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Morphological abnormalities of blood and bone marrow leukocytes and age-related changes in different leukocyte counts in the American mink (*Neovison vison*)

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Abstract: The sapphire mink is a unique animal model to study cellular morphology. It demonstrates abnormal leukocytes like in patients with Chédiak–Higashi syndrome. We aimed to determine the morphologic features of blood and bone marrow leukocytes in the sapphire mink and dark-brown mink (wild type). In addition, were assessed age-related changes in total white blood cell (WBC) and differential WBC counts. We conclude that the sapphire mink has a morphological leukocyte defect (abnormal granules). We observed that abnormal granules formed at an early stage of bone marrow leukocyte maturation. It was shown that the leukocyte profile of young mink differs from that of adult animals. The levels of abnormal segmented neutrophils and abnormal lymphocytes were higher in adult mink as compared with puppies (4-, 10-day-old).

Key words: Abnormal granules, Chediak–Higashi syndrome, differential WBC counts, *Neovison vison*

1. Introduction

At present, the American mink (*Neovison vison*) is the most popular animal for fur breeding. Mink breed well in cage farming and are suitable as an experimental model for studying the effects of the environment, age, and some pathologies. There are differences in body size, breeding performance, and susceptibility to infections between mink of different coat colors (1). One of the common colors of farm-bred mink is sapphire (*aapp*)—a specific blue-gray pigmentation of the fur. The sapphire mink (mutant form of the American mink) is characterized by lower resistance to diseases and the presence of enlarged granules in leukocytes. These features are similar to clinical manifestations in human Chédiak–Higashi syndrome (CHS) (2). Enlarged abnormal granules are often described as lysosomes (3). Our previous report demonstrated the activity of lysosomal markers esterase and peroxidase in enlarged granules in the sapphire mink (4). These abnormal granules were found in different cell types: leukocytes; melanocytes of skin, hair, and eyes; cells of the liver and thyroid and pancreatic glands; and juxtaglomerular cells (5–8). Structural and biochemical defects involved in CHS lead to impairment of the functional activities of leukocytes and disturbance of some physiological systems. The physiological features, functional damage, and molecular mechanisms in CHS were studied most extensively in humans and beige mice. CHS and Chédiak–Higashi-like disease in animals represent a hereditary pathology due to mutation in the LYST gene. This gene encodes a membrane-associated protein that regulates intracellular protein trafficking (9). In that study Anistoroaei et al. found the LYST gene to be responsible for the mink phenotype and the CH-like syndrome in sapphire mink. Thus, humans and sapphire mink have similar clinical manifestations and a common genetic mechanism. The sapphire mink can be used as a potential animal model for studying this pathology. It seems that the abnormality of leukocytes is one of the reasons for low viability and increased embryonic and early postnatal mortality in sapphire mink.

Copious information is available about morphological and functional defects in humans and the most popular model of CH-like syndrome—the beige mouse. We are, however, aware of only a few studies in which the morphological features of leukocytes and hematological data from mink with CHS traits were described (10,11). There are no data about age-related changes in leukocyte defects in humans with CHS and animals with traits of CHS. Accordingly, the aim of the present study was to examine the morphology of blood and bone marrow leukocytes, as well as to evaluate age-related changes in the level of white blood cells (WBCs) and differential leukocyte counts in sapphire mink with CHS traits and dark-brown mink (wild type).

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2. Materials and methods

2.1. Animals and sampling

The study was performed on dark-brown mink similar to wild mink and sapphire (genotype *aapp*) strains of both sexes at the fur farm "Pryazhinskoe" Ltd. (Republic of Karelia, Russia). All the animals were fed a standard diet and water ad libitum. The mink were housed in conventional outdoor facilities and exposed to ambient temperatures and light conditions. During the study period, the animals were observed daily for any clinical signs of diseases. This was based on physical examinations of each animal and on the presence of normal physical activity and normal food and water intake, and the absence of signs of discomfort during the observation period. The results of hematological and serum chemistry tests of these animals (including RBC, HGB, total protein) were within normal ranges. A standard production diet was provided to the mink twice each day. The puppies were allowed to suckle until 6 weeks of age and thereafter separated from the females and received only standard diet. All the experiments were conducted according to EU guidelines on the use of animals for biochemical research (86/609/EU).

