Effect of Dimethylsulfate on the Structure and Cytoarchitectonics of Rat Thymus

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Changes of structure and cell ratios of different functional zones in the thymus under conditions of long-term dimethylsulfate administration at 0.1 and 2.0 mg/m³ on the organism are studied morphometrically on histological preparations from Wistar rats. Cyclic reactions of the thymus to the toxic substance are found to be due to compensatory processes. It is shown that the nature of the reaction does not depend on the concentration of toxic substance but that the extent of changes is a function of the dimethylsulfate concentration.

Key Words: *thymus; cytoarchitectonics; dimethylsulfate*

It is well known that the action of an array of ecological factors, including toxic substances, intereferes with immunological homeostasis [2,7]. In this connection the alterations in the thymus for the development of intoxications are of great interest, especially in chronic action. In view of the wide industrial application and extremely high toxicity of dimethylsulfate (DMS), we undertook an experimental study of the structure and cell composition of the thymus from rats exposed to DMS.

MATERIALS AND METHODS

The study was carried out on 72 male Wistar rats aged from 3 to 3.5 months. The animals were daily exposed to a 4-h inhalation of DMS for 14 days. Control tests were performed in parallel. The animals inhaled air saturated with DMS vapor in a concentration of 0.1 mg/m³ (the maximum permissible concentration (MPC) of DMS in the air of a work zone) and 2.0 mg/m³. The second concentration was chosen to imitate the emergencies and departures from MPC levels common in industry [1]. The material was collected after 2, 4, 8, and 14 exposures and fixed in Carnoy fluid. Paraffin sections were stained with hematoxylin-eosin, azure II-eosin and after Van Gieson. Morphometric analysis was performed on histological sections with subsequent statistical processing of the data.

RESULTS

It was established that DMS in a concentration of 0.1 mg/m³, which is equal to the MPC, in exposure for 2, 4, 8, and 14 days results in changes of various nature not only in the structure but also in the cytoconstruction of the thymus. For example, we noted an increase of the area of the cortical and, particularly, medullary zone on histological sections after 2-day inhalation of DMS. The explanation may lie in an alteration of lymphocyte migration out of the thymus at this time point of the experiment, this being confirmed by elevation of the percentage of small and medium lymphocytes in the cortical and medullary zone (Fig. 1, *a*, *c*), attesting to lymphocyte accumulation in the thymus. In addition, we noted enhanced processes of cell destruction and reutili-

