Eur J Cancer Clin Oncol, Vol. 17, No. 8, pp. 925–931, 1981.
Printed in Great Britain.

0014-2964/81/080925-07$02.00/0

Effects of Megestrol Acetate on Growth and Secretion of a Pituitary Tumor*

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Abstract—*The effect of the administration of the 6-methylated progestagen megestrol acetate on the growth of the estrogen-induced transplantable PRL/ACTH-secreting rat pituitary tumor 7315a was investigated. A significant inhibition of tumor growth was observed only during the last week of a four week period of drug administration* (6 mg/kg). *Administration of megestrol acetate suppressed the total content and the ability to release PRL, ACTH and LH of the pituitary gland of tumor bearing rats. Further, a significant suppression of the anterior pituitary, ovarian and uterine weights was observed in the megestrol acetate treated animals. Megestrol acetate did not exert a direct effect on PRL, ACTH and LH secretion by normal rat pituitary glands incubated in vitro.*

We conclude that megestrol acetate exerts in the model used a suppressive effect on PRL, ACTH and LH release and led to a diminution of the pituitary content of these hormones, while it has also anti-estrogenic and anti-androgenic properties. The effects of the drug help further explain its beneficial effects in metastatic breast, endometrial, renal and prostatic cancer in man.

INTRODUCTION

ADMINISTRATION of high dosages of megestrol acetate (17α-acetoxy-6-methylpregna-4,6-diene-3,20-dione) or medroxyprogesterone acetate (6α-methyl-17α-acetoxy progesterone) have been shown to induce objective remission with improved survival in 25–33% of post-menopausal women with metastatic breast cancer who are resistant to cytotoxic drugs and endocrine therapies, including treatment with tamoxifen [1–7]. It has been suggested, therefore, that these progestational agents are useful compounds in the treatment of end-stage patients with advanced mammary carcinoma [2, 6]. The 6-methylated progestins possess high progestational properties and in addition demonstrate anti-estrogenic, anti-gonadotropic and anti-androgenic activity, while some degree of adrenal atrophy was observed during long-term use of high dosages of the compounds [8–10]. They have also successfully been used in the treatment of cancer of the endometrium [11], kidney [12] and prostate [13]. At present, it is unknown how these progestational drugs accomplish tumor regression in these different types of carcinomas.

In the present study we investigated the effect of the chronic administration of megestrol acetate on the growth of the estrogen-induced transplantable prolactin (PRL) and adrenocorticotropin (ACTH) secreting rat pituitary tumor 7315a in the rat. Additionally, the effect of megestrol acetate on the ability of the pituitary gland of tumor bearing rats to secrete PRL, ACTH, luteinizing hormone (LH) and follicle stimulating hormone (FSH) *in vitro* and on the total pituitary content of these hormones was compared with those in control rats without tumours and in control rats with tumors. Finally, a possible direct effect of megestrol acetate on PRL, ACTH, LH and FSH release by the rat pituitary gland *in vitro* was investigated.

MATERIALS AND METHODS

Animals, tumors and related procedures

Female rats (offspring of male Buffalo and female Wag/Rij rats) with a body weight varying between 100 and 120 g were inoculated subcutaneously (s.c.) between the scapulae with the PRL/ACTH secreting pituitary tumor

Accepted 16 April 1981.
*This work was supported by grants from the Dutch organization for fundamental research (FUNGO/ZWO) and from the Dutch National Cancer League, the "Koningin Wilhelmina Fonds."
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Abbreviations; PRL, prolactin; ACTH, adrenocorticotropin; LH, luteinizing hormone; FSH, follicle stimulating hormone; GH, growth hormone; TSH, thyroid stimulating hormone.

7315a (obtained from Dr. R. M. MacLeod, Charlottesville, Virginia). Five grams of tumor tissue were chopped and minced to a homogeneous solution in 10 ml 0.9% saline and 0.2 ml of this suspension was injected. The animals were housed six per cage under artificial light from 06.00 to 19.00.

