern. However, these changes do not seem to have any direct effect on the Km and Vmax profile.

Cytochrome oxidase (C.O.) has been found to have a variable composition of polypeptides depending on the evolutionary stage of the organism studied, the bacteria have 3, while the mammalian species have 13 subunits [31-33]. Of these the first three are coded by mitochondrial DNA and the others by nuclear DNA [29,33]. The first three subunits have a catalytic function, while the latter do not have any specific catalytic function attributed to them. These nuclear DNA coded peptides have tissue-specific differences in size and N - terminal sequences [33,34], as well as kinetic and structural properties [29]. Mitochondrial myopathy patients

also show alterations and deficiency in C.O. activity in different tissues [36]. Such changes in the polypeptides synthesized in the cytoplasm could also occur during development [33]. Although no catalytic function is governed by these subunits, they are important since they are thought to play an important role in regulating the enzyme activity. Hence such variations in the subunits during development could also affect the enzyme characteristics. In addition to this there are several other factors which are known to effect the enzyme characterstics eg. ATP, which induces conformational change by affecting the cytochrome c binding site [36], in vitro alterations in nucleotide concentrations using liposomes [37], and peroxidation of mono-unsaturated PC and PE [38].

The Arrhenius kinetics of the SMP ATPase was followed to study the phase transition temperature T_t and the energies of activation above (E_1) and below (E_2) the transition temperature in the different age groups. The T_t gives an idea about the membrane characteristics and fluidity of the membrane while the energies of activation show the amount of energy required to carry out the activation and reaction in the high and low temperature ranges.

The absence of phase transition in the 14-and 35-day groups led to absence of E_1in both the age groups.

It has been shown that the existence of a phase transition point is due, not to any effect on the protein moiety, but, to

the changes in the physical properties of the membrane lipids surrounding and interacting with the enzyme [32]. Nemat-Gorgani and Meissami [33] have found absence of phase transition in the young, 5-day-old, rat brain Na^+-K^+-ATPase Arrhenius kinetics.

The adult has the least energies of activation which may aid in more efficient functioning of the enzyme.

Thus on the whole, activity of all the enzymes is seen to increase with age and the functioning becomes more effecient. However,as is seen, [1], the liver enzyme activities increase tremendously within one hour after birth. Hence,this increase observed in our present studies, rather than a maturation phenomena, could be a means of aiding the liver to fulfill its metabolic functions more effeciently.

SUMMARY

The developmental changes in the enzymes of liver mitochondrial ETC in rats of various age groups i.e. from 2 weeks to adult rats were examined.

The activity of the primary dehydrogenases was the lowest in the 14- day group and continued to increase till the adult stage. However, the GDH activity decreased slightly between 21- and 35- days.

The ATPase under all conditions, i.e. basal as well as Mg^{2+} and/or DNP stimulated, was lowest in the 14- day group while in the adults, the activity was highest with greatest stimulation by Mg^{2+}/DNP.

Kinetic characteristics of the cytochrome oxidase, i.e. the Km and Vmax revealed that these values were highest in the 28-day group. The adults had the lowest Km and an intermediate Vmax.

The SMP ATPase Arrhenius kinetics showed absence of a phase transition in the 14- and 35- day old rats. In the adults, E_1 and E_2 were the lowest. In the 28- day group, the T_t decreased by 4.5°C compared to the adults.

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