Eur J Cancer Clin Oncol, Vol. 25, No. 12, pp. 1843–1850, 1989.
Printed in Great Britain

0277–5379/89$3.00 + 0.00

The Effect of Pregnant Mares Serum Gonadotrophin on the Response of DMBA Induced Rat Mammary Tumours to Tamoxifen or Oestrogen

EDWARD R. CLARK,* SIMON P. ROBINSON,* ELAINE M. WHITAKER† and JANUSZ A. ZOLTOWSKI*

*Departments of *Pharmacology and †Physiology, University of Leeds, Leeds LS2 9JT, U.K.*

Abstract—*Ovariectomized and intact rats bearing mammary tumours, induced with 7,12-dimethylbenz(a)anthracene, were treated with diethylstilboestrol (DES), tamoxifen and pregnant mares serum gonadotrophin (PMSG) alone or in various combinations.*

In ovariectomized animals, PMSG (100 iu daily) alone, or with DES (50 μg) or tamoxifen (50 μg), slowed tumour regression. Mean tumour volume after 5 weeks was larger than control ($P < 0.05$) in animals treated with PMSG + DES but not with PMSG or PMSG + tamoxifen.

In intact animals, PMSG (200 iu bd, but not 2 or 20 iu bd) slowed tumour growth ($P < 0.05$) and PMSG (2 or 200 iu bd) reversed the tumour inhibiting effect of tamoxifen (200 μg). PMSG (2 iu bd), in contrast to 20 and 200 iu doses, showed no evidence of oestrogen stimulation. It is suggested that the reversal of the tumour inhibiting effect of tamoxifen by this low dose of PMSG may be of relevance to failure of tamoxifen treatment and to 'tamoxifen flare'.

INTRODUCTION

THE PART played by oestrogens in the aetiology of breast cancer has long been recognised. As early as 1836 Cooper [1] observed fluctuations in the growth of breast cancers during different phases of the menstrual cycle and in 1896 Beatson [2] used ovariectomy to treat young women with breast cancer, a form of treatment which was revived in 1941 to treat premenopausal women with advanced disease. The higher incidence of breast cancer in postmenopausal women [3], in whom atrophy of the ovaries results in a reduction in the ovarian production of oestrogen, might thus seem surprising but bilateral adrenalectomy [4], hypophysectomy [5], the use of drugs such as aminoglutethimide [6], which blocks the aromatization of androstenedione [7], and anti-oestrogens such as tamoxifen [8] have been found to produce remarkable remissions in postmenopausal women with advanced breast cancer.

Accepted 5 September 1989.
Author to whom correspondence and reprint requests should be addressed: Dr E. R. Clark, Department of Pharmacology, Worsley Medical and Dental Building, University of Leeds, Leeds, LS2 9JT, U.K.
The financial support of the Yorkshire Cancer Research Campaign and the free gift of animals and tamoxifen from Imperial Chemical Industries plc, Pharmaceuticals Division, are gratefully acknowledged.

Unfortunately, the overall response rate to hormonal manipulation in breast cancer is only approximately 30% and even if the cases considered are limited to those in which the tumours have been shown to possess significant levels of oestrogen receptors the response rate is only 57% and 77% for those patients with tumours possessing both oestrogen and progesterone receptors [9, 10].

Since the level of cytoplasmic oestrogen receptors in tumours of postmenopausal patients is, in general, higher than in premenopausal patients, one might expect the overall response rate to hormonal manipulation to be better in post- rather than in premenopausal patients, particularly so when using anti-oestrogens such as tamoxifen, which raises plasma oestradiol levels two- to eightfold when administered daily (20 mg) to premenopausal women during the follicular phase of the cycle [11, 12]. Contrary to expectation, tamoxifen produces similar remission rates in premenopausal and postmenopausal patients with advanced disease [13].

A further apparent anomaly is the observed regression of postmenopausal breast cancers on the administration of large doses of oestrogen [14, 15], a treatment which would have a disadvantageous effect in premenopausal patients, though Kennedy [16] achieved tumour regression in pre- and postmenopausal women using massive doses of oestro-

gens. The reasons for the discrepancy are not understood but one obvious difference between the two groups of patients is the different hormonal milieu of postmenopausal women, atrophy of the ovaries with the accompanying reduced production of oestradiol leading to an increased output of gonadotrophins. Indeed the hypothalamic–pituitary axis in postmenopausal women remains intact and an excellent response of the pituitary to gonadotrophin-releasing hormone can be observed, the synthesis and release of FSH and LH being unimpaired by the ageing process [17]. The question arises, therefore, whether or not the levels of gonadotrophins in the circulation affect tumour response to oestrogen or anti-oestrogen.