Megestrol acetate (Novo Industries, Bagsvaerd, Denmark) was dissolved in 0.2 ml peanut oil and administered s.c. between 09.00 and 10.00. Control rats and control tumor bearing rats received an injection with oil alone. The administration of megestrol acetate was started on the 3rd day after tumor implantation and daily injections were continued until the animals were killed on day 28.

Growth of the transplanted pituitary tumors was evaluated by expressing tumor size in centimeters squared (maximum length × maximum width). The actual tumor weight has been shown to be significantly correlated ($P < 0.01$) with tumor size when expressed according to these criteria [14].

Control animals without tumors were sacrificed when in diestrus, while the tumor bearing and megestrol acetate treated rats did not cycle. Control rats with and without tumors and the megestrol acetate treated tumor bearing rats were sacrificed simultaneously and the pituitary incubations were carried out on the same morning.

In order to evaluate the effects of the chronic administration of megestrol acetate on the ability of the pituitary gland to release PRL, ACTH, LH and FSH *in vitro* and on the total concentration of these hormones in the pituitary gland [15], the animals were killed by decapitation at 10.30 on day 28, 90 min after the last injection and plasma was collected. Anterior pituitary glands were quickly removed. One complete anterior pituitary gland or three hemipituitary glands from different rats were incubated *in vitro* without preincubation in 1 ml medium 199 (Gibco BioCult, Glasgow, Scotland). The flasks were incubated at 37°C in a Dubnoff shaker in an atmosphere of 95% O_2–5% CO_2 (v/v). After incubation for 5 hr the medium was removed and the glands were rinsed quickly and homogenized in 1 ml distilled water. Part of the medium and pituitary gland extract were taken apart for the ACTH-assay and brought to pH 1 with 1 N HCl.

The hormone content of the anterior pituitary glands was calculated by addition of the hormone concentration found in the medium and that found in the pituitary gland after the 5 hr-incubation. This assumes that during this period no important breakdown or biosynthesis of these hormones has occurred [14, 15].

Hormone assays

The level of PRL in the incubation medium, the pituitary extract and the plasma was measured by a double-antibody radioimmunoassay using materials and protocols supplied by the NIAMD Rat Pituitary Hormone Distribution Program. The results are expressed in terms of NIAMD rat prolactin RP-1. LH and FSH were estimated by double-antibody RIA's using NIAMD-rat LH I-6 and NIAMD-rat FSH I-4 for iodination and NIAMD-rat LH RP-1 and NIAMD-rat FSH RP-1 as standards. Antisera (anti-ovine LH 610 and anti-ovine FSH 619) were obtained by immunization of rabbits with NIH-LH S17 and NIH-FSH S9 respectively, as described elsewhere [16]. ACTH in the medium and the pituitary extract was measured with a bioassay using the steroidogenic response of isolated adrenal cells as described by Lowry *et al.* [17].

All data are expressed as means ± S.E.M. Statistical analysis was done with Student's unpaired *t*-test.

RESULTS

Megestrol acetate (6 mg/kg) did not affect tumor growth between the 3rd and 22nd day after implantation (Fig. 1). From then on, however, control tumors increased between day 22 and 28 in size by $12.7 \pm 2.4\ cm^2$, while the megestrol acetate injected tumors grew only by $4.2 \pm 2.1\ cm^2$ ($P < 0.01$). On day 28 the size of the tumors in the control animals amounted to

