

MECHANISM OF PERMANENT ESTRUS IN RATS AFTER
TRANSPLANTATION OF THE OVARIES INTO A
HYPOTHERMIC ENVIRONMENT

P. A. Vunder and M. D. Smetanina

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Disturbance of the sex cycle in the form of permanent estrus (PE) is observed in rats in several cases, for example, after round the clock illumination [9], neonatal androgenization [4], aging [6], and after transplantation of the ovaries into the tail [1]. The present writers found that PE readily arises in adult rats after autotransplantation of the ovaries into the conchae auriculae, i.e., into a hypothermic environment.

The establishment of PE after neonatal androgenization has been shown to be preceded by a period with normal cycle lasting many weeks [5, 11]. The impression is created that the onset of PE is facilitated once the cyclic center has reached a certain age.

The object of this investigation was to study the role of the animal's age in the appearance of PE after transplantation of the ovaries in the conchae auriculae, and also to study the mechanism of establishment of PE after such an operation.

EXPERIMENTAL METHOD

Experiments were carried out on two groups of noninbred albino rats: 1) adult animals with a normal estrous cycle, with a mean body weight of 220-240 g, and 2) infantile, with body weight of 57-60 g. Under pentobarbital (or less frequently, ether) anesthesia both ovaries were removed and transplanted into the conchae auriculae or beneath the capsule of both kidneys. In control rats, a skin incision was made on the dorsal surface under anesthesia.

In a separate series of experiments exchange operations were performed, i.e., the ovaries of adult rats were transplanted into the conchae auriculae or beneath the renal capsule of infantile animals and, conversely, ovaries of infantile animals were transplanted into the conchae or into the kidney of adult females. To study hormonal functions of the ovary grafted into the concha auriculae, the degree of compensatory hypertrophy of the ovary remaining *in situ* (only one ovary was transplanted) was investigated. In all animals the sex cycle was monitored by examination of vaginal smears. The rats were decapitated at the end of the experiment. The transplanted ovaries, the uterus, and pituitary gland were weighed. The ovaries were investigated histologically (fixation in Bouin's fluid, embedding in paraffin wax).

EXPERIMENTAL RESULTS

Experiments on Adult Females. Autografting of the ovaries into the conchae auriculae led to the appearance of vaginal smears of diestrous character for 5 days. Starting with the 6th day permanent vaginal estrus was established and it continued throughout the period of observation. After transplantation of the ovaries beneath the renal capsule, a normal sex cycle was established starting with the 6th day after the operation. Only the estrous stage was lengthened a little (2.39 ± 0.17 compared with 1.48 ± 0.11 days in animals of the control group).

The experiments with autografting of the ovaries into the conchae auriculae of adult female rats were repeated. The results confirmed that 5-6 days after the operation the animals went into PE. In our experiments, persistent estrus was observed for 200 days.

Department of Physiology of Man and Animals, N. G. Chernyshevskii Saratov University.
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TABLE 1. Compensatory Hypertrophy of Right Ovary after Removal of Left or Its Autotransplantation into Concha Auriculæ

Character of procedure	Number of animals	Body weight, g	Weight of ovaries, mg/100 g		
			left, at time of transplantation or removal	right	increase, %
Left-sided castration	21	244	16,90±0,73	24,28±0,09 $P<0,001$	43,67
Autografting of left ovary into concha auriculæ	21	233	17,15±1,47	21,19±0,93 $P<0,025$	23,56

Legend. Reaction of intact ovary was determined 20 days after transplantation of the other ovary.

Permanent vaginal estrus induced by transplantation of the ovaries into the conchæ auriculæ was accompanied in most rats (80%) by signs of psychological estrus. During mating with males the females exhibited typical sexual behavior: they responded by lordosis to introduction of the males.

Since autografting of the ovaries beneath the renal capsule did not induce PE it can be tentatively suggested that the latter is the result of transplantation of the ovaries into a hypothermic environment, although other particular features distinguishing the conchæ auriculæ as transplantation site likewise cannot be ruled out. PE also was readily induced by transplantation of only one ovary into the concha auriculæ if the other ovary was removed. In that case the phenomenon appeared on average after 7 days. Autopsy of the animals showed that the transplanted ovaries were below their initial weight, especially if transplanted into the conchæ auriculæ. For instance, 20 days after autografting the weight of the ovaries was reduced by 62%. The weight of the uterus in the experimental animals was normal. In rats with PE the weight of the pituitary was significantly increased: by 20% 20 days, and by 39% 200 days after autografting of both ovaries; 80% of ovaries transplanted into the conchæ auriculæ compared with those transplanted into the kidney did not contain recent corpora lutea. Very large follicles were found in 60% of ovaries.

