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A STUDY OF DELAYED IMPLANTATION CAUSED BY PARABIOSIS IN PREGNANT RATS

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Summary. Parabiosis of rats on Day 1 of pregnancy with Day-1 pregnant females, non-pregnant females or male rats resulted in delayed implantation of ova. The delayed implantation was not caused by the surgery involved nor by the stress of restricted movement, for when surgery was performed without parabiosis, or when rats were sutured together without parabiosis, pregnancy proceeded normally. Delayed implantation did not occur when parabiosis was of the skin to skin type, but did occur when skin and muscles were joined. Parabiosis on Day 1 or Day 3 caused delayed implantation, but parabiosis on Day 5 did not. Parabiosis on Day 1 which was terminated after 24 hr did not result in delayed implantation, but 72 hr of parabiosis did. Experiments on adrenalectomized rats suggest that adrenal secretions are not involved. The decidual reaction of rats in parabiosis was inhibited. Ova recovered from rats in parabiosis were delayed in development, but some implanted normally when transferred to normal pseudopregnant recipients. Treatment of parabiotic rats with 0·1 μg of oestradiol on Day 4 of pregnancy resulted in normal implantation in some individuals.

INTRODUCTION

Delayed implantation occurs in rats and mice only under rather special circumstances. For example, Lataste (1891) observed that ovum implantation is delayed in rats during lactation. It was later demonstrated by Canivenc & Mayer (1955) that the number of suckling young must be at least five if implantation is to be delayed, although it may be delayed in mothers suckling less than five if the mother is subjected to the stress of a severe burn. A similar burn does not cause delayed implantation in pregnancies not involving lactation (Canivenc, personal communication). Superovulated mice have been observed to have delayed implantation when subjected to crowded conditions (Dicksen, 1964), but no report has been made of an effect of crowding on implantation in pregnancies not involving superovulation. Failure of implantation in rats occurs in pregnancies not involving lactation or superovulation if the rats are ovariectomized on Day 4 of pregnancy, and implantation in such rats may be induced by the injection of progesterone (Canivenc, Laffargue & Mayer, 1956).

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Autotransplantation of the pituitary may also cause delayed implantation, and this may be prevented by injection of oestradiol (Mayer, 1963). Certain drugs, such as reserpine (Mayer & Meunier, 1959) and chlorpromazine (Chambon, 1955), also can cause delayed implantation, apparently by altering the hormonal production of the pituitary (Mayer, 1963). It has been suggested that nutritional stress might also cause delayed implantation (Bruce, 1963).

During the course of experiments designed for another purpose, it was observed that delayed implantation occurred in normal pregnant rats placed in parabiosis. The present report describes experimental findings which indicate the nature of this delayed implantation.

METHODS AND MATERIALS

Adult female Sprague-Dawley rats weighing 200 to 250 g were placed in mating cages in the evening with normal males to obtain pregnant females, or with vasectomized males to obtain pseudopregnant females. Pregnancy or pseudopregnancy was inferred from the presence of a vaginal plug early the following day, which was designated Day 1. Pairs of rats were matched for weight and were joined in parabiosis as described by Wilson (1961) and, except where otherwise specified, this involved union of skin, cut surface of muscle and abdominal cavity. After parabiosis, each pair was housed in a separate cage with food and water *ad libitum*. Except where otherwise specified, the rats were killed on the 8th day of pregnancy and the uteri examined for implantations.

Comparisons of decidual reactions were made between groups of rats placed in parabiosis on Day 1 or Day 3 of pseudopregnancy and groups of control rats which were not placed in parabiosis. On Day 5, a laparotomy was performed on each rat and 2–0 cotton surgical thread was inserted into the left horn of the uterus from a point near the uterotubal junction to a point near the cervix. On Day 8, the rats were killed and the uterine horns were dissected free of adherent tissue and weighed individually. The weight of the stimulated horn was divided by the weight of the control horn of the same rat to obtain a ratio designated as decidual response.

