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Influence of age on adrenomedullary catechol hormones content in twenty-five avian species

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ABSTRACT

Catechol hormones (Norepinephrine and Epinephrine) content in the adrenal medulla of 25 species of the newly-hatched and adult birds were investigated. Norepinephrine (NE) and Epinephrine (E) were found to be the major hormones in the adrenal medulla of the newly-hatched passerine and non-passerine birds respectively. E was the preponderant hormone in the adrenal medulla of the adult passerine birds. The adrenal medulla of the adult non-passerine birds produced either NE as the predominant catechol hormone or NE and E in almost equal proportion as the adrenomedullary hormones.

KEY WORDS: Catechol hormones; adrenal medulla.

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INTRODUCTION

In mammals, it is known that the adrenal medulla produces Epinephrine (E) as the major hormone in adults (*see* von Euler, 1967) while the foetal and post-natal adrenal medulla produce quite a high proportion of Norepinephrine (NE) which gradually diminishes with advancing developmental age (Shepherd & West, 1951; West *et al.,* 1953).

Ghosh (1977, 1980) from his exhaustive work on 32 avian species belonging to different phylogeny had reported that birds with a more remote ancestry (namely Cormorant, Chicken and Egret) have more NE, whereas recently evolved birds (e. g., passerine birds) have more E in their medulla. Birds occupying an approximately intermediate position (e. g., Cuckoos, Pigeons) on the avian evolutionary scale have both catechol hormones in almost equal proportions. He suggested that in orders with a primitive heritage, the mechanism of hormonal methylation is not so efficient. The process of methylation of catechol hormones becomes progressively complete during evolutionary ascent. However, the reports concerning the proportion of catechol hormones in the juvenile birds are variable. Thus, Ghosh & Ghosh (1964) found no difference in proportion of the two amines in juvenile and adult birds investigated by them, while others (Ljunggren, 1969; Wassermann & Bernard, 1971) had reported the preponderance of E in the adrenal medulla of juvenile birds. In this investigation, therefore, we have studied the catechol hormones content of the adrenal medulla in 25 species of newly-hatched and adult birds with a view to justify and strengthen the previous reports and also to generalize the situation in birds.

MATERIALS AND METHODS

Twenty-five species of the newly-hatched (6 hours to 3 days post-hatching) and adult birds were collected during the same period from a local bird dealer. They were left for 2 to 4 hours and were given food and water. Then the birds were killed by cervical dislocation and the adrenals were quickly dissected and processed for histofluorescence and biochemical observations as follows.

Histofluorescence method

15 μm cryostat sections of the adrenals were cut and processed according to the 'Modified Glyoxylic Acid Technique: The SPG Method' (de la Torre & Surgeon, 1976). The fluorescence was studied and photographed with the help of Zetopan-Binolux (Reichert, Austria) with the exciter filter 13 (Transmission 48%: Wave length 400 nm) and absorption filter 3 (Transmission 90%: Wave length 550 nm).

Biochemical method

In order to support our histofluorescence findings we have chosen a simple and rapid spectrophotometric method as described by von Euler (*see* Camichael et al., 1983) for estimation of NE and E. All the samples vere read by using a UV/VIS Spectrophotometer (Perkin-Elmer, Model 550S).

RESULTS

Histofluorescence

An intense green fluorescence was observed in the medullary cord of the newly-hatched and adult birds. Based on the characteristics of fluorescence, the hitherto investigated birds were broadly classified into 4 categories as described below.

