2. M. A. Dolgov and V. V. Darsht, in: *Physiology of the Autonomic Nervous System* [in Russian], Kuibyshev (1979), Vol. 1, pp. 176-177.
3. N. I. Kas'yanov, *Klin. Med.*, **46**, № 11, 95-100 (1968).
4. M. I. Sarin, N. V. Sarina, N. D. Topolyanskii, *et al.*, *Klin. Med.*, **50**, № 1, 99-101 (1972).
5. V. A. Safonov, V. N. Efimov, and A. A. Chumachenko, *Neurophysiology of Respiration* [in Russian], Moscow (1980).
6. O. S. Sergeev, *Neuronal Organization of the Respiratory Center in the Medulla Oblongata, and Regulation of Its Activity* (Doctoral dissertation) [in Russian], Moscow (1984).

Respiratory and Circulatory Effects of Inhalation Exposure to Air Mixtures with Low Concentrations of Hydrogen Sulfide-Containing Natural Gas

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The importance of unraveling the mechanisms by which hydrogen sulfide-containing gases act on living organisms stems from the fact that hydrogen sulfide is highly active both chemically and biologically and can cause serious and even irreparable damage to various organs and systems of the body [2,5]. Although the deleterious effects of hydrogen sulfide-containing gaseous mixtures have been under study for a long time, there is still no agreement among investigators as to how such mixtures act on the vital systems, including the respiratory and cardiovas cular systems. In fact, the available information about the mechanisms of the damaging action of natural gases on these systems is rather fragmentary.

In this study, we measured parameters of pulmonary hemodynamics and respiration in cats inhaling gaseous mixtures with relatively low concentrations of the hydrogen sulfide-containing gas from the Astrakhan condensed gas deposit.

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MATERIALS AND METHODS

The study was carried out on 19 nembutal-anesthetized (35 mg/kg i.p.) random-bred cats of both sexes 2-4 kg in weight. Rectal temperature was measured in the cats at the start of the tests and then maintained close to the initial level (±0.5°C) throughout the study period by means of an electric heater. Tracheotomy was performed at the level of the upper third of the trachea; systemic arterial pressure (AP) was measured via a cannula inserted into the femoral artery. In the course of the study, AP, heart rate, breathing rate, and minute volume were recorded with a Russian-made MKh-01 polygraph. Oxygen tension in arterial blood (pO_2) and its reactions (pH) were recorded continuously using a DS67101 flow-through cuvette that contained fixed electrodes and was thermostatically controlled at 37.5°C with a VTS-136 thermostat. Oxygen tension was determined with an E-5046 electrode

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TABLE 1. Respiratory and Circulatory Parameters Measured in Anesthetized Cats Inhaling an Air Mixture Containing 250 mg/m³ Hydrogen Sulfide under Conditions of Spontaneous Respiration (*a*) and Artificial Respiration (*b*). The Values are Means±SEM

Respiration	Parameter	Time, min									
		0	1	3	5	10	15	20	25	30	*n*
a	MV, ml/min	805±130	779±110		1250±350	960±160	1090±140	1130±120		860±120	8
	BR, breaths/min	28.1±4.6	29.0±4.1	28.9±4.9	30.5±4.7	31.5±4.5	30.6±4.2	30.6±3.3	32.6±4.9		8
	HR, beats/min	226±14	226±14	226±15	228±15	225±16	230±14	229±13	230±23	247±20	8
	Mean systolic AP, mm Hg	92.9±4.8	89.4±4.8	90.8±6.2	96.6±5.2	98.3±5.7	97.6±6.0	99.3±6.2	86.3±1.2	74.3±6.0	49'
b	CO, ml/min	212±36	210±35	199±35	205±40	194±56	158±73				7
	HR, beats/min	222±10	213±12	215±12	206±10	206±10	172±17'				6
	Mean systemic AP, mm Hg	78±12	75±12	75±11	71±12	50±11	38±23				5
	Mean pulmonary AP, mm Hg	17.5±1.6	18.8±2.1	19.0±2.5	16.3±2.0	16.5±2.0	11.1±1.1"				8
	Blood flow in left lobar pulmonary artery, ml/min	58±12	56±10	56±10	55±10	57±12	59±14				7

Note. One and two asterisks denote a significant difference at $p<0.05$ and $p<0.01$ levels, respectively; *n*: number of tests; MV: minute volume; BR: breathing rate; HR: heart rate; AP: arterial pressure; CO: cardiac output.