RESULTS AND DISCUSSION

The results of a series of experiments in which rats were joined in parabiosis on Day 1 of pregnancy are shown in Table 1. In a group of untreated control rats (Group 1), nine out of ten had implantation sites on Day 8. However, in a group of six rats, each of which was joined in parabiosis with a non-pregnant female, only one had implantation sites on Day 8 (Group 2). Only one rat in a group of ten, each of which had been joined in parabiosis on Day 1 of pregnancy with a male rat, had implantation sites on Day 8 (Group 3).

In order to determine whether or not the observed interference with pregnancy in parabiotic rats was caused by dilution of the hormones of pregnancy with the blood of the non-pregnant partner, rats on Day 1 of pregnancy were joined in parabiosis with other rats on Day 1 of pregnancy (Group 4). Of the fourteen such rats in seven parabiotic pairs, only three had implantation sites on Day 8. In no case did both members of the same parabiotic pair have

implantation sites. This experiment indicates that the failure of implantation caused by parabiosis cannot be explained as a dilution of the hormones of pregnancy.

It is a popular belief that stress—physical or emotional—can interfere with early pregnancy. Group 5 in Table 1 consists of seven rats which were subjected to the same surgical procedures as those used in joining rats in parabiosis, except that the rats were not joined together. As seen in Table 1, five of these seven rats were pregnant on Day 8, indicating that the interference with normal pregnancy caused by parabiosis cannot be attributed to surgical stress alone.

The inability to move as independent individuals must be extremely uncomfortable to animals in parabiosis. To determine whether or not the stress caused by restriction of movement could account for the observed interference with pregnancy, nine rats on Day 1 of pregnancy were sutured without further

TABLE 1

EFFECT OF PARABIOSIS ON DAY 1 OF PREGNANCY ON IMPLANTATION IN RATS

Group No.	*Treatment*	*No. assumed pregnant on Day 1*	*No. pregnant on Day 8*	*Mean implantation sites/ pregnant rat*
1	No treatment—controls	10	9	11·4
2	Parabiosis—pregnant to non-pregnant	6	1	10·0
3	Parabiosis—pregnant to male	10	1	9·0
4	Parabiosis—pregnant to pregnant	14	3	11·0
5	Surgery alone	7	5	9·0
6	Tied up alone	9	7	9·3
7	Skin to skin parabiosis—pregnant to pregnant	6	6	12·3
8	Muscle to muscle parabiosis—pregnant to pregnant	12	3	12·6
9	Adrenalectomy and cortisone, without parabiosis	7	5	10·8
10	Adrenalectomy and cortisone and parabiosis—pregnant to pregnant	12	1	11·0

surgery to other rats in a manner simulating parabiosis (Group 6). These rats were thus subjected to the stress caused by a loss of freedom of movement, but not to surgical and other stresses of parabiosis. Of the nine pregnant rats so treated, seven were pregnant on Day 8, indicating that the stress caused by loss of freedom of movement did not account for the observed interference with pregnancy in rats in parabiosis.

Parabiosis may involve joining only the skin flaps of the two rats, in which case there is little circulation of blood between them, or it may also involve the cut surfaces of muscle, in which case there is a continual exchange of blood between the members (Wilson, 1961). In the rats of Groups 2, 3 and 4, the skin flaps, cut muscle surfaces and peritoneal cavities were joined (coelioanastomosis). The effects of joining only the skin flaps of rats on Day 1 of pregnancy (Group 7) are compared in Table 1 with parabiosis which included both skin flaps and cut surfaces of muscle (Group 8), as well as coelioanastomosis (Group 4). All six rats (three pairs) joined in skin to skin parabiosis were pregnant on Day 8, while only three out of twelve rats (six pairs) which had cut surfaces of muscle joined were pregnant on Day 8.