Category 'I': An intense green fluorescence was found to be restricted to small areas in the medullary cords of the newly-hatched birds (Fig. 1). In the adults, however, an increase in area and intensity of fluorescence was observed (Fig. 2). This characteristic of fluorescence was found in *Anas platyrhynchos, Bubulcus ibis, Ardeola grayii* and *Gallus domesticus.*

Category 'II': Very bright green fluorescence was observed in small areas in the medullary cords of the newly-hatched birds (Fig. 3). In the adults, the medullary cords were found to have increased in size and the fluorescence showed an increase in area and in intensity (Fig. 4). It was evident in *Otus bakkamoena, Treron phoenicoptera, Columba livia, Spilornis cheela, Spizaetus cirrhatus, Eudynamys scolopacea, Megalaima asiatica, Megalaima haemacephala* and *Dinopium benghalensis.*

Category 'III': An intense green fluorescence was encountered in the medullary cords of the newly-hatched birds. The intensity of fluorescence was found to be higher in the medullary cords of the peripheral region of the adrenal gland. Diminution in area and intensity of fluorescence was, however, noticed in the medullary cords of the adult birds except *Sturnus contra* where the fluorescence characteristics of the adrenal medulla were almost similar in the newly-hatched and adult birds. This type of fluorescence was evident in

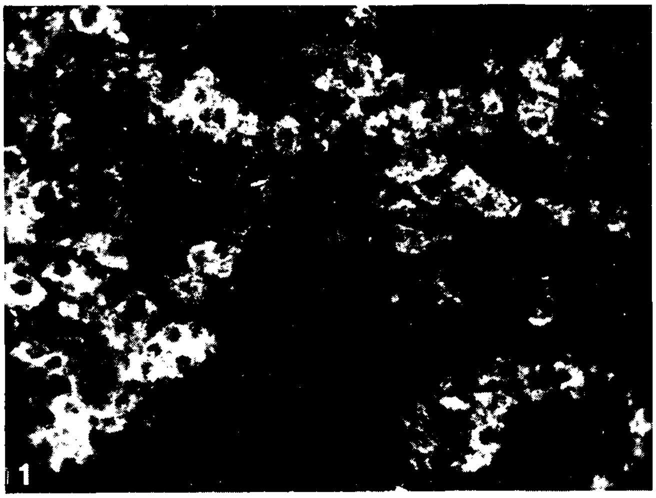

Fig. 1 - Fluorescence micrograph of the adrenomedullary cords in the newly-hatched *Bobulcus ibis.* Note the bright fluorescence is restricted to small areas in the medullary cords. × 400.

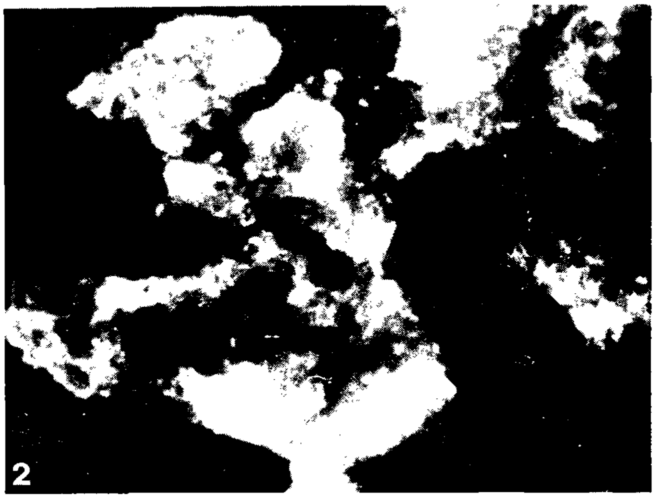

Fig. 2 - Fluorescence micrograph of the adrenomedullary cords in the adult *Bubulcus ibis.* Note the increase and in area in intensity of fluorescence (Compare with Fig. 1). × 400.