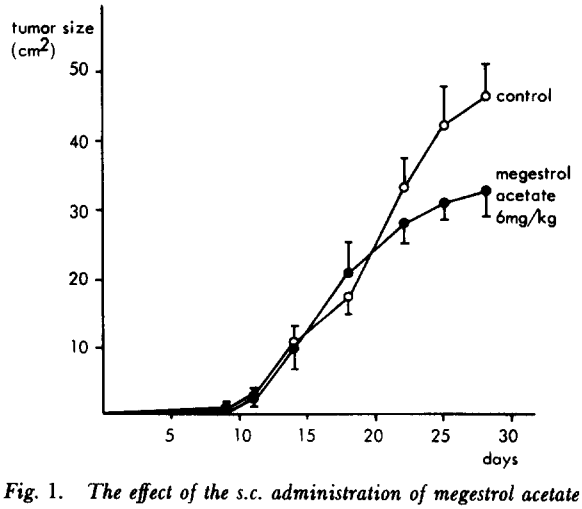

Fig. 1. The effect of the s.c. administration of megestrol acetate (6 mg/kg) *from* day 3 *until* day 28 *after tumor implantation on the growth of the transplantable PRL/ACTH secreting rat pituitary tumor 7315a. Between* day 22 *and* day 28 *a significantly slower growth of the tumor was observed after megestrol acetate in comparison with control tumors* ($P < 0.01$). *(Five rats per group, means ± S.E.M.).*

$46.4 \pm 5.5\,cm^2$, while that in the megestrol acetate treated animals was $33.0 \pm 4.4\,cm^2$ ($P < 0.05$). The weight of the tumors was not significantly different (Table 1). This is probably caused by the variable size of the control tumors, as the actual tumor weight and the tumor size in these 10 animals (5 control tumor bearing rats and 5 megestrol-treated tumor bearing rats) showed a statistically significant correlation ($P < 0.01$). The inhibition of tumor growth by megestrol acetate was not accompanied by a significant diminution of the greatly elevated plasma PRL concentrations (Table 2). The megestrol acetate treated animals had the same body weight as the tumor-bearing control rats (data not shown).

Implantation of the tumor resulted in a diminution of the pituitary and uterine weights in comparison with control non-tumor bearing rats ($P < 0.01$ and $P < 0.01$, resp.; Table 1). The adrenal weights, however, were greatly elevated ($P < 0.01$ vs control non-tumor bearing rats), while ovarian weights remained unchanged (Table 1). After chronic administration of megestrol acetate a decrease in pituitary, ovarian and uterine weights was observed in comparison with the weights in the control tumor bearing rats ($P < 0.05$; $P < 0.01$ and $P < 0.01$ resp.; Table 1).

Hyperprolactinemia in tumor-bearing control animals was accompanied by a significant suppression of the circulating LH ($P < 0.01$) and FSH ($P < 0.01$) levels (Table 2). Chronic administration of megestrol acetate to tumor bearing rats did not further change the suppressed plasma gonadotropin concentrations.

Implantation of the PRL/ACTH secreting pituitary tumor resulted in a considerable decrease of the total contents of PRL and ACTH of the anterior pituitary glands of tumor bearing animals in comparison with those of control rats without tumors ($P < 0.01$ and $P < 0.05$, resp.; Fig. 2). The ability of the pituitary gland of tumor bearing control rats to release PRL *in vitro* was also significantly suppressed ($P < 0.01$ vs control non-tumor rats; Table 3), while ACTH release remained unchanged. Chronic administration of megestrol acetate to tumor bearing rats resulted in a further diminution of the already suppressed total content of PRL and ACTH in the pituitary gland ($P < 0.01$ and $P < 0.01$, resp. vs control tumor bearing rats; Fig. 2). The ability of these glands to release PRL and ACTH *in vitro*

Table 1. Effect of the daily administration of megestrol acetate (6 mg/kg *body weight*) *starting on* day 3 *after implantation of the tumor on tumor, pituitary, adrenal, ovarian and uterine weights of rats inoculated on* day 1 *with the transplantable PRL/ACTH secreting pituitary tumor* 7315a *and killed on* day 28

