COMPENSATORY OVARIAN GROWTH AND COMPENSATORY OVULATION AFTER UNILATERAL OVARIECTOMY IN RATS WITH AN OVARIAN AUTOGRAFT IN THE REGION OF THE PORTAL VEIN

By

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ABSTRACT

Compensatory growth and the compensatory increase of the number of ovulations in the remaining ovary after unilateral ovariectomy were compared in three groups of rats:
I. Rats with no ovarian graft. II. Rats with an ovarian autograft in the region of the portal vein. III. Rats with an ovarian autograft in a region draining into the general circulation.
Compensatory growth was not inhibited by ovarian grafts placed in the region of the portal vein, whereas this was inhibited with grafts of the same volume but draining into the general circulation. This finding supports the view that compensatory ovarian growth after unilateral ovariectomy is at least partially the result of an increase in the gonadotrophin level in the blood, following the decrease in oestrogen level.
In contrast, the total number of ovulations did not differ significantly in the three groups. The number of ovulations in the ovary in situ was in most cases decreased, when ovulations had occurred in the grafts, even in animals with a graft in the region of the portal vein. These findings are discussed.

After unilateral ovariectomy the remaining ovary shows compensatory growth and a compensatory increase in the number of ovulations. Two mechanisms have been suggested to explain these phenomena: (1) consumption or inactivation of an increased proportion of an unchanged amount of circulating gonadotrophins by the one remaining ovary. This has been defended by*

* The author thanks Prof. Dr. J. Moll for his active interest and helpful advice.
McLaren (1966), who found no increase in the gonadotrophin concentration of the hypophysis after unilateral ovariectomy, and Zarrow et al. (1965) (2) a compensatory increase in the output of gonadotrophins from the pituitary gland as the result of a decreased oestrogen secretion following the removal of one ovary (Edgren et al. 1965). Since such an increase in the output of gonadotrophins from the pituitary gland has recently been reported (Grady & Greenwald 1968; Benson et al. 1969), it was considered of interest to test the consumption theory again. The compensatory phenomena have been compared in three groups of unilaterally ovariectomized rats. I. Rats with no ovarian graft. II. Rats with an ovarian graft in the kidney or ovarian bursa. III. Rats with an ovarian graft in the vena porta region. This last type of graft in the adult rat contributes only a very small amount of oestrogens to the general circulation because of the metabolizing activity of the liver (Donovan & O'Keeffe 1966; Ber 1968). If the consumption theory is correct, a similar inhibition of the compensatory phenomena would be expected in rats with both types of grafts. McLaren's (1966) data from a comparable experiment in mice did not provide any conclusive evidence. This she ascribed to the rapid degeneration of the grafts.

MATERIALS AND METHODS

The experiments were performed on adult female rats of the inbred R strain of the Netherlands Cancer Institute Amsterdam. Rats were maintained in groups of five per cage with food and water ad lib. The rat room was illuminated from 6.00 a.m. to 8.00 p.m. Vaginal smears were taken daily for at least two weeks before the operations until the animals were killed. Operations and autopsies were always performed on the day of vaginal oestrus (almost all cells were cornified). All animals had a regular 5 day cycle before and after operation.

Most of the rats were unilaterally or bilaterally ovariectomized and received an ovarian autograft. In a first experiment the grafts were placed either under the kidney capsule or in the spleen. Since ovarian grafts in the spleen had a somewhat abnormal appearance with distinct fibrosis and a reduced number of small follicles and corpora lutea, a second experiment was performed in which the grafts were placed in the ovarian bursa instead of under the kidney capsule and in the greater omentum instead of in the spleen. These grafts proved to be histologically comparable. The term ovary in situ is used only for untouched in situ ovaries, not for grafts in the ovarian bursa.

In preliminary experiments it was found that during the first three cycles after unilateral ovariectomy the weight of the remaining ovary increased by 50% (see also Peterson et al. 1964). During the following cycles the increase in weight was small. On the other hand, a decrease in the number of ovulations (eventually leading to a complete absence of ovulations) was observed in ovarian grafts of unilaterally ovariectomized rats during the first five cycles after the operation. Three cycles after the operation there was both a distinct compensatory ovarian growth and an active graft. Therefore the animals were killed in the third vaginal oestrus after operation.
After killing the ovaries in situ and uteri were weighed wet. The weight of the grafts could not be determined accurately since it was very difficult to clean them from the adjacent tissue without causing damage which might cause difficulties in the histological studies. Ovaries in situ and grafts were fixed in Bouin's fluid, embedded in paraffin and sectioned. The sections were stained with haematoxylin and cosin and used for counting the number of fresh corpora lutea.

