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Steroids Production by Ovarian Non-functioning Tumors in Postmenopausal Women

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ABSTRACT. Higher serum levels of estrone, estradiol, progesterone and testosterone were observed with a high incidence in patients with ovarian non-functioning tumors in postmenopausal women. Postsurgical decreases of the serum levels of steroids in cases who underwent complete removal of tumor were found in all cases only in estradiol, in some cases in other steroids. The difference of steroid serum levels between cases with tumor volume >1000 cm^3 and <1000 cm^3 was statistically significant only in estrone. Serum levels of estrone, estradiol and value of urinary 17-KS in a case with marked hyperestrogenemia were not suppressed by dexamethasone and stimulated by hCG and decreased to normal levels after surgery. Positive localizations of estradiol and testosterone were seen not only in stromal cells but also in tumor cells. Ovarian tumor tissues which recurred in Douglas pouch in the case with hyperestrogenemia stated above and were considered possibly not to contain ovarian stromal cell produced higher levels of estrone and estradiol in the medium of tissue culture. These facts suggested that ovarian non-functioning tumors could produce estrogen, progesterone and androgen with a higher incidence, that production site of estradiol might be ovary and of estrone, progesterone and testosterone might be ovary in some cases and be ovary and/or extragonad in other cases and that tumor cell itself might be able to produce estrogen.

Key words : steroids production — ovarian non-functioning tumor — postmenopause

The cases of ovarian non-functioning tumors in postmenopausal women with endometrial hyperplasia suggesting estrogen production were reported by Hughesdon,[1] Brown *et al.*[2] and thereafter, many cases with endocrinological activity in ovarian non-functioning tumors are reported. The steroids production by ovarian non-functioning tumors had been discussed by clinical symptoms until the development of radioimmunoassay revealed the details of serum levels of steroids, but the accurate incidences of cases with higher levels of serum steroids and the exact site of steroids production remains still in arguments. The purpose of this paper is to clarify the more detailed features and the site of steroids production in ovarian non-functioning tumors.

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SUBJECTS AND METHODS

Twenty-one patiens of ovarian non-functioning tumors in postmenopausal women from 2 to 35 years after onset of menopause, with an average of 14.3 years, as shown in Table 1, were investigated. Serum levels of LH, FSH, estrone, estradiol, progesterone and testosterone in the cases were determined by radio-immuno assays before and after surgeries. The correlations of tumor volume, tumor malignancy and clinical stage with higher serum levels of steroids were evaluated. In a case of 71 years of age of mucinous cystadenoma of low potential malignancy with marked hyperestrogenemia and higher level of urinary 17-KS, dexamethasone suppression, hCG stimulation and hexesterol suppression tests were carried out to identify the site of estrogen production. Tumor tissues in paraffin section were immunohistochemically stained by PAP method and studied to evaluate the correlations of localizations of LH, FSH, estradiol and testosterone with higher serum levels of the hormones. The antibodies used were as follows: LH (monoclonal, Immunotech), FSH (monoclonal, Immunotech), estradiol (Immulok) and testosterone (Immulok). The tumor cells from the tumor tissues which recurred in Douglas pouch one year after hysterectomy with bilateral salpingooophorectomy in the same case of mucinous cystadenoma described above were cultured and the levels of estrogens in culture medium were determined to investigate the ability to produce estrogens.

TABLE 1. Subjects, years of age and years after onset of menopause

Tumor	No. of cases	Yrs. of age (Yrs. after menopause)
serous cystadenoma	3	78(35), 87(32), 53(2)
mucinous cystadenoma	1	82(32)
serous cystadenoma (low potential malignancy)	2	62(12), 61(5)
mucinous cystadenoma (low potential malignancy)	2	60(12), 71(31)
dermoid cyst	1	58(9)
fibroma	1	50(9)
Brenner tumor	1	70(22)
serous cystadenocarcinoma	5	69(31), 56(6), 60(10), 61(7), 56(3)
mucinous cystadenocarcinoma	4	68(13), 55(4), 55(3), 70(20)
mesonephroid carcinoma	1	53(3)
	21	

RESULTS

The serum levels of estrone and estradiol before surgery were shown in Fig. 1 (levels of estrone and estradiol of normal postmenopausal women were 43.1 ± 23.2 and 24.1 ± 20.2 pg/ml [mean$\pm$SD], respectively. $n=10$). The higher serum levels of only estrone were seen in 5 cases, of both estrone and estradiol in 8 cases and normal levels of both in 8 cases as shown in Fig. 1. The incidences of cases with higher estrone and estradiol were 13 of 21 cases, 61.9% and 8 of 21 cases, 38.0%, respectively. The higher serum levels of progesterone and testosterone determined before surgery (levels of progesterone

