Examination of organs and tissues of adult sheep grazed in an area with possible intoxication with rocket fuel, Kazakhstan

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Parenchymal organs, Lymphohistiocytic proliferative, Pneumonia, Bronchospasm, Echinococcosis.

Summary
The authors have conducted experiments to study the pathoanatomical and histological pattern of organs and tissues of adult sheep affected by unsymmetric dimethylhydrazine (UDMH). This highly toxic fuel was spilled on the territory of the Karsakpay and Ulytau districts of Karaganda region, Kazakhstan, because of the fall of the rocket 'Proton-M' after an unsuccessful launch from the Baikonur cosmodrome in 2007. In the experiment, the study group was consisted of 7 adult sheep that grazed in the area of possible intoxication with rocket fuel UDMH. The main objects of the study were histological preparations obtained from "fixed structures" - live and dead cells and tissues. As the structures have a flat contrast and are poorly detected in the ordinary light microscope, the specially processed preparations were used. After preparing, the authors studied organs and tissues using a microscope, which allowed to reveal in detail the level of damage caused by intoxication and to establish the negative effect of UDMH on the internal organs. The group of sheep showed a high index of macroscopic signs of interstitial pneumonia (85.7 ± 14.3%), and histologically quite high index was granulomatous inflammation of liver (71.4 ± 18.4%). Kidneys also showed a high level of abnormalities.

Introduction

In peacetime, the damage by the rocket fuel components generally occurs in emergencies, violation of safety rules during fueling, as well as in the destruction of storage (Petrenko 2006, Nauryzbaev *et al.* 2006). The most common type of liquid fuels is two-component rocket fuels, consisting of oxidizing agent and combustible agent. Oxidizing agents are nitric tetraoxide and nitric acid, and combustible agent is unsymmetrical dimethylhydrazine (UDMH or heptyl). This composition is self-igniting upon contact of the components with each other, which simplifies the engine start system and reduces the risk of explosion in the combustion chamber (Fedorov 2005).

UDMH is a colorless, transparent, highly volatile liquid with a strong unpleasant smell. The substance is hygroscopic, readily soluble in water, hydrocarbons, alcohols, ethers. The molecular weight is 60.08. UDMH is a highly dangerous compound with strong irritant properties. Its vapors irritate the mucous membranes of the eyes and respiratory tract. The effect of UDMH at a concentration of 400 mg/m^3 causes a person to be fatal poisoning. The strong odor of UDMH is felt at its concentration in the air above 5.0 mg/m^3 in the first minutes of contact. Subsequently, olfactory adaptation may occur (Schmidt 2011, Ushakova *et al.* 2004).

The accumulation, transformation and migration of any toxicant generally depends on its chemical nature and possible variants of transformations in biological objects, as a result of which secondary and even tertiary pollutants of the environment can be formed, which are even more dangerous than the raw material itself. According to the literature data, UDMH, a derivative of hydrazine, is a sufficiently reactive substance. In chemical

nature, this substance is a strong reducing agent, the oxidation of which causes the release of more substances that have a negative effect on the biota (Clements and Stoye 2014). UDMH is readily soluble in water, alcohols, amines and mixed with petroleum products and many organic solvents. UDMH forms mixtures with water with significant heat release (Tulupov *et al.* 1991).

Materials and methods

The authors conducted experiments to study the pathoanatomical and histological pattern of organs and tissues of adult sheep affected by unsymmetric dimethylhydrazine (UDMH, heptyl). This highly toxic fuel was spilled on the territory of the Karsakpay okrug of Ulytau district of Karaganda region as a result of the fall of the rocket 'Proton-M' after an unsuccessful launch from the Baikonur cosmodrome in 2007 (Zhubatov *et al.* 2006). The experiment was conducted in 2018. Four rams and 3 ewes (animal age 5 years), which grazed in the zone of possible intoxication with UDMH rocket fuel, were studied.

