GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Demyelination of Nerve Fibers in the Central Nervous System Caused by Chronic Exposure to Natural Hydrogen Sulfide-Containing Gas

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We studied the effect of natural gas from Astrakhan' gas field on rat brain. The study revealed a specific effect of natural H_2S-containing gas on myelin sheaths in the brain, which determines its neurotoxicity even at low concentrations in the inspired air.

Key Words: *natural gas; hydrogen sulfide; myelin sheath; rats*

A gas field with high hydrogen sulfide (H_2S) content is located near Astrakhan' [1]. The study of morphology and prevalence of brain tumors in Astrakhan' region showed that the incidence of neural diseases correlates with the degree of anthropogenic environmental pollution [5]. Apart from hydrogen sulfide, natural gas contains mercaptans and hydrocarbons, while industrial gas conversion produces sulfur and sulfurous and sulfuric anhydrides. Similar to H_2S, these substances released into atmosphere, soil, or water are toxic for humans and dangerous for biocenosis [3]. Hydrogen sulfide acts as an irritant and suffocant. In concentrations >150 mg/m^3 it induces irritation of throat mucosa, metallic flavor, fatigue, headache, and nausea. The maximum permissible concentration for hydrogen sulfide in the air of working area is 10 mg/m^3 for pure gas and 3 mg/m^3 in a mixture with C_1-C_5 hydrocarbons [3].

Many experimental studies demonstrated pronounced neurotropic effect of natural H_2S-containing gas. Chronic exposure to natural gas induces neurasthenic and psychoneuropathic states characterized by headache, giddiness, emotional lability, memory and sleep disturbances, impaired concentration, pains in extremities, and paresthesia [2,7].

A universal peculiarity of hydrogen sulfide action is activation of phospholipases. Intensive degradation of phospholipids in the plasmalemma and mitochondrial membranes dramatically inhibits aerobic ATP resynthesis, because activities of cytochrome-*c*-reductase, NAD-dehydrogenase, and succinate dehydrogenase sharply decrease. Both disintegration and synthesis of phospholipids take place under normal conditions, but under pathological conditions phospholipids catabolism prevails, while reutilization of metabolites is sharply decelerated. Similar disturbances were observed during poisoning with gases containing hydrogen sulfide [2]. Hydrogen sulfide applied in small doses (30 mg/m^3, 1 h per day for 11 days) decreased LPO level in the cortex and brainstem [6].

Since CNS contains a large amount of myelin, the substance enriched with lipids acting as a solvent for hydrogen sulfide, our aim was to study the morphology of myelinated nerve fibers during chronic exposure of natural H_2S-containing gas.

MATERIALS AND METHODS

Experiments were carried out on 90 male random-bred albino rats (200-250 g). The rats were subdivided into

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3 groups. Group 1 (n=30) and group 2 (n=30) were daily (1 h per day) exposed to gas mixture containing H_2S (10 and 100 mg/m^3, respectively) for 1 month. Group 3 served as the control. Natural gas from Astrakhan' gas field was dried and then used in the study. The experiments with gas mixture were performed in a Heldinskolde gas chamber. H_2S concentration was measured on Pasport and J-813 gas analyzers.

The specimens were isolated under chloral hydrate narcosis (325 mg/kg) immediately after the last exposure to gas mixture. For light microscopy, the rats were transcardially injected with AFA-fixative (70% absolute alcohol, 20% formalin (37%), 10% glacial acetic acid), and the brain was isolated within 6 h. Fragments of the sensorimotor cortex were processed routinely [7]. Mapping of cerebral structures was performed according to Paxinos—Watson rat brain atlas. Serial sections of the brain were deparaffinized and stained with luxol fast blue according to I. V. Viktorov's rapid technique of visualization of myelinated fibers [7]. For electron microscopy, transcardiac perfusion with 2.5% glutaraldehyde on cacodylate buffer (pH 7.3) was performed under the same narcosis. Then the specimens were processed according to the method accepted in Department of Experimental Cell Pathology. The data were analyzed statistically using Statistica for Windows 5.5 software.

