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DEVELOPMENT OF PATHOMORPHOLOGIC ALTERATIONS IN THE LIVER, KIDNEY AND HEART OF RATS TREATED WITH T—2 TOXIN

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The effects of sublethal doses of T—2 toxin on the development and character of pathomorphological alterations in rat liver, kidney and heart were investigated. To this end, Wistar rats received daily doses of T—2 toxin (0.1 x LD_{50}) subcutaneously. Animals were sacrificed successively on days 7, 14, 21 and 28 of the experiment. Paraffin-embedded or frozen tissue sections were stained by using heamatoxylin eosin (HE), PAS, a trichromatic method according to Masson-Goldner and the Sudan III technique.

The alterations induced in the investigated organs were predominantly dystrophic and necrotic in nature while circulatory disorders or proliferative manifestations were seen as secondary phenomena. The character of the alternations varyied from parenchymatous dystrophy as the most common finding in the first week, to vacuolar or ballooning dystrophy and further to necrotic changes that were often seen in the third but specifically in the fourth week of exposure. The pathomorphological alterations affecting the liver, the kidney and the heart were observed in all experimental groups as a function of the time of exposure to T—2 toxin. The earliest and most prominent alterations occurred in the kidneys following parenteral introduction of the toxin.

Key words: Rat, T-2, lesions, liver, kidney, heart.

INTRODUCTION

The toxin T—2 is one of the important toxins pertaining to the phylum *Trichothecium* and is produced by mold fungi of the genus *Fusarim* (Buck and Osweiler, 1976). The toxin has strong cytotoxin activity (Humphereys, 1988) and is equally toxic to animals and men. Human intoxications were described as "red mold disease" (Yoshuzava, rf. Ueno, 1983), "corn mold toxicosis" in the US (Vesonder, 1983) or ATA (alimentary toxic aleukia) in the USSR (Joffe, 1960). However, reports on cases in domestic or laboratory animals are much more numerous (Buck and Osweiler, 1976; Wyllie and Morehouse, 1978; Humphreys, 1988).

The alterations incurred by the introduction of T—2 toxin varied owing to its complex mechanism of action. Uraguchi and Yamazaki (1978) claimed that there was no firm bond between the chemical structure of the mycotoxin and the specificity of target organs. These authors reported pathomorphological alterations in the liver, the digestive tract, the kidney, the bone marrow and the lungs while other authors also added the heart, gallbladder, bile ducts,the spleen and all haematopoietic organs and nervous tissue to this list (Buck and Osweiler, 1976; Wyllie and Morehouse, 1978; Humphreys, 1988). The intensity and character of the described alterations differ substantially from author to author but this may be ascribed to different doses of T—2 applied or the time interval of exposure, the mode of application and the observed species. Most commonly reported are dystrophic and necrotic alterations plus circulatory disorders affecting the parenchymatous organs specifically (Kosuri *et al.,* 1971; Smalley, 1973;Carlton and Scczech 1978; Schoental *et al.,* 1979; Hayes *et al.,* 1980; Krier *et al.,* 1982; Wilson *et al.,* 1982; Lidenberg *et al.,* 1985; Pang *et al.,* 1986).

Considering that alterations in parenchymatous organs such as the liver, kidneys or heart appear as a common denominator of all the reports, the present objective was to elaborate on their genesis and character. To this effect, an experimental model with rats receiving sublethal doses of T—2 toxin at determined time intervals was employed.

MATERIALS AND METHODS

Male Wistar rats 6-8 weeks of age and 210 + 5 g body weight were used for the experiment. Food and water were given *ad libitum.*

The toxin T—2 was isolated from the fungus *Fusarium sporotrichioides* cultured on a liquid (synthetic) substrate. Extraction and purification of the crude extract were carried out according to the method of Betine (1984), using the normal procedure(modification according to Bocarov-Stancic *et al.,* 1986; Bocarov-Stancic and Mutanjola-Cvetković, 1989). On the basis of the purity of the T—2 preparation, the data available in the literature and previous investigations, we decided on the mean lethal dose (LD_{50}) of 0.50 mg T—2/kg body weight.

The experiment involved 48 rats divided into two groups: an experimental group (32) and the control (16). The experimental group received daily subcutaneous doses of T—2 toxin (0.10 x LD_{50}). The control group received a mixture of ethanol and normal saline. Animals from this group were sacrificed at the beginning and at the end of the experiment. Six animals from the experimental group were sacrificed at each of the 7-, 14-, 21- and 28-day intervals.