and pH with a pair of G-265C and KS67053 electrodes (all this equipment was from Radiometer International A/S). Blood was delivered to the cuvette by means of a peristaltic pump from the femoral artery and then introduced into the femoral vein at a rate of 10 ml/min. To prevent thrombus formation, the cats were preliminarily administered heparin (200 units/kg i.v.). The pO_2 and pH values in arterial blood were registered on KSP-4 automatic recorders. Saturation of arterial blood hemoglobin with oxygen was determined by means of an OSM-1 instrument (Radiometer International A/S)/ Pulmonary and aortic blood flows were recorded with an M-46 flowmeter (Nihon Kohden, Japan) after applying flow sensors to a lobar pulmonary artery and to the aorta. The desired hydrogen sulfide concentration in the inhaled gaseous mixture was set by adding natural gas from the Astrakhan condensed gas deposit (which contains 23% H_2S) to the air. The amount of added gas was adjusted by counting gas bubbles, while the H_2S concentration in the inhaled gaseous mixture was adjusted using indicator tubes.

The parameters listed above were determined in control cats breathing spontaneously and in artificially ventilated test cats with pneumothorax. Parameters for artificial ventilation were selected taking into account the breathing rate and minute volume in the cats before opening the chest. The breathing rate was adjusted stepwise as close to the natural rate as possible (20-34 breaths/min), while the minute volume was adjusted in a continuous fashion at the natural or somewhat higher level so that the arterial pO_2 did not decrease by more than 20% (the arterial pH could increase slightly).

The results were treated statistically by standard procedures, using Student's *t* test to determine the significance of differences between the control and test animals.

RESULTS

In 8 preliminary acute tests on anesthetized cats, 5 to 150 mg/m³ of hydrogen sulfide were added to the inhaled gaseous mixture in order to determine the H_2S concentrations that exerted only minimal effects on the respiratory and circulatory parameters. These tests showed that prolonged (up to 100 min) inhalation of gaseous mixtures containing H_2S in the 5 to 150 mg/m³ range did not lead to appreciable alterations in the respiratory or circulatory parameters either in artificially ventilated cats (with open chest) or in those breathing spontaneously. It may be appropriate to note here that according to data of WHO (1986), the threshold limit value adopted in some countries for H_2S

