

# Proliferation, mitosis orientation and morphogenetic changes in the uterus of mice following chronic treatment with both estrogen and glucocorticoid hormones

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## Abstract

Glucocorticoids have been known to be involved in the regulation of some aspects of estrogen action on the uterus. However, the effect of glucocorticoids on changes in uterine morphogenesis produced by chronic estrogen exposure is not known. Therefore, the aim of this work was to examine the role of glucocorticoids on proliferative and morphogenetic uterine reactions induced by continuous estrogen treatment. Ovariectomized mice received subcutaneous injections of estradiol dipropionate in olive oil (2 µg per 100 g body weight once a week) or vehicle and drank water with or without dexamethasone (2 mg/l) for 30, 60 and 90 days. Treatment with dexamethasone caused a marked reduction in estradiol-induced changes in uterine weight, in proliferation (estimated from the proportion of mitotic and BrdU-labeled cells in all uterine tissues), and in changes in estradiol-dependent

morphogenesis, which was redirected from the formation of atypical hyperplasia in animals receiving only estradiol to the appearance of simple or cystic endometrial hyperplasia in animals receiving both estradiol and dexamethasone. Estradiol alone increased dramatically the number of perpendicular oriented mitoses in luminal and glandular epithelia, and administration of dexamethasone inhibited this effect. In the absence of estradiol, chronic treatment with dexamethasone has no effect on all uterine parameters tested. Thus, chronic glucocorticoid treatment produces a complex antiestrogenic effect in the uterus of mice. Estradiol-induced changes in mitosis orientation are probably responsible for changes in the shape of glands and development of endometrial hyperplasia.

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## Introduction

One major aspect of estrogen action on the uterus is the influence on proliferative processes. It is well known that estrogen strongly increases proliferative activity in all uterine tissues (Martin *et al.* 1973, Zhang *et al.* 1998, Couse & Korach 1999). Another important result of (especially chronic) estrogen action on the uterus is morphogenetic alterations that include changes in the type of luminal and glandular epithelia, the number and shape of glands, the glandular to stromal ratio, and the morphology of epithelial cells (Martin *et al.* 1973, Deligdisch 2000, Silverberg 2000). In the normal uterus, lumen and glands are lined with simple or pseudostratified columnar epithelium, but after continuous estrogen treatment these epithelia may be stratified and often show atypical cytology. The normal uterus has simple tubular glands separated by stroma. Estrogen increases the number of glands, and the glands are less separated by stroma. Moreover, glands with abnormal shapes and architecture appear. These changes are related to hyperplasia and cancer formation (Martin *et al.* 1973, Deligdisch 2000,

Silverberg 2000). Therefore, endometrial hyperplasia and cancer can be regarded as the final step in the development of estrogen-dependent morphogenetic changes in the uterus.

Glucocorticoid hormones are known to be involved in the regulation of some aspects of estrogen action on the uterus (Rabin *et al.* 1990, Bigsby 1993, Sahlin 1995, Burton *et al.* 1998, Ho *et al.* 1999). It has been shown that glucocorticoids have an inhibitory influence on proliferation induced by short-term estrogen treatment (Campbell 1978, Markaverich *et al.* 1981, Stewart *et al.* 1983, Rabin *et al.* 1990, Bigsby 1993). In other experiments, a single estrogen injection after long-term treatment with the synthetic glucocorticoid triamcinolone induced a progesterone-like enhancement of proliferation in stromal and myometrial cells (Gunin 1998, Gunin *et al.* 2000). However, nothing is known about proliferation or morphogenetic changes in the uterus when both glucocorticoids and estrogens act simultaneously for a long period of time. Because only the chronic action of estrogens is able to induce prominent morphogenetic changes including hyperplasia and cancer, it is especially important to know

how glucocorticoids affect the uterine response to long-term estrogen treatment. Therefore, the aim of this work was to examine the role of glucocorticoids on proliferative and morphogenetic uterine reactions induced by continuous estrogen treatment.

On the basis of studies on embryonic morphogenesis (Bowerman & Shelton 1999, Miller *et al.* 1999, Schlesinger *et al.* 1999, Jan & Jan 2000), one mechanism that could direct estrogen-dependent morphogenesis and maybe pre-cancerous changes in uterine epithelial tissues is the orientation of mitosis in uterine epithelia. Therefore, the orientation of mitoses in uterine epithelia was also determined.

## Materials and Methods

### Animals

All procedures were performed in accordance with the Guidelines on the Handling and Training of Laboratory Animals published by the Universities Federation for Animal Welfare and with the Chuvash State University Rules for Work with Laboratory Animals. White outbred female mice (20–22 g) were used. Animals were obtained from the Animal Department of Chuvash State University (Cheboksary, Russia) and were housed with free access to water and food. Mice were ovariectomized 1 week before the experiments were started. All surgical procedures were performed under anesthesia with ketamine and droperidol (70 mg/kg, 0.1 mg/kg respectively, i.p., Gedeon-Richter, Budapest, Hungary).

### Treatments

Groups of ovariectomized mice received subcutaneous injections of either vehicle alone or estradiol dipropionate in olive oil (Minmedprom, Rostov-Don, Russia) at a dose of 2 µg per 100 g body weight once a week for 30, 60 and 90 days (i.e. 5, 9 or 13 injections). During the same period they drank water with or without dexamethasone (2 mg/l; Sigma Chemical Co., St Louis, MO, USA). Water intake was monitored throughout the experiment, and the dose of dexamethasone was then calculated to be approximately 0.035 mg dexamethasone per 100 g body weight per day. There were 11 mice in each of the groups treated with estradiol with or without dexamethasone, and five mice in each of the groups treated with olive oil with or without dexamethasone.