Tissue sections from liver, kidneys and heart were fixed in 10% neutral formalin, embedded in paraffin or cryotome-cut, and Kept for histology. Preparations were stained with haematoxylin eosin (HE), a trichromatic method according to Masson-Goldner, and the PAS technique. The Sudan III technique was used for histochemical detection of fatty matter.

RESULTS

In rats from the control group that were sacrificed at the beginning and at the end of the experiment, no macroscopic alterations were noticed that would suggest a deviation from the normal histological structure of liver, kidneys or heart.

Histology in liver tissue from rats sacrificed after 7 days of the experiment most often revealed the normal size, shape and arrangement of hepatocytes with fine to coarsely granulated cytoplasm. The size and number of the granules were increased in the immediate proximity of a well-outlined cellular membrane. The majority of hepatocytes had clearly distinguishable nuclei whose diameter varied (anisokaryosis) (Figure 1). In addition to general enlargement, these nuclei were prominently hyperchromatic. In PAS-stained sections, positively reacting hepatocyte cytoplasm was noticed. PAS-positive material was not uniform and was concentrated into two poles, one staining densely.

Liver tissue from rats sacrificed on day 14 revealed densely packed hepatocytes with irregular shape and arrangement, so that their usual framelike pattern was indistinguishable in certain sinusoids. Coarsely granulated cytoplasm with irregularly shaped, and arranged granules of different sizes was seen. Some hepatocytes, also had a vacuolar structure which was foamy in appearance. While the nuclei in some cells remained preserved, they stained less in others or were missing (Figure 2). The Sudan III staining did not detect any fatty globules, thereby suggesting that the observed alterations resulted from intracellular oedema of the hepatocytes. The weak staining of parenchymatous cells made Kupffer's cells distinguishable. Their weakened multiplication was also observed.

The characther of the alterations in rat liver at 21-days was similar to the forementioned. However, the alterations were more prominent and were seen as a dystrophy characterised by coarsely granulated and vacuolated cytoplasm, hardly discernible nuclei and indistinguishable boundaries between hepatocytes. These alterations were more noticeable in the centriglobular regions of the sinusoids. In contrast to the former group characterised by dystrophic alterations only, the dystrophied parenchyma in this group also had focally disseminated nonreactive necrotic areas (Figure 3).

In rats sacrificed on day 28 of the experiment, the dystrophically altered liver parenchyma showed irregular necrotic areas differing in size and disseminated without any obvious pattern. These areas had no clear boundaries and blended areactively into the surroundings. Tissue and perivascular haemorrhagia in the liver parenchyma could be seen very often, especially in the proximity of necrotic foci (Figure 4).

The pathohistological findings in rat kidneys at 7-days were restricted to alterations affecting the renal tubules. The cytoplasm of the renal epithelial cells was granular and turbid while the nuclei were preserved and the borders between cells discernible on the major part. A small number of epithelial cells from cortical and subcortical regions showed vacuolated cytoplasm in the form of a number of PAS and Sudan III negative vacuoles whose fusion provided

