

Serotonin Levels in Blood, Heart, and Spleen of Chickens, Ducks, and Pigeons¹ (36144)

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(Introduced by P. Griminger)

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Serotonin (5-hydroxytryptamine, 5HT) is widely distributed in most animal tissues (1, 2) and the data on some tissues are voluminous. However, much of these data were obtained by questionable or nonspecific methods involving, in many instances, bioassay procedures without suitable controls. These variable techniques may account, in part, for the highly variable values that have been reported for various species and tissues. Much of the tissue work has been concerned with brain and pineal (2-4).

Less data are available on levels of 5HT in blood, spleen, and heart. In some instances, the blood data are based on serum, and in some, on whole blood. Some have reported that 5HT is carried by the blood platelets in mammals; and Bracco *et al.*, (5) claim that avian thrombocytes contain 5HT and may be considered physiologically similar to mammalian platelets. If, in fact, 5HT is carried by any of the cell types, then the data on the serum may be virtually meaningless.

Weissbach *et al.* (6) reported levels of 5HT in whole blood of chickens (sex not stated) ranging from 2 to 3 $\mu\text{g}/\text{ml}$.

This study showed significant species and sex differences for blood levels of 5HT, and that most of it is carried in the buffy coat blood fraction; also that atrial levels are significantly higher than ventricular heart levels in all three species investigated.

Materials and Methods. Tissues (spleen and heart) were taken quickly from unanesthetized decapitated animals usually in the early morning hours and frozen immediately in liquid nitrogen, weighed, and then homogenized in the tubes containing 0.4 N per-

chloric acid, EDTA, and ascorbic acid. Aliquots of the homogenates were subjected to analysis. Blood was collected quickly by heart stab from birds under minimal stress conditions in the morning hours.

Determinations of serotonin were made on tissues and blood of adult male and female chickens, ducks, and pigeons employing the spectrophotofluorometric methods of Wise (7, 8) for tissues, plasma, and blood cell fractions (red and white cells) and Weissbach *et al.* (6) for whole blood. The latter method, in our hands, is sensitive to 0.1-0.2 $\mu\text{g}/\text{ml}$ and the Wise method is sensitive to 0.01 $\mu\text{g}/\text{ml}$ of solution in the cuvette. Quantities of 5HT, ranging from 0.10 to 2.0 μg , were added to blanks or blood and carried through the various procedural steps, and recoveries averaged over 95%. For tissues such as heart and spleen, a known amount of 5HT (usually 1 μg) was added and recoveries also were above 95%.

Usually about 1 g of heart tissue/sample was processed; and the final readings in the cuvette were based on 0.2 g of tissue. The amount of blood processed was 1 ml for whole blood and more for the various fractions.

All standards and unknowns were determined on an Aminco-Bowman spectrophotofluorometer at wave lengths of 290 m μ , excitation and 543, emission, with a slit setting of 5 and sensitivity of 25. A Kodak polarizer screen [Wise (7)] was used to minimize second order scatter peak at 580 m μ . Calculations were based on recoveries of 95%.

Results. The concentration of serotonin (5HT) in the heart and spleen are shown in Table I. The levels in the spleen of the chicken are very high compared to the duck and

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TABLE I. Levels of Serotonin in Hearts and Spleens ($\mu\text{g/g}$) of Chickens, Ducks, and Pigeons (means and SE).

Species	No.	Sex	Age (months)	Ventricles		Atria		Spleen
				Right	Left	Right	Left	
Chicken	10	M	9	0.35 ± 0.04	0.32 ± 0.03	0.47 ± 0.03	0.45 ± 0.05	19.48 ± 1.64
	10	M	12	0.32 ± 0.03	0.30 ± 0.03	0.49 ± 0.04	0.45 ± 0.04	19.50 ± 1.35
	7	F	12	0.36 ± 0.03	0.35 ± 0.05	0.48 ± 0.06	0.46 ± 0.04	49.03 ± 3.93
	10	M	31	0.36 ± 0.03	0.32 ± 0.03	0.50 ± 0.04	0.47 ± 0.03	21.27 ± 2.17
Duck	8	M	2	0.32 ± 0.03	0.29 ± 0.02	0.50 ± 0.03	0.46 ± 0.04	6.04 ± 0.62
	8	F	2	0.32 ± 0.05	0.27 ± 0.04	0.50 ± 0.05	0.44 ± 0.05	11.52 ± 1.31
Pigeon	6	M	12	0.29 ± 0.04	0.28 ± 0.05	0.47 ± 0.06	0.49 ± 0.05	2.80 ± 0.21
	6	F	12	0.28 ± 0.05	0.30 ± 0.05	0.49 ± 0.06	0.46 ± 0.04	5.40 ± 0.49

pigeon and most mammals (2). There appeared to be no significant age difference, but the level in spleens of the female of all three species is significantly higher than in the male. The levels in both male and female ducks are higher than in pigeons.

