

Histamine in Blood and Tissues of Aves¹ (36795)

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(Introduced by P. Griminger)

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As in mammals, catecholamines and 5-hydroxytryptamine (5-HT) have been found distributed in the avian cardiovascular system (1, 2). Histamine is another biogenic amine which is widely distributed in mammalian tissues (3-5), but little attention has been devoted to histamine in the avian cardiovascular system. This amine has been determined in aves either by means of bioassay (6, 7) or chemically in the GI tract (8). No chemical determination has been made on histamine in blood, heart, or spleen of chickens, ducks or pigeons. The objective of this study was to determine histamine in the blood and other tissues of these avian species by employing a more sensitive fluorescent technique.

Methods. Mature 1-yr-old white leghorn chickens (*Gallus gallus domesticus* L.) and white king pigeons (*Columba livia*) of both sexes were used. In addition ca. 2-month-old white Peking ducks (*Anas platyrhynchos*) of both sexes were used. All animals were fed *ad libitum* with a conventional commercial diet containing 16% protein. They were kept in floor pens at a temperature of ca. 25°. Unanesthetized and unexcited animals were sacrificed in the early morning hours by decapitation. Muscle samples of the four cardiac chambers were obtained immediately thereafter, dipped into liquid nitrogen, and stored under freezing conditions (-10°) until histamine extraction was carried out. Whole spleens, *i.e.*, capsule and pulp (white and red), were used. Less than 30 sec elapsed between sacrificing the animals and completely freezing the tissues. Blood was with-

drawn gently from all intact, unexcited animals via heart puncture. Nine parts of blood were immediately mixed gently with one part of 0.2 M sodium citrate in siliconized centrifuge tubes. In contrast to sodium oxalate, sodium citrate was found not to hemolyze avian red blood cells. It also did not affect later histamine recovery (9). The blood was chilled until centrifugation at ca. 1000g for 30 min in a refrigerated centrifuge to obtain samples of its elements which were further chilled until histamine extraction. Histamine was extracted and determined spectrofluorometrically (10) with minor modifications in the fluorometric assay procedure by using 0.1% *o*-phthalaldehyde reagent and 1.5 M H₃PO₄ (11). This method was sensitive to 0.01 µg/ml HCl extract. Fluorescence was measured by an Aminco-Bowman spectrophotofluorometer at 450 nm resulting from activation at 360 nm, and the results are expressed in micrograms per gram of wet tissue or micrograms per milliliter of blood fraction. After correction for percentage recovery, which was 92%, unpaired observations were statistically compared and Student's *t* test was used to test for significance (12).

Results. Table I summarizes the levels of histamine in both sexes of mature chickens, ducks and pigeons. Of the three species, the chicken values were essentially the highest. The duck tissues showed higher values than those of the pigeons except for the ventricles, in which case the pigeon values were next to those of the chickens. Histamine levels in the atria of male and female chickens were higher ($p < .001$) than those of the ventricles, right compartments tending to have higher concentrations than left ones. Atrial histamine was also significantly higher than that of the ventricles in both sexes of ducks and

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TABLE I. Tissue Levels of Histamine in Mature Male and Female White Leghorn Chickens, White Peking Ducks, and White King Pigeons.

Species	Sex	No. of animals	Tissue levels of histamine ($\mu\text{g/g}$); means \pm SE				
			Ventricles		Atria		Spleen
			Right	Left	Right	Left	
Chicken	M	4	1.430 \pm 0.19	1.010 \pm 0.13	6.010 \pm 0.34	5.630 \pm 0.39	14.66 \pm 2.7
	F	4	1.800 \pm 0.40	1.300 \pm 0.06	3.930 \pm 0.86	3.350 \pm 1.03	15.21 \pm 1.7
Duck	M	9	0.249 \pm 0.04	0.134 \pm 0.01	2.487 \pm 0.15	2.342 \pm 0.24	7.21 \pm 0.5
	F	7	0.204 \pm 0.02	0.089 \pm 0.01	2.402 \pm 0.18	1.760 \pm 0.12	15.37 \pm 1.6
Pigeon	M	13	0.661 \pm 0.05	0.415 \pm 0.04	0.637 \pm 0.15	1.436 \pm 0.11	2.68 \pm 0.3
	F	9	0.537 \pm 0.24	0.473 \pm 0.11	1.347 \pm 0.31	1.103 \pm 0.48	3.17 \pm 1.5

pigeons. The right compartments showed tendencies to contain higher histamine concentrations than those of the left ones. Sex differences were observed in the atrial tissues of chickens, the males exhibiting a higher ($p < .01$) histamine level than females. This is also true in the duck tissues: left ventricles ($p < .01$), left atria ($p < .05$). In the pigeon however, the only sex difference appeared in right atrial tissues ($p < .05$) with the female having higher amine concentrations. Of all the tissues examined, the spleen contained the highest amount of histamine in both sexes. Splenic histamine was higher ($p < .001$) in female ducks than in the males.

Table II summarizes the levels of histamine in the blood fractions of both sexes of the three species. Blood fractions included plasma, red blood cells, buffy coat and packed cell volume. The buffy coat is made up mostly of leukocytes and thrombocytes. The plasma values were negligible and are not presented here. Chicken whole blood contained the highest concentration of histamine, followed by that of ducks and pigeons. Except in male pigeons, the white blood cells in all three species contained higher histamine concentrations than the erythrocytes. Female blood contained consistently higher levels of histamine than that of males. Although sex difference was not always significant in all the blood fractions examined, it was significant in the case of packed cell volume of pigeons ($p < .001$) and chickens ($p < .001$), erythrocytes of chickens ($p < .001$) and ducks ($p < .01$), and whole blood of pigeons ($p < .01$).