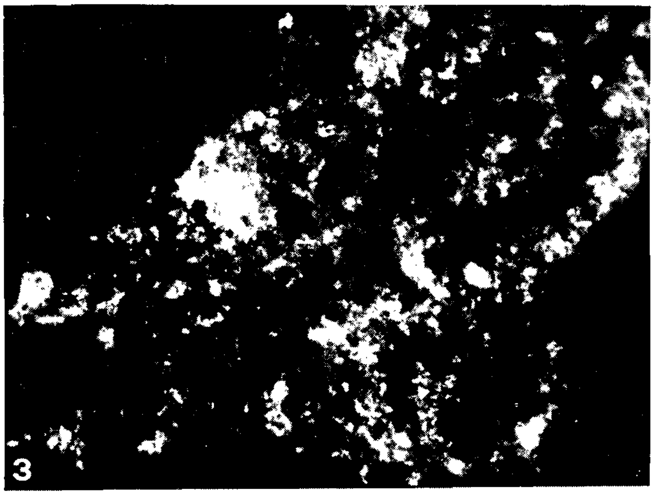

Fig. 3 - Fluorescence micrograph of the adrenomedullary cords in the newly-hatched *Otus bakkamoena.* Note the intense fluorescence is restricted to small areas in the medullary cord. × 400.

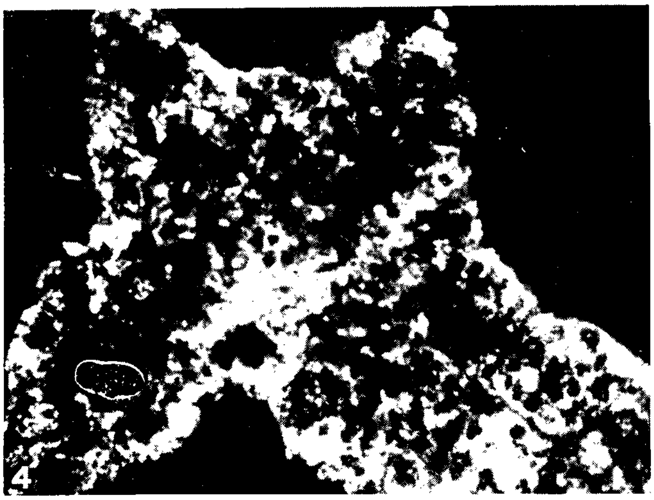

Fig. 4 - Fluorescence micrograph of the adrenomedullary cord in the adult *Otus bakkamoena.* Note an increase in area and intensity of fluorescence (compare with Fig.3). × 400.

Corvus splendens, Dendrocitta vagabunda, Sturnus malabaricus, Sturnus contra, Dicrurus adsimilis, Oriolus xanthornus, Lanius schach.

Category 'IV': Bright green fluorescence was noticed in the medullary cords of the newly-hatched birds. Although the intensity of fluorescence decreased, the area of fluorescence appeared to have increased in the medullary cords of the adult birds. This type of fluorescence was seen in *Pycnonotus cafer, Pycnonotus jocosus, Turdoides caudatus, Copsychus saularis* and *Zoothera citrina.*

Biochemical

For biochemical determinations of catechol hormones in the newly-hatched and adult birds see Table 1. For the generalised patterns of distribution of the relative proportions of NE and E in the adrenal medulla of newly-hatched and adult birds see Figure 5.

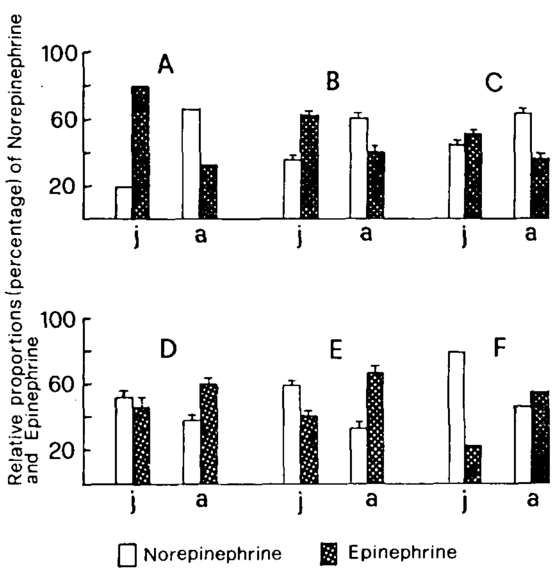

Fig. 5 - Histogram showing the generalized patterns of distribution of the relative proportions (percentage) of norepinephrine and epinephrine in the adrenal medulla of 25 species of newly hatched and adult birds. A: *A. platyrhynchos;* B: *T. phoenicoptera, C. livia, S. cirrahatus, O. bakkamoena, C. benghalensis;* C: *S. cheela, B. ibis, A. grayii, G. domesticus, E. scolopacea, M. asiatica, M. haemacephala;* D: *O. xanthornus, T. caudatus;* E: *L. schach, C. saularis, P. cafer, P. jocosus, C. splendens, D. vagabunda, S. malabaricus, S. contra, D. adsimilis;* F: *Z. citrina.* a, adult; j, juvenile.

