CHAPTER 7

STUDIES ON DEVELOPING AVIAN LIVER 7. THE CORRELATION OF FUNCTIONAL MATURITY OF THE LIVER WITH THE FUNCTIONS OF CERTAIN ENDOCRINE GLANDS IN PIGEON, DURING POST-HATCHING DEVELOPMENT

The development of avian embryo in the egg is independent from maternal influence unlike in *in utero* development of mammalian foetus. Hence, avian embryo has to accomodate in such a way that by the time of hatching almost all important organs attain the minimum level of functional status. Whereas other parts of body which are necessary in adult life (wing muscles, feathers etc.) develop during late phase of post-hatching development (O'Connor, 1977). Such spacing of development is possible only if post-hatching growth of altricial species is quite long; for example pigeon nestling takes 30 days for complete development of body. Unlike pigeon young ones, the sparrow nestlings take to wings soon after 14 days of life in the nest. In such cases almost all organs develop simultaneously. When the nestlings leave the nest, they should be not only functionally mature to lead an independent life without parental support, but also should be able to withstand the initial food crisis while learning to procure food by themselves. Hence, before leaving nest, not only the birds complete the development but are also compelled to divert major part of the energy supplied through diet as reserves. In the case of pigeon nestlings, it was observed that the liver was found to synthesise and store large amount of lipid just prior to the termination of nestling period. Thus the liver was found to attain functional maturity by 20th day of post-hatching development (Chapters 1, 2, 3, 4, 5 and 6). It was elucidated that the liver was predominantly active in cell division and growth (during which a protein rich diet i.e., crop milk was provided to the young one) till 10 days, and then it showed the development of metabolic activities similar to that of adults (when a carbohydrate rich diet i.e., grains, is supplied). However, as mentioned earlier (Chapter 4), it appears that food is not the only factor which induces these changes but thyroid hormones are also involved in controlling liver metabolism during post-hatching development. George and Naik (1964a) and Pilo and George (1970) reported that hyperactive thyroid gland could be responsible for hyperlipogenesis and stimulating birds for migratory flight. In connection with these reports, Patel (1976), observed that the thyroidectomized starlings showed a reduced fat deposition. Goodridge and Adelman (1976) in their tissue culture experiment reported that triiodothyronine along with insulin stimulated synthesis of 'malic' enzyme several folds in embryonic liver cells of chick while glucagon inhibited this effect. Similarly, thyroxine also influenced the rhythm of fattening response to prolactin in the pigeon (John *et al.*, 1972). Normally an extra dose of thyroxine results in a depletion of some important metabolites *i.e.*, glycogen, fat etc. But when its dose is less than 1.5 times of normal level, it favours the anabolic activity and thus helps in growth and differentiation (Pitt-Rivers and Tata, 1959); particularly, cells or organs of developing animal have an anabolic response instead of a catabolic one shown by adult tissues. Freeman (1964a) reported a marked increase of PBI* concentration after 15th day of incubation, which resulted finally into a high rate of thyroxine release at the time of hatching. Tanabe (1964) also reported the progressive increase of thyroxine secretory rate upto 2 weeks in cockerels.

The influence of developing adrenal gland on the metabolic activity of liver is also as significant as other endocrine glands, which again is rather complicated to understand because of its double compartments, releasing hormones of diverse influence on metabolism. The development

* PBI - Protein bound iodine

of adrenal gland is under the influence of adenohypophysis and the thyroid gland (Rascio, 1967). Girouard and Hall (1973) have reported that development of adrenal gland during second phase i.e., from 11th to the day of hatching is faster under the stimulatory effect of ACTH from adenohypophysis. Hence it is not surprising therefore that a greater proportion of the adrenal should be of cortical in nature in a newly hatched bird. In fact, Pyne (1955) noted that 90% of the adrenal gland is occupied by interrenal compartment in a newly hatched chick. It could also be easy to suspect the role of adrenal and thyroid glands not only in maturation of duodenum and intestine (Mogg, 1961; Hart and Betz, 1972; Pedernera, 1971) but also in that of liver. Moreover, along with relative weight and histological observation, the quantitative estimation of ascorbic acid content would provide the complete picture of endocrine status of adrenal gland and its effect on the liver physiology during post-hatching development.