All data of animals in which adhesions were found between the grafts in the vena porta region and the body wall or periovarian fat, were discarded.

The statistical analysis of the results was performed using Wilcoxon's two sample test. A difference was considered as statistically significant, if the double tail probability \( \leq 0.01 \). The ovarian and uterine weights in the tables are given per 100 g bodyweight. Statistical evaluation of the results, however, leads to the same conclusions when absolute weights are compared.

**OBSERVATIONS**

**Experiment 1: Comparison of the effect of ovarian grafts of different sizes in the spleen or the kidney on compensatory ovarian growth and ovulation**

After the survival period of three cycles, the ovaries of unilaterally ovariectomized rats showed a mean increase in weight of about 50%. The mean number of corpora lutea showed a two fold increase (Table 1, groups 1 and 2).

The inhibitory effect of ovarian grafts in the kidney on compensatory growth of the ovary in situ was very marked, when 1/1 or 1/2 ovary was grafted. When, however, 1/10 ovary was grafted to the kidney we found no inhibition. Absence of inhibition was also found with grafts in the spleen independently of their volume. Rough estimations of the weight of the grafts yielded values of 8–11 mg when 1/1 or 1/2 ovary was grafted and 0.5–2 mg when 1/10 ovary was grafted. We did not find a tendency for kidney ovaries to have a higher weight than spleen ovaries. Rough estimates of the volume of the grafts from histological sections too did not show any difference between spleen and kidney ovaries.

The total number of fresh corpora lutea per animal (in in situ and, if present, in grafted ovaries) did not differ significantly between the control and experimental groups, although a considerable variation was observed. The number of fresh corpora lutea in the ovaries in situ was significantly higher without grafted ovaries than with 1/1 or 1/2 ovary grafted to the kidney or 1/1 ovary grafted to the spleen (Table 1, groups 2, 3, 4 and 6).

The mean uterine weight was significantly higher in the groups with spleen ovaries than in the other groups.

**Experiment II: Comparison of the effect of ovarian grafts in ovarian bursa and greater omentum on compensatory ovarian growth and ovulation**

The histological appearance of ovarian grafts approached the normal con-
Table 1.

Inhibitory effects of ovarian autografts (of different sizes) in the kidney or in the spleen on compensatory growth and ovulation of the right ovary 3 cycles after removal of the left ovary.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Mean weight ± se of the right ovary (mg per 100 g bodyweight)</th>
<th>Mean number of fresh corpora lutea ± se in the right ovary in situ in the graft</th>
<th>Mean weight ± se of uterus (mg per 100 g bodyweight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Intact animals</td>
<td>10</td>
<td>19.6 ± 0.8</td>
<td>5.7 ± 0.9</td>
<td>—</td>
</tr>
<tr>
<td>2) Unilaterally ovariectomized animals</td>
<td>10</td>
<td>29.4 ± 2.6</td>
<td>10.6 ± 1.2</td>
<td>—</td>
</tr>
<tr>
<td>3) Unilaterally ovariectomized animals with 1/1 ovary grafted to the kidney</td>
<td>6</td>
<td>23.3 ± 2.3</td>
<td>7.2 ± 1.8</td>
<td>3.2 ± 1.1</td>
</tr>
<tr>
<td>4) Same, 1/2 ovary grafted to the kidney</td>
<td>6</td>
<td>23.5 ± 2.5</td>
<td>6.7 ± 3.1</td>
<td>2.0 ± 1.9</td>
</tr>
<tr>
<td>5) Same, 1/10 ovary grafted to the kidney</td>
<td>6</td>
<td>28.0 ± 2.8</td>
<td>10.5 ± 1.9</td>
<td>1.8 ± 1.6</td>
</tr>
<tr>
<td>6) Same, 1/1 ovary grafted to the spleen</td>
<td>6</td>
<td>31.4 ± 4.1</td>
<td>8.0 ± 1.1</td>
<td>1.5 ± 1.0</td>
</tr>
<tr>
<td>7) Same, 1/2 ovary grafted to the spleen</td>
<td>6</td>
<td>29.2 ± 4.8</td>
<td>9.8 ± 1.4</td>
<td>0.5 ± 0.8</td>
</tr>
<tr>
<td>8) Same, 1/10 ovary grafted to the spleen</td>
<td>6</td>
<td>28.9 ± 4.9</td>
<td>10.0 ± 1.4</td>
<td>0.6 ± 0.3</td>
</tr>
</tbody>
</table>

