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Changes in Ovary Following Unilateral Ovariectomy with or without "Ovarian Fragmentation" in Mice

With 1 Text-figure

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ABSTRACT Compensatory hypertrophy occurred in the remaining ovary of unilaterally ovariectomized (ULOX) mice, reaching a maximum level 90 days after the operation. However, the ovarian hypertrophy was not maintained after 120 postoperative days, reducing to the normal weight level after 180 postoperative days. In unilaterally ovariectomized, ovary-fragmented (ULOX-OF) mice, such hypertrophy never took place in the ovarian remnants during 180-day observation period. Histology of the remaining ovary of ULOX-OF mice revealed that interstitial cell hypertrophy and follicular hemorrhage appeared 90–150 days after the operation, while normal follicles and corpora lutea were lacking or decreased in number.

INTRODUCTION

Unilateral ovariectomy causes compensatory hypertrophy of the remaining ovary in rats (Emery, 1931; Takewaki, 1933; Florsheim *et al.*, 1967; Arai and Gorski, 1968; Welscher, 1970). Such ovarian hypertrophy, however, has been observed in rats only for a relatively short period (less than 3 months) following unilateral ovariectomy. For a longer period there are no observations of this phenomenon in rats and mice.

On the other hand, Bruzzone and Lipschutz (1954) and Lipschutz (1960) have demonstrated that subtotal ovariectomy ("ovarian fragmentation") gives rise to follicular hemorrhage, nodular formation of enlarged cells and luteoma-like lesion in the ovarian remnants of unilaterally ovariectomized, ovary-fragmented guinea pigs

and mice. These findings suggest that the ovarian fragmentation results in a disturbance of the feedback regulation between ovary and hypothalamo-hypophysial system. The present study, therefore, was planned to examine whether the compensatory hypertrophy takes place in the ovarian remnants of unilaterally ovariectomized, ovary-fragmented mice, and also whether it persists for a longer period in the remaining ovary of unilaterally ovariectomized mice.

MATERIAL AND METHODS

Female mice of C57BL/Tw strain at 2 months of age were divided into 3 groups of 31, 46 and 57 individuals, respectively. Mice of the first group were left intact for the normal controls. In the second group, the left ovary was removed under ether anesthesia; the right ovary was left intact during the observation period. The left ovary of the third group mice was removed, and then 3 fourths of the right ovary were removed, leaving one fourth of the tissue on the oviductal side ("ovarian fragmentation"). Vaginal smears were examined daily in mice of these groups. All mice were sacrificed 0, 30, 60, 90, 120, 150, and 180 days after the operation, respectively. At autopsy, ovaries and uteri were dissected out, weighed and fixed in Bouin's fluid. Sections, cut in paraffin at 7 μ, were stained with Delafield's hematoxylin and eosin. Six ovarian sections of the middle part of the ovary were photographed at 160 magnification, and the mean number of the interstitial cells gathering compactly without intercellular spaces were counted in 0.8×10^{-3} mm^2 area of the interstitial tissue. Index of the interstitial cell hypertrophy per mouse was calculated by the following formula: (mean number of the interstitial cells in 6 sections/0.8×10^{-3} mm^2)$^{-1}\times1{,}000$.

RESULTS

Weights of ovary and uterus in unilaterally ovariectomized, and unilaterally ovariectomized, ovary-fragmented mice.

In unilaterally ovariectomized (ULOX) mice, the contralateral ovary showed no significant weight change until 60 days after the operation (Fig. 1). The ovary was enlarged thereafter, culminating in weight on the 90th postoperative day. The enlarged ovary, however, was decreased in weight on the 120th day, abruptly. The difference in ovarian weight between mice on the 90th day and those on the 60th or 120th day was statistically significant. After 120 postoperative days, the ovary reached the weight level similar to that of a unilateral ovary of normal mice.

On the other hand, there was no significant weight change in the fragmented ovary of ULOX mice. No difference was also found in uterine weight between normal and ULOX or unilaterally ovariectomized, ovary-fragmented (ULOX-OF) mice (Table 1).