We have attempted to answer this question by examining the effects of pregnant mares serum gonadotrophin (PMSG) on the response of 7,12-dimethylbenz(a)anthracene (DMBA) induced rat mammary tumours to treatment with tamoxifen or oestrogen. Because of their difficult availability and short circulating half lives, rat pituitary FSH and LH were considered impractical for this investigation. PMSG, on the other hand, is readily available, possesses substantial levels of both FSH- and LH-type activity in the rat [18] and has a long circulating half life in rodents [19]. Intact and ovariectomized tumour bearing rats were used as models of the pre- and postmenopausal states respectively.

MATERIALS AND METHODS

Tumor induction

Method 1. A solution of 7,12-dimethylbenz-(a)anthracene (DMBA; Sigma Chemical Co. Ltd) in arachis oil, at a concentration of 10 mg/ml, was prepared by stirring at room temperature. A single dose of 20 mg (2 ml) was administered by gavage, using a blunt ended metal cannula passed into each animal's stomach, to 50-day-old (150 g) conscious, virgin, female, Sprague–Dawley rats (Charles River strain; ICI plc, Animal Breeding Unit, Alderley Park).

Method 2. Four doses (0.25 ml) of a solution of DMBA in sesame oil (20 mg/ml), prepared by stirring at room temperature, were administered, as in method 1, at weekly intervals to 50-day-old (150 g), Category IV Sprague–Dawley rats (Nottingham University Animal Breeding Unit), under light ether anaesthesia.

Rats were examined daily for acute toxic effects for 4 weeks following the first DMBA administration and then palpated for mammary tumours at weekly intervals.

Tumour measurement and response

Tumour response to hormone manipulation was assessed by following changes in tumour volume as estimated by the formula $4/3\pi r^3$, where r is the mean of two radii obtained by measuring externally with calipers, two orthogonal diameters, one measured across the greatest width. Tumours with a mean diameter <0.5 cm were considered too small for measurement with an acceptable degree of accuracy. Rats bearing tumours not less than 1.0 cm mean diameter (2–3 months after first DMBA administration) were randomly distributed into treatment groups.

Animals were housed four to a cage in a temperature controlled environment with 12 h light, 12 h dark and allowed food and water *ad libitum*.

Tumours were measured, under light ether anaesthesia, immediately before the first drug administration and then at weekly, or, in the 16 day experiments, 4 day intervals.

Drug preparation and administration

Solution of tamoxifen (ICI plc, Pharmaceuticals Division) and diethylstilboestrol (BDH Ltd) were prepared by adding aliquots of an alchoholic solution of the drug to a volume of arachis oil to give the required dosage in 0.1 ml and evaporating the alcohol by gentle warming (60°C) under a stream of nitrogen. The solutions were stored in the dark, at room temperature for the duration of an experiment.

Solutions of pregnant mares serum gonadotrophin (PMSG; Paines & Byrne Ltd) were prepared immediately before administration by adding the appropriate volume of sterile water to an ampoule containing 6000 iu. Lower concentrations, such that the required dose was contained in 0.1 ml, were obtained by serial dilution with sterile water.

All drug solutions were administered subcutaneously through loose folds of skin at the back of the neck, two different sites being used for dual drug therapy.

Ovariectomy

Rats were anaesthetised with ether, a small incision made through the skin and abdominal wall of each flank, the ovaries tied off with 3/0 surgical silk and excised. The abdominal wall was restructured with 3/0 silk surgical suture on an atraumatic needle and the skin held together with a 18/8 Michel clip.

Vaginal smears and staining

Vaginal smears were taken by saline lavage, the washings allowed to dry on a microscope slide and spray fixed prior to staining by the Papanicolaou technique [20].

Uterine, intra-uterine fluid and paired ovarian weights

Intact rats, treated for 16 days with PMSG alone or together with tamoxifen, were sacrificed on the

morning of the 17th day by ether anaesthesia and exsanguination via the abdominal aorta. Uteri and ovaries were dissected, cleaned of adhering fascia and weighed. Intra-uterine fluid weight was determined by pressing the uteri between blotting paper and reweighing.

Uteri, ovaries and a portion of each tumour from each animal were fixed in formal saline solution and sent for histology.

Plasma oestradiol and prolactin assays

Oestradiol. Saline diluent was prepared by dissolving sodium chloride (9 g), gelatin (1 g, British Drug Houses Ltd) and thiomersal (0.1 g, British Drug Houses Ltd) in distilled water (1 l) by warming at 60°C and stored at 4°C until required.

Plasma samples from control (0.5 ml) or PMSG treated animals (0.25 ml diluted to 0.5 ml with saline diluent) were made alkaline by adding 2 N sodium hydroxide solution (50 μl). Diethyl ether (5 ml) was added and, after mixing for 2 min, the aqueous layer was frozen. The ether layer was pipetted off, evaporated to dryness in a stream of air at 40°C and the residue reconstituted in 0.1 ml of saline diluent.

