SEX DIFFERENCES IN MORPHOLOGY OF THE NUCLEI OF NEUTROPHILS IN RATS

K. N. Muksinova and S. A. Rogacheva

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Sex differences in the nuclear structure of the neutrophils were first described by Davidson and Smith [7] during a study of human blood films. Subsequently these findings were confirmed by other investigators [1,2,4,5,8,12, 17]. The accessory nuclear formations characteristic of female individuals were also found in the neutrophils of monkeys [6,10], dogs [15,16], rabbits [9,13,14], rats [3,11,18], and mice [3]. However, the determination of sex chromatin in the leukocytes of rats is very difficult [11,18]. Some authors [11] actually doubt the possibility of determining the sex of the leukocytes in these animals from structural differences in the nuclei.

For this reason, a differential analysis has been made of the accessory nuclear formations in the neutrophils of the blood of male and female rats and an attempt has been made to determine their sex specificity.

EXPERIMENTAL METHOD

Rats of the Wistar line weighing 180-190 g were used. The numbers of the various nuclear appendages in the polymorphonuclear neutrophils were studied in peripheral blood films of 47 females and 37 males.

The nuclear appendages as a whole in the neutrophils of the rats were subdivided into types. Type A were intensively stained formations, round or oval in shape, attached to the nucleus by a thin pedicle. In shape these appendages resembled a drumstick or hanging drop. The diameter of the head of these formations was 1.0-1.5 μ (see figure, a, b, c). Type B were appendages resembling drumsticks in shape, but with a smaller head (less than 1 μ in diameter) and, as a rule, identical in intensity of staining with the nuclear chromatin (see figure, d, e, f). Type C were formations resembling the appendages of type A in shape and size, but with an area of translucency in the center of the head (see figure, g, h). Type D were thin appendages of different shapes, thread-like, hook-shaped, and finger-shaped. Often two or three such appendages were seen in one nucleus (see figure, i, j, k, l). Type E were nodular formations, round or oval in shape, directly in contact with the nucleus and differing noticeably from the nuclear chromatin in the intensity of their staining. In their shape, size, and staining properties, these formations were similar to the heads of the nuclear appendages of type A, and like the latter, were found very rarely – not more than one per nucleus (see figure, m, n, o).

The blood films were stained by Pappenheim's method. In the blood film of each rat, the morphology of the nuclei was studied in 500 polymorphonuclear neutrophils, paying attention to the number of different nuclear appendages. The proportion of neutrophils with accessory nuclear formation was expressed in percent. The sizes of the nuclear appendages were determined by means of an ocular micrometer.

EXPERIMENTAL RESULTS

The results of repeated counts of the nuclear appendages in the neutrophils of the blood of the same animal and the results obtained in counts of the same films from the different individuals showed no significant difference.

The leukocyte formula and the total leukocyte count in the peripheral blood of the investigated animals were within normal limits.

The study of the numbers of the various nuclear appendages in the polymorphonuclear neutrophils of the Wistar rats revealed differences in their number in male and female individuals (Table 1). In the females, nuclear appendages of type A were found in 0.8% of neutrophils, and in males in 0.2% ($P < 0.01$). The differences in the content of nodular formations (Type E) were still greater. In females their proportion was on the average 0.9%, but in males only 0.03% ($P < 0.0001$).

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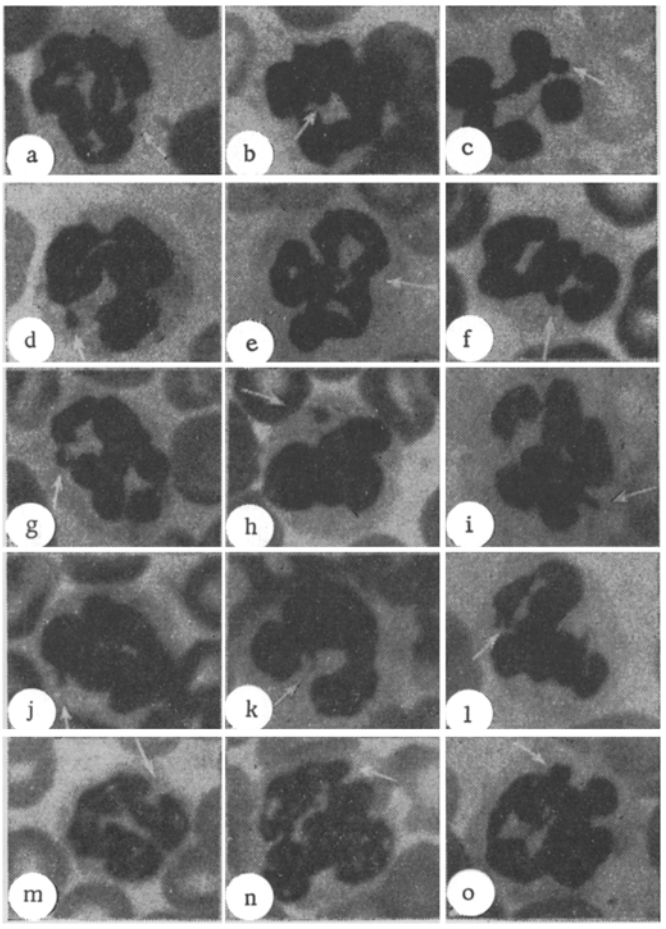

