Time Course of Neutrophilic Leukocyte Population during Healing of a Skin Wound Inflicted under Diverse Conditions of Temperature Homeostasis

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Since wound healing is often attended by altered body reactivity caused by external physical factors, it is of interest to investigate the course of this process during exposure to such factors. Neutrophilic leukocytes (NL) play an important role in the process of wound repair and in its outcome, yet, in spite of numerous studies in this field, their function is still to be elucidated.

In the present work we studied NL populations in the red bone marrow, blood, skin, and regional lymph node during healing of a skin wound inflicted under normothermia and against the background of a profound and prolonged hypothermia of the body.

MATERIALS AND METHODS

Experiments were carried out on 150 adult albino rats. Group I animals (73 rats, control) narcotized with nembutal (40 mg/kg, intraperitoneally), were inflicted a full-thickness skin wound 15 mm in diameter in the interscapular area under conditions of normothermia. Group II narcotized rats (n=73) were cooled as described previously [4] to a rectal temperature of 20°C and inflicted similar wounds, this being followed by 6 h hypothermia, after which the animals were warmed with the aid of a reflector. The rate of wound closure was assessed by planimetry [6], and the rate of intercalary growth was studied by the method of tattoo marks [5]. Specimens were taken on days 1, 3, 5, 7, 10, 15, 20, and 30 after the injury. Regenerating tissues with the adjacent skin and regional lymph node were fixed in Bouin's fluid and embedded in paraffin. Skin samples and lymph nodes taken from four intact rats were investigated for control. Sections of these organs and smears of red bone marrow and blood were stained with AzureII-eosin. NL percent share was assessed in these smears by the routine method. NL were also counted in the regenerating tissues, papillary and retinal layers of the dermis, and in the hypodermis adjacent to the wound. THe NL count in the cortex and medulla of the lymph node was determined separately. The calculations were performed using Avtandilov's morphometric grid [1], estimating the NL count per mm^2 section area. Alkaline phosphatase (AP) activity was estimated in relative units in NL, plasmocytes, and B lymphocytes of the lymph node in cryostat skin and lymph node sections [4]. The numerical data were statistically processed using nonparametric Wilcoxson-Mann-Whitney U test.

RESULTS

A delay of wound closure and decreased rate of intercalary growth were observed in the rats exposed to cooling (group II). Their wounds healed within