Standards were prepared by extracting saline diluent (4 ml), made alkaline with 2 N sodium hydroxide (0.4 ml), with diethyl ether (40 ml). Aliquots (5 ml) of the ether extract were evaporated to dryness, reconstituted in saline (0.1 ml) and 0.05 ml of oestradiol standards (0–150 pg) in saline added.

Saline diluent (0.05 ml) was added to experimental samples to maintain a fixed volume.

Saline diluent (0.1 ml) containing [2,4,6,7-^{3}H]oestradiol-17β (8000 cpm, Amersham International plc) was added to each test and standard tube and the tubes agitated to ensure thorough mixing. Antiserum (100 μl) was added to each tube and the mixtures incubated on ice for 150 min. Free ligand was removed by incubating at 0°C for 12 min with 0.5 ml dextran coated charcoal (0.5% w/v charcoal and 0.05% w/v dextran in saline diluent) prior to centrifugation at 2000 ***g***. Bound ligand was determined by decantation and liquid scintillation counting of the supernatant. Stripped plasma samples, solvent blanks and low and high oestradiol containing controls were used to monitor background and solvent effects.

Prolactin. Plasma prolactin levels were determined on 5 μl samples by the method of Whitaker and Robinson [21].

Statistical analysis

Student's *t* test was used to examine the differences in tumour volumes, uterine, intra-uterine fluid and paired ovarian weights and plasma oestradiol and prolactin levels, as appropriate. Tumour volumes were transformed to logarithms prior to calculating means and standard errors when the range of values was inconveniently large for plotting (see figures). Analyses of variance and covariance, using BMDP Statistical Software [22] packages P1V, P2V or P4V as appropriate, were carried out on tumour volumes and uterine, intra-uterine fluid and paired ovarian weights from the experiment using three different doses of PMSG and tamoxifen in order to test for overall effects and to test for possible interactions.

RESULTS

At the commencement of this work, satisfactory induction of hormone sensitive tumours, with minimal animal distress and loss, was achieved by administering a single dose of DMBA (20 mg) in arachis oil (2 ml) by gavage, but subsequently acute toxic symptoms with respiratory distress, low body temperature and high mortality in the first week following administration was observed. Following extensive investigations into DMBA purity, administration technique and origin and grade of the Sprague–Dawley rats used, the multiple dosage technique of Shimkin *et al.* [23] rather than the single dose technique of Huggins *et al.* [24] was adopted and grade IV rats used for all subsequent experiments. Using the multiple dosage technique the latent period to first tumour appearance was 59.0 ± 1.48 days, which is not markedly dissimilar to the 42.8 ± 3.16 days reported by Huggins *et al.* [24] but in marked contrast to the 118 ± 11.9 days to first tumour appearance when a single dose of DMBA (20 mg) was administered to the grade IV Sprague–Dawley rats. Hormone sensitivity of a tumour is related to its age [25], fewer tumours that develop after longer periods since DMBA insult being frank adenocarcinomas. The multiple dosage technique in our hands provided tumours of satisfactory sensitivity, seven out of eight tumours produced by this method, which were growing in size, regressed to less than 50% of their original volume in 11.2 ± 2.6 days following ovariectomy, two becoming too small to measure by day 21. On subsequent oestradiol administration, three tumours doubled in volume in 10 days, those which had become too small to measure did not reactivate and one tumour remained static in size. Though the regression on ovariectomy or tamoxifen (cf. Figs 1 and 4) was not quite as dramatic as that seen at the start of this work when satisfactory tumour induction was obtained with a single 20 mg dose of DMBA (see Figs 1, 2 and 3), at least one tumour was observed on all the animals treated by the multiple dosage method and not less than 70% developed tumours of usable size. The multiple dosage technique was therefore considered to be desirable both on grounds of an acceptable level of hormone dependency of the tumours and economy

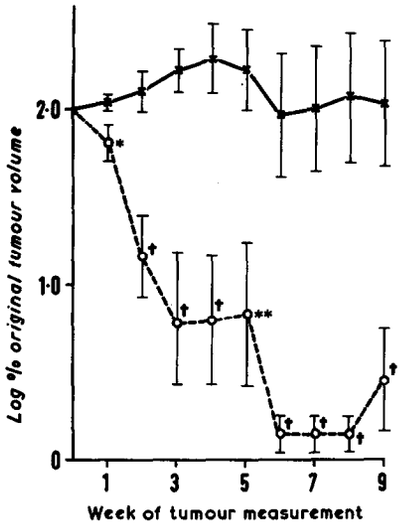

*Fig. 1. The mean (± S.E.M.) volumes off DMBA induced mammary tumours on intact rats treated for 5 days per week for 5 weeks with (a) (×—×) arachis oil (0.1 ml sc, n < 6) and (b) (O---O) tamoxifen (50 μg in 0.1 ml arachis oil sc, n = 5). Tumour volumes, calculated as $4/3\pi r^3$ where r is the average of two orthogonal radii, were determined 2 days before commencing treatment and then at weekly intervals and are expressed as a percentage of the original volume (=100%). All percentage tumour volumes were transformed to logarithms. Tumours too small to measure were arbitrarily assigned the smallest dimension (r = 2.5 mm) capable of measurement with acceptable accuracy prior to calculations. Difference from arachis oil control by Student's t test: *P < 0.05; **P < 0.02; †P < 0.01.*

