IMMUNOMORPHOLOGICAL ANALYSIS OF LONG-TERM POISONING WITH SMALL DOSES OF THE HERBICIDE SIMAZINE

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Intensive application of chemistry to production and everyday life has led to considerable pollution of the human environment. About 10,000 highly active substances are present in the biosphere. The most dangerous of them, in the opinion of the World Health Organization, are pesticides. Since they may enter the human and animal body with food products, pesticides can cause lasting changes in the immune and other systems [2, 4]. The greatest risk to man or animals is presented by long term contact with small doses of pesticides. From the economic point of view one of the most important groups of herbicides is that of the symmetrical triazines, one representative of which is simazine [3].

The aim of this investigation was to analyze immunomorphological processes in animals during long-term exposure to small doses of the herbicide simazine.

EXPERIMENTAL METHOD

Experiments were carried out on 80 male Wistar rats. For 7 months the animals were given simazine daily by gastric tube in a dose of 0.001 LD_{50}. On the last day of administration of the toxic chemical and on the 14th and 21st days after its discontinuation, animals were taken out of the experiment by decapitation. The experiment was accompanied by a corresponding control.

In all the animals the peripheral blood leukocyte count and leukocyte formula, the absolute number of lymphocytes, the number of T, B, and "null" lymphocytes [5], the lysozyme level, complement titer, titer of heterophilic agglutinins, myeloperoxidase activity of the leukocytes [6], phagocytic activity of the neutrophils relative to *Staphylococcus aureus,* the bactericidal activity of the serum [1], and the absolute and relative (compared with the mass of the right kidney) mass of the thymus were determined. Pieces of thymus were taken for histological investigation, fixed in 10% neutral formalin, and sections were stained by the usual methods. The relative area of the cortex and medullary layer, parenchyma, and stroma was determined morphometrically in the thymus by means of Weibel's planimetric grid Coefficients C (ratio of area of cortex to area of medullary layer) and P (ratio of area of parenchyma to area of stroma) were calculated.

Pieces of thymus with a volume of 1-2 mm^3 for electron microscopy were fixed in 2.5% glutaraldehyde in phosphate buffer, pH 7.4, postfixed in 1% OsO_4 solution, and embedded in a mixture of epoxide resins. Semithin and ultrathin sections were stained with a saturated aqueous solution of uranyl acetate and lead citrate, semithin sections with toluidine blue and pyronine G. Ultrathin sections were examined in ÉVM-100 and JEM-100 EX-11 electron microscopes.

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TABLE 1. Effect of Simazine on Factors of Immune and Nonspecific Resistance (M ± m)

Parameter	Control	Administration of simazine for 7 months	21 Days after discontinuation of simazine
Lysozyme level, optical density units	11,62±1,07	25,86±2,63	26,49±0,36
Titer of complement	0,027±0,003	0,033±0,008	0,073±0,003
Titer of heterophilic agglutinins	0,156±0,031	0,083±0,053	0,104±0,013
Phagocytic number, %	59,3±3,94	35,3±3,16	42,5±3,1
Phagocytic index	7,01±0,55	3,97±0,41	6,03±0,64
Bactericidal activity of serum, %	89,59±2,86	75,51±3,22	80,92±3,68
Myleoperoxidase activity of leukocytes (mean cytochemical coefficient)	2,32±0,17	1,39±0,11	1,65±0,14
Number, %, of:			
T Lymphocytes	57,5±0,99	26,5±1,43	19,5±0,67
B Lymphocytes	34,17±0,79	57,17±1,78	70,0±1,65
"Null" lymphocytes	8,33±0,56	16,33±1,63	10,5±1,36

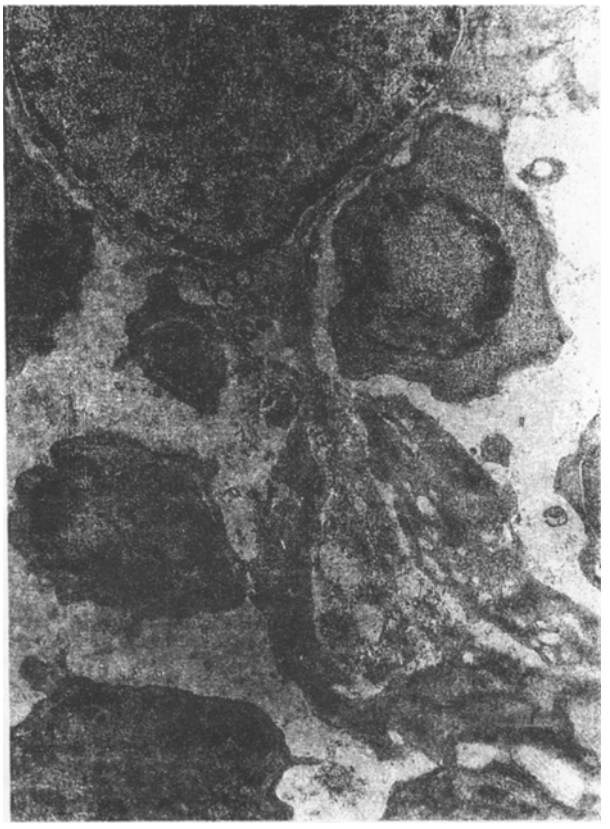

Fig. 1. Swelling of mitochondria with translucent matrix, increase in number of filaments in cytoplasm of reticuloendothelial cells with complete rupture of intercellular junctions between them. 25,000×.

EXPERIMENTAL RESULTS

Administration of simazine for 1 month was accompanied by elevation of the lysozyme level and depression of other parameters of nonspecific resistance (Table 1).