DISCUSSION

The green fluorescence in the adrenal medulla indicated the presence of catecholamines in the gland. It appeared from the present finding that the fluorescent area as well as the intensity of fluorescence were less in the adrenal medulla of newly-hatched birds. In contrast, in the adults the adrenal medulla showed considerable increase in the area and intensity of fluorescence (*see* Figs. 1 to 4). The fluorescence findings as expected, indicated lesser amount of catecholamines in the adrenal medulla of newly-hatched birds, while greater amount of catecholamines in the adrenal medulla of the adult animals. Interestingly, our biochemical data on catecholamine content (*see* Table I) supported the fluorescence findings.

The present study revealed that in the adrenal medulla of the newly-hatched passerine birds, norepinephrine was the preponderant hormone while epinephrine was the major hormone in the adults. Strikingly, E was found to be the predominant hormone in the adrenal medulla of the newly-hatched non-passerine birds, however, their adults produced either NE as the major catechol hormone or NE and E in almost equal proportions as the catechol hormones.

Our finding on catechol hormones content in the adrenal medulla of the newly-hatched and adult passerine birds agreed with that reported in foetal and adult mammals respectively (Shepherd & West, 1951; West *et al.*, 1953; von Euler, 1967). It had been demonstrated in mammals that the enzyme phenylethanolamine-N-methyl transferase (PNMT) regulated the biosynthesis of E both in the adult and in the foetal adrenal medulla (Wurtman & Axelrod, 1965; Margolis *et al.*, 1966). Passerine birds possibly simulated the mammalian pattern of PNMT activity (*cf.* Jost, 1966).

Further, our finding on the preponderance of E in the adrenal medulla of the newly-hatched non-passerine birds in accordance with the previous reports on chick (Wassermann & Bernard, 1971; Turner & Bagnara, 1976) and on pigeon (Ljunggren, 1969). Besides marked PNMT activity in the embryonic chick (Wassermann & Bernard, 1971; Accordi *et al.*, 1975) also supported our present finding. In contrast, the production of either more NE or both the catechol hormones in equal proportions in the adrenal medulla of the adult non-passerine birds posed a problem. Why the proportion of methylated catecholamine in the adrenal gland declined in non-passerine avian species between the newly-hatched and adult animals is a moot question. However, quantitation of PNMT in the adrenal glands of the newly-hatched and adult non-passerine birds may be one of the steps to solve this problem.

In addition, Wassermann & Bernard (1971) demonstrated the influence of adrenocorticosteroids on PNMT activity and epinephrine-norepinephrine concentration in the adrenals of pre- and post-natal chicks. They observed a correlation between the maturity of the synthesis of epinephrine between the eleventh and fifteenth day of embryonic development. Furthermore, embryos injected with dexamethasone on day 8 of development show a significant increase in epinephrine at 13, 14 and 15 days of embryonic life. Similar findings have also been recorded in the 30 to 60 days old chicken (Wassermann & Bernard, 1971). Besides, Manelli *et al.* (1973) reported that adrenocorticosteroids in chick embryo's (18 days old) adrenal gland stimulated epinephrine synthesis in organ culture, as it was observed in extra adrenal chromaffin tissue in mammals (Coupland, 1968). Further, in the adult pigeons, adrenocorticosteroids elevated epinephrine synthesis (Sitaraman & Ghosh, 1977a, b). The literature thus stressed the importance of adrenocorticosteroids in augmenting epinephrine synthesis through increased synthesis of PNMT. Therefore, quantitation of adrenocorticosteroids in the adrenal glands of newly-hatched and adult non-passerine birds may be a worthwhile step to solve the alleged problem.

