2. E. B. Burlakova, A. A. Konradov, and I. V. Khudyakov, *Ibid.*, № 2, 184-194 (1990).
3. T. A. Voronina, in: *Pharmacology of Nootropics (Experimental and Clinical Studies)* [in Russian], Moscow (1989), pp. 8-19.
4. A. N. Inozemtsev and L. L. Pragina, *Vestn. Mosk. Universiteta,* Ser. *Biologiya,* № 4, 23-31 (1992).
5. T. V. Lelekova, P. N. Aleksandrov, P. Ya. Romanovskii, and I. P. Ashmarin, *Byull. Eksp. Biol. Med.,* **108**, № 7, 8-10 (1989).
6. T. V. Lelekova, I. P. Ashmarin, and P. N. Aleksandrov, *Vestn. Mosk. Universiteta, Ser. Biologiya,* № 3, 14-18 (1992).
7. L. L. Pragina, T. A. Voronina, A. N. Inozemtsev, *et al., Farmakol. Toksikol.,* **53**, № 3, 8-9 (1990).
8. N. A. Tushmalova, *Zh. Vyssh. Nervn Deyat.,* **44**, № 1, 3-7 (1994).
9. N. A. Tushmalova, V. G. Bezlepkin, F. F. Kokaeva, and A. I. Gaziev, *Dokl. Akad. Nauk SSSR,* **320**, 761-763 (1991).

Study of the Hemostatic Properties of Gaseous Ozone

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The hemostatic properties of ozone were studied on models of parenchymatous (from a wound in the liver) and stem (from the stump of the tail) bleedings in rats. An air-ozone mixture at a flow rate of 1 liter/min and a concentration of 2 mg/ml was found to exert a pronounced hemostatic effect. Our findings indicate that the arrest of bleeding under the influence of ozone is due to the formation of a fibrin membrane on the surface of the flowing blood, this leading to rapid and effective hemostasis. Preliminary drying of the wound still further speeds the onset of hemostasis.

Key Words: *gaseous ozone; parenchymatous bleeding; stem bleeding; hemostasis; rats*

Despite the ever-growing arsenal of medicamental (matrix) agents for local hemostasis [1], remote methods of arresting bleeding are still preferred in abdominal surgerry, methods that are based on the use of physical factors, such as cryo, thermo-, aerothermo-, laser, and plasma coagulation. However, hemostasis created by means of the majority of these methods involves the formation of a film of necrotic tissue of varying thickness on the surface of the wound, and this may adversely affect subsequent healing [2,6,9].

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For this reason, the influence of other, less damaging products, appears to be interesting, gaseous ozone being one of them [3]. Ozone, an allotropic modification of oxygen, is present in large quantities in the upper layer of the Earth's atmosphere [7] and forms naturally in stormy air and during some physicochemical processes [4,5]. The medical use of ozone is predicated upon its antibacterial and oxidative characteristics. It is used in the treatment of wounds and burns, in detoxication therapy, for the decontamination of water, and for the sterilization of medical instruments. Its immunomodulating, antiinflammatory, and antistress properties are also being studied. Published reports about the effects of ozone on whole blood provide information only about the correction of anemia and normalization of blood rheology during artificial circulation.

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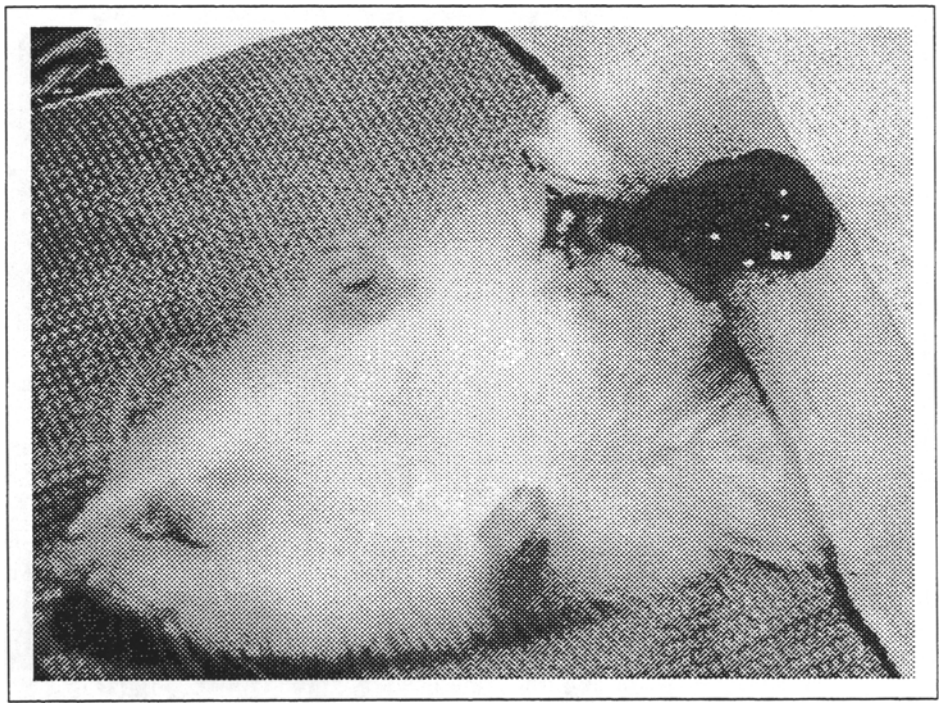