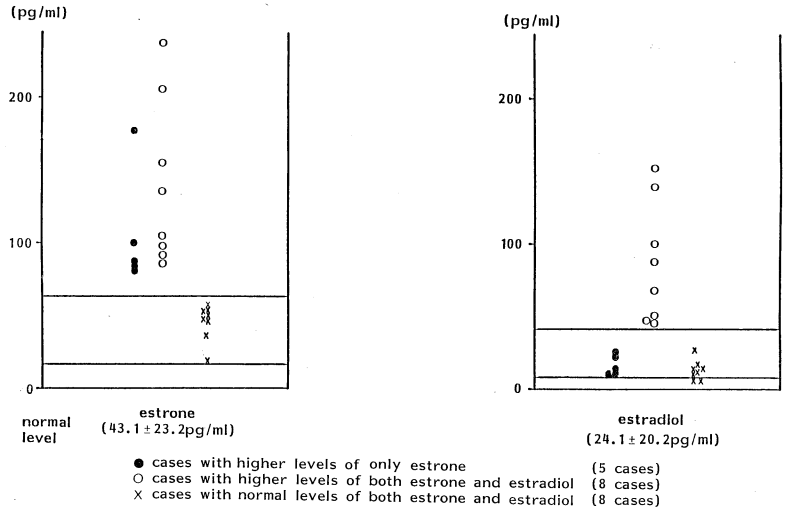

Fig. 1. Serum levels of estrone and estradiol before surgery

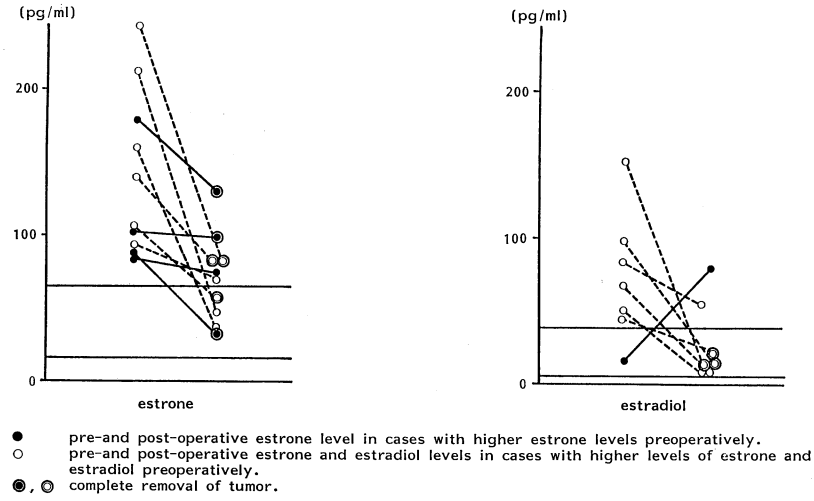

Fig. 2. Serum levels of estrone and estradiol before and after surgery

TABLE 2. Average levels of serum steroids in two groups of tumor volume

Tumor volume	Estrone (pg/ml)	Estradiol (pg/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
>1000 cm³ (n=14)	122.89±58.66*	54.76±48.20	1.06±1.00	0.95±0.67
<1000 cm³ (n=7)	50.29±29.12	20.39±13.80	0.83±0.58	0.82±0.41

(mean±SD)

* $p<0.01$ compared with the level of tumor volume <1000 cm³

TABLE 3. Levels of hormones by suppression and stimulation test in a case of mucinous cystadenoma (low potential malignancy) with hyperestrogenemia

	LH (mIU/ml)	FSH (mIU/ml)	estrone (pg/ml)	estradiol (pg/ml)	estriol (pg/ml)	cortisol (μg/ml)	ACTH (pg/ml)	urinary 17-KS (mg/day)
	75.9	42.2	248.7	153.0	<5.0			
	72.1	39.1	232.9	121.0	<5.0	12.1	<20.0	14.8
dexamethasone suppression 6 mg/day	24.9	20.7	334.4	161.0	<5.0	1.1	<20.0	22.5
	25.9	15.9	322.3	87.9	<5.0	1.6	<20.0	21.3
dexamethasone+hCG 5000 IU	89.5	19.4	835.3	241.0	<5.0	1.9	<20.0	33.0
dexamethasone+hexestrol 5 mg	13.0	3.7	257.1	125.0	<5.0	2.2	<20.0	30.4
postoperative	143.4	113.0	81.7	10.7	<5.0			