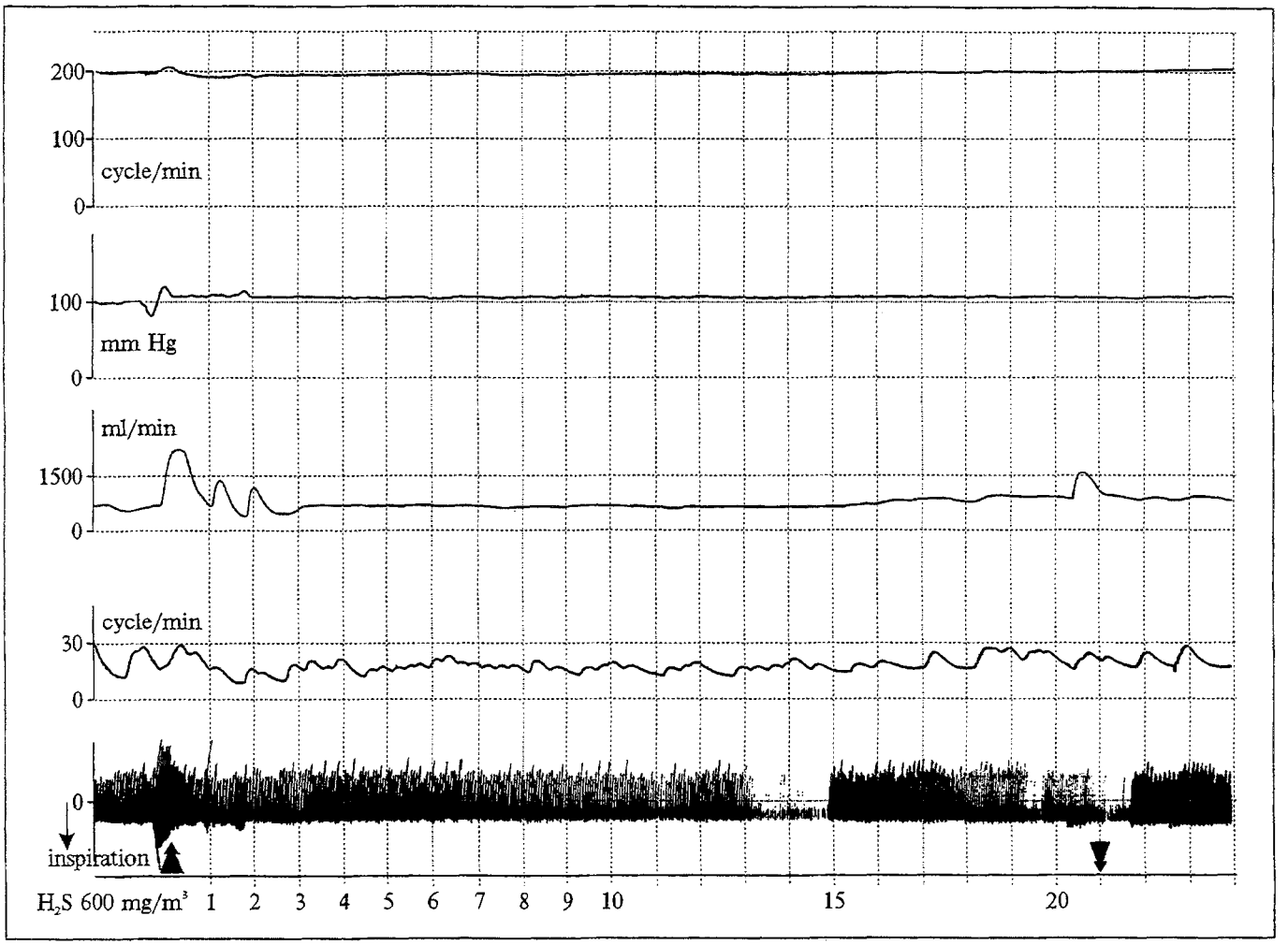

Fig. 1. Effect of hydrogen sulfide (250 mg/m^3) on pulmonary hemodynamics and ventilation in an anesthetized cat breathing spontaneously. From the top down: heart rate, beats/min; systemic arterial pressure, mm Hg; minute volume, ml/min; breathing rate, breaths/min; pneumotachogram (inspiration); time marks (min). The two arrows indicate the beginning and end of gaseous mixture supply.

in work environments is 15 mg/m^3, which is 1.5-fold higher than the maximum permissible concentration (MPC) adopted for this gas in Russia.

In spontaneously breathing cats, a gaseous mixture containing 250 mg/m^3 H_2S did not cause substantial changes in the ventilatory parameters (Table 1 and Figs. 1 and 2), although by minute 30 of exposure the systemic AP had decreased and a tendency toward an increased heart rate was observed, possibly reflecting the developing reflex tachycardia directed at the maintenance of AP. In artificially ventilated cats with pneumothorax (Table 1 and Fig. 3), an appreciable reduction of the pulmonary AP had already occurred by minute 15 of inhalation, together with a marked reduction of the systemic AP and decreases in the heart rate and minute volume, although blood flow in the lower lobar artery of the left lung had not diminished.

In general, the comparison of the effects of H_2S in spontaneously breathing and artificially ventilated cats showed that in the latter cats cardiovascular disturbances developed more rapidly than in the former and no signs of compensation were in evidence.

In spontaneously breathing animals, optimal ventilation-perfusion relationships can probably be maintained both through alterations in pulmonary ventilation and owing to adequate perfusion of the lungs, so that the overall load on the systems regulating respiration and circulation is alleviated. In animals on artificial respiration, during which the ventilatory regimen is rigidly fixed, the ventilation-perfusion relationships can only be regulated through alterations in the pulmonary hemodynamics, which increases the overall load on the cardiovascular system, thereby diminishing its capacity for adaptation.

In several tests, parameters of the oxygen regime were also registered for arterial blood in addition to those of external respiration and hemodynamics. A quantitative analysis of these experi-