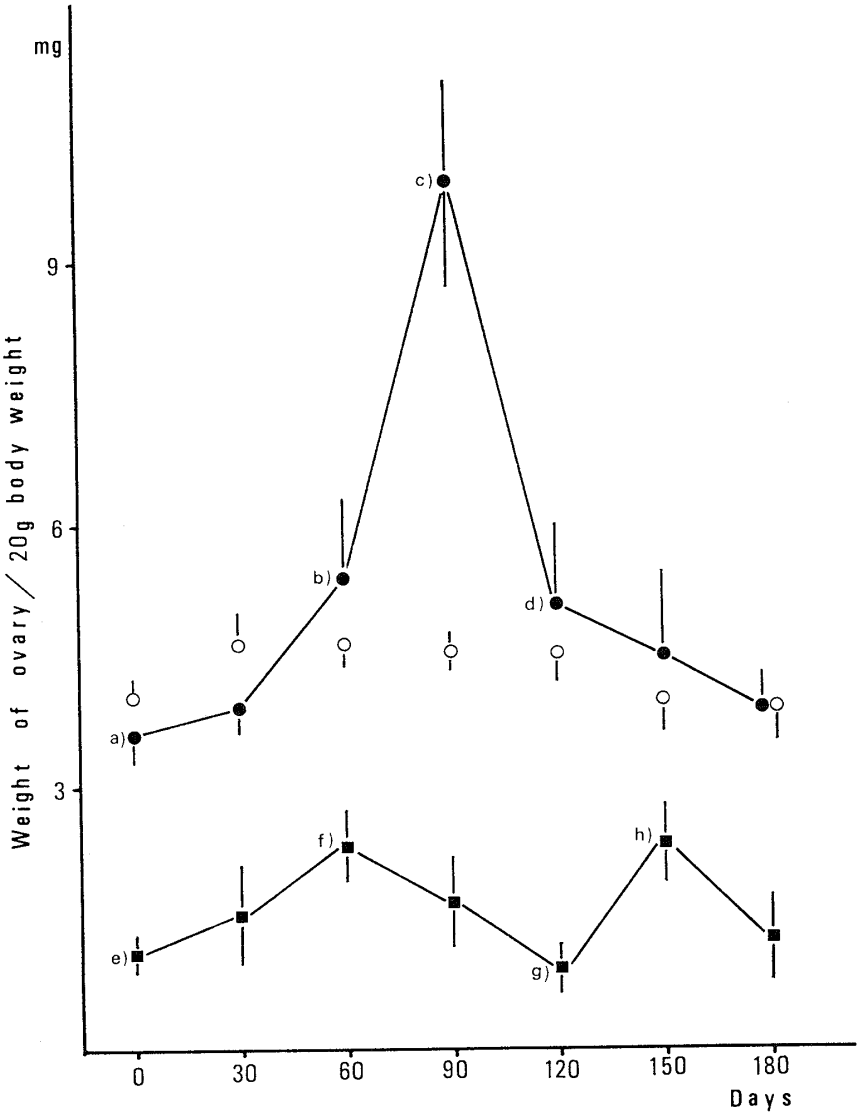

Fig. 1. Changes in weight of one of a pair of ovaries in normal (○), the remaining ovary in unilaterally ovariectomized (●) and unilaterally ovariectomized, ovary-fragmented (■) mice. Five to eleven mice were used for each of the mean ovarian weights as shown in Table 1. Bars indicate standard errors of the mean. b vs c, c vs d: $0.02<P<0.05$; a vs b, e vs f, f vs g, g vs h: non-significant.

Histology of ovary and uterus in unilaterally ovariectomized, and unilaterally ovariectomized, ovary-fragmented mice.

Daily examination of vaginal smears indicated that irregularly prolonged estrus and diestrus lasting for 5–14 days frequently appeared in ULOX and ULOX-OF mice. Ovaries of both ULOX and ULOX-OF mice showed no histological changes in follicles, corpora lutea and interstitial tissue until 60 days after operation. In ULOX-OF mice on the 90th postoperative day, atrophic change was observed in the

Table 1
Histology of ovary in normal, unilaterally ovariectomized and unilaterally ovariectomized, ovary-fragmented mice.