Age-related changes in the leukocyte profile were examined in 4-, 10-, 60-, 120-, and 180-day-old mink (n = 5–8) and adult mink (over 1 year-old, n = 29 dark-brown mink, n = 34 sapphire mink). Except for those of adult age, all animals were weighed. Blood and bone marrow were obtained in the morning after night fasting. Blood of 4- and 10-day-old mink was collected by decapitation; in 60-day-old and older puppies and adult animals (over 1 year old) it was taken from the caudal vein. Bone marrow sampling was performed from the epiphysis of the thigh bone in 180-day-old mink (n = 6) postmortem after pelting during the standard fur production process in late November. Bone marrow and peripheral blood smears were prepared on glass slides after sampling following a standard procedure.

Hematological values for red blood cells (RBCs) and hemoglobin were determined spectrophotometrically. Total protein concentration was determined using a refractometer.

All investigations were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institute of Health (NIH publication No. 85–23, 1996). It conforms to the principles outlined in the Helsinki Declaration. The Ethics Committee of the Institute of Biology, Karelian Research Centre, approved all animal care procedures prior to initiation of the experiment.

2.2. Light microscopy

The blood smears were stained with May-Grünwald and Romanowski stains (MiniMed, Russia). The smears of bone marrow were stained by the same method. The counting of total WBC and differential leukocyte counts was performed manually under a light microscope (Axioscop 40, Zeiss, Germany). Differential leukocyte counts were performed by identifying 200 leukocytes in each blood smear. The absolute numbers of each type of leukocyte were calculated from the WBC and differential leukocyte counts. In addition, the number of normoblasts (nuclear erythrocytes) and the size of erythrocytes were measured in blood smears using imaging software (VideoTesT 4.0, Video Test Inc, Russia).

Blood smears were observed for evaluation of leukocyte morphology; abnormal leukocyte types, including abnormal granules, were determined among all examined leukocytes. Abnormal granules were identified as "giant" oxyphilic rounded inclusions. The percentages and absolute values of abnormal leukocyte types were assessed in smears from sapphire mink. Bone marrow smears were investigated to study the morphology of bone marrow leukocytes.

2.3. Statistical analysis

Age-dependent changes in hematology parameters were tested by nonparametric Mann–Whitney U-test for comparison between adult mink and other ages. Nonparametric tests were used because of the small sample. The data are represented as mean ± SEM. Differences at the $P < 0.05$ level were considered statistically significant. The statistical analyses were performed using MS-Excel (Microsoft Corp., Inc., USA) and Sigma-Stat 2.03 (SPSS Science Software Ltd., USA).

3. Results

The hemoglobin level, total protein concentration, and RBC, WBC, and differential leukocyte counts are presented in Tables 1–3.

In both color forms of mink the hemoglobin level and RBC counts increased to 120 days of age, whereas total protein concentration increased to 180 days of age (Table 1). The WBC and differential leukocyte counts were also not stable during the ontogenesis of dark-brown and sapphire minks (Tables 2 and 3).

The mean WBC count in 4- and 10-day-old mink was lower than that for adult mink ($P < 0.05$) (Tables 2 and 3). There was a significant increase in WBC at 60 days, after which WBC count stabilized within the adult mean. Age-related changes in mean WBC count in dark-brown and sapphire mink were similar.

In differential leukocyte counts, lymphocytes and eosinophils were at low levels in 4- and 10-day-old mink ($P < 0.05$); the number of lymphocytes increased up to the age of 60 days in both colors of mink and thereafter insignificantly varied during the experimental period. Eosinophil counts in puppies increased during the first

Table 1. Age-related changes in erythrocyte counts (RBC), hemoglobin levels, and total protein concentrations in dark-brown and sapphire mink.