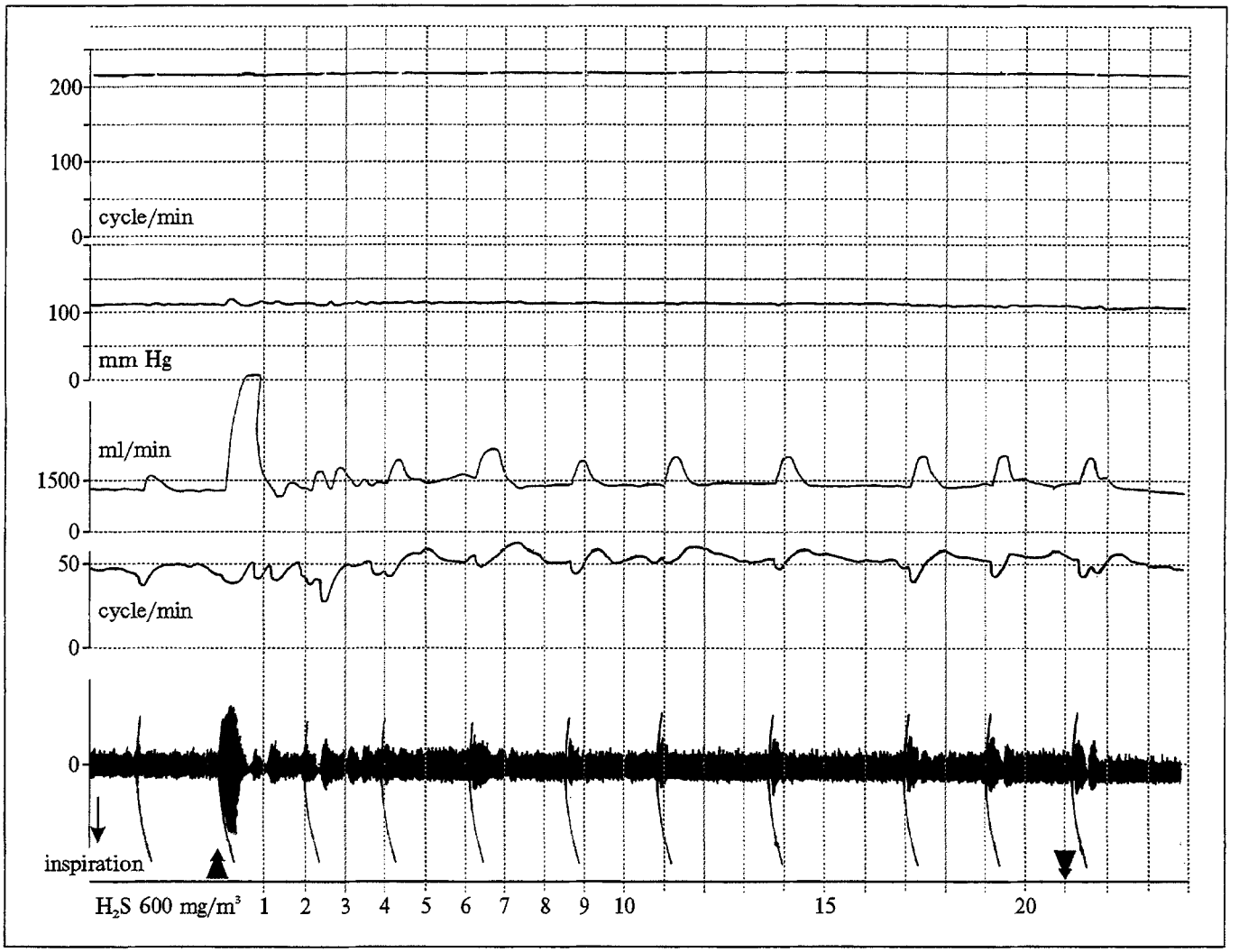

Fig. 2. Effect of hydrogen sulfide (250 mg/m^3) on pulmonary hemodynamics and ventilation in another anesthetized cat breathing spontaneously. Same designations as in Fig. 1.

mental data showed marked changes of the arterial blood reaction (pH) toward acidosis in artificially ventilated cats inhaling gaseous mixtures with various H_2S concentrations. It is noteworthy that the rate of blood acidification (drop of pH) in such cats was directly proportional to the H_2S concentration in the inhaled gaseous mixture. This is not surprising given that H_2S gas converts to a weak acid by dissolving in water, which results in an elevated hydrogen ion concentration in the solution.

In spontaneously breathing cats, not only the acidification rate in arterial blood but also the direction in which its reaction was changed were found to depend on how much H_2S the inhaled gaseous mixture contained; at relatively low concentrations (up to 250 mg/m^3 H_2S), a shift toward alkalosis was usually observed. Analysis of the oxygen regime in the arterial blood of artificially ventilated cats showed that low H_2S concentrations did not elicit changes in oxygen tension.

