HISTOLOGIC STUDY OF OVARIAN REPRODUCTIVE FUNCTION IN MATURE MICE AFTER ANTENATAL TREATMENT WITH OXYTETRACYCLINE

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The risk of late consequences of the action of biologically active factors is a problem which necessitates a careful study of reproductive function of the ovaries of the sexually mature progeny of mammals after antenatal exposure during times of greatest sensitivity of the sex cells, which include stages of proliferative activity of the oogonia and their premeiotic transformations [6, 8]. Isolated studies in this direction have demonstrated the late effect of such powerful agents as ionizing radiation, causing death in the F_2 progeny [6], and the antitumor compound musulban, the action of which leads to ovarian dysgenesis in F_1 progeny [11].

The aim of this investigation was a histologic study of the oocyte pool in the ovaries and oviducts of the mature F_1 progeny after antenatal chemotherapy with a relatively mild agent, namely oxytetracycline (OTC) which, with a broad spectrum of action, inhibits protein synthesis in cells of prokaryotes and eukaryotes in concentrations arresting proliferation [1]. Antibiotics of the tetracycline group are known to have fairly high transplacental properties. A mutagenic effect of tetracycline has also been demonstrated in a culture of human fibroblasts [4].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice. Pregnant females, 12 and 13 days after mating for 1-3 h, were given an intramuscular injection of 5 mg OTC in 0.5 ml of physiological saline. This dose is at the upper limit of effective doses, in which tetracyclines exhibit a chemotherapeutic action on mice [7]. Animals of the control group received 0.5 ml of physiological saline at the corresponding times of pregnancy.

The progeny were sacrificed 4 months after birth in the early metestrus phase by cervical dislocation. The ovaries together with the oviducts were fixed in Bouin's fluid. Paraffin sections (7 μm) were stained with Ehrlich's hematoxylin and counterstained with eosin. The pool of oocytes and follicles was counted in serial sections through the left ovaries and the proportion of atretic oocytes and follicles was expressed as a percentage. The absolute number of oocytes per ovary (allowing for the thickness of the sections) was calculated by Abercrombie's formula [5]. The total number of ovulated oocytes in the ampullary zone was determined in serial sections through the paired oviducts in each female of the sexually mature progeny. The mean diameter of the paired oviducts from each female also was determined. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Counting the total number of oocytes in the ovaries of mature mice after two antenatal injections of OTC at the 12th and 13th days of development showed a significant decrease in this number compared with the control, mainly on account of the oocyte pool of the primordial follicles (Table 1), the level of which in the experimental group was 69% of that in the control group. There was a significant increase in the proportion of oocytes and follicles with signs of degeneration, which was most marked in oocytes of primordial follicles, as shown by eosinophilia of the ooplasm and further development of pycnosis in the nucleus of the oocyte itself.

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TABLE 1. Cytologic Parameters of Ovarian Reproductive Function in Mature Progeny of Mice Receiving OTC Antenatally at the Age of 12 and 13 days (M ± m, n = 4-5)

Parameter	Group of animals	Values of M ±m	P
Total oocyte pool in ovaries	Control	1907 ± 125	<0.05
	Experimental	1398 ± 184	
Oocyte pool of primordial follicles	Control	1317 ± 89	<0.05
	Experimental	907 ± 138	
Proportion of atretic oocytes and follicles, %	Control	10.7 ± 1.5	<0.01
	Experimental	26.3 ± 3.6	
Proportion of degeneration of oocytes among primordial follicles, %	Control	6.2 ± 1.5	<0.01
	Experimental	21.3 ± 4.3	
Number of ovulated oocytes in oviducts	Control	14.2 ± 0.9	<0.001
	Experimental	7.2 ± 0.8	

