CHAPTER 5

HISTOCHEMICAL RESPONSE OF THE DEVELOPING GIZZARD TOWARDS ACETYL AND BUTYRYL CHOLINESTERASES

As a probable relationship between nervous tissue and development, which has been, by now, well recognised by a number of workers, the role of nerve mediators such as Acetyl Choline (ACh) and Butyryl Choline (BuCh) was found worthwhile to investigate during the post-natal development, differentiation and functional maturity of pigeon gizzard indirectly through their esterases. Biochemical studies on these esterases are mostly carried out in the individual tissues of amphibians by Hey (1960), of birds by Julia & Bryan (1967), Pilo (1969) and Asnani (1971) and of mammals by Ballyntyne & Burwell (1965) and Koelle (1950). Such studies to determine the possible and/or the extent of involvement of these substances in the post-natal development of organs specially those of avian alimentary canal are completely lacking. Few of these studies in this field are of ultrastructural and light microscopical nature chiefly to understand the histomorphological and anatomical relationship between nervous tissue and the developing and differentiating systems of birds (Bennett & Cobb, 1969; Maynard, 1965; Mumenthaler & Engel, 1961) and mammals (Yamamoto, 1961; Yamauchi & Burnstock, 1969a & b; Leeson & Leeson, 1965a).

With reference to alimentary canal in general and gizzard in particular, only innervation studies are made so far (Mangold, 1906; Nolf, 1934a & b; Malinovsky, 1961, 1962). These studies have shown that in both chick and pigeon, gizzard is innervated by vagi as well as by nerve trunks arising from the sympathetic coeliac plexus which run with the coeliac artery. Anastomoses between the vagus and the sympathetic nerve at the level of the coeliac plexus has been noticed in chick by Campounhout (1932) and Nolf (1934a & b) and in pigeons by Malinovsky (1961, 1962). Though there are a few reports on the distribution of cholinesterases in the adult alimentary canal of fishes (Desai, 1967) and mammals (Gerebtzof & Betrand, 1957; Koelle et al., 1950; Glick,1938), there are hardly any in the alimentary canal of aves especially during its development excepting that of Bennett (1969). The present histochemical study on cholinesterases is, thus, an attempt to understand, though indirectly, the role of nerve mediators during the post-natal development of gizzard and also to gain some knowledge on smooth muscle physiology.

MATERIALS AND METHODS

Healthy young pigeons of different age (in days) of post-natal development and adult pigeons from a well maintained

aviary were used for the present study. Pigeons, young and old, were decapitated under mild anaesthesia and the gizzards were soon separated, blotted well to remove their contents, blood and other tissue fluids and fixed on a chuck of a cryostat microtome maintained at -20°C. Frozen sections of 10 - 15 μ thickness were cut and fixed in 10% formol saline (Gurr, 1956) for 2 - 3 hours at 4°C. After fixation, the sections were washed thoroughly in distilled water and incubated in the respective media as suggested by Koelle & Friednwald (1949) and modified by Coupland & Holmes (1957).

Ingredients	AChE	BuChE
Acetylthicholine iodide (substrate) (23 mg per 1.2 ml)	1.2 ml	-
Butyrylthiocholine iodide (substrate) (23 mg per 1.2 ml)	-	1.2 ml
Acetate buffer, 0.2 M, pH 5.5	5 ml	5 ml
Copper glycine (3.75 gms of glycine and 9.52 gms of copper sulphate in 100 ml of distilled water)	0.6 ml	0.6 ml
Magnesium chloride (9.52%)	0.6 ml	0.6 ml
Sodium sulphate (40%)	7.6 ml	7.6 ml
Copper sulphate, 0.1 M	0.4 ml	0.4 ml

After incubation, the time of which was kept constant for 14 hours at 37°C, the sections were washed thoroughly in

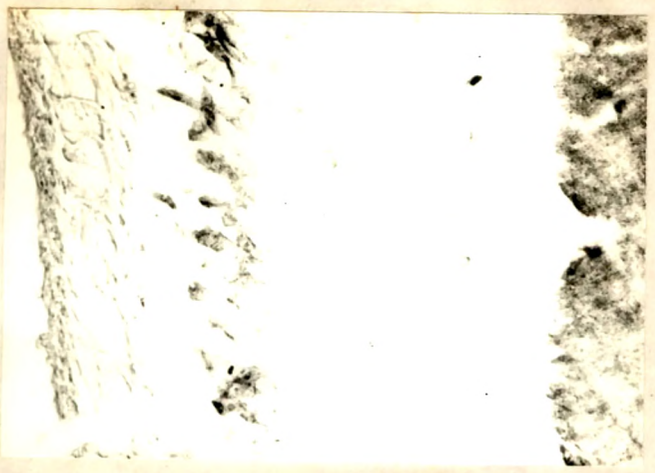
distilled water and treated with dilute yellow ammonium sulphide. They were then washed again in distilled water, dehydrated through alcohol grades and mounted in canada balsam.

