ON THE TRANSFORMATION OF ERYTHROCYTES IN THE PERIPHERAL BLOOD OF THE FROG

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The blood of all vertebrate animals, except for the mammals, contains nucleated erythrocytes. However, there is data in the literature that non-nucleated red cells may also be encountered among the nucleated ones. A number of authors [2, 3, 6-8] have pointed out the existence of similar erythrocytes in birds. There is mention of the presence of non-nucleated erythrocytes in the circulation even of the lower animals [10, 13].

We have studied the blood of the frog (Rana esculenta), the sazan, the common zheltopuzik, monitor lizard, and golden eagle. Non-nucleated erythrocytes were observed in the peripheral blood of all these animals.

Frog blood has been well studied by many authors [1, 9, 11]. It contains erythrocytes which clearly shows a characteristic anisocytosis.

METHODS

The work was carried out on five batches of frogs (consisting of seven animals each). Blood smears were taken and fixed with methyl alcohol then stained with Giemsa-Romanowsky. The nucleus was placed centrally in the erythrocytes, as a rule. Their size was usually of the order 5.5 · 9.2 microns. The violet karyoplasm contains large, almost black, lumps of chromatin. Non-nucleated erythrocytes are also found in the blood. This contradicts the opinion that similar cells are present only in the peripheral blood of tailed amphibia [13]. The form of such erythrocytes in our preparations was varied—from round to oval. Their size varied from 11 · 27.6 to 16.3 · 25.8 microns, i.e., the size of the non-nucleated cells and the normal (according to our data, their length was 18.4-27.6 microns and width 11-12.9 microns) did not differ essentially (Fig. 1).

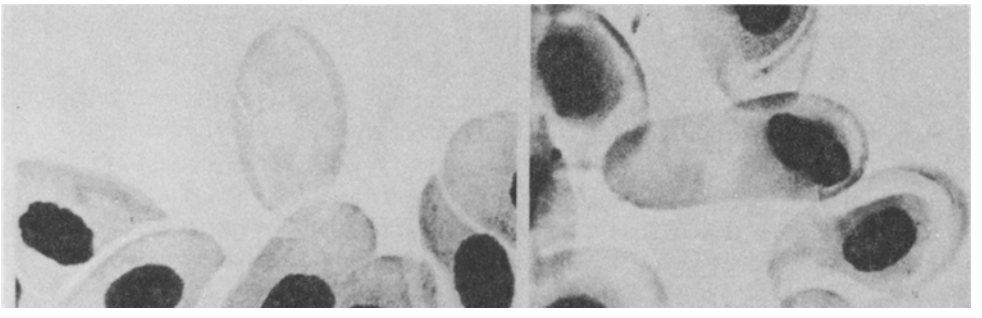

Fig. 1 Fig. 2

Fig. 1. Non-nucleated erythrocyte in the peripheral blood. Smear. Fixation: methyl alcohol; stain; Giemsa-Romanowsky. Magn. 15 ×90.

Fig. 2. Polar position nucleus. Smear. Fixation: methyl alcohol; stain; Giemsa-Romanowsky. Magn. 15 ×90.

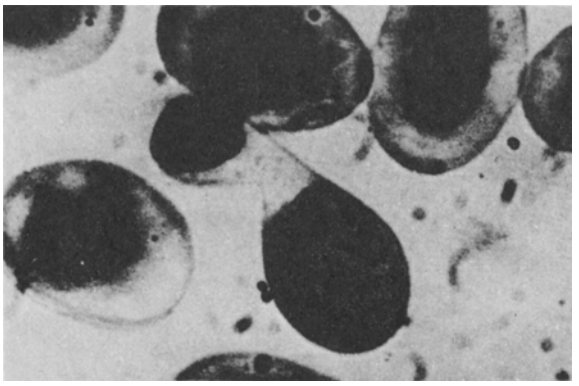

Fig. 3. Division of erythrocyte into nucleated cell and non-nucleated cell. Smear; Fixation: methyl alcohol; stain: Giemsa-Romanowsky. Magn. 15 x 90.

TABLE 1. Number of Altered Erythrocytes in the Blood of Normal Frogs

No. of animal	Erythrocytes counted			
	total	with displaced nuclei	dividing	non-nucleated
		(in %)		
1	8,025	1.89	0.04	0.00
2	21,620	1.80	0.07	0.09
3	16,280	1.98	0.15	0.03
4	24,165	1.75	0.10	0.00
5	14,840	1.48	0.10	0.07

TABLE 2. Number of Altered Erythrocytes in the Blood of Frogs with Respiration Via the Lungs