Parameters		4-days	10-days	60-days	120-days	180-days	Adult mink
	Dark-brown mink						
n		6	6	8	8	7	26
RBC	10^{12}/L	4.61 ± 0.14	4.49 ± 0.11	6.16 ± 0.19^*	9.37 ± 0.19^*◊	7.74 ± 0.22^*◊◆	8.94 ± 0.18^*◊#
Hemoglobin	g/dL	9.91 ± 0.67	9.00 ± 0.44	12.30 ± 0.36^*	21.42 ± 0.49^*◊	18.07 ± 0.69^*◊◆	17.83 ± 0.042^*◊◆
Total protein	g/dL	3.28 ± 0.12	4.88 ± 0.06^	6.78 ± 0.21^*	7.84 ± 0.13	8.34 ± 0.14^*◊◆	8.42 ± 0.18^*◊
	Sapphire mink						
n		5	6	5	5	10	34
RBC	10^{12}/L	4.57 ± 0.16	4.78 ± 0.15	6.59 ± 0.22^*	9.44 ± 0.09^*◊	7.97 ± 0.19^*◊◆	8.85 ± 0.16^*◊◆#
Hemoglobin	g/dL	9.83 ± 0.71	8.94 ± 0.31	14.32 ± 0.26^*	22.21 ± 0.49^*◊	17.52 ± 0.36^*◊◆	17.00 ± 0.51^*◊◆
Total protein	g/dL	3.74 ± 0.28	4.53 ± 0.19^	6.52 ± 0.34^*	8.12 ± 7.85^*◊	10.17 ± 2.38^*◊	8.03 ± 0.09^*◊◆

Numbers here and in Tables 2 and 3 are mean ± SEM; ^ significant difference from 4-day-old, * from 10-day-old, ◊ from 60-day-old, ◆ from 120-day-old, # from 180-old-day mink.

Table 2. Age-related changes in body weight and hematologic parameters in dark-brown mink.

Parameters		4-days	10-days	60-days	120-days	180-days	Adult mink
n		6	6	8	8	7	26
Body weight	g	20.25 ± 0.95	64.83 ± 1.62	513.13 ± 36.06	1873.00 ± 95.56	1370.14 ± 179.91	NM
Normoblasts	10^9/L	3.59 ± 1.02	0.35 ± 0.16^	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
WBC	10^9/L	3.92 ± 0.18	3.95 ± 0.41	6.18 ± 0.69^*	5.04 ± 0.50	6.31 ± 1.01^	5.68 ± 0.55
Lymphocytes	%	14.67 ± 1.73	22.5 ± 2.43	29.88 ± 3.98^	38.86 ± 4.95^*	27.0 ± 4.95^	35.5 ± 2.31^*
	10^9/L	0.58 ± 0.08	0.87 ± 0.10	1.73 ± 0.22^*	1.73 ± 0.40^	1.70 ± 0.34^	1.95 ± 0.29^*◆
Metamyelocytes	%	0.17 ± 0.17	0.17 ± 0.17	0.00 ± 0.00	0.14 ± 0.14	0.00 ± 0.00	0.00 ± 0.00
	10^9/L	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
Band neutrophils	%	19.0 ± 8.27	6.17 ± 1.45	2.75 ± 0.53^*	0.43 ± 0.20^*◊	1.14 ± 0.40^*	1.42 ± 0.30^*◊
	10^9/L	0.78 ± 0.37	0.24 ± 0.07^	0.17 ± 0.05^	0.02 ± 0.01^*◊	0.08 ± 0.03^*◊	0.06 ± 0.02^*
Segmented neutrophils	%	46.00 ± 5.84	60.8 ± 3.56	59.88 ± 3.54^	54.00 ± 4.38◊	65.0 ± 3.52^	54.11 ± 2.25◊
	10^9/L	1.76 ± 0.20	2.41 ± 0.32	3.78 ± 0.59^	2.42 ± 0.48	4.13 ± 0.72^	2.64 ± 0.29
Monocytes	%	20.00 ± 2.38	8.70 ± 1.00	5.88 ± 0.77^	5.43 ± 0.95^	5.29 ± 1.49^	5.46 ± 0.61^*
	10^9/L	0.78 ± 0.10	0.35 ± 0,05^	0.38 ± 0,08^	0.24 ± 0,05^	0.32 ± 10,10^	0.28 ± 0.05^
Eosinophils	%	0.17 ± 0.17	0.50 ± 0.34	1.13 ± 0.35*	0.71 ± 0.29	1.29 ± 0.52	2.6 ± 0.43^*◆
	10^9/L	0.01 ± 0.01	0.03 ± 0.02	0.08 ± 0.03^	0.03 ± 0.01	0.07 ± 0.02^	0.13 ± 0.03^*◆
Basophils	%	0.17 ± 0.17	1.17 ± 0.3^	0.38 ± 0.18^	0.43 ± 0.20^*	0.29 ± 0.18^	0.96 ± 0.49^*
	10^9/L	0.01 ± 0.01	0.05 ± 0.01^	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.08 ± 0.05^*

NM – parameter was not measured

60 days of life; mean eosinophil count (absolute number and percentage) was the highest in adult animals in dark-brown mink, whereas in sapphire mink the highest mean eosinophil count was observed in 60-day-old animals.