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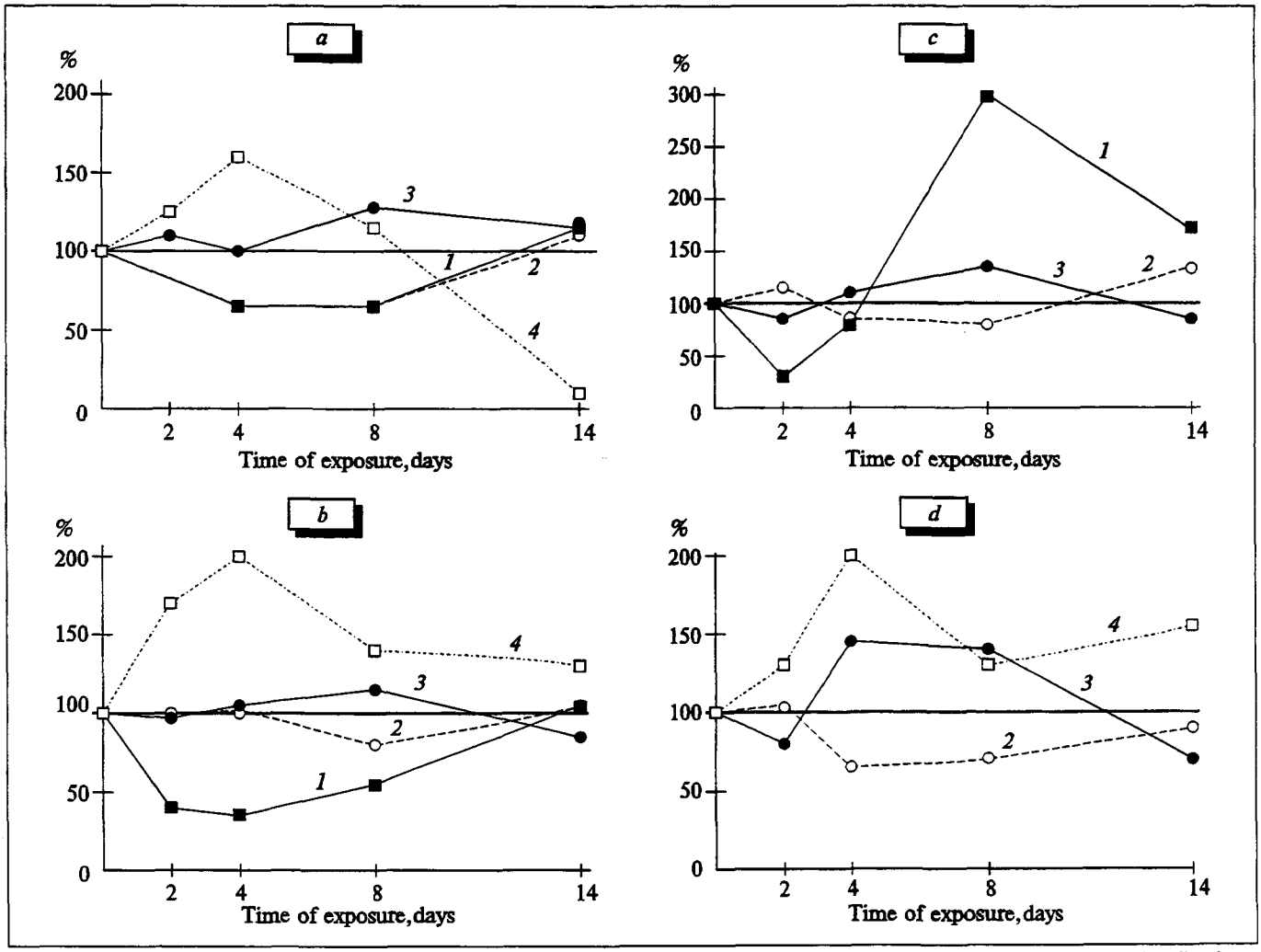

Fig. 1. Time course of changes in the percentage content of cells in cortical zone (*a*, *b*) and medullary zone (*c*, *d*) of rat thymus related to the control (100%) for exposures of different duration and DMS concentrations of 0.1 mg/m^3 (*a*, *c*) and 2.0 mg/m^3 (*b*, *d*). *1*) mitotic cells; *2*) medium lymphocytes; *3*) small lymphocytes; *4*) destroyed cells.

zation in the thymus: an increase of the relative number of destroyed lymphocytes and macrophages (by 23 and 25%, respectively, in the cortex and by 36 and 112% in the medullary zone, $p<0.05$). Along with this, at this time a lowered percentage of blasts, large lymphocytes, and mitotic cells was found in all functional zones (Fig. 1, *a*, *c*), which may be interpreted as an inhibition of lymphopoiesis. A decrease of the relative area of thymic (Hassall's) bodies, which in the view of some authorities [6] attests to a reduced functional activity, may be added to the above-described array of changes.

A diminution of the area of the cortical zone occupied on the histological section and an increase of the area of the capsule and interlobular connective tissue in comparison with the control were found after DMS inhalation for 4 days at the minimal concentration (0.1 mg/m^3) tested. Chains of mast cells are found in the interlobular septa.

The vascular bed of the medullary zone at this time occupied on the histological section an area much larger than in the control, this being morphologically manifested as a widening of the vascular lumen and vessel congestion. Devastation of the perivascular zones was also noted, testifying to intensified migration processes (Fig. 2).

