QUANTITATIVE EVALUATION AND HISTOCHEMICAL PROFILE OF MYOGLOBIN IN THE STOMACH COMPLEX OF SOME DEVELOPING AND ADULT BIRDS.

CHAPTER IV

QUANTITATIVE EVALUATION AND HISTOCHEMICAL PROFILE OF MYOGLOBIN IN THE STOMACH COMPLEX OF SOME DEVELOPING AND ADULT BIRDS.

Myoglobin is a reddish brown muscle sarcoplasmic chromoprotein consisting a heme group attached to a globin. Its role as an oxygen store in diving mammals has long been known. Kühne (1863) was the first person to show spectroscopically that myoglobin is present in large quantities in the dark red muscles. Millikan (1939) has shown that myoglobin occurs in animals that require vigorous and sustained muscular activity. Lawrie (1952) also drew attention to the higher concentration of myoglobin in the red muscles. Myoglobin, if present in sufficient quantity, is capable of taking over a substantial part of the burden of oxygen transport in the cells whenever the pressure of oxygen drops (Wymann, 1966). Bokdawala and George (1967) and Grinyer and George (1969) adduced evidence to the effect that the occurrence of more myoglobin, fat and lipase in the red muscle in contrast to the white, is a rejection of the higher metabolic activity of the former. Involuntary or smooth muscle is morphologically characterized by an absence of cross striations and a pale pink colour due to lack of myoglobin (Blessing and Müller, 1974). While smooth muscle usually lacks myoglobin the ventriculus of some birds, particularly members of the family galliformes, is a notable exception (Patak and Baldwin, 1988).

When histochemically demonstrated, Morita *et al.* (1969) observed in pig striated muscle that myoglobin is responsible in large part for the colour of muscle and serve as a storage organ for oxygen, being therefore important to metabolism of the cell. Various attempts have been made to localize myoglobin in skeletal muscles of vertebrates (Drews and Engel, 1961; Chinoy, 1963; Goldfisher, 1967; Kagen and Gurevich, 1967; James, 1968 and Morita *et al.*, 1969). The purpose of this study was therefore, to investigate differences, if any, in the myoglobin concentration and distribution in proventriculus and ventriculus of two developing and adult precocial and few adult altricial birds and to compare and comment on the importance of these in the general physiology of the organs.

MATERIALS AND METHODS

Newly hatched chicks of Domestic fowl (RIR variety) were obtained from Government Poultry

Farm, Baroda and newly hatched chicks of Japanese quails were obtained from Poultry Farm, Mualia, Dahod respectively. They were reared in a well maintained aviary of Department of Zoology, M.S. University of Baroda till adulthood was attained. Care and maintenance of adult birds (as mentioned in chapter I) were also taken.

Young ones of fowl and quail of different age groups *viz.*, 0, 5, 10, 20 and 30 days as well as adults of fowl, quail, swift, sparrow and pigeon were used for the present study.

I. Biochemical Analysis

For the biochemical analysis known amount of proventriculus ventriculus and muscle (pectoralis) were homogenized in cold chilled mortars following the method of Reynafarge (1962).

II. Histochemical Analysis

Proventriculus and ventriculus from the young ones of different age groups (as mentioned above) as well as adults were separated after decapitation, blotted well to remove their contents, blood and other tissue fluids and fixed on a chuck of a cryostat microtome maintained at - 20°C. Sections of 12 and 15 μ thickness were cut and processed as suggested by James (1968) for the histochemical observation of myoglobin.

RESULTS

<u>Proventriculus</u> (Tables 1, 2; Figures 1a, 1b).

Biochemical analysis showed that in fowl there was a slow and gradual increase in the myoglobin concentration from the day of hatching till day 20, but thereafter this increase was sudden and reached the peak level in adult. In case of quail, the low level of myoglobin registered on the day of hatching gradually increased till day 30 and reached the highest level in the adult. On a comparative basis, the level on day 0 was more in fowl than in quail and in the adulthood, a slightly higher elevated concentration was recorded in quail than in fowl. Among adult birds the precocial ones exhibits more myoglobin concentration than the altricial birds with pigeon, sparrow and swift registering such low activities in that order.

Ventriculus (Tables 1, 2; Figures 1a, 1b).

