CHAPTER 6

STUDIES ON DEVELOPING AVIAN LIVER 6. ACTIVITIES OF SUCCINATE DEHYDROGENASE, ADENOSINE TRIPHOSPHATASE AND LACTATE DEHYDROGENASE IN THE LIVER OF PIGEON DURING POST-HATCHING DEVELOPMENT

The development of pigeon liver during post-hatching period could be arbitrarily divided into two phases; (1) a period of rapid growth (first 10 days after hatching) and (2) a period (next 10 days) of rapid acquisition of metabolic machinery, complementary not only to food but also to the need of body (Chapter 1). During the first phase, the food (crop milk) provided to the nestlings is very much akin to that available in in ovo, consisting more of protein and fat. Hence, the metabolic machinery need not undergo drastic changes from what was already established in in ovo phase of development. During the second phase, the food (grains) provided to the young ones is almost similar to the food of adult and hence the metabolic machinery also undergoes rapid changes so as to confer metabolic maturity

to the liver. During this period, due to heavy influx of carbohydrate rich food and also due to hormonal interplay, an increased glucose uptake and lipogenesis manifest in the pigeon liver (Chapter 1). The activity pattern of non-specific acid and alkaline phosphatases changes over to the adult pattern (Chapter 5) and the lipogenic enzymes such as 'malic' enzyme and G-6-PDH also show peak activity levels during this second phase of post-hatching development (Chapter 4). Along with this functional maturity, structural maturity in terms of increased cholinergic plexus (Chapter 2) and connective tissue (Chapter 3) also develops during the second period (between 10th and 20th day).

Since the liver metabolism has to show varying degrees of adjustment to meet different priority activities, the energy utilization and energy generating pathways also may show variations. High or low levels of operations of aerobic or anaerobic glycolysis, Krebs cycle and the ATP utilization could be deduced from the activities of enzymes such as LDH, SDH and ATPase. The degree of release of lactate into the blood could also be judged from the LDH level in the serum. A quantitative survey of succinate dehydrogenase and adenosine triphosphatase in the liver and lactate dehydrogenase in the liver and serum was undertaken with a view to understand the patterns of oxidative phosphorylation and anaerobic glycolytic

metabolism in the liver of pigeon during post-hatching development.

MATERIALS AND METHODS

The young ones of 1, 5, 10, 15, 20, 25 and 30 days as well as adult pigeons were collected from an open aviary maintained by the department and were sacrificed by decapitation immediately after collecting blood from jugular vein. A piece of liver was quickly excised from the bird, homogenized in chilled glass distilled water and the homogenate was used for enzyme and protein estimations. Blood was centrifuged and serum was collected for the purpose of LDH and protein estimations.

ENZYME ASSAYS

SUCCINATE DEHYDROGENASE (SDH):

Activity of succinate dehydrogenase (E.C. 1.3.99.1) was estimated according to method of Kun and Abood (1949), using triphenyl tetrazolium chloride (TTC) as a hydrogen acceptor. The optical density of colour developed was read at 420 mμ on a Klett-Summerson photoelectric colorimeter. Protein content was estimated by the Biuret method according to the procedure described by Layne (1957). The enzyme activity is expressed as μg formazan formed/mg protein/30 minutes.

ADENOSINE TRIPHOSPHATASE (ATPASE):

Activity of Mg^{++}-ATPase (E.C. 3.6.1.4) was assayed according to the method described by Umbreit et al. (1957). Disodium salt of Adenosine-5-triphosphate (from equine muscle, Sigma chemical Co., U.S.A.) was used as a substrate. Inorganic phosphorus (Pi) released was estimated by the method of Fiske and SubbaRao (1925). The optical density of colour was read at 660 mμ on Klett-Summerson photoelectric colorimeter. The protein content of homogenate was estimated by the Biuret method and the activity is expressed as μg phosphorus released/mg protein/10 minutes.

LACTATE DEHYDROGENASE (LDH):

Activity of lactate dehydrogenase (E.C. 1.1.1.27) was assayed by colorimetric method of King as described by Varley (1975) and optical density of colour developed was read at 440 mμ on a Bausch & Lomb spectronic-20 colorimeter while protein was estimated by method of Layne (1957). The activity is expressed as μ Moles lactate oxidised/mg protein/15 minutes.

RESULTS

The data on the levels of activities of SDH and ATPase in the liver and LDH in the liver and serum of developing pigeon during post-hatching period is given in Table 1 and Fig.1.

CHAPTER 6

EXPLANATION FOR FIGURE

Fig. 1. Graph showing quantitative analyses of activities of succinate dehydrogenase and Mg^{++}-adenosine triphosphatase in the liver and lactate dehydrogenase in liver and serum of pigeon during post-hatching development.

