

## Effect of vitamin C on adrenomedullary hormones in gonadectomised juvenile pigeons

SHILLA CHATTERJEE, DHANANJAY PAL and ASOK GHOSH

Histophysiology Laboratory, Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India

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**Abstract.** Administration of vitamin C to juvenile castrated pigeons leads to a fall in the adrenal epinephrine level, but no significant change in the norepinephrine level is noted. Since the role of vitamin C in converting dopamine to norepinephrine is anticipated, it has been surmised that the bursa of Fabricius (thymolympathic organ) might be responsible for the inability of these young birds to respond to vitamin C treatment.

**Keywords.** Vitamin C; adrenal medulla; epinephrine-norepinephrine content; thymolympathic system; gonadectomy.

### 1. Introduction

The activity of the avian adrenal gland is considerably influenced by ascorbic acid or vitamin C (Ghosh and Chatterjee 1981). The adrenal glands contain the highest concentration of this vitamin. The latter appears to be one of the cofactors involved in the formation of norepinephrine (NE) from Dopamine (Levin *et al* 1960). The final step of biosynthesis, i.e. the methylation of NE to epinephrine (E) is dependent on the enzyme phenylethanolamine N methyl transferase (PNMT), which, in turn, is influenced by adrenal corticosteroids (Pohorecky and Wurtman 1971; Wasserman and Bernard 1971). Interestingly, it has been found that adrenal corticosteroid biosynthesis has been reported to be inhibited by vitamin C (Kitabchi 1967). Lower yield of corticosteroids would therefore retard the methylation of NE to E by PNMT.

Gonadal steroids are also alleged to affect the methylation mechanism of NE to E in birds (Sitaraman and Ghosh 1977; Chaudhuri *et al* 1966) and mammals (Lupulescu 1958). Ljunggren (1969) has noted that in *Columba palumbus*, the degree of methylation of NE to E is perhaps greatest during the periods of lowest reproductive activity. Since these data are relatively old and based mostly on cytochemical observations, it seems essential to reinvestigate the problem by taking a more modern approach. As the first step in this direction, we have tried to investigate the effect of vitamin C on adrenomedullary catecholamines from a biochemical point of view. The experiment has been performed by eliminating gonadal influence in young adult pigeons, *Columba livia*.

### 2. Materials and methods

Thirty six young adult pigeons (about 2 months old) were procured from a local bird dealer and housed under conditions of the laboratory for a number of days. Young

adult pigeons were chosen for convenience in surgical gonadectomy. Twelve birds were divided into two groups. While distilled water was administered intramuscularly to one group (Group I) at a dose of 0.8 c.c. water/bird/day for 7 days, the remaining birds (Group II) received intramuscular injections of vitamin C at a dose of 100 mg/100 gm body weight for 7 days. Of the remaining 24 birds, 6 were left intact and 18 birds were castrated. The wound was allowed to heal prior to the commencement of experimental therapies. The birds were divided into 3 equal groups (Groups IV, V and VI). While Group IV was left untreated, Groups V and VI received intramuscularly water and vitamin C, respectively, at a dose of 0.8 c.c. water/bird/day for 7 days and 100 mg/100 gm body weight for 7 days.

At the termination of the experimental period, the birds were killed by cervical dislocation, their adrenals were dissected out and either fixed in Wood's fixative for cytological procedures, or processed for biochemical quantitation of adrenomedullary catecholamines and adrenal ascorbic acid following von Euler's (1950) and Bessey *et al's* (1947) methods, respectively.

### 3. Results

#### 3.1 Biochemical

The biochemical data are summed up in table 1. Though not much change has been achieved in different groups, vitamin C therapy causes a fall in the epinephrine level of castrated young adult birds.

#### 3.2 Cytological

The sections of adrenal glands fixed in Wood's fixative were observed under the light microscope after staining them with Wood's stain.

Castration leads to no change in the adrenomedullary picture. While slight preponderance of red (E) cells is seen in all control sections, predominance of yellow (NE) cells, and reduced staining intensity of red cells is seen in vitamin C treated sections. It is noteworthy that the cortex is atrophied in castrated pigeons, in contrast to previous reports of hypertrophied cortex in castrated ducks and fowls (Assenmacher 1973). Vitamin C treatment leads to further cortical atrophy.

### 4. Discussion

The present investigations reveal that castration does not affect adrenal catecholamine levels in young adult pigeons. Though adrenomedullary catecholamine levels do not change in ascorbic acid treated normal birds, in castrated birds, the administration of vitamin C causes a preferential release of epinephrine from the adrenal gland. The NE level, however, remains unchanged. The presence of vitamin C perhaps prevents the release of NE (cf. Subramaniam 1977). Secondly, there might be further retardation in the conversion of NE to E in gonadectomised birds, therefore indicating an inhibitory effect of vitamin C on PNMT activity. The retardation of PNMT activity by vitamin C seems to be more pronounced in the absence of gonads in young adult birds.

Table 1. Alteration in adrenomedullary hormones by vitamin C in intact and castrated young adult pigeons?