RESULTS

On paraffin sections stained with luxol fast blue we found the following abnormalities in the structure and

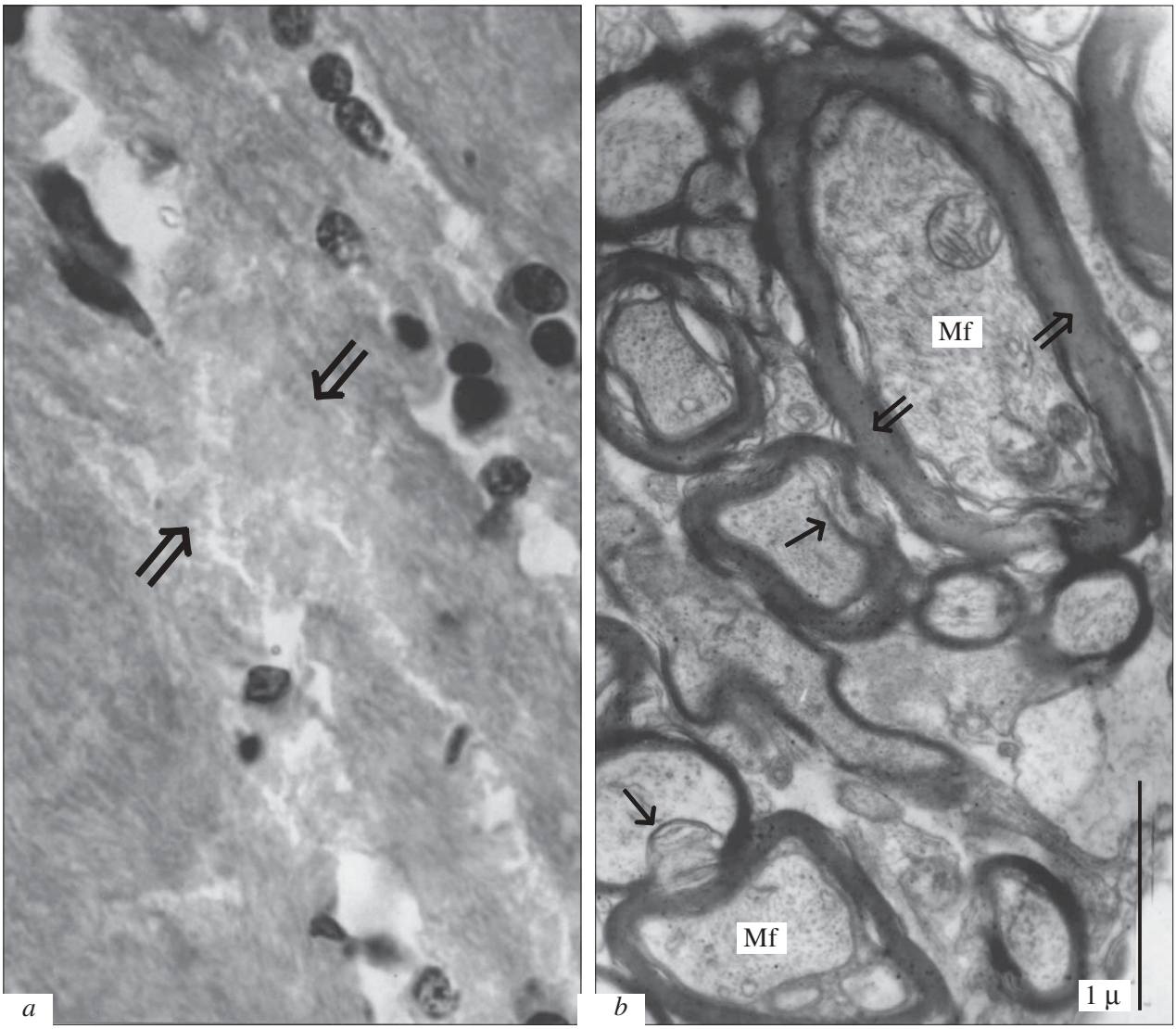

Fig. 1. Effect of natural H_2S-containing gas (100 mg/m^3 daily 1-h exposure for one month) on myelinated fibers (Mf) in corpus callosum, ×128. *a*) Luxol fast blue staining. *b*) Ultrastructure of myelinated fibers. The sites of focal demyelination and decreased osmiophilia are shown by single and double arrows, respectively.