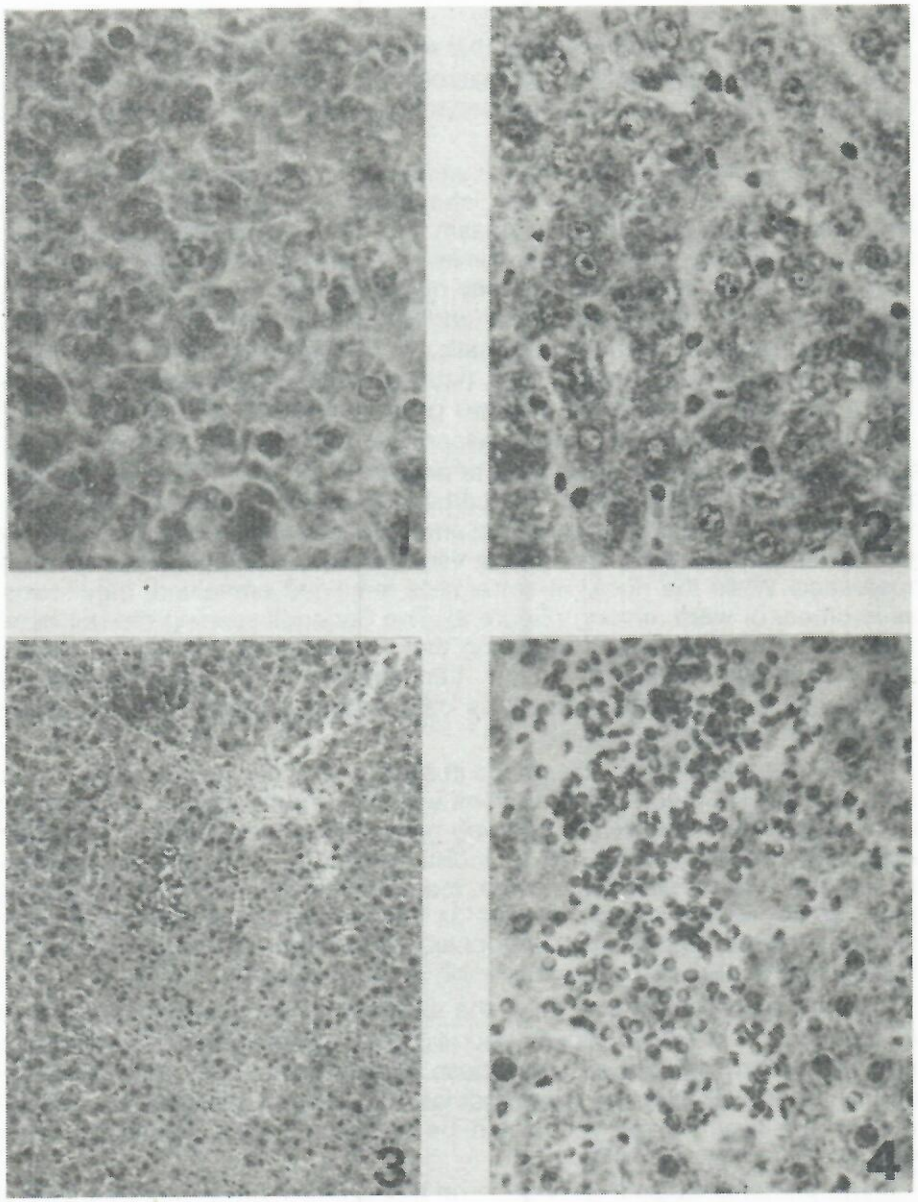

Figure 1. Liver. Granulated cytoplasm of the hepatocytes. Anisokaryosis. (HE x 252)
Figure 2. Liver. Foamy cytoplasm of the hepatocytes and the activation of Kupffer's cells. (Masson-Goldner x 252)
Figure 3. Liver. Areactive necrotic areas. (HE x 63)
Figure 4. Liver. Hepatocyte necrosis and extensive heamorrhagia. (Masson-Goldner x 252)

