

THE EFFECT OF BLOOD AND ITS COMPONENTS ON THE HEALING PROCESS IN EXPERIMENTAL FRESHLY-INFECTED WOUNDS

A. A. Vodyannikova

From the Sverdlov Research Institute of Plastic Surgery, Traumatology and Orthopedics (Director -
Corresponding Member of the Academy of Medical Sciences of the USSR, Professor F. R. Bogdanov)

(Received April 3, 1956. Submitted by Active Member of the Academy of Medical Sciences of the
USSR, Professor N. N. Zhukov-Verezhnikov)

The problem of the local effect of blood on the course of the traumatic process remains unsolved.

The blood, together with the lymph and tissue fluid, is the internal medium of the organism which washes all the cells and tissues of the human body and which has many different functions. Among them, the "defense function" of the blood, evident as its bactericidal and phagocytic activity, is of substantial significance.

The solution of the problem of the degree of bactericidal activity possessed by the various blood components has great significance not only in theoretical medicine but also in practice, in particular in connection with the local effect of blood and its components on the healing process of infected wounds.

The purpose of the present work was the study of the effect of local application of blood and its components plasma, serum and erythrocyte suspension, on the healing process of wounds infected with aerobic microorganisms.

EXPERIMENTAL METHODS

The experiments were carried out on white mice, guinea pigs and rabbits according to the following method, proposed by Professor L. G. Perets.

A horizontal incision 2 cm in length was made in a shaved section of the skin on the flank of a rabbit or guinea pig, a little below the vertebral column, then the skin, together with the subcutaneous tissue, was separated from the muscles with a blunt instrument (a spatula). In mice, the subcutaneous pocket was made on the dorsal surface in the direction of the occiput. A suspension of microorganisms was introduced into the subcutaneous pocket thus formed with a graduated pipette.

In each series of experiments, one of the following substances was administered to four out of five animals 40-45 minutes after infection: serum, plasma, erythrocyte suspension and whole blood. The fifth animal served as a control—it only received the suspension of microorganisms.

The greatest number of experiments was set up using hemolytic Staphylococcus aureus as the infecting agent.

In all, 517 animals were used in the experiments: 426 white mice, 60 rabbits, 31 guinea pigs.

EXPERIMENTAL RESULTS

The evaluation of the experimental results on the mice was carried out on the basis of the general condition and of a count of the number of dead and surviving animals. The results of the experiments on rabbits and guinea pigs were evaluated on the basis of: 1) the general condition of the animals, 2) local changes, 3) bacteri-

ological investigation of the contents of the subcutaneous pocket, 4) histological investigation, 5) cytological investigation of smears. The results of the experiments carried out on 426 mice are presented in Fig. 1.

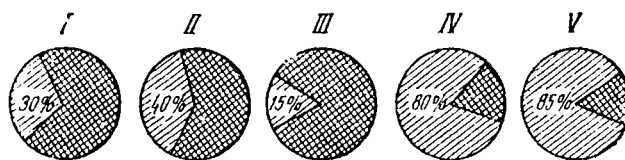


Fig. 1. Results of administering whole blood and its components during the healing of infected wounds of white mice.

I) control (infectious agent + physiological solution); II) infectious agent + whole blood; III) infectious agent + erythrocyte suspension; IV) infectious agent + plasma; V) infectious agent + serum. Lined sector—percent of surviving mice, cross-hatched—percentage of mice succumbing.

As is evident from Fig. 1, when only the suspension of microorganisms was administered into the subcutaneous pocket of the mice, 70% of the animals died; when whole blood was added, 60% died. When erythrocyte suspension was administered into the subcutaneous infected pocket of the mice, 85% of the animals died. The best effect was obtained with the administration of serum or plasma: only 15-20% of the animals died. Also, the death of the mice occurred later in comparison with the application of whole blood or suspension of erythrocytes.

In experiments on rabbits and guinea pigs, aside from a record of the general condition and local changes, histological examination was carried out of material biopsied from the region of the subcutaneous pocket certain intervals of time (24 hours, 3, 7 and 12 days) after the administration of this or that blood component into the infected wound. At the same times, bacteriological investigations were carried out by seeding the contents of the wound on a nutritive medium and the smears were investigated by the method of Prof. M. P. Pokrovskaya and M. S. Makarov.