TABLE 4. Immunohistochemical findings in tumor tissues

Case	yrs. after menopause	Tumor	Higher level in serum			LH		FSH		Estradiol		Testosterone	
						tumor cell	stroma	tumor cell	stroma	tumor cell	stroma	tumor cell	stroma
I.M.	13	mucinous cystadenocarcinoma	E_1			−	−	−	−	−	−	−	−
Y.T.	12	mucinous cystadenoma (LPM)	E_1		T	−	−	−	−	−	−	++	++
S.F.	35	serous cystadenoma	E_1			−	−	−	−	−	++	−	−
U.S.	31	serous cystadenocarcinoma	E_1			−	−	++	−	++	−	+	−
N.S.	32	mucinous cystadenoma	E_1			−	−	++	+	++	−	++	−
Y.T.	3	mesonephroid carcinoma	E_1	E_2		−	−	−	−	−	−	−	−
Y.K.	3	mucinous cystadenocarcinoma	E_1	E_2	T	−	−	−	−	−	−	−	−
A.H.	20	mucinous cystadenocarcinoma	E_1	E_2		−	−	−	−	++	−	+	−
S.S.	31	mucinous cystadenoma (LPM)	E_1	E_2	T	−	−	−	−	−	−	+	−
S.T.	5	serous cystadenoma (LPM)	E_1	E_2	T	−	−	−	−	+	−	+	−
E.F.	3	serous cystadenocarcinoma	E_1	E_2		−	−	+	++	−	−	++	+
M.N.	7	serous cystadenocarcinoma				−	−	−	−	++	−	−	−
K.K.	6	serous cystadenocarcinoma				−	−	−	−	−	−	−	−
T.Y.	10	serous cystadenocarcinoma				−	−	++	−	−	−	++	−

L.P.M.; low potential malignancy (E_1 : estrone, E_2 : estradiol)

and testosterone in normal postmenopausal women were <2.0 and 0.2 ± 0.2 ng/ml [mean$\pm$SD], respectively.) were seen in 15 of 17 cases, 88.3% and 17 of 19 cases, 89.5%, respectively.

The changes of serum levels of estrone and estradiol before and after surgeries were shown in Fig. 2. In 10 cases with higher levels of estrone, 6 cases underwent a complete removal of tumor, but only 2 cases among them showed decreasing levels of estrone to normal after surgery. In 6 cases with higher levels of estradiol, 3 cases underwent a complete removal of tumor and all 3 cases showed decreasing levels of estradiol to normal after surgery. The cases of decreasing serum levels of progesterone and testosterone among the cases who underwent a complete removal of tumor were found in 1 of 3 cases and in 2 of 5 cases, respectively.

The average levels of serum steroids in the cases with tumor volume >1000 cm^3 and <1000 cm^3 were shown in Table 2. Among 4 steroids determined only difference of levels of estrone between two groups was statistically significant ($p<0.01$). The differences of serum levels of estrone, estradiol, progesterone and testosterone among the cases with clinical stage I, II and III and among the cases with benign, intermediate and malignant tumors were statistically not significant.

In a case of 71 years of age of mucinous cystadenoma (low potential malignancy) with marked hyperestrogenemia and higher level of urinary 17-KS, 6 mg/day dexamethasone suppression during 5 days and 5000 IU hCG stimulation test and hexestrol 5 mg suppression under continuing dexamethasone suppression were carried out before surgery to identify the site of steroids production. The levels of hormones and metabolites were shown in Table 3. The levels of serum estrone, estradiol and of urinary 17-KS did not decrease by dexamethasone, but increased markedly by hCG and did not change by hexestrol.

The localizations of LH, FSH, estradiol and testosterone in tumor tissue of 14 cases stained immunohistochemically by PAP method were studied to clarify their correlations with higher serum levels of these hormones. The result was shown in Table 4. The localizations of hormones were observed not only in stroma cells, but also in tumor cells. However, the correlation between higher serum levels of hormones and the histological localizations was not confirmed. The stroma luteinized cell clusters were observed only in a small focus of 2 cases with multiple blocks preparations studies by hematoxylin eosin staining on 21 cases.

