CHAPTER 2

STUDIES ON DEVELOPING AVIAN LIVER 2. ACTIVITIES OF ACETYLCHOLINESTERASE IN THE LIVER AND SERUM AND THE IONIC CONCENTRATIONS IN THE LIVER OF POST-HATCHING DEVELOPING PIGEONS

The development of birds after hatching, although genetically oriented to follow a predetermined pattern, specific for each species, is influenced by environmental, physiological and behavioural factors. These factors, such as food and feeding habits, nest and nesting behaviours of both caring parents and cared young ones, and the length of duration of parental care have profound influence over tissues and organs of the post-hatched developing birds. In Chapter 1, it is reported that the concentrations of the metabolites in the liver as well as the rate of growth of liver are delicately interrelated with food and feeding habits of the growing young ones of pigeon. As the liver is concerned with storage and interconversions of metabolites as well as release of them into blood stream according to the demand, changes in the quality and quantity of food supplied to or ingested by the young ones, will greatly influence the metabolic functions of the organ. Besides, the liver should show functional maturity at an approximate time of development to adapt to the changes in the diet. It has been already recorded that the metabolic contents of the liver show fluctuations, when (1) parents stop feeding the young ones with crop milk only (5th day) and (2) when the young ones are fed with only grains (20th day). Apart from the changes in the concentration of metabolites, the liver weight to body weight ratio was also found to fluctuate during development (Chapter 1).

In this context it is conceivable that, the rate of growth of the liver and the development of full complements of machinery related to the metabolic and other functions of it, are as a result of the interplay of both environmental (dietary) and physiological (hormonal) factors. In Chapter 1, it is suggested that the pigeon liver, more or less, attained full competancy to cope up with the changes in the quality and quantity of food by 20th day of post-hatching development. In other words, the enzyme machinery concerned with the liver functions should reach the level and state comparable to that of the adult condition by 20th day of post-hatching period. In order to corroborate this contention, a series of studies on key enzymes in the liver of developing pigeon was carried out along with the liver composition analysis. In the present chapter, the results of quantitative and histochemical studies on hepatic cholinesterase, quantitative determinations of serum cholinesterase and hepatic cation concentrations in the developing pigeons during post-hatching period, are recorded.

MATERIALS AND METHODS

The developing pigeons selected for the studies were from the departmental aviary where a large colony of domestic pigeons is maintained. The young ones at different days of post-hatching development (viz., 1, 5, 10, 15, 20, 25 and 30 days old) and adults were sacrificed and liver and serum were removed immediately for various analysis. The liver and serum cholinesterase was estimated quantitatively using acetylcholine chloride as the substrate following the method of de la Huerga *et al.* (1952). The readings were taken in a Klett-Summerson photoelectric colorimeter at 540 $m\mu$ and the results are expressed as μ mole acetylcholine hydrolyzed/100 mg liver or ml serum/60 minutes. The histochemical methods employed for the localization of cholinesterase enzyme was that of Koelle and Friedenwald (1949) as modified by Coupland and Holmes (1957) using Acetyl thiocholine iodide (Sigma Chemical Co., U.S.A.) as the substrate.

The sodium, potassium and calcium concentrations in the fat free dry tissue were determined using 'EEL' flame photometer.

RESULTS AND DISCUSSION

Data on quantitative analysis are presented in Table 1. Acetylcholinesterase activity in the liver of 5 day old pigeon increased considerably from 16.56 μ mole ACh hydrolyzed/ 100 mg liver/60 minutes (value of one day old pigeon) to 26.26. The liver of 20 day old pigeon showed the maximum activity (35.26 units). Similarly, the maximum cholinesterase concentration in the serum was observed in 20 day old pigeon. Interestingly, the ionic concentrations (sodium, potassium and calcium) in the liver of 20 day old pigeon were at the maximum level. Incidently, the 20 day old pigeons invariably feed on whole grains only and hence the food availability is much more than during earlier period when they were fed with crop milk plus broken grains. This change of diet resulted in an increase in the deposition of carbohydrate and fat (the latter synthesized de novo) in the liver (Chapter 1). The increase in the activity of acetylcholinesterase in the liver as well as in the serum could be then related to the increased carbohydrate intake. Since the level of activity of AChE is an indication of acetylcholine secreted by the nerve endings, it could be suggested that cholinergic nerves

TABLE 1

Hepatic and serum acetylcholinesterase activities and concentrations of electrolytes in the developing pigeons. Mean ± S.D.

