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Serum Oestradiol-17β and Prolactin Concentrations during the Luteal Phase in Women with Benign Breast Disease*

PATRICK V. WALSH,†‡ IAN W. McDICKEN,§ RICHARD D. BULBROOK,|| JOHN W. MOORE,||
WILLIAM H. TAYLOR¶ and WILLIAM D. GEORGE†**

Departments of †Surgery, Pathology and ¶ Chemical Pathology, Royal Liverpool Hospital, Liverpool, Merseyside, U.K. and ||Department of Clinical Endocrinology, Imperial Cancer Research Fund, London, U.K.

Abstract—*Serum profiles of both oestradiol and prolactin were measured during the luteal phase in normal women, in women with cyclical breast pain and in women with recently biopsied benign breast disease. The results suggest that, in women with benign breast disease, the concentration of both hormones may be increased, when compared to the normal controls, during the evening in the latter part of the luteal phase.*

INTRODUCTION

ALTHOUGH the causes of benign breast disease remain obscure, the clinical features of some of these conditions suggest the possibility of an underlying endocrine abnormality. This has prompted several groups of workers to investigate ovarian and pituitary hormones in women with several forms of benign breast disease. The principal hormones of interest have been progesterone, oestradiol-17β and prolactin. These previous endocrine studies have yielded conflicting results. Most authors have found a normal concentration of progesterone in women with benign breast disease [1–3], although one group of workers in particular reported a pronounced luteal deficiency for this hormone in these patients [4–9]. An earlier report from this study [10] found a normal concentration of progesterone in women with benign breast disease. There have been reports of both normal [1, 2, 4–6, 11–14] and an increased concentration of oestradiol in patients with various forms of benign breast disease [2, 7–9, 11]. Similarly, there are reports of an increased concentration of prolactin, particularly in patients with painful forms of breast disease [13, 15], while other authors have found a normal concentration in these patients [8, 16, 17]. There are several possible causes for the conflict between these reports. The nomenclature of benign breast disease has been inconsistent and the methods of hormone analysis have varied. More important, however, are differences between the methods of blood sampling. In particular, in studies where daily blood sampling has not been carried out, the accuracy with which blood samples have been 'dated' with reference to the menstrual cycle has been very variable. In addition, some studies have not taken account of possible circadian variations in concentrations of the hormones under investigation.

The aim of this study was to investigate the luteal phase concentrations of oestradiol and prolactin in relation to cyclical breast pain (mastodynia) and to biopsied benign breast disease, classified according to histological appearance.

MATERIALS AND METHODS

Subjects

Four groups of women were studied (Table 1).

Group A. This group comprised 288 self-referred women who attended a breast screening clinic. Each of these women answered a detailed questionnaire, including a full history of breast symptoms, and provided one blood sample between 8.45 and 11.30 a.m. on a day calculated to

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‡To whom requests for reprints should be addressed at: Gartnavel General Hospital, Glasgow G12 0YN, U.K.

**Present address: Department of Surgery, Western Infirmary, Glasgow, U.K.

Table 1. Subjects studied

	n	Age (yr) Mean	Median	Range	Oestradiol concentrations (pg/ml)	Prolactin concentrations (μU/ml)	
Group A (screened women)	288	41.9	44	22–52	288	272	(morning)
Group B (control group)	14	42.5	43	37–47	51	47	(evening)
Group C (mastodynia patients)	33	33.4	34	21–50	102	102	(evening)
Group D (biopsied women)	63*	38.8	40	23–50	200	182	(evening)

*Group D includes nine women from group C, aged 31–50, mean age 35 yr, who provided 26 blood samples.

be in the luteal phase of her cycle. These women were separated into those who gave a history of moderate or severe cyclical mastodynia ($n = 82$) and those with trivial discomfort or no symptoms at all ($n = 206$).

Three smaller groups, B, C and D, provided at least three luteal phase blood samples in the evening between 4.15 and 6.30 p.m.