The uteri were removed on the third day after the last injection of estradiol or vehicle. All animals were injected intraperitoneally with bromodeoxyuridine (BrdU; 5 mg per 100 g body weight; Sigma) dissolved in saline 2 h before the tissues were removed. The middle segment of a uterine horn, adrenal glands, and thymus were removed under deep ether anesthesia. Uteri were weighed, placed

in modified Bouin's fixative (Gunin *et al.* 2000) for 6 h at room temperature, then dehydrated in ethanol and embedded in paraffin. Uteri were transversely oriented and cut at 5–7 µm. To monitor general glucocorticoid effects on the organism, adrenal glands and thymus were weighed immediately after removal.

### Determination of the incidence of mitotic and BrdU-labeled cells

Proliferative processes were assessed from the incidence of mitotic and BrdU-labeled cells. Mitoses were counted in sections stained with iron hematoxylin. BrdU was detected using immunohistochemical staining (Gunin *et al.* 2000) using anti-BrdU mouse monoclonal antibody conjugated with alkaline phosphatase (Boehringer Mannheim GmbH, Mannheim, Germany) diluted to 1:20. Alkaline phosphatase activity was revealed through the use of naphthol AS-BI-phosphate and new fuchsin as chromogens. In control sections, the antibody was replaced with normal goat serum. All results were expressed as the proportion (%) of mitotic or labeled cells.

To determine the incidence of mitotic and BrdU-labeled cells in luminal and glandular epithelia, all cells of luminal epithelium and epithelium of endometrial glands (separately) in whole transverse sections were counted, and dividing or BrdU-labeled cells were also counted. Not less than 3000 cells were assessed per mouse. The proportion of mitotic or labeled cells was then calculated. The incidence of mitotic cells in endometrial stroma and myometrium was calculated as follows. Whole transverse sections were viewed and all dividing cells were counted in endometrium and myometrium, separately. The total number of stromal and myometrial cells per section was then calculated. For this, the number of stromal and myometrial cells per field of view of the microscope was counted. Then, the area of microscopic field of view, the area of endometrium and myometrium respectively in transverse section were found by measuring the dimensions of the field of view, the endometrium and myometrium respectively, using an ocular micrometer. The number of stromal cells and myometrial cells per section was then calculated separately. The proportion of mitotic cells for stromal and myometrial cells was calculated by dividing the number of mitosis per section into the total number of stromal or myometrial cells respectively per section. Not less than 10 000 stromal cells and myometrial cells were analyzed per mouse. The incidence of BrdU-labeled endometrial stromal cells and myometrial cells was determined similarly. In a section, the number of labeled cells and the total number of stromal or myometrial cells respectively per microscopic field of view were counted in different regions of endometrium or myometrium. At least ten fields of view were analyzed per section. The labeling index for stromal or myometrial cells was calculated by dividing the number of labeled cells by the total number of

stromal or myometrial cells respectively per field of view. Not less than 3000 cells were analyzed per animal.

#### Orientation of mitosis

In the tissue sections stained with iron hematoxylin, mitotic figures were viewed using a light microscope, and the orientation of mitoses (mitotic spindle) in luminal and glandular epithelia was determined. Mitoses with poles aligned from 0° to 45° to the basement membrane of epithelium were regarded as parallel oriented. Mitoses with poles aligned from 45° to 90° to the plane of basement membrane were regarded as perpendicular oriented. Parallel and perpendicular mitoses were counted. Only mitoses in which the orientation was clear (>80% of the total) were counted.

#### Uterine histology

Histological changes in the uterus were analyzed and diagnosed according to Scully *et al.* (1994). Chronic estrogen action leads to hyperplasia and cancer formation in the uterus (Niwa *et al.* 1998, Liehr 2000). Hyperplastic and neoplastic changes are well known from their microscopic appearance, but are poorly characterized by quantitative criteria. Uterine glands were therefore subdivided into a number of morphological types to estimate the extent of any hyperplastic or neoplastic changes in the endometrium.

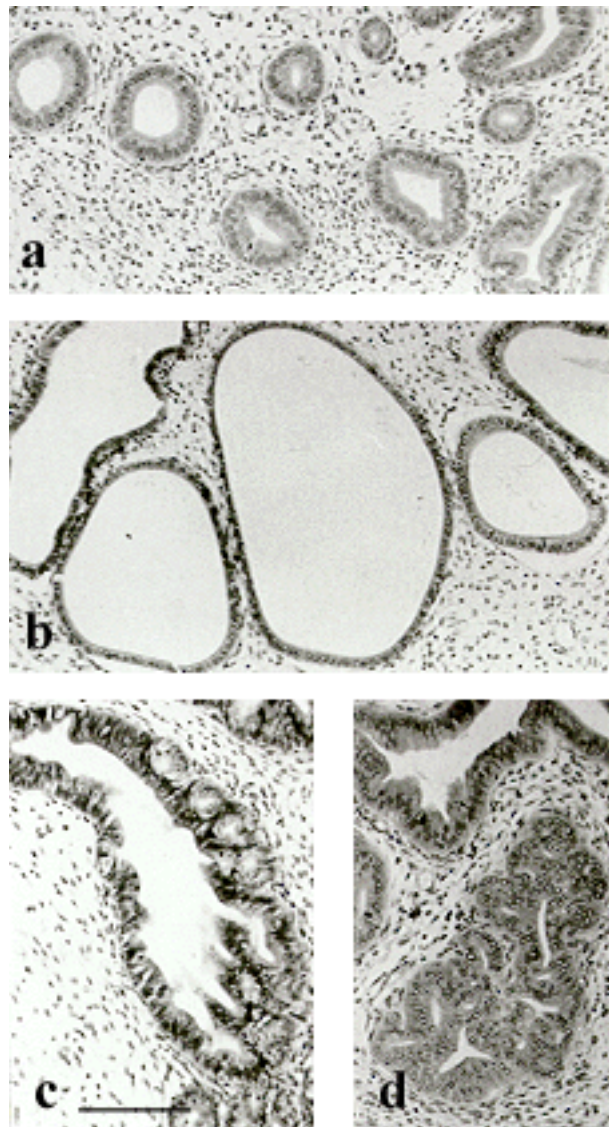
**Uterine gland typing** All sections were histologically examined to define the types of endometrial glands which were subdivided into four groups according to the following criteria (Fig. 1):