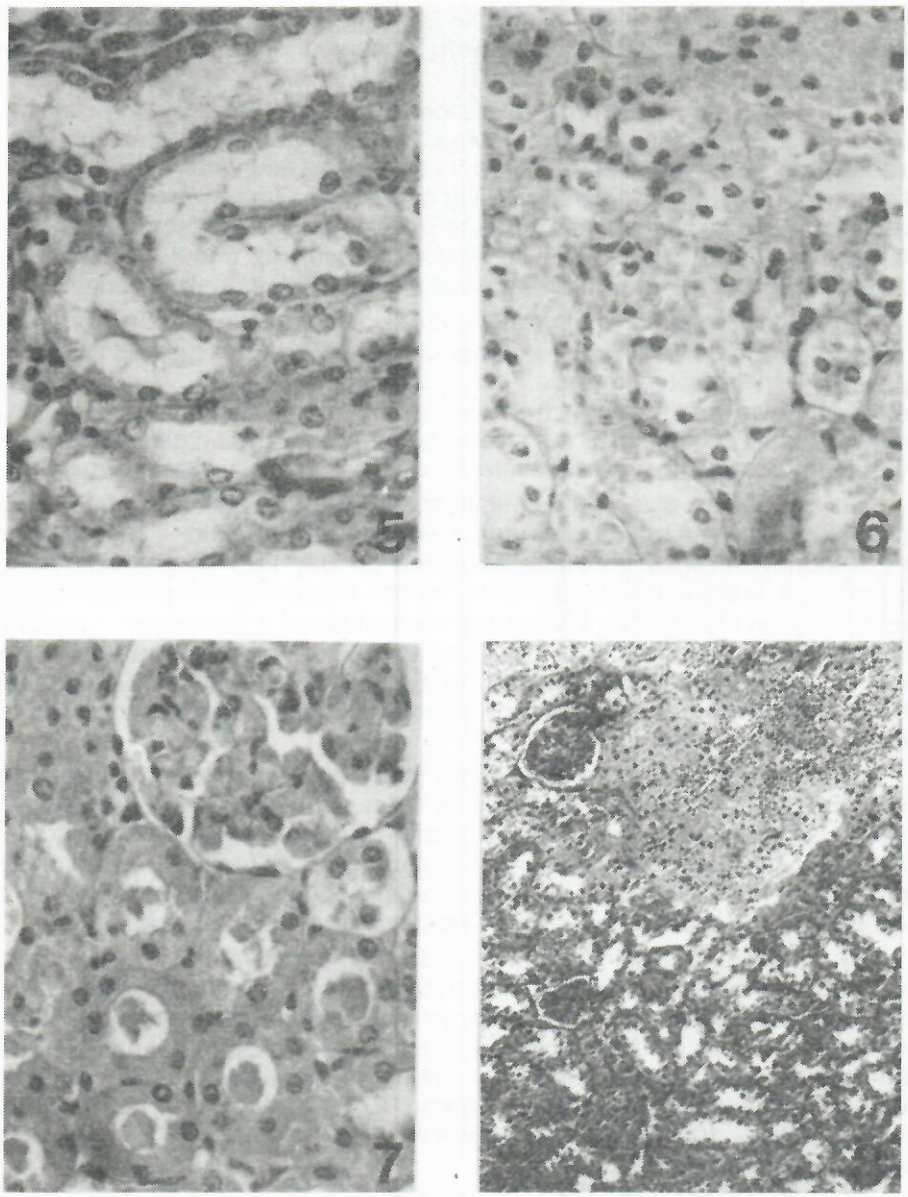

Figure 5. Kidney. Vacuolar netlike appearance of epithelial cells of the cortex. (HE x 252)
Figure 6. Kidney. Large number of epthelial cells of the proximal tubules without the nuclei. Damaged basal membranes of the tubulocytes. (HE x 252)
Figure 7. Kidney. Protein cylinders in the lumen of the proximal and distal tubules. (HE x 252)
Figure 8. Kidney. Necrotic haemorrhagic area in the cortex. (HE x 63)

the cells with a netlike outlook (Figure 5). The nuclei of these cells were either pyknotic or completely missing. In some collecting ducts, fresh cellular cylinders made of 3-4 cells fused together were observed. Their contours, however, remained distinguishable.

The most prominent renal alterations in animals sacrificed on day 14 affected the proximal tubules of the cortex where, in addition to coarsely granulated cytoplasm, a vacuolar and ballooning degeneration was seen. The nuclei of some tubulocytes were either missing or pyknotic. There were no clear boundaries between individual cells, and the basal membrane of some tubules had disintegrated, indicating the initial necrotic processes (Figure 6). The distal tubules were extended and often contained cylinders of cellular origin.

In 21-day treated animals, dystrophic and necrobiotic alterations assumed a greater proportion than in the former group. The cytoplasm was netlike and speckled with necrobiotic nuclei. No clear border could be seen between individual tubules, and their structure was indistinguishable, thereby aiding theformation of small necrotic foci. The structure of the surrounding glometules stayed preserved. Some renal tubules were filled with protein cylinders (Figure 7).

In addition to dystrophic alterations exhibited in different forms — from intracellular oedema to reticular and ballooning degeneration — the major finding in kidney sections from rats sacrificed on day 28 was characterised by necrotic foci. In the cortex, these foci were often minute but voluminous necroses could be seen also. Such necrotic regions contained a centralpart in which full homogenisation of the tissue had taken place, followed by the frequent appearance of fragmented necrotic mass and the finding of fresh erythrocytes. On the periphery, cellular debris could still be seen, mostly as fragmented or pyknotic nuclei (Figure 8).

The cardiac muscles of animals sacrificed on day 7 showed no alterations in contrast to day 14 when pale muscle fibres were seen either individually or assembled into small groups of fibres, with prominently granulated cytoplasm and no cleary distinguishable nuclei (Figure 9). The interstitium located in the immediate proximity of alterated fibres was moderately extended due to the accumulation of serous fluid but no increased cellularity was observed.

In addition to muscle fibres affected with parenchymatous dystrophy and markedly granulated cytoplasm, the myocardium of rats sacrificed on day 21 also contained cells with pale, homogenised and glassy cytoplasm, with no nuclei or with their pyknotic debris, suggesting a hyaline degeneration. These fibres, either separate or grouped in 2-3 fibre groups, were randomly distributed among unchanged muscle fibries, giving a patched up appearance of the muscle (Figure 10). Blood vessels were often hyperaemic. Occasional mild haemorrhagiae were seen also.