The changes in thymus cytoconstruction show individual peculiarities in each functional zone. In the subcapsular zone an increase of the relative number of blasts, large lymphocytes, and mitotic cells was noted after 4 days of DMS inhalation (0.1 mg/m^3), evidently indicating enhanced lymphopoietic function. At this time the cortical zone showed, together with a significant percentage rise of lymphoblast number, a drop in the relative number of mitotic cells (Fig. 1, *a*). The percent content of medium-size and small lymphocytes was similar to that in the control. Taken together, these observations suggest an impairment during this period of

lymphocyte proliferation and differentiation in the cortical zone of the thymus. In the medullary zone epithelioreticulocytes were hypertrophic (Fig. 3, *a*) and a higher percentage of them was noted as well as marked growth of the area of thymic bodies subsequent to DMS inhalation for 4 days. According to the literature [9,10], hypertrophy of thymic epithelial cells and an increase of the number of Hassall's bodies are signs of boosted secretory activity of the thymus. On the other hand, activation of the epithelial structures in the thymus is a manifestation of the defense reaction of the organism [4,11].

As a result of long-term (8 days) exposure to DMS in a concentration of 0.1 mg/m^3 the number of mitotic cells as well as of blasts and large lymphocytes diminished in the subcupsular zone of the thymus. The deep layers of the cortical zone demonstrated the same changes in cell ratios as those in the subcapsular one (Fig. 1, *a*). The percentage of mitotic cells increases while that of blasts and large lymphocytes decreases following 8-day DMS (0.1 mg/m^3) inhalation. Along with these changes, in all functional zones of the thymus the relative number of small lymphocytes and macrophages was found to increase while that of medium lymphocytes and cells with destructive alterations decreased (Fig. 1, *a*, *c*).

Capsule and interlobular connective tissue as well as the vascular bed of the medullary zone were increased in volume on histological sections from animals after 14-day inhalation of DMS (0.1 mg/m^3). In addition, the area of thymic bodies increased (Fig. 3, *b*). In the subcapsular zone at this time the percentage of mitotic lymphocytes was lower compared to the control, whereas the number of blasts (which act as a reserve for lymphocyte differentiation) was significantly higher [13,14]. At the same time, in the deep layers of the cortical and medullary zones a high percentage of blasts, large lymphocytes, and mitotic cells (Fig. 1, *a*, *c*) was found, evidently as a compensatory boost for lymphopoiesis in these zones in response to its lowered level in the subcapsular zone of the thymus. Hand in hand with the alterations described above, in all functional zones of the thymus a decrease of the percentage of destroyed cells was revealed against the background of a high number of macrophages (Fig. 1, *a*, *c*) upon exposure for 14 days. In the medullary zone, unlike the other thymus regions, the relative number of epithelioreticular cells is increased along with the appearance of hypertrophic forms.

Thus, the structural-functional alterations in the thymus induced by DMS are related to manifestations of the main function of the central organ of the immune system such as proliferation, differentiation, and replenishment of the T-cell population at the periphery, as well as secretion of factors regulating these processes. In assessment of the thymus reaction, the stressor action of DMS should be taken into account, namely the probable elevation of the blood corticosteroid level (especially at thr early stages of the experiment) with a consequent reinforcement of destructive processes