Accessory appendages in nuclei of neutrophils in the peripheral blood of rats of the Wister line: a,b,c) drumsticks, diameter over 1 μ (Type A); d,e,f) drumsticks, diameter under 1 μ (Type B); g,h) drumsticks with area of translucency in center of head (Type C); i,j,k,l) finger-shaped and hook-shaped appendages (Type D); m,n,o) nodular formation (Type E). Magnification ×2000.

TABLE 1. Content of Various Accessory Appendages in Nuclei of Neutrophils from Peripheral Blood of Rats (in %)

Type of appendages	Females (n 47)	Male (n 37)	P
A	0,8±0,04	0,2±0,02	<0,01
B	1,2±0,09	2,2±0,16	
C	0,1±0,02	0,3±0,03	
D	0,3±0,03	2,8±0,25	
E	0,9±0,07	0,03±0,008	<0,0001
A + E	1,7±0,11	0,23±0,03	<0,0001

The differences in the numbers of neutrophils containing appendages of type C in the males and females were not significant. Accessory nuclear formations of types B and D in most cases were found in the neutrophils of the male rats. According to some authors, these formations are seen more often in the nuclei of male blood cells in other species of animals [14] and in man [7,8]. However, the nature of these appendages is not clear, and as a rule they cannot be used to indicate the sex specificity of the cells. In contrast to this, appendages of types A and E, evidently associated with the female sex chromosomes [7,8], are found in much larger numbers in females. As the

TABLE 2. Content of Accessory Nuclear Appendages (A + E) Characteristic of Females in Neutrophils of Irradiated Male Rats Treated with Bone Marrow of Female Donors

Experimental condition	Group of animals	Number of animals	Number of bone marrow cells injected	Cells with types A and E appendages in donors (in %)	Cells with types A and E appendages (in %) in recipients at different times after treatment			
					Immediately after treatment	10 days after	20 days after	30 days after
				M ± m	M ± m			
Accute irradiation (900 R)	Treated	6	5 × 10^7	1.8 ± 0.14	0.1 ± 0.03	0.7 ± 0.09	1.3 ± 0.19	0.7 ± 0.09
	Control	3	—	—	0.1 ± 0.03	0	0.2 ± 0.1	0
Daily repeated irradiation (total dose 2100-3600 R)	Treated	7	1 × 10^8	1.8 ± 0.11	0.15 ± 0.06	0.7 ± 0.06	0.5 ± 0.02	0.4 ± 0.04
	Control	5	—	—	0.1 ± 0.06	0.15 ± 0.06	0.2 ± 0.07	0.2 ± 0.01

results of the present experiments (see Table 1) and investigations by other authors [8,10] show, more reliable results may be obtained by counting the total number of these two types of nuclear appendages. The use of the total number reflecting the content of appendages of types A and E together is further justified by the fact that the nodular formations are possibly prestages of the drumsticks [8], or the differences in the morphology of these formations are due to their spatial arrangement [10].

After determining the accessory nuclear formations characteristic of female neutrophils, it was interesting to determine whether this label could be used to identify female bone marrow cells injected into male rats. Experiments in which bone marrow from female donors was injected into male recipients were carried out on animals subjected to a single acute (6 rats) or repeated daily (7 rats) irradiation with γ-rays from radioactive cobalt (Co^{60}). The dose of acute irradiation was 900 R ($LD_{90/30}$), and the dose rate 20 R/min. Repeated irradiation in a dose of 60 R was given daily until the animals developed hypoplasia of the bone marrow (total dose of irradiation 2100-3600 R).

The number of neutrophils containing nuclear appendages of types A and E in the male rats receiving injections of bone marrow from female donors after a single acute or repeated daily irradiation is given in Table 2.

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