

ESTRAL CYCLE AND THE OVARIAN STRUCTURE IN WHITE RATS WITH CHRONIC CHLOROPRENE INTOXICATION

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The clinical, experimental, and anatomicopathological findings of Soviet and foreign authors [1] indicate the toxic effect of chloroprene on various organs and systems of the body. Chloroprene intoxication is manifested in functional disorders of the central and autonomic nervous systems, cardiovascular system, gastrointestinal tract, in disorders of hematopoiesis, metabolism, in eczemas, rhinitis, trichorrhea, loss of libido, amenorrhea, etc.

According to the findings of V. G. Mkhitarian [5, 6] the functional and morphological changes observed in the organism in chloroprene intoxication in many respects resembles the picture of radiation lesions.

Many researchers who studied the toxic effect of chloroprene have omitted such an important sphere as the female sexual organs.

Taking into account the importance of the problem of the effect of chloroprene intoxication on the state of the sexual organs of the female in particular on the generative functions of the ovaries (especially in Armenia where the production of synthetic chloroprene rubber is widely organized), we carried out an experimental study of the morphological structure of the ovaries and the estral cycle of rats under conditions of chronic chloroprene intoxication.

The work was supervised by Z. A. Ryabinina.

METHOD

In order to approximate the experiments to conditions of chronic chloroprene intoxication we exposed the animals to small concentrations of chloroprene (0.5 ± 0.001 mg/liter) for 4 (I series of experiments) and 7 (II series) months with a daily 5-h exposure (inhalation of chloroprene vapors). The concentration of chloroprene was checked chemically—by the method of microcombustion [7].

The investigations were carried out on 173 sexually mature white female rats weighing 160-180 g. Each series had its control (rats not subjected to the effect of chloroprene vapors).

The estral cycle of the rats was studied by the usual method of investigating the vaginal content. Smears were stained by the Giemsa-Romanovsky method.

The left ovary was fixed in Carnoy's fluid, embedded in paraffin, and cut serially into sections 6μ thick.

The absolute quantity of primordial follicles and follicles with a single layer of cells were counted in each tenth section and maturing follicles having from 2 to 4 layers of granulosa cells or more and the graafian follicles were counted in each fifth section. We counted only those follicles which contained oocytes with a nucleus and nucleolus. Atretic follicles were counted only in each fifth section.

Average Number (absolute) of Generative Elements of Rat Ovaries in Chronic Chloroprene Intoxication (with 7 month observation)

Structural components of ovaries	Control	Experiment	Significance of differences (P)
Primordial follicles and follicles with one layer of granulosa cells	723.1 \pm 47.1	390.9 \pm 47.0	P < 0.001
Maturing follicles having from 2 to 4 layers of granulosa cells and more	33.0 \pm 9.62	52.0 \pm 5.3	P > 0.891
Graafian follicles	7.5 \pm 1.6	13.25 \pm 1.59	P < 0.0093
Atretic follicles	1444.0 \pm 175.3	1907.5 \pm 157.2	P < 0.0574
Corpora lutea	14.9 \pm 2.1	18.9 \pm 1.28	P > 0.1096
Total number of structural components	2222.5 \pm 189.8	2382.6 \pm 63.05	P > 0.4237

The absolute number of corpora lutea was counted by means of a projection camera.

In addition, we determined the area of the connective-tissue stroma: 1 middle section of the ovaries was photographed; the area occupied by the stroma was cut out and weighed.

RESULTS

Both in the I and the II series of experiments we observed during the first month of exposure to chloroprene a prolongation of the estral cycle which was especially marked during the first two months. In the I series of experiments during the first month it was equal to 6.04 days vs. 4.19 in the control (P < 0.0001) and in the II series, 6.7 vs. 4.11 in the control (P < 0.0002). Later the cycle somewhat shortened; however, it did not reach the initial value.

The estral period was prolonged from the first month of the experiment, reaching a maximum in the I series after 2 months—1.3 days in the experiment and 1.06 days in the control—and in the II series after 6 months—1.5 days in the experiment and 1.04 days in the control, which is statistically significant.

Disruption of the interestrual period was also established. In the latter it was prolonged and reached a maximum after a month in both experimental series: 5.1 and 6.24 vs. 2.9 days in the control (P < 0.0001); later, this period also remained high and the variations were insignificant.

At the same time chloroprene intoxication led to a reduction of the total number of estral cycles, especially of normal ones. In the first month of experiments in the I series there were 4.42 cycles per female as opposed to 6.41 in the control (P < 0.0001) and in the experiments of the II series there were 3.79 cycles vs. 6.5 in the control (P < 0.0001).

The drop in the number of normal cycles was especially pronounced. Whereas in the control there were 6.4 such cycles per female, under the effect of chloroprene there were 2.86 cycles in the I series of experiments and 1.9 in the II series. The differences are statistically significant. Analogous changes were observed also during subsequent months of the experiment.

The reaction of the animals to the effect of the poison was dissimilar. Some animals reacted to chloroprene by a prolonged estrus lasting up to 19 days, with a shortening of the resting period to one day, and in others the same concentration of chloroprene caused a prolongation of the interestrual period, reduction in the number of estral cycles, or cessation of the cycles.

Chloroprene intoxication also caused atypical estrus, when in the vaginal content leukocytes were admixed with the scales, or a scanty estrus with few scales.

The disturbances of the estral cycle during the 4-month period of the effect of chloroprene in the experiments of the II series were the same with respect to the qualitative and quantitative indexes as those during the same period in the I series of experiments. Later, during the remaining three months, the disorders in the estral period were more intense in the II series of experiments.

The ovaries of 60 of the 173 rats were subjected to a morphological study. The structure of the ovaries of

the control group of animals and the ovaries of the experimental rats subjected to chloroprene for four months differed little. Substantial changes occurred in the ovary with the 7-month exposure (see Table).

The absolute weight of the ovaries of the rats in the experimental group was 32.35 g vs. 25.6 g in the control ($P < 0.001$) and the relative weight respectively 0.012 and 0.009%, i.e., we noted a significant increase in the absolute and relative weights of the ovaries.

The decrease in the number of primordial follicles and follicles with one layer of granulosa cells in the experimental animals was statistically significant.

The number of maturing follicles having 2-4 layers of granulosa cells and more was somewhat greater in the experimental rats, but the difference in comparison with the control was insignificant.

The number of graafian follicles in the ovaries of the experimental animals increased in comparison with the controls.

The number of atretic follicles also increased in the investigated animals.

There were almost as many corpora lutea in the follicles of the experimental animals as in the control.

We noted an increase in the area occupied by the stroma of the ovary in the experimental animals. Thus, the absolute index of the increase in the area of the stroma of the control animals was 0.086 mg whereas in the experimental animals it was 0.20 mg; in the former case the stroma occupied 16.5% of the ovarian area and in the latter case, 33.02% ($P < 0.001$).

Thus, the effect of chloroprene at first caused disturbance of the estral cycle and later there were changes in the structure of the ovaries.

We tend to explain the drop in the number of primordial follicles and follicles with one layer of granulosa cells by the increased entry of the latter into the stage of intense growth when their rapid destruction occurs when exposed to chloroprene, as a result of which the number of atretic follicles increases, i.e., the processes of atresia are enhanced.

The pathological changes in the follicular apparatus and stroma of the rat ovaries which were detected during the 7-month exposure to chloroprene vapors we consider to be a development similar to those observed by a number of authors [2, 8, 9, et al.] in other parenchymatous organs when exposed to chloroprene.

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