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Distribution of Serotonin Among Blood Cells of the Domestic Fowl[1] (38346)

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Localization of serotonin within mammalian platelets is well established (13) and Sturkie *et al.* (11) have shown that 5-hydroxytryptamine (5-HT) is found within the buffy coat fraction of avian whole blood. This blood fraction contains a number of cell types which may be responsible for serotonin transport, but in the chicken there has been speculation that most of the blood serotonin is localized in the thrombocyte, a nucleated analogue of the mammalian platelet (1, 3, 4). However, there is no quantitative work relating to the distribution of serotonin in avian blood and the object of this research was to isolate purified fractions of cells, determine their serotonin content and relate this to whole blood levels.

Methods. The cell separation techniques of Sengar *et al.* (8) involve the differential centrifugation of buffy coat fractions of plasma in the presence of ethylenediaminetetracetate (EDTA). The low speed centrifugation of blood with EDTA at 4° provides a medium in which a pure lymphocyte fraction (97%) and a pure lymphocyte–thrombocyte fraction (98%) may be isolated with negligible loss of 5-HT (2%). Total and differential counts of lymphocytes, thrombocytes, polymorphs, and monocytes were performed in a hemacytometer employing the stain of Natt and Herrick (7) and thus, permitting specific amounts of serotonin to be related to particular cell types. Approximately 30 ml of blood were removed via heart puncture from adult male and female White Leghorn chickens and treated with 10% EDTA in siliconized glassware. Differential cell counts were performed on whole blood and 1 ml was removed for the 5-HT blood determination by the technique of Weissbach *et al.* (12).

Eight milliliters of blood were added to a 10 ml graduated test tube and centrifuged at 80*g* for approximately 7 min at 2–4° or until layers of plasma and buffy coat were formed. This removes virtually all of the red cells and concentrates the buffy coat fraction containing lymphocytes, thrombocytes, polymorphs, and monocytes at the top of the test tube. The top 2 ml of this buffy coat were removed and added to the modified pasteur pipettes (8). The remaining 6 ml were resuspended, and 1 ml of this mixture was immediately removed for 5-HT analysis.

The pasteur pipette was centrifuged at 80*g* for 5 min or until the red tint (red cells) was removed from the upper lymphocyte layer. This separates the mixture into a pure lymphocyte fraction in the upper three quarters of the tube and a cloudy portion of lymphocytes–thrombocytes in the lower quarter of the tube. One ml of the lymphocyte-rich plasma was removed from the pasteur pipette and assayed for 5-HT content and a lymphocyte count of this fraction was performed in a hemacytometer. Lymphocyte–thrombocyte fractions were prepared using a slower centrifugation speed, which allowed the thrombocytes to remain in the upper lymphocyte fraction and gave the mixture a cloudy appearance.

Results. The number of pure lymphocytes/ml in the blood of females was determined and the concentration of 5-HT in this same 1 ml was also measured. The concentration of 5-HT/lymphocyte was determined by dividing the concentration of 5-HT in the pure lymphocyte fraction by the number of lymphocytes (Table I)

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TABLE I. Serotonin Content of Lymphocytes and Thrombocytes in Female Chickens.

	Pure Lymphocytes							
	Lymphs/ml × 10^7		μg 5-HT/ml lymph		μg 5-HT/lymph × 10^{-7}		μg 5-HT in whole blood (1ml)	
Mean	1.596		1.6		1.01		5.2	
± SE	0.422		0.411		0.165*		0.48	
	Thrombocytes							
	Lymphs/ml × 10^7	μg 5-HT/lymph × 10^{-7}	μg 5-HT/ml lymph[a]	μg 5-HT/ml lymph + throm[a]	μg 5-HT/ml throm	throm/ml × 10^7	μg 5-HT/throm × 10^{-7}	μg 5-HT/ml blood
Mean	1.81	1.01	—	—	3.13[c]	3.265	0.952	6.7
± SE	0.316	0.165	—	—	0.693	0.512	0.178*	0.844

* Coefficient of variation.

[a] Individual tallies obtained by multiplying (lymph/ml) (μg 5-HT/lymph × 10^{-7}).

[b] Individual values determined by direct assay.