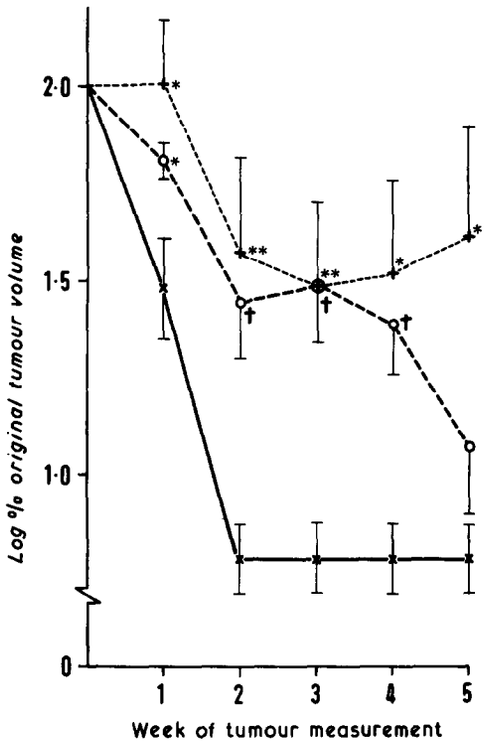

*Fig 2. The mean (± S.E.M.) volumes of DMBA induced mammary tumours on ovariectomized rats treated for 5 days per week for 5 weeks with (a) (×—×) arachis oil (0.1 ml sc; n = 7), (b) (O---O) diethylstilboestrol 50 μg in 0.1 ml arachis oil; n < 7), c, (+···+) diethylstilboestrol (50 μg in 0.1 ml arachis oil sc) plus pregnant mares serum gonadotrophin (100 iu in 0.1 ml sterile water sc; n = 9). Tumour volumes, calculated as $4/3\pi r^3$, where r is the average of two orthogonal radii, were determined 2 days before treatment commenced and then at weekly intervals and are expressed as a percentage of the original tumour volume (=100%). Tumours too small to measure were arbitrarily assigned the smallest dimension capable of measurement with reasonable accuracy (r = 2.5 mm). All percentage tumour volumes were transformed to logarithms prior to statistical calculation. Differences from arachis oil control by Student's t test: *P < 0.05; **P < 0.02; †P < 0.01.*

of animal usage. It was used for all the experiments involving intact rats with PMSG and/or tamoxifen.

Administration of low doses (2 or 20 iu twice daily) of PMSG to intact tumour bearing rats for 16 days produced no significant effect ($P > 0.2$ by Student's t test) on the rate of tumour growth (Fig. 4) but increasing the dose to 200 iu twice daily produced a significant slowing in tumour growth rate ($P < 0.05$), four out of nine tumours showing regression. This tumour regressing effect of high doses of PMSG was not seen in the presence of tamoxifen. Tumours on intact rats subjected to a daily administration of tamoxifen (200 μg) and PMSG (200 iu twice daily) continued to grow and even 2 iu of PMSG twice daily reversed the tumour regressing effect of tamoxifen (Fig. 4). Indeed three out of eight tumours on rats treated with tamoxifen and 2 iu PMSG twice daily grew very rapidly and the rats were sacrificed before the scheduled 16 day administration period was completed. Intermediate doses of PMSG (20 iu twice daily) with tamoxifen (200 μg) appeared to increase tumour growth rate compared with tamoxifen treated controls, but unlike the 2 and 200 iu doses of PMSG did not produce a statistically significant reversal of the tumour inhibitory effect of tamoxifen ($P > 0.2$ compared with $P < 0.05$ and < 0.02 respectively for the 2 iu and 200 iu doses).

Highly significant increases in uterine wet weight, intra-uterine fluid weight and ovarian paired weights occurred in intact tumour bearing rats treated twice daily for 16 days with either 20 iu or 200 iu of PMSG in the presence or absence of tamoxifen ($P < 0.001$). The only exception to this general increase was that of intra-uterine fluid in rats treated with 20 iu PMSG alone, which showed no significant increase on controls ($P > 0.5$) (Fig. 5). Vaginal smears taken on days 7, 8 and 9 from PMSG treated animals exhibited some cornification with few leucocytes and some nucleated cells on each day whereas those from all control rats were characteristic of rats undergoing normal oestrus cycles. Oestradiol and prolactin levels in plasma from animals treated with the highest dose of PMSG for 14 days were significantly increased ($P < 0.05$) compared with controls, oestradiol being 281 ± 6.9 pg/ml and prolactin 660 ± 160 ng/ml compared with 55 ± 6.6 pg/ml and 160 ± 85.6 ng/ml respectively for the controls ($n < 4$).

