Endometrial histology and biochemistry in climacteric women during oestrogen and oestrogen/progestogen therapy[1]

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Introduction

The effects on the endometrium of long-term exogenous oestrogens, given for the relief of menopausal symptoms, remain controversial. An increase in the risk ratio for endometrial carcinoma of between 4.6:1 and 8.0:1 was reported independently by three retrospective, case-control studies carried out in the United States (Mack *et al.* 1976, Smith *et al.* 1975, Ziel & Finkle 1975). Because of defects in methodology, however, these studies have been criticized (Cooke 1976, Feinstein & Horwitz 1976, Greenblatt 1976, Studd 1976). The criticisms have not only undermined the validity of the results but have also demonstrated the ascertainment bias inherent in this type of investigation. Therefore it is unlikely that further retrospective studies will be able to determine the exact relationship between exogenous oestrogens and endometrial carcinoma and prospective investigations are required to elucidate this problem.

Serial endometrial biopsy is desirable in prospective investigations. The recent introduction into clinical practice of the Vabra Aspirator (Lewis Laboratories) has allowed substantial specimens of endometrium to be obtained in the outpatient department without general anaesthesia (Holt 1970, Whitehead & Campbell 1978).

In the United Kingdom, over 90% of exogenous oestrogens are prescribed at present in cyclical regimens (Bye 1978). With cyclical therapy the urinary and plasma oestrogen levels are maintained from day to day during tablet ingestion (J Hutton, H Jacobs & V H T James, personal communication; M I Whitehead & M Sharples, unpublished), and do not follow the premenopausal oestrogen pattern which peaks when ovulation occurs at mid-cycle; also the oestrogenic stimulus is not opposed by a progestin. Therefore cyclical therapy cannot be considered physiological as during the reproductive years progesterone opposes the proliferative effect of oestrogen. In the secretory phase oestrogen-induced endometrial cell biosynthesis, as measured by the concentrations of cytoplasmic progesterone receptor, is reduced (Bayard *et al.* 1978, Kontula 1975). Oestradiol 17β-dehydrogenase, the enzyme which converts the more potent intrauterine oestrogen, oestradiol, into the less active oestrone, is also produced as a result of progesterone stimulation (Pollow *et al.* 1975, Tseng & Gurpide 1974).

To determine the endometrial response to exogenous oestrogens, and the modifying effect of progestogens on this response, we have monitored endometrial proliferation during cyclical oestrogen therapy and sequential oestrogen/progestogen therapy in a longitudinal study. Cytoplasmic progesterone receptor levels and oestradiol 17β-dehydrogenase activity were also measured in some endometrial samples. These results are now presented and their significance is discussed.

Patients and methods

One hundred and seventy-seven patients were recruited between 1973 and 1977. When the study commenced curettage was not performed before therapy was started unless specifically

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indicated. To establish baseline endometrial values this policy was later changed and subsequently a pretreatment inpatient or outpatient curettage was performed on every patient.

One hundred and eight patients entered the study without a pretreatment curettage. Not one had previously experienced abnormal vaginal bleeding. Sixty-nine patients, all with normal endometrium at pretreatment curettage, were also admitted to the study. Normal endometrium was defined as either proliferative, secretory, menstrual or atrophic endometrium, benign polypi or 'no curettings'. One hundred and fifteen patients (65%), with a mean age of 54.1 years, were postmenopausal (i.e. no spontaneous menstruation in the previous 12 months); and 62 patients (35%), with a mean age of 49.9 years, were perimenopausal (i.e. spontaneous menstruation within the previous 12 months but with at least three months between menses).

Clinical therapy

All symptomatic patients were prescribed either cyclical oestrogen or sequential oestrogen/progestogen therapy. With cyclical therapy the oestrogen was given daily in 3-week cycles with one treatment-free week between cycles. Patients received one of three oestrogen preparations and high- and low-dose regimens of each were prescribed depending upon the severity of symptoms. High-dose regimens were: conjugated oestrogens (Premarin) 1.25 mg daily; piperazine oestrone sulphate (Harmogen) 3 mg daily; and oestradiol valerate (Progynova) 2 mg daily. Low-dose regimens were the above halved. All dosages are recommended by the respective manufacturers.

With sequential therapy the oestrogen preparations and dosages prescribed were as for cyclical therapy. The progestogens were either: norethisterone (Primolut) or medroxyprogesterone acetate (Provera) and the dosages were 5 mg daily with a high-dose oestrogen regimen, and 2.5 mg daily with a low-dose regimen.

