Comparative Biochemistry and Physiology, Part A 156 (2010) 351–356

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Age-correlation of blood values in the Rock Pigeon (Columba livia)

Roland Prinzinger *, Andrea Misovic

Department Metabolic Physiology, FB 15 (Biowissenschaften), Johann-Wolfgang Goethe University, Siesmayerstrasse 70, 60323 Frankfurt am Main, Germany

ARTICLE INFO

Article history:
Received 13 May 2009
Received in revised form 16 July 2009
Accepted 28 July 2009
Available online 23 February 2010

Keywords:
Rock pigeon
Blood components
Plasma chemistry
Age
Sexual variation
Season
Columba livia

ABSTRACT

Hematological adaptations to age (1–17 years) and about variability *per se* for free-living rock pigeons *Columba livia* are presented. Increasing age is correlated with decreasing values of hematocrit and hemoglobin. A marked reduction of lactate dehydrogenase (EC 1.1.1.27) activity in the first 2–3 years may be caused by a training-based increase of the relative portion of the aerobically working red breast muscles (responsible for endurance) at the expense of the proportion of anaerobically working white breast muscles. The age-correlated increase in glucose could indicate a decreasing tolerance for carbohydrates. Optimal flight performance is achieved by the doves at an age of about 2–3 years; the high performance is retained until an age range of 7–9 years.

1. Introduction

The usefulness of bird plasma biochemistry and blood composition as a complementary tool (not only) in the diagnosis of disease and in monitoring the progress of the clinical condition of birds is widely recognized. However, it is also useful in captive breeding programs, medical management of endangered bird species, and as an important physiological parameter for many comparative considerations, as well as for inter- and intraspecific analyses in different fields of biology. Nevertheless, for the classification of measured blood parameters in birds, it is necessary to know the factors that can influence blood in its composition, e.g. sex, season and – as yet relatively less considered in the literature – the age of the animal.

Age can be an important source of variability of blood parameters in birds. However, studies on avian blood parameters have mainly focused on the comparison between chicks or juveniles and adult birds (e.g. Casado et al., 2002; Alonso-Alvarez, 2005; Wolf et al., 1985; Howlett et al., 2002). Studies which describe a more detailed approach are very scarce. Jurani et al., 2004) described changes in plasma composition in starling *Sturnus vulgaris*) chicks during the complete period in the nest (1–15 days of age). Bailey et al. (1999) showed changes in plasma parameters from a few weeks of age to adulthood (1 year-old) in captive houbara (*Chlamydotis undulata)* and Kori bustards (*Ardeotis kori*). But there does not exists any experimental data which describes possible age-correlated changes of blood parameters from grown up birds until their death.

Pigeons, especially the domestic rock pigeon (*Columba livia f. domestica*), are widely distributed throughout the whole world (except arctic regions) and they may be a good model species to study age-correlated changes in blood composition during adulthood, because many blood reference values have recently been reported (Gayathri et al., 2004; Kalomenopoulou & Kolakios, 1989; Kasprzak et al., 2006; Palomeque & Planas 1977; Scope et al., 2002; Viscor et al., 1985). On the other hand, there are still a lot of open questions concerning the "gerontology" of blood parameters in this species.

For this reason we analyzed the age-related dynamics of blood and plasma parameters in sexually mature pigeons from 1 to 17 years old.

2. Materials and methods

2.1. Birds tested and sampling methods

For this study we used the rock pigeon *Columba livia*, of which we have enough individuals with different basic characters to obtain general information on avian hematology and to determine the variations in blood parameters according especially to age. Additionally, our doves were very accustomed to human handling, so that the data should have a high significance.

The pigeons have been bred and housed in an outdoor aviary in our institute for more than 40 years. They can leave the aviary for outdoor flights whenever they want. Each bird is ringed immediately after hatching, so that it is possible to determine the exact age. We chose 79 individuals of different hatching years 1991–2007 (Table 1). All tested birds were adult, sexually mature (≥1 year) and apparently healthy. Blood samples were obtained between November 2006 and November 2008 during two seasons: in winter (October–January), when the

* Corresponding author. Tel.: +69 798 24749; fax.: +69 798 24820.
E-mail address: prinzinger@bio.uni-frankfurt.de (R. Prinzinger).

1095-6433/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2009.07.028

Table 1
Number and sex of tested rock pigeons (*Columba livia*).

Year of hatching	Number of males	Number of females	Sex unknown
1991	1		1
1992	1		1
1993		2	
1994			1
1995		3	1
1996		2	
1997	2	1	3
1998	3	2	1
1999	4	1	1
2000	2	6	
2001	5	5	
2002		1	
2003	5	5	
2004			
2005	5	5	
2006	5	5	
∑	33	37	9

birds didn't show any signs of courtship display or egg-laying, and in summer (March–September) during the breeding season.