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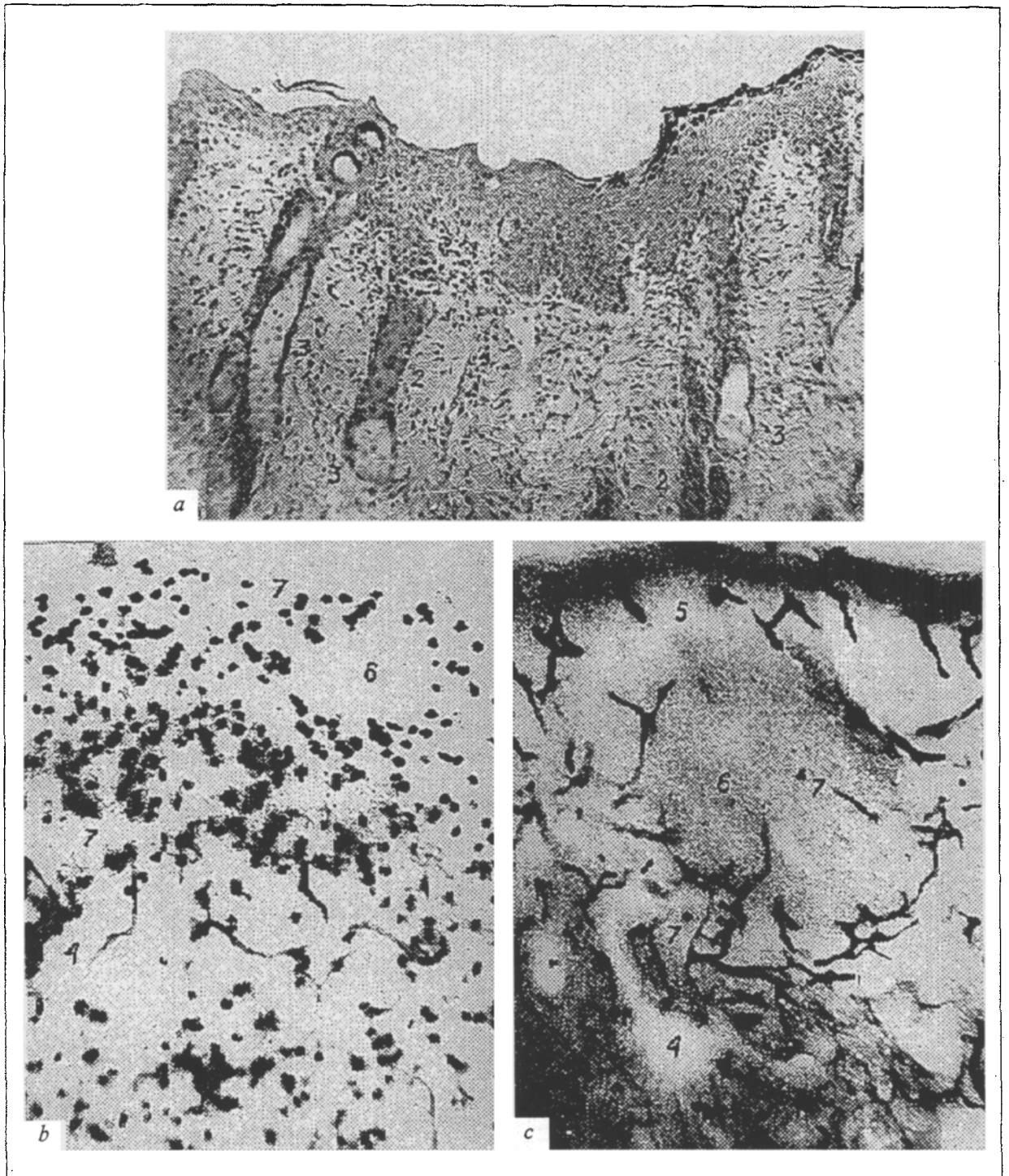

Fig. 1. Histological examination of tissues of rats exposed to hypothermia. *a*) hair and sebaceous glands formed de novo in regenerating tissues of rats exposed to hypothermia 15 days after the wound was inflicted (×100); *b*) abundant reaction of NL in medullary rays of lymph node in intact rats with poor plasmocytosis (×200); *c*) intensive plasmocytosis in medullary rays of lymph node in rats exposed to cooling 15 days after the wound was inflicted (in the absence of neutrophilic infiltration) (×200). 1) regenerating epidermis; 2) hair follicles formed de novo; 3) sebaceous glands formed de novo; 4) medullary rays; 5) apex of lymph nodule; 6) reactive center; 7) neutrophilic leukocytes. Staining: *a*) AzureII–eosin, *b* and *c*) alkaline phosphatase test after Burstone.

11.6 days, whereas in group I rats healing took only 9.5 days. At the end of the follow-up organotypic regeneration of tissues with complete restoration of the hair and without scar tissue was observed at the site of the wound in group II animals, whereas in group I just partial restoration of the hair and scar formation were observed.

Histological investigations revealed a decreased number of young fibroblasts with hyperbasophilic cytoplasm at the early stages of wound repair and the

absence of coarse fibrous tissue at the later stages of this process in the rats exposed to hypothermia as against group I animals. Beginning from the 10th-15th day of observation, epidermal ingrowth into the regenerating dermis and development of hair follicles and sebaceous glands were seen in these animals (Fig 1, *a*).

Neutrophilia was found in the red bone marrow of rats of both groups, developing later in group II. The increase of the NL count in this group was short-term but sharp; two peaks of cell count were noted: after days 5 and 20 (Fig. 2, *a*). The differences between the two groups did not disappear even at the end of the follow-up period. A lowered NL count on days 1 and 15 and a raised count on days 5 and 20 after the injury were detected in the blood of group II animals as against group I (Fig. 2, *b*). The number of NL increased both in the red bone marrow and in the blood at the expense of young as well as mature cellular forms.