	Tumor weight (g)	Pituitary weight (mg)	Adrenal weight (mg) ($n = 2$)	Ovarian weight (mg) ($n = 2$)	Uterine weight (mg)
Control (no tumor)	—	12.0 ± 0.3	40 ± 4	113 ± 4	547 ± 48
Control (tumor)	45.8 ± 6.3	8.4 ± 0.2*	238 ± 14*	101 ± 9	308 ± 10*
Tumor + megestrol acetate	33.8 ± 4.6	7.4 ± 0.2*†	202 ± 30*	38 ± 12*‡	222 ± 21*‡

*$P < 0.01$ vs control rats without tumor.
†$P < 0.05$ vs control rats with tumor.
‡$P < 0.01$ vs control rats with tumor.
Values are means ± S.E.M., five animals/group.

Table 2. Effect of megestrol acetate (6 mg/kg *daily*) *on plasma concentrations of PRL, LH and FSH in rats bearing the transplantable PRL/ACTH-secreting pituitary tumor* 7315a

	Plasma PRL (ng/ml)	Plasma LH (ng/ml)	Plasma FSH (ng/ml)
Control (no tumor)	21 ± 4	46 ± 13	154 ± 10
Control (tumor)	14300 ± 1115*	14 ± 2*	114 ± 11*
Tumor + megestrol acetate	14200 ± 355*	15 ± 3	110 ± 4*

Plasma samples were obtained by decapitation 90 min after the last injection on day 28.
*$P < 0.01$ vs controls without tumor.
Means ± S.E.M., $n = 5$ in each group.

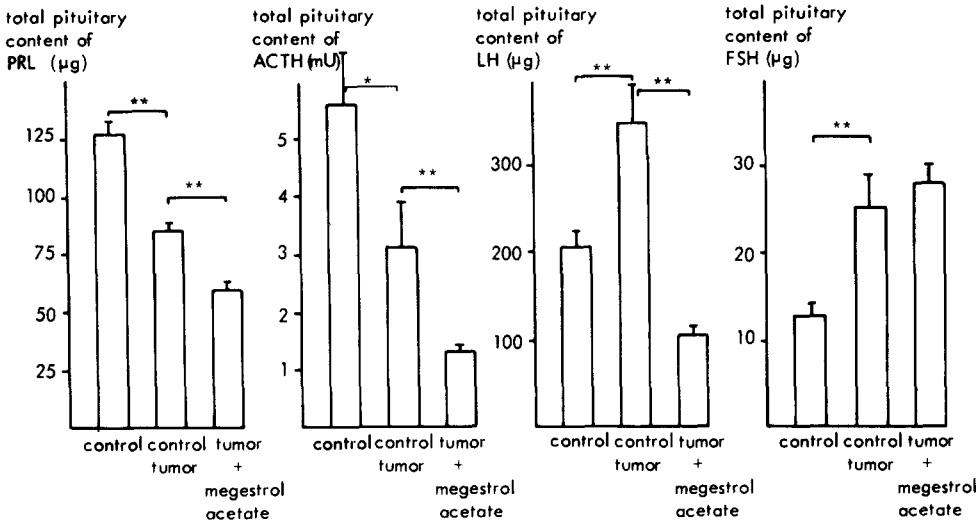

Fig. 2. The total PRL, ACTH, LH and FSH content of pituitary glands obtained from control rats, PRL/ACTH secreting tumor bearing rats and tumor bearing rats treated with megestrol acetate (6 mg/kg) *for* 25 days. (*Five pituitary glands per group; mean ± S.E.M.*). $^{*}P < 0.05$ $^{**}P < 0.01$

Table 3. Effects of the daily administration of megestrol acetate (6 mg/kg *body weight*) *starting on* day 3 *after implantation of the tumor on the ability of the pituitary gland to release PRL, ACTH, LH and FSH of rats bearing the transplantable PRL/ACTH-secreting pituitary tumor* 7315*a*