Heart. The levels of 5HT in the atria and ventricles of each of the three species, respectively, were not significantly different; but, in all species, the atrial levels were considerably higher than the ventricular ones.

Blood. The concentration of 5HT in whole blood of chickens, ducks, and pigeons is significantly higher in females than males (Table II). In general, the levels are highest in chickens, intermediate in ducks, and lowest in pigeons. Age from 6 to 20 months in chickens did not influence serotonin levels appreciably.

TABLE II. Serotonin Levels ($\mu\text{g/ml}$) in Whole Blood of Chickens, Ducks and Pigeons (means and SE).

Species	Age (months)	No.	Whole blood	
			Male	Female
Chicken	6	10	2.72 ± 0.15	4.53 ± 0.15
	8	10	2.56 ± 0.13	4.33 ± 0.14
	12	7	2.54 ± 0.12	4.49 ± 0.21
	20	10	2.68 ± 0.13	4.31 ± 0.12
Ducks (Peking)	5.5	13	1.73 ± 0.156	2.05 ± 0.193
Pigeons (white king)	36	10	0.70 ± 0.06	1.52 ± 0.14

Direct determinations on plasma and erythrocytes revealed very low levels of serotonin (Table III), indicating that most of it (97%) is located in the buffy coat fraction, which includes thrombocytes and leukocytes. As a further check, determinations were made not only on red cells and plasma but packed celled volume (PCV) which includes red cells and white cells. Subtracting the value for red cells ($\mu\text{g/ml/whole blood}$) from the PCV value gives a value for the buffy coat fraction of the same order of magnitude (92–99%) as reported above.

Our data on blood fractions for ducks and pigeons are meager, but they show that at least 95% of the serotonin is also in the buffy coat fraction.

Discussion. Interest attaches to the higher levels of 5HT in atria than ventricle of the heart. This might suggest a causal relationship between serotonin level and sympathetic innervation and catecholamine levels (9) where the levels are also higher in atria than ventricles. That sympathetically innervated structures can take up serotonin has been reported (11). We have data, to be reported elsewhere, which suggests that such a relationship does exist, based on uptake studies of isolated perfused avian hearts. The right atria takes up considerably more 5HT than the ventricle in normally innervated hearts, and the sympathetically denervated hearts (particularly atria) take up about one-half of the serotonin that the innervated heart does. It is well known that heart denervation depletes the heart of catecholamines, particular-

TABLE III. Serotonin Levels in Different Fractions of Chicken Blood (7 males and 6 females).

Sex	Whole blood (WB) ($\mu\text{g}/\text{ml}$)	Plasma		Red cells		Buffy coat ^a white cells	%
		1 ^b	2 ^c	1 ^b	2 ^c	2 ^c	
M	2.54 ± 0.12	0.082	0.05	0.047	0.018	2.47	97.2
F	4.49 ± 0.079	0.079	0.0057	0.031	0.008	4.42	97.9

^a Whole Blood (red cells and plasma in whole blood).

^b $\mu\text{g}/\text{ml}$.

^c $\mu\text{g}/\text{ml}/\text{WB}$.

ly norepinephrine which is bound normally to sympathetic nerve terminals (12). The nature of the binding of the 5HT in the avian heart is not known, but apparently it is a loose one, because perfusion of the isolated heart washes out all 5HT in a short time, but not tightly bound norepinephrine (9, 10).

The very high levels of 5HT in the spleen are yet to be explained. Whether or not it is synthesized *in situ* or taken up from the blood and stored remains to be determined.

The fact that most of the blood 5HT is located in the buffy coat fraction suggests that it is bound with thrombocytes or leukocytes. In chickens, the total number of thrombocytes is about the same as the number of leukocytes and there is no sex difference (13). The significantly higher level of blood 5HT in females may be related to the greater number of leukocytes, but not thrombocytes in the female (13). Since 60–70% of the total leukocytes in chickens are represented by the lymphocytes, this cell type may figure prominently in the sex difference of blood 5HT. This, however, remains to be determined.

Summary. Serotonin was determined in heart, spleen and blood of chickens, ducks, and pigeons. The levels in spleens of chickens are very high compared to ducks, pigeons, and most mammals and the concentration is higher in the female sex of all three species. The levels of each of the species were not significantly different; but, in all species, the

atrial levels were considerably higher than the ventricular ones.

The concentration of 5HT in the whole blood was highest in chickens, intermediate in ducks, and lowest in pigeons; and the level was significantly higher in females than males. The data show that approximately 95% of the 5HT is located in the buffy coat fraction of blood.

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