No. of animal	Erythrocytes counted			
	total	with displaced nuclei	dividing	non-nucleated
		(in %)		
1	17,530	6.25	0.32	0.14
2	18,980	5.57	0.54	0.27
3	16,840	5.93	0.65	0.12
4	11,180	5.98	0.25	0.26
5	11,840	5.99	0.62	0.14
6	14,090	7.20	0.49	0.18
7	11,270	5.26	0.32	0.15
8	7,250	5.93	0.27	0.13
9	9,750	7.56	0.51	0.25
10	18,730	6.40	0.40	0.08

The results of studying the smears indicated that non-nucleated erythrocytes arise from the nucleated cells directly in the peripheral blood. This process begins with the movement of the nucleus, which makes a turn, is displaced to the side and later to a polar position. Then, from one or both sides of the cell grooves are formed; initially they are small, later becoming deeper (Figs. 2 and 3). It is evident how the protoplasm with the hemoglobin almost entirely migrates into the forming oval bag. After formation of the body of the non-nucleated erythrocyte the connecting strand grows thinner and breaks off. This leads to the division of the erythrocyte into two portions—a larger and a smaller. The latter contains the nucleus, which does not differ in form from the nucleus of normal erythrocytes. It is surrounded by a layer of protoplasm of 4.6 to 7.4 microns in thickness. The larger portion of the divided erythrocyte is round.

The number of cells with displaced nuclei, which are dividing, and the non-nucleated cells in the peripheral blood of normal animals is presented in Table 1. Displaced nuclei are comparatively frequent and non-nucleated cells very rarely encountered, which testifies that the formation of such erythrocytes in the majority of instances is incomplete. A similar picture has been described by Semashkevich [10], who called this process "internal budding," during which the nucleus is displaced to one pole of the cell, while at the other appears "a lump of dense protoplasm." Then the erythrocyte divides in half—one part receives the normal nucleus and the other remains without a nucleus.

The suggestion that the above described phenomenon is an artifact is without basis, since blood smears taken from the cardiac ventricle have been prepared with observance of all precautions [12]. In addition, the leucocytes, which were completely unaltered, served as a criterion for the proper treatment of the blood smears. Thus, it remains to be clarified whether to regard the formation of non-nucleated cells as a progressive phenomenon which arises in the amphibia and reaches greatest development in the mammalia, or whether it is a degenerative process. To resolve this question a very simple experiment was performed.

Frogs were placed for two weeks in water which had been well boiled. To avoid the natural diffusion of gases the water was changed for fresh twice a day. The animals were placed in the water so that their entire body was submerged in liquid with only the head emerging. Such maintenance must force the frog to manage breathing with its lungs, which, as a rule, are poorly developed. The exclusion of cutaneous respiration creates the conditions for development of hypoxemia.

The results of study of blood from frogs kept in boiled water showed a marked change in the processes of formation of non-nucleated erythrocytes. In Table 2 are presented the data concerning the composition of red cells in these frogs.

Upon comparing the data in Tables 1 and 2 a marked difference is evident in the number of erythrocytes with displaced nuclei as well as in dividing and non-nucleated cells. In the experimental animals the number of erythrocytes with displaced nuclei is increased four to six times; erythrocytes found in stages of division are increased four to seven times; the content of non-nucleated erythrocytes is also increased approximately five times.

Consequently, the positive result of the experimental influence on the erythrocytes is unquestionable, and the appearance of non-nucleated erythrocytes in the peripheral blood of the experimental frogs must be considered as one of the reactions of the organism to hypoxemic conditions.

In the literature it is indicated that non-nucleated red blood cells of mammals require only tenths and even hundredths as much oxygen as the nucleated erythrocytes of amphibia and reptiles. As a consequence, the non-nucleated elements are more economical. Their appearance in vertebrates, possibly, is related with the struggle of the organism for economic expenditure of oxygen in its blood [4, 5].

It may be thought that the appearance of non-nucleated erythrocytes in the blood of experimental frogs is one the types of adaptation of their body to low oxygen supplies. This adaptation is carried out to a certain degree by a decrease in the oxygen requirement of the blood itself.

SUMMARY

The blood of the frog contains nucleated and non-nucleated erythrocytes. The latter appear in the peripheral blood by division of the cell into a smaller, nucleated, and a larger, non-nucleated part. During experimental hypoxia the number of nucleated erythrocytes in the blood stream of the frog increases by the same method five times over. It is known that the consumption rate in nucleated erythrocytes in cold-blooded animals is scores of times higher than that of non-nucleated erythrocytes as one of the body reactions to hypoxemic conditions, and the erythrocytes as cells capable of reacting to unfavorable conditions and forming non-nucleated elements better adapted to transmission of oxygen.

Our experiment warrants the supposition that the appearance of non-nucleated erythrocytes in invertebrates is apparently one of the types of adaptation of their bodies to a relatively decreased oxygen supply. This is effected by a reduction of oxygen consumption in the blood itself.

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