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DIFFERENCES IN THE COMPOSITION OF VENOUS AND CARDIAC BLOOD FROM WHITE-CROWNED SPARROWS

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We have examined the blood composition of White-crowned Sparrows (*Zonotrichia leucophrys*) for several years. In the process, we frequently measured the composition of venous and cardiac samples from the same birds. Some relationships between the composition of the two have come to light and are potentially useful to avian biologists. These pertain specifically to plasma-free (nonesterified) cholesterol and total calcium levels, and to the packed cell volume (hematocrit) of the blood.

Mammalian physiologists have collected information about arteriovenous (AV) differences in blood for many years (see Dittmer 1961:169–176). Avian physiologists have apparently done less work in this area (e.g. Sykes 1960, Cohen 1969). On the whole, little comparative information is available concerning the composition of avian blood in different parts of the body, and virtually no literature appears to exist for small passerines. Even among larger birds, reports are limited to such measurements as differences between the "whole body" and venous hematocrits of the Pintail (*Anas acuta*; Cohen 1969); or calcium uptake by the shell gland of domestic fowl (*Gallus domesticus*) determined by means of AV differences in this cation (Winget et al. 1958, Hunsaker and Sturkie 1961). We have been unable to find reports of AV differences in the plasma cholesterol of birds.

Arteriovenous, or more correctly cardiovenous, differences can be important if they are predictable, because they then permit direct comparisons to be made between blood studies in which samples have been collected at different sites. In small songbirds, there are three such sites: the heart (Utter et al. 1971, de Graw 1972, Temple 1974); the brachial artery or vein (Follett et al. 1974, 1975, Wingfield and Farner 1976); and the point at which the vena tibialis postica passes over the intertarsal joint of the leg (Kern et al. 1972).

MATERIALS AND METHODS

We measured the blood composition of female White-crowned Sparrows (*Z. l. gambelii*) captured in mist nets near Pullman, Washington, and held in large outdoor aviaries at Washington State University under natural conditions of temperature and photoperiod. Females, as determined by laparotomy (Bailey 1953), were selected randomly from this captive group for use in our experiments. All of them were photosensitive. The experiments have been presented in detail in a previous paper concerning temporal changes in blood composition (Kern et al. 1972) and will be outlined here only briefly.

Birds were held by pairs indoors in small cages (23 × 36 × 28 cm) under controlled photoperiods (LD 8:16 or 16:8) and ambient temperatures (21–25°C). Groups of at least six sparrows were given intramuscular injections of estradiol, testosterone, progesterone, or cottonseed oil for 12–15 days and sacrificed three days later.

On the day of sacrifice, a blood sample (ca. 100 µl total volume) was collected from the vena tibialis postica of each sparrow in two heparinized microhematocrit tubes (Clay-Adams, Parsippany, N.J.). These were heat-sealed at one end and put aside while a larger sample of blood (1.0–2.0 ml total volume) was removed from the bird's heart. Cardiac samples were collected in a siliconized 2.0-ml syringe fitted with a 1.5-inch, 20-gauge hypodermic needle. Syringe and needle were rinsed with 1% heparin (California Corporation for Biochemical Research, 100 U/mg) in 0.9% NaCl and drained briefly before use. The volume of heparinized saline retained in the needle-syringe unit is less than 0.041 ml and for a blood sample of 1.0–1.5 ml causes an error smaller than one hematocrit unit (1% de Graw 1972). On the basis of color, the majority of cardiac samples came from the right ventricle (mixed venous blood). However, we did not control for this and have assumed that little change occurs in blood cholesterol, calcium, and hematocrit during circulation of blood through the pulmonary circuit. The fact that there were consistent differences between shank and cardiac blood, whether the latter came from the left or right side of the heart, appears to confirm this assumption.

Blood was quickly withdrawn from the heart and transferred to a dry, heparinized centrifuge tube. A sample of it was immediately taken up in a third microhematocrit tube, which was heat-sealed at one end. All three microsamples were then centrifuged at 5130 *g* for 10 min, a period adequate for complete packing of the erythrocytes (de Graw 1972). The hematocrit of each tube was subsequently determined with a microhematocrit tube reader. The plasma in each tube was then separated off and chemically analyzed. Nonesterified (free) cholesterol in the plasma was measured with a miniaturized version of the Searcy-Bergquist method (1960) and total calcium by flame photometry (Perkin-Elmer Atomic Absorption Spectrophotometer, Model 303).