Group B. This control group comprised 14 women who gave no personal history of breast disease, no family history of breast cancer and no history of involuntary infertility. In addition, their breasts were normal, clinically and radiologically.

Group C. This group comprised 33 patients with persistent, severe cyclical mastodynia, which was defined as pain lasting for at least 7 days before menses.

Group D. This group comprised 63 women who had undergone breast biopsy for a benign condition between 3 months and 2 yr previously. Nine of this group of women also belonged to group C. This group of women (D) was identified from the records of the Department of Pathology. The histology slides of each patient were reviewed by one of the authors (I.W.M.) and the microscopic findings were graded according to the presence or absence of epithelial proliferation [18].

All subjects studied satisfied the protocol which stipulated that all were pre-menopausal and that none was taking any medication likely to affect hormone concentrations, either at the time of the study or during the preceding 3 months.

Every blood sample was 'dated' according to the number of days that elapsed between venepuncture and the date of the next onset of menstruation as determined from a menstrual calendar.

Blood samples were drawn from an antecubital vein using a Sarstedt Monovette syringe, centrifuged at 4°C and the serum separated within 2 hr of venepuncture. All serum aliquots for oestradiol estimation were stored at -20°C until the completion of the study, when they were assayed in duplicate in 14 batches at the Department of Clinical Endocrinology, Imperial Cancer Research Fund, London. Serum aliquots for prolactin assay were stored at 4°C for up to 2 weeks before inclusion in one of 16 routine assay batches in the Department of Clinical Pathology, Royal Liverpool Hospital.

Methods

Analytical. The assay batches for both hormones were composed of a random selection of samples from all parts of the study.

Oestradiol assay. Oestradiol assay was performed according to the method of Bulbrook *et al.* [19]. Interassay variation was 10.1%; overall variation was 14.2%. Results are quoted in pg/ml of serum.

Prolactin assay. Prolactin assay was performed by a double antibody solid phase radioimmunoassay technique [20]. One assay batch was unsatisfactory and the results were discarded, with the consequent loss of 38 results. Interassay variation was 12%; within-batch variation was 8%. Results are quoted in μU/ml of serum.

Statistical. The data for each hormone were log-normally distributed, and the statistical analysis was performed after logarithmic transformation of the data. Hormone profiles were derived by calculating the mean, plus or minus one standard error of the mean, for values in each interval of 2 days before menstruation. For group A, hormone profiles were derived for the women with and without significant symptoms of cyclical breast pain. Hormone profiles were derived for each of groups B, C and D, and subsequently profiles were derived for the proportions of patients within group D who demonstrated specific features of epithelial proliferation.

Oestradiol profiles were compared by two-way analysis of variance for non-orthogonal data. The titres assayed on samples obtained more than 12 days before menstruation were few in number and also displayed a very wide scatter of values. This has been attributed to inconsistent detection of high or low concentrations during the mid-cycle 'peak' of oestradiol. Therefore statistical comparison of profiles was performed only on the data obtained from samples drawn 12 days or less before menstruation.

Prolactin profiles are presented as for oestradiol.

However, as there was no significant difference between the concentrations in each two-day interval (one-way analysis of variance), the mean concentration was calculated for each complete profile and compared by Student's t test.

RESULTS

The data obtained from the women attending the breast screening clinic, who provided single morning blood samples, were initially analysed in four separate age subgroups and according to differing degrees of severity of cyclical breast symptoms. No discrepancy was found with respect to either age or differing degrees of pain. For this reason the hormone profiles of women with moderate or severe cyclical mastodynia ($n = 82$) were compared with those obtained from women with negligible symptoms ($n = 206$).

Hormone profiles from the three groups of women who provided multiple evening blood samples were compared as follows. The control group (B) were compared with the women with cyclical mastodynia (group C). The control group were next compared with the women who had undergone breast biopsy (group D), but excluded from this comparison were the data from the nine women who also appeared in group C. Finally, profiles were derived according to the histological appearance of the breast biopsy specimens; nine women were found to have fibroadenoma only and these women, whose hormone titres were unremarkable in the context of this group, were excluded. The remaining 54 women were separated into those whose biopsies demonstrated epithelial proliferation and those which did not.

