

EXPERIMENTAL BIOLOGY

EFFECT OF DISTURBING THE INNERVATION OF THE UTERUS ON THE COURSE OF IMPLANTATION IN RATS

P. G. Svetlov and G. E. Korsakova

From the Institute of Obstetrics and Gynecology of the Academy of Medical Sciences of the USSR,
Leningrad

(Received February 22, 1955)

Implantation of a rat embryo is performed with the help of a fairly complicated combination of local changes in the uterine mucosa. These changes represent a shaping process typical of the organ, in which the implanted blastocyst plays the role of the stimulus and the uterus that of the reacting system [3]. In order to discover what significance the normal innervation of the uterus has in this reaction, and to approach understanding of its mechanism in this way, we undertook experiments on the denervation of the rat uterus during the first days of pregnancy.

The operations were carried out with ether narcosis and consisted of three stages: 1) extirpation of the lower visceral nerves which lie on the aorta and of the inferior mesenteric ganglion together with the hypogastric nerves and the accessory ganglia (g. accessoria) lying along their path; 2) extirpation of the lumbar area of the sympathetic nerve trunk by 3-4 ganglia; 3) extirpation of the solar plexus. All three excisions were carried out one after the other through an incision in the abdominal wall along the media line from the xiphoid process of the sternum to the cranial edge of the urinary bladder.

As is known, 4 paths of innervation of the uterus exist: through the preaortic plexus, the ovarian plexus, branches of the sympathetic trunk (directly or through the preaortic or ovarian plexus) and the sacral plexus. The above operation had as its purpose the interruption of the first three of these paths of uterine innervation. It was necessary to resort to such a radical operation as the excision of the solar plexus because we did not succeed in interrupting the ovarian pathway. As yet, we have not carried out any operations on the nerves which pass through the sacral plexus (which form the major part of the parasympathetic innervation of the uterus). It should be stated that until now, the topography of the nerve trunks and plexus of the rat's abdomen has been little known, and the described operation only became possible after special anatomical investigations, which were carried out for this purpose.

The described operation undoubtedly achieved a great degree of sympathectomy of the uterus. But since we removed the solar plexus, i.e., the nerve plexus which plays a major role in the innervation of all the organs in the abdominal cavity, our operation should be regarded not only as the denervation of the uterus but also as a serious interference with the function of a number of other organs.

The operation was carried out on the first and fourth day of pregnancy (the method of producing pregnancy in rats and of determining the time of conception was described earlier by us [1]). The implantation process usually occurs during the 6th day of development. The results were measured on the 10th day of development. By this time, the results of implantation were sufficiently clear, while the embryos which died after implantation and their decidua did not have time to be resorbed. On the 10th day of development, the rats uterus, together with the ovaries, was excised under anesthesia, the uterus being first observed for hyperemia and the nature of the egg cylinders in the live state. In order to take into account the state of the internal parts of the decidua and

and embryos, the uteri were fixed with Bouin's fluid and desiccated with alcohol. The basic method of considering the state of the embryos was excision of parallel sections about 1 mm in thickness from the fixed egg cylinders with a razor blade. In medial sections under a binocular microscope the unsegmented embryos in the amnion, the incipient placentas and other parts of the embryonic membranes and decidua could be seen well. In some cases, we resorted to the usual method of imbedding in paraffin and studied the embryos in sections after staining them with Ehrlich's hematoxylin and eosin.

We observed the following peculiarities of development: 1) the number of embryos in the uterus in comparison with the number of corpora lutea; the percentage of implantation was determined in this way; 2) the distribution of the egg cylinders, in the uterus; 3) the degree of development of the embryo and other parts of the decidua; 4) pathological phenomena and necrosis. All the embryonic peculiarities which were found among the embryos developing in the denervated uteri were compared with the symptoms of embryos which developed in the uteri of control animals. In addition, we carried out control operations, i.e., laparotomies, which differed from the operations carried out on the experimental animals only in that denervation was not carried out. The effect of ether in the dosages used during the operations on implantation was also studied.