1) All animals weighed 130–150 g.
2) No significant differences were found between weights and ovulation rates of right and left ovaries.
3) Significantly different from data of groups 1, 2, 5, 6, 7 and 8.
4) Significantly different from data of groups 1, 2, 5, 7 and 8.
5) Significantly different from data of groups 1, 2, 3, 4 and 5.
dition more closely in the greater omentum than in the spleen. Nevertheless
the number of fresh corpora lutea was somewhat lower in the ovaries in the
omentum than in the ovaries grafted to the ovarian bursa. This difference,
however, was not significant. Moreover rough estimates of the weight and
volume of both types of grafts showed no significant differences. The results
are given in Table 2. This experiment confirmed the three major results of
the first experiment: (1) an ovarian graft in the vena porta region did not
inhibit compensatory ovarian growth, whereas this occurred with ovarian
grafts draining into the general circulation. This effect was quantitatively
small, but statistically convincing. (2) Both types of grafts are able to »inhib-
it« to some extent the increase in the number of ovulations in the ovary
in situ after unilateral ovariectomy. (3) The mean uterine weight is signi-
ficantly higher in unilateral ovariectomized animals bearing an ovarian graft
in the vena porta region than in animals with no ovarian graft or with a graft
draining into the general circulation.

In order to get more information about the oestrogen metabolizing activity
of the liver we included two groups of bilaterally ovariectomized rats, one
with no graft and one with a graft in the greater omentum. The grafts were
large and looked healthy after a survival period of 15 days. Nevertheless, none
of the animals showed vaginal cornification during the postoperative period.
On the other hand, the mean uterine weight in bilaterally ovariectomized
rats bearing an ovarian graft in the vena porta region was higher than in bilaterally ovariectomized rats with no graft. In both groups, however, the
mean uterine weight was markedly decreased in comparison with the other
groups in Table 2.

DISCUSSION

As pointed out in the introduction we tried to decide between the two sug-
gested mechanisms of compensatory growth and ovulation after unilateral
ovariectomy. It seemed reasonable to assume that otherwise comparable ovarian
grafts in the vena porta region and in a region draining into the general cir-
culation would have the same inhibitory effect on the compensatory growth
and ovulation providing that the consumption theory was correct but a dif-
ferent inhibitory effect if the feedback theory was correct.

Firstly it is desirable to make some remarks on the testsystem used. The
system has to fulfil two conditions (1) that both types of grafts are fully
comparable and (2) that grafts in the vena porta region contribute little or no
oestrogens to the general circulation, and in consequence do not exert a major
feedback action on the hypothalamo-hypophyseal system. The first condition
is not fulfilled in the case of ovarian grafts in the kidney and spleen since