In contrast, lymphocyte and eosinophil total counts and percentages of young neutrophilic forms of cells, metamyelocytes and band neutrophils, were significantly were higher in 4- and 10-day-old mink than other ages ($P < 0.05$). The highest values of metamyelocytes and band neutrophils were detected in 4-day-old sapphire mink. In both colors of mink, band neutrophil counts leveled off with age, while metamyelocytes disappeared

Table 3. Age-related changes in body weight and hematologic parameters in sapphire mink.

Parameters		4-days	10-days	60-days	120-days	180-days	Adult mink
n		5	6	5	5	10	34
Body weight	g	17.00 ± 0.45	48.83 ± 2.34	806.8 ± 26.18	2091.6 ± 141.24	1456.8 ± 132.18	NM
Normoblasts	10^9/L	20.05 ± 4.01	0.66 ± 0.20^	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
WBC	10^9/L	4.26 ± 0.59	3.33 ± 0.21	8.65 ± 1.14^*	5.61 ± 0.53^*◊	6.96 ± 0.63^*	8.52 ± 0.44
Lymphocytes	%	9.40 ± 1.94	21.5 ± 2.29^	40.0 ± 3.21^	35.6 ± 3.98^*	26.90 ± 2.20^	37.8 ± 2.21^*
	10^9/L	0.43 ± 0.18	0.55 ± 0.13	3.46 ± 0.58^*	2.05 ± 0.38^*	1.86 ± 0.24^*◊	3.09 ± 0.28^*◊
Abnormal lymphocytes	%	0.00 ± 0.00	0.00 ± 0.00	1.20 ± 0.49	1.20 ± 0.58	0.78 ± 0.28	2.66 ± 0.96
	10^9/L	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.03	0.08 ± 0.04	0.05 ± 0.02	0.38 ± 0.08
Metamyelocytes	%	1.80 ± 0.37	1.17 ± 0.79	0.20 ± 0.20^	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	10^9/L	0.08 ± 0.03	0.04 ± 0.02	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Band neutrophils	%	20.2 ± 5.56	7.67 ± 0.92	3.0 ± 0.95^	0.80 ± 0.80^*	0.40 ± 0.22^*◊	1.77 ± 1.34^*
	10^9/L	0.97 ± 0.36	0.22 ± 0.06^	0.26 ± 0.10	0.04 ± 0.04^	0.03 ± 0.01^*◊	0.44 ± 0.20^*◊
Segmented neutrophils	%	59.6 ± 7.40	56.5 ± 3.55	40.4 ± 2.25^*	54.8 ± 4.88	59.3 ± 2.80◊	48.17 ± 2.52#
	10^9/L	2.38 ± 0.14	1.56 ± 0.35	3.52 ± 0.52^	2.98 ± 0.12^*	4.12 ± 0.43*^	3.93 ± 0.34^
Abnormal segmented neutrophils	%	14.80 ± 3.33	15.33 ± 4.5	21.4 ± 3.04^*	21.6 ± 5.33*	27.33 ± 2.14^*	17.44 ± 1.67#
	10^9/L	0.60 ± 0.13	0.39 ± 0.16	1.85 ± 0.33^	1.16 ± 0.24	1.78 ± 0.30	1.37 ± 0.14^*
Monocytes	%	8.20 ± 2.15	11.83 ± 2.21^	8.40 ± 1.87	6.20 ± 2.20	7.90 ± 0.75^	6.73 ± 0.66^*
	10^9/L	0.37 ± 0.13	0.37 ± 0.10	0.68 ± 0.15	0.38 ± 0.16	0.56 ± 0.09	0.56 ± 0.08
Abnormal eosinophils	%	0.60 ± 0.25	0.17 ± 0.17	7.20 ± 1.59^*	2.20 ± 1.31	5.2 ± 1.40^*	4.79 ± 0.59^*
	10^9/L	0.02 ± 0.01	0.01 ± 0.01	0.64 ± 0.19^*	0.15 ± 0.10	0.36 ± 0.10^*	0.39 ± 0.05^*
Abnormal basophils	%	0.20 ± 0.20	1.17 ± 0.31^	0.40 ± 0.25	0.40 ± 0.25	0.40 ± 0.16	0.35 ± 0.13
	10^9/L	0.01 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01*