The distribution of the egg cylinders in the uterus of rats operated on the 4th day of pregnancy was often anomalous. While in normal animals the embryos are at some distance from each other in the uterus, with approximately equal intervals between them (3% of the embryos are exceptions), the embryos of females operated in the indicated way on the 4th day of pregnancy often drew together in groups of two or three, with some of the neighboring egg cylinders fused completely with each other (Fig. 1, for numerical data see Table, right column).

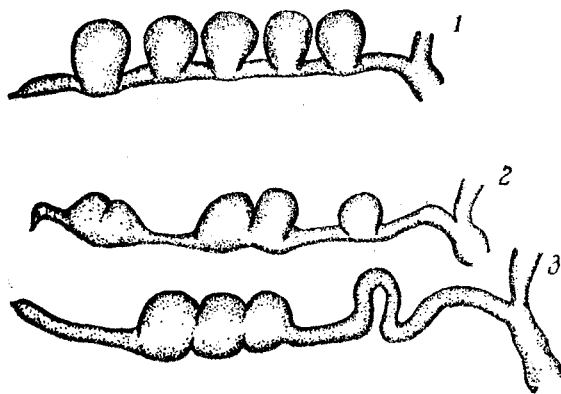


Fig. 1. Schematic representation of the rat uterus on the 10th day of pregnancy.

1) development under normal conditions, 2 and 3) development with denervation of the uterus and excision of the solar plexus; the egg cylinders are approximated and fused in groups of two and three. The mesometrial side is turned up, the vagina at the right, the ovary at the left.

In cases when the egg cylinders are adjacent in the uterus, normal development does not occur: usually the embryos in these cases were dead already on the 10th day, the rest were very retarded in their development; everything indicated that these embryos later die, since the double and triple egg cylinders are never found at the late stages of development. We note that the drawing together of the cylinders after operations on the first day of pregnancy is observed no oftener than normal.

The number of implantations which did not take in the experimental and control animals is presented in Fig. 2. (see average statistical errors in Table). The operation carried out on the 4th day of pregnancy proved especially effective. The control laparotomy without denervation, carried out at this period of development (8 animals, 54 embryos), also involved a considerable amount of unsuccessful implantations. Ether narcosis without operation (6 animals, 68 embryos) did not change the percentage of implanted blastocysts (the decrease

indicated in Fig. 2, right, is statistically insignificant). Similar experiments, carried out on the first day of pregnancy had a smaller, but nevertheless significant number of unsuccessful implants (difference from the control of $15.9 \pm 5.2\%$). The control operations and one ether narcosis on the first day of pregnancy had no effect on the number of implanted embryos.

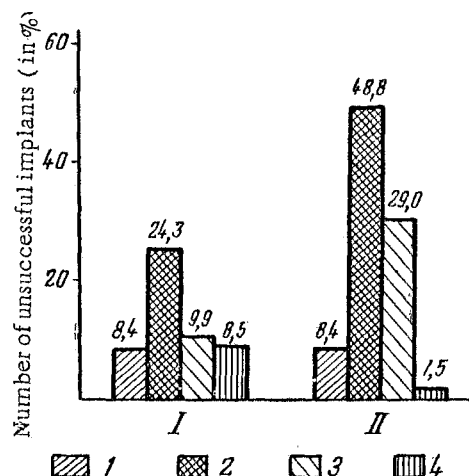


Fig. 2. Number of unsuccessful implants, determined by the difference between the number of corpora lutea and embryos on the 10th day of development. 1) control, 2) denervation, 3) laparotomy without denervation, 4) narcosis, I) operation on the first day of pregnancy, II) operation on the fourth day of pregnancy.