Age in days	Body wt. gm.	Liver wt./100g body wt.	Acetylcholinesterase[1] activity Liver	Acetylcholinesterase[1] activity Serum	Electrolytes[2] Na^+	Electrolytes[2] K^+	Electrolytes[2] Ca^{++}	Liver[3] Glycogen	Liver[3] Fat	Liver[3] Water
1	17.5 ± 0.5	2.518 ±0.455	16.56 ± 3.18	154.15 ± 13.13	500.0 ± 32.0	1362.0 ± 89.3	170.0 ± 6.9	0.2616 ±0.0848	9.970 ±0.2237	76.22 ±0.77
5	56.0 ± 5.7	6.527 ±0.395	26.26 ± 1.84	170.45 ± 16.01	485.0 ± 39.8	1270.0 ± 78.4	184.0 ± 9.1	0.5072 ±0.1300	8.427 ±0.722	75.38 ±1.04
10	144.0 ±19.3	5.405 ±0.401	26.41 ± 2.13	174.24 ± 13.98	487.0 ± 37.6	1293.0 ± 83.7	153.0 ±11.3	1.345 ±0.091	8.835 ±1.063	74.15 ±1.76
15	168.0 ± 7.4	3.946 ±0.946	26.50 ± 3.32	193.21 ± 14.87	537.0 ± 44.7	1060.0 ± 98.4	113.0 ± 9.3	2.53 ±0.31	8.235 ±1.293	74.15 ±1.38
20	202.0 ± 6.1	5.008 ±0.305	35.55 ± 3.59	235.36 ± 15.07	631.0 ± 43.7	1493.0 ± 87.6	155.0 ±12.3	3.04 ±0.14	13.525 ±1.813	73.78 ±2.08
25	217.0 ±19.0	3.954 ±0.407	32.94 ± 2.98	207.02 ± 19.33	601.0 ± 49.1	1368.0 ± 81.3	138.0 ±10.6	3.21 ±0.15	10.77 ±2.21	73.12 ±2.35
30	235.0 ±12.2	3.630 ±0.363	32.51 ± 2.84	194.73 ± 17.71	490.0 ± 22.7	1330.0 ± 93.2	100.0 ± 8.3	4.42 ±0.33	12.59 ±2.83	71.31 ±2.07
ADULT	278.0 ±17.0	3.278 ±0.312	33.70 ± 1.89	194.50 ± 18.46	533.0 ± 42.8	1360.0 ± 90.1	105.0 ± 9.4	4.86 ±0.33	12.13 ±1.03	70.61 ±1.90
*Significant at the level	$p<.001$	$p<.01$	$p<.001$	$p<.02$	$p<.001$	$p<.001$	$p<.02$	$p<.001$	$p<.001$	$p<.001$

*p values refer to differences between 1 day and 20 day stages. The Student's 't' test was used to analyse differences in means; 1-μ mole ACh hydrolyzed/100mg liver or ml serum/hr; 2-mg/100 gm liver; 3-gm/100gm fresh tissue.

