ELECTRON-MICROSCOPIC INVESTIGATION OF THE EFFECT OF BORIC ACID ON THE SEMINIFEROUS TUBULES OF ALBINO RATS

A. A. Silaev, A. A. Kasparov,
V. V. Korolev, and V. V. Nebstrueva

UDC 612.617.1.014.46:546.27-32]-087.45

Boric acid was given by mouth in a dose of 1 g/kg body weight daily for 2 weeks to noninbred albino rats. Changes were found in the nuclei and cytoplasm of the spermatocytes and spermatids in the early stages of their formation. The appearance of many multinuclear cells with an even and odd number of nuclei (from 2 to 10 or more) was observed. The formation of these multinuclear cells can probably be explained by the action of the boric acid on the prolonged processes of meiotic division of cells of the spermatogenic epithelium (spermatocytes, spermatids).

KEY WORDS: *spermatocytes; spermatids; multinuclear cells; boric acid.*

One of the compounds with a specific action on the male reproductive system is boric acid [5, 6, 8], which is used not only in medicine but also in the chemical and glass industries and in nonferrous metallurgy. There is experimental evidence [5, 6] of the development of functional and morphological disturbances in the testes of noninbred albino rats following oral administration of boric acid in a dose of 1 g/kg for 2 and 4 weeks or after its inhalation in concentrations 10 and 50 mg/m^3 for 4 months. The morphological changes are expressed as a decrease in the index of spermatogenesis, an increase in the number of tubules with desquamated spermatogenic epithelial cells, an increase in the number of spermatogonia, and an increase in the number of destructive forms of spermatozoa. One result of these changes is a sharp decrease in fertility, or even total sterility of the experimental animals. Atrophy of the spermatogenic epithelium of the testes is observed. At the same time, multinuclear structures can be seen.

To clarify the changes observed in the spermatogenic epithelium following exposure to boric acid an electron-microscopic investigation was undertaken.

EXPERIMENTAL METHOD

A solution of boric acid in a dose of 1 g/kg was given by mouth for 2 weeks to 12 sexually mature male albino rats; six rats served as the control. Pieces of the testes for investigation were embedded in Araldite. Ultrathin sections were examined in the electron microscope. A parallel series of histological sections was cut for a survey study at the ordinary light-optical level: These sections were stained with hematoxylin—eosin and toluidine blue.

EXPERIMENTAL RESULTS

In most tubules, besides normal spermatogenic cells, cells were seen with a ring-shaped nucleus with the almost total absence of chromatin from its center, and with vacuolation and granulation of their cytoplasm. It can tentatively be suggested that these cells were spermatids in the early stages of formation. In some of the convoluted tubules the number of layers of cell associations at certain stages of spermatogenesis was reduced. In some

Laboratory of Electron Microscopy and Department of Work Hygiene, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 4, pp. 496-499, April, 1977. Original article submitted November 12, 1976.

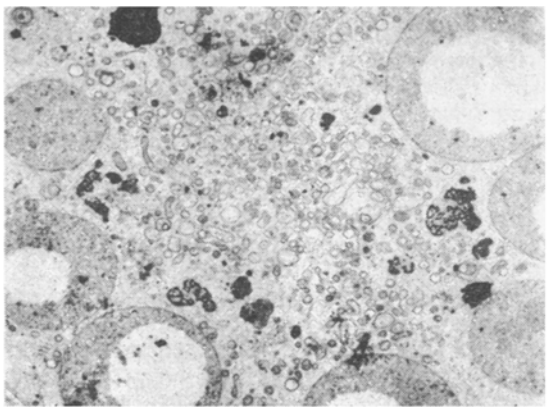

Fig. 1

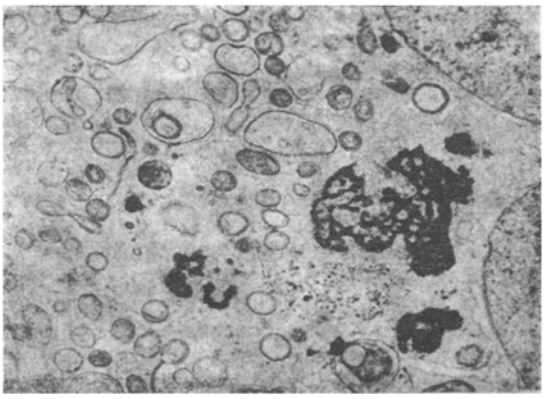

Fig. 2

Fig. 1. Multinuclear cell with seven nuclei (2300×).

Fig. 2. Cytoplasm of multinuclear cell: Myelin figures and chromatoid bodies can be seen (11,600×).

tubules, the lumen of which was appreciably reduced in diameter, germinative cells were completely absent and only sustentacular cells still remained.