1. Normal glands. Simple tubular glands which can appear in sections as round, oval or elongated with a narrow lumen. This type has no branches or daughter glands (Fig. 1a).

2. Cystic glands. Round glands of more than average or large size (Fig. 1b).

3. Glands with daughter glands. These glands have various shapes (round, elongate, tortuous) and sizes, and have forming or formed daughter gland or glands inside the epithelium or inside the mother gland lumen, or on the outer surface of the mother gland (like budding gland) (Fig. 1c).

4. Conglomerate of glands. This type has a very complex architecture in which individual glands are closely disposed to each other almost without intervening stroma (Fig. 1d) and have multiple interconnecting lumens. This type may develop from glands with daughter glands. It is similar to the focus of adenoma or cancer development, but these can be diagnosed only by other characteristics of the epithelium such as cellular and nuclear polymorphism, disrupted basement membrane, and myometrial invasion. Both type 3 and 4 glands are



**Figure 1** Photomicrographs demonstrating types of endometrial glands (see Materials and Methods). a, normal glands; b, cystic glands; c, gland with multiple daughter glands; d, glands forming conglomerate. Iron hematoxylin stain. Scale bar 100  $\mu$ m.

considered to be closely related to the development of malignancy in the endometrium.

The number of each type of glands was calculated in randomly selected sections. No less than three sections from each animal were examined. Results were expressed as the proportion (%) of each type of gland.

**The type of glandular epithelium** The epithelium of all glands in randomly selected sections was examined and typed as simple, pseudostratified or stratified (multi-layered) epithelia. The proportion (%) of glands with each type of epithelium was calculated.

### Statistics

Data were checked for normality of distribution. Arithmetic means and standard errors were calculated for each data group. The significance of the influence of dexamethasone treatment on proliferation, orientation of mitoses at different time points was evaluated by two-way ANOVA. The significance of differences was determined using the chi-square test.

## Results

### General glucocorticoid action

The effectiveness of the treatment protocols was controlled by weighing the thymus and adrenal glands. In the three groups of mice treated with estradiol (E) the co-administration of dexamethasone (E+D) caused a reduction in the weight of the thymus glands (30 days:  $60.1 \pm 4.1$ , E+D vs  $175 \pm 7$ , E; 60 days:  $71 \pm 5$ , E+D vs  $174 \pm 11$ , E; 90 days:  $69 \pm 9$ , E+D vs  $191 \pm 10$ , E; mg per 100 g body weight; mean  $\pm$  s.e.m.;  $n=11$  for each comparison;  $P<0.001$ , two-way ANOVA). There was a similar marked reduction in the weight of the adrenal glands ( $4.4 \pm 0.2$ , E+D 30 days vs  $7.1 \pm 0.5$ , E 30 days;  $2.8 \pm 0.2$ , E+D 60 days vs  $8.6 \pm 0.4$ , E 60 days;  $3.3 \pm 0.4$ , E+D 90 days vs  $7.3 \pm 0.3$ , E 90 days; mg per 100 g body weight; mean  $\pm$  s.e.m.;  $n=11$  for each comparison;  $P<0.001$ , by two-way ANOVA) under combined treatment with estradiol and dexamethasone.

In mice treated with vehicle (olive oil) plus dexamethasone for 1 month, weights of thymus ( $87 \pm 7$  vs vehicle control  $188 \pm 19$  mg per 100 g body weight; mean  $\pm$  s.e.m.;  $n=5$ ) and adrenal glands ( $4.3 \pm 0.3$  vs vehicle control  $7.2 \pm 0.3$ ; mg per 100 g body weight; mean  $\pm$  s.e.m.;  $n=5$ ) were also significantly reduced. Similar differences in the weight of thymus and adrenal glands were found in mice treated with olive oil with and without dexamethasone for 60 or 90 days. Two-way ANOVA showed a significant ( $P<0.01$ ) influence of dexamethasone treatment for 30, 60 and 90 days.

### Uterine weight

Uteri of ovariectomized mice treated with olive oil for 30 days were very light ( $52 \pm 8$  mg per 100 g body weight;  $n=5$ ) and further olive oil injections for 60 or 90 days and dexamethasone ingestion did not change this. Treatment with estradiol caused the expected increase in the uterine weight, and ingestion of dexamethasone markedly attenuated this increase at 30, 60 and 90 days of treatment (30 days:  $507 \pm 18$ , E+D vs  $821 \pm 76$ , E; 60 days:  $618 \pm 22$ , E+D vs  $870 \pm 37$ , E; 90 days:  $585 \pm 38$ , E+D vs  $963 \pm 41$ , E; mg per 100 g body weight;  $n=11$  for each comparison; mean  $\pm$  s.e.m.;  $P<0.001$ , by two-way ANOVA).

