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TO THE STUDY OF HEMATOLOGICAL INDEXES (THE SIZE OF ERYTHROCYTES) OF A PALLAS COLUBER *(ELAPHE DIONE),* 1773 (SQUAMATA, SERPENTES)

К изучению гематологических показателей (размеров эритроцитов) у узорчатого полоза Elaphe dione Pallas, 1773 (Squamata, Serpentes)

Наарду сойлоктун Elaphe dione Pallas, 1773 (Squamata, Serpentes) гематологиялык керсеткучтерун (эритроциттердин елчемдеру) изилдее

Abstract: *the article is dedicated to the study of one of the most widespread types of snakes from the genus of creeping colubers-to the Pallas Coluber (Elaphe dione), to its morphology and such indexes, as size of red corpuscles, reflecting the physiological state of organism depending on ecological conditions (in this case in the Kochkor Valley, Kyrgyzstan). In this research, the method of intravital determination of blood composition was applied, which is important for saving the population.*

Аннотация: *статья посвящена изучению одного из широко распространенных видов змей из рода ползающих полозов - узорчатому полозу Elaphe dione Pall, его морфологии и такому показателю, как размеры эритроцитов, отражающему физиологическое состояние организма в зависимости от экологических условий (в данном случае условий Кочкорской долины Кыргызстана). В исследовании применялся метод прижизненного определения состава крови, что важно для сохранения численности популяции вида.*

Аннотация: *бул макала жыландардын арасынан сойлоктор уруусуна кируучу кецири тараган тур - наарду сойлокко Elaphe dione Pall., анын морфологиясына жана экологиялык шарттарга жараша организмдин физиологиялык абалын чагылдыруучу эритроциттердин влчвмдврунв арналган. (Кочкор врввну Кыргызстан). Изилдвдв турдун популяциясынын санын сактоо учун маанилуу болгон, кандын курамын аныктоодо тируу абалында изилдее методу колдонулду*

Keywords: *hematological indexes; morphology and physiology of Pallas Coluber; physiological state; quantity and diameter of erythrocytes; activity; artificial hibernation; method of a intravital research.*

Ключевые слова*: гематологические показатели; морфология и физиология узорчатого полоза; физиологическое состояние; количество и*

диаметр эритроцитов; активность; искусственная спячка; метод прижизненного исследования.

***Негизги свздвр**: гематологиялык кврсвткучтвр; наардуу сойлоктун морфологиясы жана физиологиясы; физиологиялык абалы; эритроциттердин саны жана диаметри; активдуулук, жасалма чээнге киргизуу; тируулвй изилдвв методу.*

Among innoxious snakes of Kyrgyzstan, the Pallas Coluber *(Elaphe dione)* (Genus of rat snakes Elaphe, Firz, 1832) is considered to be the most widespread type of Elapheand, of very changeable coloration. [13.6]). Its length is up to 1.2 m. If we take a look at the head pholidosis of this Pallas Coluber, it is visible that prefrontal scutes adjoin with supraorbital ones, and the only jugal scute is trapezium-shaped. As a rule, there are from one to three postorbital scutes, eight supralabial scutes, subcaudal ones - 44-76. Females are bigger than males, and their coeliac scutes reach up to 184-215 in quantity. Proctal scute is usually split; the fourth labial scute adjoins the eyes. Scutes on the back are with weak ribs and two apikal pores, and on the sides of the body they are smooth. On the top surface of the head, a drawing familiar to this type of Pallas Coluber is located, which changes with age. A dark temporal strip passes through the eyes to the neck. The belly part is usually grayish or yellowish; rarely with dark spots. After a molt the coloring becomes more contrast and scutes start to shine.

It lives in the most various conditions in several natural zones of the republic, i.e. it is very ecologically pliant genus. It is spread from steppes and deserts of the Chuy and Talas valley plains up to the mid-mountain and mountain steppes, meadows, deserts and semi-deserts of the Kochkor, Kara-Kuzhur, Dzhumgal, Toktogul and other valleys. They can be found at the floodplains and valleys of the rivers, lakes, in the juniper forests and on stony slopes of the mountains, on the suburbs of the swamps, which rise up to 3200 meters above the sea level. Pallas Coluber may be found in arid landscapes, on saline soils. It often inhabits anthropogenic landscapes: gardens, kitchen gardens, crops and irrigated lands. It perfectly climbs and quickly moves on branches of trees and bushes and on the ground, perfectly swims and dives. It eats small mammals (voles, mice), amphibians, fishes and insects, eats baby birds and bird eggs, easily climbs at steeps seeking for bird nests. It is usually active during the day.

