SYNOPSIS

The process of development which leads to the emergence of a complete organism is in itself a fascinating subject. This is a many faceted process where different systems work in conjunction. The developmental alterations are regulated by various factors such as enzyme levels, hormone concentrations and energy potential etc. The changes in characteristics and concentrations of the hormones and the enzymes during development further complicate the situation.

Since energy is an important requirement for development, the proper functioning of the mitochondria during development, is extremely important. Hence the changes occuring in the mitochondrial functions during development need to be studied. The pattern of development in different organs is different and the same applies for the development of mitochondrial functions.

In view of this, it was decided to study the developmental pattern of mitochondrial functions from two organs known to differ with respect to the stage of life at which they become fully mature i.e. liver and brain. In the rats the liver attains maturity within the first few hours after birth while in the brain the major development starts only after birth and continues during the first few weeks during which the level of arborization and dendritic network formation increases.

Male albino rats of the Charles-Foster strain of different age groups i.e. 14-day-old, 21-day-old, 28-day-old, 35-day-old and adults were used for the present studies. The mitochondrial functions studied were : a) the activities of the primary dehydrogenases, i.e. glutamate dehydrogenase (GDH), malate dehydrogenase (MDH) and succinate-DCIP-reductase (SDR), which serve as the entry ports for the electrons into the electron transport chain, b) the activities of basal, Mg^{2+}, DNP and DNP + Mg^{2+}- stimulated ATPase - an enzyme which in situ functions towards the synthesis of ATP, c) the substrate kinetics of cytochrome oxidase using N,N,N',N' tetramethyl-p-phenylene diamine (TMPD) as the artificial electron donor in the ascorbate + TMPD system and d) the lipid content and profiles of the different phospholipid classes, and the membrane fluidity measurements. In the brain, additionally, the developmental profile of mitochondrial oxidative phosphorylation was traced (using different substrates i.e. glutamate, pyruvate + malate, succinate and ascorbate + TMPD), since this would reflect upon the energy required by the brain during it's postnatal development.

The developmental profile of liver mitochondrial functions was as follows :

GDH activity was the lowest on day 14 and gradually increased to reach the adult values which were about 4 fold higher. MDH was highest on day 35 but in the adults the values

were comparable to those in the 14-day-old pups. SDR activity gradually increased till the adult age where it was about 5 fold higher than on the 14th day.

In the adults the basal ATPase activity was about 3 fold higher compared to the 14-day group. However on day 35 the values were about half of the adults. The pattern for Mg^{2+}-ATPase was similar though the extent of variation in the 35-day-old and adult group was larger. The DNP-stimulated activity increased gradually with the adults showing 9 times higher activity. The increase in DNP+Mg^{2+}-stimulated activity followed a similar pattern.

For cytochrome oxidase the K_m (TMPD) was fairly constant except for the 28-day-old group where it was about 50% higher. The V_{max} increased from second week and reached maximum levels in 28-day group before decreasing once again and the adult value was less than half of the 28-day value.

The Arrhenius kinetics of the rat liver sonic mitochondrial particle (SMP) ATPase revealed absence of phase transition on day 14 and 35; only the energy of activation in the higher temperature range (E_2) could be discerned. The phase transition temperature (T_t °C) in the other two age groups differed by 4.5°C and was higher in the adults. In case of the E_2 values the highest was on day 21 while in all the other age groups the values were comparable.

The total phospholipid (TPL) content did not vary with age, while the cholesterol (CHL) content increased till the 35^{th} day but decreased in the adults to half of this value.