The main stages of cytological and histological analysis were the choice of the object of the study, its preparation for microscopic examination, the use of microscopic methods of investigation, and the qualitative and quantitative analysis of images (Nurtazin *et al.* 2006). The objects of the study were live and dead (fixed) cells and tissues, as well as their images obtained as a result of light and electron microscopy.

The main objective of the study were histological preparations obtained from fixed structures. Samples can be an imprint (for example, from spleen, thymus, liver), a film from the tissue (for example, connective tissue or peritoneum, pleura) and a thin section (Bratkov *et al.* 1987). The authors selected tissue and organ sites for the experiment (liver, lungs, thyroid gland). Histological preparations can be studied without special treatment (Winter *et al.* 2015). But due to the fact that the structures have a flat contrast, they are poorly detected in the ordinary light microscope, and the use of special microscopes (phase-contrast, etc.) is required. Therefore, specially processed preparations, i.e. fixed, embedded in a solid medium and stained are used more often. The process of making the histological preparation included the following main steps: sampling and material fixing, compacting, section cutting, staining or contrasting the sections (Angell *et al.* 2015, Angell *et al.* 2018).

After preparing, the authors studied organs and tissues using a microscope. Microscopy allows to examine an object at the cellular level to determine the level of exposure to organs. Microscopy can be light and electron microscopy. Light microscopy can be carried out in transmitted light when light passes through a thin transparent histological preparation, or in reflected light, when, for example, a thick or opaque object is examined. Similarly, electron microscopy can be a transmission microscopy, when an electron beam passes through the studied ultrathin slice, or raster microscopy or scanning microscopy, when the electron beam is reflected from the surface of the studied object. The authors studied the tissues and organs of adult sheep, which allowed to reveal in detail the level of damage caused by intoxication (Back and Thomas 1963).

Results and discussion

Echinococcosis of the liver and lungs

In the pathoanatomical examination of organs and tissues in seven adult sheep, macroscopic changes were detected in four animals (No. 4235, No. 3223, No. 06931, No. 5470), which were presented as granulomatous inflammations, mainly in the liver, less often in the lungs (Figure 1).

One ewe No. 4235 had visible macroscopic changes in liver and lungs at the vesicular stage of echinococcosis. They were characterized by multiple polymorphic foci of greyish-greenish or greyish-bluish color with a size of 3 x 5 cm mainly with surface-subcapsular localization. Fibrotic folds with costal pleura were observed in places of their localization in the lungs. Among the echinococcal vesicles there were single, but denser (calcified) small gray-white areas, consisting of two or three paired foci from 1 to 2 cm. The liver in the areas of absence of granulomas was slightly indurated, gray-brown or light-brown in color; the gallbladder contained a small amount of bile (30 ml).

In one sheep, a disorder of the coordination of

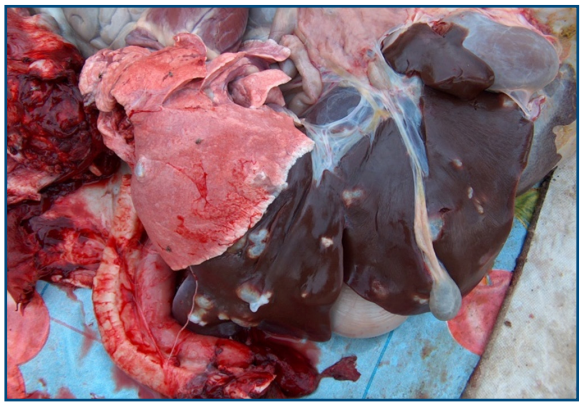

Figure 1. *Internal organs of the ewe No. 4235: multiple large-focal echinococcosis of the liver and lungs in a vesicular form in the calcification stage.*

movements in the form of a blind-staggers (a true turn sickness) was observed. However, at the autopsy, 5 larvae of *Oestrus ovis* in the frontal sinus area were found, the so-called and known veterinary disease – sheep oestrosis. The integrity of the anterior part of the cerebral cavity was broken; polycystosis was found on the right side of the brain, in three areas; the brain was atrophied, gray-dirty in color; gray and white substances were indurated, thinned and pale (Petersen *et al.* 2018). The nasal cavity in the area of the ethmoid bone was dirty-green in color, with ichorous smell.