The Kochkor Valley, is located in the Central part of Tien Shan, and differs by its orographic state and a number of ecological conditions. Dwelling of many animals in such conditions, including reptiles, to which Pallas Coluber belongs, influences morphological and physiological (hematological) indexes [6].

In the Kochkor Valley, we have found it in the village of Arakol (coordinates: height 1998 –2001m; N42^{0}11' 038", E 075^0 28' 409" and N42^{0}10' 157", E 075^0 29' 299") where it has occupied a piedmont part of the valley and was found along wetlands stretching to the north of the village and occupy scores of hectares. Abundance is little – one or two specimen per hectare. Under the conditions of mountainous semi-desert and steppes, what Shamsi is like (coordinates: height of 2397-2500 m, N42^{0}19' 711", E 075^{0}24' 348"), the Pallas Coluber is spread sporadically and occupies semi-desert and steppe sites. In a high-mountain part of the Kochkor Valley with the Chaar-Arch rift (coordinate: height 2752-3041m; N42^{0}02' 687", E 075^0 10' 492" - N42^{0}03' 951", E 075^0 10' 061") with its rugged landscape and various elevation changes, Pallas Coluber was found at the threshold of this rift, where it occupied a meadow and steppe zone. However, we should note that the population in these conditions was low, and we found only two specimen.

In the conditions of the Kochkor Valley, the Pallas Coluber falls into hibernation at the beginning of September, and it lasts till March or April. Both leaving for wintering and the beginning of copulation depends on altitudinal belt. In a plain part of the valley (the Arakol village, Kochkorka village, Cholpon, etc.), the Pallas Coluber falls into hibernation at the end of September, and it lasts till April or the beginning of May; in an alpine part (the Shamsi village, Ormonkhan, Chaar-Archa, etc.) – at the end of August, and it lasts till May. According to data, it is known that males mature before females [14]. For the first time, the blood was taken from a Pallas Coluber from the Kochkor Valley to identify the size of red corpuscles, more precisely, to identify their diameter from different habitats depending on a physiological condition of an organism.

Resaurces and work procedure. The material for this work was (as an ecologically pliant genus) **the Pallas Coluber, which was caught from different areas.**

The blood draw and sample was made, taking into account the sex, season, age and physiological conditions of animals. We applied a method of intravital blood draw, which is important for saving the population of a genus. There are several methods of intravital blood draw from snakes and lizards. Many researchers use the reptiles' tail part of the body for blood draw. It is a widespread blood draw method of dissecting or incision of a tail tip of turtles [16], snakes, small lizards and crocodiles [15, etc.]. Tail amputation allows receiving the necessary amount of blood for hematological and biochemical researches. The punctures of orbital, carotid

and sciatic arteries of snakes, offered by Olson [15] and other researchers, are not used nowadays, because of the small amount of blood received, which is insufficient for hematological researches.

We applied a method of blood draw by dissecting away a tail tip. We were convinced the amount of blood is enough for hematological research, especially for receiving good quality samples which is important while studying red corpuscle and measuring their diameter.

Determination of blood composition was made in the period of activity (June-July), i.e. in vivo and after it quits natural hibernation which corresponds to the state after keeping it at a temperature of $+1^0$C, $+ 2^0$C artificially. Smear was carried out by the standard methods in medical industry and animal ecology [11, 5], with some modifications in relation to poikilothermal animals. Duration of smear coloring was selected based on a dye. Preparations were kept fixed in the 96^0 ethylenic alcohol, fixing lasted up to one minute, the coloring was carried out by azur-eosine according to Romanovsky, and duration was 20-25 min. Smears dried usually on open air at ambient temperature until disappearance of the wet gloss, because the cells are colored in better way. [10].

The painted dry smears then were looked through with the immersion system of a microscope. In the thin place of a smear, we measured the diameter of 200 red corpuscles. As the red corpuscles of poikilothermal vertebrata are ellipse shaped with core in the center, the measurement was performed in length and width (length x width), and for calculation of the effective diameter of red corpuscles (EDRC) and for more convenient calculation of differences reliability between size groups only indexes of big diameter, i.e. length of red corpuscles were taken.