NM – parameter was not measured

from the circulation after 10 days of age. The numbers of band neutrophils, monocytes, and basophils changed significantly during early postnatal development (from 4 to 10 days of life). At day 60, the levels of monocytes and basophils in peripheral blood stabilized within the adult mean.

Sapphire mink are characterized by lower body weight during the first 10 days of life in comparison with dark-brown mink of the same age (Tables 2 and 3). At the age of 60 days and afterwards mean body weights of sapphire mink were higher than those of dark-brown mink.

Blood smears of puppies (4 and 10 days) contained normoblasts and polychromatophilic erythrocytes. We observed a significant increase in normoblasts in the peripheral blood of sapphire puppies as compared with dark-brown puppies (Tables 2 and 3). The erythrocyte-size distribution in age groups 4, 10, and 60 days is shown in Figure 1. The size of erythrocytes differed between sapphire and dark-brown mink in early postnatal development. The largest erythrocytes were found in sapphire puppies. At the age of 60 days, the size of erythrocytes was similar in both colors of mink.

The morphologies of blood leukocytes of dark-brown mink and blood abnormal leukocytes of sapphire mink are presented in Figure 2. In all sapphire mink, abnormally large leukocyte granules were observed throughout the study period. Abnormal granules in the neutrophils of sapphire mink appeared as enlarged, oxyphilic, and rounded inclusions (Figure 2F). The granules in the eosinophils of sapphire mink were larger and more varied in size than were the neutrophil granules (Figure 2G). In some sapphire mink, large azurophil granules were found in lymphocytes (Figure 2E) and monocytes. The content of abnormal blood neutrophils ranged from 1% to 41%; the number of abnormal neutrophil granules could be up to 10 granules per cell, although most commonly it was 2–3 granules per cell. Examination of neutrophils in blood smears from dark-brown mink revealed no visible granules (Figure 2B). Eosinophils of dark-brown mink demonstrated numerous small granules (Figure 2C).

