Eur J Cancer Clin Oncol, Vol. 24, No. 1, pp. 29–43, 1988.
Printed in Great Britain

0277–5379/88$3.00+0.00
Pergamon Journals Ltd.

The Role of Oestrogens and Progestagens in the Epidemiology and Prevention of Breast Cancer

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Abstract—*The protective effect of early menopause shows that ovarian hormones increase the risk of breast cancer: it is likely that this is because they stimulate breast cell division. The mitotic rate of breast cells is higher during the luteal phase of the menstrual cycle than during the follicular phase, suggesting either that progesterone and oestrogen together induce more mitoses than oestrogen alone (the 'oestrogen plus progestagen hypothesis') or that oestrogen alone induces breast cell mitoses in a dose-dependent manner and that progesterone has no effect (the 'oestrogen alone hypothesis'). Both hypotheses are consistent with the known effects of reproductive history, obesity, combined oral contraceptives and oestrogen replacement therapy (ERT) on breast cancer risk, but while the oestrogen alone hypothesis predicts that hormone replacement therapy with oestrogen and a progestagen (HRT) will cause the same increase in risk as ERT, the oestrogen plus progestagen hypothesis predicts that HRT will cause a greater increase in risk than ERT. Both hypotheses suggest that the risk of breast cancer could be reduced by delaying the onset of regular ovulatory menstrual cycles and by minimizing the therapeutic use of oestrogens, and possibly of progestagens, in postmenopausal women. It may be possible to design hormonal contraceptives that will decrease breast cancer risk.*

INTRODUCTION

EPIDEMIOLOGICAL studies of breast cancer have provided both direct and indirect evidence that ovarian hormones play a very important role in the aetiology of this disease. We believe that an understanding of the mechanism by which hormones affect risk should eventually allow us to prevent breast cancer through hormonal means.

The 'unopposed oestrogen hypothesis' explains the epidemiology of *endometrial* cancer in terms of the effects of oestrogen ('unopposed' by progesterone) on endometrial cell proliferation. The success of this hypothesis for endometrial cancer suggests that a similar hypothesis should explain the epidemiology of breast cancer. The development of this hypothesis requires an understanding of the hormonal control of breast cell proliferation.

In this review we begin with a summary of the major risk factors for breast cancer and of why these indicate that ovarian hormones affect risk. We then discuss the epidemiology of breast cancer in relation to specific hypotheses for the hormonal control of breast cell division. Direct study of breast cell division during the menstrual cycle suggests either an 'oestrogen alone hypothesis' or an 'oestrogen plus progestagen hypothesis' for breast cancer. We examine the consistency of these hypotheses with the major breast cancer risk factors and with direct studies of endogenous hormones in breast cancer cases. We then use the hypotheses to predict the effects of exogenous hormones on breast cancer risk, and finally we discuss the possibilities for preventing the disease.

RISK FACTORS FOR BREAST CANCER

Detailed reviews of the epidemiology of breast cancer have been published by Kelsey [1] and by Moore *et al.* [2]. There are several major risk factors related to reproductive history. Risk is decreased by late menarche and by early menopause or ovariectomy. The effect of childbirth on risk is more complex. The risk of developing breast cancer before about age 40 is lower in nulliparous than in parous women, but above this age the relationship is reversed so that parous women have a substantially lower lifetime risk than nulliparous women [3]. Early first birth is associated with decreased risk at these older ages, and increasing parity appears to cause further small decreases in risk.

The overall effect of obesity is to increase the risk of breast cancer [4], but this increased risk is

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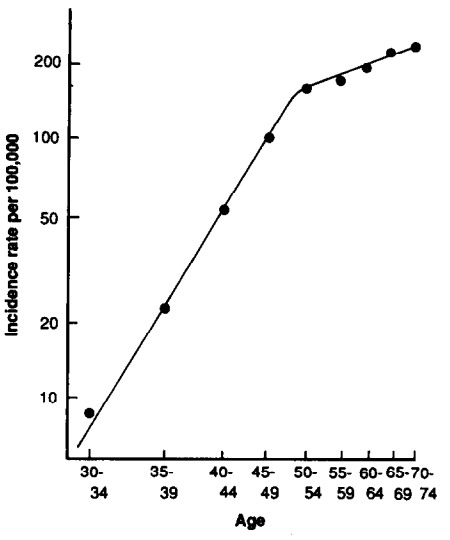

Fig. 1. Age–incidence curve of breast cancer (from data for U.S. white females 1969–1971).

restricted to older postmenopausal women [5, 6]. In premenopausal women, obesity may be associated with a small reduction in risk [7, 8].

Oestrogen replacement therapy (ERT) in postmenopausal women causes an increase in the risk of breast cancer (see below for detailed discussion), while studies of combination-type oral contraceptives (COCs) have found either no effect or an increase in risk, so it is only safe to conclude that COCs do not protect against breast cancer.

Several studies have examined the length of menstrual cycles in breast cancer cases and controls. Sherman *et al.* [9], Olsson *et al.* [10] and La Vecchia *et al.* [11] all found some evidence that cases have shorter cycles than controls: Olsson *et al.* [10] and La Vecchia *et al.* [11] found that this was due to a deficit of long and irregular cycles in cases.

The protective effect of menopause on breast cancer risk is the key factor to consider when attempting to understand the aetiology of this disease. The effect of menopause can be seen most clearly by looking at the age–incidence curve for breast cancer. When the logarithm of age is plotted against the logarithm of incidence the resultant 'curve' is approximately two straight lines (Fig. 1): the increase in incidence with age is much steeper during the premenopausal period than after the menopause. This indicates that the hormonal pattern of premenopausal women [cyclic production of relatively large amounts of oestradiol (E2) and progesterone (Pg)] causes a greater rate of increase in risk of breast cancer than the hormonal pattern in postmenopausal women (constant low E2 and very low Pg), but gives no information on the relative importance of the two ovarian hormones in determining risk.