A delayed and less intensive leukocyte reaction was noted in the regenerating tissues of group II rats: its maximum occurred on day 3 instead of day 1. Similar changes of the NL count were observed in the skin adjacent to the wound (Table 1). Differences in cortical and medullary NL reactions were detected in the regional lymph node. In group II the NL number increased over that in group I in the medulla after 3 days, and decreased after 1, 7, and 10 days. Similar changes developed in the cortex later: the parameter studied increased in group II on day 15 and decreased on days 20 and 30 (Table 1).

The changes of the NL number in the lymph node were in negative correlation with the intensity of plasmocytosis. Plasmocytosis was not pronounced in intact rats, this being confirmed by the absence of or low AP activity in these cells. AP activity is known to increase sharply during B-cell differentiation into plasmocytes [8]. Parallel with this the number of NL with maximal AP activity increased in the cortex and medulla of intact rats (Fig. 1, *b*). During plasmocytosis activation (its maximum was observed on days 1, 3, and 10 after injury in group I rats, and on days 7-30 of wound healing in group II animals), the number of NL in the lymph node was markedly lower compared to that in intact rats (Table 1). Plasmocytosis activation was accompanied by a marked activation of AP in B lymphocytes of the reactive centers, in the apex of the lymph nodules, and, specifically, in the plasmocytes of the medullary rays (Fig. 1, *c*).

Thus, a specific pattern of wound repair was revealed in the rats exposed to hypothermia compared to the control. In the early stages of healing, delayed wound closure and its epithelialization and a decreased rature of intercalary growth were noted. The morpho-

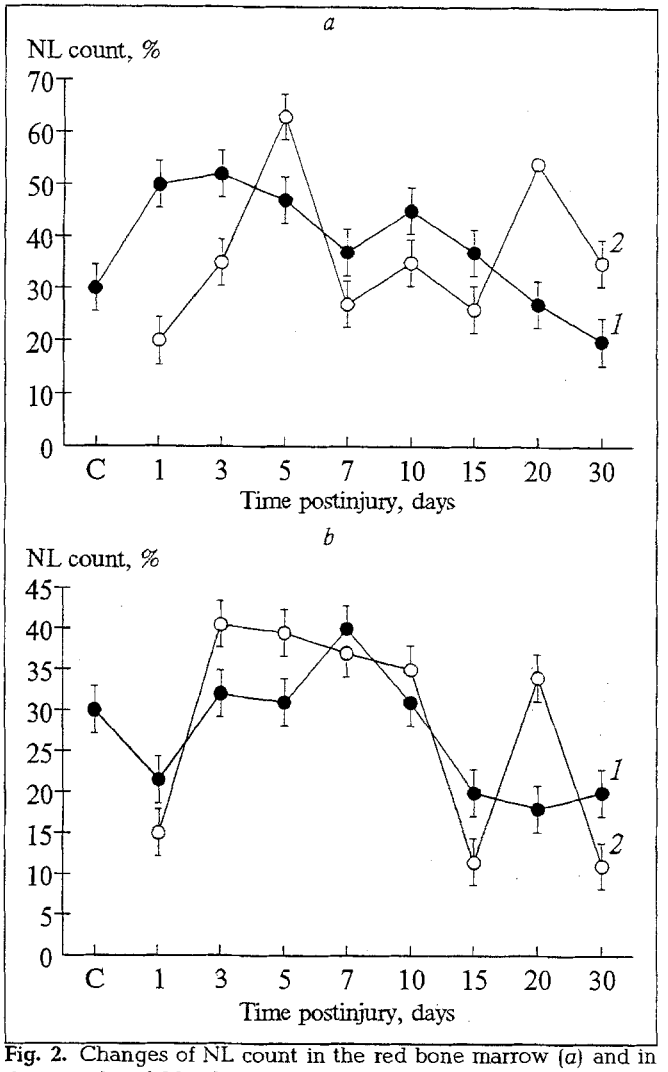

Fig. 2. Changes of NL count in the red bone marrow (*a*) and in the peripheral blood (*b*) during the healing of a skin wound inflicted under conditions of normothermia (1) and hypothermia (2).