[c] Individual values used for the mean and SE obtained by subtracting. (μg 5-HT/ml of lymphs and thromb) from (μg 5-HT/ml lymphs).

derived from a whole blood cell count of 16 birds. The average 5-HT concentration in lymphocytes is 1.51 μg/ml (1.01×10^{-7} (μg 5-HT/lymph)) $\times$ (1.49×10^{7} (lymphs/ml)), Table III; the average 5-HT concentration in blood taken at 10 AM is 5.96 μg/ml and is contained in the lymphocytes, thrombocytes, polymorphs, and possibly monocytes. The lymphocytes therefore contain 25.4% of the total serotonin in 1 ml of blood (1.51 μg/5.96 μg), (Table III).

The numbers of thrombocytes and lymphocytes/ml in the blood of females were determined and the concentration of 5-HT in this same 1 ml of pure thrombocytes–lymphocytes was also measured. Multiplying the average lymphocyte serotonin concentration (1.01×10^{-7} μg 5-HT) by the number of lymphocytes in 1 ml yields the 5-HT concentration of the lymphocytes in the mixture of lymphocytes and thrombocytes. Subtracting this from the total 5-HT in 1 ml yields the serotonin concentration due to thrombocytes (Table I). Dividing the thrombocyte 5-HT concentration by the number of thrombocytes in the same 1 ml yields the serotonin concentration per thrombocyte (Table I). The average 5-HT concentration in thrombocytes is 2.95 μg/ml (0.952×10^{-7} (μg 5-HT/thromb)) $\times$ (3.0956×10^{7} (thromb/ml)) and therefore thrombocytes contain 49.5% of the total serotonin in 1 ml of blood (2.95 μg/5.96 μg) (Table III). The remaining cells, polymorphs (essentially heterophils) and monocytes, account for 1.5 μg 5-HT or 25% of the total 5-HT in 1 ml of blood (Table III).

The concentration of 5-HT in lymphocytes and thrombocytes of male blood was determined in a 1 ml mixture of lymphocytes (1.525×10^{7}) and thrombocytes (2.7875×10^{7}). Employing the previously determined concentration ratio of 5-HT in lymphocytes and thrombocytes of female blood (1.01:0.952) the following equation yields the concentrations of 5-HT per lymphocyte and per thrombocyte:

$$x = \text{5-HT/lymphocyte}$$
$$y = \text{5-HT/thrombocyte}$$
$$1.725x + 3.269y = 3.41$$
$$\frac{x}{y} = \frac{1.01}{0.952}$$

Solving for x and y, $x = 0.70$ $y = 0.66$.

The average 5-HT concentration for a male lym-

TABLE II. Serotonin Content of the Lymphocyte–Thrombocyte Fraction in Males.

	lymphs/ml $\times 10^{7}$	thromb/ml $\times 10^{7}$	μg 5-HT/lymph $\times 10^{-7}$	μg 5-HT/thromb $\times 10^{-7}$	μg 5-HT/ml throm + lymph	μg 5-HT/ml blood
Mean	1.725	3.269	0.70	0.66	3.4	3.5
± SE	0.230	0.486			0.40	0.21

TABLE III. Average Concentration of 5-HT in Lymphocytes, Thrombocytes, Monocytes, and Polymorphonucleargranulocytes in Male and Female Birds.

	Cell type							
	Lymphocytes		Thrombocytes		Polymorphs & monocytes		Total buffy coat cells	
	(Male)	(Female)	(Male)	(Female)	(Male)	(Female)	(Male)	(Female)
μg/ml 5-HT	1.01	1.51	1.84	2.95	0.65	1.50	3.5	5.96
% of total 5-HT	29.0%	25.4%	52.6%	49.5%	19.4%	25.1%	100%	100%
% of buffy coat cells	31.4%	28.6%	57.2%	59.2%	11.4%	12.2%	100%	100%

	Average cell coun's (X 10^7 per ml.)			
Cell type	Males	Coefficient of variation	Females	Coefficient of variation
Lymphocytes	1.5250	8.6%	1.49326	10.6%
Thrombocytes	2.7875	5.0%	3.09560	6.1%
Polymorphs	0.3938	55.4%	0.51300	42.1%
Monocytes	0.1563	49.7%	0.12700	47.0%
All buffy coat cells	4.8625	45.0%	5.23000	28.0%

phocyte is 0.70×10^{-7} μg and for a thrombocyte 0.66×10^{-7} μg (Table II). These values are multiplied by the average value of lymphocytes and thrombocytes derived from a whole blood cell count of eight birds (Table III). The lymphocytes contain 29.0% of the total serotonin in 1 ml of blood (1.0 μg/3.5 μg) and the thrombocytes contain 52.6%of the serotonin in 1 ml of blood (1.84 μg/3.5 μg). The polymorphs and monocytes account for the remaining 5-HT (0.45 μg) or 19.4% of the total 5-HT in 1 ml of blood (Table III).