Control: Control sections were treated, prior to incubation, with 3 X 10^{-5}M solution of eserine sulphate at 37°C for 30 minutes and incubated in a medium identical for the samples.

OBSERVATIONS
(Figs. 1 to 8 and 1a to 8a)

Of the two cholinesterases studied herein a positive reaction was elicited only by acetylcholinesterase (AChE) while buturylcholinesterase (BuChE) remained totally unrepresented during the gizzard development. On the day of hatching both the mucosal tubules and smooth muscle fibres showed a weak but demonstrable amount of AChE. From the 5th day onwards there was a gradual and steady increase in the activity of this enzyme in both the components of the gizzard with an ultimate maximum being attained on the 20th day. This high activity continued to be the distinct feature of gizzard development hereafter and remained so even in the adult condition. Though there was a parallel increase of enzyme activity in both the mucosal tubules as well as the muscle fibres the latter revealed a slightly higher concentration of enzyme than the former on a comparative basis.

ACETYLCHOLINESTERASE ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD



200 μ

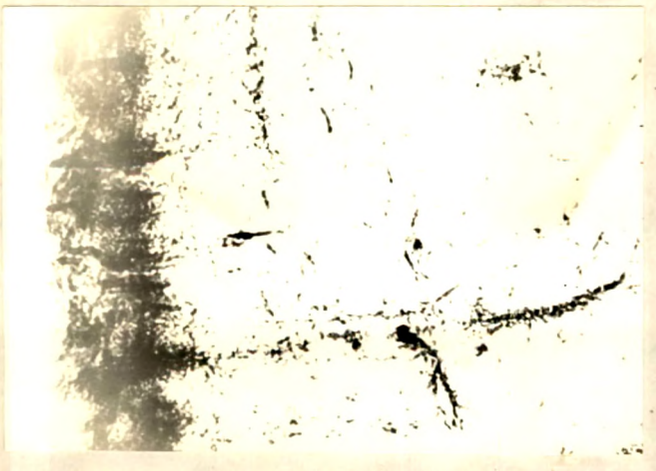
1 DAY OLD

1

200 μ

5 DAY OLD

2

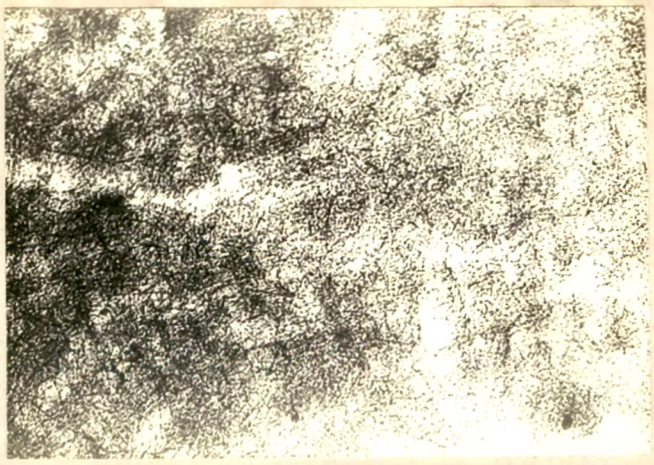


200 μ

10 DAY OLD

3

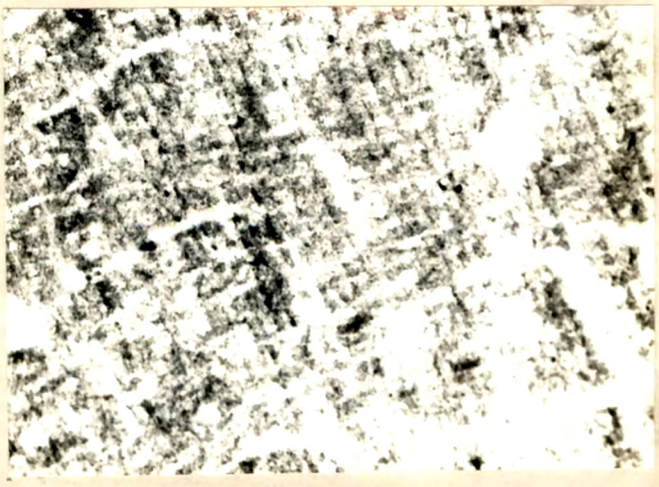
ACETYLCHOLINESTERASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200 μ

1 DAY OLD

1a



200 μ

5 DAY OLD

2a

200 μ

10 DAY OLD

3a

ACETYLCHOLINESTERASE ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD

200 μ

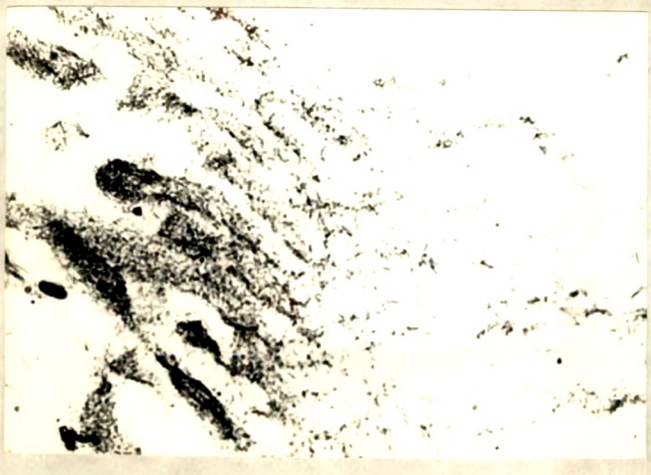
15 DAY OLD

4

200 μ

20 DAY OLD

5

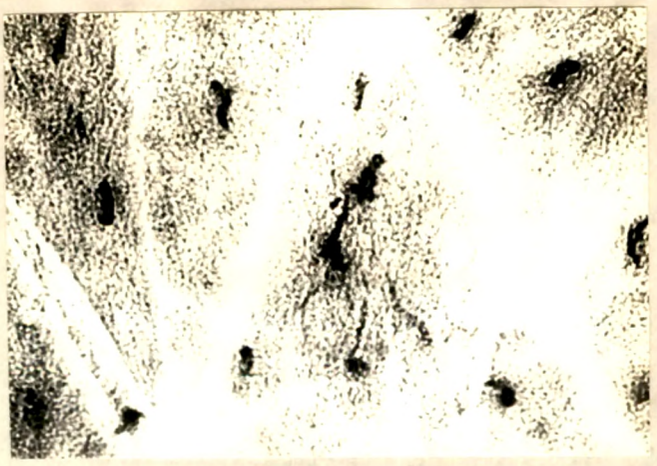


200 μ

25 DAY OLD

6

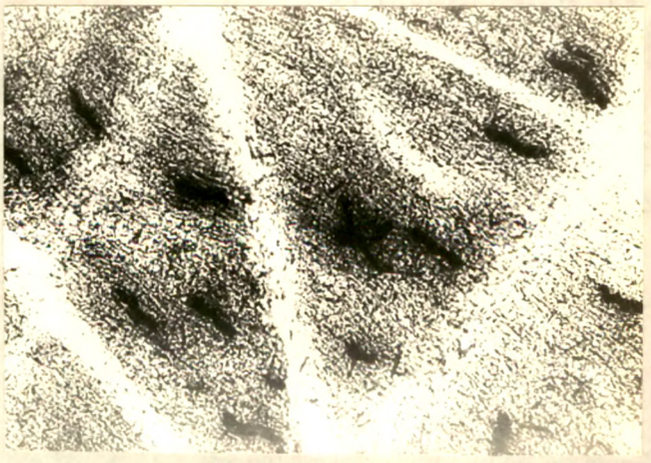
ACETYLCHOLINESTERASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200 μ

