A Chemocytological Investigation of the Avian Adrenal Medulla

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Introduction

While studying the distribution and quantity of cytochemically demonstrable catechol hormones in nine avian species, Ghosh and his collaborator (Ghosh 1962, Ghosh and Ghosh 1962) noted certain phylogenetic trends. Thus, cormorants, egrets and fowl which are possibly representatives of primitive natural orders—their adrenals preponderantly synthesise noradrenaline. The recently evolved passerine birds, on the other hand, are principally adrenaline-producers. The cuckoos and pigeons occupying almost an intermediate rank in the evolutionary scale, manufacture both methylated and non-methylated catechol hormones in practically equal proportions. These findings led the authors to suggest that an efficacious accomplishment of methylation from noradrenaline (NA) to adrenaline (A) was possibly established through birds' evolutionary ascent. As the data in this field were rather insufficient so far as the authors are aware, it is desirable to open up the problem once again by investigating upon few more avian species. The present report has been concerned with an attempt to explore the distribution and proportions of cytochemically detectable A and NA-containing cells in the medullary tissue of twelve avian species representing hitherto uninvestigated phylogenetic orders (barring Passeriformes) and families.

In one of the above mentioned previous papers (Ghosh and Ghosh 1962) it has been pointed out that acid phosphatase, metachromatic substances and plasmalogen may constitute the 'fundamental cytochemical unit' of the avian adrenal medulla. Their probable role in avian medullary physiology has also been emphasised by Ray and Ghosh (1961). In order to ascertain whether these microchemical entities are of universal occurrence in the avian adrenal medulla, the present writers have decided to demonstrate cytochemically the presence of acid phosphatase, metachromatic materials and plasmalogen in the chromaffin tissue of those avian species in which the catechol contents will also be estimated.

Materials and methods

Mainly adult birds (male) were used in the present study. The birds used in this

investigation may be classified (Biswas 1953, Ali 1955) under the following orders and families:

Order	*Family*	*Species*
ANSERIFORMES	Anatidae	*Anas querquedula* (Linnaeus)
FALCONIFORMES	Accipitridae	*Milvus migrans* (Boddaert)
GRUIFORMES	Rallidae	*Amaurornis phoenicurus* (Pennant)
PSITTACIFORMES	Psittacidae	*Psittacula krameri* (Scopoli)
STRIGIFORMES	Strigidae	*Athene brama* (Temminck)
CORACIFORMES	Coraciidae	*Halcyon smyrnensis* (Linnaeus)
PICIFORMES	Picidae	*Brachypternus benghalensis* (Linnaeus)
PASSERIFORMES	Dicruridae	*Dicrurus macrocercus* (Vieillot)
	Necterinidae	*Necterina asiatica* (Illiger)
	Oriolidae	*Oriolus oriolus* (Linnaeus)
	Muscicapidae	*Turdoides somervillei* (Sykes)
	Zosteropidae	*Zosterops palpebrosa* (Temminck and Schlegel)

The birds were killed by chloroform anaesthesia. The glands were taken out immediately after autopsy and were plunged into appropriate fixatives for various cytochemical studies.

Chromate-dichromate and iodate methods were employed to observe the total catechol hormone containing and noradrenaline secreting cells respectively (Hillarp and Hökfelt 1955). Finally, the outlines of these cellular areas were traced with the aid of a camera lucida and measured by means of a 'Allbrit' disc planimeter. Metachromatic substances were demonstrated in glands fixed in Bouin's fixative and stained with 0.05% toluidine blue solution buffered at a pH ranging from 6-8 (Montagna *et al.* 1951). To visualise plasmal materials, unfixed frozen sections (10–15 μ) were treated with 1% mercuric chloride solution and Schiff reagent in a sequential manner (Hayes 1949). Acid phosphatase was demonstrated in formal-aceto-alcohol fixed adrenals by the application of Gomori's lead phosphate technique (Gomori 1952).

Table 1. Percentages of NA and A-containing cells and ratio of Cortex: Medulla in 12 avian species

Species	% NA	% A	C:M
A. querquedula	65	35	5:1
M. migrans	65	35	3:1
A. phoenicurus	47	53	2:1
P. krameri	37	63	3:1
A. brama	49	51	2:1
H. smyrnensis	51	49	5:1
B. benghalensis	64	36	5:1
D. macrocercus	25	75	4:1
N. asiatica	21	79	2:1
O. *oriolus*	21	79	3:1
T. somervillei	15	85	2:1
Z. palpebrosa	15	85	4:1

Results and discussion

Camera lucida drawings of chromate-dichromate as well as iodate positive medullary tissues of all the avian species described here have been incorporated in Figs. 1–24. Planimetric measurements of these areas reveal a great variation in percentages of A and NA-containing cellular areas (Table 1). In general,

the present data also corroborate the earlier findings reported from this laboratory (Ghosh 1962, Ghosh and Ghosh 1962). Simulating these studies, a phylogenetic trend is also noticeable here when relative percentages of A