THE UNOPPOSED OESTROGEN HYPOTHESIS FOR ENDOMETRIAL CANCER

Epidemiological studies of endometrial cancer have shown that risk is reduced by early menopause [12], suggesting that ovarian hormones increase the risk of the disease. The marked increase in risk associated with postmenopausal ERT (see Kelsey [12] for references) shows that oestrogen alone is capable of increasing the risk of endometrial cancer. Since the only known effect of oestrogen on endometrial cells is to stimulate cell division, it appears very probable that oestrogen increases the risk of endometrial cancer by increasing the rate of cell division, and that a detailed understanding of the hormonal control of endometrial cell division should explain the epidemiology of the disease.

Study of endometrial cell mitoses during normal menstrual cycles [13] has shown that the endometrial cell division rate is very low during days 1–4 of the menstrual cycle, then increases rapidly to a maximum and remains at about the same maximal rate until day 19: the rate then decreases sharply to the very low levels found on days 1–4 and remains at this level for the remainder of the cycle. Endometrial cell proliferation therefore appears to be maximally stimulated (after a lag of about 4 days) by the low plasma E2 levels (50 pg/ml) found in the early follicular phase [14] and to be effectively zero in the presence of luteal phase Pg levels (there appears again to be a lag period in mitotic activity of about 4 days before the 'opposing' effect of Pg is observed; see figures in Key and Pike [15]). Pg opposes the stimulatory effect of E2 on the endometrium mainly by reducing the concentration of E2 receptors, but also by increasing the metabolism of E2 to the less active oestrone (E1) and by stimulating differentiation of endometrial cells to a secretory form (see Henderson *et al.* [16] for references). These observations have led to the unopposed oestrogen hypothesis for endometrial cancer, which maintains that oestrogen unopposed by a progestagen increases the risk of endometrial cancer by stimulating endometrial cell division [16, 17].

This hypothesis provides a very satisfactory explanation of the major risk factors for endometrial cancer. Early menopause reduces risk by reducing the unopposed E2 concentration, and the associated cell division rate, from the relatively high level during the follicular phase of every menstrual cycle to the low level of the postmenopausal period. ERT increases the effective plasma oestrogen concentration and hence the endometrial cell division rate above the usual postmenopausal level. COCs reduce the risk of endometrial cancer (see Weiss *et al.* [18] and Henderson *et al.* [19] for references) because they reduce the period of endometrial exposure to unopposed oestrogen from the 14 days of a normal

follicular phase to the 7 days per 28 day cycle during which the COC is not used, and because endogenous oestrogen concentrations, and therefore cell division rates, are very low during these 7 days [20].

The explanation for the increased risk of endometrial cancer associated with obesity is less straightforward. In postmenopausal women obesity is related to increased concentrations of E2 and increased concentrations of non-protein bound E2 [21] and therefore is related to increased exposure of the endometrium to unopposed E2. In premenopausal women obesity is not clearly related to E2 concentrations [22] but is strongly related to an increase in non-protein bound E2 [21]. As we noted above, however, increases in plasma E2 above 50 pg/ml do not appear to cause any further increases in endometrial mitotic rate, and since E2 concentrations are above this upper limit throughout the follicular phase, the small increases in E2 or in free E2 caused by obesity should not increase cell division (see Key and Pike [15] for discussion). The effect of obesity on risk in premenopausal women is therefore likely to be due not to an increased concentration of unopposed (follicular phase) E2 but to an extended exposure to unopposed E2 due to underproduction of Pg. This conclusion is supported by evidence that obesity is associated with amenorrhoea [23], with subnormal luteal phase Pg concentration [24], and with irregular menstrual periods [8, 25], although not all investigators have found this [26].

An important aspect of the unopposed oestrogen hypothesis is that it can be used to suggest how various factors will modify the 'normal' age–incidence curve for endometrial cancer, and hence can allow one to predict the effect of these factors on lifetime risk. As we noted above with breast cancer, if the logarithm of age is plotted against the logarithm of incidence for endometrial cancer, the resultant 'curve' is approximately two straight lines (see Fig. 2a): a steeply sloping line during the premenopausal period, when unopposed E2 and cell division rates (during the follicular phase) are high, and a line with a much shallower slope in the postmenopausal period when unopposed E2 and cell division rates are low. Figure 2 shows this normal curve and how it is likely to be modified by the alterations in unopposed E2 and cell division rates caused by early menopause (Fig. 2a), and by ERT and COCs (Fig. 2b). Early menopause advances the age at which the postmenopausal slope begins. ERT delays the onset of the postmenopausal slope, and 'high dose' ERT in fact increases the slope above that of the premenopausal line because the endometrium is exposed to unopposed oestrogens for more than 14 days out of each 28 day cycle. COCs produce a temporary

Fig. 2a. Age–incidence curve of endometrial cancer showing the normal curve (from data for the West Midland Region, U.K., 1968–1972) and the predicted effect of early menopause at age 40.

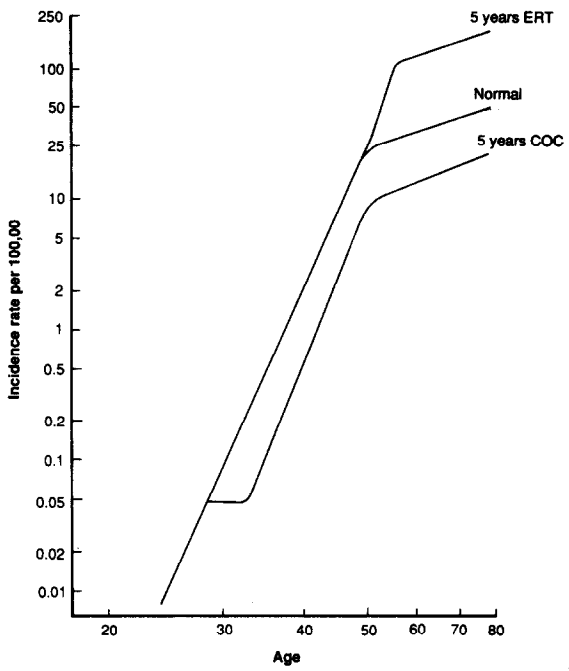

Fig. 2b. Age–incidence curve of endometrial cancer showing the normal curve and the predicted effects of 5 years of combination-type oral contraceptives (COC) starting at age 28 and of 5 years of high dose oestrogen replacement therapy (ERT) starting at age 50.

plateau in the premenopausal line, because endometrial exposure to unopposed oestrogens is minimal during COC use and endometrial cell division is rare. The most important point to note from Fig. 2 is that the predicted changes in incidence caused by hormonally controlled changes in cell division rates persist throughout life: these predicted lifelong effects have all been observed (to the extent possible) in epidemiological studies.