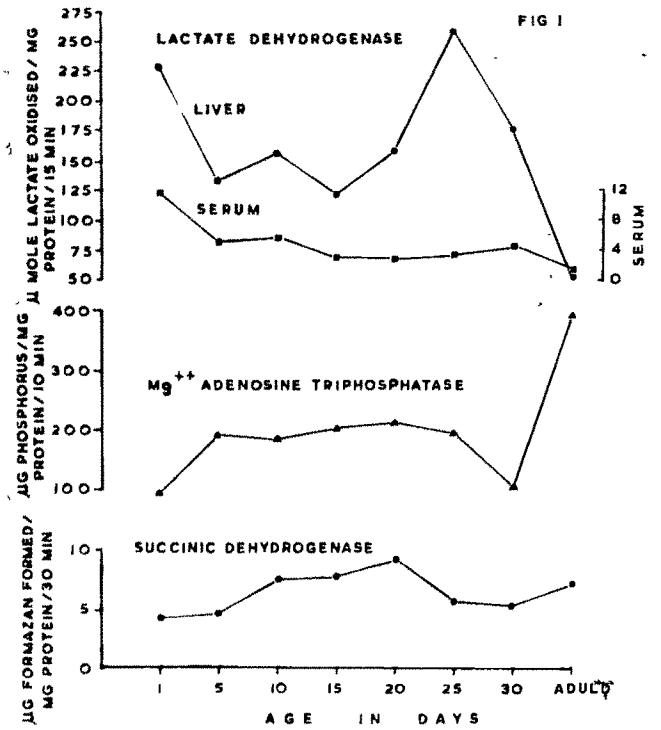
FIG I
LACTATE DEHYDROGENASE
LIVER
SERUM
µ MOLE LACTATE OXIDISED / MG PROTEIN / 15 MIN
275
250
225
200
175
150
125
100
75
50
12
8
4
0
SERUM
µG PHOSPHORUS / MG PROTEIN / 10 MIN
400
300
200
100
Mg++ ADENOSINE TRIPHOSPHATASE
µG FORMAZAN FORMED / MG PROTEIN / 30 MIN
10
5
0
SUCCINIC DEHYDROGENASE
1
5
10
15
20
25
30
ADULT
AGE IN DAYS

SUCCINATE DEHYDROGENASE: The activity of SDH in the liver was lowest on 1st day. It increased significantly on 10th day after hatching and was maintained so even on 15th day. However, its activity reached a peak level on 20th day (9.277 units) and then it declined during 25 and 30 days. The liver of adult pigeon showed a high value of SDH but was lower than that was at 20th day.

ADENOSINE TRIPHOSPHATASE: Activity of ATPase was at the lowest level on the 1st day (97.76 units), thereafter it sharply increased on 5th day to 190.90 units/mg protein. Tenth day onwards it remained at a more or less the same level upto 25th day with a small peaked activity at 20th day (210.56 units). Then it showed a decline on 30th day, however, the liver of adult pigeon registered a very high value (389.00 units).

LACTATE DEHYDROGENASE: Activity of lactate dehydrogenase showed a different pattern during development. Its activity was remarkably high (227.10 units) in the liver of one day pigeon. On the 5th day, however, the level decreased and thereafter the level registered a small peak on the 10th day and a high peak on the 25th day (261.86 units). The liver of adult pigeon showed the lowest (53.25 units) activity.

TABLE 1

Activities of succinate dehydrogenase and adenosine triphosphatase in liver and lactate dehydrogenase in liver and serum of pigeon during post-hatching development. Mean ± S.D.

Age in days	LIVER			SERUM
	SDH[1]	Mg^{++}ATPase[2]	LDH[3]	LDH[3]
1	4.285 ±0.710	97.76 ±17.21	227.10 ± 13.04	11.92 ± 2.92
5	7.493 ±0.739	190.90 ±12.90	137.81 ± 13.69	5.13 ± 1.22
10	7.572 ±1.110	188.06 ±25.47	161.39 ± 39.50	5.77 ± 2.19
15	7.987 ±0.940	201.73 ±11.13	121.81 ± 36.03	3.24 ± 0.10
20	9.277 ±1.080	210.56 ±13.13	167.51 ± 31.52	3.20 ± 0.50
25	5.610 ±0.850	194.32 ±10.14	261.86 ± 25.28	3.79 ± 0.34
30	5.447 ±1.401	104.97 ± 9.34	175.84 ± 27.21	4.41 ± 0.13
Adult	7.239 ±1.130	389.00 ±37.00	53.35 ± 12.23	1.60 ± 0.53
*Significant at the level	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.01$

*p values refer to differences between 1 day and 20 day stages. The Student's 't' test was used to analyze differences in means.