Least squares analyses were done on each blood parameter in order to determine if venous and cardiac values were related. Student's *t*-tests were performed to determine the statistical significance of these relationships (Snedecor 1956).

TABLE 1. Composition of plasma samples from the shank and the heart of individual White-crowned Sparrows. Pairs of values are representative of a larger pool of information on which equations presented in the text of this paper are based.

Plasma total calcium (mEq/L)				Plasma free cholesterol (mg/ml)				Hematocrit (%)			
Shank	Heart	Shank	Heart	Shank	Heart	Shank	Heart	Shank	Heart	Shank	Heart
3.67	3.40	6.60	6.14	.307	.350	.758	.664	28	26	48	43
3.75	4.11	6.82	5.98	.425	.400	.767	.694	30	24	48	48
3.92	4.58	7.29	6.56	.433	.506	.788	.807	32	27	49	43
3.93	4.02	7.61	5.65	.467	.412	.795	.707	32	30	49	44
3.97	4.11	7.83	7.45	.485	.331	.806	.756	33	28	50	40
4.06	4.14	7.88	6.47	.493	.475	.826	.655	33	32	50	43
4.10	3.91	8.59	5.26	.500	.506	.833	.812	34	30	51	45
4.22	3.91	24.44	20.00	.513	.444	.847	.773	35	28	51	45
4.38	4.06	28.08	21.92	.527	.362	.861	.906	35	30	52	45
4.44	4.05	31.54	24.62	.533	.662	.871	.673	36	31	52	48
4.55	4.24	33.33	31.94	.559	.765	.883	.781	38	24	53	47
4.61	5.04	34.62	22.31	.560	.569	.906	.847	38	32	53	49
4.79	5.47	37.69	28.46	.589	.462	.917	.906	39	31	54	52
4.83	4.83	38.06	33.33	.593	.438	.941	.985	40	35	55	44
4.90	4.33	39.95	25.00	.600	.694	.995	.840	41	33	55	51
5.00	5.42	40.83	39.72	.607	.662	1.011	.938	42	37	56	45
5.14	4.58	43.06	37.22	.611	.719	1.196	.990	42	39	57	47
5.21	4.65	46.94	45.83	.624	.573	1.208	1.075	43	31	58	46
5.62	5.16	47.22	41.67	.633	.569	1.312	.930	43	40	58	48
5.77	5.48	62.50	55.00	.667	.812	1.431	.775	44	39	61	52
5.92	5.92	65.22	50.43	.672	.781	2.741	2.668	44	42	61	52
6.04	5.10	77.50	68.33	.688	.765	3.945	3.818	45	43	61	52
6.06	4.23	80.87	59.13	.700	.660	4.118	5.405	46	42	63	53
6.09	5.05	84.35	71.30	.719	.640	4.624	4.071	47	43	64	55
6.23	6.15	89.56	66.96	.733	.694	5.259	5.704	47	45	66	57