Sequential therapy was given in two ways. In the first, which the majority of patients received, the oestrogen was taken daily continuously and the progestogen was added for one week in each calendar month. In the second the oestrogen was given in 21-day cycles with one treatment-free week between cycles. The progestogen was added from the 16th to the 21st days of treatment. In addition a small number of patients received oestradiol valerate 2 mg daily for 21 days with dl-norgestrel 0.5 mg from 12th to 21st day (Cyclo-Progynova).

Frequency of curettage

Between 1973 and 1975 Vabra Suction curettage was performed routinely at intervals of 12 to 18 months and on any patient following abnormal vaginal bleeding. The high incidence of hyperplasia during cyclical therapy caused a change in policy in 1975, and thereafter patients taking unopposed oestrogens were curetted at six-monthly intervals. With sequential therapy curettage was performed at intervals of 12 to 15 months. The 10% of patients in whom Vabra curettage was unsuccessful were admitted for inpatient curettage. Endometrial samples for histology were usually obtained in the third week of treatment with cyclical therapy and in the week before the progestogen was taken with sequential therapy; samples for biochemical studies were obtained during all 4 weeks of treatment with both cyclical and sequential regimens.

Reporting of endometrial histology

All endometrial tissue was reported by one of two consultant pathologists. Nonpathological endometrium required the opinion of only one pathologist, but when hyperplasia was diagnosed by one the opinion of the second pathologist was sought and they reported jointly.

Biochemical studies

Cytoplasmic progesterone receptor levels were measured as described previously (King *et al.* 1978) in some of the endometria obtained from patients receiving high- or low-dose conjugated oestrogens, either alone cyclically or in combination with norethisterone, sequentially.

Oestradiol 17β-dehydrogenase activity was also determined during sequential high- and low-dose conjugated oestrogens/norethisterone therapy by the method of Tseng & Gurpide (1974).

Results

Endometrial histology related to type of therapy (Table 1)

Cyclical therapy ranged from 2 months to 47 months (mean duration 15.1 months). With high-dose oestrogen, cystic glandular hyperplasia was diagnosed in 16 patients (23%) and atypical hyperplasia in 6 patients (9%); with low-dose regimens, cystic glandular hyperplasia was diagnosed in 4 patients (12%) and atypical hyperplasia in 2 patients (6%).

Table 1. Endometrial histology related to type of therapy in 177 patients

Type of therapy	No. of patients	Normal endometrium	Cystic glandular hyperplasia	Atypical hyperplasia	Incidence of cystic glandular and atypical hyperplasia
Cyclical high-dose therapy	69	47	16	6	32%
Cyclical low-dose therapy	33	27	4	2	18%
Sequential high-dose therapy	46	44	1	1	4%
Sequential low-dose therapy	29	28	—	1	3%

Sequential therapy ranged from 2 months to 50 months (mean duration 16.2 months). With high-dose regimens one patient (2%) was found to have cystic glandular hyperplasia and one patient (2%) atypical hyperplasia; with low-doses atypical hyperplasia was diagnosed in one patient (3%).

It was not always possible to time the onset of hyperplasia exactly as curettage was not performed on all the patients at regular intervals. With cyclical therapy hyperplasia was diagnosed as early as two months, and as long as 35 months, after treatment started. The condition was found in 11 patients in the first 12 treatment months; in 12 patients between the 13th and 24th treatment months and in 5 patients receiving therapy for more than 25 months. In 11 of the 28 patients normal endometrium had been obtained at first curettage and hyperplasia was found at second or subsequent curettage.

Both patients in whom hyperplasia was found during sequential high-dose therapy had received treatment for only 3 months. In one, atypical hyperplasia had been diagnosed 13 years previously following abnormal vaginal bleeding. A pretreatment curettage had been performed and it is probable that this patient had a predisposition towards hyperplasia. During sequential low-dose therapy, atypical hyperplasia was diagnosed at fourth curettage in one patient after 50 months of treatment.

Follow-up of patients with endometrial hyperplasia

Twenty of the 28 patients in whom endometrial hyperplasia was diagnosed during cyclical therapy subsequently received sequential regimens. In all cases but one normal endometrium was obtained at repeat curettage. Two of the three patients in whom hyperplasia was diagnosed during sequential therapy subsequently received a combined oestrogen/progestogen regimen (norethisterone 2.5 mg given in combination with the oestrogen every day), and in both cases normal endometrium was obtained at repeat curettage.