In the light of several strong reports suggesting secondary influence of insulin in avian carbohydrate metabolism it is easy to consider its hypoglycemic effect because when injected in an intact chicken it suppressed blood glucose level (Hazelwood and Berksdale, 1970). Moreover, Benzo and de la Haba (1972) have reported that chick embryonic liver cells require Zn-insulin for the maturation. Maraud *et al*. (1965) claimed that insulin enhances the thyroid activity where the latter's influence on lipogenesis in birds is well known. In fact, not only the synthesis and release of pancreatic hormones but the structure and development of islets are also greatly influenced by the nature of food and therefore the level of glucose and other metabolites present in blood. Hence, information about its development would atleast help in correlating its influence on the physiology of liver during post-hatching development.

Thus, keeping these information in view, the weight of adrenal and thyroid glands, histology of adrenal, thyroid and pancreas as well as quantiative estimation of ascorbic acid content of adrenal glands have been undertaken and an attempt has been made to establish a possible correlation of development and functions of these glands with the functional maturity of pigeon liver during post-hatching development.

MATERIALS AND METHODS

Young ones having different stages of development *i.e.*, 1, 5, 10, 15, 20, 25 and 30 days as well as adult domestic pigeons (*Columba livia*) were collected from an open aviary maintained by the Department. Body weight as well as weights of adrenal and thyroid glands were taken, using 'Mettler' balance and then the endocrine glands were fixed in Bouin's fixative for histological study. Five μ thick paraffin sections were stained with Haematoxylin-Eosin. Four to five adrenal glands of all stages were processed for estimation of ascorbic acid content according to the method of Roe and Kuether, (1943) as described by Roe (1954). The values of AA is expressed as mg/100 gm fresh weight of tissue. Among the lobes of pancreas, splenic lobe was selected for histological study of populations of large and small islets.

RESULTS

The data of the adrenal and thyroid glands (weights and relative weights), the populations of large and small islets in the splenic lobe and the ascorbic acid content of adrenal gland of all the stages of post-hatching development are recorded in Table 1.

ADRENAL GLAND

The weight of adrenal gland was minimum (5.11 mg) in 1-day pigeon and increased remarkably during first 10 days attaining 3 times the weight of that of 1-day pigeon.

This weight remained almost at the same level upto 20th day, though it increased slightly during 25th and 30th day reaching the same level as in adult pigeon. On the other hand its relative weight was maximum (23.02 mg%) in 1-day old pigeon and gradually declined along with increasing age and attained the lowest value (4.18 mg%) on 20th day which again increased nearly to double during further development. In histological study it was noticed that majority of the gland was occupied by interrenal tissue or cortical cells as compared to very few chromaffin (Medullary) cells migrating slowly from well developed sympathetic ganglia during further development and attained the pattern of adult adrenal gland by 20th day (Figs. 1 & 2). The ascorbic acid concentration was maximum (246.90 mg/100 gm) in the adrenal gland of 1-day old pigeon, which decreased significantly during further development except on 20th day where it showed a comparatively higher concentration (Table 1).

THYROID GLAND

The weight of thyroid gland was also minimum (4.50 mg) in 1-day pigeon which increased significantly from 5th day (13.40 mg) to 30th day (26.80 mg), however, it was lower (17.10 mg) in the adult pigeon. On the contrary the relative weight of the gland was maximum (20.61 mg%) in 1-day pigeon

TABLE 1

Relative and direct weights of adrenal and thyroid glands, Ascorbic acid content of adrenal gland and islet population of splenic lobe of pancreas of pigeon during post-hatching development. Mean ± S.D.