In fowl the concentration of myoglobin was negligible on day 0 of development and rose by half on day 5 and further on day 10 of development. This level was maintained on day 20 however, the concentration almost doubled on day 30 and this high concentration was retained in adulthood. In case of quail, the concentration was negligible during the initial days which gradually increased during the rest of the developing periods and registered a moderate concentration in the adult. This level of myoglobin concentration was appreciably lower than the concentration observed in fowl. Among the precocial birds, it was observed that, the concentration of myoglobin was higher in fowl than in quail. In altricial birds, the content was low in swift and sparrow and medium in pigeon.

Pectoralis muscle (Tables 1, 2; Figures 1a, 1b).

The low (7.30 mg/gm) value of myoglobin concentration registered on the day of hatching in the fowl gradually rose and reached a moderate content (26.83 mg/gm) in the adult. In quail also such a slow and gradual increase was noted, but the values were far lower than those observed for fowl. In case of precocial birds, the quail recorded 13.64 mg/gm of myoglobin concentration whereas the amount was almost double the concentration in fowl (26.83 mg/gm). Among the altricial birds, swift and sparrow recorded the highest value when compared to pigeon.

Histochemical Observations (Plates I, II, III; Tables 3, 4).

When histochemically demonstrated, it was observed that the myoglobin activity was more or less uniformly distributed in all the elements and components of the proventriculus and ventriculus of all the developing and adult birds. However, on a comparative basis the multiple glands and outer layer of the proventriculus of fowl registered slightly more enzyme reaction than one observed in quail. In case of adult altricial birds, highest enzyme reaction was observed in the multiple glands and outer layer of pigeon followed by sparrow and swift. As far as

Table 1. Myoglobin* concentration in proventriculus, ventriculus and muscle** of developing and adult fowl and quail.

AGE (DAYS)	PROVENTRICULUS		VENTRICULUS		MUSCLE	
	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL
0	14.98 ± 2.50@	8.48 ± 0.70	6.25 ± 0.32	6.15 ± 0.40	7.30 ± 0.42	6.00 ± 0.20
5	20.23 ± 1.67	10.56 ± 1.14	9.27 ± 0.57	6.99 ± 0.52	10.71 ± 0.40	6.69 ± 0.31
10	21.72 ± 1.59	18.75 ± 1.51	12.94 ± 0.97	8.94 ± 0.50	15.42 ± 1.51	6.89 ± 0.60
20	24.55 ± 2.30	19.79 ± 1.18	14.08 ± 1.14	9.67 ± 0.51	18.40 ± 1.06	7.04 ± 0.54
30	42.86 ± 2.50	24.95 ± 1.79	14.23 ± 1.42	10.11 ± 0.42	18.55 ± 1.02	10.02 ± 0.52
ADULT	45.63 ± 3.60	49.35 ± 3.40	25.00 ± 1.55	13.88 ± 0.50	26.83 ± 2.24	13.64 ± 0.73

* mg/gm wet tissue.
** Pectoralis.
@ Values expressed as mean ± SEM of six animals.

n = ?

Table 2. Myoglobin* concentration in proventriculus, ventriculus and muscle** of adult birds.

