

EFFECT OF DISTURBANCES OF UTERINE INNERVATION IN PREGNANT RATS ON SUSCEPTIBILITY OF EMBRYOS TO HYPERTHERMIA AND MYLERAN

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Denervation of the uterus in rats on the 1st, and in particular, the 4th day of pregnancy leads to pathological changes in fetal development. Transient hyperthermia of rats with a denervated uterus and simultaneous administration of myleran increase the incidence of developmental disturbances in embryos.

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During examination of the harmful action of external agents on development of the Placentalia and man [7] the question arises of the extent to which they act directly on the developing organism and to what extent their action is transmitted indirectly through the mother. The study of the importance of the maternal nervous system is of particular interest.

A number of embryological studies of the effects of denervation of the uterus in pregnant animals on fetal development have been undertaken [3-7]. In all these investigations a definite increase in the number of various types of developmental anomalies was observed in the fetuses of the experimental animals. However, to study the question stated above it is of the greatest interest to investigate the combined action of uterine denervation and other external agents. There is some information in the literature on the action of harmful agents on embryogenesis after disturbance of innervation of the maternal uterus [2, 3]. The object of the present investigation was to study the action of hyperthermia and myleran on embryos of rats whose uterine innervation had been disturbed.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats weighing 180-200 g. The agents chosen to be combined with denervation were transient hyperthermia of the pregnant rats, as a relatively nonspecific agent, and the antitumor drug myleran, whose high biological activity is due to its ability to take part in alkylating reactions with nucleic acids and also with nucleophilic groups of proteins [1].

The following nerves and ganglia were excised under ether anesthesia: part of the sympathetic trunk in the lumbar region, the solar plexus, the lesser splanchnic nerve with the hypogastric nerves and accessory ganglia.

Operations were carried out on the 1st and 4th days of embryonic development depending on the character of the supplementary procedure. The experimental results were read at autopsy on the experimental females on the 17th day of pregnancy, when formed fetuses were present, and on the 10th day, when the implanted embryos had just started to undergo segmentation.

Of anomalies of a general character, hematomas were found, most frequently in the region of the head and trunk, together with edema, stasis, and delay in development. As a measure of the effects produced, the following indices were calculated (in percent): the number of implanted blastocysts relative to the number of ovulations (by counting the corpora lutea in the ovary and the number of fetuses formed), the number of living embryos relative to the number of implanted embryos, and the number of normal embryos as a proportion of the total number of living embryos. The statistical significance of each index was also determined. As Fig. 1 shows, disturbance of the normal uterine innervation caused a significant yet small de-

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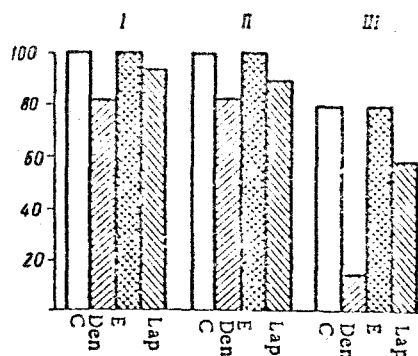


Fig. 1. Development of rat embryos after partial denervation of the uterus on the 4th day of embryonic development. Results read on 17th day. Number (in percent) of implanted blastocysts (I) and of living (II) and normal (III) embryos. C) Control; Den) denervation; E) ether anesthesia; Lap) laparotomy.

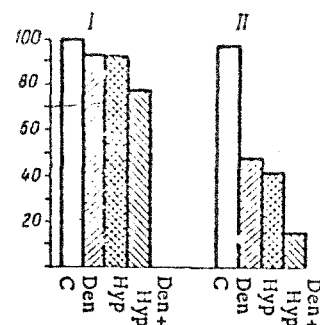


Fig. 2. Development of embryos after denervation of uterus on 1st day of pregnancy followed by hyperthermia on 4th day. Number (in percent) of living (I) and normal (II) embryos on 17th day of development. Hyp) Hyperthermia. Remainder of legend as in Fig. 1.