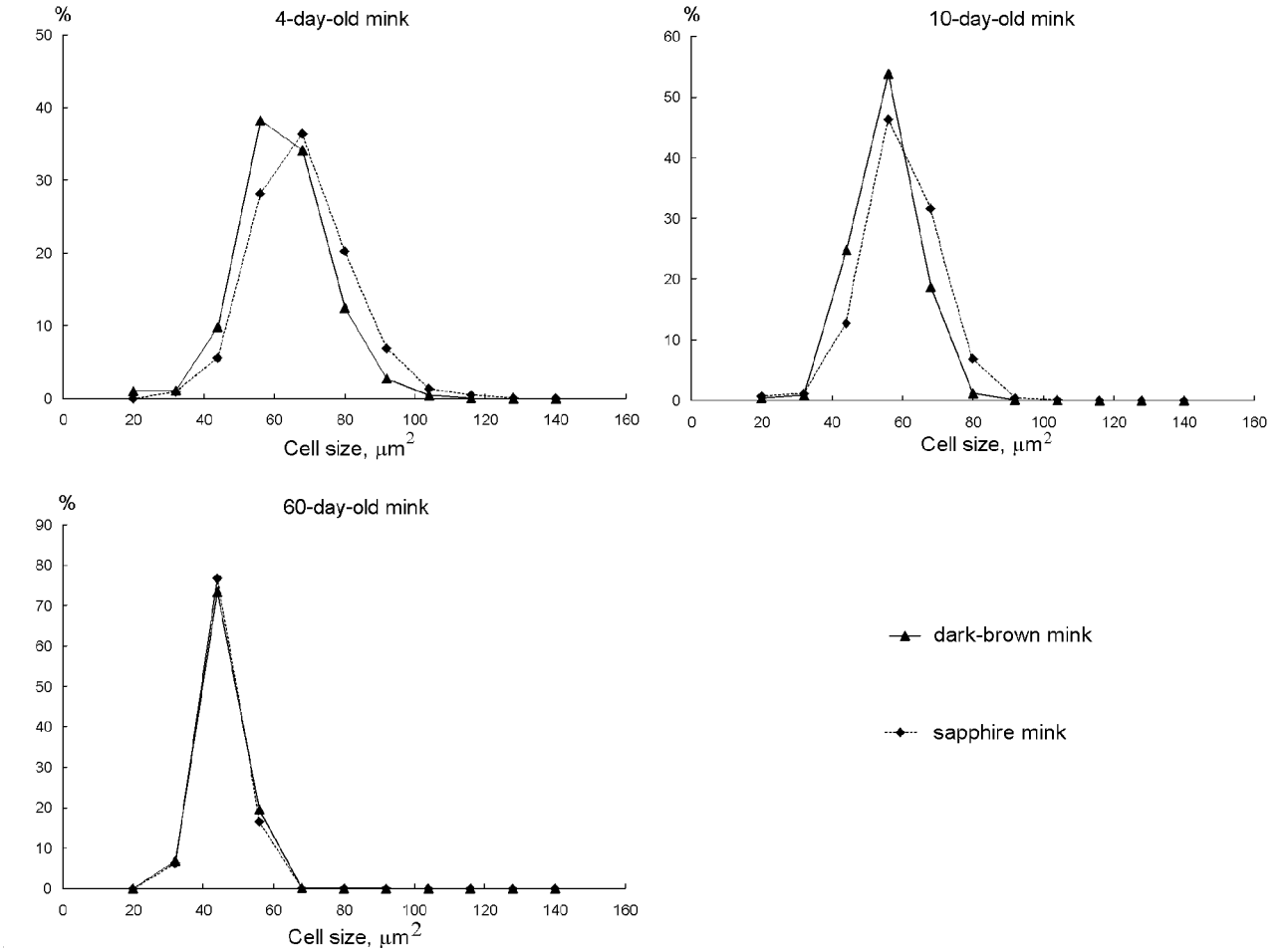

Figure 1. Histogram of erythrocyte size distribution for dark-brown and sapphire mink aged 4, 10, and 60 days.

There was a significant increase in abnormal neutrophil content in adult sapphire mink as compared with 4- and 10-day-old sapphire mink. No abnormal lymphocytes were detected in 4- and 10-day-old mink and the level of abnormal lymphocytes increased in adults compared with 60-day-old sapphire mink ($P < 0.05$).

Light microscopy of bone marrow leukocytes in dark-brown mink showed that neutrophils had faintly discernible granules and eosinophils had numerous homogeneous small size granules (Figures 3A and 3B). By contrast, bone marrow neutrophils and eosinophils in sapphire mink contained enlarged granules. Visual observation of bone marrow smears revealed that granule size was greater in sapphire mink as compared with dark-brown mink, whereas the number of granules was smaller than in dark-brown mink. Morphological analysis of bone marrow eosinophils and neutrophils demonstrated that all stages of granule formation in sapphire mink were abnormal (Figures 3C and 3D). The defect progressed from myelocyte to mature cell. The largest granules in bone marrow were found in mature cells (Figure 3D).

4. Discussion

Our choice of mink ages was determined by the features of growth, development, and maturation of physiological systems in the given ontogenetic periods. Early postnatal development was represented by 4- and 10-day-old ages. Mink are considered to be immature-born animals. The age of 4 days in mink is currently thought to be in the neonatal period of development. Until the age of 10 days puppies are covered in a downy fur coat; their eyes and acoustic meatuses remain closed until 30 days of age. Based on the data presented here and the results of previous published studies we can conclude that the hemopoietic system is not mature enough in early postnatal development (12,13). The presence of normoblasts and large erythrocytes in peripheral blood confirmed the immaturity of the puppies' hemopoietic system. Age-related changes in WBC and differential leukocyte counts in mink are similar to those of most other mammals. In the present study, the reduced level of WBCs in early postnatal mink (4- and 10-day) is in agreement with previous studies of other mammalian species—rats, rabbits, and pigs (14–16). Like in other