To receive a reliable image, the calculation was made from one preparation of diameter of 100 red corpuscles for calculation of EDRC [12, 2, 3], and minimum 2-3 smears were taken from one animal. We measured the red corpuscles of 13 preparations, which were received from snakes of different inhabitation and at a different physiological state – in the period of activity and during the hibernation, i.e. after artificially created hibernation in a cold storage room. The sizes of erythrocytes received this way are divided into three size groups – red corpuscles from 8 to 10 microns in recalculation turned out to be 10.3-12.8 microns, from 11 to 15 microns or 14.9-17.5 microns and from 15 to 17 or 18.6 – 21.3 microns or above. It should be noted, that human's red corpuscles are distributed by their sizes to microcytes, normocytes and macrocytes (it is necessary to consider that the size do not coincide with reptiles' red corpuscles), we also will adhere to

such distribution and call red corpuscles nominally in the same way. The obtained data was processed by a statistical method with finding the reliability of distinctions – t. For establishment of distribution of red corpuscles in their magnitude, we calculated the ratio of different size groups, expressed in a percentage (%).

The effective diameter of red corpuscles (EDRC) is calculated by results of red blood cell count, by multiplication of each percent of cells with a particular diameter on its value in microns, and by summing up these products and dividing it by 100 [11,9]. For example, 10% of red corpuscles with a diameter of 10 microns, 60% - with a diameter of 13 microns and 30% - with a diameter of 16 microns. Thus, EDRC is calculated as follows:

$$EDRC = \frac{(10x10) + (60x13) + (30x16)}{100} = \frac{100+780+480}{100} = 13.6 \text{ microns}$$

The results and discussion. Diameters of red corpuscles vary differently at different genus. The least diameter of red corpuscles have mammals, the largest red corpuscles are of batrachians. Fish red corpuscle diameters are equal to 10.5x19.7 microns and 6.0x12.0 microns [9]. According to G.N. Kalashnikov [8], the size of red corpuscles of fish are higher, than of the majority of mammals, but less than of the other representatives of poikilothermal animals. The quantity of red corpuscles in a unit of blood volume is linked to the intensity of an animal's metabolism. The greatest number of red corpuscles is in mammals' blood, the least – of reptiles, amphibians and fish, and in particular of cartilaginous fishes [4].

The respiratory properties of reptiles' blood research are of interest, because for the first time they have exclusively pulmonary respiration and skin respiration loses its importance [1]. The author has determined that a Caucasian agama has more red corpuscles in its blood than a turtle. Reptiles have lower number of red corpuscles than birds, though their red corpuscles are smaller in size: lizard's (Lacerta muralis) length of red corpuscle ranges from 12.5 to14.0 microns, width – from 5.5 to 7.0 microns; turtle's (*Emys orbicularis*) –15.5 – 18.5 and 10.0 – 12.5 microns respectfully [4].

The shape and size of red corpuscle are the function of metabolism, and thus torins the shape, which helps to fulfill the function. The research of the size and shape of red corpuscle and their variability has to lead to understanding of the delicate mechanisms of its function [13]. According to our data, for Palla Coluber (*Elaphe dione*=) from different landscape zones with different ecological conditions EDRC ranges widely and the percentage ratio of size groups of red corpuscles is unequal (see Table 1).

Table 1. Red corpuscle size of Pallas Coluber during the activity and hibernation periods

№ preparation	Place of catching	Quantity of counted cells	Effective diameter of red corpuscles (EDRC) according to the biggest diameter (in microns)					
			M ± m 11,3 - 13,8	% EDRC	M ± m 11,3 - 13,8	% СДЭ	M ± m 11,3 - 13,8	% EDR C
1	Arakol village	100	13,2 ± 0,15	34	16,2 ± 0,16	57	19,2 ± 0,24	9
2		100	13,2 ± 0,18	22	16,3 ± 0,13	60	19,6 ± 0,28	18
3		100	13,2 ± 0.16	32	16,3 ± 0,13	57	18,5 ± 0,09	11
4*		100	13,0 ± 0,19	30	16,1 ± 0,15	54	19,0 ± 0,10	16
5*		100	13,2 ± 0,18	31	16,5 ± 0,14	57	19,9 ± 0,12	15
6	Chaar-Archa	200	13,8 ± 0,18	22	16,4 ± 0,13	64	19,1 ± 0,16	14
7		200	13,8 ± 0,24	25	16,3 ± 0,12	68	19,3 ± 0,30	8
8*		100	13,9 ± 0,25	24	16,5 ± 0,13	67	18,9 ± 0,29	11
9	Shamsi	200	13,5 ± 0,13	20	16,2 ± 0,12	70	18,7 ± 0,03	10
10		100	13,0 ± 0,16	24	16,3 ± 0,10	66	18,8 ± 0	10
11		100	13,6 ± 0,09	24	16.2 ± 0,13	68	18,7 ± 0,03	8
12*		100	13,3 ± 0,16	25	16,3 ± 0,12	57	19,3 ± 0,20	18
13*		100	13,5 ± 0,14	21	16,4 ± 0,13	59	19,4 ± 0,21	17
	Total:	1600						