Fig. 1. Final arrest of bleeding after application of ozone to a wound in rat liver modeling parenchymatous bleeding. The surface of the blood discharged is covered with a fibrin membrane.

Our experiments have revealed a hemocoagulating activity of ozone, which, to our knowledge, has not been reported in the literature. This paper presents the results of our study of the hemostatic properties of gaseous ozone in animal experiments.

MATERIALS AND METHODS

The studies were carried out on 81 outbred male albino rats weighing 180 to 220 g in which parenchymatous and stem bleedings were induced. Acute experiments were carried out under ether narcosis under thermostatically controlled conditions (at 25°C) with the animals fixed in a special apparatus which did not disturb the hemodynamics.

The model of parenchymatous bleeding was created as follows; after depilation of the abdominal wall, the latter was dissected and the left lobe of the liver was removed and placed on a gauze cloth moistened with normal saline. A bleeding wound of a standard size and depth was then created using a stencil with a 7-mm hole and a razor. The model of stem bleeding was created by amputation of the tail at a specified level using a stencil with a 7-mm hole and a razor.

Three series of experiments were carried out. In the first series the hemostatic activity of gaseous ozone was demonstrated on the model of parenchymatous bleeding in 27 rats divided into 3 groups, 9 animals in each. The wounds in these animals (groups 1, 2, and 3) were exposed to a flow of air, pure oxygen, and an air-oxygen mixture, respectively, at a flow rate of 1 liter/min. The ozone concentration in the mixtures was 2 mg/li-

TABLE 1. Results of Experimental Studies of the Hemostatic Properties of Gaseous Flows of Different Composition in Rats

Experimental series	Group of rats	Wound model*	Composition of gaseous mixture	Time needed to arrest bleeding, sec
I	1	Liver	Air	244.5±11.3
	2	Liver	Pure oxygen	209.4±10.8
	3	Liver	Air–ozone	45.0±3.0
II	4	Liver	Air–ozone	46.1±7.0
	5	Tail	Air–ozone	40.1±3.8
III	6	Liver	Air–ozone	33.0±3.0
	7	Tail	Air–ozone	35.0±2.8
Control	8	Liver	–	305.5±12.3
	9	Tail	–	710.5±15.4

Note. *Parenchymatous bleeding was modeled by a wound in the liver and stem bleeding by amputation of the tail.