Analysis of variance and covariance of the data summarized in Figs 4 and 5 demonstrated PMSG

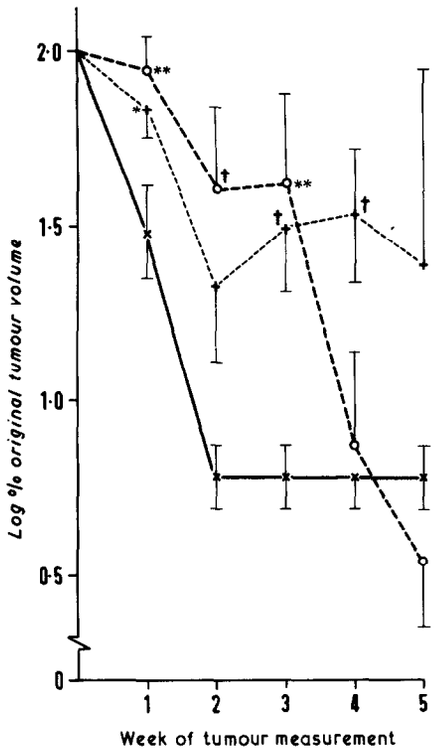

*Fig. 3. The mean (± S.E.M.) volumes of DMBA induced mammary tumours on ovariectomized rats treated for 5 days per week for 5 weeks with (a) (×—×) arachis oil (0.1 ml sc; n = 7), (b) (O---O) pregnant mares serum gonadotrophin (PMSG, 100 iu in 0.1 ml sterile water sc; weeks 1–3 n = 7, week 4 n = 5, weeks 5 n = 3), (c) (+..+) PMSG (100 iu in 0.1 ml sterile water sc) plus tamoxifen (50 μg in 0.1 ml arachis oil sc; weeks 1–4 n = 10; week 5 n = 4). Tumour volumes, calculated as $4/3\pi r^3$ where r is the mean of two orthogonal radii, were estimated 2 days prior to commencing treatment and are expressed as a percentage of the original tumour volume (=100%). Tumours too small to measure were arbitrarily assigned the smallest dimension capable of measurement with an acceptable level of accuracy (r = 0.25 mm). All percentage tumour volumes were transformed to logarithms prior to statistical calculation. Differences from arachis oil control by Student's t test: *P < 0.05, **P < 0.02, †P < 0.01.*

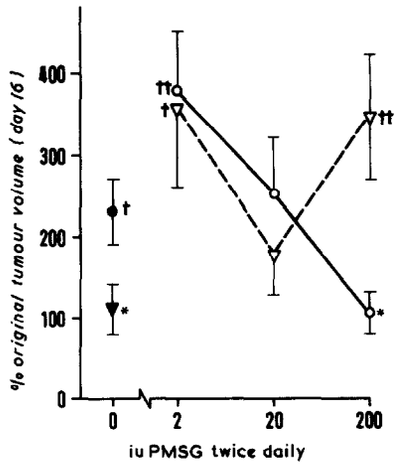

*Fig. 4. Changes in volume (mean ± S.E.M.) of DMBA induced mammary tumours on intact rats treated (sc) for 16 days with the stated doses of pregnant mares serum gonadotrophin (PMSG) in sterile water (0.1 ml) alone or together with tamoxifen (200 μg per day) administered in arachis oil (0.1 ml). All rats received three injections per day, PMSG in sterile water or sterile water alone (0.1 ml) at 9.00 and 16.30 h, and tamoxifen in arachis oil or arachis oil alone (0.1 ml) at 9.00 h. Controls received sterile water and arachis oil (●) or sterile water and tamoxifen in arachis oil (▼). Responses to PMSG plus arachis oil are designated (○), and responses PMSG plus tamoxifen in arachis oil (▽). Tumour volumes, calculated as $4/3\pi r^3$ where r is the mean of two orthogonal radii, were estimated immediately prior to commencing treatment and are expressed as a percentage of the original tumour volume (=100%). Tumours too small to measure were arbitrarily assigned the smallest dimension capable of measurement with an acceptable level of accuracy (r = 0.25 mm). Differences from sterile water/arachis oil control are indicated (*P < 0.05) and differences from sterile water/tamoxifen control (†P < 0.05, ††P < 0.02).*

to have a significant dose related effect on tumour volume ($P = 0.025$), uterine weight ($P < 0.001$), intra-uterine fluid weight ($P < 0.001$) and paired ovarian weights ($P < 0.001$) but tamoxifen in the presence of PMSG had a significant effect on uterine weight only ($P = 0.0005$). The interaction between PMSG and tamoxifen was significant only on tumour volume ($P = 0.049$; 0.0033 if tumour volumes were transformed to logarithms prior to statistical analysis).