Oestradiol

The oestradiol profiles of the screened women (group A) with and without cyclical breast pain are shown in Fig. 1. There was no significant difference between these profiles.

When the profiles for the control women (group B) and the patients with cyclical mastodynia (group C) were compared (Fig. 2) a significant difference emerged. The women with pain had a higher concentration of oestradiol [$F(1,114) = 4.2$, $P < 0.05$] than the control women during the latter part of the luteal phase.

Similarly, the women who had only undergone breast biopsy also showed a significantly different oestradiol profile from that seen in the control group [Fig. 3; $F(1,205) = 7.2$, $P < 0.01$], with a higher concentration during the latter part of the luteal phase.

In the biopsied group, oestradiol profiles were derived according to histological appearances. No difference was found between the oestradiol profiles of women with and women without

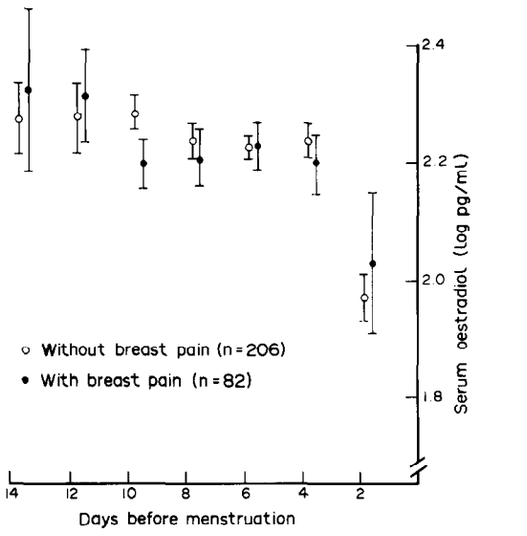

Fig. 1. Screened women (group A) with and without cyclical breast pain: oestradiol.

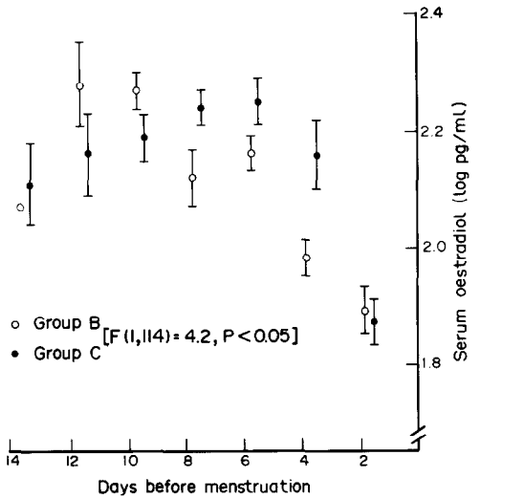

Fig. 2. Control group and patients with severe cyclical mastodynia: oestradiol.

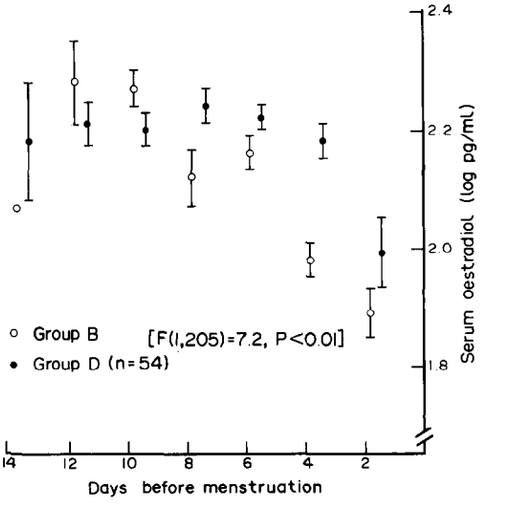

Fig. 3. Control group and patients who have undergone breast biopsy: oestradiol.

epithelial proliferation. The profiles for women whose biopsy did ($n = 15$) or did not ($n = 39$) demonstrate papillomatoid proliferation of the ductal epithelium, for example, are shown in Fig. 4, and there is no difference between these profiles and certainly no suggestion that this form of florid proliferation was associated with a higher concentration of oestradiol.