Experiments on Infantile Animals. When the ovaries were autografted into the conchæ auriculæ of young rats opening of the vagina and the first estrus began 10 days after the operation. In control rats and also in animals whose ovaries were transplanted beneath the kidney capsule, these parameters of sexual maturation did not appear until the 18th day. Transplantation of the ovaries into a hypothermic environment thus stimulated sexual maturation. In all animals of these three groups a normal sex cycle was established. As the rats whose ovaries were transplanted into the conchæ auriculæ reached adulthood, the duration of the estrus stage began to lengthen gradually. For instance, during the first 8 weeks after the operation the duration of estrus was 2.00 ± 0.11 days (1.68 ± 0.07 days in the control). During the next 8 weeks the duration of estrus was 2.23 ± 0.12 and 1.70 ± 0.10 days, respectively. PE was established 16 weeks after transplantation of the ovaries into the conchæ auriculæ, i.e., when the body weight of the animals reached 220-230 g.

The response of female rats to autografting of the ovaries into the conchæ auriculæ thus differed in infantile and adult animals. The presence of PE, due to transplantation of the ovaries into a hypothermic environment, cannot be regarded as the result of hypersecretion of estrogens. Such an explanation is contradicted by the considerable decrease (by 61-63%) in weight of the transplanted ovary. Moreover, to induce PE it was sufficient to transplant only one ovary into the concha auriculæ, having removed the other. Secretion of androgen by the transplanted ovary [10] is a further indication of weakening of estrogen synthesis.

Finally, a reduction in estrogen production by the ovary when transplanted into the concha auriculæ is further confirmed by hypertrophy of the other ovary, left *in situ*. This hypertrophy, however, was less than that obtained after total removal of one ovary (Table 1).

It can be concluded from all the facts described above that ovaries, transplanted into the concha auriculæ, are characterized by reduced estrogen biosynthesis, although they do give rise to the PE phenomenon. Incidentally, a decrease in the excretion of total estrogens and an increase in the excretion of estriol were found previously [2] in rats in which persistent estrus was induced after transplantation of the ovaries into the tail.

What can the mechanism of establishment of PE be after autotransplantation of the ovaries into a hypothermic environment? What can lie at the basis of age differences in the response to such an operation?

In our opinion, in adult rats when secretion of estrogen by the transplanted ovary is insufficient the threshold of stimulation of the center for the sex cycle is not reached. Positive feedback from the estrogen is not triggered. Ovulation does not take place. Estrogen secretion becomes continuous, although inadequate. PE is established. Absence of response of the center for the cycle may also be due to an increase in androgen secretion by ovaries transplanted into the conchae auriculae [10]. Injection of testosterone at the diestrus II stage is known to inhibit the onset of ovulation [3]. This inhibition is based on blockade of preovulatory secretion of luteinizing hormone (LH) [8]. According to data in the literature [5] testosterone propionate reduces LH secretion induced by estradiol benzoate. Potentiation of androgen secretion by ovaries grafted into the conchae auriculae could thus be responsible for the absence of ovulation and for the appearance of persistent estrus.

In the writers' view, the center for the sex cycle in infantile rats is evidently more sensitive to the stimulating action of estrogen than in adult individuals. In infantile females, it can be tentatively suggested, despite reduced estrogen production and potentiation of androgen secretion by the ovary transplanted into the concha auriculae, the threshold for stimulation of the center for the sex cycle is nevertheless reached. Ovulation begins and a normal sex cycle is established. With the development of adulthood the sensitivity of the center for the sex cycle to the stimulating action of estrogen begins to fall and the quantity of estrogen secreted by the ovary transplanted into the concha auriculae is no longer sufficient to excite the center. Persistent estrus accordingly arises.

If this explanation of age differences in the response of the ovary to transplantation into the conchae auriculae is correct it can be expected that these differences will persist when exchange transplantations are carried out, i.e., when ovaries of infantile rats are transplanted into adult females and ovaries of adult rats into sexually immature animals. This expectation was fully vindicated. In adult rats in which, after castration, the ovaries of infantile animals were transplanted into the conchae auriculae, PE was established 10 days after the operation, just as in adult rats when their own ovaries were transplanted into the ears.

In the experiment in which the ovaries of adult females were transplanted into the conchae auriculae of infantile females, the first estrus began 29.5 ± 4.1 days later, and thereafter a normal sex cycle was established. However, after 10-11 weeks PE was established. The animals had, as it were, grown up. Exchange transplantations of ovaries beneath the renal capsule were followed in all cases by establishment of a normal sex cycle.

Exchange transplantations of the ovaries into the conchae auriculae thus confirmed the role of the recipients' age and not that of the transplanted ovaries in determining the specific character of the response to transplantation of the gonads into a hypothermic environment.

The results of these experiments, in the writers' view, are evidence in support of their hypothesis that age differences exist in the sensitivity of the center for the sex cycle to the stimulating action of estrogen.

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