It should be mentioned that bradycardia and cardiac arrest in animals intoxicated with H_2S were first observed nearly a century ago, as were biphasic changes in AP - an initial rise followed by a fall to zero [6]. Much more recently, triphasic rather than biphasic changes of AP were found to occur in humans inhaling this gas: a pressor phase, a depressor phase, and a phase of recovery [3,4]. As regards respiration, the initial respiratory arrest under the action of H_2S has been found to be followed by a resumption of respiratory movements, though in a pathological form (Cheyne-Stokes respiration) [7]. Other authors, however, have reported initial increases in the frequency and amplitude of respiratory movements followed by their inhibition [1,8].

In our studies, the regulation of respiratory and cardiovascular functions appears to have been impaired in some of the cats acutely exposed for up to 100 min to H_2S concentrations of 200-250

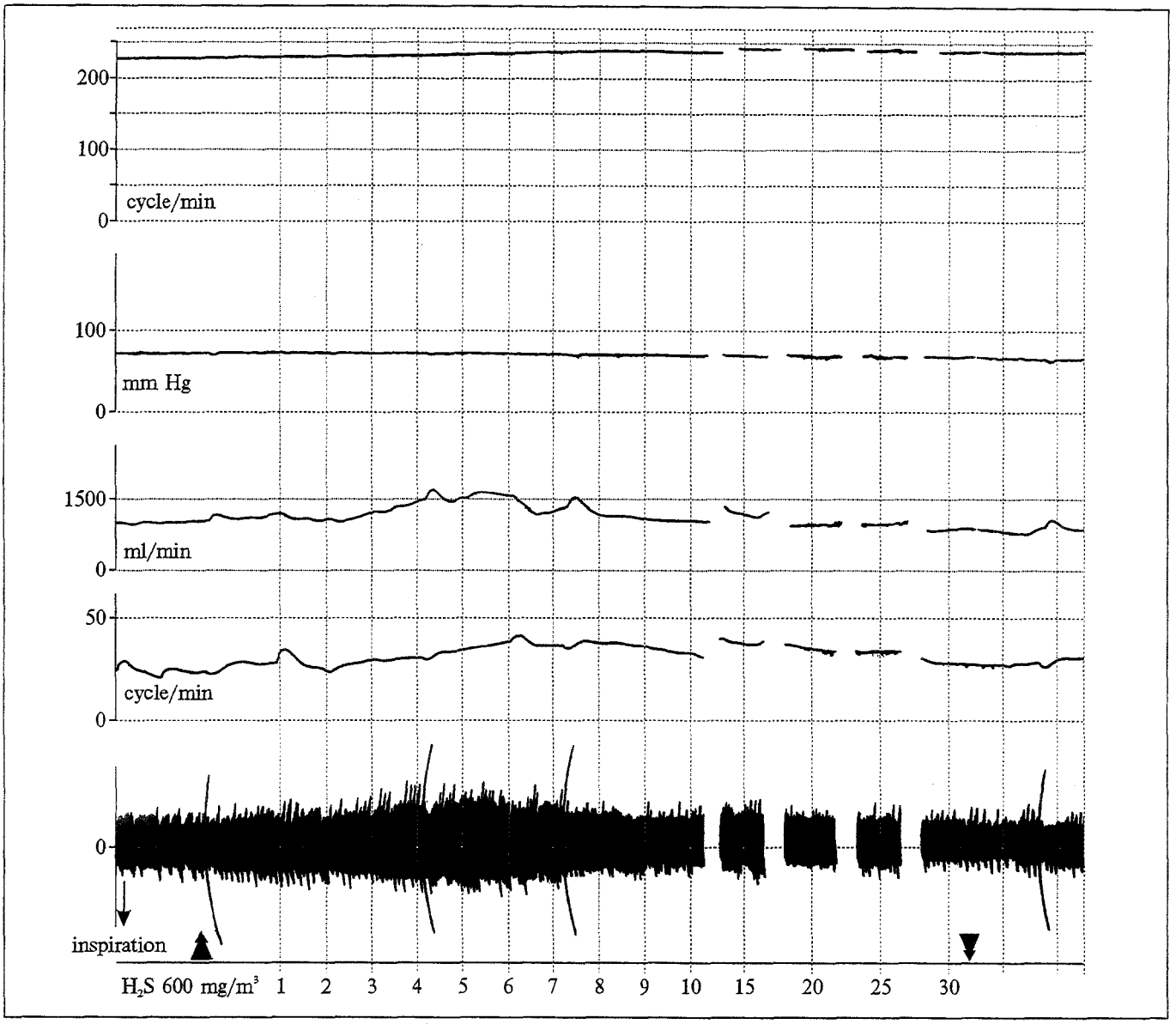

Fig. 3. Effect of hydrogen sulfide (250 mg/m^3) on systemic and pulmonary hemodynamics and on ventilation in an anesthetized and artificially ventilated cat with pneumothorax. Same designations as in Fig. 1.

mg/m^3. Some disturbances of regulation were present in a latent, compensated form, but compensatory reactions only occurred in animals inhaling gaseous mixtures with low to medium H_2S concentrations and, moreover, were incomplete because of the acidosis developing in the arterial blood.