A sharply evident inflammatory focus developed 24 hours after the administration of erythrocyte suspension in the subcutaneous infected pocket. Massive infiltration of the tissues by polymorphonuclear leucocytes was established on histological examination.

Muscle fibers were found to be degenerated, falling apart into discs and fragments. When the contents of the subcutaneous pocket was seeded on a nutritive medium, hemolytic *Staphylococcus aureus* grew. Smears from the wound showed a predominance of degenerated neutrophils, phagocytosis was incomplete, a great number of staphylococci were outside the cells. In three days, widespread necrosis of the skin developed in the region of the infected subcutaneous pocket and an ulcer formed. On histological examination, inflammation in the tissues was observed, accompanied by necrotic changes. Hemolytic *Staphylococcus aureus* was isolated from the wound. The cytological picture in the smears remained unchanged. In seven days, the inflammatory signs increased. Accumulations of degenerating neutrophils were found among the erythrocytes and fibrin. The muscular fibers were found to have degenerated. Hemolytic *Staphylococcus aureus* was found on seeding. Cytological examination indicated the unfavorable course of the wound process—a predominance of neutrophils in a state of degeneration, many free-lying staphylococci, absent or incomplete phagocytosis (Fig. 2).

In 12 days, ulceration was observed in the center of the cutaneous wound. Morphological examination showed that the ulcerous surface was covered with detached necrotic masses with a large number of neutrophils. The focus of infection was surrounded by a granulated wall, along whose periphery the formation of young fibrous tissue could be observed. Metastatic abscesses were near the basic focus. Hemolytic *Staphylococcus aureus* was isolated from the wound. Degenerated neutrophils with incomplete phagocytosis predominated in smears from the wound. In addition, isolated phagocytic neutrophils, monocytes, and profibroblasts were present.

An entirely different course of the wound healing process was observed when serum or plasma was administered into the subcutaneous infected pocket. In 24 hours, the inflammatory phenomena were insignificant. Limited infiltration of the dermis by polymorphonuclear leucocytes and detachment of necrotic tissues from the

surface bordered by a wall of small round cells, was found on morphological examination. Degenerative changes in the muscles were insignificant. Hemolytic *Staphylococcus aureus* was isolated from the contents of the wound. In smears from the wound, neutrophils with active phagocytosis predominated. Neutrophils with incomplete phagocytosis were absent, free-lying staphylococci occurred singly.

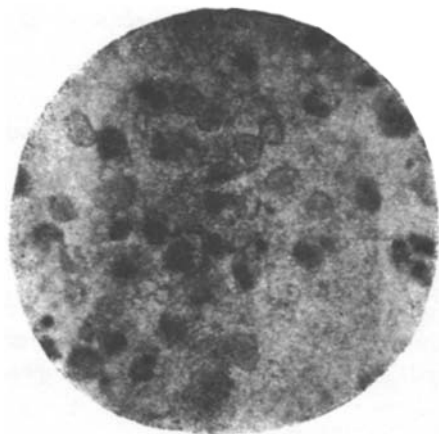


Fig. 2. Smear of the contents of the wound 7 days after the administration of erythrocyte suspension.

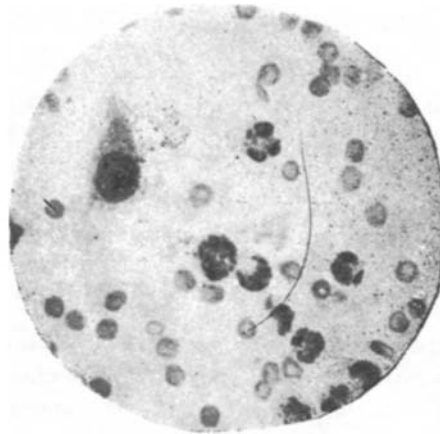


Fig. 3. Smear of the contents of the wound 7 days after plasma administration.