	Radioimmunoassayable PRL (μg/mg pituitary gland)		bio-assayable ACTH (μU/mg pituitary gland)	
	medium	pituitary gland	medium	pituitary gland
Control (no tumor)	4.39 ± 0.19	6.38 ± 0.48	13.5 ± 1.9	464 ± 47
Control (tumor)	3.41 ± 0.14*	6.53 ± 0.23	10.5 ± 1.0	363 ± 53
Tumor + megestrol acetate	2.94 ± 0.17*	5.18 ± 0.25*‡	8.8 ± 1.1*	169 ± 13*‡
	Radioimmunoassayable LH (μg/mg pituitary gland)		Radioimmunoassayable FSH (μg/mg pituitary gland)	
	medium	pituitary gland	medium	pituitary gland
Control (no tumor)	0.30 ± 0.03	17.1 ± 1.5	0.25 ± 0.02	0.79 ± 0.08
Control (tumor)	0.73 ± 0.07*	40.7 ± 5.7*	0.28 ± 0.04	2.79 ± 0.16*
Tumor + megestrol acetate	0.34 ± 0.06†	14.0 ± 2.1†	0.27 ± 0.07	3.59 ± 0.31*

Incubation of one pituitary per flask for 5 hr. Pituitary glands were obtained 90 min after the last injection of megestrol acetate on day 28. Values are means ± S.E.M.; five animals/group.
†$P < 0.05$ vs control rats with tumor.
‡$P < 0.01$ vs control rats with tumor.

was also suppressed in comparison with that in control non-tumor bearing rats ($P < 0.01$ and $P < 0.01$, resp.), but equivalent with the release of the pituitary glands obtained from control tumor rats (Table 3).

The anterior pituitary glands from control tumor bearing rats contained a significantly higher total amount of LH ($P < 0.01$) and FSH ($P < 0.01$) than those from control rats (Fig. 2). Incubation of these glands *in vitro* showed that the release of LH was increased in comparison with control glands, while FSH release was unchanged (Table 3). After chronic administration of megestrol acetate a decrease in the total LH content and in the ability to release LH *in vitro* was observed in comparison

with those in control tumor bearing rats ($P < 0.01$ and $P < 0.01$, resp. Fig. 2 and Table 3), but the total FSH content and the release of FSH by the pituitary glands *in vitro* remained unchanged.

Finally, the direct effect of megestrol acetate on hormone secretion by normal rat anterior pituitary glands *in vitro* was investigated. Megestrol acetate (0.1 and 1 μM) did not affect basal PRL release and also did not change the dopamine-mediated inhibition of PRL release *in vitro* (Table 4). 0.1 and 1 μM megestrol acetate did not influence basal LH, FSH and ACTH release (data not shown). 5 μM megestrol acetate also did not suppress basal ACTH release, while it did also not influence the lysine vasopressin mediated ACTH stimulation of the rat pituitary gland *in vitro* (Table 4).

DISCUSSION

In previous studies we have shown a strong suppressive action of the administration of the estrogen-receptor blocking agent tamoxifen and the long-acting LHRH-analog ICI 118,630 on the growth of the estrogen-induced transplantable rat pituitary tumor 7315a [18, 19]. These inhibiting effects of tamoxifen and the LHRH-analog on tumor growth are presumably mediated via a blockage of the estrogen receptors on the tumor and chemical castration, respectively. When administered from the 3rd day after implantation of the tumor tamoxifen (200 μg/kg body weight each day) prevented its growth completely[19], while in a later stage of its development tumor growth was inhibited by the administration of tamoxifen by 74 ± 6% [18]. The LHRH-analog (5 μg s.c. twice daily for 12 days) inhibited pituitary tumor growth by 68 ± 11% [18]. Megestrol acetate exerted a less marked effect on the growth of this tumor: a significant inhibition of tumor growth was only present during the last 7 days of a period of 25 days during which the drug was administered. The inhibition of tumor growth on the 28th day of megestrol acetate injections amounted to 29% in comparison with the size of the control tumors, while no significant differences were noted in the actual tumor weights.