Group of mice	Days after operation	No. of mice	Weight of ovary (mg/20 g Body weight)	Weight of uterus	Ovarian histology
NR		6	4.5±0.20	62±8.8	F, CL
ULOX	30	7	3.9±0.24	47±16.1	F, CL
ULOX-OF		9	1.6±0.45	50± 6.4	F, CL
NR		5	4.5±0.17	53± 3.3	F, CL
ULOX	60	9	5.4±0.93	46± 3.3	F, CL
ULOX-OF		9	2.3±0.42	59± 6.2	F, CL
NR		5	4.4±0.14	55±13.7	F, CL
ULOX	90	8	9.9±1.26	63± 7.3	F, CL
ULOX-OF		10	1.7±0.45	42± 8.3	F, aCL, hypISC
NR		5	4.4±0.29	48± 9.6	F, CL
ULOX	120	7	5.1±0.93	47± 9.2	F, CL
ULOX-OF		10	0.9±0.24	39± 2.2	sF, hemF, hemCL, hypISC
NR		5	3.9±0.23	51± 7.2	F, CL
ULOX	150	7	4.5±1.02	64±15.2	sF, aCL, shypISC
ULOX-OF		11	2.3±0.42	45± 4.6	hemF, hypISC
NR		5	3.8±0.36	60±17.7	F, CL
ULOX	180	8	3.9±0.48	53± 8.8	sF, hemF, shypISC
ULOX-OF		8	1.2±0.51	47± 7.2	hemF, hypISC

NR: Normal mice, ULOX: unilaterally ovariectomized mice, ULOX-OF: ULOX and ovary-fragmented mice, F: Varying sizes of matured follicles, sF: Small follicles with antra, hemF: Hemorrhagic follicles, CL: Varying sizes of well-developed corpora lutea, aCL: Atrophic corpora lutea, hemCL: Hemorrhagic corpora lutea, hypISC: Hypertrophied interstitial cells, shypISC: Slightly hypertrophied interstitial cells.

luteal tissue, resulting in a decrease in size and number of corpora lutea. The interstitial cells were hypertrophied as shown by the hypertrophy index (Table 2).

On the 120th day, relatively large follicles and corpora lutea underwent hemorrhage in ULOX-OF mice, although a few small normal follicles with antra were still present in the fragmented ovary with hypertrophied interstitial tissue (Table 1). The ovaries of ULOX mice were similar to those of normal mice on the 120th day.

In ULOX mice on the 150th day, small normal follicles and atrophic corpora lutea were present in the ovaries with slightly hypertrophied interstitial tissue. By contrast, the fragmented ovaries contained only a few hemorrhagic follicles and hypertrophied interstitial tissue, lacking normal follicles and any type of corpora lutea on the 150th day.

On the 180th day, the fragmented ovaries were similar to those on the 150th day; the ovaries of ULOX mice lacked corpora lutea, consisting of small normal follicles, varying sizes of hemorrhagic follicles and slightly hypertrophied interstitial

Table 2
Index of ovarian interstitial-cell hypertrophy in normal, unilaterally ovariectomized and unilaterally ovariectomized, ovary-fragmented mice.

Group of mice	Index of interstitial-cell hypertrophy (Days after operation)				
	60	90	120	150	180
NR	1.70 ± 0.10 (5)	1.68 ± 0.11 (5)	1.61 ± 0.07 (5)	1.80 ± 0.05^{g} (5)	1.63 ± 0.04^{j} (5)
ULOX	1.74 ± 0.09^{a} (9)	1.63 ± 0.06^{c} (8)	1.78 ± 0.09^{e} (7)	2.00 ± 0.11^{h} (7)	1.96 ± 0.10^{k} (8)
ULOX-OF	1.95 ± 0.05^{b} (9)	2.34 ± 0.18^{d} (10)	2.53 ± 0.12^{f} (10)	2.40 ± 0.11^{i} (11)	2.77 ± 0.12^{l} (8)

Numbers of mice are given in parentheses. c vs d: $0.02<P<0.05$; e vs f, h vs i, k vs l: $P<0.01$; a vs b, g vs h, j vs k: non-significant (Student's t test).

tissue. From the 90th day, the interstitial cells of ULOX-OF mice always showed significantly higher hypertrophy index than those of ULOX mice (Table 2).

Uteri of all ULOX and ULOX-OF mice persistently possessed the high columnar epithelium and well-developed glands from the 120th day as those of normal estrous mice did.