Prolactin

The profile and mean concentrations of prolactin were compared in the screened women (group A) with and without cyclical breast pain (Fig. 5). These two profiles were very similar and the mean concentrations were virtually identical.

The prolactin profiles of the control women (group B) and the patients with cyclical mastodynia are shown in Fig. 6. The mean concentrations are shown for these two profiles and in addition the patients' mean concentration was also calculated excluding results earlier than day -11 in order to avoid the bias of comparing a relative excess of results obtained from blood samples drawn from the mastodynia patients earlier than day -11. The mean concentration of the patients was significantly higher when compared with the mean for controls ($t = 3.14$, d.f. = 132, $P < 0.01$), reflecting the evident difference between these two prolactin profiles.

Although a similar divergence is seen (Fig. 7) between the prolactin profiles of the control women and the biopsied women (group D), the mean concentrations are identical.

As with oestradiol, there was no difference between the prolactin profiles according to histological appearances. The example shown (Fig. 8) is of the profiles for women whose biopsy demonstrated adenosis ($n = 39$) or no adenosis ($n = 15$). The mean prolactin concentrations are almost identical.

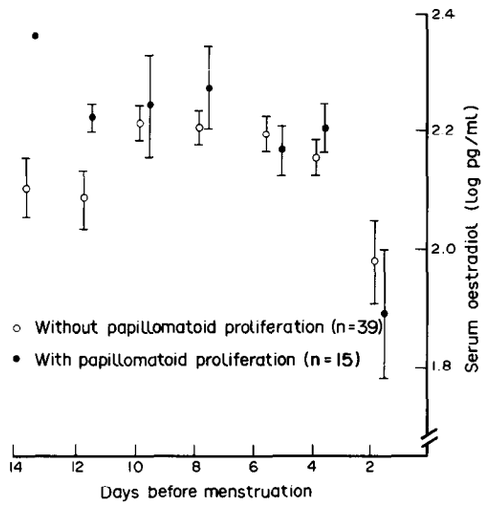

Fig. 4. Biopsied women (group D) with and without papillomatoid proliferation: oestradiol.

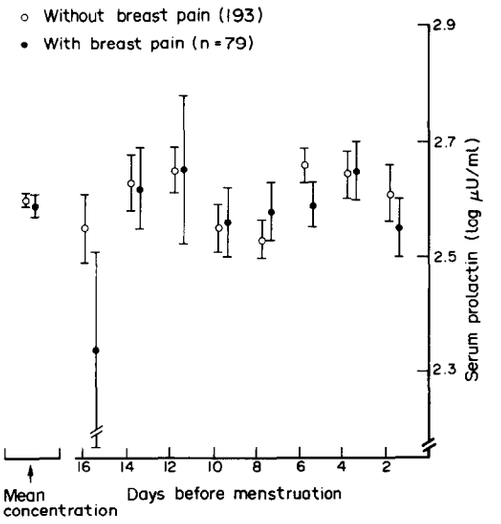

Fig. 5. Screened women (group A) with and without cyclical breast pain: prolactin.