Table 4. The direct effect of megestrol acetate on PRL and ACTH secretion by normal female rat pituitary glands in vitro

Exp.1	PRL in medium (μg/mg pituitary)
Control	3.99 ± 0.28
megestrol acetate (0.1 μM)	3.60 ± 0.24
(1 μM)	3.41 ± 0.51
dopamine (500 nM)	2.12 ± 0.39*
dopamine (500 nM + meg. acet. (1 μM)	1.80 ± 0.26*
Exp.2	**ACTH in medium (μU/mg pituitary)**
Control	6.78 ± 1.61
megestrol acetate (5 μM)	6.68 ± 1.37
lysine vasopressin (40 ng/ml)	21.74 ± 2.74*
LVP + megestrol acetate	26.81 ± 5.11*

Incubation of three hemipituitary glands per flask for 5 hr; four flasks per group.
*$P < 0.01$ vs control.

Progesterone and its derivatives have long been considered to antagonize estrogen action, as it was shown to inhibit both estrogen-induced uterine growth [20] and uterine protein synthesis [21]. The anti-estrogenic effects of progestins are probably not mediated via a blockage of estrogen receptors via competition with 17β-estradiol for binding with its receptors, but they are more likely to be caused by interference with estrogen receptor availability resulting in a decreased number of available receptor sites [22, 23]. This difference of action of progestagen, like megestrol acetate, with that of tamoxifen may well account for its less impressive inhibiting effect on the growth of the pituitary tumor used in this study.

Chronic administration of megestrol acetate to tumor bearing rats resulted in a variety of changes in the hormone content and release of the pituitary glands of these rats. The ACTH-secreting pituitary tumor suppressed, either directly or via the increased secretion of corticosterone, the total ACTH content of the host's pituitary gland. Megestrol acetate even further suppressed the total ACTH content. 6-Methylated progestagens have been reported to cause atrophy of the adrenal glands of normal rats [10], while medroxyprogesterone was reported to induce depletion of the pituitary ACTH concentration and of the ACTH release in response to acute stress in rats [24]. The further suppressed total ACTH content of the pituitary glands of megestrol acetate treated tumor rats was not accompanied by a change in the weight of the adrenal gland. The presumably high circulating plasma ACTH levels of tumor bearing control rats, which were not measured in this study, were probably (like the plasma PRL levels) not diminished by the slight decrease in tumor size. This suggests that the suppression of the ACTH content of the pituitary gland by megestrol acetate is mediated via a negative glucocorticoid feedback effect of the drug either on the hypothalamus, or on the pituitary gland itself. The

results of our study on the direct effect of megestrol acetate on basal and lysine vasopressin stimulated ACTH release do not support the latter site of action.

Administration of megestrol acetate was also shown to suppress the total LH content of the pituitary gland and the ability to release LH *in vitro*. This effect is probably a direct anti-gonadotropic effect of the drug on the hypothalamus and/or pituitary gland, as it occurred in the presence of a significant decrease in ovarian weight. During short-term incubation of pituitary glands *in vitro* no direct effect of megestrol acetate on LH secretion was noted.

With regard to the regulation of PRL secretion, it has been shown that progesterone partially inhibits the stimulating effect of estrogens on PRL secretion [25] and also has an inhibitory influence on PRL release in pregnant rats [26]. Progesterone receptors have been described in the brain and in the anterior pituitary gland [27]. Progesterone inhibited the stimulatory effects of estradiol on PRL release by a clonal strain of rat pituitary cells, but only after several days of incubation [28]. In short-term incubation no direct effect of megestrol acetate on PRL release by normal rat pituitary glands was seen in the present study.