Age in days	Direct weight (mg)		Relative weight (mg/100gm body wt.)		Ascorbic acid content of Adrenal gland (mg/100 g tissue)	Islet population of splenic lobe of pancreas	
	Adrenal	Thyroid	Adrenal	Thyroid		Small	Large
1	5.11 ±0.56	4.50 ±0.58	23.02 ± 1.90	20.61 ± 1.59	246.90 ± 9.76	23.0 ± 7.0	6.0 ±2.0
5	10.34 ±1.05	13.40 ±1.10	16.09 ± 1.31	19.94 ± 1.37	137.96 ± 8.70	23.0 ± 5.0	6.0 ±2.0
10	17.11 ±2.00	15.80 ±1.38	12.46 ± 0.93	10.84 ± 1.93	146.55 ± 11.93	24.0 ± 4.0	7.0 ±2.0
15	15.30 ±1.30	16.30 ±1.78	8.60 ± 0.71	8.89 ± 0.91	162.20 ± 9.85	35.0 ± 6.0	7.0 ±2.0
20	15.45 ±1.47	23.10 ±2.01	4.18 ± 0.35	12.12 ± 0.98	208.90 ± 10.45	39.0 ± 6.0	11.0 ±3.0
25	17.55 ±1.80	23.10 ±1.93	8.10 ± 0.78	9.84 ± 1.01	141.60 ± 9.67	41.0 ± 5.0	11.0 ±3.0
30	20.28 ±1.93	26.80 ±2.12	8.69 ± 0.42	11.89 ± 1.12	114.70 ± 11.37	43.0 ± 7.0	11.0 ±3.0
Adult	18.50 ±1.78	17.10 ±1.97	9.35 ± 0.67	8.70 ± 0.71	143.40 ± 10.17	43.0 ± 6.0	11.0 ±3.0
*Significant at the level	P<.001	P< .001	P< .001	P< .02	P< .001	P< .01	P< .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.

CHAPTER 7

EXPLANATIONS FOR FIGURES

Figs. 1 to 6. Photomicrographs of sections of adrenal and thyroid glands and splenic lobe of pancreas stained with haematoxylin-eosin of pigeon during post-hatching development. Each one is of 200X magnification.

Fig. 1. Section of adrenal gland of 1 day old pigeon.