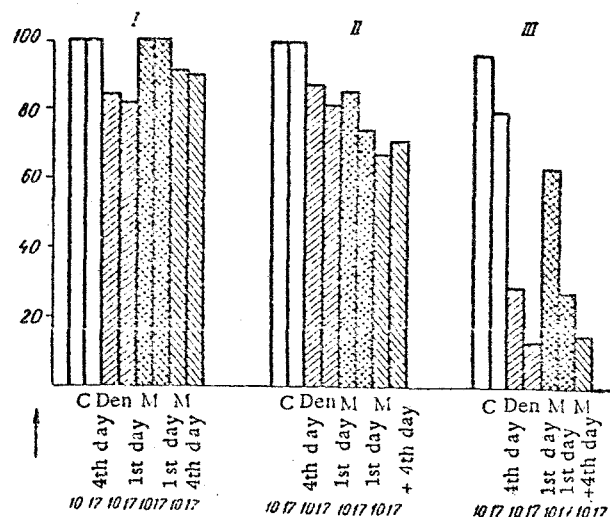


Fig. 3. Comparison of results of experiment on 10th day and 17th days of embryonic development. M) Myleran. Remainder of legend as for Fig. 1.

crease in the percentage of implantations and of living fetuses, but very considerably increased the number of general developmental anomalies.

To study the combined effect of temperature and denervation of the uterus on development the rats were kept in an incubator at 41-42° for between 50 min and 3 h. The principal criterion of the state of the experimental animals was its rectal temperature. This was 34.6-36.9° before the experiment and 39.8-41.3° after the experiment. The effect of hyperthermia was studied in intact females and compared with its effect on animals undergoing operation. The results of both series of experiments were read on the 10th day of pregnancy (Fig. 2). In these series all hundred percent of ovulating ova were implanted and deciduomas formed around them. The action of hyperthermia of the females to 41° for a single exposure on the 4th day of pregnancy, as is clear from Fig. 2, was

considerable: of the total number of implanted embryos only 42.7% developed normally. The rest showed various anomalies; the mortality was not significantly different from the control ($6.8 \pm 2.4\%$). After combined action of denervation and hyperthermia the mortality rose significantly, reaching $22.1 \pm 3.9\%$. The number of normally developing embryos, however, fell to a greater degree than after hyperthermia without denervation (15.2%), and still more so compared with the control ($81.3 \pm 4.1\%$). Denervation thus reduced the percentage of normally developing embryos by almost two-thirds. On the 1st day of pregnancy at the same time of day (in the morning) Myleran was administered to all the animals in a dose of 10 mg/kg; autopsy was performed on the 10th and 17th days (Fig. 3).

Myleran itself did not reduce the percentage of implanted embryos but had a marked pathogenic action on the embryos themselves. The number of living embryos fell to 74.5%, and the percentage of normally developing embryos also fell, reaching 20.7% (difference from control $59 \pm 5.8\%$).

After administration of myleran on the 1st day of pregnancy combined with denervation on the 4th day the percentage of implanted embryos fell significantly (90.3 ; difference $9.7 \pm 2.9\%$). The number of dead embryos rose to 33.4%, compared with 14.1% on the 10th day of development in experiments with myleran alone (difference $19.3 \pm 5.8\%$). The percentage of normally developing embryos fell particularly sharply

when determined on the 17th day of development: 100% of the embryos were injured. Consequently, the effect of myleran on fetal development was very greatly increased when combined with denervation.

Comparison of the results of these experiments when read on the 10th and 17th days of development shows that the effect of all harmful agents described above increases until the 17th day, and only rarely remains at the same level (Fig. 3).

Hence, after disturbance of the normal uterine innervation, the harmful action of exogenic hyperthermia and myleran on rat fetal development is considerably intensified.

The uterus receives its innervation by so many and different pathways that its nerve supply cannot be completely isolated from other parts of the nervous system surgically. Furthermore, many uninjured neurons were still left in the uterus itself, where they presumably took part in reflexes involving short pathways within its wall. However, disturbance of the connection between these neurons and the abdominal autonomic centers by itself has a harmful action on the course of embryogenesis, although absence of this connection was manifested particularly strongly when combined with other external influences on the denervated animals. Comparison of the action of hyperthermia and myleran showed that, contrary to expectation, the effect of the specific agent myleran was potentiated by denervation no less than the effect of transient exogenic hyperthermia. The action of myleran as an alkylating agent with highly specific action should in theory have been directed on the synthesis of nuclear products in the embryonic cells. In fact, its action on the embryos proved to be highly dependent on the condition of the maternal organism. Disturbance of the normal uterine innervation somehow or other modifies this state. We cannot yet say which of the changes taking place in the mother after denervation of the uterus are responsible for increasing sensitivity of the embryo to the action of hyperthermia and myleran. However, it may be postulated that the existence of the autonomic nervous connections of the uterus protects the embryo against the harmful action of agents with completely different mechanisms of action.

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