TABLE I - *Influence of age on adrenomedullary catechol hormones content in 25 species of birds*.*

Species, Orders and Families	Newly-Hatched			Adult		
	NE mg/gm of adrenal	E mg/gm of adrenal	Total CAM mg/gm of adrenal	NE mg/gm of adrenal	E mg/gm if adrenal	Total CAM mg/gm of adrenal
Anas platyrhynchos (6) O: Anseriformes F: Anatidae	0.38 ± 0.04**	1.58 ± 0.13	1.96 ± 0.17	1.43 ± 0.17 p < 0.005	0.71 ± 0.05 p < 0.005	2.14 ± 0.22 N.S.
Bubulcus ibis (7) O: Ciconiiformes F: Ardeidae	0.52 ± 0.09	0.72 ± 0.14	1.24 ± 0.18	1.85 ± 0.07 p < 0.001	0.86 ± 0.07 N.S.	2.72 ± 0.01 p < 0.001
Ardeola grayii (7) *O: Ciconiiformes* *F: Ardeidae*	0.47 ± 0.05	0.59 ± 0.08	1.06 ± 0.08	2.01 ± 0.04 p < 0.001	0.84 ± 0.07 N.S.	2.86 ± 0.11 p < 0.001
Gallus domesticus (6) O: Galliformes F: Phasianidae	0.72 ± 0.07	0.84 ± 0.07	1.56 ± 0.14	2.04 ± 0.18 p < 0.005	1.65 ± 0.15 p < 0.01	3.69 ± 0.04 p < 0.001
Columba livia (10) O: Columbiformes F: Columbidae	0.92 ± 0.05	1.28 ± 0.11	2.20 ± 0.07	1.65 ± 0.11 p < 0.001	1.34 ± 0.15 N.S.	2.99 ± 0.08 p < 0.001
Ireron phoenicoptera (8) O: Columbiformes F: Columbidae	0.65 ± 0.31	1.05 ± 0.13	1.71 ± 0.21	1.61 ± 0.15 p < 0.05	1.27 ± 0.10 N.S.	2.89 ± 0.19 p < 0.01
Spilornis cheela (5) O: Falconiformes F: Accipitridae	0.83 ± 0.11	1.10 ± 0.07	1.93 ± 0.13	1.73 ± 0.19 p < 0.05	1.02 ± 0.11 N.S.	2.75 ± 0.08 p < 0,025
Spizaetus cirrhatus (5) O: Falconiformes F: Accipitridae	0.93 ± 0.12	1.31 ± 0.18	2.24 ± 0.06	1.91 ± 0.20 p < 0.05	1.01 ± 0.05 N.S.	2.92 ± 0.15 p < 0.025
Otus bakkamoena (8) O: Strigiformes F: Strigidae	0.71 ± 0.07	1.57 ± 0.17	2.09 ± 0.24	2.04 ± 0.10 p < 0.001	1.40 ± 0.20 N.S.	3.44 ± 0.25 p < 0.01
Coracias benghalensis (7) O: Coraciiformes F: Coraciidae	0.71 ± 0.05	1.38 ± 0.10	2.09 ± 0.07	1.77 ± 0.12 p < 0.001	0.88 ± 0.13 p< 0.05	2.65 ± 0.25 N.S.
Eudynamys scolopacea (8) O: Cuculiformes F: Cuculidae	0.93 ± 0.03	1.19 ± 0.01	2.13 ± 0.03	1.67 ± 0.10 p< 0.001	0.99 ± 0.11 N.S.	2.63 ± 0.04 p< 0.001
Megalaima asiatica (6) O: Piciformes F: Capitonidae	1.08 ± 0.06	1.30 ± 0.01	2.39 ± 0.07	1.72 ± 0.05 p< 0.001	1.12 ± 0.02 p< 0.005	2.83 ± 0.06 p< 0.01
Megalaima haemacephala (7) O: Piciformes F: Capitonidae	0.79 ± 0.03	0.91 ± 0.03	1.71 ± 0.05	2.55 ± 0.06 p< 0.001	1.62 ± 0.04 p< 0.001	4.18 ± 0.09 p< 0.001
Turdoides caudatus (6) O: Passeriformes F: Muscicapidae	0.94 ± 0.07	0.82 ± 0.03	1.77 ± 0.04	1.61 ± 0.09 p< 0.005	2.54 ± 0.07 p< 0.001	4.16 ± 0.15 p< 0.001
Copsychus saularis (7) O: Passeriformes F: Muscicapidae	1.05 ± 0.06	0.72 ± 0.09	1.77 ± 0.13	0.57 ± 0.12 p< 0.025	1.34 ± 0.18 p< 0.05	1.92 ± 0.31 N.S.
Zoothera citrina (5) O: Passeriformes F: Muscicapidae	0.68 ± 0.28	0.19 ± 0.09	0.87 ± 0.34	1.13 ± 0.04 N.S.	1.37 ± 0.05 p< 0.005	2.50 ± 0.02 p< 0.025
Pycnonotus cafer (8) O: Passeriformes F: Pycnonotidae	1.51 ± 0.28	1.11 ± 0.18	2.62 ± 0.44	1.63 ± 0.22 N.S.	3.13 ± 0.72 p< 0.05	4.77 ± 0.49 p< 0.025
Pycnonotus jocosus (8) O: Passeriformes F: Pycnonotidae	1.50 ± 0.01	1.07 ± 0.05	2.57 ± 0.06	0.94 ± 0.45 N.S.	2.40 ± 0.33 p< 0.025	3.35 ± 0.12 p< 0.005
Corvus splendens (9) O: Passeriformes F: Corvidae	1.12 ± 0.04	0.74 ± 0.09	1.86 ± 0.06	1.15 ± 0.40 N.S.	2.47 ± 0.32 p< 0.005	3.62 ± 0.16 p< 0.001
Dendrocitta vagabunda (9) 0: Passeriformes F: Corvidae	1.50 ± 0.05	1.11 ± 0.08	2.62 ± 0.06	1.07 ± 0.13 p< 0.025	3.14 ± 0.52 p< 0.01	4.21 ± 0.66 p< 0.05