Group	Adrenal ascorbic acid (mg/100 gm tissue)	Epinephrine ( $\mu\text{g}/\text{mg}$ tissue)	Norepinephrine ( $\mu\text{g}/\text{mg}$ tissue)	Total catecholamine ( $\mu\text{g}/\text{mg}$ tissue)
I	Intact control (6) <sup>a</sup>	2.29 $\pm$ 0.19 (61%)	1.45 $\pm$ 0.19 (39%)	3.74 $\pm$ 0.22
II	Vitamin C (6)	2.33 $\pm$ 0.21 (57%)	1.78 $\pm$ 0.25 (43%)	4.11 $\pm$ 0.28
III	Intact control (6) <sup>b</sup>	1.36 $\pm$ 0.15 (56%)	1.07 $\pm$ 0.23 (44%)	2.43 $\pm$ 0.39
IV	Castrated control (6) <sup>d</sup>	1.44 $\pm$ 0.14 (50%)	1.44 $\pm$ 0.41 (50%)	2.88 $\pm$ 0.55
V	Castrated Sham control (6) <sup>d</sup>	1.46 $\pm$ 0.23 (57%)	1.12 $\pm$ 0.16 (43%)	2.58 $\pm$ 0.40
VI	Castrated + vitamin C (6) <sup>d</sup>	0.66 $\pm$ 0.12 (34%) <sup>e</sup>	1.28 $\pm$ 0.21 (66%)	1.94 $\pm$ 0.32

Number in parentheses indicates number of specimen.

<sup>a</sup>Intact control for acute experiment with vitamin C.

<sup>b</sup>Intact control for other castrated groups, which had to be caged for a longer period.

<sup>c</sup>Mean  $\pm$  Standard error.

<sup>d</sup>Castrated group.

<sup>e</sup>P value < 0.025.

Our previous experiments (unpublished results) with vitamin C administration to adult pigeons (where the bursa of Fabricius normally atrophies) and bursectomised young pigeons led to rise in the norepinephrine level. This suggests that Dopamine  $\beta$ -hydroxylase, which occurs in the chromaffin granules of the adrenal medulla, accumulates ascorbate efficiently, and utilises it in the conversion of dopamine to norepinephrine. No significant rise in the NE level of young adult intact or castrated pigeons after vitamin C administration suggests that in these birds the homeostatic mechanism concerning adeno-hypophysis-adrenal axis is somehow not yet perfected for effective functioning. The bursa of Fabricius (a thymolymphatic organ), which modifies many normal physiological responses of adult birds, is perhaps involved in the inability of young birds to respond to vitamin C treatment (compare Bhattacharya and Ghosh 1970). Probably many interesting facts might come out on repeating these experiments on adult and bursectomised young adult birds.

### References

- Assenmacher I 1973 The peripheral endocrine glands; in *Avian Biology* (eds) D S Farner and J R King (New York: Academic Press) Vol. 3, pp. 184-262
- Bessey O A, Lowry O H and Brock M J 1947 The quantitative determination of ascorbic acid in small amounts of white blood cells and platelets; *J. Biol. Chem.* **168** 197-205
- Bhattacharya T K and Ghosh A 1970 Influence of surgical and steroidal bursectomy on the behaviour of adrenal ascorbic acid during stress in juvenile pigeons; *Gen. Comp. Endocrinol.* **15** 420-424
- Chaudhuri D, Ghosh I and Ghosh A 1966 Steroidal influence on adrenomedullary catechol hormones of the pigeon; *Acta Morphol. Acad. Sci. Hung.* **13** 245-252
- Ghosh A and Chatterjee S 1981 Influence of ascorbic acid on adrenomedullary catecholamine content in the Red vented Bulbul; *Nineth International Symp. on Comp. Endocrinol*, Hong Kong (in press)
- Kitabchi A E 1967 Inhibitory effect of ascorbic acid on steroid hydroxylase systems of beef adrenal cortex; *Fed. Proc.* **264** 484
- Levin E Y, Levenberg B and Kaufman S 1960 The enzymatic conversion of 3, 4 dihydroxy phenyl ethylamine to norepinephrine; *J. Biol. Chem.* **235** 2080-2086
- Ljunggren L 1969 Seasonal studies of Wood pigeon population II. Gonads, crop glands, adrenals and the hypothalamo-hypophysial system; *Viltrevy* **6** 41-126
- Lupescu A 1958 Modificarile glandelor endocrine dupa administrate de estrogeni, timus si Testesteron; *Stud. Cercet. Endocrinol.* **1** 91-101
- Pohorecky L A and Wurtman R J 1971 Adrenocortical control of epinephrine synthesis; *Pharmacol. Rev.* **23** 1-35
- Sitaraman S and Ghosh A 1977 Steroidal control of catechol hormone production in the pigeon; *Columba livia Folia Biol.* **25** 21-25
- Subramaniam N 1977 Minireview on the brain ascorbic acid and its importance in metabolism of biogenic amines; *Life Sci.* **20** 1479-1484
- von Euler U S 1950 Estimation of adrenaline and noradrenaline in Tissue Extracts; in *Methods in Medical Research* (Chicago: Year Book Publisher) Vol. 3, pp. 130-135
- Wassermann G F and Bernard E A 1971 The influence of corticoids on the phenylethanolamine-N-methyl transferase activity in the adrenal glands of *Gallus domesticus*; *Gen. Comp. Endocrinol.* **17** 83-93