The areas affected with parenchymatous and hyaline dystrophy in the hearts of 28-day treated animals were greater and more clearly distinguishable than in the former group (Figure 11). The surroundings of these altered areas were characterised by the accumulation of serous fluid and mononuclear cellular elements seen as one or more small foci (Figure 12).

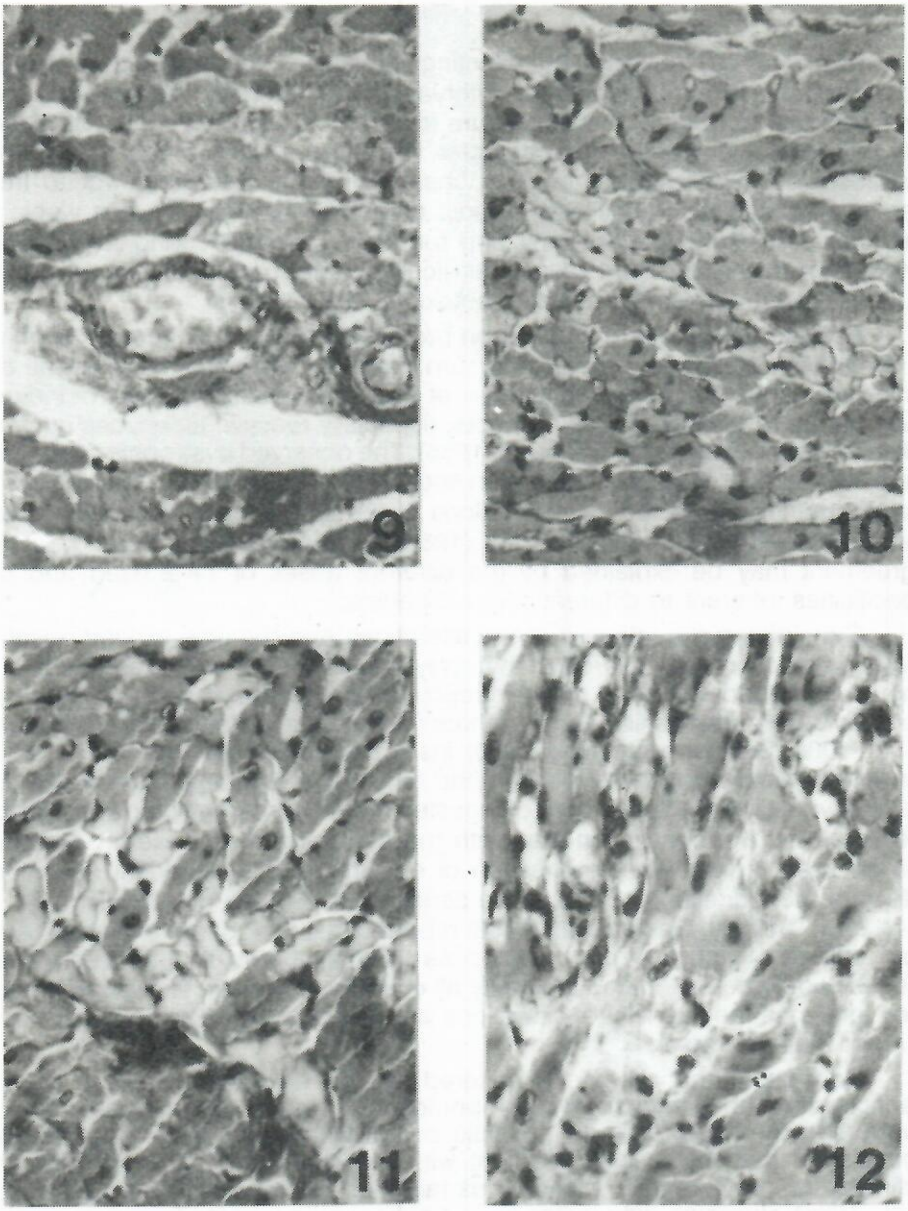

Figure 9. Heart. Granular cytoplasm of muscle fibres, without the nuclei. Accumulation of serous fluid. (HE x 252
Figure 10. Heart. Granular cytoplasm of muscle fibres. Some fibres without the nuclei; glassy appearance (HE x 252)
Figure 11. Heart. Hyaline appearance of the cytoplasm of some muscle fibres. (HE x 252).
Figure12. Heart. Mononuclear cellular infiltrate between altered muscle fibres. (HE x 386)