All blood samples were taken alternately from the right and the left brachial vein at nearly the same hour (9–10 h), to minimize variations in blood components caused by circadian rhythm (Schaub et al., 1999). Blood samples were drawn with a 2 mL syringe fitted with a 0.6 mm-gauge-needle, filled with a defined amount of heparin (20 µL Heparin-Natrium-7500 ratiopharm® FS). The amount of blood was precisely determined from the syringe's scale to correct the dilution with heparin. The samples for plasma biochemistry analyses were centrifuged immediately to avoid changes, for example, in glucose concentration) after collection at 2 000 U/min for at least 5 min. Then the plasma was stored at −20 °C until analysis. The counting of blood cells and estimation of hemoglobin and hematocrit were done immediately after sampling.

2.2. Blood components

Blood cell counts were made according to (Natt & Herrick 1952). Aliquots of blood were diluted 100-times in hematological pipettes with Natt & Herrick solution and five big squares of the Thomae-chamber were counted for erythrocytes as well as all 16 big squares for leucocytes.

Hemoglobin content was estimated colorimetrically using a Hemo-Cue 201$^+$ (Hemocue AB, Ängholm, Sweden). Blood was drawn up into a cuvette, where it underwent a modified azide-methemoglobin reaction. The erythrocyte membranes were disintegrated by sodium deoxycholate, resulting in the release of hemoglobin. Sodium nitrite converted the hemoglobin iron from the ferrous to the ferric state to form methemoglobin, which then combined with azide to form azide-methemoglobin (Vanzetti 1966). The photometer used a dual wavelength measuring method, 570 nm and 880 nm, for compensation of turbidity. The amount of hemoglobin was given in the display after 1–2 min.

For the determination of packed cell volume, heparinised capillary tubes were filled with blood and centrifuged at 10,000 g for 8 min. The packed cell volume was then read directly on a micro-hematocrit reader.

For the measurement of the erythrocytes a blood smear was prepared and dyed according to Pappenheim. The length and width of the erythrocytes and their nucleus were measured using an ocular micrometer.

The amount of the mean concentration of hemoglobin (MCH = pg/erythrocyte and MCHC = percent/erythrocyte) and cell volumes (MCV) were calculated from the obtained data.

2.3. Plasma biochemistry

The following 19 biochemical parameters in plasma were investigated: glucose, total protein, triglycerides, cholesterol, urea, uric acid, Ca^{2+}, Mg^{2+}, PO_4^{3-}, Na^+, Cl^-, K^+, Fe^{2+}, alkaline phosphatase (AP; EC 3.1.3.1), creatine-kinase (CK; EC 2.7.3.2), aspartate aminotransferase (AST; EC 2.6.1.1), alanine aminotransferase (ALT; EC 2.6.1.2), lactate dehydrogenase (LDH; EC 1.1.1.27), and cholinesterase (CHE; EC 3.1.1.7/3.1.1.8).

All parameters were determined by a dry chemical system (Kodak Ektachem DT 60 Analyzer) using the modules DT 60 II, DTSC II and DTE II Johnson & Johnson Clinical Diagnostics). For each analyte given above Clinical Diagnostic provides special small multilayer films, which contain all necessary chemicals for an appropriate and fast determination of corresponding quantitative content. Onto each such film strip 10 µL blood plasma was dropped, and then the strips were incubated in the associated incubator. The analyzer is equipped with a special photo-detector that transmits the measured parameter-concentration directly to a printer.

2.4. Statistics

Mean ± SEM, and range of each blood parameter were calculated separately for each season and within the season for each sex. We used the *t*-test for analysis of any significant differences between the groups.

To analyze age-correlated changes in blood composition, we used the non-parametric test according to Kruskal–Wallis (GraphPad Prism) after testing that the distribution of our results conformed to Gaussian distribution. We used nonparametric statistics because we did not have enough old pigeons (>12 years) available to get more than two samples in each age category. When we determined which of the parameters varied significantly with age, we summarized all these parameters measured in pigeons older than 12 years in one group and used one-way-ANOVA and Tukey's post-test to confirm the results previously obtained.

3. Results

3.1. Mean and range values over the whole sample range

An overview of the mean data over all age-groups is given in Table 2. No differentiation between seasons and sexes was made. Data on the variabilities depending on sex, season, breeding, and different other parameters are published at another place (Prinzinger & Misovic, 2010).

3.2. Variabilities depending on age

To minimize variations in blood components caused by the breeding cycle, only blood samples taken during the winter were tested for variability depending on age, because the differences between male and female pigeons were too great during the summer measurement owing to the egg-production by females. All results conformed to Gaussian distribution, and after checking our results with the non-parametric test, we found clear age-related changes in blood components in the following parameters: hemoglobin ($p = 0.0467$), glucose ($p = 0.0132$), hematocrit ($p = 0.0045$), chlorides ($p = 0.0103$), and LDH ($p = 0.0011$); body mass did not differ among the age-groups.

3.2.1. Hematocrit (Hc)

The highest value for Hc [%] can be found in 2 year-old pigeons, about 65.3 ± 1.3%. During the first 6 years the hematocrit remained relatively stable, with a mean value of 64.9 ± 0.3%. The correlation with age is very weak: Hc = 65.0–0.09 · A [years], $r = 0.45$. From the age of 7 years on there is a clear decrease, which can be described by the linear correlation Hc = 68.9–0.86 · A, when we do not take into account the single values