The tumor cells obtained from the recurrent tumor in Douglas pouch one year after a hysterectomy with bilateral salpingooophorectomy in the case with marked hyperestrogenemia described above were cultured in Eagle's minimal essential medium with 10% fetal calf serum. Estrone and estradiol in the medium were determined on the 59th and the 90th day of primary culture. The cultured tissues were thought not to contain the ovarian stroma cell because they recurred after a complete removal of internal genitalia tissue. The cultured cells showed morphologically epithelial type as shown in Fig. 3. The levels of estrone and estradiol in the medium were 227.0 pg/ml and 104.0 pg/ml on 59th day and 37.6 pg/ml and 9.4 pg/ml on 90th day respectively, and both were markedly higher than those in the medium of the cultured cells from the control described below. The levels of estrone and estradiol in the

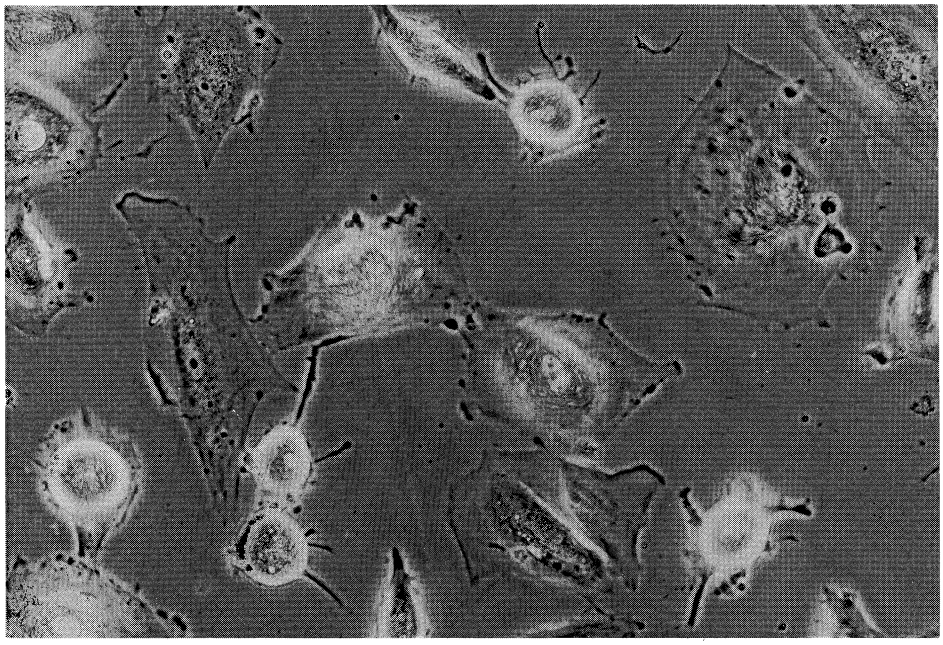

Fig. 3. Cells in culture on the 90th day obtained from the recurred tumor of the case with hyperestrogenemia. (×400)

TABLE 5. Levels of estrone and estradiol in culture medium of recurred ovarian tumor cells (pg/ml)

	Tumor cells		Control (cells from lig. sacrouterina)
Day of culture	59	90	28
Estrone	227.0 (<5.0)	37.6 (7.1)	<5.0 (<5.0)
Estradiol	104.0 (<5.0)	9.4 (<5.0)	8.1 (7.4)

( ) : level of steroid in medium with 10% fetal calf serum as control.

medium of the control with cells obtained from the sacrouterine ligament from a patient of myoma of 45 years of age with regular menstruation were shown in Table 5.

DISCUSSION

Among 21 cases of ovarian non-functioning tumor in postmenopausal women higher serum levels of estrone were observed in 13 cases (61.9%), of estradiol in 8 cases (38.1%). Higher serum levels of progesterone were seen in 15 of 17 cases (88.2%) and of testosterone in 17 of 19 cases (89.5%). The average higher serum levels of estrone in cases of postmenopausal ovarian non-functioning tumor than in cases without the tumor were reported by Obata *et al.*,[3] Pelusi *et al.*[4] and MacDonald *et al.*[5] and of estradiol by Heinonen *et*

al.,[6] Jeppsson *et al.*,[7] Mählch,[8] Obata *et al.*,[3] Suzuki *et al.*,[9] Carlström *et al.*[10] and of progesterone by Jeppsson *et al.*,[7] Mählch[8], Obata *et al.*,[3] Carlström *et al.*[10] and Bäckström *et al.*[11] and of testosterone by Aiman *et al.*[12] The incidences of cases with higher serum levels of steroids, however, were reported only by Obata *et al.*[3] with the result that those of estrone was 9 of 16 cases (56.3%), of estradiol was 22 of 29 cases (75.9%) and of progesterone was 14 of 20 cases (70.0%) and by Jeppsson *et al.*,[7] with the result those of estradiol and of progesterone were 8 of 10 cases (80.0%) in both. The incidences of higher serum levels of steroids in the present study agreed approximately with that of other authors except that for estradiol.