In animals that had been inhaling gaseous mixtures in which the H_2S concentrations were not high, the abnormalities in vital functions were usually reversible and disappeared some time after the discontinuation of exposure. This time was comparable to the duration of exposure that had caused the observed abnormalities.

In conclusion, this study, in which several parameters characterizing the functioning of vital systems such as the respiratory and circulatory systems were recorded simultaneously in anesthetized cats breathing spontaneously or ventilated artificially, has enabled us to detect signs of impaired activity of these systems caused by the inhalation of hydrogen sulfide-containing gaseous mixtures.

REFERENCES

1. *Harmful Substances in Industry* (N. V. Lazarev and I. D. Gadaskina, eds) [in Russian], Leningrad (1977).
2. V. V. Darsht, *Pathophysiological Analysis of the Damaging Action of a Natural Gas from the Orenburg Gas Field* (Author's synopsis of dissertation) [in Russian], Orenburg (1986).
3. E. A. Drogichina, *Occupational Diseases of the Nervous System* [in Russian], Leningrad (1968).

4. V. K. Zburzhinskii, *Involvement of Reflex Mechanisms in the Toxic Effects of Hydrogen Sulfide and Sodium Sulfide* (Dissertation) [in Russian], Moscow (1955).
5. V. K. Zburzhinskii, *Trudy Leningradskogo Sanitarno-Gigienicheskogo Instituta*, **62**, 58-65 (1960).
6. P. A. Skvortsov, *Effect of Hydrogen Sulfide on Lung Tissues in Animals Poisoned with This Gas* (Doctoral Dissertation) [in Russian], St. Petersburg (1896).
7. G. A. Smirnov, *Impact of Hydrogen Sulfide on the Animal Organism, with Some Considerations Regarding the Pathological Aspects of Cheyne-Stokes Respiration* (Doctoral dissertation) [in Russian], St. Petersburg (1984).
8. J. A. Madden, Ch. Dawson, and D. R. Harder, *J. Appl. Physiol.*, **59**, № 1, 113-118 (1985).

Relationships between Catecholamine Levels in the Salivary Glands, Oral Mucosa, and Saliva of Rats with Experimental Staphylococcal Sialadenitis

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Since the condition of the oral mucosa and periodontium depends on the level of secretion by the salivary glands, the question arises as to how they interact. It has been shown that altered levels of catecholamines, mainly norepinephrine, in saliva are often associated with periodontitis, caries, aphthous stomatitis, and other oral diseases [2,4,5]. Severe disorders of salivation occurring in various forms of sialadenitis are frequently linked with abnormalities of the oral mucosa.

This study was undertaken to assess how catecholamine levels change in the salivary gland parenchyma, saliva (after stimulation of its secretion), and oral mucosa of animals during the development of staphylococcal sialadenitis.

MATERIALS AND METHODS

For the experiments, 95 random-bred rats of both sexes weighing 150.4±11.3 g were used. They were divided into six groups: *1*) intact rats with background (basal) saliva secretion; *2*) intact rats with saliva secretion stimulated by pilocarpine injected subcutaneously at 1 mg/kg body weight; *3* and *4*) rats with background saliva secretion at an early (2 h) and late (24 h) stage, respectively, of acute experimental sialadenitis produced by injection of a staphylococcal toxin (LH-0.18, series 33, manufactured at the Gamaleya Institute of Experimental Medicine) under the capsule of the left submandibular gland under sterile conditions; *5 and 6*) rats with pilocarpine-stimulated saliva secretion in the early and late stages of acute sialadenitis, respectively.

To synchronize salivary gland activities, all rats were deprived of food for 24 h before the acute experiment while being allowed to drink water *ad libitum*. Tissue pieces from the left submandibular gland and oral mucosa were taken under Nembutal anesthesia (40 mg/kg). In rats in the early or late stage of sialadenitis, tissue pieces were also taken from the right submandibular gland. In rats with stimulated saliva secretion,

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