Cytoplasmic progesterone receptors (Figure 1)

With both cyclical and sequential therapies the receptor levels with high-dose conjugated oestrogen regimens did not differ significantly from those with low-dose regimens and

therefore the high and low-dose data have been combined. Endometria were obtained during all four weeks of the cyclical and sequential treatment cycles. For each week, the number of samples assayed and the mean (±s.e.) receptor levels are shown. With cyclical therapy, weeks 1–3 represent the treatment cycles and week 4 is the treatment-free week; with sequential therapy conjugated oestrogens were given daily continuously (weeks 1–4) and norethisterone was added in week 4. The mean (±s.e.) premenopausal proliferative and secretory phase levels from the same laboratory are included for comparison.

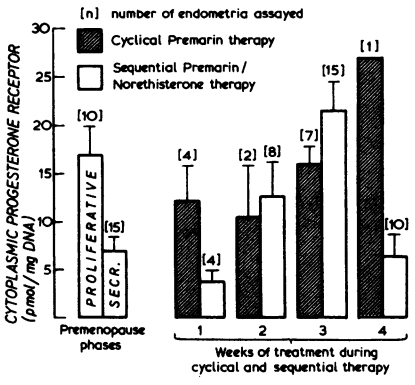

Figure 1. Mean (±s.e.) cytoplasmic progesterone receptor levels: premenopause proliferative and secretory phases and during cyclical and sequential oestrogen (Premarin) therapy

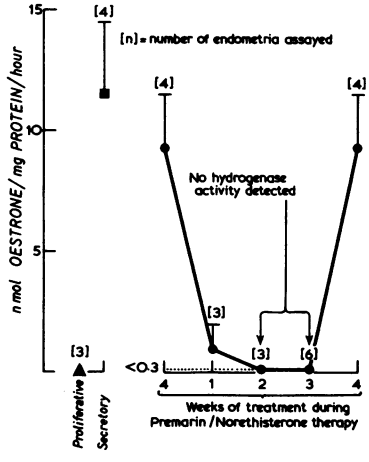

Figure 2. Mean (±s.e.) oestradiol 17β-dehydrogenase activity: premenopause proliferative and secretory phases and during sequential oestrogen (Premarin)/norethisterone therapy

Cyclical therapy: During the three treatment weeks the mean levels were more comparable with the premenopausal proliferative than secretory range. At this time therefore the endometrium was subjected to a potent oestrogen stimulus. Although the data for the treatment-free week are sparse, the level did not fall and this observation, if confirmed subsequently, would indicate that the endometrium had little respite from stimulation at this time.

Sequential therapy: The mean receptor levels increased from weeks 2 to 3, and again were more comparable with the premenopausal proliferative range. However, the mean levels were depressed both during the week of norethisterone ingestion (week 4) and in the week following (week 1) and were respectively in and below the premenopausal secretory range. In week 4 this depression could be due to the receptor being lost to the assay, either by binding to the norethisterone or by translocating to the nucleus. With the week 1 data, however, this explanation cannot apply. The linear increase in mean levels from weeks 1 to 3 was statistically significant ($P<0.01$, $r=0.527$).

Oestradiol 17β-dehydrogenase (Figure 2)

This activity was measured during sequential high- and low-dose conjugated oestrogens/norethisterone therapy. Again, endometria were obtained during all four weeks of the treatment cycle and for each week the number of samples assayed, and the mean (±s.e.) activity is shown. Included for comparison are the mean (±s.e.) premenopausal proliferative and secretory ranges from the same laboratory.

In all samples from weeks 2 and 3 no activity was detectable (<0.3 nmol oestrone formed per mg protein per hour). During norethisterone ingestion (week 4) the activity rose to the premenopausal secretory range and was significantly higher than that in weeks 2 and 3 ($P<0.001$ and $P<0.001$ respectively: Student's t test). In the week following (week 1) the activity fell dramatically.

Discussion

Cyclical oestrogen therapy is associated with both cystic glandular and atypical hyperplasia and the incidence of hyperplasia is related to the oestrogen dose.

The development of hyperplasia is to be expected. We have reported previously that exogenous oestrogens are being given in pharmacological doses. During therapy the 24-hour urinary total oestrogen excretion is within and above not only the premenopause mid-cycle ovulatory range (Whitehead *et al.* 1978), but also the range known to be associated with the development of hyperplasia (Brown *et al.* 1959). The term 'hormone *replacement* therapy' is thus inappropriate. Cytoplasmic progesterone receptor levels are an index of oestrogen stimulation and the high levels observed in this study confirm that during high- and low-dose conjugated oestrogen therapy the oestrogen stimulus being applied to the endometrium is potent. Preliminary receptor measurements during oestradiol valerate and piperazine oestrone sulphate therapy give similar results (King, Whitehead & Campbell, unpublished).