ultrastructure of sensorimotor area of cerebral hemispheres in group 2 rats: focal edema under meninges, hemorrhage into the area of myelinated fibers located in the white substance, and extensive demyelination sites in the white substance of corpus callosum and caudate putamen (Fig. 1, *a*). The neurons surrounded by edematous astroglia processes underwent chromatolysis. Karyocytolysis involved single neurons and was accompanied by neuronophagia. The glial elements penetrated dying nerve cells. Dead neurons were replaced by gliosis nodes. The destructive alterations were generally mosaic: combination of irreversible and reactive changes.

Ultrastructural analysis also revealed alterations in neurons: cisterns of the granular endoplasmic reticulum were dilated, nucleoli were displaced to the periphery, and perinucleolar chromatin clusters appeared. The number of lipid inclusions in neuron cytoplasm increased (Fig. 2). Their diameter varied from 0.3 to 2.5 μ, their area per 100 μ^2 cytoplasm increased 6-fold in comparison with the control. These lipid granules differed in optical density from homogenous gray to highly osmiophilic. Moreover, hyperosmic inclusions had many round and optically empty sites. The neurons with these inclusions were often located near blood vessels. Lipid granules contained lipofuchsin; the exposure to natural H_2S-containing gas dramatically increased the content of lipofuscin. Lipofuscin granules were located in the perinuclear area of neurons. Different electron densities indicated various stages of the development of these granules. These pathological changes were accompanied by the formation of collagen fibers (Fig. 2).

Electron microscopy of myelinated fibers in the sensorimotor area of cerebral hemispheres revealed focal deformation and demyelination of these fibers. Ultrastructure of the white substance (corpus callosum and caudate putamen) was also characterized by decreased osmiophilia of myelinated fibers, widening of periaxial spaces, and focal demyelination (Fig. 1, *b*). Pronounced neurodegeneration induced by natural hydrogen sulfide-containing gas (100 mg/m^3) was partially determined by damage to axons. Axons were edematous, separation of myelin sheaths progressed toward the axial cylinder. Severe damage to axons led to vacuolation, lysis, and pyknosis of nerve cells. In