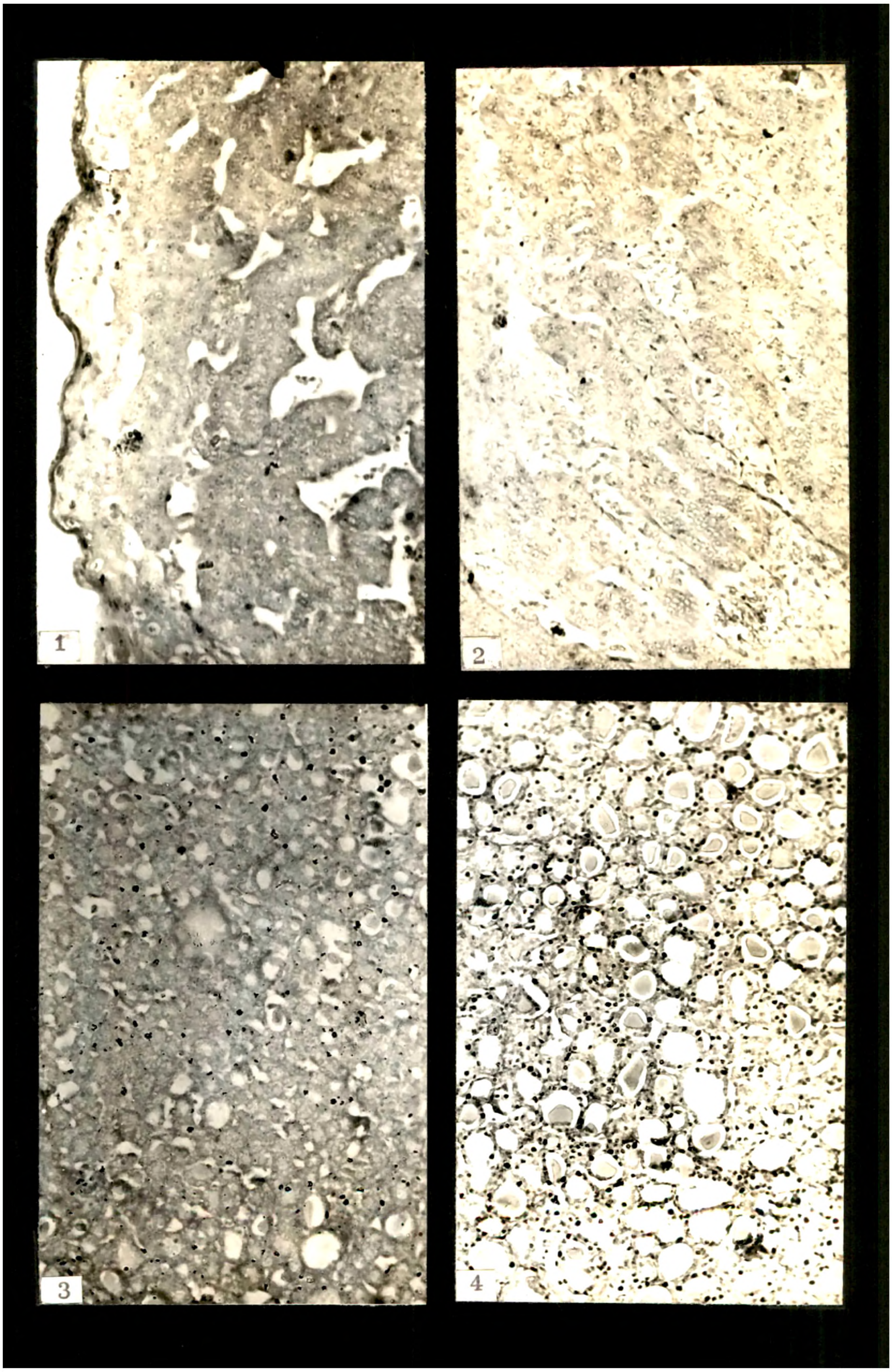
Fig. 2. Section of adrenal gland of 20 day old pigeon.

Fig. 3. Section of thyroid gland of 1 day old pigeon.

Fig. 4. Section of thyroid gland of 20 day old pigeon.

Fig. 5. Section of pancreas (splenic lobe) of 1 day old pigeon.

Fig. 6. Section of pancreas (splenic lobe) of 20 day old pigeon.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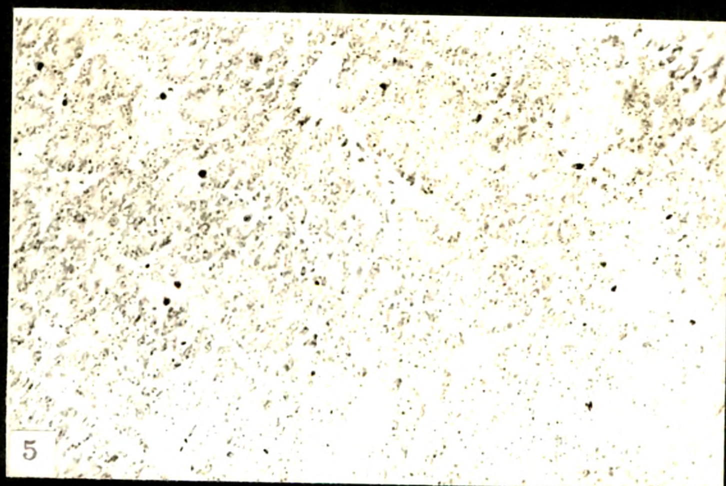