In all the experimental animals multinuclear formations, lying both freely in the lumen of the seminiferous tubules or among the spermatogenic epithelium, were found in most of the seminiferous tubules, even if the structures of the germinative epithelium were relatively intact. The appearance of these cells in the seminiferous tubules in rats has been observed by other workers following exposure to chemicals with a selective gonadotropic action, such as ethylenimine [2], lead [1], etc.

Among the spermatogenic cells many type 1 spermatocytes, and also spermatids in the early stages of formation, with severe degenerative changes in the cytoplasm and nucleus, exhibited as chromatolysis and vacuolation, were found electron microscopically among the spermatogenic cells. Similar changes were observed in the structure of the multinuclear cells. The multinuclear cells were distributed both among the germinative cells and also freely in the lumen of the seminiferous tubules, surrounded by desquamated spermatogenic cells. Their cytoplasmic membrane still remained in contact to some extent with the sustentacular cells. Under normal physiological conditions definite means of communication exist between spermatogenic and sustentacular cells, in the form of close contact between the cytoplasmic processes of the latter with the germinative cells [7].

The binuclear cells located in the basal part of the seminiferous tubules were surrounded by cytoplasmic processes of sustentacular cells and were in contact with other cells. In many cases these contacts were completely absent, especially in cells with many nuclei (over four).

The multinuclear cells contained different numbers of round nuclei, mostly odd numbers: 7, 9, and 11 (Figs. 1 and 2). The nuclear membrane was intact over its whole length, and the perinuclear space was irregularly widened. Chromatin was either diffusely distributed in the nucleus or localized as a narrow band along the inner surface of the nuclear membrane. In some multinuclear cells, in which the nuclear membrane was intact, only "ghosts" of the nuclei with a few grains of chromatin remained. The cytoplasm of these cells was characterized by numerous tiny vesicles, varying in shape and density, often lying directly against the inner surface of the cytoplasmic membrane. In the cytoplasm of the binuclear and tetranuclear cells (Fig. 3) the mitochondria were vacuolated or were dumbbell-shaped with a dense matrix and with cristae in the form of arcs, connected to the inner surface of the outer membrane. These organelles were mainly located around the nucleus. In some multinuclear cells chromatoid bodies and numerous distinctive vesicles concentrated near the nuclei were clearly seen. They were presumably remnants of some destroyed organelles, especially of the lamellar complex. These disturbances were more severe in the cytoplasm of cells containing more than six nuclei. Only solitary unchanged round or elongated mitochondria could be seen. The matrix of most of the altered mitochondria was condensed and the

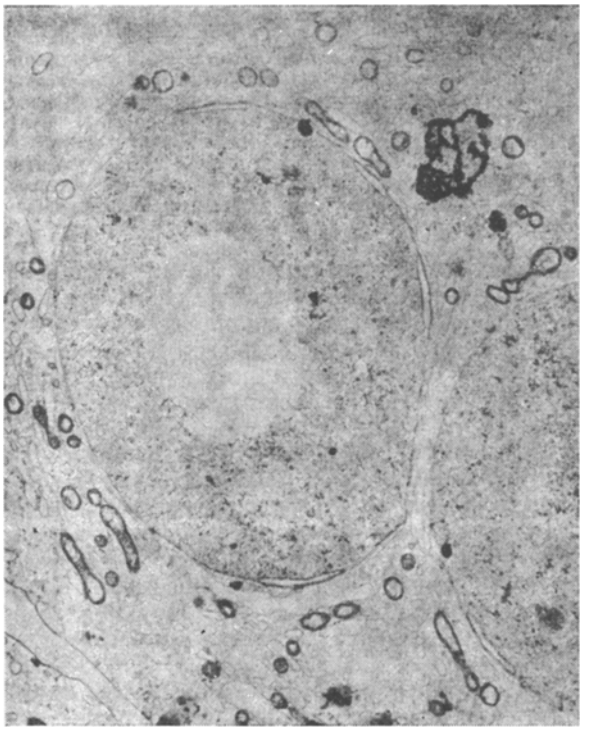

Fig. 3. Binuclear cell: various forms of mitochondria and widening of a perinuclear space (5600×).

integrity of the membranes and cristae was disturbed. Such cells (Fig. 2) contained numerous myelin-like structures. These multinuclear cells were presumably degeneratively changed spermatocytes or spermatids in the early stages of spermatogenesis.

In the modern view, under normal physiological conditions, multinuclear cells may arise as a result of incomplete division of spermatogenic elements, namely spermatogonia in the last spermatogonial division [7]. The binuclear spermatogonia thus formed subsequently undergo further differentiation, to produce binuclear primary spermatocytes, tetranuclear secondary spermatocytes, and spermatids with 8 or 16 nuclei which, in turn, could give rise to 8 or 16 biologically perfect spermatozoa. The reason for this phenomenon, according to the authors cited above [7], evidently depends on endogenous factors. In some animals, with severe general disturbances, the number of these formations rose sharply and multinuclear structures not normally found appeared [3, 4].