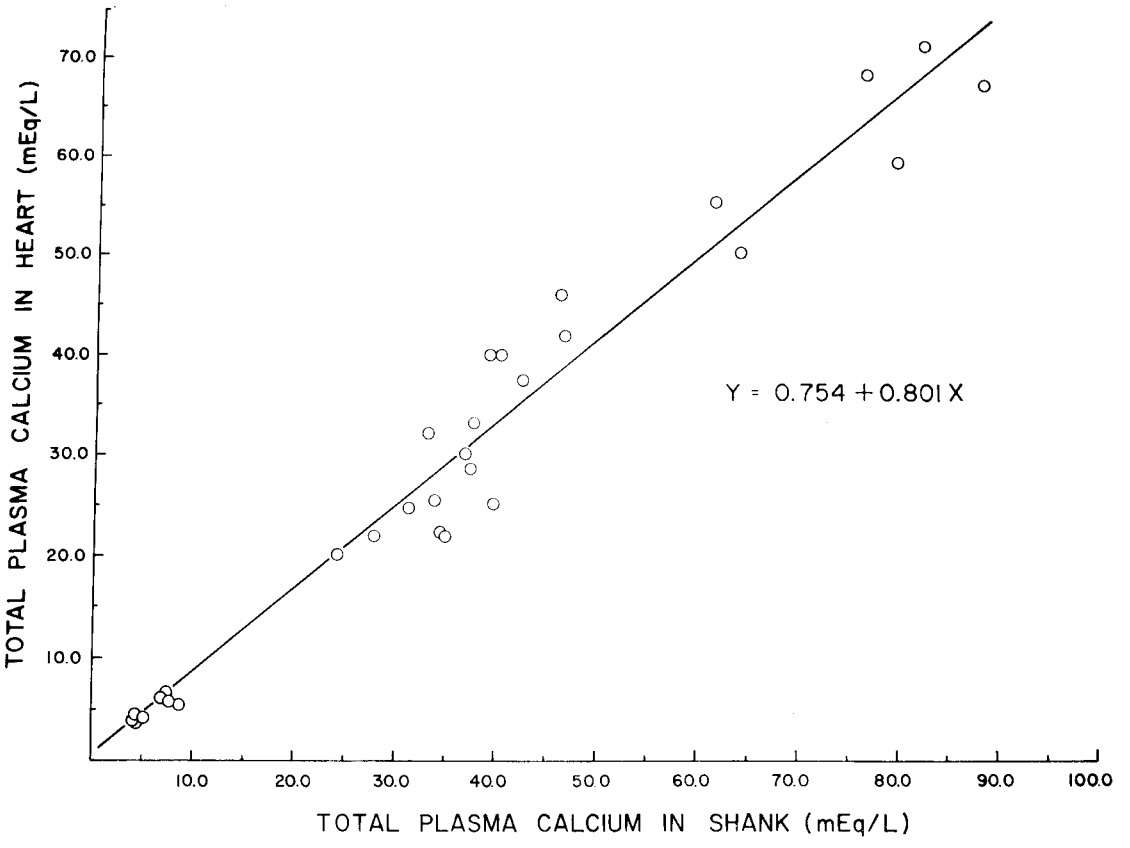

FIGURE 1. The relationship between the total plasma calcium content of blood from the shank and the heart is linear in female White-crowned Sparrows (*Zonotrichia leucophrys gambelii*), and described by the equation which appears in the figure. The correlation coefficient (r) for this line is 0.990. Open circles are representative values from among the 107 on which the regression line is based.

RESULTS

We found statistically significant linear relationships between the composition of blood in the shank and heart (Table 1). The relationship shown in Figure 1 between the calcium concentration of blood in the leg and heart is representative.

The hematocrit of blood from the shank was consistently higher than that of cardiac blood throughout the normal range of hematocrits for this species (Table 2; also see Kern 1970, de Graw 1972) and even in cases where estrogen severely depressed the hematocrit (as low as 33.8%). The relationship is linear (the correlation coefficient, r, is 0.885; $n = 111$ sparrows), statistically significant ($P < 0.001$), and described by the equation

$$Y = 1.899 + 0.828\ X \qquad (1)$$

in which X and Y are the shank and cardiac hematocrits (in %), respectively.

Similarly, the total calcium content of venous blood from the shank generally exceeds the calcium content of cardiac blood unless the calcium level is below 3.79 mEq/L (Table 1 and Fig. 1). The relationship is linear ($r = 0.990$; $n = 107$ sparrows) and applicable to calcium values normally found in captive and free-living White-crowned Sparrows (Table 2), as well as the extreme hypercalcemia (approaching 80.00 mEq/L) induced by estrogen. The relationship is statistically significant ($P < 0.001$) and can be described by the equation

$$Y = 0.754 + 0.801\ X \qquad (2)$$

in which X and Y are the total calcium concentrations (in mEq/L) of shank and cardiac blood plasma, respectively.

The free cholesterol levels of shank and cardiac samples are also linearly related ($r = 0.968$; $n = 81$ sparrows). The relationship is again significant ($P < 0.001$) and can be expressed mathematically by the equation

$$Y = -0.074 + 1.045\ X \qquad (3)$$

in which X and Y are the free cholesterol values (in mg/ml) of shank and cardiac plasma, respectively. The equation is applicable to the entire range of cholesterol levels

TABLE 2. Normal values of calcium, cholesterol, and hematocrit for the cardiac blood of captive and free-living White-crowned Sparrows.