Note: * preparations were drawn during the artificial hibernation period

Red blood cells count for Pallas Coluber blood during the different physiological states - the period of activity and hibernation - has showed the following picture: the puberal Pallas Coluber, with a total body length of 710 mm from Arakol village, has the average size of red corpuscles of 0.1-0.5 microns, which is bigger than of a Pallas Coluber's from Shamsi village. However, we noted red corpuscles with narrow width. Therefore, red corpuscles with effective diameter of 12.9x7.2 microns make 34%, with sizes of 16.0x8.2 microns - 57% and 18.8x9.7 microns - 9%. After staying in hibernation, this specimen shows the red corpuscles dilatation due to the expansion of their small diameter. Thus, microcytes with sizes of 13.2x8.4 microns make 30%, normocytes - 16.2x9.5 microns - 54% and macrocytes - 19.1x10.3 microns - already 16%. The reliability of differences between the second and the third size group is higher, than during the active period and it makes 16.3 against 10.4.

In June, the quantity of red corpuscles of Pallas Coluber from Chaar-Archa alpine, is 770 thousand/mm^3, the size of big diameter of red corpuscles ranges within 13.1 - 19.3 microns, of small diameter - 7.9 - 9.8 microns. Microcytes with a diameter of 13.1x7.8 microns make 21-25%,

with EDRC of 16.3 x 9.5 microns and 16.4x9.3 microns - 64-68% and with EDRC -19.1 x 9.8 microns – 19.3x9.1 microns make only 8-14%. The reliability of differences between the first and second size group is equal to 15.4 and between the second and the third – to 12.8.

The puberal Pallas Coluber (total body length is 670 mm) from Shamsi area in August has 495 thousand/mm^3 red corpuscles in blood. After the wintering, the number of red corpuscles has considerably increased and made 950 thousand/mm^3. In the period of activity, the volume of EDRC is equal to 13.5x9.0 microns and makes 20%, at the average size of 16.2x9.9 microns – 70% and 18.7x10.5 microns – only 10%. After being in hibernation, it is noted that the quantity of red corpuscles has increased, and the size make 13.6x10.1 microns (occurrence of these red corpuscles is 24%); with size of 16.2x10.3 microns, the occurrence makes 68%, and with size of 18.7x10.1 microns –8%; so, between the size groups the distinctions reliability of *t* is equal to 16.3 and 15.6.

Thus, by studying Pallas Coluber in different ecological conditions and in different physiological states, we may trace the adaptive character of reptiles to different altitudinal belts at the tissue level. Blood, being an important index of a physiological condition of an organism, especially of poikilothermal animals, which are the reptiles, is a feedback response to the changing of habitat conditions. As we noticed, Pallas Coluber's size group of normocytes in blood is greater than, for example, of micro- and macrocytes, and there is a big reliable difference between size groups. The red corpuscles' size change shows their volume; hence the response of an organism to the changing environment conditions depends on altitudinal belts.

For the first time the method of intravital capture and blood composition determination applied for the study allowed revealing a physiological condition of an organism, without doing an essential harm to the ecology, while keeping the genus population.

Thus, during the summer period Pallas Coluber species from different habitats have more normocytes in their blood, at effective diameter of 16.1 – 16.5 microns, which accounts for about 54-70%. Macrocytes in all cases range within 18.5 – 19.9 microns, and the % of EDRC makes 8 - 18%. In general, the considered Pallas Colubers' blood has more of the first and the second size groups, in comparison with the third group.

On the basis of the foresaid, it is possible to come to a conclusion that red corpuscle size changes in animals have an adaptive value and depend on a physiological condition of an organism. In the period of activity, Pallas Colubers have rather low content of red corpuscles in blood, and during hibernation period, their quantity increases. Along with the last one, EDRC changes towards the growth, in average of 0.1 – 0.6 microns and more.

iterature

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