The success of the unopposed oestrogen hypothesis for endometrial cancer in explaining and predicting the epidemiology of this disease has stimulated the effort to develop an equivalent

hypothesis for breast cancer. This requires an understanding of the control of breast cell division.

THE HORMONAL CONTROL OF BREAST CELL DIVISION

The biopsy studies of Anderson and Ferguson [27, 28] and the recent autopsy study of Longacre and Bartow [29] show clearly that breast epithelial cell division rates are low during the follicular phase and high during the luteal phase, with a peak at days 23–25. These findings are in agreement with the earlier studies of Meyer [30] and Masters *et al.* [31]; the contrary results of Vogel *et al.* [32] are inexplicable.

The effects of oestrogens and progestagens on breast cell division have also been studied in cell culture systems. In their study of human breast tissue transplanted to athymic mice, McManus and Welsch [33] found that E2 alone caused a large increase in DNA synthesis, while Pg alone caused a small increase, and Pg with E2 had a similar effect to that of E2 alone. The E2 dose McManus and Welsch used was physiological in the sense that the dose was in the range in which a cell division dose–response was observed, but no explanation of the Pg dose was given and it is not clear that it was near the physiological luteal phase peak. Longman and Buehring [34] found that E2 alone stimulated breast cell division in primary culture, and that Pg alone had no significant effect, but they did not examine the effect of E2 and Pg together. The media concentrations of Pg and of E2 were some 3–4 times the serum concentrations found in late pregnancy, with a Pg concentration 10 times that of E2; these concentrations were chosen on the basis of significant *malignant* breast cell proliferation (in their system both E2 and Pg stimulated *malignant* cell growth). The luteal phase Pg concentration is some 50 times the E2 concentration (and one-sixth the late pregnancy Pg concentration) so it is again not clear that the experimental situation is mimicking the luteal phase of the cycle as is required. The synthetic progestagens Longman and Buehring tested had no significant effect on division of normal cells, either alone or in combination with ethinyl oestradiol. In contrast, in similar *in vitro* studies reported by Mauvais-Jarvis *et al.* [35] some synthetic progestagens inhibited the mitogenic effect of E2: the media concentrations used by these investigators were in the physiological range, and much lower than those used by Longman and Buehring. Further laboratory studies are needed, although there will always be uncertainties in extrapolating their findings to *in vivo* physiological conditions.

Two interpretations of the evidence relating to the hormonal control of breast cell division have to be seriously considered. A simple explanation of the biopsy and autopsy studies is that E2 alone (in the follicular phase) induces some cell division, but that E2 and Pg together (in the luteal phase) induce much more cell division. The failure of the laboratory studies to show a significant mitogenic effect of Pg suggests an alternative explanation: breast cell division is induced only by oestrogens with Pg having little or no effect. This alternative explanation requires there to be a dose–response relationship between cell division and E2 plasma concentration in the range of E2 concentrations occurring during the normal menstrual cycle, and also a 4–5 day lag time between changes in E2 concentration and the induced changes in cell division.

As we discussed above, ovarian hormones appear to affect the risk of endometrial cancer through their effects on endometrial cell division (the 'unopposed oestrogen hypothesis'), and we consider it very likely that they affect the risk of breast cancer similarly by their effects on breast cell division. Because of our incomplete knowledge of the hormonal control of breast cell division, we need to consider the two hormonal explanations of breast cell division rates which we discussed above. The 'oestrogen plus progestagen hypothesis' is that increased exposure to oestrogen alone causes some increase in breast cancer risk but that risk will be increased much more by exposure to oestrogen and a progestagen together. The 'oestrogen alone hypothesis' is that oestrogen alone increases risk and that progestagens are irrelevant.

Many authors have considered an 'unopposed oestrogen hypothesis' for breast cancer, that is, that the effect of oestrogens and progestagens on the breast is the same as their effect on the endometrium, with unopposed oestrogen increasing risk and progestagens (including progesterone in the physiological range) being protective. The direct observational studies show that in the physiological range Pg cannot be effectively opposing the mitogenic effects of oestrogen on breast cells.

In the following sections we examine current knowledge of endogenous and exogenous hormonal exposure in breast cancer cases, and then consider the consistency of this evidence with the alternative hypotheses.

ENDOGENOUS OESTROGENS AND PROGESTERONE IN BREAST CANCER CASES AND CONTROLS

Tables 1–3 summarize the results of many case-control studies and two prospective studies which have investigated exposure to endogenous hormones by making measurements of hormones in urine or plasma. E2 is the most potent endogenous oestrogen and is quantitatively dominant in premenopausal women, but in postmenopausal women