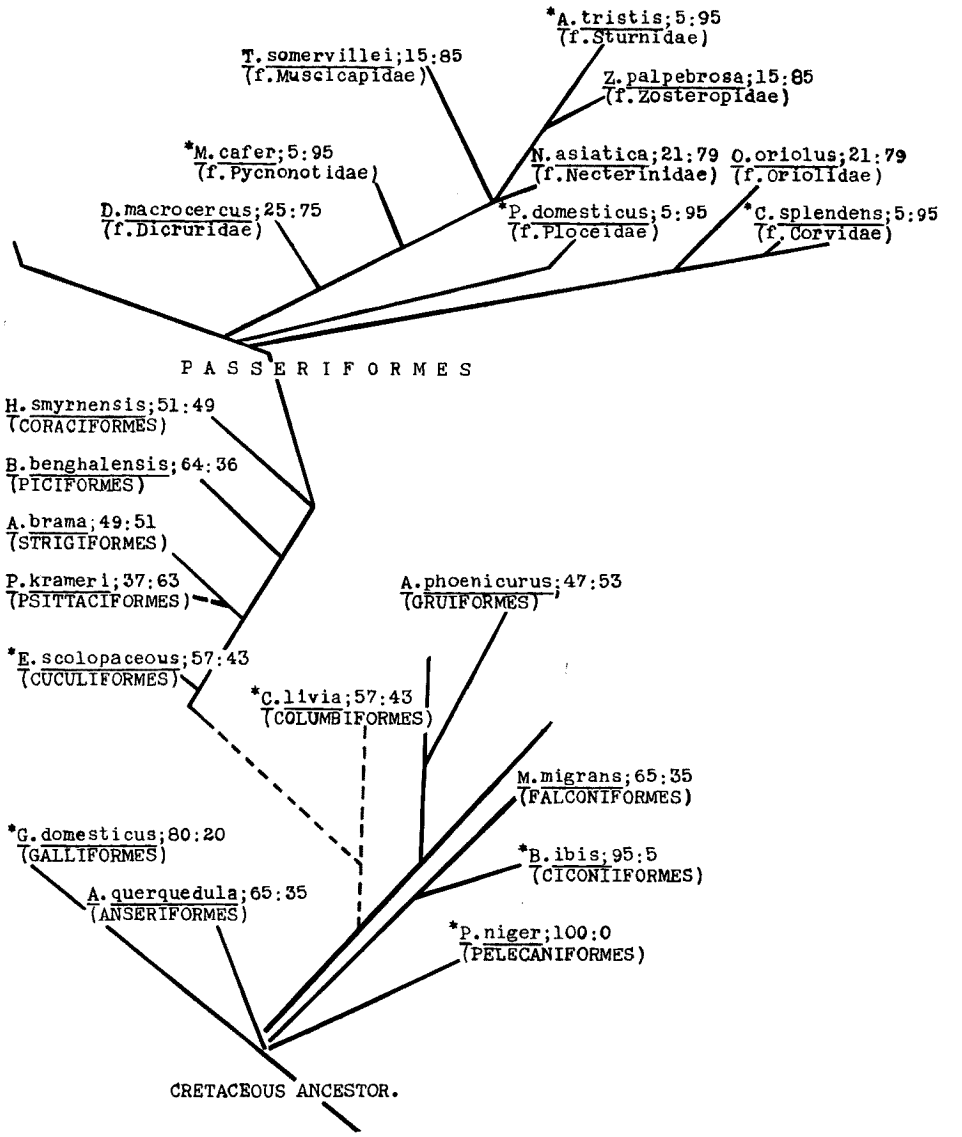

Chart 1. Phylogeny of those birds included in this investigation (After Gregory, 1957). The relative proportions of adrenaline and noradrenaline have also been shown against each species. Quantitative data of catechol hormones of birds with asteriks (*) are incorporated from Ghosh and Ghosh, 1962. Numericals on left indicate noradrenaline.

and NA-containing cells are plotted against the suggested avian family tree (Chart 1). Howevr, *B. benghalensis* (Ord. Piciformes) and *H. smyrnensis* (Ord. Coraciformes) manifest definite divergence from the proposed taxonomy

based upon the degree of hormonal methylation. According to this concept, these two species representing somewhat recent origin in avian ancestry should not contain so much of non-methylated medullary hormones. Though this is an inconvenient fact, yet biochemical diversity of similar nature is not at

Figs. 1-24. Camera lucida drawings of chromate-dichromate and iodate positive areas in 12 avian species. Odd numbered figs. in the serial order represent chromaffin patterns of *A. querquedula, M. migrans, A. phoenicurus, P. krameri, A. brama, H. smyrnensis, B. benghalensis, D. macrocercus, N. asiatica, O. oriolus, T. somervillei* and *Z. palpebrosa* respectively; even numbered ones show the iodate positive medullary tissues of these species.

all uncommon in the phylogenetic history of animals (Florkin 1960).