It has been reported previously that implantation of this transplantable PRL-secreting pituitary tumor causes a suppression of the weight and function of the host's pituitary gland [15]. These pituitary glands synthesize and release considerably less PRL than the glands of control animals. It was concluded that this is presumably mediated through an autofeedback mechanism due to the greatly elevated circulating level of PRL [29]. The further diminution of the already suppressed total PRL content of the pituitary glands of tumor bearing rats after megestrol acetate injections could be mediated via an inhibiting effect at the hypothalmic level, or it could also merely represent the further lowering of estrogen activity in these animals as evidenced by the suppression of ovarian and uterine weights.

In conclusion, it was shown in the present study that megestrol acetate results in a diminution of the pituitary content and/or ability to release several hormones of the anterior pituitary gland including PRL, ACTH and LH. No data are available on a possible effect on GH and TSH secretion. In addition, anti-estrogenic and anti-androgenic properties of the drug have been described [8]. This wide spectrum of endocrine effects of megestrol acetate in this model of a pituitary tumor which contains estrogen and possibly other hormone receptors [30] could be of help in explaining the beneficial effect of high dosages of 6-methylated progestational drugs in about one third of the patients with advanced breast cancer resistant to cytotoxic and endocrine therapy [2–7] and of its positive effects in other "hormonally" sensitive carcinomas like those of the endometrium, kidney and prostate [12, 13, 15].

REFERENCES

1. Ansfield FJ, Davis HL, Ellerby RA, Ramirez G. A clinical trial of megestrol acetate in advanced breast cancer. *Cancer*, 1974; **33**: 907–910.
2. Ansfield FJ, Davis HL, Ramirez G, Davis TE, Borden EC, Johnson RO, Bryan GT. Further clinical studies with megestrol acetate in advanced breast cancer. *Cancer*, 1976; **38**: 53–55.
3. Buzdar AU, Tashima GK, Blumenschein GR, Hortobagyi GN, Yap H-Y, Krutchik AN, Bodey GP, Livingston RB. Mitomycin-C and megestrol acetate in treatment of breast cancer refractory to hormonal and combination therapy. *Cancer*, 1978; **41**: 392–395.
4. De Lena M, Brambilla C, Valagussa P, Bonadonna G. High dose medroxy-progesterone acetate in metastatic breast cancer previously treated with chemotherapy. *Cancer Chemother Pharmacol*, 1979; **2**: 175–180.
5. Klaassen DJ, Rapp EF, Hirte WE. Response to medroxy-progesterone acetate as a secondary hormone therapy for metastatic breast cancer in post menopausal women. *Cancer Treat Rep*, 1976; **60**: 251–253.
6. Mattsson W. High dose medroxyprogesterone acetate treatment in advanced mammary carcinoma. A phase II investigation. *Acta Radiol Oncol*, 1978; **17**: 387–392.
7. Muggia FM, Cassileth PA, Oshoa M, Flatow FA, Gellhorn A, Hyman GA. Treatment of breast cancer with medroxyprogesterone acetate. *Ann Intern Med*, 1968; **63**: 326–337.
8. David A, Edwards K. Fellowes KP, Plummer JM. Anti-ovulatory and other biological properties of megestrol acetate. *J Reprod Fertil*, 1963; **5**: 331–346.