must be assisting in the uptake of glucose and other materials ~~in~~by the hepatic cells. The acetylcholine influences the permeability of cell membrane (Augustinsson, 1950) resulting in an influx of ions into the hepatic cell. In fact the concentrations of sodium, potassium and calcium were found to be increased in the liver of 20 day old pigeon along with the increase in the activity of AChE. Since corresponding increase in the water content was not observed in the liver of 20 day old ones (Chapter 1), the increase in the ionic concentration may be (1) due to changes in the permeability of cell membrane and (2) for facilitating the uptake of metabolites. It is common knowledge that the deposition of ions, especially potassium, increases in the cell along with the enhanced deposition of glycogen. Perhaps, acetylcholine by increasing the movement of ions assists in the uptake of glucose. Addition of acetylcholine with insulin is found to elevate the uptake of glucose by the liver (Mondon and Burton, 1971). The stimulation of cholinergic (vagus) nerve results in an increased activity of glycogen synthetase (Shimazu, 1967). In general the transport of glucose across cell membrane can be through flow coupled transport (ionic transport) or through chemiosmotic coupling (phosphorylation) (Wilbrandt, 1975). Hence it is to be believed that acetylcholine secreted at the nerve endings of hepatic nerve plexus could actively facilitate the uptake of glucose by the hepatic cells either alone or in close association with insulin. Bertrand (1954) observing cyclic variations in the intralobular localization of hepatic cholinesterase during feeding and fasting suggested that acetylcholine-acetylcholinesterase (ACh-AChE) system might be involved in the assimilation process. Since maximum AChE was found in the liver of 20 day old pigeon (when carbohydrate intake was high) ACh-AChE complex must be facilitating maximally the glucose uptake by the liver. Shah et al. (1972), while studying histochemical distribution of cholinesterases in the livers of birds with varying dietary preferences, observed that these esterases were found more in the liver of graminivores like pigeon and dove. Pilo (1969) observed an increase in the AChE activity in the liver of migratory starling, Sturnus roseus, during pre-migratory period compared to the level of the enzyme activity in the post migratory period. Since Sturnus roseus changes its diet, from a mixed one (grains and insects) in post migratory period, to carbohydrate rich food (grains and fruits) during premigratory period, a correlation between AChE activity and carbohydrate rich diet could be established.

The histochemical studies on the AChE activity in the liver of developing pigeon (Figs. 1-8) revealed that the AChE was localized in the sinusoidal linings and there was a gradual increase in the histochemical reactivity of the enzyme as the development progressed. In the liver of 20 days old pigeon, the enzyme was more concentrated in the portal areas (Fig. 6). It is this increase of AChE in the portal areas that has resulted in the overall increase in the AChE observed quantitatively (Table 1). The parasympathetic nerve plexus are situated along the blood vessels and sinusoidal linings (Sutherland, 1964) and hence the acetylcholine is invariably released in these regions. The presence of AChE in the sinusoidal linings (Fig. 7) is then to degrade the acetylcholine released there. Invariably some amount of acetylcholine may find its way to the blood flowing through the sinusoids. The effective removal of ACh released into the blood is carried out by the serum cholinesterase. In fact the serum acetylcholinesterase also comes from the liver (Augustinsson, 1950). The increase in the serum cholinesterase in the 20 days old pigeon (Table 1) could be to inactivate the increasingly released ACh from the liver.

CHAPTER 2

EXPLANATIONS FOR FIGURES

Figs. 1 to 8. Photomicrographs of the liver sections of young ones and adult pigeons showing acetylcholinesterase activity.

Fig. 1. 1 day old pigeon. 75X.

Fig. 2. 5 day old pigeon. 75X.

Fig. 3. 10 day old pigeon. 75X.