### Proliferation

In ovariectomized mice injected with olive oil but without dexamethasone for 30 days, very few mitoses were found in luminal and glandular epithelia, and no mitotic figures were seen in stroma or myometrium. The percentage of mitotic cells was  $0.0025 \pm 0.0009$  (mean  $\pm$  s.e.m.;  $n=5$ ) for luminal epithelium and  $0.006 \pm 0.003$  for glands. There were also only a few BrdU-labeled cells in the uterus of these animals ( $0.11 \pm 0.04\%$  in luminal epithelium,  $0.14 \pm 0.05\%$  in glands,  $0.004 \pm 0.001\%$  in stromal cells,  $0.002 \pm 0.001\%$  in myometrial cells). Further treatment with olive oil with or without dexamethasone did not alter the proliferation in these tissues.

In mice injected with estradiol but without glucocorticoid, the number of mitotic and BrdU-labeled cells was markedly greater than in animals injected with vehicle alone (Fig. 2). Dexamethasone administration markedly reduced the incidence of mitotic and BrdU-labeled cells in all uterine tissues at all periods of observation (Fig. 2).

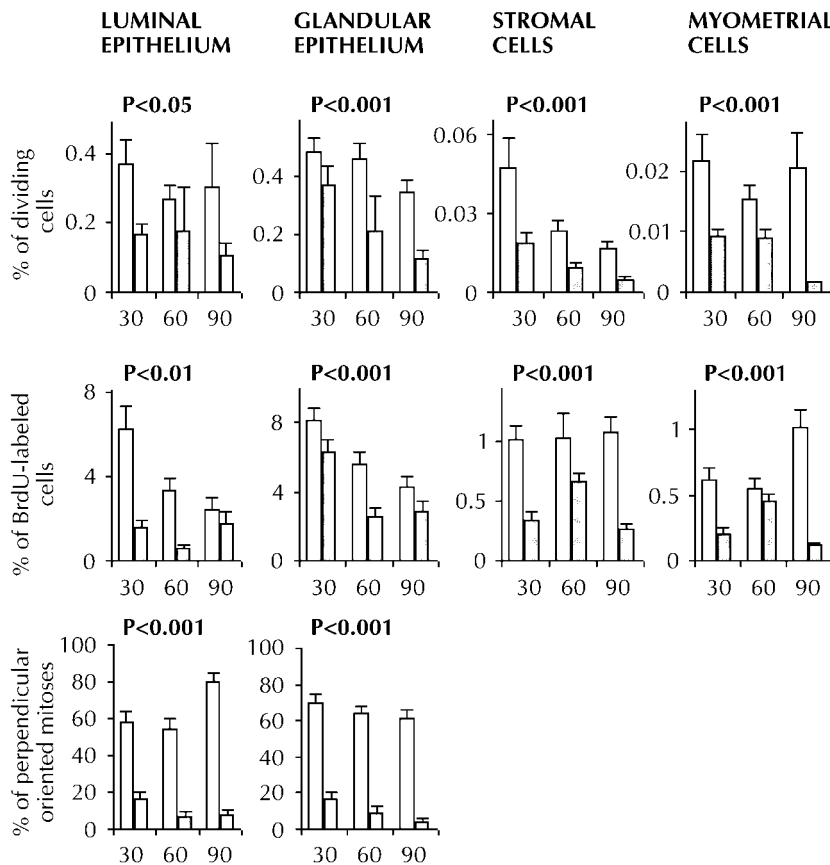
The dexamethasone-induced reduction in both proliferative parameters was greatest in luminal epithelium at 30 days, and in stromal and myometrial cells at 30 and 90 days.

### Uterine histology

Microscopical examination of uteri of ovariectomized mice which were not subjected to estrogen treatment revealed atrophic endometrium in all cases independent of the duration of treatment with olive oil with or without dexamethasone. All the endometrial glands in all these uteri had a narrow lumen, and a round, oval, or elongated shape, a microscopical reflection of simple tubular glands which were regarded as normal. All glands were lined with simple cuboidal epithelium.

In mice injected with estradiol but not ingesting dexamethasone, the number of glands, their size and the number of atypical glands, especially glands with daughter glands and glands forming conglomerates, were increased (Figs 3 and 4). The number of glands lined with pseudostratified or stratified epithelium, which often contained atypical cells and nuclei, was also increased by estradiol treatment (Fig. 3). The histological analysis of the uteri is summarized in Fig. 3. The incidence of atypical hyperplasia is increased following estrogen action at all time points.

The uterine histology of mice administered both estradiol and dexamethasone differed markedly from that of control animals that had not received dexamethasone. The number of glands with daughter glands and glands forming conglomerates was not increased, and the number of these types of glands was significantly lower. However, treatment with estradiol and dexamethasone did cause an increase in the number of cystic glands. The incidence of glands with stratified epithelium was not increased.



Duration of treatment with estradiol and dexamethasone (days)

**Figure 2** The incidence of mitotic, BrdU-labeled cells and of perpendicular oriented mitoses in the various morphological compartments of the uteri of ovariectomized mice that received injections of estradiol (2 µg per 100 g body weight once a week, s.c.) and dexamethasone (2 mg/l) administered in the drinking water for 30, 60 and 90 days (shaded bars) in comparison with those of mice that received injections of estradiol and tap water without dexamethasone to drink (open bars). There were no changes in proliferative parameters and mitosis orientation in control mice that received injections of vehicle (olive oil) and water to drink with or without dexamethasone for the same periods of time. There were 11 mice in each estradiol-treated time group and five mice in each olive oil-treated time-group. Values are means ± S.E.M. *P* values indicate the significance of the influence of dexamethasone treatment for 30, 60 and 90 days (two-way ANOVA).