For 3 days, the focus of inflammation remained small in size. Histologically, a zone of young granulation tissue, which changed without sharp boundaries into the tissue of a fibrous capsule, was visible around the focus of inflammation. Seeding on nutritive medium grew hemolytic *Staphylococcus aureus* weakly. Cytoscopy uncovered in addition to neutrophils with active phagocytosis, basophilic plasmocytes, monocytes and profibroblasts. In 7 days, detachment of the necrotic masses from the surface was observed. The bottom of the wound was filled with mature granulation tissue, rich in collagen fibers; epithelialization of the wound occurred from the sides. Seeding on nutritive media uncovered no microorganisms. A small number of actively phagocytosing neutrophils, single monocytes, basophilic plasmocytes, profibroblasts and Metchnikoff's macrophages were observed on cytoscopy (Fig. 3). In 12 days, an epithelialized scar formed where the skin defect had been.

Histological, bacteriological and cytological investigation in experiments with administration of whole blood showed that healing of an infected wound occurred with greater difficulty than when plasma and serum were administered, but more favorably than when erythrocyte suspension was administered.

The general condition of the animals in the experiments with the administration of plasma and serum was good throughout the days of observation. A slightly depressed condition and a slight weight decrease were noted in animals in experiments with the administration of whole blood. Among the control animals and especially in experiments with the administration of erythrocyte suspension, the general condition was characterized by lassitude, anorexia, and considerable weight loss.

Thus, the experiments which were carried out on the local effect of blood and its components on the healing of experimental freshly-infected wounds showed that serum and plasma give the best effect.

The disappearance of microorganisms from the wound, active phagocytosis and the early organization of the focus of inflammation, noted from the first days when plasma and serum were administered, indicate the increased reactivity of the injured tissues in the animal's organism. This can be explained by the following.

It is known, that immunobiological components of plasma and serum: normal antibodies, complement, properdin, ~~enzymes~~ and hormones - determine bactericidal action. In addition, possessing certain physico-chemical properties, serum and plasma create a favorable medium for the cells which participate in regeneration and phagocytosis.

All this facilitates the increased resistance of the tissues to the infectious agent and more rapid healing of the wound.

Local application of serum and blood plasma speeds the healing of experimental decreased inflammation, activation of phagocytosis with freshly-infected wounds, which is evidenced as the simultaneous disappearance of microorganisms from the wound and shortening of the regeneration times. Erythrocyte suspension, a good nutritive medium for microorganisms, deteriorates the course of the healing process, which is accompanied by sharply evidenced destructive processes in the tissues, the suppression of phagocytosis and the presence of a large number of microorganisms in the wound.

The data obtained in the experiment substantiate the advisability of local administration of serum and blood plasma in the form of irrigation of bandaging during the initial treatment of freshly-infected wounds when a suture cannot be applied.

SUMMARY

The influence of local application of blood and its components (serum, plasma, erythrocyte suspension) on the healing of wounds infected by aerobic microbes was studied.

Experiments on rabbits, guinea-pigs and white mice have shown that local application of serum and blood plasma accelerates the healing of experimental fresh-infected wounds and injection of erythrocyte suspension aggravates the wound process and delays regeneration.

- [1] Z. V. Ermolyeva, *Antibiotiki*, 1956, vol. 1, No. 1, pp. 5-10.
- [2] A. A. Efremenko, *Zhurnal Mikrobiology, Epidemiology i Immunobiology*, 1955, No. 1, pp. 108-113.
- [3] O. B. Lepeshinskaya, *The Genesis of Cells from Living Substances and the Role of Living Substance in The Organism*,* Moscow, 1950.
- [4] I. I. Mechnikov, *Nonsusceptibility in Infectious Diseases*,* Moscow, 1947.
- [5] L. G. Perets, *Zhurnal Mikrobiology, Epidemiology i Immunobiology*, 1941, No. 10-11, pp. 20-26.
- [6] M. P. Pokrovskaya and M. S. Makarov, *The Cytology of the Exudate from a Wound as an Indicator of the Healing Process of the Wound*,* Moscow, 1942.
- [7] L. V. Yudina, *Experiments in Increasing the Bactericidal and Bacteriostatic Activity of Blood Serum*,* Dissertation, Sverdlovsk, 1949.

* In Russian.