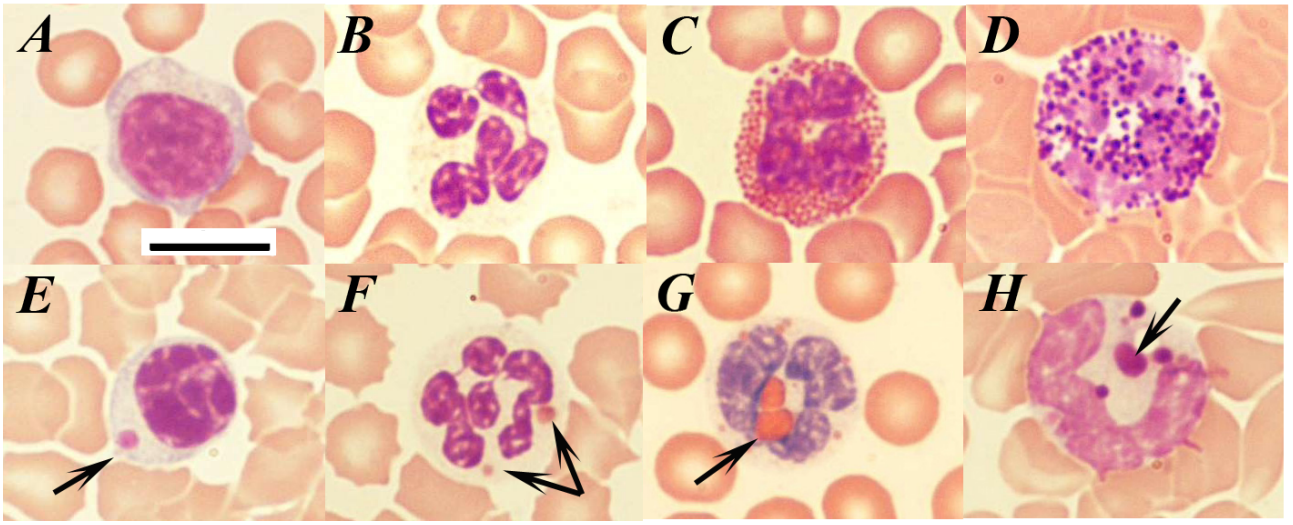

Figure 2. Blood lymphocytes (A, E), segmented neutrophils (B, F), eosinophils (C, G) basophils (D, H) of dark-brown (top) and sapphire mink (bottom) stained with May-Grünwald, Romanowsky. Leukocyte sapphire mink demonstrate abnormally large granules (arrows). Bar = 10 μm.

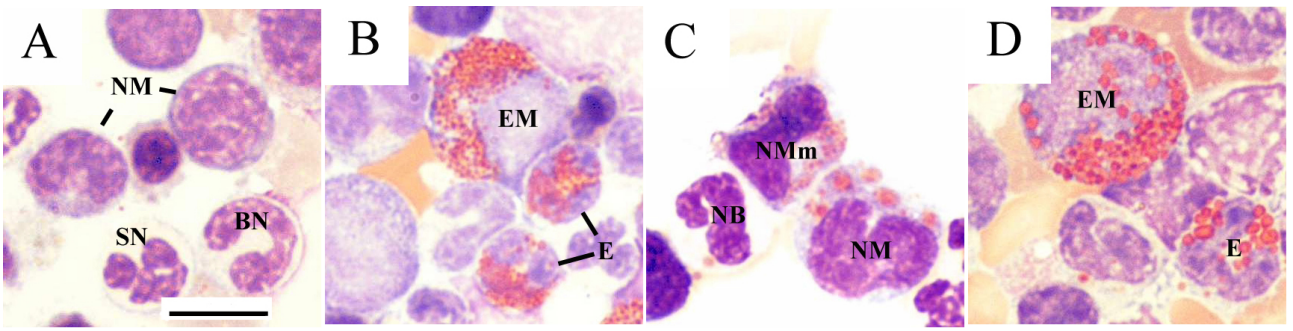

Figure 3. Bone marrow of dark-brown (A, B) and sapphire (C, D) mink. Different stages of leucocyte maturation (NM, neutrophil myelocyte; EM, eosinophil myelocyte; NMm, neutrophil metamyelocyte; BN, band neutrophil; SN, segmented neutrophil; E, mature eosinophil) are presented in photomicrographs. Neutrophils of dark-brown mink demonstrate faintly discernible granules or no visible granules (A); eosinophils at various stages of differentiation in dark-brown mink demonstrate numerous small size granules (B). Neutrophils of sapphire mink have large granules that vary greatly in size (C). Immature eosinophils (myelocytes) have more large granules as compared to dark-brown mink. Eosinophils at later stages of maturation have very large granules. May-Grünwald, Romanowsky stain. Bar = 10 μm.