DISCUSSION

Pathohisological alterations affecting the rat liver, kidneys and heart were observed at all four experimental intervals. Their intensity differed and was proportionate to the length of exposure to T—2 toxin. Predominant alterations were dystrophic and necrotic in character. Circulatory disorders and proliferative manifestations were secondary. The character of the alterations varied from parenchymatous dystrophy as the most common finding in the first week, to vacuolar or ballooning dystrophy and further to necrotic changes that were often seen in the third but more specifically in the fourth week of exposure. Such findings indicate the strong cytotoxicity of T—2 toxin.

Besides its complex influence on protein synthesis, it is known that T—2 toxin affects fat peroxidation which in turn produces adverse effects on the cell which are either direct such as the loss of its membranous structure, or indirect such as the formation of toxic products, especially reactive aldehydes such as alkenes (Segal *et al.,* 1983; Camporti, 1985). The observed dystrophic alterations were attributable to disorderly water and protein metabolism. The Sudan III staining did not detected any alterations of the type of fatty dystrophy which was described by LIndenberg *et al.* (1985) or Glavits *et al.* (1989). This disagreement may be explained by the different doses of T—2 used and the specificities inherent to different animal species.

Considering the intensity of the alterations and the time of their onset in the examined organs, it is clear that they first affect the liver and kidneys. It should be added, however, that dystrophy in the kidneys is stronger, and that necrotic foci appear earlier. Many authors (Uraguchi and Yamazaki, 1978; Vanyi *et al.*, 1988; Sandor and Vanyi, 1990) insist on the liver as the central organ for detoxication owing to its location and function, i. e. its exposure to the first wave of virtually the total amount of ingested and absorbed mycotoxins. Hence, they come to the conclusion that both the initial and most severe alterations will be seen in liver. The explanation of our finding of substantial alterations affecting the kidneys, too, should be sought in the mode of administration of T—2 toxin. Namely, Gaines *et al.* (1966) reported that after parenteral administration, only 27.5% of the toxin would pass through the liver immediately while the rest would be distributed uniformly all over the organism. This would, therefore, influence the intensity of alterations and the order in which they will occur but not their character.

Comparing the alterations suffered by the different groups of rats, it transpired that their intensity was proportionate to the length of exposure, thus supporting the hypothesis od possible cumulative effects of T—2 toxin. This speculation may be in disagreement with the fact that T—2 toxin tends to disintegrate into less toxic compounds fairly fast (Bata et al., 1983; Johanson *et al.,* 1986). However, data on the limited ability of the liver for T—2 detoxication and its 80% decrease within 10 days (Vanyi *et al.*, 1988; Sandor and Vanyi, 1990), testify to the limited possibilities for T—2 degradation and, therefore, its possible cumulative action.

Eventually, it should be emphasized that during examination of T—2 toxin-effected alterations, especially those produced with low doses at short

exposure, possible postmortem alterations must be taken into account. The investigations by Rousseaux *et al.* (1990) highlighted the considerable problems that may be encountered while trying to discriminate between T—2 and postmortem alterations in some organs more than 6 hours after death.

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PATOMOROLOŠKE PROMENE U JETRI, BUBREZIMA I SRCU PACOVA TRETIRANIH T—2 TOKSINOM

SINOVEC SNEŽANA i JOVANOVIĆ M.

SADRŽAJ

Ispitivan je uticaj subletalnih doza T—2 toksina na razvoj i karakter patomorfoloških promena u jetri, bubrezima i srcu pacova. U tu svrhu pacovima Wistar soja svakodnevno je subkutano aplikovano 0,1XLD_{50} T—2 toksina. Životinje su sukcesivno žrtvovane 7., 14., 21 i 28. dana. Za bojenje parafinskih ili smrznutih isečaka tkiva korišćene su HE, PAS, trihromna po Masson—Goldner-u i Sudan III metoda.

U ispitivanim organima dominirale su promene distrofično-nekrotičnog karaktera, dok su cirkulatorni poremećaji i proliferativne manifestacije bili u drugom planu. Patomorfološke alteracije u jetri, bubrezima i srcu, ustanovljene u svim ogledним grupama bile su direktno zavisne od vremena izloženosti dejstvu T—2 toksina. Najranije i najmarkantnije promene uočene su u bubrezima što je objašnjeno parenteralnim unošenjem T—2 toksina.