1. µg Formazan formed/mg protein/30 minutes.
2. µg phosphorus released/mg protein/10 minutes.
3. µ mole lactate oxidised/mg protein/15 minutes.

In serum, the activity of lactate dehydrogenase was at the highest level (11.92 units) on the first day, which declined sharply on the 5th day showing more than 50% reduction. This level was maintained thereafter all throughout the development as well as in the adult condition.

DISCUSSION

Being a key enzyme of TCA cycle, the measurement of SDH activity could be a most reliable index of the oxidative metabolism and the production of ATP molecules of any metabolically active organ. In the liver of pigeon minimum activity of SDH during early phase of development are indicative of three possibilities; (1) the existing mitochondrial population shows low oxidative metabolism, (2) the mitochondria are still undergoing biogenesis or (3) TCA cycle itself operates at a minimum rate. From the prominent increase of SDH activity on 10th day and maximum activity on 20th day, it could be purported that the mitochondrial function, particularly TCA cycle operation, increases and attains a maximum momentum at this stage of development in order to supply the adequate amount of ATP molecules necessary for the active synthesis of important metabolites, such as lipids, glycogen etc. In fact maximum activities of $NADPH_2$ generating

enzymes such as G-6-PDH and ~~NADP~~-malic enzyme (Chapter 4) and consequently maximum lipid deposition (Chapter 1) have been reported in the liver of developing pigeon on 20th day. Further, the increase in the activity of SDH is not difficult to explain when one considers the effects of some hormones such as growth hormone or thyroxine on mitochondrial population or mitochondrial activity (TCA cycle). Jakovcic et al. (1971) have suggested that mitochondrial content approximately doubles in the immediate postnatal period of the new born rat and respiratory enzyme activities of the foetal liver were 1/4th to 1/20th of that of adult liver which constantly increases till 30 days after birth. Similarly Lang and Herbener (1972) have also suggested that there is 1.4 times net increase in the mitochondrial compartments, while specific activities of the mitochondrial enzymes increased 1.8 to 2.1 times in the liver of weaning rats than that of the new born. Probably all these effects are under the influence of growth hormone, as Dimitrov and Kalchev (1975) have observed a stimulatory effect of growth hormone on respiration and oxidative phosphorylation in the liver mitochondria of both normal and hypophysectomized rats. It is also well known that a higher concentration of thyroxine but below 1.5 times of the normal physiological concentration in adult and even

higher concentration than this in developing animals favours the oxygen consumption as well as anabolic activities in liver and other organs. Increased RQ of liver and muscle of Rosy Pastor (Sturnus roseus) during premigratory period (Pilo, 1967) and total lipid concentration (Chapter 1) as well as activities of G-6-PDH and ~~NADP~~-malic enzyme in the liver of developing pigeon (Chapter 4) concomitant with the increased activity of thyroid gland, lends credulance to the suggestion that the increased oxidative metabolism in the liver of pigeon is definitely under the stimulatory influence of somatotropin and thyroxine and probably prolactin which is physiologically more important in the avian metabolism.

At the same time it is worthwhile to mention that the hyperactive TCA cycle operating in the liver of developing pigeon particularly at 20th day might be due to the availability of large quantity of carbohydrate rich diet i.e. grains, supplied by parents. Patel et al. (1976) have also observed similar changes in SDH activity in the liver of the migratory starling (Sturnus roseus) when they were found to take large amount of fruits and seeds during premigratory period.

ADENOSINE TRIPHOSPHATASE:

An active synthesis of ATP and its enzymatic hydrolysis is the characteristic feature of the metabolically active

tissue and both the phenomena normally run together in the tissue or organ which is engaged in synthesis of essential metabolites, active transport of metabolites across plasma membrane or active mitotic activity. It has been known that there exists a physiological polymorphism of ATPase in terms of pH optima, activators or inhibitors and site of intracellular localization (Potter et al., 1953). The total activity of ATPase in the liver includes mainly two types (1) Na^{+}-K^{+}-ATPase or membrane bound which is involved in active transport of Sodium and Potasium ions (in nerve fibres, kidney etc.) as well as essential metabolites like glucose and amino acids (in intestine, liver, brain and kidney) and (2) Mg^{++}-ATPase (E.C.3.6.1.4) also termed as mitochondrial ATPase, which is involved in chemiosmotic mechanism in the mitochondrial membrane. The quantitative survey of Mg^{++} ATPase could easily give an insight into the physiology of energy metabolism of any metabolically active tissue.