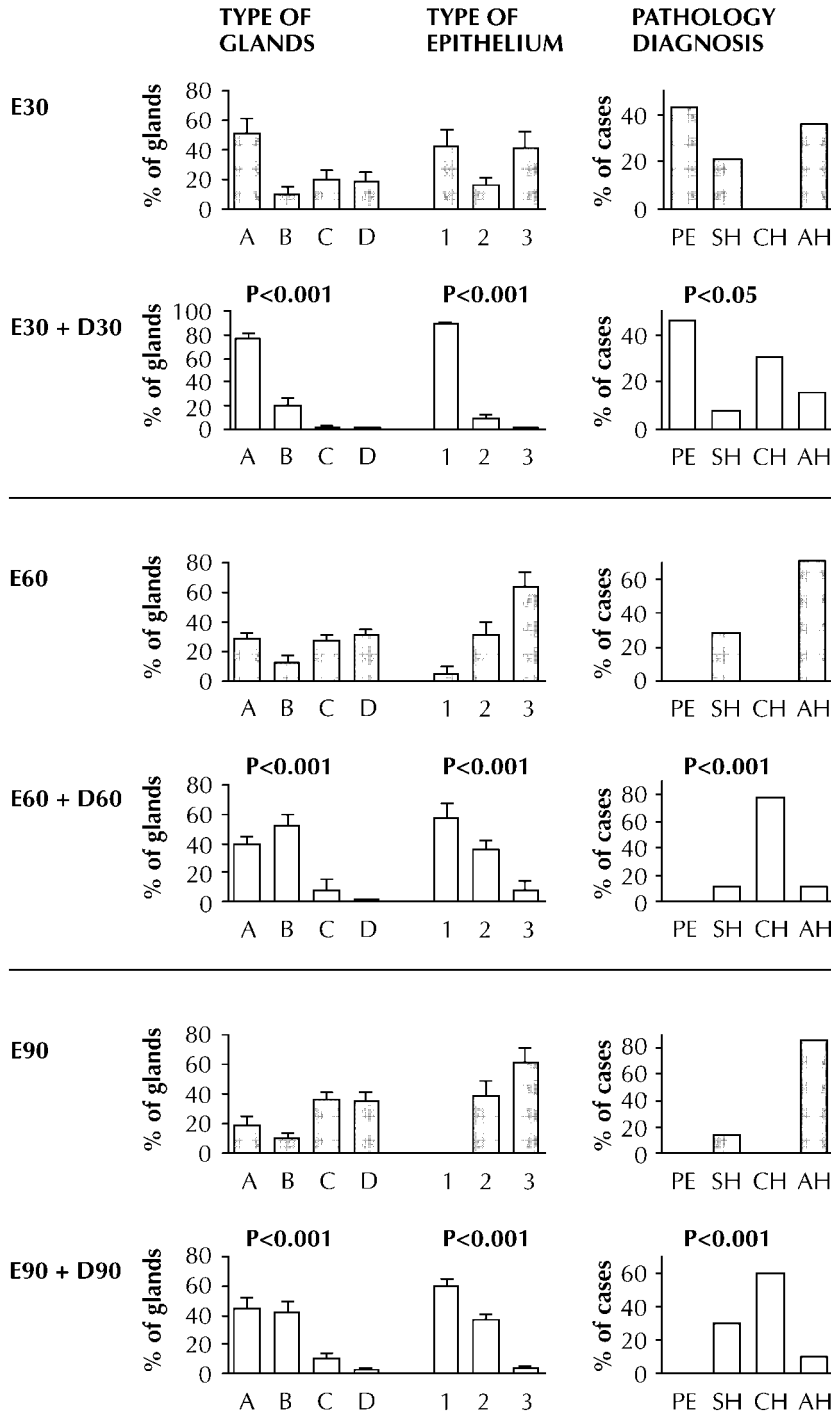
Finally, the incidence of atypical hyperplasia was markedly reduced, but the incidence of cystic hyperplasia was increased (Figs 3 and 4)

*Mitosis orientation*

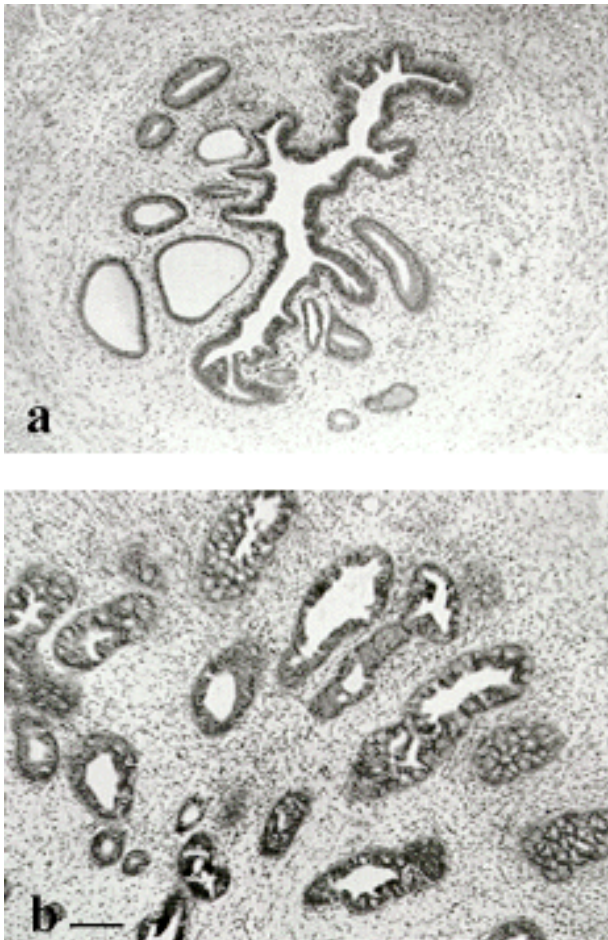
In the uterus of ovariectomized mice treated with olive oil without dexamethasone for 30 days, mitoses are very rare and were found only in luminal and glandular epithelia where they were all oriented parallel to the basement membrane. Ingestion of dexamethasone did not change the orientation of mitoses at any time point.