animal species, the percentage and total numbers of metamyelocytes and band neutrophils were higher and the levels of lymphocytes and eosinophils were lower during early postnatal development. The reduced content of lymphocytes in neonatal animals has different causes: hypoxia, higher concentrations of corticosteroids, and participation of lymphocytes in regulation of organ growth (14,17). White et al. (18) demonstrated the immaturity of lymphoid cell production during neonatal development. The especially low content of lymphocytes and an elevated amount of immature neutrophils in sapphire puppies can be evidence of a delay in hemopoietic system development. It is known that sapphire puppies are characterized by increased embryonal and early postnatal mortality as well as low weight at birth (2). In this connection it is important that the phagocytic ability of leukocytes in neonatal animals correlates with birth weight (19). Sapphire puppies in the first 10 days of life demonstrated retarded growth. Retarded body weight, immaturity of the hemopoietic system, and presence of abnormal granules in leukocytes in sapphire puppies reduce their chances of successful postnatal adaptation.

At the age of 60 days mink enter the period of intensive growth, and at the same time the weaning period is completed and the puppies begin the transition from their mother's milk to solid foods. After that, sapphire mink demonstrated an increased growth rate; mean body weight at 60 days of age was higher than that in dark-brown mink

until adult age. In both colors of mink, WBC, lymphocytes, and eosinophils increased significantly in 60-day-old mink and reached the level typical of adult animals; this process concurred with intensive weight gain. There is a high level of neutrophils in mink older than 60 days. The growth and evolvement of mink are accomplished at the age of 180 days, when the formation of the skull and a majority of physiological systems is completed. At the age of 7–8 months, mink growth stops, and there are only seasonal changes in weight (20).

Like in other studies with animals having CH traits, we show that blood leukocytes in sapphire mink contains abnormal granules (6,7,21). There are major differences in the severity and clinical manifestations of the disorders between species with CH traits. We compared our data with those of other reports where CHS-like defect of leukocyte granules was examined (5,20,22). The defect of eosinophils in sapphire mink was more pronounced than in humans, foxes, mice, and cats. The order of increase of the degree of the defect in neutrophils was beige mice, sapphire mink, and then humans.

Information about age-related changes in leukocyte abnormality in CHS and CHS-like diseases is lacking. There are conflicting reports regarding the age effect on other tissues (8). As revealed in our study, abnormal leukocyte count changed during postnatal development. The total amounts of abnormal lymphocytes and segmented neutrophils increased with aging.

In the present study, light microscopy showed differences in bone marrow leukocyte morphology between dark-brown and sapphire mink. Bone marrow leukocytes in dark-brown mink were similar to leukocytes of most other mammalian species (23). Granules in bone marrow leukocytes of sapphire mink appeared massively enlarged. Previous reports demonstrated that bone marrow smears had giant inclusions in leukocyte precursor cells in other species with CH traits (5,7,24). Abnormal granules formed throughout leukocyte maturation in bone marrow in sapphire mink; "giant" granules were detected at different stages of maturations—from myelocyte to mature granulocyte. Morphological findings indicate that enlarged granules in neutrophils and eosinophils of sapphire mink's bone marrow are the result of aberrant fusion. Similarly, Davis et al. reported the size of abnormal granules to grow through fusion (25).

In summary, dark-brown mink and sapphire mink have common patterns of hemopoietic system maturation similar to that described for other mammals. We can conclude that WBC and differential leukocyte counts in mink of both colors undergo significant postnatal development; these age-related changes are linked with postnatal adaptation. We found considerable differences in leukocyte morphology and differential blood cell counts between dark-brown mink and sapphire mink. Sapphire puppies are characterized by immaturity of the hemopoietic system. Another feature of sapphire mink is abnormal morphology of leukocyte granules. The abnormality of leukocytes in sapphire mink apparently leads to impairment of the organism's immune defense.

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