The stud ram No. 06931 had changes that differed somewhat from the previous cases in that among the gray-yellow indurated foci there were gray, relatively large, tallow-like, considerably protruding above the liver surface, multiple growths 3 x 4 cm in size with soft consistency. In addition, along with the above described pathology of granulomatous inflammation, in two areas of the liver of stud ram No. 06931 there were small-focal, round-oval, exploded immature granulomas with infiltrative growth, wherein an occupied area was about 10 x 5 cm. The stud ram had ruffled hair, fatness below the average, frank thinning, pallor of the eye sclera and change in vesicular breathing before the slaughter.

Against the background of more frank granulomatous inflammations, perifocal encapsulation, proliferation of connective tissue, slight indurations, as well as flabbiness and congestion were observed in the liver of animals. Also, slight changes in the lung tissue were detected. In this case, a slight corrugation of the capsule, pink-reddish color of the tissue and monotonicity, despite the bleeding by decapitation, were detected.

Liver granulomatosis

The most significant granulomatous inflammation was found in the liver of two adult sheep. The stud ram No. 06931 had small multiple microscopic visual intralobular or extralobular granulomas. Histologically granulomas were characterized by a rounded shape, of different size with necrosis or without necrosis in the center. Some of them contained lymphohystocytes, fibroblasts and fibrocytes with a tendency to form connective tissue. Generally, such granulomas did not have foreign bodies, fragments or detritus of parasitic origin and cholicosis in the center. The granulomas were larger, partially visualized without a microscope, and had a more complex, layered structure and consisted of 4 layers, i.e. with necrosis in the center and the distinctiveness of cellular proliferates in the direction from the center to the periphery. In the center of the granuloma there was a cell necrotic mass with an admixture of groups of hepatocytes in the form of islets (Ferreira *et al.* 2016). Following the necrotic mass there was a layer with a perifocal, rich cell composition of polynuclears, histiocytes and a small number of giant cells of Pirogov-Langhans (Figure 2). The third layer was a capsule of dense connective tissue, consisting of fibrocyte-fibroblastic cells from groups of modified, multinucleated hepatocytes. The fourth layer (densely adjacent to the third layer) was consisted of homogeneous cellular structures, but also characterized by heterogeneity, multinuclear cells-hepatocytes with a symplastic and dense layer growth. The layer of pericapsular hepatocytes is very different from the comparatively distant, described hepatocytes. Giant multinucleated liver cells are formed from aggregated cells 3 x 4, round, sometimes oval in shape and contain 3, 4 or 5 nuclei with condensed basophilic-eosinophilic cytoplasm.

A large and variant number of described macroscopic granulomas indicate a development in the liver of more significant processes with a tendency to form connective tissue and cirrhosis (Figure 1). The histostructure of the section from more distant from granulomas site of animal liver No. 06931 characterizes the cytolysis of hepatocytes with significant disturbance of the hemodynamics of the microcirculatory bloodstream, a significant extension of the sinusoids, the deposition of the fibrillar structure of fibrin, the discomplexation and atrophy of the hepatic tubules with the proliferation of the stellate endothelial cells and endothelium. In histopreparations from more distant site of the liver in animal No. 5470, small granulomas with a size of 15 x 25 μm could be seen intralubularly. In the perivascular vessels and ducts of extralobular zones, small granulomas with lymphohystocyte cells and fibroblasts were found. There were also lymphoid infiltration and erythrocyte stasis, dystrophic-necrobiotic changes in hepatocytes in the swelling state in some lobules. The other adult animals (No. 5550, No. 5056, No. 3223, No. 4235)