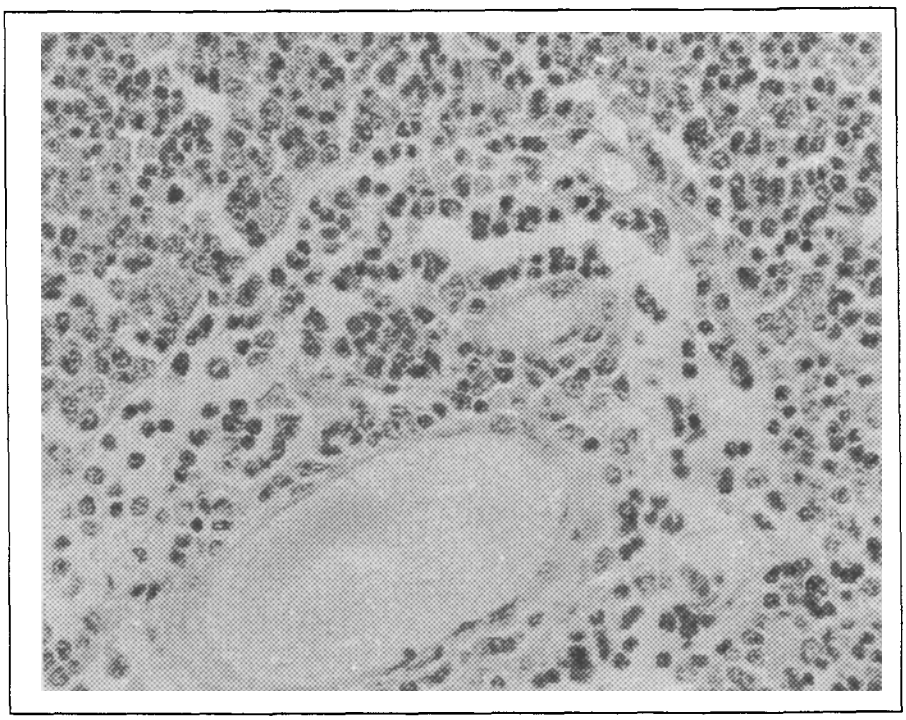

Fig. 2. Devastated perivascular region in medullary zone of rat thymus following 4-day exposure of DMS in a concentration of 0.1 mg/m^3. ×400. Here and on Fig. 3: staining with azure II–eosin.