DISCUSSION

The results recorded here are conflicting—the differences found among women studied in the evening were not found in the screened women who provided morning blood samples. It is possible that the method used in the morning, obtaining only one blood sample from each woman, was too imprecise to permit the detection of relatively subtle differences. Also, it is

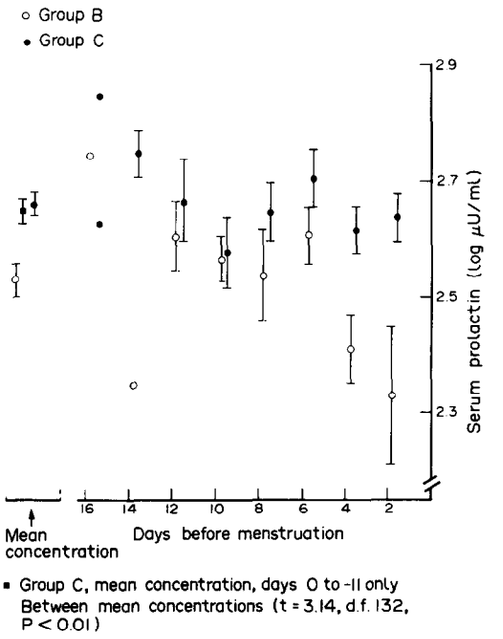

Fig. 6. Control group and patients with severe cyclical mastodynia: prolactin.

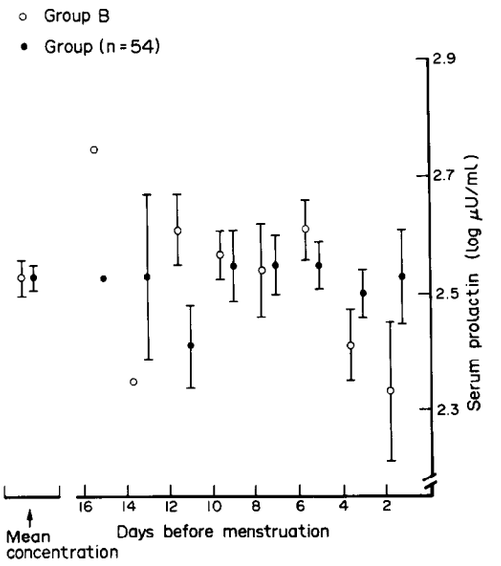

Fig. 7. Control group and patients who have undergone breast biopsy: prolactin.

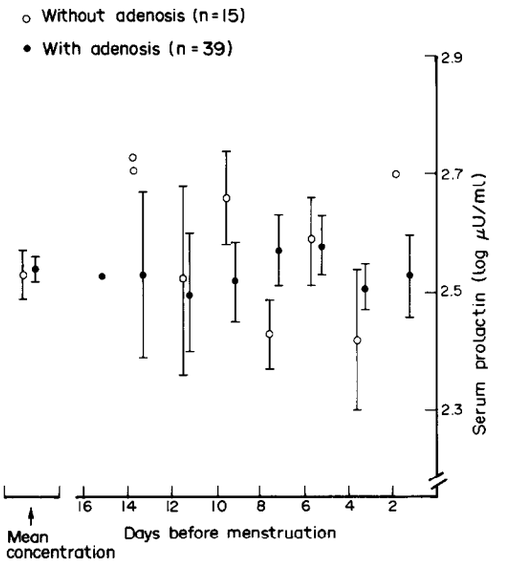

Fig. 8. Biopsied women (group D) with and without adenosis: prolactin.

recognised that the method used to 'date' blood samples within the putative luteal phase was subject to the variation in cycle length which may have occurred in each woman studied. However, this method successfully demonstrated the cyclical variation of prolactin concentration described by Vekemans *et al.* [21]. This cyclical variation, which was detected in all four age subgroups of the screened women (group A), supports the validity of deriving luteal-phase hormone profiles from single blood samples. Similarly, subtle differences between the four age subgroups in relation to age were detected in the profiles of oestradiol and progesterone [22] but, as noted above, there was no discrepant difference between women with and women without cyclical mastodynia within any age subgroup; hence the results have been presented together. The method used in the evening to study the women of groups B, C and D (multiple rather than single blood samples) may be open to the same criticism that data may be distorted by variation in cycle length. The hormone profiles shown here and those of progesterone [22] appear to validate the method, however, and suggest that any variation in cycle length occurs principally in the follicular rather than the luteal phase [23]. The results of the evening part of the study are in some agreement with those of England and his colleague [2, 11, 15]. Women with cyclical mastodynia ('fibroadenosis') had an increased concentration of prolactin during the latter part of the luteal phase, and both these women and women with biopsied breast disease had a higher concentration of oestradiol in the latter part of the luteal phase when compared with normal women. When the biopsied women (group D) were subdivided according to different histological appearances, however, no differences in either oestradiol or prolactin profiles were found within this group. Two examples, adenosis and papillomatoid proliferation, are shown.