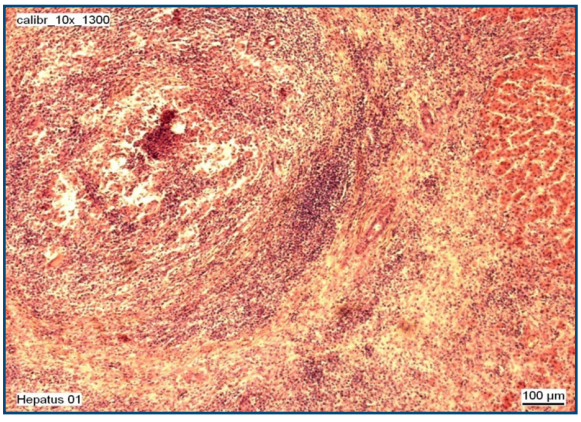

Figure 2. *The liver of stud ram No. 06931: granuloma with necrosis in the center, a giant cell such as Pirogov-Langhans cell, hypertrophic cirrhosis.*

had less significant changes in the liver. They were characterized by the presence of small single granulomas with activation of stellate cells with slight expansion of sinusoids and maintenance of the normal structure; macroscopic findings were echinococcosis and oestrosis of sheep with a lesion of the nervous system and a slight exacerbation of vascular disorders.

Interstitial pneumonia

As a result of scanning the sections of the lung parenchyma of the lungs of sheep No. 069131 and No. 5056 with light-optical microscope, we saw the development of focal-spread interstitial pneumonia (Figure 3). In this case, the alveolar septa were unevenly thickened (650 x 200 µm) and accompanied by a frank congestion of the capillaries, diapedesis hemorrhage in the perifocal zones. In sheep No. 5056 we observed activation and proliferation of alveolar epithelium with conversion into macrophages.

Respiratory epithelium is not straightened, more often is dentiform. A mild peribronchial fibrosis, was noted bronchospasm and the formation of small outgrowths of up to 100 µm in the inside of the lumen of the bronchi and bronchioles of the respiratory epithelium. In the perifocal sites, we could see the thinning and breaking of the septum, and the increase in the size of the alveoli with the activation of regional processes of emphysema. In some places they reached a size of up to 200 x 100 µm. These processes were somewhat less significant in the ram No. 5056, but there was a frank vascular disorder, provoking old foci of interstitial pneumonia.

Changes in foci of interstitial pneumonia were found in ewes No. 06980, No. 3232, No. 4235. The results of histological changes indicate the development of interstitial pneumonia processes with periods of exacerbation against a background of mild vascular disorders of hemodynamics in the microcirculatory bloodstream of the lungs.

Subacute glomerulonephritis

Many animals (No. 5056, No. 5470, No. 4235) had an extracapillary serous edema expressed in varying degrees; less often they had mild plasmorrhage with expansion of the extracapillary space, compression and atrophy, necrosis and necrobiosis of the glomerulus with eccentric localization. In addition, quite often the studied adult sheep had intracapillary hyperemia, palmateness, expansion of the interstitium, plasmorrhage, less often they had edema; some of the sheep had granular dystrophy and excretory insufficiency of tubules (Figure 4). The dimensions of the glomerular capsule were 150 x 90 µm, and the dimensions of the glomeruli were 65 x 120 µm. Morphological criteria were collectively classified as subacute glomerulonephritis.

As a rule, in the vast majority of cases, we observed a hypoexcretory function with tubulopathy processes with a total delay of eosinophilic, homogeneous and small (from 0 to 1.5 x 2 µm) substance in the center in the lumens, with simultaneous rejection of the apical part of the nephrocyte of the straight and convoluted tubules of the kidney.

Disorders of the normal structure of the thyroid gland

Endocrine glands, including the thyroid gland, are actively involved in the period of adaptation of the organism to adverse environmental influences. In functional terms, the thyroid gland performs regulation of metabolic, thermoregulatory, reparative and other processes, which under a certain pathology undergo a change in their coloidal secretory structural-morphological unit.