DISCUSSION

Administration of PMSG to both intact and ovariectomized tumour bearing rats appears, in general, to be disadvantageous. In ovariectomized animals it produced an early slowing of tumour regression when given alone or together with either diethylstilboestrol or tamoxifen (Figs 2 and 3). However, when given to ovariectomized animals together with diethylstilboestrol, it produced a longer term disadvantage, mean tumour volume at the end of 5 weeks being significantly larger than in control animals whereas the mean for animals treated with diethylstilboestrol alone was not significantly different from control (Fig. 2). The tumour regression pattern produced by PMSG plus tamoxifen was very similar at first to that seen with PMSG plus diethylstilboestrol, but mean tumour volume at the end of 5 weeks was not significantly different from control. However several animals in the PMSG plus tamoxifen treatment group died during the 5th week markedly reducing the number of tumours available for comparison (Fig. 3).

The interaction between PMSG and tamoxifen was even more marked when the two drugs were administered to intact tumour bearing rats, the complete reversal of the tamoxifen induced inhibition of tumour growth produced by a twice daily administration of 2 iu of PMSG (Fig. 4) being particularly dramatic. With this low dose of PMSG there was no evidence of stimulation of oestradiol output from the ovaries, there being no increase in uterine wet weight, ovarian weight or intra-uterine fluid weight (Fig. 5). In contrast, larger doses of PMSG (20 or 200 iu twice daily), either alone or

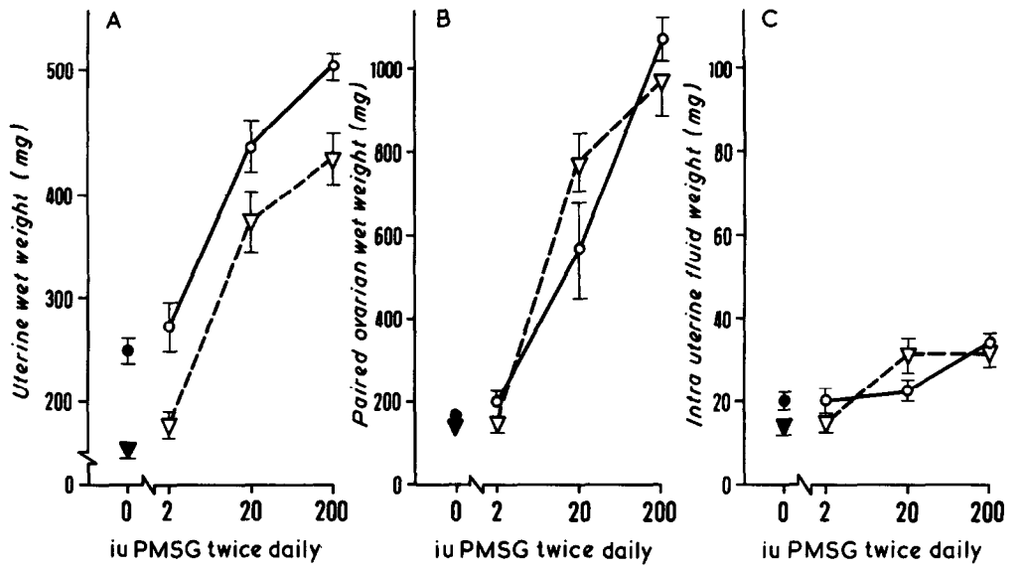

Fig. 5. (A) Uterine wet weight, (B) paired ovarian weight and (C) intra-uterine fluid weight of DMBA induced tumour bearing rats treated (sc) for 16 *days with the indicated doses of pregnant mares serum gonadotrophin (PMSG) in sterile water* (0.1 ml) *alone or together with tamoxifen* (200 μg *per day) in arachis oil* (0.1 ml). *All rats received three injections per day, PMSG in sterile water or sterile water* (0.1 ml) *at* 9.00 *and* 16.30 h, *and arachis oil alone* (0.1 ml) *or tamoxifen in arachis oil at* 9.00 h. *Controls received either sterile water and arachis oil* (●) *or sterile water and tamoxifen in arachis oil* (▼). *Mean* (± *S.E.M.*) *responses to PMSG plus arachis oil are indicated* (○) *and to PMSG plus tamoxifen in arachis oil* (▽).

together with tamoxifen, produced highly significant increases in uterine and ovarian weights consistent with an increase in oestradiol output. For the highest dose of PMSG the highly significant increase in plasma oestradiol was accompanied by an increase in prolactin levels, both increasing to approximately four times control. It is possible that the high level of oestradiol in the circulation might be responsible for the significantly smaller tumour volumes observed on day 16 on intact rats treated twice daily with 200 iu of PMSG alone, the high level of oestradiol in the circulation being equivalent to the administration of supramaximal doses of diethylstilboestrol. The reversal of this control of tumour growth by a daily administration of tamoxifen was surprising but serves to emphasize the complexities inherent in the hormonal control of mammary tumour growth.