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Sturnus malabaricus (7) O: Passeriformes F: Sturnidae	2.82 ± 0.12	1.83 ± 0.15	4.65 ± 0.28	1.34 ± 0.23 p< 0.005	2.97 ± 0.15 p< 0.005	4.31 ± 0.15 NS.
Sturnus contra (6) O: Passeriformes F: Sturnidae	1.31 ± 0.13	0.82 ± 0.26	2.13 ± 0.13	1.66 ± 0.22 NS.	2.03 ± 0.13 p< 0.025	3.70 ± 0.34 p< 0.025
Dicrurus adsimilis (8) O: Passeriformes F: Dicruridae	1.24 ± 0.08	0.86 ± 0.11	2.10 ± 0.19	0.86 ± 0.15 NS.	1.84 ± 0.24 p< 0.01	2.70 ± 0.35 NS.
Oriolus xanthornus (7) O: Passeriformes F: Oriolidae	0.43 ± 0.10	0.41 ± 0.12	0.85 ± 0.22	0.79 ± 0.06 p< 0.05	1.18 ± 0.01 p< 0.005	1.98 ± 0.70 NS.
Lanius schach (8) O: Passeriformes F: Laniidae	1.07 ± 0.10	0.76 ± 0.06	1.83 ± 0.16	1.16 ± 0.05 NS.	2.19 ± 0.01 p< 0.001	3.36 ± 0.06 p< 0.001

Figures in parentheses indicate the number of observations.
* The statistical analyses were done by Student's t' test.
** Values are given as Mean ± Standard Error of the Mean.
NE: Norepinephrine, E: Epinephrine, CAM: Catecholamines, NS: Not significant, O: Order, F: Family
P values refer to comparison with the newly-hatched birds.

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