The phospholipids (PL) were separated into the individual classes i.e. lysophosphatidic acid (Lyso), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG). The individual contribution by each of these as % of TPL at different ages was as follows :

Lysophosphatidic acid was highest on day 35 but decreased in the adults to about 20 %. SM also was highest on 35^{th} day and yhis too decreased in the adults. PC, a major phospholipid, remained almost constant in all the groups. PI increased till day 35 but decreased in the adults. PS decreased on day 21, but increased 10 fold on 35^{th} day before decreaseing to half in the adults. Content of PE-another major PL- was highest on the 14^{th} day and decreased by about 40% on day 35 but once again increased in the adults. DPG decreased till day 35 but once again increased in adults with values higher than those on the 14^{th} day.

The membrane fluidity measured using diphenylhexatriene (DPH) as the probe was highest on day 35 while the adult values were similar to the 14-day-old group.

Parallel studies were carried out in the brain, with the additional measurement of mitochondrial oxidative phosphorylation.

With glutamate as the substrate the state 3 respiration rates increased gradually with age; adult rates being about 5 fold higher compared to the 14 day value. The ADP-phosphorylation rates mirrored this pattern. The profile with the other substrates i.e. pyruvate + malate and succinate and ascorbate + TMPD as electron donor system resembled closely with that seen with glutamate and the final net increase in the respiration rates in the adults was about 5-7 fold.

The GDH activity increased tremendously (25 fold) in the adults as compared to the 14 day group. MDH activity was maximum on the 14^{th} day itself and decreased marginally by 20% in the adults. SDR activity increased gradually with the highest values being seen in the adults (5 fold increase) although a slight decrease was seen in the 35-day-old rats.

The basal ATPase was fairly constant till 35^{th} day but in the adults this activity decreased by 30 %. The Mg^{2+} - ATPase activity was highest on day 14, but decreased by day 21 but again increased slightly till the adult stage, though the values were still 30 % lower. DNP and DNP + Mg^{2+} -stimulated ATPase activities were lowest on day 14 but increased on day 21. This value was similar to adult values although on day 35 the activity decreased slightly.

For cytochrome oxidase, the Km did not change in any age group except on day 28 whereas the Vmax increased till day 28 after which it again gradually decreased till the adult stage.

Brain SMP ATPase substrate kinetics studies (with ATP) revealed presence of two sites: a high affinity and a low affinity site. The Km of high affinity site was the least on day 35 while the low affinity site Km progressively decreased with age. The Vmax in both the groups had a similar pattern : lowest value on day 35 and highest in the adults.

From the Arrhenius kinetics of brain SMP ATPase it is evident that on day 35 and in adults the phase transition was absent while in the other groups it did not change much. E_1 on day 21 was only 31 % of adult while the E_2 value of the 14-day- group increased by 20 KJ till day 21.

The content of lysophosphatidic acid was lowest on day 14 and highest on day 35, the values in adults were 30% lower than on day 35. SM showed a similar pattern. PC, a major PL, decreased from the 14 day levels to the lowest values on day 35, however the levels in the adults were slightly higher. PI was lowest on day 14 but doubled on day 21; this value decreased in the adults. PS levels were highest on day 35 but once again decreased in the adults (-25%). In adults PC and PE levels were almost comparable which is characteristic of the brain mitochondria; this pattern was seen for all the age groups except in the 21-day-group where the PE levels were the

lowest and much lesser than the PC content. DPG was highest on day 14 but decreased on day 21 which remained constant at this level till the adult stage.

The P values for membrane fluidity measurement were maximum on day 21 and minimum on day 35.

The studies thus delineated the developmental profiles of liver and brain mitochondrial functions.

Extending the above studies we examined the effect of Cd toxicity on mitochondrial functions after exposure at different ages i.e. in young and adult rats. Due to the increased use of metal based materials, electronic instruments and rechargable batteries allof which contian Cd, the possibility of an individual getting exposed to Cd from the environment has increased. While clinical and histochemical studies on Cd exposure are carried out, not much work has been done to examine the biochemical effects of Cd on the tissue/subcellular functions. Hence such investigations are warranted. Cd is known to get maximally accumulated in the liver, whereas brain is known to be the most protected. Except for some work on metallothionein induction, chelation therapy for the alleviation of the Cd toxicity and on the enzymes such as the aminotransferases not many biochemical studies are reported. It has been seen that Cd toxicity results in complete loss of mitochondrial membrane integrity and