It is interesting to compare the results of the present study with respect to cyclical mastodynia with those reported for the distinctly separate but related condition of pre-menstrual syndrome. The concentration of prolactin is reported to be increased in patients with pre-menstrual tension [24] and the concentration of oestradiol is also reported to be increased in these patients during the latter part of the luteal phase [25, 26].

The disagreement between the morning and the evening results of this study may arise from possible differences between patients and 'normals' in the circadian variation of the hormones studied, with divergence appearing in the evening rather than in the morning. There are already reports suggesting an altered circadian rhythm of prolactin in breast cancer [27] and in relation to certain breast cancer risk factors [28–32], and in women with benign breast disease [33]. While there is as yet insufficient data regarding a circadian variation of oestradiol in normal non-pregnant women, there are reports indicating such a rhythm during the third trimester of pregnancy [34, 35], with a higher concentration in the morning and a lower concentration in the evening, as was apparently found in this study in the women studied in the morning and in the control group studied in the evening. Moreover, there is a close relationship between the circulating concentrations of

prolactin and oestradiol [36, 37], the prolactin secretion response being enhanced by oestrogenic stimulation [38]. This prolactin secretion response has been demonstrated to be increased in patients with benign breast diseast [39, 40] and also, interestingly, in patients suffering from cyclical oedema [41].

The results of the present study suggest that an abnormality of oestrogen metabolism may be responsible for some forms of benign breast disease and that, when detected, an increased concentration of prolactin may simply be a marker for increased underlying oestrogenic activity. The differences that exist between the results of various studies may be due in part to circadian variation in the hormones studied. Since there is already evidence that the secretion of pituitary gonadotrophins and ovarian steroids is not only pulsatile but also that the rhythm of secretion varies with the menstrual cycle [42], further study of ovarian steroid hormones and pituitary gonadotrophins and their rhythms in relation to benign breast disease may be fruitful.