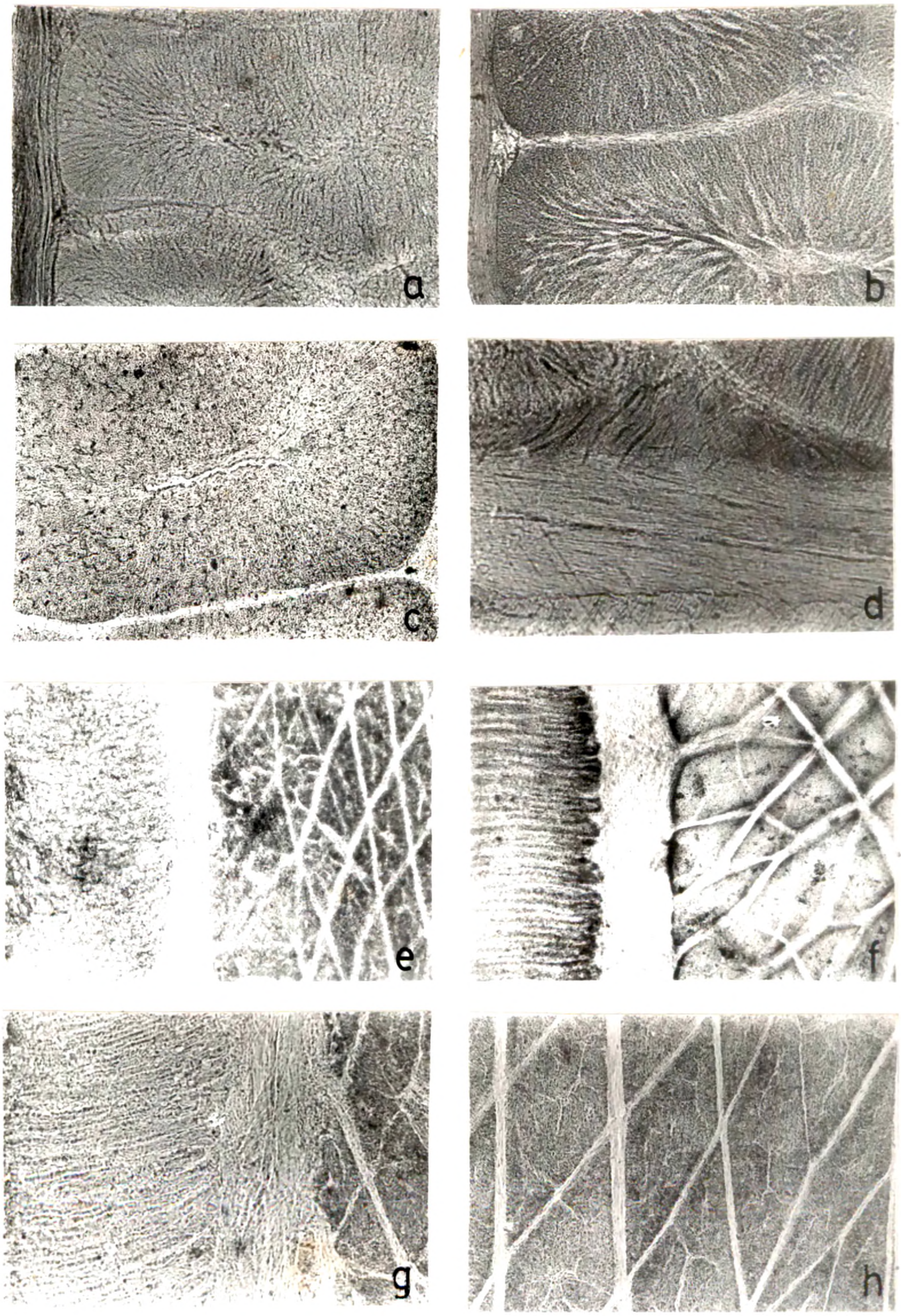
ANIMAL MODEL	PROVENTRICULUS	VENTRICULUS	MUSCLE
SWIFT	25.04 ± 1.05@	3.62 ± 0.22	108.38 ± 5.36
SPARROW	28.97 ± 2.60	5.02 ± 0.29	101.76 ± 4.32
QUAIL	49.35 ± 3.40	13.88 ± 0.50	13.64 ± 0.73
PIGEON	35.91 ± 1.65	7.53 ± 0.36	29.46 ± 2.28
FOWL	45.63 ± 3.60	25.00 ± 1.55	26.83 ± 2.24

* mg/gm wet tissue.
** Pectoralis.
@ Values expressed as mean ± SEM of six animals.

EXPLANATION TO PLATE

Plate I: Photomicrographs of sections of the proventriculus and ventriculus of developing and adult domestic fowl showing myoglobin activity.

a. 0 day old proventriculus (64x).
b. 20 day old proventriculus (64x).
c. Adult proventriculus [Multiple gland] (64x).
d. Adult proventriculus [Outer layer] (64x).
e. 0 day old ventriculus (64x).
f. 10 day old ventriculus (64x).
g. Adult ventriculus [Mucosal tubules] (64x).
h. Adult ventriculus [Muscles] (64x).