Multinuclear structures may be formed through the action of pathological agents by two different mechanisms. First, they may arise as a result of degenerative changes. Second, these multinuclear formations may be the result of a disturbance of the special intercellular content found between the various spermatogenic elements and the sustentacular cells [4] or from fusion of the germinative cells [3]. These are good grounds for suggesting that the crucial stage in the mechanism of origin of the multinuclear structures is the direct action of boric acid on processes of spermatogenesis and spermiogenesis, especially on processes such as meiotic division of spermatocytes and the formation of spermatozoa, especially in its early stages.

LITERATURE CITED

1. E. Ya. Golubovich, M. M. Avkhimenko, and M. M. Chirkova, in: The Toxicology of New Industrial Chemicals [in Russian], No. 10, Moscow (1968), pp. 64-73.
2. G. N. Zaeva, L. A. Timofievskaya, I. V. Silant'eva, et al., in: The Toxicology of New Industrial Chemicals [in Russian], No. 9, Moscow (1967), pp. 59-71.
3. V. G. Kondratenko, "Changes in the testis and epididymis after denervation," Candidate's Dissertation, Moscow (1960).
4. V. V. Nevstrueva, in: Problems in Nervous Regulation of Tissue Processes. Proceedings of the Second Moscow Medical Institute [in Russian], Moscow (1970), pp. 148-154.

5. O. M. Strongina, "The effect of boric acid on the generative function," Author's Abstract of Candidate's Dissertation, Moscow (1971).
6. N. Yu. Tarasenko, A. A. Kasparov, and O. M. Strongina, Gig. Tr., No. 11, 13 (1972).
7. M. H. Burgos and D. W. Fawcett, J. Biophys. Biochem. Cytol., 1, 287 (1955).
8. R. Truhaut, Nguen Phu-Lich, F. Loisillie, et al., C. R. Acad. Sci. (Paris), 258, 5099 (1964).

SOME STRUCTURAL FEATURES OF VITAL DYES

M. G. Iogannsen

UDC 57.086.15:54

Lymphocytes and macrophages of healthy rats, cells of lymphosarcoma and ovarian tumors (strain OYa) of rats, and cells of sarcoma 37 and lymphosarcoma Nk/Ly of mice were stained intravitally with $1 \cdot 10^{-5}$-$3 \cdot 10^{-2}$ M solutions of 86 dyes. The presence of alkylating amino groups in the molecule of the dye substantially increases its ability to penetrate to living cells and to be deposited in their cytoplasm. Acid radicals considerably reduce the ability of the dye to stain cells intravitally. This has been shown for thiazine, oxazine, triphenylmethane, acridine, diazine, and xanthene compounds. The degree of basicity of the dye molecule and also of its amino group does not play a decisive role in the process of vital staining.

KEY WORDS: *vital dyes; intravital microscopy; cell membranes; permeability.*

The problem of interaction between living cells and dyes has been studied for many years. Nevertheless, many aspects of the chemistry of vital staining still remain unexplained. It has been noted that methylation of the amino groups of some basic dyes can increase their ability to stain cells intravitally [13]. However, the choice of vital dyes is still made empirically.

The object of this investigation was to demonstrate the existence of a definite relationship between the chemical structure of dyes and their ability to stain normal and, in particular, tumor cells intravitally.

EXPERIMENTAL METHOD

Cells of lymphosarcoma and ovarian tumors (strain OYa) and lymphocytes of rats, and also cells of sarcoma 37 and lymphosarcoma Nk/Ly of mice were stained intravitally *in vitro* with $1 \cdot 10^{-5}$-$3 \cdot 10^{-2}$ M solutions of 86 basic and acidic dyes (in Hanks's solution) at 18-20°C at pH 6.8-7.2. The list of dyes tested is given in Table 1.

The results of staining were read 1-3 min after the beginning of contact between the cells and the dye by visual examination under the microscope. The rat macrophages were stained by injecting the dye into the animal in a special chamber for intravital microscopy *in vivo* [9].

To determine the toxicity of the various dyes, the viability of stained lymphocytes and lymphosarcoma cells was tested in tissue culture, and the viability of the tumor cells was tested also by subcutaneous inoculation of animals and in special chambers for intravital microscopy *in vivo* [6, 9].

Laboratory of Chemical Carcinogenic Agents, N. N. Petrov Scientific-Research Institute of Oncology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 4, pp. 499-503, April, 1977. Original article submitted August 3, 1976.