15 DAY OLD

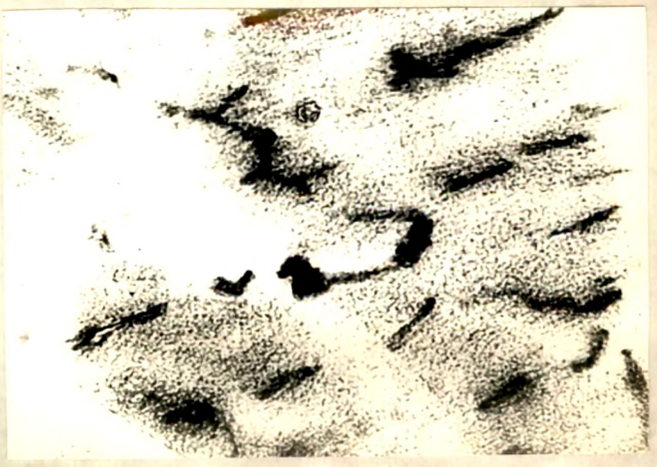
4a



200 μ

29 DAY OLD

5a

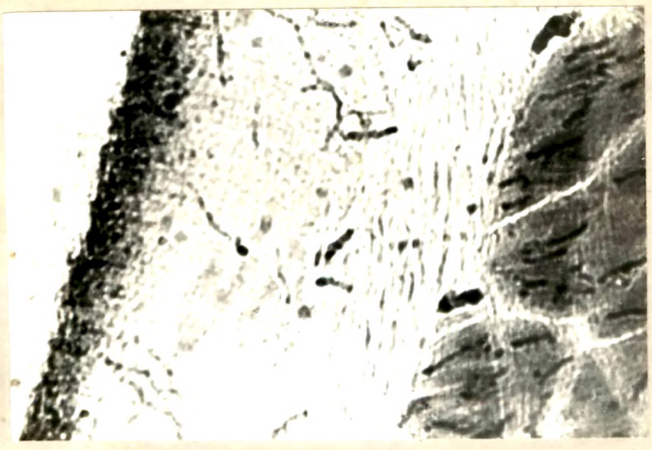


200 μ

25 DAY OLD

6a

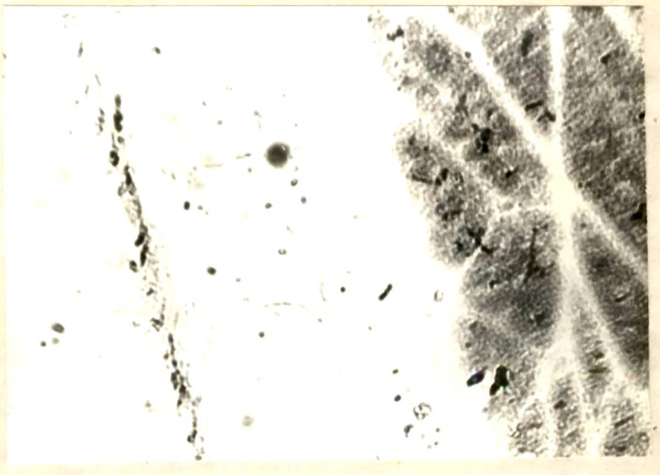
ACETYLCHOLINESTERASE ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD



