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COMPARATIVE BIOCHEMICAL AND ELECTRON-MICROSCOPIC STUDY OF MEMBRANE DAMAGE TO THE ENDOPLASMIC RETICULUM BY A CHEMICAL CARCINOGEN IN VIVO AND IN VITRO

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It has recently been shown that the unfavorable action of chemical environmental pollutants, including nitroso compounds, has a membrane-damaging effect [5, 7], which is characterized by increased permeability of biomembranes coupled with inhibition of activity of specific enzymes contained in intracellular structures. The study of the membrane-damaging effect on experimental models in vivo and in vitro, using modern biochemical, morphological, electron-microscopic, and other methods of investigation, could shed light on some of the general principles governing metabolic changes and could provide an approach to the solution of an urgent problem of medico-biological importance in the field of hygiene, namely rapid testing of biological effects.

This paper gives the results of a comparative biochemical and electron-microscopic study of the membrane-damaging action of the chemical carcinogen N-nitrosodimethylamine (NDMA), as a result of exposure in vivo and in vitro.

EXPERIMENTAL METHOD

Experiments in vivo were carried out on noninbred mature male albino rats weighing 180-250 g, kept on a standard diet. Tests were carried out during development of the biological effect of NDMA, 12, 24, 48, and 72 h after administration of the carcinogen by the intragastric route (30 mg/kg, 0.75 LD_{50}).

The experiments in vitro were carried out on a culture of human amnion cells, grown in flasks on medium 199. On the 2nd day of culture, during monolayer formation, nutrient medium containing activated NDMA was added to the cell culture in a dose of 2.5, 5, and 10 mg/liter. Activation of NDMA and subsequent treatment of the cellular material followed the description given previously [8].

Activity of the following membrane-bound enzymes of the nedoplasmic reticulum was determined in a liver tissue homogenate and in the culture of human amnion cells: UDP-glucuronyltransferase (UDP-GCT), catalyzing the reaction of the second phase of xenobiotic metabolism, and glucose-6-phosphatase (G-6-P), which plays an important role in glycogenolysis and gluconeogenesis; the methods used were described in [4, 5].

Material for electron-microscopic study was fixed in a 2% solution of glutaraldehyde in phosphate buffer, pH 7.4, followed by fixation in 1% OsO_4 solution. Sections were stained with an aqueous solution of uranyl acetate and with lead hydroxide by Reynolds' method. The sections were examined in the UMV-100 electron microscope with instrumental magnification of between 10,000 and 25,000.

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EXPERIMENTAL RESULTS

A characteristic feature of development of the biological effect of NDMA, both in vivo and in vitro is a decrease in activity of the membrane-bound enzymes of the endoplasmic reticulum chosen for study. For instance, 24 h after intake of NDMA by the animals in a dose of 30 mg/kg, UDP-GCT activity fell to 1.25 ± 0.16 μmole/min/g tissue (on average by 53%, $P < 0.001$), and G-6-P activity fell to 5.9 ± 0.5 μmole/min/g liver tissue (on average by 39%, $P < 0.01$). A similar trend of the change in activity of these enzymes also was discovered in experiments in vitro. However, in this case, a significant fall in enzyme activity was observed at later stages of the investigation (after 96 h). For instance, after administration of the carcinogen in a concentration of 5 mg/liter, UDP-GCT activity reached 0.19 mmole/mg protein, or 45% of its activity in intact cells. Incidentally, the fall in total G-6-P activity (determined with the aid of the nonpolar detergent Triton X-100) was accompanied by a significant increase in its free activity (not bound with membrane structures) on average by 60%. Such a combination of increased permeability of endoplasmic reticulum membranes and inhibition of activity of the enzymes located in it is the most significant manifestation of structural and functional disturbances in the membranes [7].