Discussion. These results show that 5-HT of whole blood is distributed among at least three cell cypes as follows: 50% in the thrombocytes, 25–29% in the lymphocytes and 19–25% in polymorphs and monocytes.

The average 5-HT concentration per lymphocyte however is higher than that of the thrombocytes, but the number of lymphocytes is significantly less in both sexes. The concentration per thrombocyte of the chicken cell is comparable to that in the rabbit platelet (12), a mammalian species with high blood level of 5-HT.

A possible function of the 5-HT in these cells is the removal of the large amounts of serotonin that are released into the blood from the intestine. This is evidenced by the significant diurnal changes in 5-HT levels (6) and may exceed the capacity of the degradative enzymes in the kidney, lungs, and liver where high concentrations of 5-HT are also found. Our unpublished results suggest that there is no monoamine oxidase activity in chicken plasma that would be able to degrade the released amine.

Significant sex differences in the concentration of blood and tissue 5-HT have been described by Sturkie *et al.* (11) and Meyer *et al.* (6). The sex difference also exists in the blood levels of catecholamines (10) and histamine (2). Our data suggest that the sex difference in 5-HT levels is primarily due to a higher concentration of 5-HT in each of the female cell types. Since blood 5-HT is essentially derived from the synthesis and release of intestinal 5-HT it is quite probable that the enterochromaffin cells of females have a relatively higher rate of synthesis-release than those of males. The possibility also exists that female cells have a greater number of active sites for serotonin uptake, although this remains to be proven.

Another contributing factor to the sex difference may be the reported greater number of lymphocytes in the female. Although our data show that male and female lymphocyte counts are approximately equal, Lucas and Jamroz (5) show a female to male ratio of 22,000:10,500. However, this discrepancy is not unusual since many factors can influence leucocyte counts such as different environmental conditions of temperature, photoperiod, single or group caging and the normal diurnal variation in some cell

counts (9).

Summary. The 5-HT content of whole blood of chickens is distributed mainly among the following cell types: (a) thrombocytes (50%), (b) lymphocytes (25–29%) and (c) polymorphs and monocytes (19–25%).

The significantly higher number of thrombocytes in male and female blood largely accounts for the high level of whole blood 5-HT (50%) even though the average concentration per cell type is higher in the lymphocytes than any other type. The concentration of 5-HT per lymphocytes and thrombocytes of female blood was higher than in male blood and this accounts for the sex difference in whole blood 5-HT.

1. Bracco, M., Curti, P. C., and Giuliano, V., Experimentalis. **12,** 31 (1956).

2. El-Ackad, T. M., and Sturkie, P. D., Proc. Soc. Exp. Biol. Med. **141,** 448 (1972).

3. Inouye, A., Kataaoka, K., Sorimachi, M., and Hori, S., Eur. J. Pharmacol. **8,** 200 (1969).

4. Kimura, E., Protein Nucl. Acid Enzyme **12,** 338 (1967).

5. Lucas, A., and Jamroz, C., "Atlas of Avian Hematology," Agriculture Monograph 25, U.S. Department of Agriculture, Washington, D.C. (1960).

6. Meyer, D. C., Sturkie, P. D., and Gross, K., Comp. Biochem. Physiol. **46A,** 619 (1973).

7. Natt, M. P., and Herrick, C., Poultry Sci. **31,** 735 (1952).

8. Sengar, D. P. S., Jerome, F. N., and Douglas, R. J., Can. J. Comp. Med. **22,** 593 (1968).

9. Sturkie, P. D., "Avian Physiology," Cornell Univ. Press, Ithaca (1965).

10. Sturkie, P. D., Poorvin, D., and Ossorio, N., Proc. Soc. Exp. Biol. Med. **135,** 267 (1970).

11. Sturkie, P. D., Woods, J. J., and Meyer, D., Proc. Soc. Exp. Biol. Med. **139,** 364 (1972).

12. Weissbach, H., Waalkes, T. P., and Udenfriend, S., J. Biol. Chem. **230,** 865 (1958).

13. Zucker, M. B., Friedman, B. K., and Rapport, M. M., Proc. Soc. Exp. Biol. Med. **85,** 282 (1954).

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