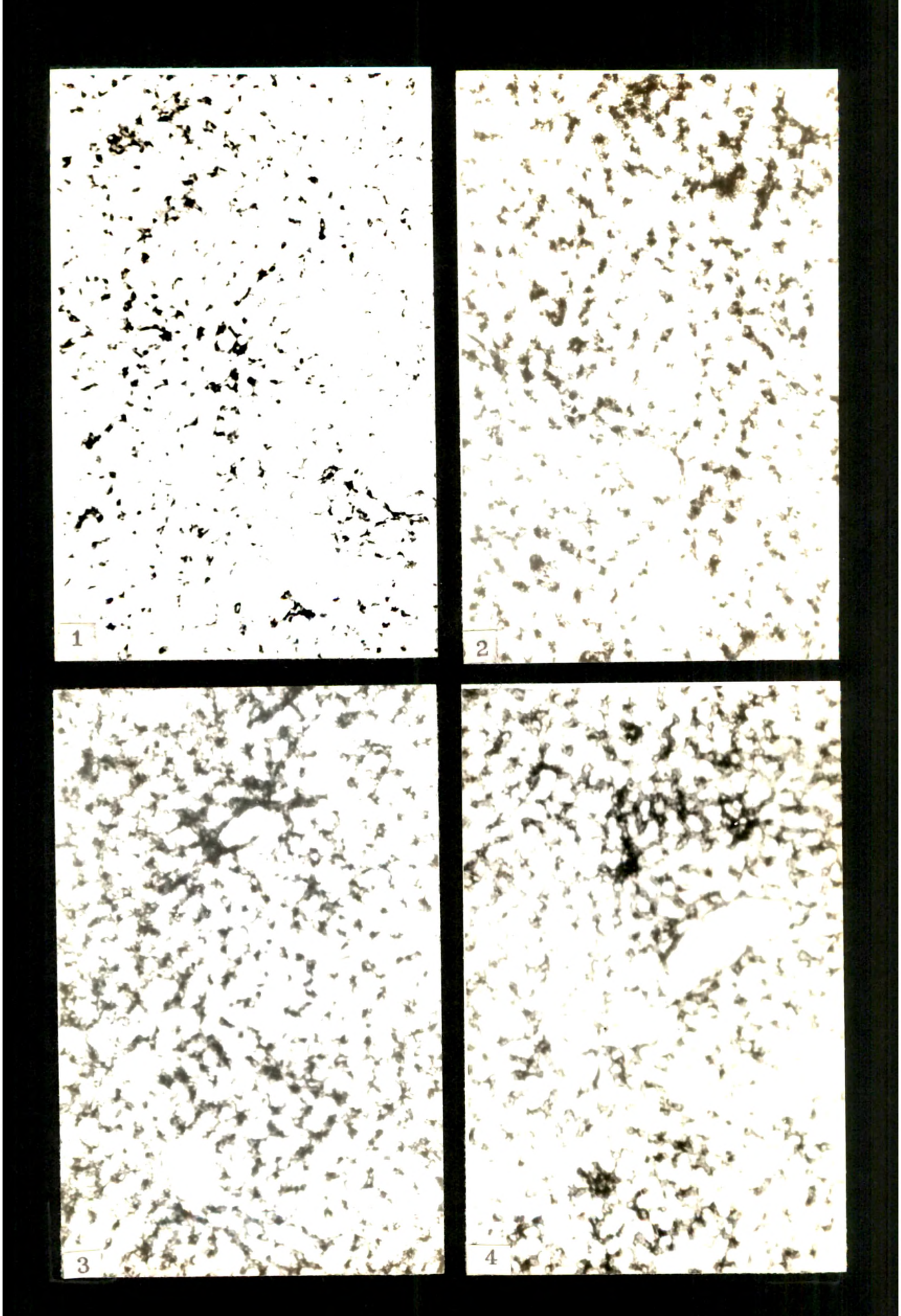
Fig. 4. 15 day old pigeon. 75X.

Fig. 5. 20 day old pigeon. 75X.

Fig. 6. 25 day old pigeon. 75X.

Fig. 7. 30 day old pigeon. 75X.