Table 1. *Urinary hormone excretion in breast cancer cases and controls**

First author [reference]	Date	Cases	Controls	Day of sample	E	*P*	Preg	*P*	T	*P*
Premenopausal										
Persson [36]	1964	15	9	Foll	1.3	NS	—	—	—	—
		8	11	Lut	1.1	NS	—	—	—	—
Marmorston [37, 38]	1965	5	12	18–23	0.7	NS	0.6	NS	—	—
Arguelles [39]	1973	47	8	14	0.7	0.05	—	—	—	—
Kodama [40]	1977	43	58	7	—	—	0.7	0.01†	—	—
		35	135	20	—	—	0.7	0.01†	—	—
Cole [41]	1978	73	55	10	1.1	NS	—	—	—	—
				21	1.1	NS	0.8	NS	—	—
MacMahon [42]	1983	94	70	10	>1	0.05	—	—	—	—
				21	>1	0.05	—	—	—	—
Secreto [43]	1983	18	22	18–22	—	—	—	—	1.8	0.01
Meyer [44]	1986	41	119	6	1	NS	1	NS	—	—
		40	110	20–22	<1	NS	<1	NS	—	—
Postmenopausal										
Persson [36]	1964	35	11	—	1.5	NS	—	—	—	—
Marmorston [37, 38]	1965	21	20	—	1.9	0.05	—	—	—	—
Gronroos [45]	1968	14	10	—	0.7	0.05	—	—	—	—
Arguelles [39]	1973	25	10	—	1.1	NS	—	—	—	—
Grattarola‡ [46]	1974	17	12	—	1.9	NS	—	—	2.9	0.001
Thijssen§ [47]	1975	41	48	—	1.0	NS	—		—	
Morreal [48]	1979	35	22	—	2.2	0.0001	—	—	—	—

Abbreviations: E = total urinary oestrone, oestradiol and oestriol; Foll = follicular; Lut = luteal; Preg = pregnanediol; T = testosterone.

*Hormone excretion is expressed as the mean rate in cases divided by the mean in controls.

†Significance of logarithmically transformed values.

‡E is oestrone and oestradiol only.

§E is oestrone and oestriol only.

E1 may also be important. The relationship between plasma oestrogens and urinary oestrogens is complex [69] but excretion rates of E1, E2 and oestriol (E3) give some indication of oestrogen production rates, plasma concentrations, and tissue exposure. Many recent studies have measured the distribution of E2 binding to plasma proteins, following the suggestion of Siiteri *et al.* [68] that the risk of breast cancer may be increased by an increased percentage of non-protein bound E2 (% free E2), or an increase in the percentage of E2 not bound to sex hormone binding globulin (% non-SHBG bound E2; this is approximately equivalent to the percentage of E2 bound to albumin).

The results in Tables 1–3 strongly suggest that postmenopausal breast cancer cases are exposed to more endogenous oestrogen than controls. Five out of seven urinary studies found evidence of greater oestrogen excretion in cases, three out of five studies found higher plasma E1, eight out of 11 found higher plasma E2, and eight out of eight found either higher % free E2 or total free E2 in cases. It should be noted however that only two of these studies were prospective: of these two studies, that by Moore *et al.* [67] did not report E2 concentrations, and that by Wysowski *et al.* [58] found no significant differences between cases and controls.

Measurements of urinary and plasma oestrogens in premenopausal women (Tables 1 and 2) have produced inconsistent results. The studies of E2 binding (Table 3) are more consistent, with four out of five studies reporting a higher % free E2 in cases. Again only one of these studies [67] was prospective, and there appears to be a decrease in the magnitude of the difference found in subsequent studies, which may reflect improvements in study design, in particular matching case and control samples on storage time [65]. We conclude that there is little evidence of increased E2 exposure in premenopausal cases.

Several studies (Tables 1 and 2) have measured Pg, or its major urinary metabolite pregnanediol, in premenopausal women. All four studies of pregnanediol, and five out of six studies of Pg, found lower levels in cases than in controls. One of these studies was prospective. An additional study which reported an indirect measurement of Pg production was that of Grattarola [70]: he examined endometrial biopsies in the late luteal phase and found evidence of deficient Pg production (with or without anovulation) in stage I and II breast cancer cases. Three prospective studies have examined breast

*Table 2. Plasma hormone concentrations in breast cancer cases and controls**

First author [reference]	Date	Cases	Controls	Day of sample	E1	*P*	E2	*P*	Pg	*P*	T	*P*
Premenopausal												
Swain [49]	1974	25	88	18–24	—	—	—	—	1.1	NS	—	—
England [50]	1974	10	32	Follic†	—	—	>1	0.05	—	—	—	—
				Luteal‡	—	—	>1	0.05	—	—	—	—
Malarkey [51]	1977	5	9	Luteal	—	—	0.9	NS	0.8	NS	2.0	0.05
Drafta [52]	1980	25	41	12–16	1.4	0.05	1.0	NS	0.9	NS	—	—
				19–24	1.1	NS	0.3	0.001	0.6	0.01	—	—
Sherman [53]	1979	13	17	All§	—	—	1.0	NS	—	—	—	—
Moore [54]	1982	38	32	Any	—	—	1.1	NS	—	—	—	—
Secreto [55]	1984	23	55	20–23	—	—	—	—	0.6	0.05	1.3	0.005
Bruning [56]	1985	17	16	18–24	—	—	1.1	NS	—	—	—	—
Meyer [44]	1986	36	103	20–22	—	—	>1	NS	<1	NS	—	—
Siiteri [57]	1986	36	36	Any	1.3	NS	1.1	NS	—	—	—	—
Wysowski‖ [58]	1987	17	68	Matched	0.8	NS	0.8	NS	0.7	NS	1.0	NS
Postmenopausal												
England [50]	1974	25	25	—	—	—	1.3	0.05	—	—	—	—
McFadyen [59]	1976	6	6	—	—	—	1.2	NS	—	—	1.7	0.05
Malarkey [51]	1977	12	9	—	—	—	1.1	NS	1.0	NS	0.7	NS
Adami [60]	1979	122	122	—	1.2	0.004	—	—	—	—	1.1	0.042
Drafta [52]	1980	39	23	—	1.1	NS	3.0	0.001	0.5	NS	—	—
Moore [54]	1982	38	38	—	—	—	2.1	0.001	—	—	—	—
Reed [61]	1983	26	32	—	1.1	NS	1.0	NS	—	—	—	—
Secreto [62]	1983	28	30	—	—	—	0.9	NS	—	—	1.7	0.001
Reed [63]	1985	9	8	—	—	—	1.3	0.05	—	—	—	—
Bruning [56]	1985	38	67	—	—	—	2.1	0.02¶	—	—	—	—
Hill [64]	1985	33	59	—	—	—	—	—	—	—	1.6	0.01
Siiteri [57]	1986	38	38	—	1.0	NS	1.1	NS	—	—	—	—
Wysowski‖ [58]	1987	39	156	—	1.0	NS	1.0	NS	1.0	NS	1.1	NS

Abbreviations: E1 = oestrone; E2 = oestradiol; Pg = progesterone; T = testosterone.
*Hormone concentrations are expressed as the mean value in cases divided by the mean value in controls.
†Mean of 11–14 days pre-ovulation.
‡Mean of 4–12 days post-ovulation.
§Mean of alternate days during one cycle.
‖Prospective study.
¶Significance of logarithmically transformed values.

cancer incidence in women with clinical evidence of progesterone deficiency (Table 4). Although the total number of cases in the three studies is only 30, and the results are not consistent with regard to any relationship with menopausal status at diagnosis, all three studies found an increased risk of breast cancer in the progesterone deficient group. All these studies are thus consistent in finding decreased progesterone exposure in breast cancer cases.