9. EDGREN RA, HAMBOURGER WE, CALHOUN DW. Production of adrenal atrophy by 6-methyl-17-acetoxy-progesterone, with remarks on the adrenal effects of other progestational agents. *Endocrinology*, 1959; **65:** 505–511.
10. ELTON RL, EDGREN RA, CALHOUN DW. Biological activities of some 6-methylated progesterones. *Proc Soc Exp Biol Med*, 1960; **103:** 175–177.
11. MALKASIAN GD JR, DECKER DG, MUSSEY E, JOHNSON CE. Progestagen treatment of recurrent endometrial carcinoma. *Am J Obstet Gynecol*, 1971; **110:** 15–23.
12. BLOOM JHC. Medroxyprogesterone acetate (provera) in the treatment of metastatic renal cancer. *Br J Cancer*, 1971; **25:** 250–265.
13. RAFLA S, JOHNSON R. The treatment of advanced prostatic carcinoma with medroxyprogesterone. *Curr Ther Res*, 1974; **16:** 261–267.
14. LAMBERTS SWJ, MACLEOD RM. The inability of bromocriptine to inhibit prolactin secretion by transplantable rat pituitary tumors: observations on the mechanism and dynamics of the autofeedback regulation of prolactin secretion. *Endocrinology*, 1979; **104:** 65–70.
15. MACLEOD RM, ABAD A. On the control of prolactin and growth hormone synthesis in rat pituitary glands. *Endocrinology*, 1968; **83:** 799–810.
16. WELSCHEN R, OSMAN P, DULLAART J, DE GREEF WJ, UILENBROEK JThJ, DE JONG FH. Levels of follicle-stimulating hormone, luteinizing hormone, oestradiol-17β and progesterone and follicular growth in the pseudo pregnant rat. *J Endocr*, 1975; **54:** 37–47.
17. LOWRY PJ, MCMARTIN C, PETERS J. Properties of a simplified bioassay for adrenocorticotrophic activity using the steroidogenic response of isolated adrenal cells. *J Endocr*, 1973; **59:** 43–55.
18. LAMBERTS SWJ, UITTERLINDEN P, ZUIDERWIJK JM, BONS EG, DE JONG FH. Effects of a LHRH-analog and tamoxifen on the growth of an estrogen-induced prolactin-secreting rat pituitary tumor and its influence on pituitary gonadotrepins. *Endocrinology*, 1981; **108**: 1878–1884.
19. DE QUIJADA M, TIMMERMANS HAT, LAMBERTS SWJ. Tamoxifen suppresses both the growth of prolactin secreting pituitary tumours and normal prolactin synthesis in the rat. *J Endocr*, 1980; **86**: 109–116.
20. LERNER LJ. Hormone antagonists: inhibitors of specific activities of estrogen and androgen. *Recent Progr Horm Res*, 1964; **20:** 435–476.
21. BHAKOO HS, KATZENELLENBOGEN BS. Progesterone antagonism of estradiol-stimulated uterine induced protein synthesis. *Mol Cell Endocrinol*, 1977; **8:** 105–120.
22. HSUEH AJW, PECK EJ, CLARK JH. Progesterone antagonism of the oestrogen receptor and oestrogen-induced uterine growth. *Nature*, 1975; **254:** 337–338.
23. HSUEH AJW, PECK EJ, CLARK JH. Control of uterine estrogen receptor level by progesterone. *Endocrinology*, 1976; **98:** 438–444.
24. HOLUB DA, KATZ FH, JAILER JW. Inhibition by 6-methyl-17-acetoxy-progesterone of ACTH synthesis and release in the rat. *Endocrinology*, 1961; **68:** 173–177.
25. CHEN CL, MEITES J. Effect of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. *Endocrinology*, 1970; **86:** 503–505.
26. AMENOMORI Y, CHEN CL, MEITES J. Serum prolactin levels in rats during different reproductive states. *Endocrinology*, 1970; **86:** 506–510.
27. KATO J, ONOUGHI T. Specific progesterone receptors in the hypothalamus and anterior hypophysis of the rat. *Endocrinology*, 1977; **101:** 920–928.
28. HAUG E, GAUTVIK K. Effects of sex steroids on prolactin secreting rat pituitary cells in culture. *Endocrinology*, 1976; **99:** 1482–1489.
29. MACLEOD RM, LEHMEYER JE. Suppression of pituitary tumor growth and function by ergot alkaloids. *Cancer Res*, 1973; **33:** 849–855.
30. MCGUIRE WL, DE LA GARZA M, CHAMNESS GC. Estrogen receptor in a prolactin-secreting pituitary tumor (MtTW15). *Endocrinology*, 1973; **93:** 810–815.