Wachstein et al. (1960 and 1962) in their histochemical studies have demonstrated ATPase activity in the liver mitochondria. Moreover, from the richest population of metabolically highly active mitochondria in the liver cells it could be easily suggested that majority of the ATPase activity in the liver cells could be the Mg^{++}-ATPase or mitochondrial ATPase. In

fact, Potter et al. (1953) have reported that a latent activity of ATPase in the liver cells of rat is dominated by Mg^{++}-ATPase. Further, it is necessary to consider atleast that ATPase is also present in mitotic apparatus which normally remains stabilized by Mg ion concentration (Mazia et al., 1961). Hence, high activity of Mg^{++}-ATPase could be expected in the tissue or cells engaged in active mitosis or in the organ with high rate of growth.

With this background information concerning the role of ATPase in different physiological functions, a quantitative study was carried out in the liver of developing pigeon which revealed that the ATPase activity is very low (97.76 units) at one day. The lower activity of ATPase in the liver of one day old pigeon indicates a slow hydrolysis of ATP and thus a poor demand of energy, while remarkable increase in the enzyme activity on 5th day and maintenance of this high level till 10th day could be due to the energy requirement for mitotic activity. The results thus, strengthen the previous work (Chapter 1) where it is suggested that liver is metabolically less active during first 10 days after hatching but is predominantly engaged in growth. However, high activity of ATPase during the period between 10th and 25th days with the highest level on 20th day of post-hatching pigeon liver is

suggestive of an active hydrolysis of ATP in mitochondria so as to provide energy for the synthetic processes such as glycogenesis, lipogenesis etc. which are reported to be at peak level on 20th day (Chapters 1 and 4). Moreover, the Mg^{++}-ATPase is known to be sensitive to the concentration of Na^{+} and K^{+}, and concentration of these ions in the liver are at maximum level at this stage of development i.e. 20th day (Chapter 2), which might have an additional stimulatory effect on the activity of ATPase. It is also reported that Mg^{++}-ATPase is also found to be localized on the plasma membrane of liver cells which is sensitive to bovine growth hormone in hypophysectomized rats (Aizono et al., 1974). Hence an increase in the total activity of Mg^{++}-ATPase in the liver of developing pigeon could be also due to the increase in the activity of this membrane bound Mg^{++}-ATPase, apart from that of other cellular locations. A very high activity of ATPase in the liver of adult pigeon probably suggests that adult liver needs tremendous amount of energy to carry out several metabolic activities requiring energy.

From the observation of the activities of both SDH and ATPase it could be seen that oxidative metabolism including active synthesis of ATP (TCA cycle) and hydrolysis of ATP (ATPase), are highly active when liver is in an active phase of synthesis of essential metabolites during post-hatching development.

LACTATE DEHYDROGENASE:

Lactate dehydrogenase which catalyzes reversible reaction of interconversion of lactate and pyruvate by using either NAD or $NADH_2$ depending on the direction of reaction, could be taken as an index of anaerobic glycolysis as well as a reliable criterion to study the capacity of liver to convert lactate formed in other part of body or inside liver into pyruvate and finally the utilization of latter for synthesis of important metabolites. A high LDH activity in liver (227.10 units) on the first day of post-hatching life is probably an indication of the existence of anaerobic glycolysis, which is the characteristic feature as well as dominant pattern of embryonic (in ovo) metabolism. At the same it is also possible that a large amount of lactate, produced by muscles (shivering thermogenesis), is actively converted into pyruvate in the liver and is utilized in other essential metabolic activity or probably converted into glucose and released into blood. But the liver showed a sudden decrease of LDH activity on 5th day and this low level was more or less maintained upto 20th day. It could be suggested that the anaerobic glycolysis gave way to oxidative metabolism as the liver acquired the requisite enzyme machinery. However, the liver showed a prominent peak in the activity of LDH on 25th day which is indicative of the fact that at this stage

of development, as the young ones have been observed leaving the nest and learning to fly and to run quickly for food, naturally a large amount of lactate produced in wing and leg muscles is available to the liver. However, in the adult liver the LDH activity is quite low perhaps due to the fact that (1) other extrahepatic tissue like cardiac muscles are competing for lactate uptake or (2) lactate production as such falls in the muscles due to the highly vascularized condition of the avian musculature. The changes in the activity of LDH from one day to adult could be due to either the change in the type of glycolysis adopted by the organ (aerobic or anaerobic) or due to the change in isozymic pattern of LDH. It could be suggested that the LDH activity in the liver of one day old pigeon might be predominently of M-type or anaerobic type and the increase in the activity in liver by 20-day onwards might be due to the activity of predominantly H-type or aerobic type of the enzyme. In fact Fine et al. (1963) have reported that adult domestic pigeon liver contains 72% of H-subunits in the total LDH enzyme molecules.

In serum of pigeon during post-hatching development LDH activity showed a gradual decrease from one day to adult age. It is possible that lactate release and LDH activity in the serum is quite high in the embryonic condition and at hatching and the concomitant increased activity of tissues maintains a low level

of lactate in the serum. Apparently the serum LDH activity also comes down to a low level soon after hatching.