structure. Some in vitro studies have shown that Cd inhibits oxidative phosphorylation. Hence, it is of interest to check for the effects of Cd on the mitochondrial functions. Cd (as Cd acetate) was injected intraperitoneally at a dose of 0.84 mg/Kg body wt. to the rats. For studies on liver the animals were killed 48-hours later (48 hr group). In order to check for the effectof Cd-insult after a longer period of time has elapsed, another group of animals was injected and killed one month later (1 month group).

As is clear from the foregoing, there is a developmental pattern to the mitochondrial functions which is tissue specific, and since exposure to Cd can occur at any age,its effect was checked in young and adults rats. The tissues studied were liver and brain since these two represent the most susceptible and the most protected organs respectively. For studies on liver 21-day-old and adult rats were given the single i.p. Cd injection and then killed after either 48 hours or 1 month. In case of brain, the rat pups were injected on the 7th day after birth to ensure entry of Cd into the brain, since the blood-brain-barrier closes between the 8th to the 16th day. The experimental groups for studying the effect of Cd on brain were different from liver. The young Cd-treated rats were killed either 2 weeks or 2 months later. While in case of the adults the Cd injected and control rats were killed either 48 h., 2 weeks or 2 months after injection.

The parameters studied were oxidative phosphorylation primary dehydrogenases, ATPase activities and the -SH content.

In the liver of 21 day old rats, mitochondrial oxidative phosphorylation employing glutamate as the substrate showed an increase in the state 3 respiration rate 48 h. after Cd treatment. ADP/O ratio remained unchanged whereas the ADP-phosphorylation rates mirrored the increase in the state 3 rates. With the other two substrates i.e. succinate and ascorbate + TMPD results similar to those obtained with glutamate were seen.

The GDH and MDH activities, both were unaffected in the 48 h. group, whereas SDR activities increased. The basal, Mg^{2+}, DNP- and DNP + Mg^{2+} - ATPase activities increased in the 48 h. group. The free and total -SH contents were comparable to the controls.

At the end of the 1 month period, the state 3 respiration rates with all the substrates decreased significantly. GDH and MDH activities increased but SDR activity decreased. The ATPase activities were generally lower in spite of the fact that the -SH content returned to the control levels.

In case of the adults, the state 3 respiration rate decreased significantly with all the substrates in both the experimental groups (48 h. and 1 month) although the decrease was much more pronounced in the 1 month group.

The dehydrogenase activities were inhibited in the 48 h. group and decreased further in the 1 month group. The ATPase activities were also inhibited in the 48 h. group and no improvement was seen in the 1 month group. The free -SH content was undetectable in the control and the two experimental groups. However the total SH content was significantly higher than the 1 month group compared to the controls.

Similar studies were also carried out on the brain mitochondria to study the effects of Cd exposure.

All the oxidative parameters were relatively unaffected in the 2 week group while in comparison, the 2 month group had suffered much more damage to the activity. In case of the adults all groups including the 48 h. group showed impaired activity.

GDH activity was unaffected in the 48 h. and 2 month groups while only the 2 week group in both young and adults showed inhibition. SDR activity was in fact stimulated by Cd in all groups except the adult 2 month group.

ATPase activity on the whole showed inhibition although the levels varied depending on the stimulation condition and age group studied.

The soluble -SH groups were undetectable in all the groups, while in the total -SH content was maximum in the 2

week group and minimum in the 2 month group, in both the young and the adult rats.

The adverse effects observed in the 2 month group could give an insight into the late onset and manifestation of the neuropathological effects of Cd.

Thus the overall results imply that effects of Cd-exposure are age-dependent and tissue-specific and can lead to impaired energy metabolism even in a well protected tissue such as brain.