The experiments described above indicate that neither surgical stress nor the stress caused by the loss of freedom of movement in parabiosis could, when administered individually, account for the loss of pregnancy in parabiotic rats. These experiments do not explore the combined effect of these stresses, nor do they provide information on other stresses which may result from the disturbance of homeostasis associated with parabiosis. The experiments of Robson & Sharof (1952) and of Velardo (1957) indicate that excess adrenal activity may have a deleterious effect on pregnancy. To determine whether or not the adrenals of parabiotic rats were playing a role in the disturbance of pregnancy, the following experiments were performed. Rats were adrenalectomized on Day 1 of pregnancy and administered cortisone acetate daily at various specific doses to determine the amount required for the maintenance of normal pregnancy. As shown in Table 1, five rats of a group of seven maintained normal pregnancies after adrenalectomy and daily injections of 3 mg of cortisone acetate (Group 9). Group 10 in Table 1 consists of twelve rats

TABLE 2

EFFECT ON IMPLANTATION OF PARABIOSIS WITH ANOTHER PREGNANT RAT AT VARIOUS SPECIFIC TIMES IN PREGNANCY AND FOR VARIOUS LENGTHS OF TIME

Group No.	*Treatment*	*No. of rats surviving on Day 8*	*No. pregnant on Day 8*	*Mean implantation sites/ pregnant rat*
11	Parabiosis on Day 3 of pregnancy	6	0	–
12	Parabiosis on Day 4 of pregnancy	10	4	12·5
13	Parabiosis on Day 5 of pregnancy	6	6	12·3
14	Parabiosis on Day 1, separated after 24 hr	7	5	8·0
15	Parabiosis on Day 1, separated after 48 hr	7	3	9·7
16	Parabiosis on Day 1, separated after 72 hr	7	0	–

which were adrenalectomized and placed in parabiosis with another member of the same group (six pairs). Each rat was injected with 3 mg of cortisone acetate daily. Of the twelve rats in Group 10, only one had implantation sites on Day 8. Since the only adrenal hormone present was an amount found adequate to maintain pregnancy, the loss of pregnancy in parabiotic rats appears not to be related to adrenal hyperactivity.

The results of a series of experiments designed to determine the stage of pregnancy sensitive to parabiosis are shown in Table 2. None of the six rats (three pairs) joined in parabiosis with other pregnant rats on Day 3 of pregnancy was found to be pregnant on Day 8 of pregnancy. However, when five pairs of rats on Day 4 of pregnancy were joined in parabiosis (Group 12), four out of the ten rats were found to be pregnant. All six of the rats were pregnant on Day 8 in three pairs of rats joined in parabiosis on Day 5 of pregnancy (Group 13).

The relationship between the length of time the rats remain in parabiosis and interference with pregnancy is also shown in Table 2. Group 14 consists of rats on Day 1 of pregnancy joined in parabiosis for 24 hr with other rats on

Day 1 of pregnancy and then separated from the parabiotic union. Five of seven rats which survived had implantation sites on Day 8 of pregnancy. When the rats were maintained in parabiosis for 48 hr before being separated (Group 15), three out of seven which survived were found to have implantation sites on Day 8. When the time in parabiosis was increased to 72 hr (Group 16), no rats in a group of seven which survived were found to have implantation sites on Day 8.

In view of the failure of implantation to occur in rats placed in parabiosis on Day 1 of pregnancy, reproductive tracts were examined to determine whether or not ova were present. In four rats placed in parabiosis on Day 1 of pregnancy, ova were recovered on Day 8. These ova appeared to be normal, although at a stage of development normally occurring on Day 4 or Day 5 of

TABLE 3

RELATIONSHIP BETWEEN THE NUMBER OF DAYS PREGNANT RATS WERE MAINTAINED IN PARABIOSIS AND THE LENGTH OF THE GESTATION PERIOD