a
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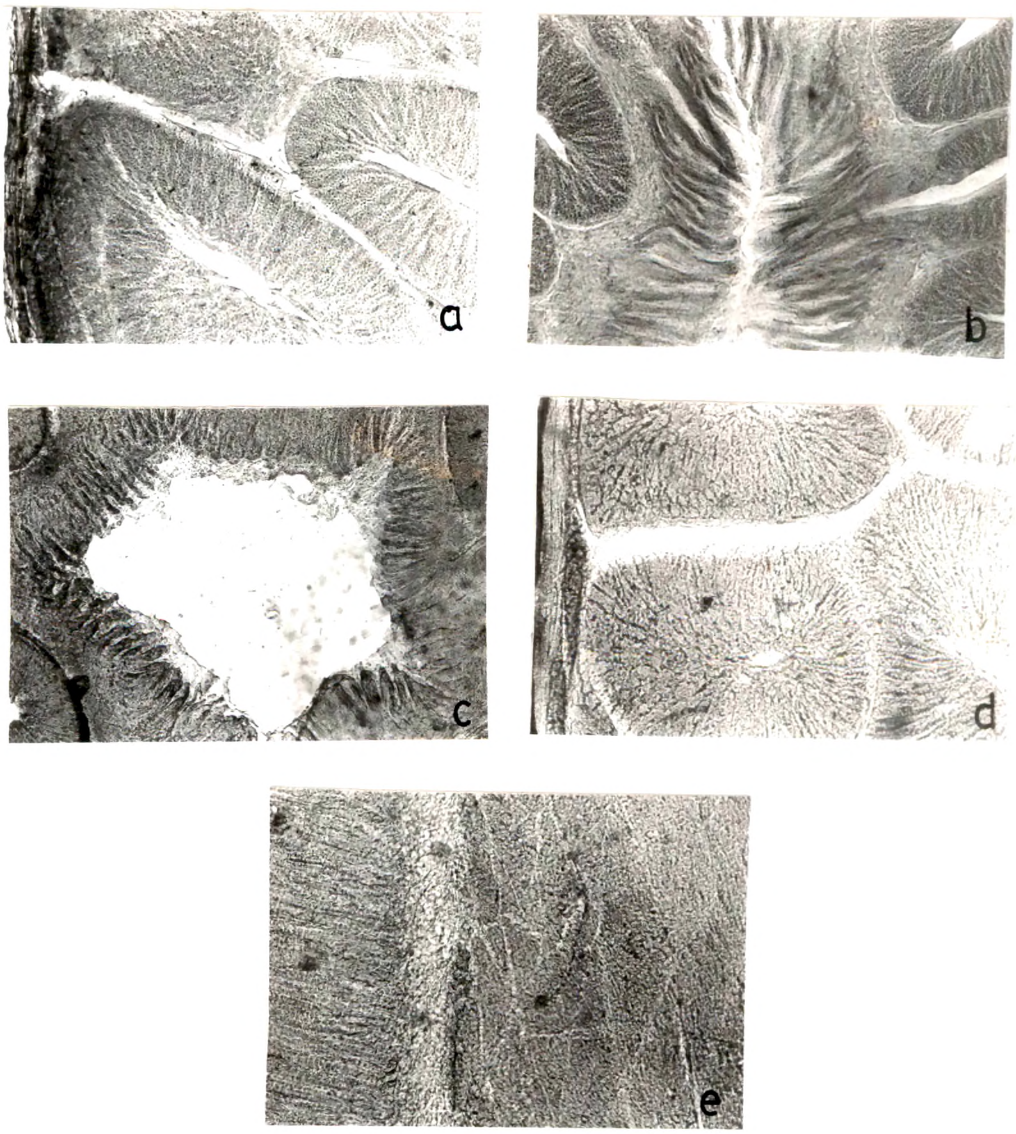
EXPLANATION TO PLATE

Plate II. Photomicrographs of sections of the proventriculus and ventriculus of the developing and adult Japanese quail showing myoglobin activity.

a. 0 day old proventriculus (64x).
b. 0 day old proventriculus [Note the narrow lumen space] (25x).
c. 20 day old proventriculus [Note the wide lumen space] (25x).
d. Adult proventriculus (64x).
e. 20 day old ventriculus (64x).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EXPLANATION TO PLATE

Plate III. Photomicrographs of sections of the proventriculus and ventriculus of certain adult species showing myoglobin activity.

a. Swift proventriculus (128x).
b. Swift ventriculus (128x).
c. Sparrow proventriculus (128x).
d. Sparrow ventriculus (128x).
e. Pigeon proventriculus (104x).
f. Pigeon ventriculus (64x).