Several of the studies summarized in Tables 1 and 2 included measurements of testosterone, and most of these found higher testosterone in cases than in controls. Little is known of the effects of testosterone on the female breast. These findings require further investigation.

ERT AND BREAST CANCER RISK

Table 5 lists studies of ERT and breast cancer risk which have used population controls (those studies which used hospital controls appear to suffer from unknown biases and we have therefore not discussed them here). The results clearly show that an increase in exposure to oestrogen (unopposed by a progestagen) causes a small increase in breast cancer risk.

The studies shown in Table 5 relate essentially to ERT given as conjugated equine oestrogens (CEE) at daily doses of 0.625 mg or 1.25 mg for 21 to 28 days in each 28 day cycle. The 0.625 mg dose produces a peak plasma E2 concentration at approximately the early follicular level (50 pg/ml), with a non-protein bound E2 and a non-SHBG bound E2 at approx. 70% of the early follicular level. CEE doses of 1.25 mg produce a 75 pg/ml peak E2 level, but only a small further increase in free E2 and in non-SHBG bound E2 (see Key and Pike [15] for references). The table shows that 20 years of such ERT use produces a relative risk of between 1.5 and 2 for breast cancer; in Fig. 3 we have used an estimated relative risk of 1.75 for 20 years ERT use (see below).

*Table 3. Oestradiol distribution and binding protein concentrations in breast cancer cases and controls**

First author [reference]	Date	Cases	Controls	Day of sample	% free E2	*P*	Total free E2	*P*	% albumin bound E2	*P*	Albumin	*P*	SHBG	*P*
Premenopausal														
Moore [54]	1982	41	32	Any	1.34	0.001	1.58	0.01†	—	—	0.97	0.05	1.05	NS
Secreto [55]	1984	23	55	20–23	—	—	—	—	—	—	—	—	1.04	NS
Bruning [56]	1985	17	16	18–24	1.09	NS	0.98	NS	—	—	1.04	NS	0.91	NS
Langley [65]	1985	7	32	Any	—	—	—	—	—	—	0.98	NS	0.91	NS
Ota [66]	1986	7	7	Any	1.19	0.05	—	—	1.32	0.05	—	—	0.80	0.05
Moore[67]	1986	12	107	Any	1.08	0.003	—	—	1.07	NS	—	—	0.87	NS
Meyer [44]	1986	36	103	20–22	—	—	—	—	—	—	—	—	>1	NS
Siiteri [57]	1986	36	36	Any	0.89	NS	1.00	NS	—	—	—	—	1.18	NS
Postmenopausal														
Moore [54]	1982	38	38	—	1.27	0.001	2.63	0.001†	—	—	0.94	0.001	0.82	0.05
Reed [61]	1983	58	32	—	1.22	0.001	—	—	—	—	—	—	1.05	NS
Reed [63]	1985	9	8	—	1.13	NS	1.63	0.01	1.03	NS	—	—	0.86	NS
Bruning [56]	1985	38	67	—	1.05	NS	1.87	0.01†	—	—	1.04	0.01	0.99	NS
Langley [65]	1985	31	39	—	1.13	0.05	—	—	—	—	0.97	NS	0.92	NS
Ota [66]	1986	15	15	—	1.23	0.05	1.37	<0.05	1.35	0.05	—	—	0.76	0.05
Moore[67]	1986	12	105	—	1.17	0.0002	—	—	1.15	0.03	—	—	0.78	0.03
Siiteri [57]	1986	38	38	—	1.01	NS	1.50	NS	—	—	—	—	1.02	NS
Pre and post														
Siiteri [68]	1981	17	17	Any	1.41	0.001	—	—	—	—	—	—	—	—
Langley [65]	1985	20	35	Any	1.10	0.025	—	—	1.14	0.025	—	—	0.86	NS

Abbreviations: E2 = oestradiol; SHBG = sex hormone binding globulin.
*Oestradiol distribution and protein concentrations are expressed as the mean value in cases divided by the mean value in controls.
†Significance of logarithmically transformed values.
‡Prospective study.

*Table 4. Breast cancer incidence in women with clinical evidence of progesterone deficiency**

First author [reference]	Date	Progesterone deficiency subjects	Comparison group	Menopausal status at diagnosis	*n*	RR	*P*
Cowan [71]	1981	Evidence of deficient Pg production	Non-hormonal infertility	Premenopausal Postmenopausal	9 1	5.4 0.3	<0.05 NS
Coulam [72]	1983	Chronic anovulation syndrome	Olmsted County incidence rates	Premenopausal Perimenopausal Postmenopausal	4 3 5	1.3 0.9 3.6	NS NS <0.05
Ron [73]	1987	Evidence of E production. No evidence of Pg production	Israel Cancer Registry incidence rates	Premenopausal	8	1.8	NS

*Abbreviations: E = oestrogen; *n* = number of cases of breast cancer; Pg = progesterone; RR = relative risk of developing breast cancer in progesterone deficiency subjects relative to comparison group.

COCS AND BREAST CANCER RISK

Recent studies which included a substantial number of long term users of COCs (see McPherson and Drife [86] and *Lancet* Editorial [87] for summary and references) have found either that COCs have no effect on breast cancer risk or that they increase risk. For the purposes of this discussion it is sufficient to note that none of these studies has found that COCs are protective.