In five sheep out of seven, the thyroid gland functionally and morphologically had definite changes, in particular in the secretory-colloidal sector and its cell proliferative components. In general, the gland had follicles of round-oval

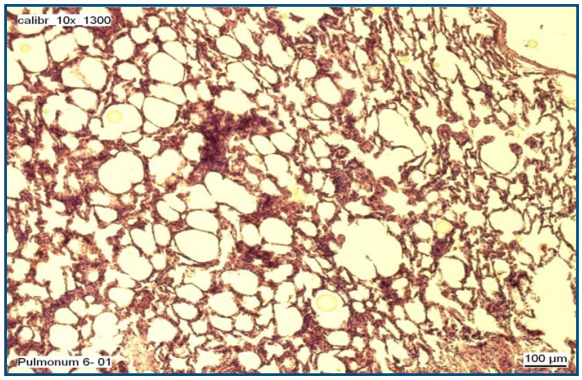

Figure 3. *Lung of the ram No. 5056: small-focal interstitial pneumonia.*

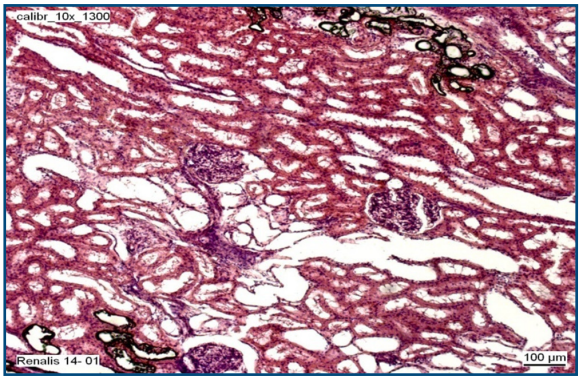

Figure 4. *Kidney of the ewes No. 4235: acute glomerulonephritis.*

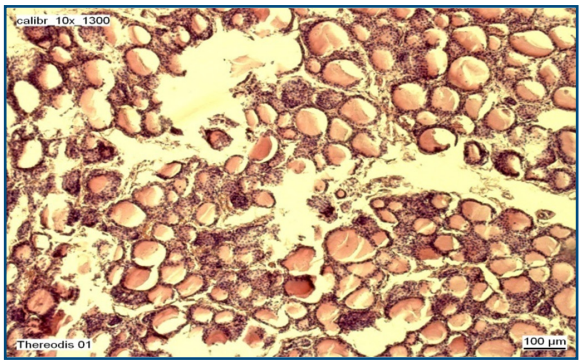

Figure 5. *Thyroid gland of the ram No. 06931: interstitial serous edema, total hypofunction of the gland.*

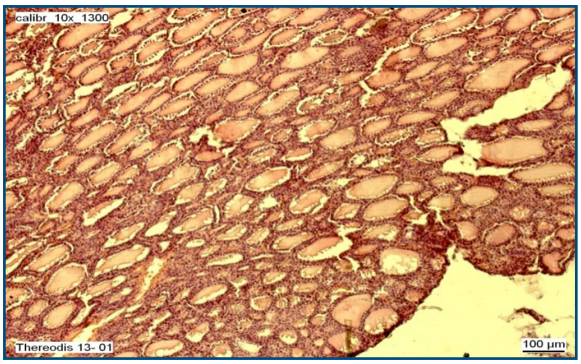

Figure 6. *Thyroid gland of the ewe No. 3223: gland dysfunction, small-follicular hypoxecretion*

shape and varied size. Despite the age and sex characteristics of animals, large forms and microfolliccles, expressed in interstitium, were dominant. The stud rams No. 06931, No. 5470 and ewes No. 3223 and No. 06980 can be classified as such animals. Most animals had a moderate, and the animal No. 066931 (Figure 5) had a more significant interstitial edema of the septum with a disturbance in the normal structure.

Despite the dominance of large follicles, we observed many generating microfollicles. Lymphohistiocytic proliferates were in the interfollicular zones. We observed activation of cell flattening from low cubic epithelium, a marked decrease in secretion properties of thyroid cells, indicating the detachment of the parietal part of the colloids or the absence of colloids and diffusely-spotty vacuolization of colloid-unfilled follicles. Some animals had mild violation of the integrity of the follicle, desquamation and proliferation of the epithelium in the intra-follicular fluid against the background of the proliferates of the interfollicular zone (Figure 6).