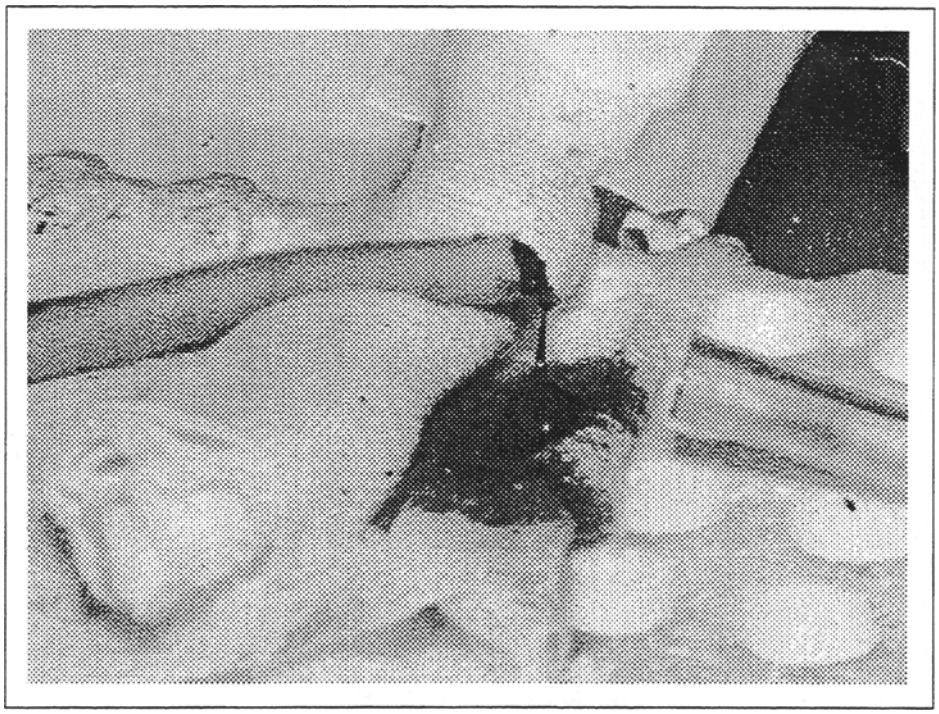

Fig. 2. Final arrest of bleeding after application of an air–ozone flow to a wound on the tail stump modeling stem bleeding. The last drop of blood discharged forms an "icicle," the surface of which is covered with a fibrin membrane.

ter. In the second series the effects of the air-ozone mixture on parenchymatous and stem bleedings were compared in 18 rats divided into 2 groups (groups 4 and 5). In the third series of experiments the wounds of rats (n=18) with parenchymatous (group 6) and stem (group 7) bleedings were dried by a single blotting with filter paper directly after the wounds were inflicted before exposure to the air-ozone mixture. Eighteen rats (groups 8 and 9) with bleeding wounds were controls administered no treatment, in which spontaneous hemostasis was observed.

An ozone generator (ozonator) of an original design was the source of ozone. The ozone concentration in the ozone-air mixture at the preset flow rate was monitored by a calibrated UV detector. A system of exhaust ventilation with a filter helped maintain the ozone concentration lower than the maximal permissible (0.1 mg/m^3) in the laboratory where the experiments were carried out.

Qualitatively, the effect of ozone on the model bleeding wounds was assessed visually. Chronometry of bleeding arrest was carried out using a timer with light and sound signaling and a stopwatch.

RESULTS

In the first series of experiments the time needed to arrest bleeding from wounds in the liver exposed to air or oxygen flow (groups 1 and 2) was 244.5±11.3 and 209.4±10.8 sec, respectively. In group 3, exposed to the ozone-containing mixture, hemostasis was attained much sooner: in 45±3 sec (Table 1). Visually, immediately after the wound had been inflicted and the air-ozone flow aimed at the appearing blood, we observed a marked reduction in the intensity of blood discharge. The surface of the blood appearing in the wound became transformed from shiny to mat within just a few seconds and acquired the appearance and properties of a membrane. If the ozonator jet was directed at an angle to the plane of the wound, the surface of a blood drop became "wrinkled" and its color gradually turned scarlet. Further treatment did not lead to appreciable changes, and therefore was stopped after the complete arrest of bleeding (Fig. 1).

In the second series of experiments, stem bleeding from the stump of the amputated tail (group 5) was arrested somewhat sooner, within 40.1±3.8 sec in comparison with parenchymatous bleeding from a liver wound (group 4), which stopped after 46.1±7 sec (Table 1). Despite the profuse bleeding observed at the moment of cutting the tail, which was several times more intensive than parenchymatous bleeding from the liver, treatment with the ozone-containing gaseous mixture led to a pronounced hemostatic effect. The constant, sometimes spouting, discharge of blood diminished to a drip, which became slower and slower as the drops became larger and larger. As a rule, the dripping gave way to the appearance of an "icicle" forming as a result of gravity with a blob suspended at the end, formed from the last drop fixed on the surface of the section. The transition from the dynamic phase