As the present interpretation can not explain certain anomalies, it is our desire to look for other probable interpretations:

In some animal species, Shephered and West (1951) have observed correlation between proportions of A and NA and the ratio of cortical size to medullary size. Our data on the ratio of cortical and chromaffin areas (Table 1) does not disclose such a fact. To exemplify this, the cortex-medulla ratio of *M. migrans* is 3 : 1 and its relative percentage of NA and A-containing cells is 65 and 35 respectively, while *O. oriolus* having the same cortico-medullary ratio possesses only 21% noradrenaline cells and 79% adrenaline cells. It may not be out of place to mention here that the degree of intermingling of cortical and medullary tissues does not also in any way determine the relative percentage of avian catechol hormones (Ghosh 1962).

An association between aggressiveness and high rate of noradrenaline secretion has been indicated by von Euler (1958). Previous to this, Goodall (1951) suggested that in aggressive animals, such as cat and the lion, the relative noradrenaline content is higher than in non-aggressive species like primates, rodents and ruminants, which obviously are in greater need of the emergency hormone — adrenaline. Possibly keeping Goodall and von Euler's views in mind, Etkin (1962) seemed to believe in a correlation between the A/NA ratio and the predatory behavior of birds. According to him, preponderance of NA in medulla of Ibis and Cormorants may be related to their predatory nature, while dominance of A in small passerine birds may be due to a non-predatory behavior of these species. Our findings as well as that of Ghosh and Ghosh (1962) are not in agreement with the concept of previous authors. Thus, non-predators like fowl, pigeon, woodpeckers, *etc.* are principally noradrenaline secretors, while a complete predator, *e.g.*, the owl (Wallace 1955) and a partial predator like the crow (Ali 1955) are preponderantly adrenaline producers. Therefore, the extent of methylation of avian medullary hormones is in all probability independent of behaviourial pattern (with particular reference to aggressiveness and predation) of a species.

In view of the above discussion, the variability of catechol hormones throughout the avian class may possibly be best interpreted (though not in a completely satisfactory manner), if the phylogenetic history of this group is taken into account.

Our observations on the concentration and distribution of plasmalogen, acid phosphatase and metachromatic substances in adrenal medulla of birds under report have been summarized in Table 2. A persual of this table leads us to support the previous concept of Ghosh and Ghosh (1962). In the nine avian species studied by these authors, the acid phosphatase, metachromatic materials and plasmalogen are alleged to constitute the 'basic cytochemical unit' of their medullary tissue. The present data also show an almost universal occurrence of these materials in the chromaffin tissue of birds under report. The two species (*A. phoenicurus* and *H. smyrnensis*), however, are characterised by a complete absence of metachromatic substances. These findings should possibly be considered as exceptions — and

the generalized view of Ghosh and Ghosh may not be discarded. The negative metachromasia in these two species gives us a further insight as to the chemical nature of the medullary metachromatic material. The suggestion that metachromasia may be due to the presence of plasmal materials (Ray and Ghosh 1961) may be invalidated at least in cases of the waterhen and kingfishers.

Table 2. Distribution and concentration of cytochemical components (including NA and A) in medullary cords of 12 avian species

Species	Acid phosph.	Metachrom.	Plasmal	Chr. -dichr.	Iodate
A. querquedula	3+	3+	4+	3+	3+
M. migrans	2+	2+/p	3+	3+	3+
A. phoenicurus	2+	—	3+	4+	3+
A. brama	2+	2+	3+	2+	2+
B. benghalensis	3+/pb	2+/c	2+	3+	2+/c
H. smyrnensis	2+/b	—	2+	2+/c	2+
P. krameri	2+/p	2+/cb	2+	4+	©3+
D. macrocercus	©2+	©2+	2+	3+	©2+
N. asiatica	3+/b	©2+	3+	2+/p	2+
O. oriolus	©2+	2+/p	3+	3+/p	2+/p
T. somervillei	2+/p	2+	2+/p	3+/p	2+
Z. palpebrosa	2+/p	2+	3+	3+/p	+/p

Legends: 4+=Very intense. 3+=Intense. 2+=Moderate. +=Very faint. p=More intense reaction in the periphery of the gland. c=More intense reaction in the central region of the gland. ©=Reaction only in the centre of the gland. b=More reaction towards the basement membrane in an individual cord.

Summary

The relative proportions of adrenaline (A) and noradrenaline (NA) containing celluler areas have been planimetrically estimated in the twelve avian species representing several orders and families. The study reveals a great variability in percentages of A and NA-containing cells in the suprarenal medulla of these species. Three possibilities have been forwarded to explain this hormonal diversity. These are. i) methylation of medullary hormones is dependent on the cortico-medullary ratio, ii) relative abundance of A and NA in a species is determined by the degree of aggression and predation behavior manifested by it and iii) differing association of A and NA in this class of vertebrates may bear some relation to the suggested avian phylogeny. Amongst these probabilities, the last one appears to fit in rather closely to our data. This assumes an efficacious accomplishment or hormonal methylation in the course of avian evolutionary ascent.

The present report also indicates that the chromaffin tissue of birds under investigation is rich in acetal phosphatides, metachromatic substances

and acid phosphatase. Two species (*H. smyrnensis* and *A. phoenicurus*), however, fail to reveal any trace of medullary metachromatic materials. This as well as the concept of 'basic cytochemical unit' in avian adrenal medulla have been pointed out and briefly discussed.

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