Rat No.	*Days in parabiosis*	*Days gestation delayed*
1	3	3
2	3	4
3	3	(Not pregnant)
4	3	(Not pregnant)
5	3	1
6	3	2
7	3	(Not pregnant)
8	3	4
9	3	4
10	8	(Not pregnant)
11	8	(Not pregnant)
12	8	(Not pregnant)
13	8	(Not pregnant)
14	8	(Not pregnant)

pregnancy. In a further four rats placed in parabiosis on Day 1 of pregnancy and killed on Day 4, thirty-three ova were recovered. Twelve were in the uterotubal junction, where they are normally found on Day 4 of pregnancy, but the remainder were still in the middle third of the Fallopian tubes. Nine of the thirty-three ova recovered were still in the 8-cell stage, eighteen were in the 4-cell stage and six were in the 2-cell stage. The ova in a normally pregnant rat at this time in pregnancy are in the 32- or 64-cell stage. The viability of the ova recovered from parabiotic rats on Day 4 of pregnancy was tested by transferring them to the uterine horns of rats made pseudopregnant 4 days earlier by mating to vasectomized males. Of twenty-five ova transferred, seven implanted normally.

The retarded, but otherwise normal, development of ova removed on Day 4 of pregnancy from rats which had been in parabiosis indicated that pregnancy at that time was retarded rather than lost. Table 3 records the results of an experiment in which rats established in parabiosis on Day 1 of pregnancy were

separated 3 or 8 days later and observed for the duration of gestation. Of the nine rats which survived the separation after 3 days of parabiosis, three did not have litters, and the remaining six delivered litters from 1 to 4 days later than is usual for our strain of rats (22 days). None of the five rats which survived after 8 days of parabiosis produced young.

It has been demonstrated that oestrogen is required for the implantation of ova (Psychoyos, 1962). In an attempt to determine whether or not delayed implantation in parabiosis results from inadequate support by the ovary, we placed eight rats in parabiosis on Day 1 of pregnancy and injected each rat with 0·1 μg of oestradiol on the afternoon of Day 4. Five of these eight rats had implantations on Day 8 of pregnancy.

TABLE 4

DECIDUOMA FORMATION ON DAY 8 IN RATS PLACED IN PARABIOSIS ON DAY 1 OR DAY 3 OF PSEUDOPREGNANCY AND IN NON-PARABIOTIC RATS

Parabiotic rats

Day of parabiosis	*No. of pairs*	*Mean weight (g) ± S.E.* *A Control horn*	*B Stimulated horn*	*Ratio B/A**
Day 1	5	126·9 ± 19	486·4 ± 41	3·8/1
Day 3	5	122·3 ± 6	656·9 ± 56	5·4/1

Non-parabiotic rats

No. of rats	*Mean weight (g) ± S.E.* *A Control horn*	*B Stimulated horn*	*Ratio B/A**
6	137·7 ± 8	942·0 ± 69	6·8/1
6	132·2 ± 5	878·7 ± 72	6·7/1

* Calculated as the mean of the ratio for each rat.

In view of the finding in parabiotic rats of ova capable of development when transferred to normal recipients, an experiment was performed to compare the size of the decidual response in pseudopregnant parabiotic rats and normal pseudopregnant controls. The results in Table 4 show that the average decidual response was 3·8 when parabiosis was effected on Day 1 of pseudopregnancy and 6·8 in non-parabiotic controls ($P<0·001$). When parabiosis was established on Day 3 of pseudopregnancy, the decidual response was 5·4 in the parabiotic rats and 6·7 in the controls ($P<0·001$). It seems clear that parabiosis partially inhibits the normal decidual reaction, and that inhibition is greater if parabiosis is established on Day 1 of pseudopregnancy than it is if parabiosis is established on Day 3 of pseudopregnancy ($P<0·01$).

As a result of parabiosis on Day 1 of pregnancy, the development of ova is retarded, and the maternal reproductive tract fails to implant them. Either removal of the rats from parabiosis on Day 3, or the administration of oestradiol on the afternoon of Day 4 to rats maintained in parabiosis, results in a re-establishment of a normal pregnancy. These results suggest that the ovary is not

functioning adequately to maintain the pregnancy, but further experiments are required to understand the nature of the interference of parabiosis with the normal course of pregnancy.

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