Journal of Steroid Biochemistry, Vol. 10, pp. 423 to 429
Pergamon Press Ltd 1979. Printed in Great Britain

AROMATASE INHIBITORS—IV. REGRESSION OF HORMONE-DEPENDENT, MAMMARY TUMORS IN THE RAT WITH 4-ACETOXY-4-ANDROSTENE-3,17-DIONE*†

ANGELA M. H. BRODIE, DAVID MARSH and HARRY J. BRODIE
Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, U.S.A.

(*Received* 28 *March* 1978)

SUMMARY

An aromatase (estrogen synthetase) inhibitor, 4-acetoxy-4-androstene-3,17-dione (4-acetoxy-A)‡, was effective in causing regression of DMBA (7,12-dimethylbenz(a)anthracene)-induced, hormone-dependent, mammary tumors in the rat. At a similar dose (15 mg/day), treatment was more effective when 4-acetoxy-A was administered by silastic wafer supplemented with injections than by injections alone. When 4-acetoxy-A was administered by wafers to 7 rats for 4 weeks, 64% of the tumors completely regressed. The mean ovarian estradiol secretion rate measured at the end of treatment with 4-acetoxy-A was reduced to 28% of the control value. Estrone and progesterone secretion rates were reduced but not significantly. Gonadotropin levels were unchanged. Tumor regression with 4-acetoxy-A was counteracted when estradiol-17β was administered concomitantly. The above findings indicate that 4-acetoxy-A probably acts to cause tumor regression by reducing estrogen production via aromatase inhibition.

INTRODUCTION

Over the past few years, we have developed a number of compounds that inhibit estrogen biosynthesis *in vitro* [1]. Some of these have marked *in vivo* activity as well and thus have potential as treatment for estrogen dependent cancer [2].

Although biosynthesis of estrogen occurs in the ovaries and adrenal glands, some also occurs in adipose tissue and muscle [3]. This peripheral aromatization of androgens to estrogens increases after menopause and becomes the main source of estrogen [4]. Peripheral aromatization may similarly increase following ovariectomy which is frequently performed for the treatment of breast cancer. Some breast tumors themselves are reported to synthesize estrogens [5–7]. In endometrial cancer, peripheral conversion to estrogen appears to be important in the etiology of the disease [8]. Thus, inhibition of estrogen production by compounds which could act at all aromatizing sites might be an effective alternative to surgical removal of the ovaries and adrenals in patients with breast cancer and a useful treatment for endometrial cancer. Other inhibitors such as aminoglutethimide which inhibits P-450 enzymes [9] and cyanoketone [10], a 3β-hydroxy dehydrogenase inhibitor, are more generalized inhibitors and interfere with biosynthesis of other steroids as well.

We recently reported that 4-acetoxy-4-androstene-3,17-dione (4-acetoxy-A) acts as an aromatase inhibitor and inhibits reproductive process *in vivo* [11]. In the present study we report antitumor activity of 4-acetoxy-A and results of further experiments which indicate that this compound probably acts *in vivo* by inhibiting estrogen biosynthesis.

EXPERIMENTAL

Preparation and purification. 4-Acetoxy-A was synthesized by acetylation of 4-OH-A and purified as described elsewhere [2].

Radioimmunoassay procedures. Methods for measuring estrone, estradiol [12, 13] and progesterone [14] were used with the same modifications as previously reported [2, 11]. LH, FSH and prolactin were assayed by the method of Odell *et al.* [15].

Animal studies

All rats were of the Sprague–Dawley strain from Charles River Breeding Labs. The animals were fed Charles River RMH 3000, *ad lib.* They were housed under conditions of controlled temperature, humidity and lighting (12 h light:12 h dark).

Bioassay of 4-acetoxy-A for androgenic activity

Male rats, 22 days old, were castrated and injected subcutaneously (s.c.) daily for 10 days with 0.1 ml

* This is paper 4 in a series on aromatase inhibitors. Papers 1, 2 and 3 are Ref. [1, 2, 11].