Acta endocr. 65, 3
**Table 2.**

Effects of ovarian autografts (of a whole ovary) in the ovarian bursa or greater omentum on uterine weight, compensatory growth and ovulation of the right ovary 3 cycles after removal of the left ovary or 15 days after removal of ovaries.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Mean weight ± SE of the right ovary (mg per 100 g bodyweight)</th>
<th>Mean number of fresh corpora lutea ± SE in the right ovary in situ in the graft</th>
<th>Mean uterine weight ± SE (mg per 100 g bodyweight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Unilaterally ovariectomized animals</td>
<td>10</td>
<td>24.9 ± 1.9</td>
<td>10.8 ± 0.74</td>
<td>223 ± 7</td>
</tr>
<tr>
<td>2) idem + ovary grafted to the ovarian bursa</td>
<td>10</td>
<td>21.1 ± 1.53</td>
<td>8.6 ± 0.4</td>
<td>1.9 ± 1.7</td>
</tr>
<tr>
<td>3) idem + ovary grafted to the greater omentum</td>
<td>10</td>
<td>24.8 ± 2.1</td>
<td>9.4 ± 0.4</td>
<td>1.1 ± 1.0</td>
</tr>
<tr>
<td>4) bilaterally ovariectomized3)</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5) bilaterally ovariectomized3) + ovary to the greater omentum</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) All animals weighed 155–180 g.
2) Since these animals were not cycling, they were killed on the 15th day after operation (comparable with 3 cycles).
3) Significantly different from values of the groups 1 and 3.
4) Significantly higher than the numbers of groups 2 and 3.
5) Significantly higher than values of groups 1 and 2.
6) Significantly lower than values of groups 1, 2 and 3.
7) Significantly higher than that of group 4.
ovaries in the spleen showed a distinct fibrosis (see also Biskind et al. 1950). Omentum and bursa ovaries, on the other hand, seemed to be fully comparable in this respect. With regard to the second point it may be remarked that Ber (1968) and many other investigators found an effect of ovarian grafts in the vena porta region of bilateral ovariectomized rats on uterine weight, indicating that some oestrogens pass through the liver. We found the same effect in both bilaterally and unilaterally ovariectomized rats. Some of the investigators mentioned by Ber (1968), however, conclude from the progressive tumour-formation in ovarian grafts in the spleen of bilaterally ovariectomized rats, that there is no major feedback action by these oestrogens on the hypothalamo-hypophyseal system. This may be due to two factors, i.e. either the higher threshold of this system for oestrogens or the fact that the oestrogens which pass through the liver produce metabolites which influence the uterus but not the hypothalamo-hypophyseal system. Byrnes & Meyer (1951a,b), Maekawa & Imai (1954) and Miyake (1961) showed that in immature rats more oestrogen was required to produce positive stimulation of the uterus than to inhibit the pituitary gland; the evidence for a similar differential in adult rats, however, was equivocal (Byrnes & Meyer 1951b). On the basis of these considerations we believe that the test system allows of valid conclusions.

In this system the ovarian weight seems to be a much more sensitive parameter for the gonadotrophin level than the number of ovulations. The ovarian weight adds the responses of all types of ovarian tissue over a period of many days, whereas the number of ovulations gives information on the gonadotrophin secretion over a few days only. Compensatory growth will therefore first be considered. Ovarian grafts in the vena porta region did not inhibit compensatory growth of the ovary in situ, whereas inhibition occurred with comparable grafts draining into the general circulation. This indicates that compensatory ovarian growth depends on diminution of the amount of oestrogen producing ovarian tissue and not on the diminution of the amount of gonadotrophin consuming ovarian tissue. This excludes the first concept, mentioned in the introduction, as a plausible mechanism of compensatory ovarian growth. It is in agreement with the findings of Benson et al. (1969) that unilateral ovariectomy induces an increase in gonadotrophin levels and ovarian weight, which can be reduced by oestrogen injections.

The total number of fresh corpora lutea, indicating the number of ovulations, is the same in unoperated and unilaterally ovariectomized rats. From this it can be argued that the increase in gonadotrophin levels after unilateral ovariectomy is too small to cause more ovulations than occurs normally.

The distribution of the ovulations between the ovaries in situ and grafts is the same in unilaterally ovariectomized animals, whether they bear an ovarian graft in the vena porta region or in a region draining into the general circulation. The most plausible explanation for this finding is that the hormonal
feedback agent of large preovulatory follicles is not important for the determination of the number of ovulations. This last suggestion may be correct, provided that Greenwald's (1962) finding in the golden hamster, that the number of ovulations depends on the FSH level in the blood in the first days of the cycle, is also true for rats.

This interpretation of our findings comes very close to the view of McLaren (1966) and suggests that the increase in gonadotrophin levels after unilateral ovariection is not responsible for all the compensatory phenomena.

In conclusion we may say that in rats, the decrease in the oestrogen level of the blood after unilateral ovariection results in an increase in gonadotrophin levels, sufficiently large to cause an increase in ovarian weight but probably too small to cause an increase in the total number of ovulations.

REFERENCES


Received on January 21st, 1970.