Decrease of higher serum levels of steroids to the normal levels after a complete removal of ovarian tumor was considered the evidence that steroids were produced in tumor tissues. The decreases of higher estrone, estradiol, progesterone and testosterone after surgeries were observed in 2 of 6, 3 of 3, 1 of 3, and 2 of 5 cases, respectively. The decreases of steroids after surgeries were reported by Takamizawa *et al.*[13] in estradiol in 7 of 11 cases, by Obata *et al.*[3] in estrone, estradiol and progesterone, by Heinonen *et al.*[6] in estrone and estradiol, by Pelusi *et al.*[4] in estrone and by MacDonald *et al.*[5] in estrone.

The decreases of estradiol after surgeries in all cases in this study suggested it was produced fully in ovarian tumor tissues and other steroids, estrone, progesterone and testosterone, were considered to be produced partially in tumor tissues and partially in the other sites than ovarian tissues, that is, in the adrenal and/or in the periphery by conversion of steroids precursors.

The data about the correlations of serum steroid levels with tumor volume, clinical stage and benign, intermediate and malignant, indicated that only the levels of estrone between tumor volume >1000 cm^3 and <1000 cm^3 differed statistically significantly and estradiol considered fully produced in ovarian tumor tissues did not differ significantly as for tumor volumes. The reason for this fact remains in obscure and needs to be analysed, further, after accumulation of many cases.

The marked higher serum levels of estrone, estradiol and urinary 17-KS in the case with hyperestrogenemia were not suppressed by dexamethasone and showed further their increases by hCG and decreases to normal levels after surgery. These facts strongly suggested that the production site of estrone, estradiol and the precursors of urinary 17-KS were ovarian tumor tissues. Obata *et al.*[3] reported the similar results in estrone and estradiol. Higher levels of steroids in ovarian vein of tumors than in periphery suggested that the production site of steroids is ovarian tumor tissues. The results supporting this fact were reported by Aiman *et al.*[12] in estrone, estradiol and testosterone and by Jeppsson *et al.*[7] in estradiol and progesterone.

It is still in arguments which components of ovarian tumor tissues are contributed for the production of steroids. The possible sites of steroids production are proposed as follows: 1) Stroma luteinized cells are postulated by Scully *et al.*[14] who found that the incidence and grade of occurrence of stroma luteinized cells are consistent with clinical symptoms of excess steroids. 2) Theca cells like cells around tumor cells are propounded by Woodruff *et al.*[15] with existence of enzyme reactions in them. 3) Hyperplastic stromal cells are asserted by Heinonen *et al.*,[6] who agreed with the hypothesis of Hughesdon[1] that tissue expansion makes stroma cells endocrinologically active, and by Obata

et al.[3] demonstrating that the higher levels of estrone and estradiol correlate with the frequency of stroma cell hyperplasia and that estradiol was immunohistochemically positive in hyperplastic cells. 4) Tumor cells are advocated by Bäckström *et al.*,[11] who found that the recurred tumor without ovarian stroma cells which metastasized from ovarian carcinoma produced higher level of progesterone and by Edward *et al.*[16] reporting that the metastasized tumor yielded higher urinary estriol level and by Takamizawa *et al.*[13] who stated that estradiol was positive in tumor cells immunohistochemically. Stroma luteinized cells were observed in a small focus only in 2 cases of 21 cases and did not consistent with the higher levels of steroids in the present study. Estradiol and testosterone were immunohistochemically positive not only in stroma cells, but also in tumor cells and this fact suggested both components could produce the steroids, although there are still some arguments concerning whether the localization of positive immunohistochemical steroid staining could be valid or not because of the difficulty to immobilize steroids in the processing of paraffin preparation. As another hypothesis MacDonald[5] proposed that the ovary produced androstenedione and it was converted at the periphery.

The higher levels of estrone and estradiol in the medium of primary culture of cells, morphologically with epithelial appearance, from tumor tissues which recurred in Douglas' pouch and were considered without ovarian stroma cell as described above than in the medium of the control cells culture suggested that tumor cell itself could produce estrone and estradiol.

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