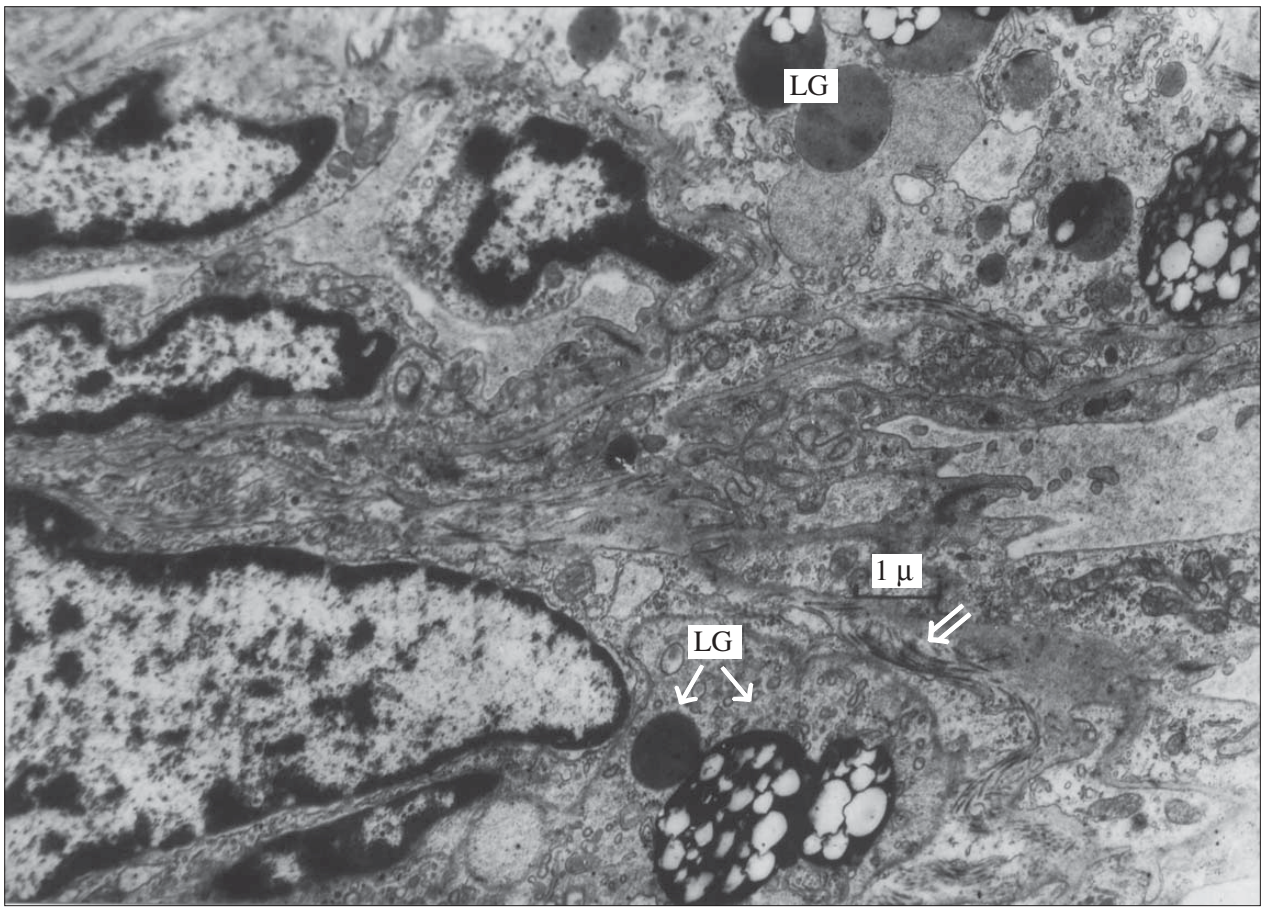

Fig. 2. Effect of natural H_2S-containing gas (100 mg/m^3, daily 1-h exposure for one month) on lipid granules (LG). Double arrows show formation of collagen fibers.