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REFERENCES

1. Swain MC, Hayward JL, Bulbrook RD. Plasma oestradiol and progesterone in benign breast disease. *Eur J Cancer* 1973, **9**, 553–556.
2. England PC, Skinner LG, Cottrell KR, Sellwood RA. Sex hormones in breast disease. *Br J Surg* 1975, **62**, 806–809.
3. Malarkey WB, Schroeder LL, Stevens VC, James AG, Lanese RR. Twenty-four hour preoperative endocrine profiles in women with benign and malignant breast disease. *Cancer Res* 1977, **37**, 4655–4659.
4. Mauvais-Jarvis P, Ware R, Sterkers N, Ohlgiesser C, Tamborini A. Corps jaune inadéquat et mastopathies. *Ann Endocrinol (Paris)* 1976, **37**, 347–354.
5. Mauvais-Jarvis P, Mutten F, Mowszowics I, Sitruk-Ware R. Mastopathies bénignes: étude hormonale chez 125 malades *Nouv Presse Med* 1977, **6**, 4115–4118.
6. Sitruk-Ware LR, Sterkers N, Mowszowics I, Mauvais-Jarvis P. Inadequate corpus luteal function in women with benign breast diseases. *J Clin Endocrinol Metab* 1977, **44**, 771–774.
7. Sitruk-Ware R, Sterkers N, Mauvais-Jarvis P. Benign breast disease I: hormonal investigation. *Obstet Gynecol* 1978, **53**, 457–460.
8. Mauvais-Jarvis P, Sitruk-Ware R, Kuttenn F, Sterkers N. Luteal phase insufficiency: a common pathophysiologic factor in development of benign and malignant breast diseases. In: Bulbrook RD, Taylor DJ, eds. *Commentaries on Research in Breast Diseases, I.* New York, Alan R. Liss, 1980, 25–59.
9. Martin PM, Kuttenn F, Sergent H, Mauvais-Jarvis P. Studies on clinical, hormonal and pathological correlations in breast fibroadenosis. *J Steroid Biochem* 1978, **9**, 1251–1255.
10. Walsh PV, Moore JW, Clark GMG, Quinlan MK, Stell PM, George WD. Serum progesterone and oestradiol-17β levels in women with cyclical breast pain. *Br J Surg* 1982, **68**, 812.
11. England PC, Skinner LG, Cottrell KM, Sellwood RA. Serum oestradiol-17β in women with benign and malignant breast disease. *Br J Cancer* 1974, **30**, 571–576.
12. Rolland PH, Martin PM, Rolland AM, Bourry M, Serment H. Benign breast disease: studies of prostaglandin E, steroids and thermographic effects of inhibitors of prostaglandin biosynthesis. *Obstet Gynecol* 1979, **54**, 715–718.
13. Watt-Boolsen S, Andersen AN, Birchent-Toft M. Serum prolactin and oestradiol levels in women with cyclical mastalgia. *Horm Metab Res* 1981, **13**, 700–702.
14. Manchesoni D, Gangemi, Mozzanega B, Patenesten D, Gragiottin A, Maggino T. Inadequate luteal phase and benign breast disease. *Clin Exp Obstet Gynecol* 1981, **8**, 160–163.
15. Cole EN, Sellwood RA, England PC, Griffiths K. Serum prolactin concentrations in benign breast disease throughout the menstrual cycle. *Eur J Cancer* 1977, **13**, 597–603.
16. Mansel RE, Preece PE, Hughes LE. A double blind trial of the prolactin inhibitor bromocriptine in painful benign breast disease. *Br J Surg* 1978, **65**, 724–727.
17. Gollinger RC, Krebs J, Fisher ER, Danowski TS. Hormones and the pathophysiology of fibrocystic mastopathy: elevated luteinizing hormone levels. *Surgery* 1978, **84**, 212–215.
18. Azzopardi JG. Terminology of benign diseases and the benign epithelial hyperplasias. In: *Problems in Breast Pathology*. London, W. B. Saunders, 1979, 23–38.