genetic potential of regenerating epidermis with ingrowth foci formation was manifested at later stages, this leading to organotypic skin regeneration with complete restoration of the hair. Possibly, the slowed rate of wound contraction could be one of the factors contributing to complete restoration of the skin integument [6]. Another possible cause could be the following. NL is known to release fibroblast-activating factors [3]. The less marked leukocyte reaction in hypothermic animals was associated with a decreased number of active fibroblasts and the absence of coarse fibrous tissue in the regenerating tissue. This circumstance, undoubtedly, created favorable conditions for the organotypic regeneration of the skin. Activation of morphogenetic processes in the regenerating tissue of rats exposed to hypothermia could shift plasmocytosis in the lymph node to a later period, because it has been proved that the immune system participates in the regulation of proliferation, differentiation, and adaptive growth [2]. It can be assumed that in the rats exposed

TABLE 1. Neutrophilic Leukocyte Counts in the Skin and Regional Lymph Node during Healing of a Skin Wound Inflicted under Conditions of Normothermia and Hypothermia (10^3 cells/mm^2)

Time postinjury, days		Skin				Lymph node	
		regenerating tissue	papillary layer	retinal layer	hypodermis	cortex	medulla
intact rats		–	0.2	0.1	0.05	0.5	0.6
1	I	3.3	0.3**	1.0**	0.6**	0.3**	0.8
	II	1.8*	0.2*	0.4*	0.6	0.3	0.4*
3	I	3.0	0.3**	0.8**	0.5**	0.2**	0.1
	II	2.6	0.3	0.3	0.4	0.2	0.6*
5	I	1.6	0.1	0.2**	0.2**	0.1**	0.5
	II	2.6*	0.2	0.3	0.3	0.2	0.5
7	I	1.1	0.1	0.2**	0.2**	0.1**	0.2**
	II	0.7	0.1	0.4	0.3	0.1	0.1*
10	I	0.5	0.1	0.1**	0.2**	0.1**	0.1**
	II	0.3	0.04*	0.1	0.1	0.03	0.02*
15	I	0.2	0.1*	0.1	0.2**	0.03**	0.1**
	II	0.1*	0.1	0.05*	0.1*	0.1*	0.04
20	I	0.1	0.04	0.05	0.1	0.2**	0.3
	II	0.02*	0.004	0.1*	0.1	0.1*	0.3
30	I	0.04	0.04**	0.05	0.1**	0.2**	0.4
	II	0.02	0.1	0.1	0.1	0.1*	0.3

Note: asterisk: reliable differences in group II vs. group I ($p<0.05$); two asterisks: reliable differences in group I vs. intact animals ($p<0.05$).

to hypothermia the lowered NL number revealed in the regional lymph node at later stages of wound healing potentiated plasmocytosis activation, because these cells are known to release a factor suppressing B-cell differentiation into plasmocytes [3].

REFERENCES

1. G. G. Avtandilov, *Morphometry in Pathology* [in Russian], Moscow (1973).
2. A. G. Babaeva and E. A. Zotikov, *Immunology of the Processes of Adaptive Growth, Proliferation, and Their Disturbances* [in Russian], Moscow (1987).
3. N. M. Berezhnaya, *Neutrophils and Immunological Homeostasis* [in Russian], Kiev (1988).
4. M. S. Burstone, *Enzyme Histochemistry and Its Application in the Study of Neoplasms*, Academic Press, New York - London (1962).
5. V. A. Borisov, O. D. Myadelets, and V. N. Brinkevich, Kriobiologiya, № 4, 49 (1989).
6. E. A. Efimov, *Posttraumatic Regeneration of the Skin* [in Russian], Moscow (1975).
7. Wounds and Wound Infection, edited by M. I. Kuzin and B. N. Kostyuchenok [in Russian], Moscow (1990).
8. C. Marquez, M. Toribio, M. Marcos, *et al.*, *J. Immunol.*, **142**, № 9, 3187-3192 (1989).