The injection of estradiol without dexamethasone led to dramatic changes in the orientation of mitoses in the uterine epithelium. More than half of these mitoses were oriented perpendicular to the basement membrane (Fig. 2). Estrogenized mice ingesting dexamethasone had markedly fewer perpendicularly oriented mitoses (Fig. 2).

Thus, chronic dexamethasone administration to estradiol-treated ovariectomized mice caused a decrease in uterine weight, a decrease in the proliferation of all uterine tissues and a decrease in the number of perpendicularly oriented mitoses in uterine epithelia. It also caused changes in estrogen-dependent morphogenesis, the



**Figure 3** Type of endometrial glands, type of glandular epithelium, and pathology diagnosis of the uteri of ovariectomized mice that received injections estradiol (2 µg per 100 g body weight once a week, s.c.) and dexamethasone (2 mg/l) administered in the drinking water for 30, 60 and 90 days in comparison with those of mice that received injections of estradiol and tap water without dexamethasone to drink are presented. There were 11 mice in each estradiol-treated group and five mice in each olive oil-treated group. A, Normal glands; B, cystic glands; C, glands with daughter glands; D, glands forming conglomerate (see Fig. 1); 1, simple columnar epithelium; 2, pseudostratified columnar epithelium; 3, stratified columnar epithelium (see Fig. 2); PE, proliferative endometrium; SH, simple hyperplasia; CH, cystic hyperplasia; AH, atypical hyperplasia. Values are means ± S.E.M. *P* values indicate the significance of differences (chi-square test).



**Figure 4** Photomicrographs demonstrating effect of dexamethasone on the morphology of the uterus from an estradiol-treated mouse with (a) or without (b) dexamethasone for 60 days. In (a) the uterine lumen is lined with simple columnar epithelium, endometrial glands have normal (round or oval) and cystic shapes, and are lined with simple columnar or cuboidal epithelium. (b) Many glands having little daughter glands inside their walls; almost all glands are lined with stratified columnar or cuboidal epithelium; the area of endometrium is much larger in this uterus. Iron hematoxylin stain. Scale bar 100  $\mu$ m.

development of which is redirected from the formation of atypical hyperplasia to the appearance of simple or cystic endometrial hyperplasia. In the absence of estradiol, chronic treatment with dexamethasone had no detectable effect on the uterine parameters tested.

## Discussion

The effect of glucocorticoid administration on estradiol-induced reactions in the uterus was examined. It is well known that long-term glucocorticoid action has a variety of general effects on the organism, including a decrease in

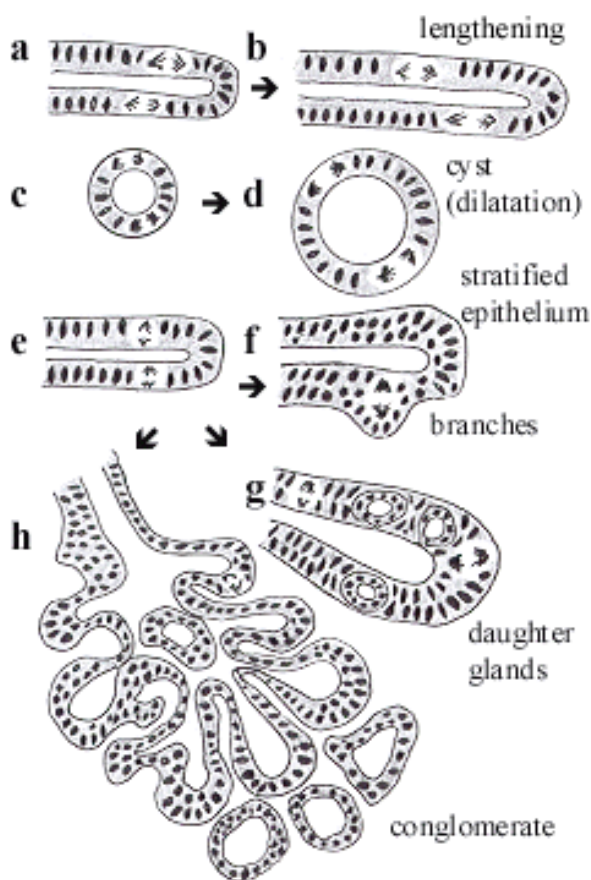
the weight of thymus and adrenal glands. This was confirmed at 30, 60 and 90 days exposure to dexamethasone. Therefore, the treatment regime used was appropriate to induce a general chronic hypercorticosteroid effect. The analysis of the uterine response to estradiol revealed the expected changes in classic parameters, such as uterine weight, proliferation and morphogenetic reorganization. A further parameter, mitosis orientation, which had not been reported previously, was also influenced by estrogen and glucocorticoid action.

Chronic ingestion of dexamethasone for 30–90 days caused a sharp decrease in estradiol-induced proliferation in the uterus, assessed by the number of mitotic and BrdU-labeled cells in luminal and glandular epithelia, in stromal and myometrial cells, indicating that the number of cells in the S and M phases of the cell cycle is reduced in these tissues. Glucocorticoids can affect progress of cells through the G1 phase of the cell cycle (King & Cidlowski 1998). Hence, a decrease in the number of cells in the S and M phases is probably due to the lengthening of the G1 phase of the cell cycle. These data extend previous reports of a decrease in proliferation in the uterus caused by acute exposure to glucocorticoids (Markaverich *et al.* 1981, Rabin *et al.* 1990, Sahlin 1995).