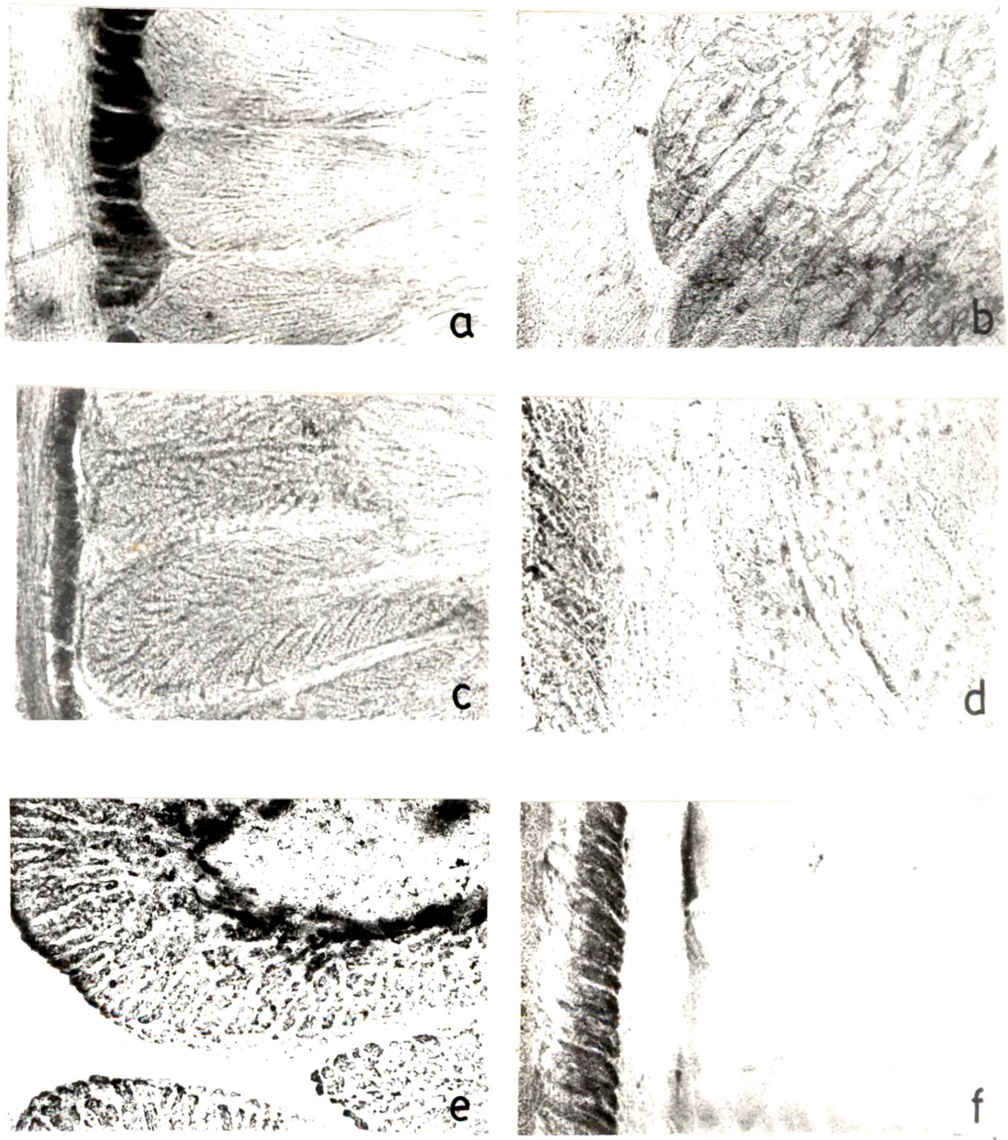
a
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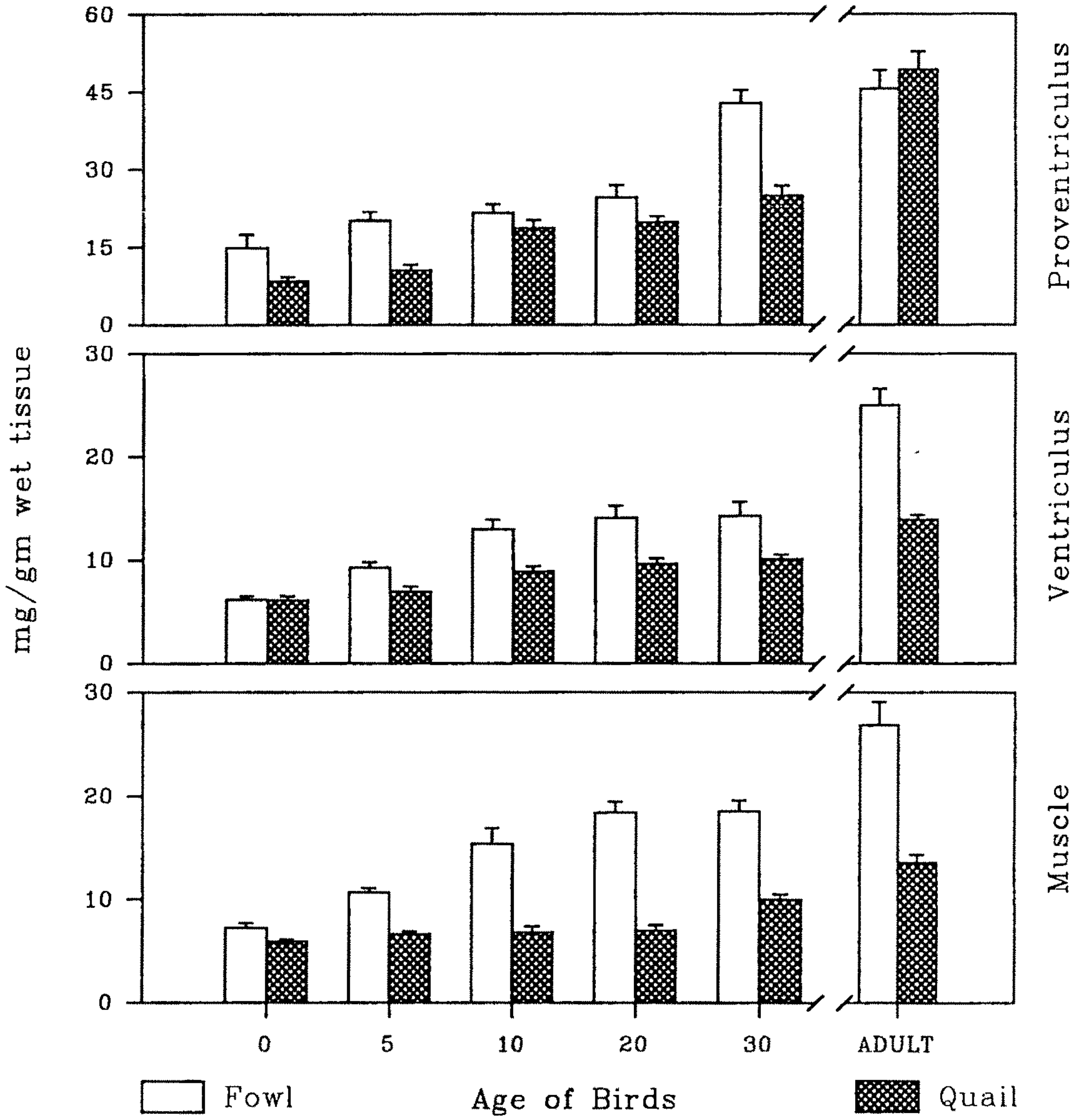
Table 3. Intensity of histochemical reactivity of myoglobin activity in proventriculus and ventriculus of developing and adult fowl and quail.