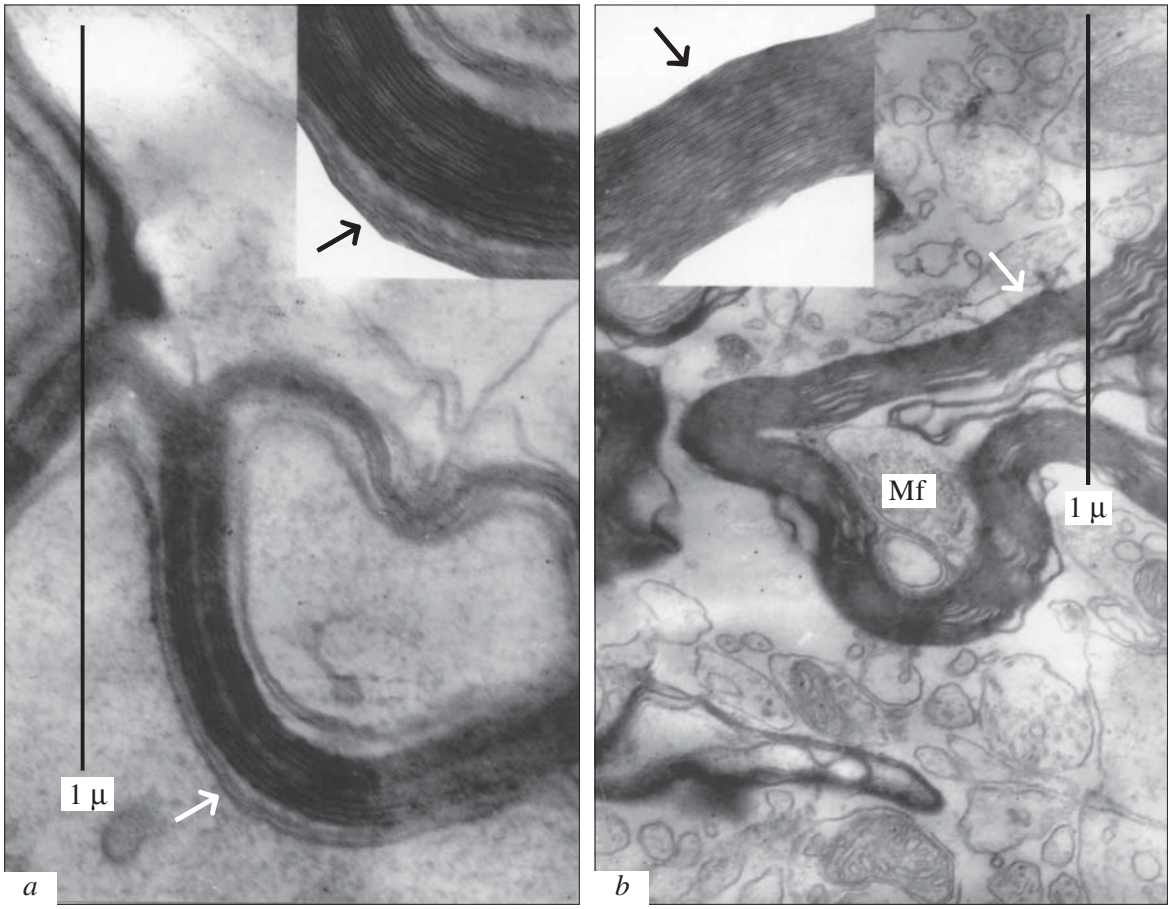

Fig. 3. Effect of hydrogen sulfide in low doses on myelinated fibers in sensorimotor cortex. *a*) myelin sheaths of nerve fibers in control rat. Arrows show fragments of myelin sheath. *b*) effect of natural gas containing hydrogen sulfide (10 mg/m^3, exposure time 2 week) on myelin sheaths in rats. Arrows mark the site in myelin sheath with diffusely decreased osmiophilia and fragmental disappearance of the main lines.

many cases, myelin sheaths were loosened or absent over a long distance. More severe alterations were accompanied by destruction of axial cylinder and marginal ruptures in myelin sheath.

Low concentrations of hydrogen sulfide-containing gas (10 mg/m^3 H_2S, maximum permissible concentration of H_2S mixed with hydrocarbons is 3 mg/m^3 [3]) produced no pathological changes detected by light microscopy. However, electron microscopy revealed ultrastructural changes attesting to certain dynamic in metabolic processes: after 2 weeks the contours of neuronal nuclei had irregular shape, endoplasmic reticulum was enriched with bound ribosomes, and nucleolus migrated to the periphery of the nucleus. At this term, local loosening of myelin sheath was noted in some myelinated fibers. Outside these sites myelin sheath had normal structure (Fig. 3, *a*) with electron-dense lines of main periods alternating with brighter parallel bands. The following alterations in myelin sheath were observed at different periods of gas exposure: while lamellar structure was preserved in some parts, other parts were characterized by total exfoliation towards the axial cylinder. However, there was no strict parallelism characteristic of normal myelinated fibers. In some fragments of myelinated fibers osmiophilia of intraperiod bands decreased, and the main periods had multiple interruptions (Fig. 3, *b*). Daily inhalation of natural H_2S-containing gas induced focal myelin loosening in many myelinated fibers. In damaged fragments myelin was completely absent and electron-dense main periods lost parallelism and became tortuous. However, this exposure produced no visible changes in axon structure, which were observed at higher content of H_2S in gas mixture.

Thus, even minor increase in H_2S concentration above the maximum permissible concentration induces destructive changes in myelin sheaths. Chronic exposure to natural H_2S-containing gas is a risk factor leading to demyelination in CNS.

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