Tamoxifen is known to affect the pituitary–gonadal axis but the overall picture is complex and differs in pre- and postmenopausal women. Significant increases in serum oestradiol occur on administration of tamoxifen to premenopausal women [11, 26–28] but findings on gonadotrophin levels vary. Numerous workers have reported that little change occurs [11, 26] or only slight elevation [27]. In a review on the pharmacology and clinical use of tamoxifen, Furr and Jordan [8] suggested that, though it may be generally accepted that tamoxifen produces little change in gonadotrophin levels overall, there may yet be subtle effects on pulse amplitude and frequency. However, an early report by Boyns and Groom [29] claimed significant increases in both plasma FSH and LH in volunteer premenopausal subjects. More recently Manni and Pearson [27] reported increased levels of gonadotrophins in two premenopausal women with stage IV breast cancer treated for a prolonged period with higher than normal doses of tamoxifen and Jordan *et al.* [28] reported three- to fivefold increases in circulating FSH compared to control cycles of normal female volunteers in young patients who had resumed menstrual cycles after receiving a short (4 months) course of chemotherapy followed by long term tamoxifen therapy.

In postmenopausal women the basal levels of both FSH and LH fall following treatment with tamoxifen but remain within the postmenopausal range [30–32]. After one month, however, Golder *et al.* [31] found that plasma FSH levels in patients who responded to tamoxifen treatment returned to pretreatment levels whereas they remained lower than pretreatment levels in both responders and non-responders.

The overall picture is complex. The menopausal status of the patients, the oestrogen/progesterone receptor content of their tumours, the nature and duration of any treatment prior to tamoxifen administration, the levels of oestrogenic hormones, progesterone and prolactin and the absolute levels of gonadotrophins in the circulation may all be of significance in determining if tamoxifen therapy is effective and changes in circulating hormone levels may contribute to the phenomenon of 'tamoxifen

flare' [33, 34]. The results described in this paper suggest that an increase in gonadotrophin levels, even though very small and leaving the overall level within the 'normal range', may be disadvantageous in relation to tamoxifen treatment. The positive response of patients with advanced breast cancer to total endocrine ablation following tamoxifen flare [35] is consistent with this view of the possible relevance of gonadotrophin levels to tumour response to tamoxifen.