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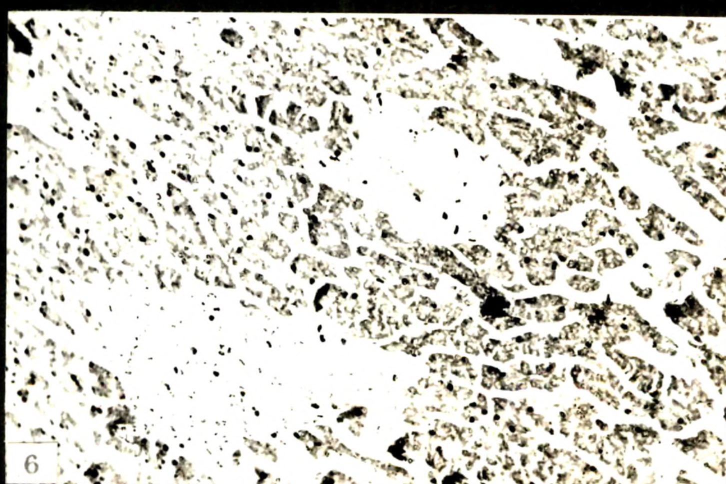

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which decreased as the age of nestlings increased and showed a slightly higher value (12.12 mg%) on 20th day. Histological observation revealed that follicles as well as follicular cells were well developed in 20th day pigeon as compared to that of 1-day old (Figs. 3 & 4).

ENDOCRINE PANCREAS

From the histological structure and counts of the populations of small and large islets in the splenic lobe of pancreas of 1-day, 20th day as well as adult pigeons, it was observed that there were only 6 large and 23 small islets in 1-day pigeon, and small islets showed a poor histological structure (Fig. 5). There were 11 large and 39 small well developed islets on the 20th day (Table 1 & Fig. 6). In the adult pigeon there was no significant change in the number of islets but they showed well developed histological structure.

DISCUSSION

ADRENAL GLAND

The maximum relative weight of adrenal gland with ascorbic acid rich densely packed interrenal tissue is indicative of high secretion of adrenocorticosteroid hormones which are essential in the early phase of post-hatching development. Several authors have reported that corticosterone, which is believed to be the main corticosteroid hormone of avian cortical cells, induces hyperglycaemia with accumulation of glycogen (Brown et al., 1958; Greenman and Zarrow, 1961; Snedecor et al., 1963). Moreover, Brown et al. (1958) reported that the rise in blood glucose is the result of active protein catabolism (gluconeogenesis). In fact, gluconeogenesis is found to be operating at a high level during the early days of post-hatching development, because nestlings are provided with protein rich carbohydrate-free diet (i.e., crop milk) by the parents. On the 20th day relative weight of adrenal gland was minimum (4.18 mg%) due to the comparatively heavy body weight. However, it increased to double the weight during 25 and 30 days probably as a result of the stress such as less availibility of food and heavy muscular strain developed due to unpracticed or unexperienced flying exersise. In adult it remained 9.33 mg% which is nearly 2.5 times less than that of 1-day pigeon.

Similarly from the data of ascorbic acid content of the gland it could be suggested that interrenal cells are very active in the synthesis of corticosteroid hormones which play a key role in the regulation of water and mineral metabolism as well as carbohydrate and lipid metabolism.

Thus in the young nestlings corticosterone could induce gluconeogenesis and glycogenesis in the liver and also helps in keeping a high blood glucose level during post-hatching development by its catabolic effect on protein which is abundantly supplied by parents. Again, high level of corticosterone is also found to be essential for lipid synthesis. Nagra and Meyer (1963) and Baum and Meyer (1960) have reported a lipogenic effect of corticosterone in chick. Thus the high concentration of ascorbic acid in adrenal gland of 1-day pigeon could be due to two reasons 1) dense population of cortical cells and 2) highly active corticosteroid secretion. The progressive decrease during the later period of development could be due to a gradual increase of chromaffin cells in the gland which are comparatively poor in AA content. However, the peak observed on 20th day is probably indicative of an active synthesis of steroid hormones for boosting the action of thyroxine in active lipogenesis which is reported to be operating in full swing at this stage of development (Chapter 4). Hence it could be suggested that adrenal gland plays an important role in controlling the function of liver in such a way that liver could utilize available food in a precise way and also could attain the functional maturity by the age of 20th day after hatching. Similar kind of influence of these hormones on maturity of intestine and duodenum has been suggested by some authors (Moog and Richardson, 1955; Hart and Beitz, 1972).