200 μ

30 DAY OLD

7

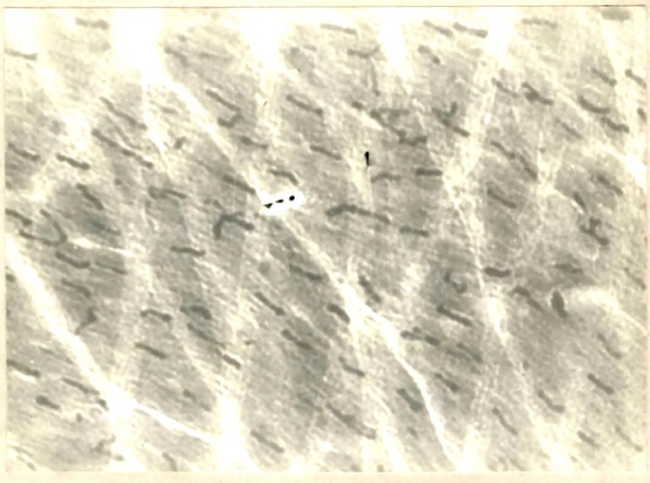


200 μ

ADULT

8

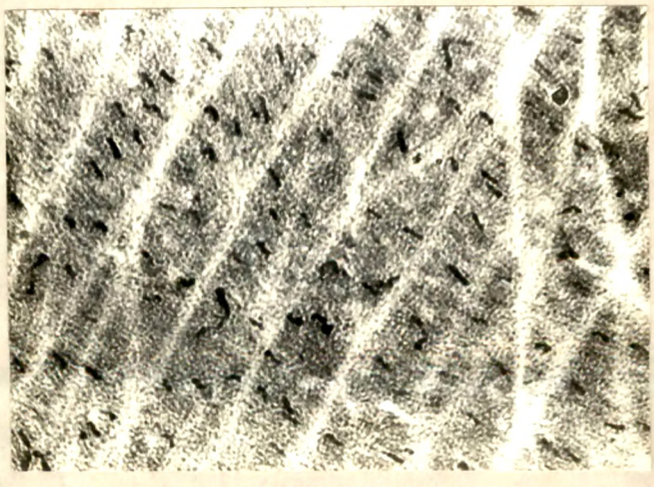
ACETYLCHOLINESTERASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200 μ

30 DAY OLD

7a



200 μ

ADULT

8a

DISCUSSION

The present investigation on cholinesterases in the developing (post-natal) pigeon gizzard seems to highlight two points: (1) a complete absence of BuChE all throughout the development and in the adult and (2) a gradual and steady increase of AChE from the day of hatching till the attainment of the adult level on the 20th day. On a comparative basis the activity of AChE appeared to be slightly more in the smooth muscle fibres than in the secretory tubules. Based on their studies on cholinesterases Chinoy and George (1965, 1966) claimed that AChE is characteristic of active tonic and narrow fibres and that BuChE is associated with the tetanic and quick contracting white fibres of pigeon pectoralis muscle. Though such a distinction has been made on pigeon pectoralis, Radhakrishnan (1972) could observe more AChE activity compared to that of BuChE in the caudal muscles of Scincid lizard, Mabuya carinata. It is interesting to note at this juncture a predominance of AChE activity in the smooth muscle fibres of the gizzard in the wake of the above observations.

It has been reported (~~Rom.aoff~~ Kuo & Shen, 1936, ~~1960~~) that the peristaltic contraction of the smooth muscle complex of proventriculus and gizzard of chick embryos first occur

on the 8th or 9th days of incubation. But Bennett and Cobb (1969), based on their work on developing chicken gizzard, are of opinion that the myoblasts at that stage are still primitive in appearance and contain few myofilaments. It is not known as to when the innervation of gizzard becomes effective but the ultrastructural relationship between axon and myoblasts in the gizzard of chick embryos appears to be as intimate as those between axons and smooth muscle cells in the gizzard of mature animals. The observation of these workers on the AChE activity of the nerves associated with myoblasts of chicken gizzard revealed the fact that the innervation may not be functional in terms of transmission before that time although the nerves may have trophic effects and influence on the developing musculature. Yamauchi and Burnstock (1969b) have made similar observations on the post-natal development of innervation of the vas deferens of the mouse and likewise concluded that the innervation may not be functional in the early stages.

The presently observed gradual increase of AChE from the day of hatching till the 15th day may be ascribed to a similar function indicative of a possible role of this enzyme in the progressive structural differentiation of the smooth muscle towards the attainment of increasing functional

maturity. Such an increase of AChE activity corresponding to myogenesis during the muscle development has been reported by Verga et al., (1957). Similarly a close association of AChE in the process of differentiation during the development of various embryos and in regeneration were reported by Sawyer (1955); Boelle (1948, 1955) and Radhakrishnan (1972). The highest activity of AChE is recorded during the 20th day of development and this level is found to be maintained not only through the rest of the days of development but also in the adult condition. The period of attainment of this high level of AChE activity, i.e., during the 20th and 25th days of development, is also the period when solid grains are taken for the first time by the bird. This coincidence of attainment of the peak value of AChE and the intake of solid grains at the same period are indicative of a probable function of nerves in the full attainment of the mechanical function of the gizzard. Since the same level of activity is more or less maintained both hereafter as well as in the adult condition, it appears that in the working of the gizzard there is a nervous involvement. Thus, it may be safely assumed from herein that in the pigeon gizzard during its post-natal development, acetylcholine has a trophic effect on development and when the adult threshold level of AChE is reached i.e., about 20th day the gizzard commences its mechanical function in full swing.