AGE (DAYS)	MYOGLOBIN ACTIVITY									
	PROVENTRICULUS				VENTRICULUS					
	MULTIPLE GLAND		OUTER LAYER		MUCOSAL TUBULE		SUBMUCOSAL CONNECTIVE TISSUE LAYER		MUSCLE LAYER	
	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL	FOWL	QUAIL
0	+±	+	++	+	+	+	±	±	+	+
5	++	+	++	+	+	+	±	+	+	+
10	++	+±	++±	+±	+	+	+	±	+	+
20	+++	++±	++±	++±	+±	+	+	+	+±	+
30	+++	++±	+±	++±	+±	+±	+	+	+±	+±
ADULT	+++	+++	+++	+++	+++	++	+±	+±	++	++

Table 4. Intensity of histochemical reactivity of myoglobin activity in proventriculus and ventriculus of certain adult birds.

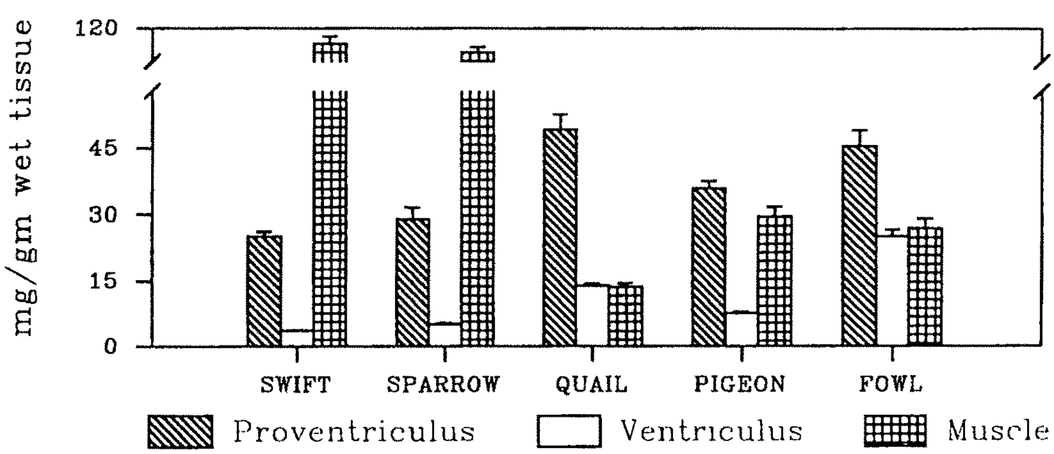
ANIMAL MODEL	MYOGLOBIN ACTIVITY				
	PROVENTRICULUS		VENTRICULUS		
	MULTIPLE GLAND	OUTER LAYER	MUCOSAL TUBULE	SUBMUCOSAL CONNECTIVE TISSUE LAYER	MUSCLE LAYER
SWIFT	+±	+	±	-	+
SPARROW	+±	±	-	-	+
QUAIL	+++	+++	++	+±	++
PIGEON	++±	+±	+	±	+±
FOWL	+++	+++	+++	+±	++

Fig 1a. Myoglobin concentration in growing birds.



n = ?

Fig 1b. Myoglobin concentration in adult birds.



n = ?

ventriculus of developing birds is concerned, myoglobin concentration was low in all the components of the ventriculus on the day of hatching and registered a progressive increase during the successive developing stages. In the ventriculus of adult altricial birds, an identical pattern to the one observed in proventriculus was the feature.

DISCUSSION

The concentration of myoglobin in a muscle is generally regarded as an index of capacity of the muscle for aerobic metabolism and sustained activity. Furthermore, the myoglobin containing muscle is physiologically slow or tonic muscles and the myoglobin lacking muscle is fast or phasic muscle (George and Berger, 1966; McPherson and Tokanaga, 1967). Such a contention has also been made by Burke *et al.* (1971) and Peter *et al.* (1972).

The skeletal muscles performing sustained work depend on aerobic adenosine triphosphate (ATP) production (Hochachka and Somero, 1984). The availability of ATP for various muscular activities and the metabolism of glycogen and lipids in proventriculus and ventriculus of developing and adult precocial and altricial birds have been discussed in chapters III and V. When viewed in the wake of above observations, the steady increase in the concentration of myoglobin noticed in the stomach complex of fowl and quail is rather self explanatory and points to the present contention that higher elevated myoglobin concentration is to meet the extra oxygen demand the organ needed to grow and attain functional maturity. Such an increase in the myoglobin content in the penguins has been reported during the transition from terrestrial to sea-life which coincides with the onset of swimming and diving practice (Webber *et al.*, 1974). In addition to the oxygen storage function, the myoglobin may serve to furnish oxygen to the cells during the fluctuations in oxygen demands between the contractions and relaxations (Barcroft, 1934; Millikan, 1939) or may serve to facilitate the diffusion of oxygen from the capillary blood to the muscle mitochondria (Butler and Jones, 1982; Pages and Planas, 1982; Gayeski and Honig, 1986).