Fig. 8. Adult pigeon. 75X.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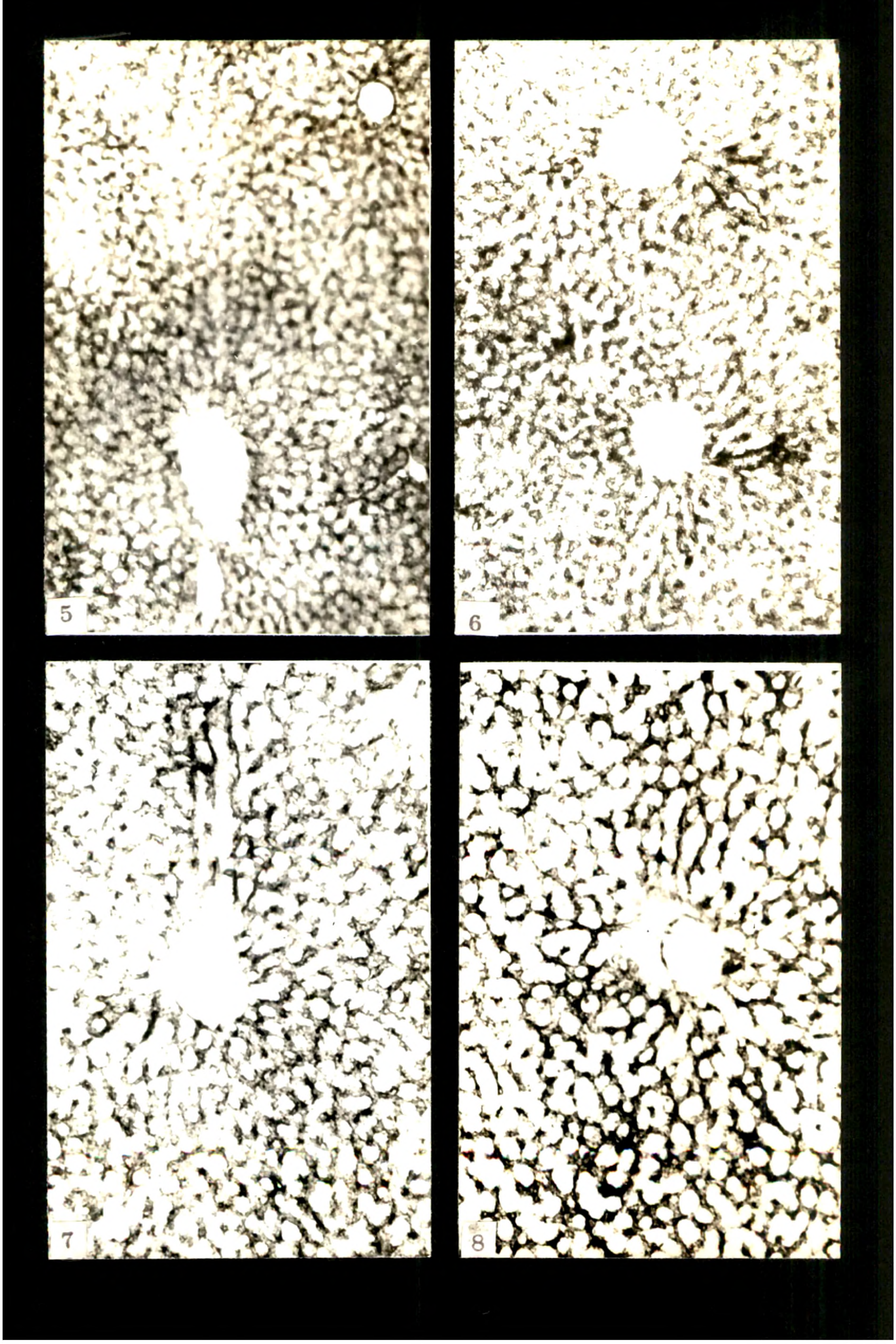
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After 20th day stage, the activity of acetylcholinesterase in the liver and serum was found to stabilize to a level characteristic of that of adults. Actually, the enzyme level in the liver and serum of adults (Table 1) was much lower than that of 20 day old pigeon. As the pigeon young ones, after 20th day come out of the nest and feed by themselves, the requirement of glucose by other tissues also goes up, and hence the liver is not burdened with heavy influx of glucose. This has resulted in the decreased level of AChE in the liver as well as serum of pigeons having more than 20 days of post-hatching development as well as of adult ones.

In conclusion, it could be said that acetylcholine-acetylcholinesterase system together with insulin plays a significant role in the uptake of glucose by the hepatic cells. The liver of 20 day old pigeon, which is fed with whole grains, has to accomodate large quantity of carbohydrates digested and absorbed and hence the increased AChE in the liver. However, in the 20 day old pigeon liver the amount of glycogen was not very high, probably due to its ready conversion to fat, resulting in the tremendous increase in the fat content of the liver (Chapter 1) at this time. Perhaps, the major part of the uptake of glucose by the pigeon liver is facilitated by coupling the transport of glucose with that of ions in which case release of acetylcholine at the sinusoidal linings and the AChE activity there go hand-in-hand with glucose transport.