Discussion

It has been accepted that compensatory hypertrophy occurs in the remaining ovary of unilaterally ovariectomized (ULOX) rats, resulting from a newly established, feedback regulation between the remaining ovary and the hypothalamo-hypophysial system (Welscher, 1970; Marton and Endröczi, 1974). The present study, however, indicated that a unilateral ovary of mouse lost the responsiveness to the lack of the contralateral ovary 120–180 days after the unilateral ovariectomy, whereas the remaining ovary was hypertrophied to a maximal level 90 days after the operation. On the 180th postoperative day, ovarian weight of ULOX mice was reduced to the level of an ovary of normal mice. Thus, the compensatory hypertrophy was observed only within 120 postoperative days. The ovarian histology of ULOX mice revealed that on the 150th postoperative day, the interstitial cells became hypertrophic without development of follicles and corpora lutea, and that on the 180th day, hemorrhagic follicles appeared without luteinization. In this context, the present findings may suggest that the compensatorily established hormonal balance following unilateral ovariectomy is not able to persist beyond a period of 4 months, resulting in an impairment of normally regulated gonadotropin secretion.

Dunlap *et al.* (1972) and Marton and Endröczi (1974) demonstrated that in neonatally androgenized rats whose ovaries secrete estrogen constantly, unilateral ovariectomy caused less degree of compensatory hypertrophy than in neonatally

non-treated ULOX rats. According to Kawashima (1960, 1977) and Aschheim (1976), the hypothalamo-hypophysial system of normal female rats lowers the responsiveness to estrogen after a long-lasting exposure to estrogen. From these results, it is conceivable that in ULOX mice, the responsiveness of hypothalamo-hypophysial system to estrogen was altered when the system had been exposed to estrogen secreted abnormally from the remaining ovary for more than 3 months as demonstrated by the vaginal-smear observation.

In contrast to the unilateral ovary of ULOX mice, the ovarian remnants of ULOX, ovary-fragmented (OF) mice never responded compensatorily to the reduction of 7 eighths of a pair of ovaries. The remaining tissue showed hypertrophy of the interstitial cells and atrophy of corpora lutea as early as on the 90th day, and then hemorrhage of follicles on the 120th day. After 150 postoperative days, the ovarian remnants lost normal follicles and any type of corpora lutea, containing only hemorrhagic follicles and strongly hypertrophied interstitial cells. These results are in accord with those in ULOX-OF mice reported by Lipschutz (1960), although the luteoma-like lesion was not detected within 6 postoperative months. The histological observation also indicated that changes in the ovarian tissue appeared in ULOX-OF mice earlier than in ULOX mice, suggesting the earlier impairment of hormonal regulation in the hypothalamo-hypophysio-gonadal system.

The present results, therefore, conclusively indicate that a unilateral ovary of ULOX mice is able to respond to the lack of the contralateral ovary only for a certain period but not capable of maintaining the response for a longer period, and also that a small amount of ovarian tissue has no capability to respond compensatorily to the subtotal ovariectomy, resulting instantly in the failure of restoring the impaired hormonal balance.

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REFERENCES

Arai, Y., and R. A. Gorski, 1968. *Endocrinology*, **82**: 871.
Aschheim, P., 1976. Everitt, A. V., and J. A. Burgess (eds.), Hypothalamus, Pituitary and Aging. C. C. Thomas Publ. Illinois, 376.
Bruzzone, S., and A. Lipschutz, 1954. *Brit. J. Cancer*, **8**: 613.
Dunlap, J. L., L. K. Preis Jr. and A. A. Gerall, 1972. *Endocrinology*, **90**: 1309.
Emery, F. E., 1931. *Physiol. Zool.*, **4**: 101.
Florsheim, W. H., P. Rudko, N. L. Corcorran and R. E. Bodfish, 1967. *Endocrinology*, **81**: 771.
Kawashima, S., 1960. *Annot. zool. Japon.*, **33**: 226.
———— 1977. *Zool. Mag., Tokyo*, **86**: 153.

Lipschutz, A., 1960. *Acta Unio Int. c. Cancrum*, **16**: 149.
Marton, I., and E. Endröczi, 1974. *Endokrinologie*, **63**: 409.
Takewaki, K., 1933. *J. Fac. Sci., Imp. Univ. Tokyo*, (IV), **3**: 129.
Welscher, R., 1970. *Acta endocr.*, **65**: 509.