Chronic dexamethasone administration markedly attenuated the estradiol-induced increase in uterine weight. Uterine weight depends on the number of uterine cells, on cell sizes, and on water content of the uterus. The dexamethasone-induced inhibition of proliferation must have caused a decrease in the quantity of cells in the uterus. The water content of the uterus may also have been reduced by dexamethasone, because glucocorticoids decrease vascular permeability in many tissues, including the uterus (Campbell 1978, Stewart *et al.* 1983).

Estrogens are known to cause morphogenetic shifts in the uterus (Martin *et al.* 1973, Niwa *et al.* 1998, Couse & Korach 1999, Deligdisch 2000). Our results show that the number of glands with abnormal shapes and abnormal type of epithelium, and the incidence of atypical endometrial hyperplasia, which has a non-favorable prognosis and is regarded as a pre-cancerous process (Deligdisch 2000, Silverberg 2000), is gradually increased from 30 to 90 days of estradiol treatment. Dexamethasone produced marked reductions in these estrogen-induced morphogeneses in the uterus. It can be concluded that glucocorticoid turns the estrogen-dependent changes from the direction of pre-cancerous, atypical hyperplasia formation to the more favorable development of simple or cystic hyperplasia. The mechanisms of this effect of glucocorticoids are not clear, but probably include changes in proliferation (see above), in cell differentiation, apoptosis activity (King & Cidlowski 1998). In addition, dexamethasone also blocks estrogen-induced changes in mitosis orientation.

To understand how mitosis orientation affects the shape of glands, it is necessary to understand how abnormal glands (cystic, with daughter glands, conglomerate of



**Figure 5** A proposed model showing how an orientation of cell division can influence the shape of a gland in the uterus. Cell divisions aligned parallel to both the basement membrane and the long axis of a gland (a) will probably lead to lengthening of the gland (b). Cell divisions oriented parallel to the basement membrane and perpendicular to the long axis of a gland (c) will probably result in an increase in diameter of the gland (dilatation) and will form cystic gland (d). Cell divisions in a plane oriented perpendicular to the basement membrane (e) will probably lead to formation of stratified epithelium and/or branches, papillae (f), and/or daughter glands (g), and/or conglomerates of glands (h).

glands) are formed from normal simple tubular glands following estrogen-induced morphogenesis. If epithelial cells of a gland divide parallel to both the basement membrane and the long axis of a gland, this should lead to lengthening of the gland (Fig. 5). If the cells divide parallel to the basement membrane and perpendicular to the long axis of a gland, this should cause an increase in diameter of the gland (dilatation) and formation of a cystic gland. If the plane of cell division is perpendicular to the basement membrane, it will probably lead to the formation of stratified epithelium or/and branches, papillae, or/and daughter glands, or/and conglomerates of glands (Fig. 5). Our results show that estradiol-induced changes in the

architecture of glands and in mitosis orientation are correlated. They strongly suggest that the orientation of mitoses perpendicular to the basement membrane is responsible for formation of the pre-cancerous changes, and may be more important than estrogen-dependent changes in proliferation. In mice treated with estradiol and dexamethasone, the pre-cancerous changes were not observed and there were almost no perpendicular oriented mitoses in the endometrial glands. This reinforces the view that mitosis orientation is responsible for the development of pre-cancerous morphological changes.

The mechanism(s) by which estradiol influences mitosis orientation is unknown and we can only speculate on possible pathways. Estrogen receptors are likely to be involved, and the effects observed are probably mediated via estrogen-dependent genes and their products. Possible candidate are genes that encode Wnt family proteins because the Wnt signaling pathway controls mitosis orientation in yeast, *Drosophila* and *Caenorhabditis elegans* and in some mammalian tissues (Bowerman & Shelton 1999, Miller *et al.* 1999, Schlesinger *et al.* 1999, Jan & Jan 2000). The Wnt pathway has been shown to be involved in embryonic development, postnatal morphogenesis of the uterus, and activity of these genes is changed during the estrous cycle (Sassoon 1999), and following endometrial carcinogenesis (Abu-Jawdeh *et al.* 1999, Nei *et al.* 1999, Ikeda *et al.* 2000). Another possible pathway is an influence of estrogens on mitotic machinery (Poelzl *et al.* 2000).

The mechanism(s) of the antiestrogen action of dexamethasone is also not clear. Its effects are probably mediated by glucocorticoid receptors, which are present in the uterus (Atkinson & Adams 1988, Ho *et al.* 1999). The effect of glucocorticoids may also involve the estrogen signaling pathway, because dexamethasone had no effect on the uterine parameters treated in the absence of estradiol. A decrease in the number of estrogen receptors in the uterus following short-term glucocorticoid treatment has also been documented (Atkinson & Adams 1988, Rabin *et al.* 1990, Sahlin 1995). However, further studies are needed to define the mechanisms involved.

Thus, chronic glucocorticoid treatment produces complex antiestrogenic effects in the mouse uterus. We hope that this research will lead to a better understanding of the origin and progression of estrogen-dependent cancer of the female reproductive system.