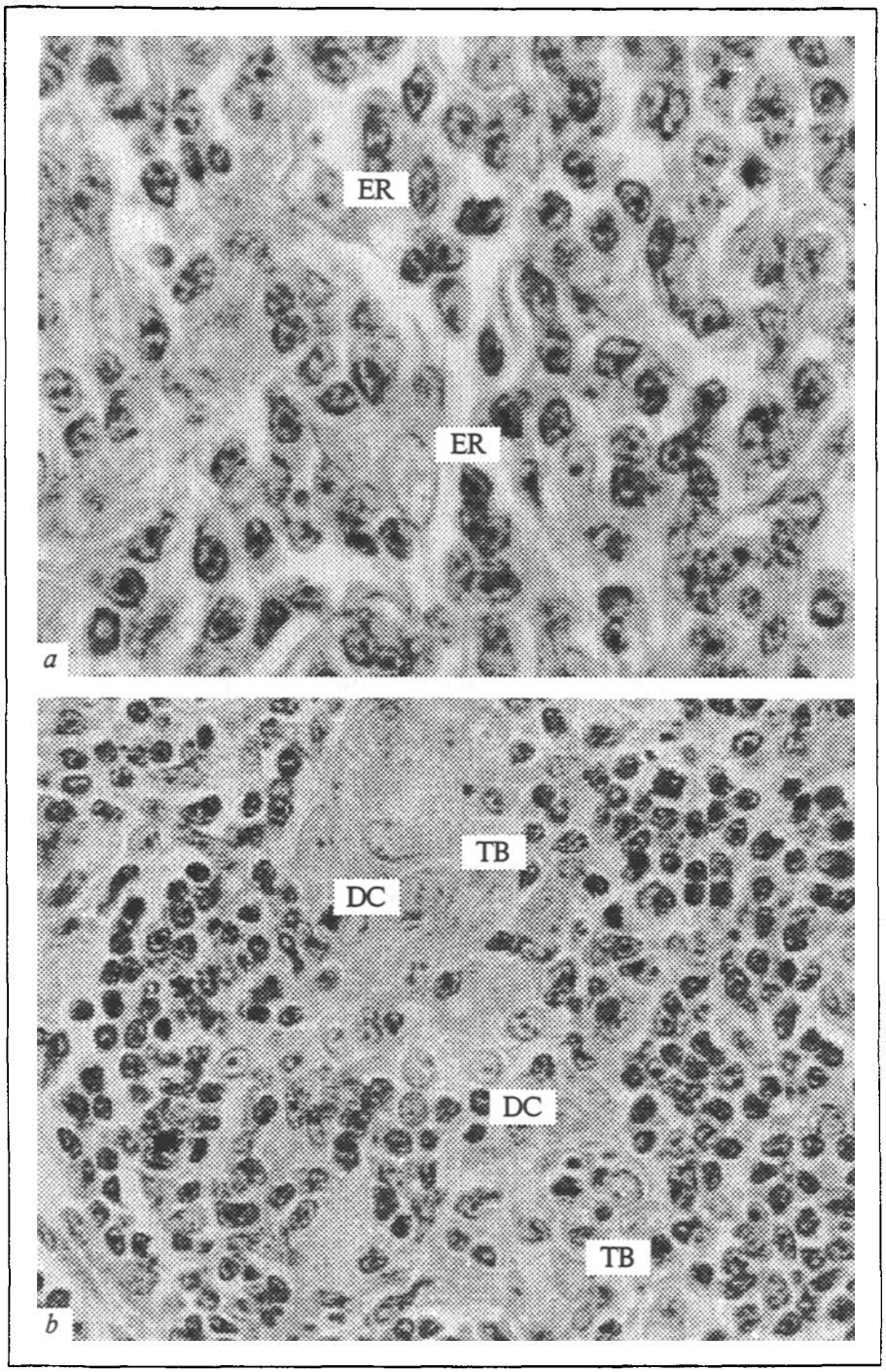

Fig. 3. Medullary zone of thymus after exposure of DMS in a concentration of 0.1 mg/m³. *a*) hypertrophic epithelioreticulocytes (*ER*) after 4-day inhalation. ×1000; *b*) a large thymic body (*TB*), infiltrated with destroyed cells (*DC*) after 14-day inhalation. ×600.

and intensification of the macrophagal and epithelioreticulocyte reaction in the thymus [12].

The results of our study show that for a greater strength of the irritant (DMS in a concentration of 2.0 mg/m³) qualitatively similar but more pronounced changes take place in the thymus. It should be noted that the pattern of development of the thymus reaction to toxic substances as a function of exposure duration is similar for the high dose and the minimal concentration tested (Fig. 1).

Thus, after 2-day exposure to DMS in a concentration of 2.0 mg/m³ a certain increase of the cortical and medullary areas was noted, the relative nature of which is explained by disturbances of the lymphocyte outflow due to drastic vasoconstriction. The area of thymic bodies in this case is diminished.

More prolonged exposure to DMS (4 or 8 days) leads to a decrease of the cortical zone area on histological sections and to a decline of the percentage of mitotic and blast cells in this region

as well as to lymphocyte depletion due to a marked decrease of the number of medium cells (Fig. 1, *b*). On the other hand, the area of the medullary zone increases at this time. Thickening of the capsule occurs due to its loosening and edema, as well as congestion of medullary vessels.

Alterations of the cytological profile of the thymus as a result of exposure to a massive concentration (20 times higher than the MPC) attest to reduced mitotic activity and to stepped-up destruction. Delymphatization of the cortical layer occurs. A steep drop in the relative number of blasts, large lymphocytes, and dividing cells is observed in all structures throughout the experiment (2, 4, 8, and 14 days). An exception is the medulla, where on the 4th day of DMS inhalation there is an increase in the number of mitotic cells, evidently the result of the increased number of blasts and large lymphocytes in this zone during the 2nd day of the experiment.

Thus, along with published data [3,5,8], our findings suggest that the structural changes described in the thymus are of a nonspecific nature and attest to a depressed immune function.

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