COC use exposes the breast to 3 weeks of exogenous oestrogen plus progestagen and 1 week of low concentrations of endogenous E2 during each 4 week treatment cycle [20]. In comparison with hormonal exposure during a normal menstrual cycle, COC use drastically reduces breast exposure to unopposed oestrogen, but probably leaves total oestrogen exposure largely unchanged, and increases progestagen exposure.

THE RELATIONSHIP OF REPRODUCTIVE RISK FACTORS AND OBESITY WITH EXPOSURE OF THE BREAST TO OESTROGENS AND PROGESTAGENS

Late menopause (as discussed above) and early menarche both increase the duration of breast exposure to cyclical E2 and Pg, but whether these risk factors are related to the level of exposure to either hormone is not clear. There is evidence that

*Table 5. ERT and breast cancer risk in studies using population controls**

First author [reference]	Date	Cases	Controls	Duration or dose of ERT	RR	P†	Adjustment for age at and type of menopause	Comments
Hoover [74]	1976	49	National Cancer Survey rates	Ever <5 Y‡ 5–9 Y 10–14 Y 15+ Y	1.3 0.9 1.2 1.3 2.0	0.05 Trend 0.02		High prevalence of surgical menop in cases did not produce low breast cancer incidence
Ross [75]	1980	138	281	Ever 1–1499 mg accumulated dose 1500+ accumulated dose	1.1 0.8 1.9	NS Trend 0.06	Type of menop: yes Results not altered by adjusting for age at menop	Increased RR in naturally postmenopausal women
Hoover [76]	1981	345	611	Ever ≤4 Y 5+ Y	1.4 1.4 1.7	0.05 Trend 0.02	Type of menop: yes Results not altered by adjusting for age at menop	An unspecified number of subjects were premenopausal
Hulka [77]	1982	152	620	0.5–3 Y 4–9 Y 10+ Y Injected E	2.1 1.5 1.7 4.4	0.05 NS NS 0.05	Results not altered by adjusting for age at menop	Similar results using hospital controls. Results are for naturally postmenopausal subjects only
Gambrell [78]	1983	53	National Cancer Survey rates	Untreated E E + progestagen	1.4 0.7 0.3	0.05 NS 0.05		Possible problem with woman-years calculations
Hiatt [79]	1984	119	119	Ever ≥5 chart notations ≥3 Y	0.7 2.1 1.8	NS 0.05 NS	Type of and age at menop: matched	Mean duration of follow-up 5 Y. All subjects had bilateral ovariectomy before age 55
McDonald [80]	1986	183	531	1–5 Y§ 6+ Y	0.8 0.7	Trend 0.06	Type of menop: yes Age at menop: −47,48+	Large effect of age at menop Bilateral ovariectomy RR = 1.3
Brinton [81]	1986	1960	2258	Ever <5 Y 5–9 Y 10–14 Y 15–19 Y 20+ Y	1.0 0.9 1.1 1.3 1.2 1.5	NS Trend 0.02	Type of menop: yes Age at menop: yes, but age groups not stated	
Nomura [82]	1986(i)	160	159	<1 Y 1–5 Y 6+ Y	0.9 0.7 1.3	NS NS NS	Age at menop: by logistic regression	21% of subjects premenopausal. Caucasion
	1986(ii)	181	181	<1 Y 1–5 Y 6+ Y	2.4 0.7 1.9	0.05 NS NS	Age at menop: by logistic regression	21% of subjects premenopausal. Japanese
Wingo [83]	1987	1369	1645	Ever 1–4 Y 5–9 Y 10–14 Y 15–19 Y 20+ Y	1.0 1.1 1.1 0.8 1.3 1.8	NS Trend 0.7	Type of and age at menop: logistic regression	Age range 25–54
Buring [84]	1987	221	33335	Ever <5 Y 5–9 Y 10+ Y	1.1 1.0 1.5 0.9	NS Trend 0.27	Results not altered by adjusting for type of and age at menop	
Hunt [85]	1987	50	Cancer Registry rates	Ever	1.6	0.05		

*Abbreviations: E = oestrogens; ERT = oestrogen replacement therapy; menop = menopause; RR = relative risk; Y = years.
†*P* values refer to RRs in use categories relative to never users, or to the trend with increasing duration of use where indicated.
‡Figures represent years of follow-up, which were highly correlated with years of use.
§Never category includes less than 1 Y ERT.

late menopause and *late* menarche are associated with an excess of long and/or anovular cycles [88, 89] which would indicate reduced Pg production, and may also indicate reduced E2 production, as occurs in athletic or anorexic amenorrhoea [90, 91]. There is some evidence that early menarche is associated with increased oestrogen production both in adolescence and in women as old as 30–39 years [92].

As discussed above in the section on endometrial cancer, obesity in postmenopausal women increases E2 and % free E2. Severe obesity can result in an

increase in E2 from approx. 10 pg/ml in non-obese postmenopausal women to approx. 25 pg/ml: the fall in SHBG with obesity results in rises in free E2 and non-SHBG bound E2 from approx. 0.2 to 0.5 pg/ml and from approx. 8 to 19 pg/ml respectively (see [15] for references). These values are close to those of the early follicular phase (approx. 0.9 and 34 pg/ml respectively). In premenopausal women obesity has little effect on total E2 but causes an increase in % free E2 and is associated with defective Pg production. Thus obesity increases breast exposure to E2 and particularly to unopposed E2.

Henderson *et al.* [93] discussed the evidence that long cycles indicate a prolonged follicular phase with a normal length luteal phase, and concluded that the deficit of long cycles found in breast cancer cases indicates that they had spent a greater proportion of each year in the luteal phase.

Pregnancy exposes the breast to a very large but short term increase in both E2 and Pg. First pregnancy causes an immediate increase in risk [3], probably due to increased hormonally induced cell division, and a long term decrease in risk, probably due to differentiation of breast cells to a less susceptible state [94]. The evidence that pregnancy causes long term changes in E2 or Pg is poor (there is better evidence for a reduction in prolactin concentration, see Musey *et al.* [95]).