The long-term significance of this induced hyperplasia and potent oestrogen stimulus are unknown. Hyperplasia arising spontaneously in untreated women carries a risk of later malignancy which is about 1% with cystic glandular hyperplasia (McBride 1959); this risk increases to 12–25% with all grades of atypical hyperplasia (Gusberg & Kaplan 1963) and to 45% with severe atypia (Campbell & Borter 1961). The oestrogen-induced and spontaneously-arising hyperplasias are indistinguishable histologically and we believe it would be unwise to regard the induced condition as carrying a lesser risk of malignancy unless proved conclusively by long-term studies.

The clinician who wishes to control menopausal symptoms is therefore placed in a dilemma. These dosages of exogenous oestrogens are required for effective relief of symptoms, especially hot flushes, but they also excessively stimulate the endometrium in an unacceptably high number of patients when given unopposed.

The addition of a progestogen such as norethisterone or medroxyprogesterone acetate for 5 or 7 days each calendar month has been shown capable of greatly reducing the incidence of hyperplasia. Therefore progestogens protect against oestrogen-induced hyperstimulation although with these regimens this protection was incomplete. The reversal of oestrogen-induced hyperplasia to normal endometrium in all cases but one in this study, and of spontaneously-arising hyperplasia to normal endometrium in another study (Whitehead & Campbell 1978), provides further evidence of this protective effect.

It is probable that progestogens exert their beneficial effects in a variety of ways. They induce withdrawal bleeding, and the importance of regular endometrial shedding has been discussed elsewhere (Whitehead *et al.* 1978). Contrary to widely-held belief, the reestablishment of vaginal bleeding was associated with high patient compliance: 90% of patients originally offered therapy were still receiving treatment on average 16 months later (Whitehead *et al.* 1978). The depressant effect of progestogens on oestrogen-induced cell biosynthesis has been demonstrated by this study and it is to be determined still whether prescribing progestogens for a longer period of time extends this depressant effect and results in a complete suppression of hyperplasia.

Norethisterone induced the production of oestradiol 17β-dehydrogenase. This enzyme, by converting oestradiol to the less active oestrone, further reduces the oestrogenic stimulus being applied to the cell. Although with all oral exogenous oestrogens the plasma oestrone to oestradiol ratio ranges from 2:1 to 5:1 in favour of oestrone (Whitehead *et al.* 1978), our preliminary measurements of intranuclear oestrogens in patients on oral therapy indicate that oestradiol is present in greater concentration than oestrone (Whitehead, King *et al.*, unpublished). Furthermore, progesterone receptor levels correlate more closely with plasma oestradiol than oestrone concentrations (King, Whitehead & Campbell, unpublished). Thus the induction of an enzyme converting oestradiol to oestrone would be protective.

With cyclical therapy curettage is required at regular intervals on every patient for as long as treatment is prescribed. This is because vaginal bleeding is wholly unreliable as an indicator of underlying endometrial pathology (Whitehead *et al.* 1978); and also because hyperplasia can develop suddenly and unexpectedly at any time during therapy. The finding of a normal

endometrium on one occasion does not exclude the subsequent development of hyperplasia. Therefore, unless serial endometrial biopsy is being performed, we recommend that all symptomatic perimenopausal and postmenopausal women with a uterus who are receiving exogenous oestrogens should also be prescribed a progestogen for at least one week in each calendar month, to protect against the development of endometrial hyperplasia and possibly carcinoma. As the receptor mechanism in breast tissue, though less sensitive, closely resembles that in the endometrium (McGuire *et al.* 1975), we believe that in women who have had a hysterectomy serious consideration should be given also to prescribing progestogens with oestrogens in order to protect the breasts.

Summary

In a longitudinal study endometrial biopsy was performed on 177 perimenopausal and postmenopausal women receiving either cyclical oestrogen or sequential oestrogen/progestogen therapy. During cyclical therapy, endometrial hyperplasia was diagnosed in 22 of 69 patients (32%) given high-dose regimens; and in 6 of 33 patients (18%) given low-dose regimens. Measurement of cytoplasmic progesterone receptor levels confirms that the oestrogenic stimulus being applied to the endometrium is potent.

During sequential therapy, hyperplasia was diagnosed in 2 of 46 patients (4%) given high-dose regimens; and in one of 29 patients (3%) given low-dose regimens. Therefore progestogens protect the endometrium against oestrogen-induced stimulation, although our regimens of progestogens did not afford complete protection. Progestogens also depressed cytoplasmic progesterone receptor levels and induced the formation of oestradiol 17β-dehydrogenase.

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