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## References

- Abu-Jawdeh G, Comella N, Tomita Y, Brown LF, Tognazzi K, Sokol SY & Kocher O 1999 Differential expression of frpHE: a novel human stromal protein of the secreted frizzled gene family, during the endometrial cycle and malignancy. *Laboratory Investigation* **79** 439–447.
- Atkinson S & Adams NR 1988 Adrenal glands alter the concentration of oestradiol-17 beta and its receptor in the uterus of ovariectomized ewes. *Journal of Endocrinology* **118** 375–380.
- Bigsby RM 1993 Progesterone and dexamethasone inhibition of estrogen-induced synthesis of DNA and complement in rat uterine epithelium: effects of antiprogesterone compounds. *Journal of Steroid Biochemistry and Molecular Biology* **45** 295–301.
- Bowerman B & Shelton CA 1999 Cell polarity in the early *Caenorhabditis elegans* embryo. *Current Opinion in Genetics and Development* **9** 390–395.
- Burton PJ, Krozowski ZS & Waddell BJ 1998 Immunolocalization of 11 beta-hydroxysteroid dehydrogenase types 1 and 2 in rat uterus: variation across the estrous cycle and regulation by estrogen and progesterone. *Endocrinology* **139** 376–382.
- Campbell PS 1978 The mechanism of the inhibition of uterotrophic responses by acute dexamethasone pretreatment. *Endocrinology* **103** 716–723.
- Couse JF & Korach KS 1999 Estrogen receptor null mice: what have we learned and where will they lead us? *Endocrine Reviews* **20** 358–417.
- Deligdisch L 2000 Hormonal pathology of the endometrium. *Modern Pathology* **13** 285–294.
- Gunin A 1998 Effect of long-term glucocorticoid treatment on oestradiol-induced proliferation in the uterus of ovariectomized rats. *Journal of Endocrinology* **157** 481–488.
- Gunin AG, Sharov AA & Nikolaev DV 2000 Two month glucocorticoid treatment increases estradiol-induced stromal and myometrial cell proliferation in the uterus of ovariectomized rats. *European Journal of Obstetrics, Gynecology and Reproductive Biology* **88** 171–179.
- Ho CK, Tetsuka M & Hillier SG 1999 Regulation of 11 beta-hydroxysteroid dehydrogenase isoforms and glucocorticoid receptor gene expression in the rat uterus. *Journal of Endocrinology* **163** 425–431.
- Ikeda T, Yoshinaga K, Semba S, Kondo E, Ohmori H & Horii A 2000 Mutational analysis of the CTNNB1 (beta-catenin) gene in human endometrial cancer: frequent mutations at codon 34 that cause nuclear accumulation. *Oncology Reports* **7** 323–326.
- Jan YN & Jan LY 2000 Polarity in cell division: what frames thy fearful asymmetry? *Cell* **100** 599–602.
- King KL & Cidlowski JA 1998 Cell cycle regulation and apoptosis. *Annual Review of Physiology* **60** 601–617.
- Liehr JG 2000 Is estradiol a genotoxic mutagenic carcinogen? *Endocrine Reviews* **21** 40–54.
- Markaverich BM, Upchurch S & Clark JH 1981 Progesterone and dexamethasone antagonism of uterine growth: a role for a second nuclear binding site for estradiol in estrogen action. *Journal of Steroid Biochemistry* **14** 125–132.
- Martin L, Finn CA & Trinder G 1973 Hypertrophy and hyperplasia in the mouse uterus after oestrogen treatment: an autoradiographic study. *Journal of Endocrinology* **56** 133–144.
- Miller JR, Hocking AM, Brown JD & Moon RT 1999 Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca<sup>2+</sup> pathways. *Oncogene* **18** 7860–7872.
- Nei H, Saito T, Yamasaki H, Mizumoto H, Ito E & Kudo R 1999 Nuclear localization of beta-catenin in normal and carcinogenic endometrium. *Molecular Carcinogenesis* **25** 207–218.
- Niwa K, Morishita S, Hashimoto M, Itoh T, Fujimoto J, Mori H & Tamaya T 1998 Effects of tamoxifen on endometrial carcinogenesis in mice. *Japanese Journal of Cancer Research* **89** 502–509.
- Poelzl G, Yasuyo Kasai Y, Mochizuki N, Shaul PW, Brown M & Mendelsohn ME 2000 Specific association of estrogen receptor with the cell cycle spindle assembly checkpoint protein, MAD2. *PNAS* **97** 2836–2839.
- Rabin DS, Johnson EO, Brandon DD, Liapi C & Chrousos GP 1990 Glucocorticoids inhibit estradiol-mediated uterine growth: possible role of the uterine estradiol receptor. *Biology of Reproduction* **42** 74–80.
- Sahlín L 1995 Dexamethasone attenuates the estradiol-induced increase of IGF-I mRNA in the rat uterus. *Journal of Steroid Biochemistry and Molecular Biology* **55** 9–15.
- Sassoon D 1999 Wnt genes and endocrine disruption of the female reproductive tract: a genetic approach. *Molecular and Cellular Endocrinology* **158** 1–5.
- Schlesinger A, Shelton CA, Maloof JN, Meneghini M & Bowerman B 1999 Wnt pathway components orient a mitotic spindle in the early *Caenorhabditis elegans* embryo without requiring gene transcription in the responding cell. *Genes and Development* **13** 2028–2038.
- Scully RE, Bonfiglio TA, Kurman RJ, Silverberg SG & Wilkinson EJ 1994 *Histological Typing of Female Genital Tract Tumours (International Histological Classification of Tumours)*, edn 2, pp. 13–18. Geneva: WHO.
- Silverberg SG 2000 Problems in the differential diagnosis of endometrial hyperplasia and carcinoma. *Modern Pathology* **13** 309–327.
- Stewart PJ, Zaloudek CJ, Inman MM & Webster RA 1983 Effects of dexamethasone and indomethacin on estrogen-induced uterine growth. *Life Sciences* **33** 2349–2356.
- Zhang Z, Laping J, Glasser S, Day P & Mulholland J 1998 Mediators of estradiol-stimulated mitosis in the rat uterine luminal epithelium. *Endocrinology* **139** 961–966.

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