THE SELECTIVE STERILIZING ACTION OF MYLERAN ON THE OVARIES OF RAT FETUSES

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Female rats were sterilized by transplacental administration of myleran in a dose of 10 mg/kg on the 14th day of embryonic development. Pituitary gonadotropic function remained completely intact, as shown by the state of constant estrus and by the stimulation of the uterus in rats sterilized with myleran, subjected to ovariectomy, and homografted with ovaries from intact females.

Investigations have been shown that the antileukemic compound myleran (busulfan) causes complete sterility in the progeny if given in a well tolerated dose to female rats on the 14-17th days of pregnancy [1, 2, 7, 8, 12]. This phenomenon attracted the writer's attention during a search for methods of stimulating neoplasms in the progeny induced by the transplacental action of carcinogens [9]. The carcinogenic effect of radiation and of certain alkylating agents on the ovaries is manifested by sterilization followed by gonadotropic stimulation from the pituitary [4-6, 10].

The possibility of development of ovarian tumors in rat progenies by a similar mechanism as a result of the sterilizing action of myleran in the antenatal period of life can be accepted if the intrinsic carcinogenic properties of this alkylating agent are also taken into account [16]. However, results indicating preservation of the gonadotropic function of the hypothalamo-hypophyseal system in the sterilized rat progenies could be of fundamental importance. The investigation described below was accordingly carried out for this purpose.

EXPERIMENTAL METHOD

Noninbred albino rats bred at the Rappolovo nursery, Academy of Medical Sciences of the USSR, were used. Myleran, dissolved in peach oil on heating, was injected intraperitoneally in a dose of 10 mg/kg on the 14th day of pregnancy. The day of discovery of spermatozoa in vaginal smears of the females after mating was taken as the 1st day of pregnancy. The scheme of the basic groups of experiments is shown in Fig. 1. Ten young rats (group 1) born from rats receiving myleran had ovaries from intact females of the same age grafted into the tail by the method described previously [3], and their own ovaries were removed and transplanted in turn into the tail of ten intact females which had acted as their donors (group 2). Ten intact, sexually mature female rats undergoing reciprocal ovarian grafting into the tail (group 3) acted as the control. All the animals were sacrificed 1.5 months after the operation, their pituitary and uterus were weighed, and their relative weights per 100 g body weight were

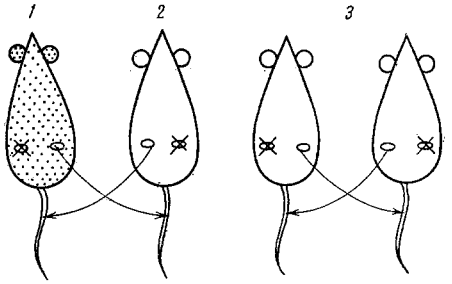

Fig. 1. Scheme showing homografting of ovaries into the tail in the various groups of experiments (explanation in text).

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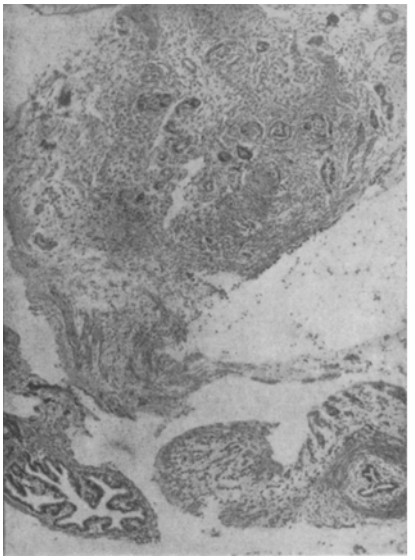