Blood variable	Captive sparrows			Free-living sparrows		
	X ± SEM	n	Range	X ± SEM	n	Range
Plasma total calcium (mEq/L plasma)	4.43 ± 0.05	(215)	2.50–6.20	4.30 ± 0.08	(267)	1.38–18.95
Plasma free cholesterol (mg/ml plasma)	0.964 ± 0.022	(205)	0.212–1.955	0.671 ± 0.012	(224)	0.181–1.322[a]
Hematocrit (% total blood volume)	47.4 ± 0.3	(205)	25–59	47.0 ± 0.3	(249)	24–58

[a] Cholesterol levels of egg-laying females have not been measured and may lie above this range.

normally found in captive and free-living White-crowned Sparrows (Table 2), as well as the extreme hypercholesteremia (up to 5.656 mg/ml plasma) caused by exogenous estrogen.

DISCUSSION

These cardiovenous relationships have some interesting and perhaps physiologically important implications. According to the equations, there are points at which the hematocrit, calcium, or cholesterol values of shank and cardiac blood are equal. For hematocrit and calcium, these *equivalence points* are 11% and 3.79 mEq/L, respectively. Values as low as this occur rarely, if ever, in *Zonotrichia* (Table 2) or any other bird. We therefore conclude that hematocrit and plasma total calcium are normally higher in the shank than in the heart.

On the other hand, the relationship for free cholesterol is more complex since the equivalence point is 1.644 mg/ml plasma. Cholesterol is higher at heart level above this point, but higher in the shank below this point. Plasma cholesterol does not reach the equivalence point in free-living White-crowned Sparrows (Table 2). In other words, less cholesterol is generally present in cardiac blood than in blood from the shank. However, the plasma cholesterol of captive sparrows is frequently above the equivalence point (Table 2) and in these birds cardiac values may exceed or fall below shank values.

We do not know why these differences exist at present. Perhaps they reflect differences in plasma volume at the two locations. Such differences would produce corresponding differences in the concentration of cholesterol-containing lipoproteins and erythrocyte volume between the same sites. However, such an explanation is less likely to account for differences in electrolyte distribution, since cations such as calcium move freely across the vascular wall in the plasma water.

The hematocrit and calcium content of venous blood in the shank may be high because calcium and erythrocytes enter the blood as it passes through the red bone marrow in the tarsometatarsus, immediately below our sampling site. Blood levels of cholesterol may be high at the level of the heart because the liver adds cholesterol-containing plasma proteins to blood in the hepatic vein (Ranney and Chaikoff 1951). However, we have no ready explanation for the disappearance of cholesterol en route to or in the lower leg.

The design of our experiments made it necessary to always sample shank blood before doing cardiac puncture. The systematic nature of the sampling method may be responsible for some of the observed differences described above, but cannot account for all of them. The fact that there are equivalence points for all three blood components argues against a sampling effect of this nature, as does the fact that cardiac values are not always above (or below) shank values (Table 1). Furthermore, the volume of blood removed from the leg is too small to introduce a significant change in blood composition or to explain the observed differences in hematocrit. The blood volume of birds is 5–13% of the body weight (Freeman 1971, Jones and Johansen 1972, Sturkie 1976). For White-crowned Sparrows with an average body weight of ca. 25 g, blood volume is accordingly 1.25–3.25 ml. Using heart puncture, we routinely obtain 1.0–2.0 ml of blood and occasionally as much as 2.5 ml. Even if we assume that the total blood volume is as small as 1.25 ml, the blood removed from the shank is only 8% of the total (3% if total blood volume is 3.25 ml). This volume is not likely to reduce blood calcium levels, given this ion's

mobility in the extracellular fluid compartments of the body and, if anything, would always reduce, rather than elevate, cardiac cholesterol levels. However, the removal of this much blood could reduce the hematocrit by 2–4 units, depending on whether blood volume is assumed to be 1.25 or 3.25 ml. Nonetheless, equation (1) predicts an hematocrit of 54.5% in the shank when cardiac hematocrit is 47% (the yearly average for White-crowned Sparrows—see Table 2), a difference of 7.5 units, or roughly double the difference attributable to the method of sampling.

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