DISCUSSION OF ALTERNATIVE HORMONAL HYPOTHESES FOR BREAST CANCER

We have described the major risk factors for breast cancer, an approach to the understanding of breast cancer risk in terms of the hormonal control of breast cell division, and the evidence concerning the hormonal exposure of breast cancer cases. We now draw some conclusions.

The 'oestrogen plus progestagen hypothesis' is consistent with the observed small increase in risk associated with the increased level of unopposed oestrogen in ERT and in postmenopausal obesity, with the increase in risk associated with early menarche, with the small protective effect of premenopausal obesity, and with the evidence for regular 'normal' cycles in cases. The hormonal content of COCs suggests that if oestrogens plus progestagens increase breast cell proliferation then COCs should cause more mitoses than in a normal cycle and hence should increase breast cancer risk; calculations suggest that 5 years COC use should give a relative risk of about 1.3 (derived using model in [96]), which is compatible with the risks observed. Against this hypothesis is the evidence for low Pg in cases and for increased risk in Pg deficiency; this conflict can only be removed by supposing that low Pg production is an early consequence of disease, which occurs some time before diagnosis. Swain *et al.* [49] found that only breast cancer cases with advanced disease had decreased Pg production and an excess of anovulatory cycles, but the other studies of Pg (Tables 1 and 2), which all found low Pg in cases, were of cases with early disease. The possibility of observing very early preclinical metabolic effects of cancer has, however, been demonstrated in studies of plasma retinol [97].

The 'oestrogen alone hypothesis' is consistent with the observed effects of age at menarche, age at menopause and COCs on breast cancer risk. The consistency of this hypothesis with the observed relatively small effects of ERT and postmenopausal obesity depends on the dose–response relationship between E2 and breast cell division. ERT and postmenopausal obesity produce an oestrogen level close to that of the early follicular phase: this level is sufficient to induce near maximal endometrial cell division, but the breast cell division rate induced by the early follicular phase oestrogen level is much less than that induced (on this hypothesis) by the late follicular and luteal oestrogen levels (as we noted above when discussing this hypothesis in the section on the hormonal control of breast cell division). The increase in breast cancer caused by ERT and postmenopausal obesity is thus much less than that observed in endometrial cancer. The increase is also less than that caused by late menopause since late menopause is associated with higher (premenopausal) oestrogen levels.

The 'unopposed oestrogen hypothesis' is consistent with observations on endogenous Pg but not with the hormonal control of breast cell division, unless one supposes that unopposed E2 causes division of susceptible stem cells whereas E2 plus Pg causes division only to differentiated, less susceptible cells. The marked increase in breast cancer risk associated with early menarche would not be predicted by this hypothesis, because early menarche is associated with relatively ample Pg production. It is difficult to make this hypothesis consistent with the small increases in risk observed with ERT and with postmenopausal obesity; much bigger risks would be expected, as are observed with endometrial cancer. Obesity is also a risk factor for endometrial cancer in premenopausal women: as we noted above we believe this is due to Pg deficiency and therefore prolonged exposure to unopposed E2. Whether this is true, or whether the risk is caused by an increase in the level of 'bioavailable' E2 due to low SHBG, the absence of an increased risk of breast cancer in premenopausal obese women is strong evidence against the unopposed oestrogen hypothesis for this disease. The other major problem for this hypothesis is that COCs have no protective effect against breast cancer, again in contrast to their marked protective effect against endometrial cancer.

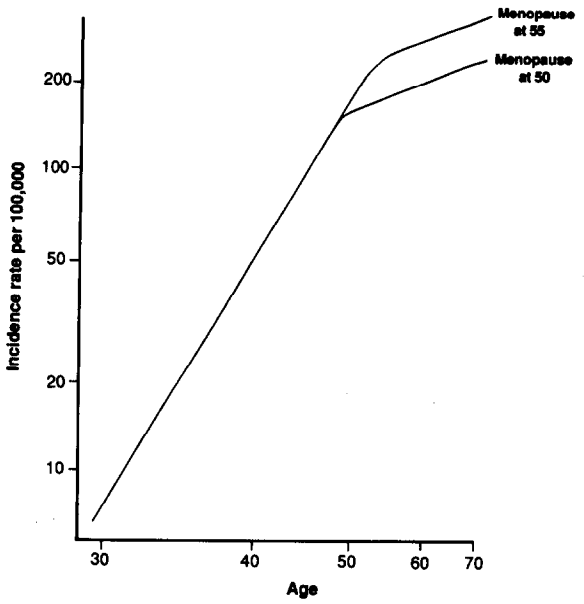

Fig. 3a. Age–incidence curve of breast cancer showing the normal curve (from data for U.S. white females 1969–1971) and the predicted effect of late menopause at age 55.

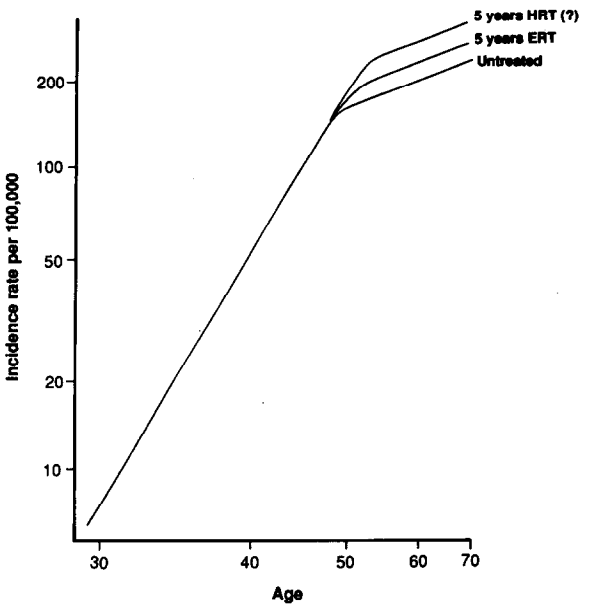

Fig. 3b. Age–incidence curve of breast cancer showing the normal curve, the predicted effect of 5 years of oestrogen replacement therapy (ERT) starting at age 50, and the effect, as predicted by the oestrogen plus progestagen hypothesis, of 5 years of hormone replacement therapy [HRT (?)] starting at age 50.