Quantitative Data From the Principle Series of Experiments

	Number of experiments	Corpora lutea	Implants	Embryos			Number of adjacent embryos
				normal	dead	anomalous	
Control							
Absolute figures	9	83	76	67	6	3	2
In %		100	91.6	88.2	7.9	4.0	3.0
Experiments							
Operation on the 4th day of pregnancy							
Absolute figures	13	127	65	8	24	33	16
In %		100	51.2	12.3	36.9	50.8	37.2
Difference from the control (in %)			40.4 \pm 5.0	75.9 \pm 5.2	29.0 \pm 6.7	46.8 \pm 6.6	34.2 \pm 7.2
Operation on the 1st day of pregnancy							
Absolute figures	11	103	78	59	10	9	2
In %		100	75.7	75.6	12.8	11.5	3.5
Difference from the control (in %)			15.9 \pm 5.2	12.6 \pm 6.1	4.9 \pm 4.4	7.5 \pm 4.2	0.3 \pm 2.8

But even if implantation occurred with a denervated uterus, further development proved impossible in a number of cases. At the same time, it is apparent that the chief reason for the death of developmental anomalies of the embryos in these cases was incomplete implantation. At present we are reporting information regarding the divergence from the normal of the development of the implanted embryos, which was found by the 10th day of pregnancy. It is apparent from the table that the development of only 12.3% of the implanted embryos was normal after denervation of the uterus on the 4th developmental day; in the rest of the egg cylinders were found either dead embryo sacs or empty embryo sacs or hemorrhages of the decidua and remaining initial uterine cavity (Svetlov and Korsakova). In some cases severe developmental retardation is observed. Since only half of the ovulated ova were implanted in these experiments (see above), it is evident that, by the 10th developmental day, development was normal in only 6.5% of the possible embryos, instead of 80% in the control. After the operation on the 1st day of pregnancy, the deviation of the implanted embryos from normal was insignificant: the calculated average deviation shows that the difference between the relative number of normal embryos in the experiment and in the control is statistically insignificant. It is possible that the increased effect when the operation was brought closer to the moment of implantation should be related to the stimuli received by the uterus from the places where the nerve trunks were cut; these stimuli die away as the distal sections of the nerve trunks degenerate.

The fates of the studied embryos which were implanted following control laparotomies without denervation and following ether narcosis without operations showed that the number of dead and anomalous embryos is greater among them than among the controls, but considerably less than after denervation of the uterus and abdominal organs. The numerical data which show this will be communicated in another paper.

Thus, the interruption of the uterine innervation (possibly in connection with the interference with the innervation of the other abdominal organs following excision of the solar plexus) causes severe disturbances in the process of implantation. As was already reported [2], partial denervation of the uterus leads to an increase (quantitative and qualitative) in the developmental anomalies which are observed after overheating the maternal body on the 10th day of pregnancy. The interruption of uterine innervation before implantation has an even greater effect on the development of the embryos. Here the very possibility of implantation and its normal course become much less probable without additional influences. This shows that the unique formative processes which develop in the uterine mucosa in response to stimuli from the blastocyst are controlled by the maternal nervous system.

SUMMARY

We have denervated the uterus in rats on the first and the fourth day of pregnancy. Experimental animals had a considerably lower number of implanted embryos than the control ones, only in the fetus 12.3% of experimental animals proved to be normal in comparison to 88.2% of the control ones.

Such pronounced changes were observed only after operations on the fourth day of pregnancy. After operations on the first day of pregnancy we observed some variations, but they were less pronounced.

LITERATURE CITED

- [1] P. G. Svetlov and G. F. Korsakova, in the book: *Reflex Reactions in the Relationships between the Mother and Embryo*,* edited by N. L. Garmasheva, 1954, Medgiz, pp. 135-161.
- [2] P. G. Svetlov and G. F. Karsakova, in the book: *Reflex Reactions in the Relationships between the Mother and Embryo*,* edited by N. L. Garmasheva, 1954, Medgiz, pp. 161-172.
- [3] P. G. Svetlov and G. F. Karsakova, *Doklady Akad. Nauk SSSR*, 1955, vol. 103, No. 3, pp. 503-506.

* In Russian.