Other evidence of dependence of the functional state of the membranes of the endoplasmic reticulum on their structural integrity is given by the close correlation found between changes in G-6-P activity and the state of the lipid components of the microsomal membranes [2]. The decrease in UDP-GCT activity indicates delay in the formation of water-soluble conjugates and,

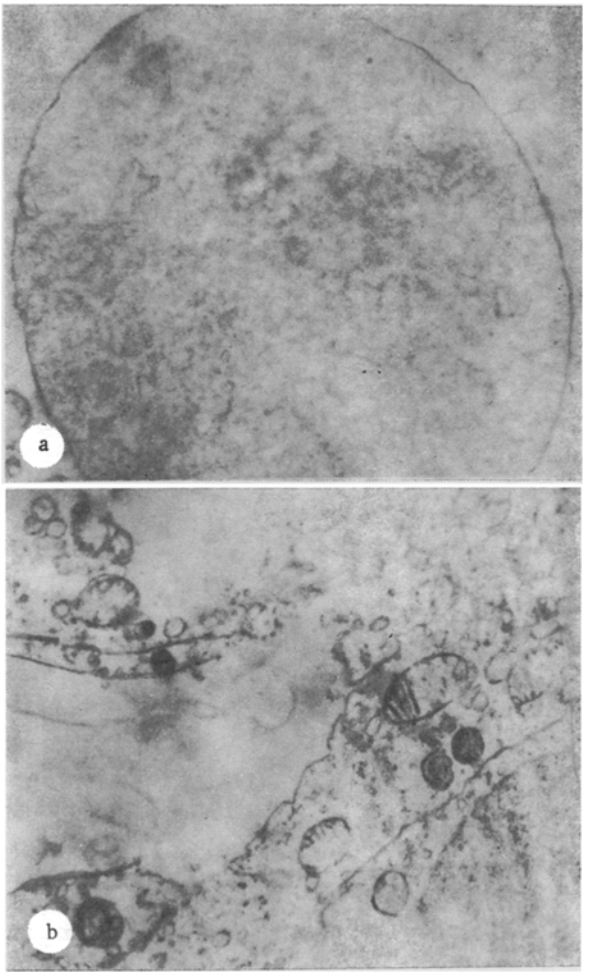

Fig. 1. Damaging action of NDMA on intracellular structures: a) edema of cytoplasm and nucleus (latter enlarged); b) destruction of mitochondrial cristae. Stained with aqueous solution of uranyl acetate and lead hydroxide by Reynolds' method, 10,000×.

consequently, delay in their elimination from the body [3]. The study of biological equivalence — an important characteristic for the establishment of hygiene regulations for chemical compounds, also revealed quantitative dependence of the change in G-6-P activity on the intensity and duration of exposure in vivo and in vitro [6].

Changes in activity of the membrane-bound enzymes of the endoplasmic reticulum studied were accompanied by morphological and functional changes in the cells and intracellular structures. For instance, under the influence of NDMA in vivo the development of foci of necrosis and destruction of hepatocytes and changes in activity of the reticuloendothelial system were observed [1]. The results of the electron-microscopic investigation of the cell components in vitro also indicated marked changes at the level of intracellular structures. Vacuolation of the cytoplasm and the presence of numerous pinocytotic vesicles and lysosomes were observed (Fig. 1a). Changes in the mitochondria were accompanied by swelling and widening of the internal septa and disorientation of the cristae (Fig. 1b). The endoplasmic reticulum exhibited considerable polymorphism and, in some cases, the integrity of the outer membrane was disturbed.

Among the general features of manifestation of the membrane-damaging effect of NDMA both in vivo and in vitro a reduction of activity of the membrane-bound enzymes of the endoplasmic reticulum (UDP-GCT and G-6-P) may thus be included, and it is accompanied by marked changes in the intracellular structure and disturbance of the integrity of their membranes.

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EFFECT OF ASCORBIC ACID ON FREQUENCY OF COLONIES RESISTANT TO COLCHICINE AND METHOTREXATE

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Data on the mutagenic and carcinogenic action of ascorbic acid, or vitamin C, are numerous [6, 10] but highly contradictory. These contradictions are largely linked with differences in the choice of test systems, the number of animals used for in vivo tests, the doses chosen, and the schedule and site of administration of the compound [6]. Tests in vitro have shown that degradation products of vitamin C formed in aqueous solutions in the presence of Cu^{++} or Fe^{+++} ions possess mutagenic activity [13]. On the other hand, prevention of the mutagenic action of nitrosamines by ascorbic acid also has been reported [13].

Experiments to determine the carcinogenicity of vitamin C both in vivo and in vitro have been undertaken recently in many laboratories. Investigations on mice [14] and rats (cited in [14]) have shown that high pharmacological doses (over 400 mg) are not carcinogenic, and that with an increase in concentration of the substance the time of appearance of tumors in-

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