The lysozyme level 21 days after discontinuation of simazine was unchanged and the remaining parameters were still below their level in the control group Differences are significant. By counting the principal subpopulations of lymphocytes, an increase was found in the number of B lymphocytes and, to some extent also, of "null" lymphocytes, against the background of a sharp fall in the number of T lymphocytes. This pathological ratio was even more marked on the 21st day after discontinuation of simazine. Differences are significant.

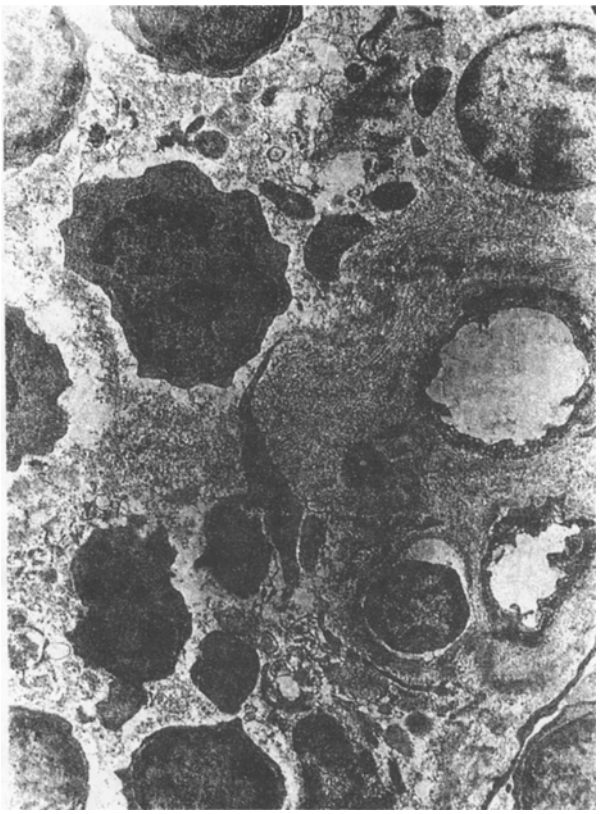

Fig. 2. Sharp increase in thickness of connective tissue layer around vessels due to accumulation of collagen fibers, among which remnants of fibroblasts are visible. 25,000×.

The absolute and relative mass of the thymus at the end of the 7th month of exposure to the toxic chemical was reduced and had not returned to the initial level 3 weeks later. Histologically, the lobules of the thymus were reduced in size and their division into layers was difficult to detect. The cortex was significantly reduced in thickness, with small foci of low density of thymocytes, resembling the "starry sky" phenomenon The number of mitoses and blast cells was much smaller than in intact animals The reticuloendothelial cells were swollen, especially in the cortical layer In their cytoplasm the quantity of PAS-positive material was reduced.

The ratio of the area of the cortex to the area of the medulla showed a marked decrease. Thymocytes were more compactly arranged in the medulla than in the control animals, and solitary large Hassall's corpuscles were found here A decrease in the relative area of the thymic lobules was determined morphometrically. On the 21st day after discontinuation of simazine the ratio of the cortex to the medulla of the thymic lobules remained low, although in some fields of vision it was close to that in the control group.

The study of serial semithin tissue sections from the thymus of animals subjected to long-term poisoning by small doses of simazine revealed an increase in the volume of stromal cells in the form of thickening of the capsule and of the connective-tissue septa radiating from it. Some arteries, present here, were in a state of spasm. Among other changes in the thymus, a decrease in the number of cells in the cortical layer and vacuolation of the pale reticuloendothelial cells must be noted.

Electron-microscopically, most reticuloepithelial cells were distinguished by degenerative changes. Cells with only slight degenerative changes were characterized by widening of the elements of the endoplasmic reticulum, partial rupture of junctions in zones of contact between processes. Cells with moderate changes were distinguished by swelling of the mitochondria with translucency of the matrix, an increase in the number of filaments, and complete rupture of intercellular junctions (Fig. 1). Among the reticuloepithelial cells described above there were some "nurse" cells in a state of necrobiosis (large vacuoles in their cytoplasm, edema of the cytoplasm, partial destruction of the cytoplasmic membranes).

Considerable pathology was found in the microvessels of the thymus (cortex and medulla), in the form of enhanced indentation of the outline of the endotheliocytes in the form of invaginations and microvilli, with very high pinocytotic activity The width of the cytoplasm of the endotheliocytes varied greatly with the presence of individual areas that were extremely thin. The connective-tissue layer around the vessels was sharply increased in volume due to the accumulation of collagen fibers, among which remnants of fibroblasts were visible (Fig. 2).

In the medullary layer, moderately severe pathology of macrophages (vacuolation of elements of the endoplasmic reticulum, a decrease in the number of thymocytes, and vacuolation of the cytoplasm of the reticuloepithelial cells) were found.

Thus the results of this dynamic immunological and comprehensive morphological study of the thymus tissue of animals exposed for long periods to small doses of the herbicide simazine, widely used in agriculture, yield evidence of the development of a secondary immunodeficiency state, with damage mainly affecting the cellular component. One reflection of the disturbance of maturation of T lymphocytes is the change in morphology of the thymus with reduction in volume of the thymic lobules, death of some thymocytes, and dystrophy of reticuloepithelial cells. Injury to and disturbance of junctions between highly specialized thymus cells lie at the basis of the developing pathology of the immune system. Parallel with death of the parenchymatous cells, an increase in volume of the stromal cells was observed, accompanied by widening of the connective-tissue formations and accumulation of coarsely fibrous structures around the vessels, sometimes leading to their total obliteration.

In our opinion, the immunodeficiency state revealed by this investigation is based on the direct toxic action of simazine on parenchymatous structures of the thymus (reticuloepithelial cells, thymocytes).

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