Discussion. The samples studied herein were from unfasted animals, and we have found that fasting overnight did not influence the histamine level of tissues examined. In other mammalian species, feeding after 48 hr of fasting did not significantly alter the levels of blood and tissue histamine (3). Histamine has been found herein to parallel the distribution of catecholamines and 5-HT (1, 2). Thus, the atria contained more of these biogenic amines than did the ventricles. It is therefore logical to assume that a possible relationship between these amines may indeed exist. The distribution of histamine in some mammalian hearts has been reported to be similar to that of norepinephrine although not on a subcellular basis (13). Norepinephrine was found to be 2–3 times greater than histamine in the dog, 3–6 times in the guinea pig, and 0.2–0.5 times in the rabbit. If we relate the catecholamine results on male chickens (1, 14) to those reported here, one obtains a histamine:norepinephrine ratio of 6.65:1 in the atria and 4.74:1 in the ventricles, with a mean cardiac ratio of 5.49:1. The results obtained herein with the other avian species indicate a similar pattern of histamine distribution. Thus, when these results are combined with 5-HT values (2), we obtain a histamine:norepinephrine:5-HT ratio of 8.15:1.43:1, respectively, histamine being the highest in the avian myocardium.

In both sexes of the three species used, the histamine values are highest in the spleen. The spleen acts as a blood reservoir. It is therefore very likely that the high histamine level in the spleen is due to its large concen-

TABLE II. Histamine Levels in Gross Blood Fractions of Both Sexes of Mature White Leghorn Chickens, White Peking Ducks, and White King Pigeons.

Histamine level in blood ($\mu\text{g}/\text{ml}$ blood fraction); means \pm SE						
Species	Sex	Packed cell vol	Erythrocytes	Leukocytes	Whole blood	
Chicken	M	(13) ^a 1.410 \pm 0.05	(10) 0.174 \pm 0.01	(10) 1.241	(7) 0.513 \pm 0.04	
	F	(7) 2.385 \pm 0.28	(6) 0.611 \pm 0.06	(6) 1.798	(7) 0.644 \pm 0.07	
Duck	M	(4) 0.273 \pm 0.02	(4) 0.036 \pm 0.001	(4) 0.238	(4) 0.102 \pm 0.015	
	F	(4) 0.318 \pm 0.05	(4) 0.054 \pm 0.004	(4) 0.266	(4) 0.109 \pm 0.012	
Pigeon	M	(4) 0.059 \pm 0.005	(4) 0.042 \pm 0.001	(4) 0.018	(4) 0.028 \pm 0.002	
	F	(4) 0.130 \pm 0.010	(4) 0.049 \pm 0.005	(4) 0.082	(4) 0.065 \pm 0.007	

^a Number of birds is given in parentheses.

tration of blood cells. According to Ewart and McMillan (15), one of the major functions of the spleen of the cowbird appeared to be hemopoiesis. The spleen is a lymphoid organ. The white pulp, which is composed of dense aggregates of lymphocytes, and the red pulp, which is made up of erythrocytes and leukocytes, are probably responsible in some way for the high histamine concentration in the spleen. It is also possible that splenic tissues independently synthesize histamine, and that histidine decarboxylase is indeed active in this organ.

Plasma and whole blood of adult female chickens were found to contain 1.8 times and 1.6 times as much 5-HT as those of males (16). Norepinephrine level was observed to be 1.44 times greater in female chickens than in males (14). On the other hand, peritoneal fluid of male rats contained higher counts of mast cells and hence higher histamine level than did females (17). However, histamine of female rats increased greatly when the animals were in estrus. Administered estradiol to ovariectomized mice increased urinary histamine (18). Placental and ovarian hormones are instrumental in increasing histidine decarboxylase activity. LH evokes the release of ovarian histamine in the rat (19). The ovarian tissue and infundibulum of the chicken were found to contain a large number of mast cells which are abundantly associated with blood vessels. Calcium, which is higher in the female chicken blood than that of males (20) was found to be indispensable for histamine release from rabbit platelets (21) and rat mast cells (22). It is likely that

higher calcium in the female blood can act directly on the mast cell within the blood vessel walls, thereby releasing histamine and showing a higher concentration in the female blood. The higher histamine value observed in the female chicken erythrocytes compared to that of the male may reflect an increased cellular uptake of histamine, since the plasma contained practically no histamine. Further work in this area is needed to explain the precise reason for the high erythrocytic histamine.

Female chicken blood has been shown to contain more lymphocytes than that of males (20). We have other qualitative results, based on histofluorescence localization and to be published elsewhere, which indicate that the chicken lymphocytes and thrombocytes contain relatively high concentrations of histamine. Since these cell types make up a very large portion of the white cell or buffy coat fraction, most of the histamine is therefore believed to be carried by these cells.

Summary. Spectrofluorometric assays were carried out on the blood fractions and some cardiovascular tissues of both sexes of chickens, ducks and pigeons to determine histamine levels. Cardiac tissue histamine ranged between 0.15 and 5.82 $\mu\text{g}/\text{g}$ in both females and males of these three species; the chicken had the highest concentration. Atrial tissue histamine was higher than that found in the ventricles; males usually had higher concentrations than females. The relevance of these values to other biogenic amines are discussed. Splenic histamine ranged from 3.17 to 15.21 $\mu\text{g}/\text{g}$; the female had higher values than the

males. Of all the blood fractions, the white blood cells contained the highest levels; the plasma contained the least. Histamine concentration in whole blood ranged from 0.33 to 0.90 $\mu\text{g/ml}$ in chickens, 0.07 to 0.14 $\mu\text{g/ml}$ in ducks, and 0.02 to 0.09 $\mu\text{g/ml}$ in pigeons. The blood fractions of female birds showed higher histamine values than those of males.

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