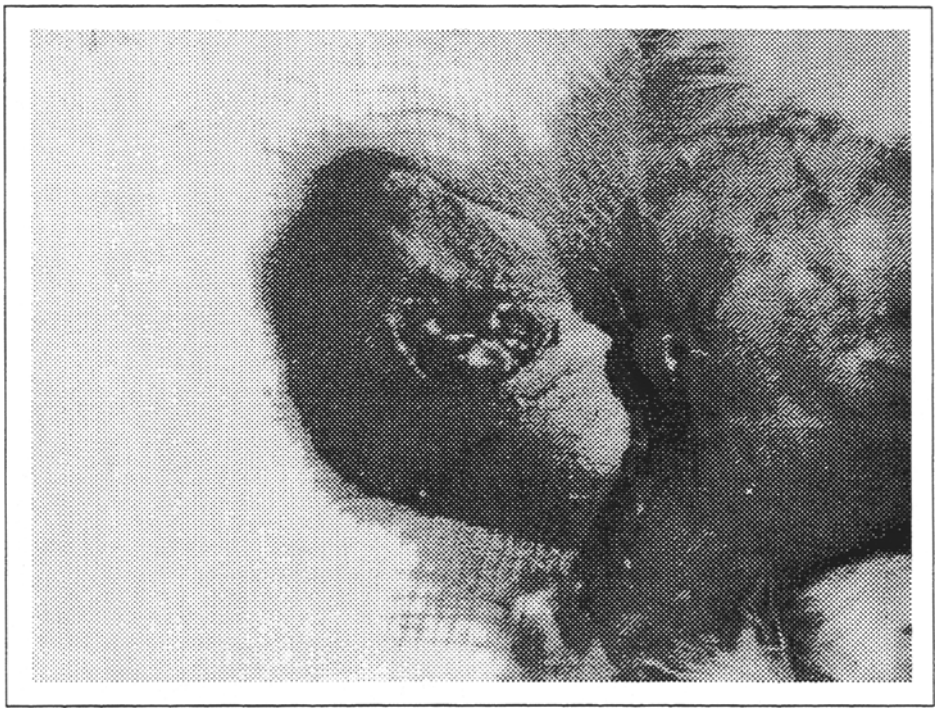

Fig. 3. Final arrest of bleeding after a single blotting of a wound in rat liver followed by exposure to an air–ozone flow. There is no blood on the surface of the wound, which is covered with a delicate, firm fibrin membrane.

of this phenomenon to the static phase was recorded as bleeding arrest. The membrane of the forming drop and the "icicle" represented a relatively firm fibrin layer consisting of a mixed clot (Fig. 2).

In the third series a preliminary single blotting of the wounds followed by exposure to the ozone-containing mixture led to a still more rapid hemostasis. In group 6 the time it took for bleeding to stop was 33±3 sec and in group 7, 35±2.8 sec (Table 1). A visible difference from bleeding arrest in group 1 animals with wounds in the liver was that several microdroplets of blood appeared in fractions of a second after wound drying, after which the wound became covered with a delicate but solid fibrin membrane and bleeding was arrested (Fig. 3). Preliminary blotting of the tail stump before treatment with gaseous ozone did not noticeably accelerate bleeding arrest.

In group 8 (control) the duration of model parenchymatous bleeding in our modification was 305.5±12.3 sec. In group 9 model stem bleeding lasted for 710.5±15.4 sec. It is noteworthy that even the best matrix hemostatics shorten the duration of model bleedings no more than two- or threefold in comparison with control groups [3].

Hence, our studies have convincingly demonstrated the hemostatic characteristics of gaseous ozone at a flow rate of 1 liter/min and a concentration of 2 mg/ml. Our findings indicate that bleeding arrest after exposure of the wounds to an air-ozone mixture is due to the formation of a fibrin membrane on the surface of the blood discharged, which, in case of a hemorrhage from nondescending liver vessels, leads to its rapid and effective arrest. The still more rapid hemostasis observed with ozone in the case of stem bleeding appears to be due to the high contractility of the vessels in the collagen-rich stump of the rat tail [8]. Preliminary drying of the wound still hastened the onset of hemostasis still further, this permitting further modifications of the technique.

These preliminary data should provide a starting point for in-depth studies of the mechanisms of the effect of ozone on blood clotting *in vivo* and *in vitro*, which will be our next task.

REFERENCES

1. A. A. Adamyan, Yu. P. Kashperskii, and S. V. Dobysh, *Khirurgiya*, № 11, 81-85 (1993).
2. V. A. Vishnevskii, *Improvement of Methods for Surgical Treatment of Focal Injuries to the Liver* [in Russian], Abstract of Ph.D. Dissertation, Moscow (1990), p. 14.
3. Yu. P. Kashperskii, *Development of a Method for Assessing the Specific Pharmacologic Activity of Local Hemostatics* [in Russian], Dissertation of Cand. Biol. Sci., Moscow (1993), p. 83.
4. A. Yu. Ishlinskii (Ed.), *Polytechnical Dictionary* [in Russian], Moscow (1980), p. 339.
5. Yu. V. Filippov, V. A. Voblikova, and V. I. Panteleev, *Electrosynthesis of Ozone* [in Russian], Moscow (1987), p. 4.
6. W. Frick and G. Frick, *General Surgery*, Ed. W. Schmitt *et al.*, [Russian translation], Moscow (1985), p. 110-113.
7. I. L. Knunyants (Ed.), *Encyclopedia of Chemistry* [in Russian], Moscow (1983), p. 397.
8. A. Arem, *Clin. Plast. Surg.*, **12**, № 2, 212 (1985).
9. J. M. Tew, Jr., and W. D. Tobler, *Clin. Neurosurg.*, **31**, 506-549 (1984).