We conclude that current evidence provides little support for the unopposed oestrogen hypothesis, but that both the oestrogen plus progestagen and the oestrogen alone hypotheses must be pursued. Several areas of research should produce valuable information for choosing between these hypotheses. The effect of depot medroxyprogesterone acetate (DMPA, Depo Provera) on risk must be established, since this progestagen causes complete suppression of ovulation and therefore of endogenous oestrogen production: currently available findings are based on small numbers and are inconclusive [98–100]. Also important is knowledge of the effects of 'progestagen only' oral contraceptives and of different formulations of COCs both on breast mitoses and on breast cancer risk. Further studies of clinically progesterone deficient women are required, and should include more measurements of endogenous hormones and longer follow up.

PREDICTION OF THE EFFECTS OF ERT AND HRT ON BREAST CANCER INCIDENCE

We discussed briefly how the incidence of endometrial cancer can be predicted using the unopposed oestrogen hypothesis. We now examine equivalent predictions using the oestrogen plus progestagen hypothesis and the oestrogen alone hypothesis for breast cancer.

We have already noted that the plot of the logarithm of age against the logarithm of incidence for breast cancer is approximately two straight lines (Fig. 1). Figure 3a shows the effect of age at menopause on this plot. Just as for endometrial cancer (Fig. 2a), a delay in the onset of menopause causes an approximately equal delay in the onset of the postmenopausal slope [96, 101]. For breast cancer a 5 year delay in menopause produces a relative risk of about 1.4 [102].

The relative risk observed with ERT is about 1.75 for 20 years of use (see Table 5 and discussion above), which is equivalent to a relative risk of 1.16 for 5 years of use. Figure 3b shows this effect of 5 years of ERT on breast cancer risk: during the period of ERT use the slope of the age–incidence plot is greater than that of the postmenopausal period but is less than that of the premenopausal period. The fact that 5 years of ERT is associated with a lower relative risk than a 5 year delay in menopause is consistent with both the oestrogen plus progestagen hypothesis and with the oestrogen alone hypothesis, because both predict that ERT will cause fewer breast cell mitoses than extended exposure to premenopausal hormones. Under the oestrogen plus progestagen hypothesis this is because of the absence of a progestagen, while under the oestrogen alone hypothesis this is because the oestrogen level is close to that of the early follicular phase and is therefore lower than the average premenopausal level.

No adequate studies of the effects of HRT on risk have yet been reported, so the effects of HRT predicted by the two hypotheses are of particular interest. Under the oestrogen alone hypothesis progestagens are irrelevant, so that the effect of HRT will be the same as that of ERT illustrated in Fig. 3b

(assuming that the oestrogen dose is the same). Under the oestrogen plus progestagen hypothesis the effect of high dose HRT will be almost the same as that of late menopause, a considerably greater increase in risk than that observed with ERT. The effect of 5 years HRT use as predicted by the oestrogen plus progestagen hypothesis is shown in Fig. 3b.

OESTROGENS, PROGESTAGENS AND THE PREVENTION OF BREAST CANCER

There is little doubt that a reduced lifetime exposure to ovarian hormones will reduce breast cancer risk. Age at menarche is related to the balance between energy intake and energy expenditure during early adolescence [103], and the onset of regular ovulatory cycles may be delayed by moderate exercise [104]. Avoidance of childhood obesity and encouragement of moderate physical exercise in adolescence should therefore reduce breast cancer risk; this prediction is supported by the report of reduced breast cancer prevalence in a cohort of college athletes [105]. The effects of changes in dietary composition on endogenous sex hormones in premenopausal women are not yet clear, but the possibility of reducing plasma oestrogens by choosing a fat diet [106] must be investigated.

Early menopause can be produced by ovariectomy. This would reduce the risk of breast cancer, even if ERT was given subsequently; such ERT would eliminate the increased risks of coronary heart disease and of osteoporosis which are caused by early menopause. Ovariectomy is probably not justified for breast cancer prevention alone, but this benefit should be considered when deciding whether to remove the ovaries during hysterectomy for other reasons.

It should be possible to select or to design a hormonal contraceptive to reduce the risk of breast cancer, by choosing a formulation which reduces the number of mitoses induced in susceptible cells to below that in an average cycle. Under the oestrogen alone hypothesis, 'progestagen only' oral contraceptives should cause no increase in risk, and DMPA should cause a substantial decrease in risk. Under the oestrogen plus progestagen hypothesis all doses of COCs which are sufficient to be effective as contraceptives would be expected to cause some increase in risk. Sequential oral contraceptives might not increase risk, but it should be possible to design them in such a way that they would not increase the risk of endometrial cancer: one week of oestrogen alone followed by 2 weeks of oestrogen plus progestagen should achieve this. Short term use of contraceptive drugs, other than oestrogens and progestagens, which prevent follicle development and ovulation, should cause a large reduction in the risk of breast cancer; 5 years of such use should be equivalent to a menopause 5 years early, producing an approx. 30% decrease in risk.

The hormonal treatment of postmenopausal women can probably only be modified to minimize the *increase* in risk of breast cancer produced. The increase in risk caused by HRT may be either the same as or more than that caused by ERT. The use of any replacement therapy must be considered in terms of its effects on coronary heart disease and osteoporosis as well as on breast and endometrial cancer. Current evidence suggests that the overall benefit will be greatest with low dose ERT, but safer means of preventing heart disease and osteoporosis (e.g. diet, exercise) should be pursued.

One other possibility for the hormonal prevention of breast cancer has been raised by the studies of Russo *et al.* [94] and of Grubbs *et al.* [107]. They have shown that, in the rat, high doses of E2 and Pg which simulate pregnancy can induce epithelial differentiation and lifelong protection against mammary tumours. Equivalent treatment of women is a long term but not impossible goal.

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