19. Bulbrook RD, Moore JW, Clarke GMG, Wang DY, Tong D, Hayward JL. Plasma oestradiol and progesterone levels in women with varying degrees of risk of breast cancer. *Eur J Cancer* 1978, **14,** 1369–1375.
20. Morris K. Oestrogen receptors, oestradiol-17β and prolactin in breast cancer. M.Sc. Thesis, University of Bradford, 1982.
21. Vekemans M, Delvoye P, L'Hermite M, Robyn C. Serum prolactin levels during the menstrual cycle. *J Clin Endocrinol Metab* 1977, **44,** 989–993.
22. Walsh PV, Bulbrook RD, Stell PM, Wang DY, McDicken IW, George WD. Serum progesterone concentration during the luteal phase in women with benign breast disease. *Eur J Cancer Clin Oncol* 1984, **20,** 1339–1343.
23. Landgren BM, Unden AL, Diczfalusy E. Hormonal profile of the cycle in 68 normally menstruating women. *Acta Endocrinol (Copenh)* 1980, **94,** 89–98.
24. Halbreich U, Assael M, Ben-David M, Bornstein R. Serum prolactin in women with premenstrual syndrome. *Lancet* 1976, **ii,** 654–656.
25. Backstrom T, Wide L, Sodergard R, Carstensen H. FSH, LH, TeBG capacity, estrogen and progesterone in women with pre-menstrual tension during the luteal phase. *J Steroid Biochem* 1976, **7,** 473–476.
26. Munday MR, Brush MG, Taylor RW. Correlations between progesterone, oestradiol and aldosterone levels in the premenstrual syndrome. *Clin Endocrinol (Oxf)* 1981, **14,** 1–9.
27. Malarkey WB, Schroeder LL, Stevens VC, James AG, Lanese RR. Disordered nocturnal prolactin regulation in women with breast cancer. *Cancer Res* 1977, **37,** 4650–4659.
28. Kwa HG, Cleton F, De Jong-Bakker M, Bulbrook RD, Hayward JL, Wang DY. Plasma prolactin and its relationship to risk factors in human breast cancer. *Int J Cancer* 1976, **17,** 441–447.
29. Kwa HG, Wang DY. An abnormal luteal phase evening peak of plasma prolactin in women with a family history of breast cancer. *Int J Cancer* 1977, **20,** 12–14.
30. Kwa HG, Bulbrook RD, Cleton F, Verstraeten AA, Hayward JL, Wang DY. An abnormal early evening peak of plasma prolactin in nulliparous and obese post-menopausal women. *Int J Cancer* 1978, **22,** 691–693.
31. Kwa HG, Cleton F, Bulbrook RD, Wang DY, Hayward JL. Plasma prolactin levels and breast cancer: relation to parity, weight and height, and age at first birth. *Int J Cancer* 1981, **28,** 31–34.
32. Bulbrook RD, Wang DY, Hayward JL, Kwa HG, Cleton F. Plasma prolactin levels and age in a female population: relation to breast cancer. *Int J Cancer* 1981, **28,** 43–45.
33. Tarquini A, Di Martino L, Malloci A *et al.* Abnormalities in evening plasma prolactin levels in nulliparous women with benign or malignant breast disease. *Int J Cancer* 1978, **22,** 687–690.
34. Townsley JD, Dubin NH, Grannis GF, Gartman LJ, Crystle CE. Circadian rhythms of serum and urinary estrogens in pregnancy. *J Clin Endocrinol Metab* 1973, **36,** 289–295.
35. Reck G, Noss U, Breckwoldt M. Circadian rhythm of free oestradiol in relation to plasma cortisol in late human pregnancy. *Acta Endocrinol (Copenh)* 1979, **90,** 519–524.
36. Franchimont P, Dowray C, Legros JJ *et al.* Prolactin levels during the menstrual cycle. *Clin Endocrinol* 1976, **5,** 643–650.
37. Robyn C, Delvoye P, Van Exeter C *et al.* Physiological and pharmacological factors influencing prolactin secretion and their relation to human reproduction. In: Crosignani PG, Robyn C, eds. *Prolactin and Human Reproduction.* London, Academic Press, 1977, 71–96.
38. Reymond M, Le Marchand-Beraud T. Effects of oestrogens on prolactoin and thyrotrophin response to TRH in women during the menstrual cycle and under oral contraceptive treatment. *Clin Endocrinol (Oxf)* 1976, **5,** 429–437.
39. Geller S, Grenier J, Nahoul K, Scholler R. Insuffisance luteale et mastopathies benignes. Etude a la lumière des donnes de l'épreuve combinée LH-RH+TRH couplée a l'étude des steroides ovariens. *Ann Endocrinol (Paris)* 1979, **40,** 45–46.
40. Kumar S, Mansel RE, Scanlon MF, Hughes LE. Secretory response of prolactin and TSH in benign breast disease before and after TRH stimulation. *Br J Surg* 1983, **70,** 293–294.
41. Young JB, Brownjohn AM, Chapman C, Lee MR. Evidence for a hypothalamic disturbance in cyclical oedema. *Br Med J* 1983, **286,** 1691–1693.
42. Backstrom CT, McNeilly AS, Leask RM, Baird DT. Pulsatile secretion of LH, FSH, Prolactin, oestradiol and progesterone during the human menstrual cycle. *Clin Encocrinol (Oxf)* 1982, **17,** 29–42.