A considerable high content of myoglobin has been reported in the chicken gizzard muscles compared with the skeletal muscle (Nishida and Nishida, 1978). In the present study, it is observed that the proventriculus registered the highest concentration of myoglobin and the ventriculus and pectoral muscles registered a slightly lower concentration of the same during different days of development. In any case the myoglobin concentration in proventriculus was never lower than the amount observed in ventriculus. Nishida and Nishida (1978, 1985) observed a higher concentration of myoglobin in the ventriculus than in the skeletal muscles. The elevated concentration of myoglobin observed in the proventriculus is related to the continuous and rhythmic contractions of the organ, whereas the slightly lower concentration of myoglobin is expected in the ventriculus, as the organ contracts at a rate of 2 to 3 powerful contractions per minute to expel the food in to the intestine (Ziswiler and Farner, 1972; Hill and Strachan, 1975; Sturkie, 1986). The skeletal and cardiac muscle contract continuously and rhythmically as do the stomach muscles when the birds take the food, but a more heavier contraction should be maintained during prolonged repetitive digestion of food. Thus, it may be required that the proventriculus and ventriculus should contain large amount of myoglobin to reserve adequate oxygen for aerobic energy rich phosphate synthesis. Myoglobin concentration in a muscle is usually associated with the active catabolic activity (Baldwin and Patak, 1988). More the myoglobin, less its capacity for glycolysis, but greater its capacity to use oxidative metabolism and to synthesize ATP (Lawrie, 1953 a, b). The variations in the concentration of myoglobin observed in proventriculus and ventriculus of these two developing and adult precocial birds are related to the species specificity and do not show any uniformity in their distribution which is conformity with the observations made by Weber *et al.*(1974) in penguin.

In the birds with precocial mode of development (fowl and quail) a very high level of myoglobin was noted in the proventriculus and ventriculus. Amongst the altricial birds, pigeon stomach registered an elevated myoglobin content. These birds basically feed on hard food items (predominantly on grains) and as reported earlier, heavier contraction of the stomach muscles should be maintained during prolonged repetitive activity for digestion of hard food. As far as the other two birds, sparrow and swift (altricial mode of development) are concerned, slightly reduced content of myoglobin was noted in stomach complex. This low concentration is in conformity with the relatively soft food these birds consume and consequently the heavy contractions noted in those birds taking harder food are not needed to process the food materials. Blessing and Muller (1974) added that the breast muscles in chicken, which are known to be almost white to the naked eye contain the lowest amount of myoglobin. The present results obtained for the pectoral muscle of pigeon depicted a higher amount of myoglobin. This higher amount is attributed to the red colour of the fibres largely due to the presence of oxygen carrying compounds myoglobin and cytochrome (which are apparently absent or very rare in white muscle) and also their higher level of flight activity (Lawrie, 1952; George and Naik, 1960; Bokdawala and George, 1967; Grinyer and George, 1969). In case of swift and sparrow, the myoglobin content in the pectoralis muscle was noted to be very high and such high content of myoglobin in the flight muscle of humming bird (Johansen *et al.*, 1987), pigeon guillemot (Haggblom *et al.*, 1988) and barheaded geese (Saunders and Fedde, 1991)has been reported. Hence the high concentration of myoglobin in these birds (swift and sparrow) is attributed to the flight activity of the pectoralis muscle.

Considering the facts described above, as well as the data presented in the present study, it could be noted that the stomach complex of precocial and altricial birds is myoglobin rich in its characterization. The relatively high concentration of myoglobin is related with the high capacity to deliver oxygen to the tissues and with the sustained contractility of this organ and also to synthesize energy rich phosphate for its effective functioning.