Fig. 2

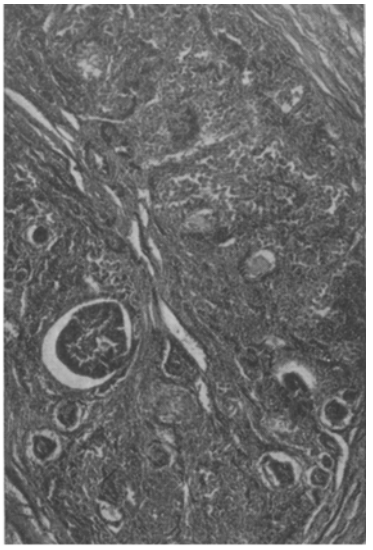

Fig. 3

Fig. 2. Ovary of rat aged 3 months treated with myleran on the 14th day of embryonic development. Here and in Fig. 3: hematoxylin-eosin, 80 ×.

Fig. 3. Ovary of intact female grafted into the tail of a rat treated with myleran on the 14th day of embryonic development (operation performed at age of 3 months; 45th day of experiment).

calculated. The grafted ovaries were studied histologically. Throughout the experiment, cytological investigations of vaginal smears from all the experimental and control rats were carried out. The experimental results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

By the 45th day of the experiment the incidence of development of permanent estrus in the rats of the control group which had undergone reciprocal homografting of the ovaries into the tail was 80%, in close agreement with the results obtained after autografting of the ovaries [3]. In the rats of group 1, sterilized with myleran in the antenatal period and with ovaries grafted from intact females, the incidence of permanent estrus was not significantly different from the control (70%). When, however, ovaries from animals sterilized with myleran were grafted into intact, castrated females (group 2), anestrus was observed in all cases.

The weight of the uterus in the animals of group 2 was much less than in the rats of group 1 (26 ± 4.5 and 44 ± 7.7 mg/100 g respectively; $P < 0.05$), while the weight of the pituitary was somewhat greater (6.4± 0.78 and 4.9 ± 0.27 mg/100 g respectively; $P > 0.05$).

Morphological investigation of the ovaries of the rats sterilized with myleran showed that follicles were rare, and oöcytes were never found (Fig. 2). These findings confirm the results of previous observations [2, 12]. Grafts of these ovaries in intact females (group 2) were usually replaced by fibrous tissue, and only in a few areas were remnants of ovarian tissue seen. Meanwhile in the intact ovaries, grafted into rats sterilized with myleran (group 1), follicles developed well, hyperplasia of the theca tissue was frequently observed and corpora lutea were absent (Fig. 3). All this indicated high functional activity of the grafts.

The results of the morphological study of the grafted ovaries and cytological examination of the vaginal smears demonstrate that the sterilizing effect of myleran is not due to its action on the hypothalamo-hypophyseal system of the fetus, but to its strictly selective action on the gonocytes in the period of their greatest mitotic activity at these times [15].

If the antenatal administration of myleran had in fact led to damage to the fetal hypothalamo-hypophyseal system, despite subsequent grafting of the ovaries from intact females, their anestrus would have persisted. On the other hand, temporary inhibition of the estrogenic function in intact females as the result of ovariectomy would have led to de-inhibition of pituitary gonadotropic activity and to stimulation of the graft taken from the rat sterilized with myleran, and hence to the development of permanent estrus. However, in the animals of this group the wieght of the pituitary was increased, the uterus was atrophied, and the results of cytological examination of the vaginal smears indicated the presence of anestrus, i.e., a castration effect was observed. The animals of group 1 showed signs of estrogenic stimulation: the uterus was large and permanent estrus was present.

In experiments on rodents ovarian tumors appeared after exposure to a wide variety of agents (x-rays, chemical carcinogens, disturbances of the hormonal balance) [4, 5, 14], and in every case the essential factor was a disturbance of the hypothalamo-hypophyseo-ovarian system due to the damage to its peripheral component while the activity of the central components remained intact or was increased. The selective action of myleran on the fetal ovaries while the gonadotropic function remains intact, as revealed by the present experiments, is evidence that, in principle, ovarian neoplasms can develop in rats as a result of the transplacental sterilizing action of this alkylating compound.

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