REFERENCES

1. Cooper AP. In: Lee A, ed. *The Principles and Practice of Surgery*. London, E Cox 1836, 333–335.
2. Beatson GT. On the treatment of inoperable cases of carcinoma of the mamma. Suggestions for a new method of treatment with illustrative cases. *Lancet* 1896, **ii**, 104–107.
3. Levin ML, Thomas DB. Epidemiology of breast cancer. In: Robinson R, ed. *Current Trends in the Management of Breast Cancer*. London, Balliere, Tindall, 1977.
4. Huggins C, Bergenstal DM. Inhibition of human mammary and prostatic cancers by adrenalectomy. *Cancer Res* 1952, **12**, 134–141.
5. Luft R, Olivecrona H. Experience with hypophysectomy in man. *J Neurosurg* 1953, **10**, 301–316.
6. Santen RJ, Samojlik E, Worgul TJ. Aminoglutethimide, product profile. In: Santen RJ, Henderson IJ, eds. *A Comprehensive Guide to the Therapeutic Use of Aminogluethethimide*. Basel, Karger, 1981, 125–134.
7. Thompson EA Jr, Siiteri PK. Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstenedione. *J Biol Chem* 1974, **249**, 5364–5372.
8. Furr BJA, Jordan VC. The pharmacology and clinical use of tamoxifen. *Pharmacol Ther* 1984, **25**, 127–205.
9. Edwards DP, Chamness GC, McGuire WL, Estrogen and progesterone receptor proteins in breast cancer. *Biochim Biophys Acta* 1979, **560**, 457–486.
10. McGuire WL. Steroid hormone receptors in breast cancer treatment strategy. *Recent Prog Hormone Res* 1980, **36**, 136–146.
11. Groom GV, Griffiths K. Effect of the anti-oestrogen tamoxifen on plasma levels of luteinizing hormone, follicle-stimulating hormone, prolactin, oestradiol and progesterone in normal pre-menopausal women. *J Endocrinol* 1976, **70**, 421–428.
12. McGuire WL, Horwitz KB, Zava DT, Garola RE, Chamness GC. Hormones in breast cancer. Update 1978. *Metabolism* 1978, **27**, 487–501.
13. Jungi WF, Alberto P, Wagenknecht L, Cavalli F, Martz G, Brunner KW. Antiostrogene: eine neue endokrine Behandlungsmoglichkeit beim metastasierenden Mammakarzinom. *Schweiz Med Wschr* 1978, **108**, 1317–1321; quoted by Vorherr H, in *Breast Cancer: Epidemiology, Endocrinology, Biochemistry and Pathology*. Urban, Schwarzenberg, eds. Baltimore-Munich, 1980, 394.
14. Haddow A, Watkinson JM, Patterson E. Influence of synthetic oestrogens upon advanced malignant disease. *Br Med J* 1944, **2**, 393–398.
15. Report to the Council on Drugs. Androgens and estrogens in the treatment of disseminated mammary carcinoma: retrospective study of nine hundred and forty-four patients. *JAMA* 1960, **172**, 1271–1283.
16. Kennedy BJ. Massive estrogen administration in premenopausal women with metastatic breast cancer. *Cancer* 1962, **15**, 641–648.
17. Greenblatt RB, Natrajan PK, Tzingounis V. Role of the hypothalamus in the aging woman. *J Am Geriatr Soc* 1979, **XXVII**, 97–103.
18. Moore WT, Ward D. Pregnant mares serum gonadotrophin; an *in vitro* biological characterisation of the lutropin-follitropin dual activity. *J Biol Chem* 1980, **225**, 6930–6936.
19. Sasamoto S, Sato K, Neito H. Biological active life of PMSG in mice with special reference to follicular ability to ovulate. *J Reprod Fertil* 1972, **30**, 371–379.
20. Drury RAB, Wallington EA. The Papanicolaou technique. In: Drury RA, Wallington EA, eds. *Carleton's Histological Technique*. New York, Toronto, 1967, fourth edition, 381–382.
21. Whitaker EM, Robinson AC. Circulating reproductive hormones and hypothalamic oestradiol and progesterone receptors in infertile Zucker rats. *J Endocrinol* 1989, **120**, 331–336.
22. Dixon WJ, chief ed. *BMDP Statistical Software*. Berkeley, Los Angeles, University of California Press, 1983, 347–412.
23. Shimkin MB, Gruenstein M, Mercuare DR, Acuff M, Thatcher D. The effects of schedule and dose of 7,12-dimethylbenz(a)anthracene on the induction and growth of carcinomas in Sprague–Dawley female rats. *Cancer Res* 1969, **29**, 503–505.
24. Huggins C, Grand LC, Brillantes FP. Mammary cancer induced by a single feeding of polynuclear hydrocarbons, and its suppression. *Nature* 1961, **189**, 204–207.
25. Griswald DP, Green CH. Hormone sensitivity of 7,12-dimethylbenz(a)anthracene induced mammary tumour in Sprague–Dawley rats. *Cancer Res* 1970, **30**, 819–826.
26. Sherman BM, Chapler FK, Crickard K, Wycoff D. Endocrine consequences of continuous

antiestrogen therapy with tamoxifen in premenopausal women. *J Clin Invest* 1979, **64**, 398–409.

27. Manni A, Pearson OH. Antiestrogen-induced remissions in premenopausal women with stage IV breast cancer. *Cancer Treat Rep* 1980, **64**, 779–785.
28. Jordan VC, Fritz NF, Tormey DC. Endocrine effects of adjuvant chemotherapy and long-term tamoxifen administration on node-positive patients with breast cancer. *Cancer Res* 1987, **47**, 624–630.
29. Boyns AR, Groom GV. Effect of tamoxifen on plasma gonadotrophin concentrations. In: *Proceedings of a Workshop ICI 46474 — Work in Progress*. ICI Pharmaceuticals Division plc, Macclesfield, 1972, 35–39.
30. Golder MP, Phillips MEA, Baum M *et al.* Hormones in breast cancer patients on tamoxifen. *Cancer* 1975, **32**, 246–247.
31. Golder MP, Phillips MEA, Fahmy DR *et al.* Plasma hormones in patients with advanced breast cancer treated with tamoxifen. *Eur J Cancer* 1976, **12**, 719–723.
32. Manni A, Trujillo J, Marshall JS, Brodkey J, Pearson OH. Antihormone treatment of stage IV breast cancer. *Cancer* 1979, **43**, 444–480.
33. Tormey DC, Simon RM, Lippman ME, Bull JM, Myers CE. Evaluation of tamoxifen dose in advanced breast cancer: a progress report. *Cancer Treat Rep* 1976, **60**, 1451–1459.
34. Plotkin D, Lechner JJ, Jung WE, Rosen PJ. Tamoxifen flare in advanced breast cancer. *JAMA* 1978, **240**, 2644–2646.
35. Hartley JW, Wong J, Fletcher WS. Response of advanced breast cancer to total endocrine ablation after exacerbation on tamoxifen: results in seven patients and possible mechanism of action. *J Surg Oncol* 1987, **34**, 182–187.