Chromaffin tissue on the other hand developed after the interrenal tissue has become well developed and by 20 days of age and onwards the chromaffin cells could become helpful to the young ones in adapting to the stress during 25th and 30th day age.

THYROID GLAND

If the weight of the thyroid gland is considered, it could be noticed that at about 20th day it attained almost the full size (23.10 mg). The increase in direct weight could be due to the active cell division and follicle formation while the increase in the height of follicular cells (Chapter 4) could be indicative of progressive increase in secretory activity which was found maximum on 20th day after hatching. Tanabe (1964) has reported that the rate of thyroxine secretion in chick increased progressively after hatching and reached maximum at the age of 2 weeks, while in altricial bird like pigeon it could be little slow and reach maximum level only on 20th day.

In addition to its growth promoting action, thyroxine is known for its lipogenic function too. Its significant role in the intermediary metabolism of birds has been reported by several authors (Dodd and Matty, 1964; Sturkie, 1965; Raheja _et al_., 1971; Snedecor _et al_., 1972; Balnave, 1973; Thapliyal _et al_., 1973, 1975).

George and Naik (1964a) and Pilo and George (1970) have reported that premigratory hyperlipogenesis and stimulus for migration in migratory birds were induced by increased thyroid secretion. While Freedland (1965), Reed and Tepperman (1968), Chandrabose and Bensadoun (1971b) as well as Snedecor _et al_. (1972) have reported that lipogenic action of thyroxine is actually the result of powerful stimulatory effect on $NADPH_2$ generating enzymes like G-6-PDH and 'malic' enzyme which are highly essential for lipid synthesis. Recently Goodridge and Adelman (1976) have also suggested that action of triiodothyronine (T_3) was potentiated by insulin in the induction of 'malic' enzyme 77 fold which was found higher than the individual effect of both hormones _i.e._, 2 fold by insulin and 23 fold by T_3 in the culture of embryonic liver cells of chick. Hence it is easy to suggest that the peak lipogenic activity on 20th day could be under the influence of insulin and thyroxine. In fact, concentration of insulin and thyroxine (from the population of small (light) B-islets in the splenic lobe of the pancreas and the height of follicular cells of thyroid gland) is inferred to be maximum at this stage. The insulin might be involved directly, or indirectly by simulating I_2 uptake by thyroid gland as reported by Mayrad *et al*. (1965), in the lipogenic activity.

ENDOCRINE PANCREAS

The importance of the splenic lobe as a source of insulin is reported by Kedinger *et al*. (1972); 66% of the total pancreatic insulin is found in this tissue which itself represents 6% of the pancreatic tissue. Hence splenic lobe of the pancreas is being selected in present investigation in which the population of small (light) and large (dark) islets have been studied. According to Smith (1974) small (light) islets are considered as the B-islets.

From the histological study of large-dark (A-islets) as well as small-light (B-islets) islets, it was noticed that B-islets were 23 and majority of them were small and poorly developed in 1-day pigeon. The number increased to 39 and also showed improved histological structure by 20th day. Similarly large (A-islets) also increased from 6 on 1-day to 11 on 20-days. Probably the development of B-islets is under the pressure of heavy carbohydrate load because nestlings are provided with grains maximally at this stage of development. Consequently a high concentration of insulin on 20th day might be useful in maximum utilization of carbohydrate rich diet for lipid and glycogen synthesis in the liver either directly by its hypoglycaemic action or through stimulating thyroid gland (Maraud *et al*., 1965).