Thus, interpreted morphological criteria indicate the manifestation of hyposecretion with a disorder of the normal structure of the thyroid gland, which occurs as a result of residual exudative-proliferative and age-related changes in the basic functional structures of the parenchyma.

As is known from literature sources (Simonyan and Khisamutdinov 1995, Ioffe *et al.* 1979, Zrelov and Seregin 1975), the adrenal gland, participating in the endocrine system of the animal organism, actively participates in the restoration of disturbed homeostasis, weakened by various disorders and damages of various body systems. The histostructure of the adrenal glands was the same in most adult sheep. Adjacent to the adrenal gland extraorganic tissue and connective tissue capsule was loosened, disorganized and had a serous edema in some areas. Quite often, the most significant

Table I. *Statistical data of sectional and histological studies of the organopathology of adult sheep.*

Pathology of organs and tissues	Small ruminants (adult sheep)			
	Number of examined	Number of affected	Percentages	
			M±	m±
Liver Echinococcosis				
a) vesicular stage;	7	2	28.6	18.4
b) calcified and uncalcified small foci.	7	2	28.6	18.4
Echinococcosis of the lungs a) vesicular stage	7	1	14.3	14.3
Liver granulomatosis				
a) multiple granulomas with necrosis, giant cells such as Pirogov-Langhans cells and foreign bodies;	7	1	14.3	14.3
b) single granulomas without necrosis;	7	5	71.4	18.4
Dystrophy and necrobiosis of hepatocytes	7	6	85.7	14.3
Expansion of sinusoids, deposition of fibrillar structures in sinusoids.	7	2	28.6	18.4
Interstitial pneumonia	7	6	85.7	14.3
Subacute glomerulonephritis	7	5	71.4	18.4
Disorders of the normal structure of the thyroid gland	7	4	57.1	20.2

changes were observed in zona fasiculata of the animals, which is due to a local-total disorders of hemodynamics. At the same time, they were characterized by large-rounded, sometimes oblong, focal degenerative-destructive changes, swelling of adrenocorticocytes, a decrease in dark cells, and increase in light cells. Discomplexation with the phenomenon of vagueness of the contours of cells, their dystrophy and necrobiosis were quite distinct. In addition, we observed a significant loosening, expansion of capillaries, plasmorrhagia and enlightenment of zona fasiculata. Statistical data of sectional and histological studies of the organopathology of adult sheep in the zone of possible UDMH intoxication after the emergency fall of the rocket 'Proton-M' are presented in Table I.

Conclusions

The authors conducted sectional and histological studies of the detection frequency of organopathology in adult sheep in the area of possible intoxication with unsymmetric dimethylhydrazine after the fall of the rocket 'Proton-M'. A high percentage of the development of macroscopic signs of interstitial pneumonia (85.7 ± 14.3%) was established, and histologically, granulomatous inflammation of the liver had a rather high index (71.4 ± 18.4%). Granulomatous inflammation was accompanied by dystrophic-necrobiotic processes (85.7 ± 14.3%) of hepatocytes. The percentage ratio of multiple granulomas in the liver with necrosis and fibrosis and the presence of giant Pirogov-Langhans cells in them was within 14.3%. In addition, the authors noted the significant expansion of sinusoids of hepatocytes in adult sheep, followed by the deposition of fibrillar structure (28.6 ± 18.4%), such as pre-stage of granulomatosis and single microgranules.

Among organ pathology, the frequency of histological changes in the kidneys was also highwhich was accompanied by extra- (71.4 ± 18.4%) and intracapillary (85.7 ± 14.3%) exudative process in glomeruli and the development of dystrophy and necrobiosis of tubular nephrocytes (71.4 ± 18.4%), granulomas and necrosis (71.4 ± 18.4%) and a decrease in the excretory function of the kidney (71.4 ± 18.4%). At the same time, it should be noted that the morphofunctional differences of the thyroid gland were decreased (57.1 ± 20.2%).

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