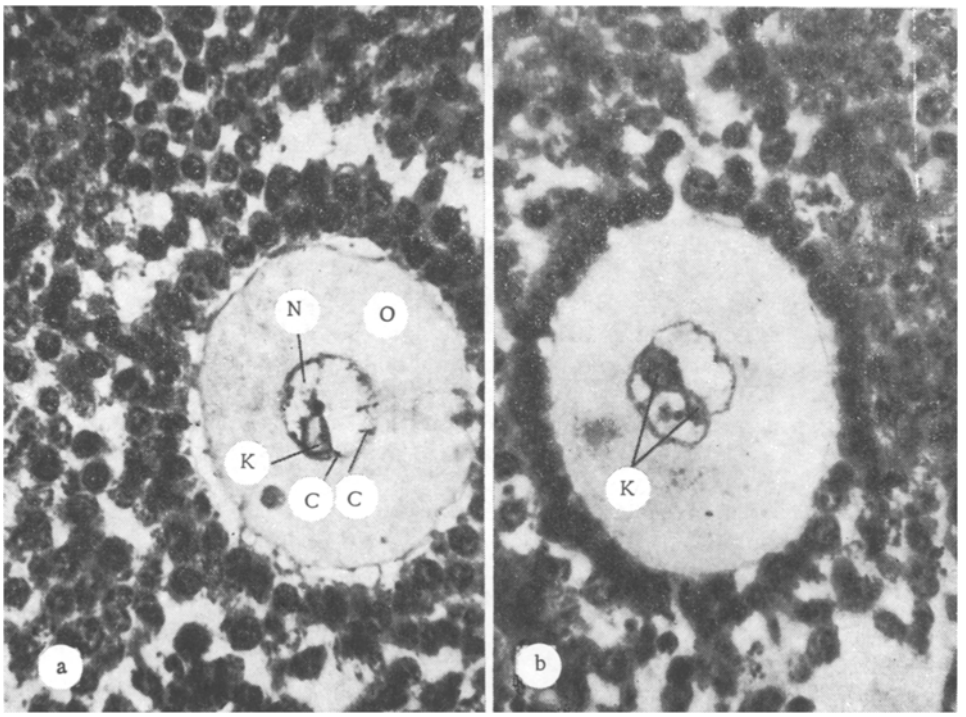

Fig. 1. Oocytes of ovaries of mature mice exposed antenatally to OTC in the initial period of karyospherogenesis, with ordinary (a) and atypical (b) structure of chromosome-nucleolar apparatus. Fixation in Bouin's fluid, stained with hematoxylin-eosin, magnification 280 ×. Legend: O) ooplasm, N) nuclear membrane, C) chromosomes, K) developing karyosphere, connected with nucleolus and nuclear membrane.

Besides ordinary atresia of the follicle, often leading to false meiotic maturation of the oocyte and even pseudocleavage, oocytes with an atypical structure of the chromosome-nucleolar apparatus [3] appeared in the antral follicles of ovaries of the experimental group, in both atretic and normal follicles, i.e., without pycnosis of the granulosa cells (Fig. 1, a, b). The proportion of these oocytes among the antral follicles was 1.2 ± 0.1%.

The number of ovulated oocytes found in the ampullary zone of the oviducts in the early metestrus phase in the experimental females was only half of that in the controls, and 26% of them, moreover, were in a deformed state (compared with 4% in the control). The mean diameter of the ovaries of the experimental females also was considerably reduced at the same phase of the estrous cycle (2216 ± 35 μm in the control, 1914 ± 46 μm in the experiment; $P < 0.001$).

When OTC was injected into the pregnant mice 12-13 days after mating, it acted during the period of proliferative activity of the oogonia and their partial transition into the preleptotene stage of condensation of the chromosomes. As the previous investigations showed, action of this kind causes a significant increase in the proportion of pathological mitoses of the oogonia. Deviations from normal found in ovaries of experimental groups of mature progeny perhaps reflect latent genetic injuries to the oocytes as a result of antenatal exposure to OTC, manifested in subsequent stages of oogenesis. For instance, it is considered that degeneration of oocytes of primordial follicles is determined by genetic factors [9], and it is therefore natural to expect changes in the level of degeneration if the factors responsible for this are injured; however, this does not rule out the probability of latent disturbance of the cytoplasmic structures of the oocyte also.

It has been suggested that the minimal threshold level of the pool of primordial follicles is necessary for the normal ovarian cycle of sexually mature individuals [10]. However even in the absence of changes in the cycle, reduction of the total oocyte pool accompanied by their increased degeneration may shorten the period of reproductive life of the individual.

The discovery of atypical structures of the chromosome–nucleolar apparatus in oocytes of the experimental mice could be, on the one hand, the result of degenerative changes starting in the nucleus or, on the other hand, a manifestation of the mutagenic effect of OTC. Evidence against the first suggestion is given by the fact that in the control group no atypical structures were found either in oocytes of atretic follicles or in oocytes of normal follicles. The reduction found in the number of ovulated oocytes confirms the presence of a late effect on reproductive function of the ovaries of the mature progeny after antenatal chemotherapy with oxytetracycline.

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