† This work was supported by grants CA 18595 and AM-14625 from the National Institutes of Health.

‡ Abbreviations used 4-acetoxy-A = 4-acetoxy-4-androstene-3,17-dione; 4-OH-A = 4-hydroxy-4-androstene-3,17-dione.

Table 1. The effect of 4-acetoxy-A on DMBA-induced mammary tumors in the rat

	Treatment	rats	Tumors: Before treatment No.	Max. produced No.	Completely regressed No.	%	Regressed to <0.02 cc No.	%	Signif.* regressed No.	%	Growth No.	%	All others† No.	%	Total regressions No.	%
1.	Control	6	19	19	1	5	2	9	1	5	10	46	8	36	4	18
	4-acetoxy-A	5	19	19	0	0	11	58	3	16	2	11	3	16	14	74
	Testosterone	6	21	22	4	18	4	18	10	45	2	9	2	9	18	82
2.	Control	6	17	25	0	0	2	9	4	16	5	20	10	40	6	24
	4-acetoxy-A	6	17	17	5	29	6	35	6	35	0	0	0	0	17	100
3.	4-acetoxy-A	7	23	28‡	18	64	4	14	5	18	0	0	1	4	27	96
	4-acetoxy-A $+E_2$	6	15	21	2	10	1	5	7	33	5	24	6	29	10	48

After tumors were induced with DMBA (see Methods) rats were: (1) treated twice daily with s.c. injections 5 mg/100 g/day of steroid in Steroid Suspending Vehicle for 4 weeks, control rats received only vehicle; (2) implanted with silastic wafers (150 mg 4-acetoxy-A + 0.5 g silastic) and injected s.c. twice daily with 1.25 mg/100 g/day 4-acetoxy-A; (3) implanted with wafers as in (2) and injected s.c. twice daily with 2.5 mg/100 g/day 4-acetoxy-A. In addition one group received 25 μg estradiol in the implant and after the second week 0.2 μg E_2/rat/day was also injected.

* Tumors regressed to less than one-half their pretreatment volume.

† Tumors showed less than 50% change in volume.

‡ Five new tumors developed in the first week of treatment; 4 regressed completely and 1 regressed to <0.02 cc.

steroid in Steroid Suspending Vehicle–Klucel* (5:1). The animals were divided into groups of 10 and each group received one of the following treatments: vehicle only, 0.6 mg, 1.2 mg, 2.4 mg total dose of testosterone or 6 mg, 12 mg, 24 mg, 48 mg total dose of 4-acetoxy-A. On the tenth day the animals were autopsied and seminal vesicles and ventral prostates weighed. All animals were weighed before and at the end of treatment.

Competition of 4-acetoxy-A for estrogen binding sites

To tubes containing [2,4,6,7 ^{3}H]-estradiol (1 Ci/mmol; approx. 40,000 d.p.m./tube) were added different concentrations (1×10^{-9} M to 1×10^{-5} M) of unlabelled 4-acetoxy-A followed by aliquots of cytosol prepared from uteri of rats in proestrous [16]. Rat uteri rather than mammary tissue were used as the former are richer in estrogen binding protein. The tubes were incubated overnight at 4°C, then dextran-coated charcoal suspension was added to each tube to absorb unbound steroid. An aliquot of supernatant after centrifugation was counted for radioactivity. For comparison, similar incubations were carried out with testosterone, with the antiestrogen Tamoxifen and with diethylstilbesterol (DES).

Induction of tumors. Female rats, 50–55 days old, were anesthetized with ether and 20 mg DMBA (Sigma Corp.) in 2 ml peanut oil was administered to each by gavage. After about 6 weeks the rats were inspected periodically for tumors as described previously [2]. The tumors were measured in 2 dimensions with a caliper and the volume of each tumor calculated ($v = 4/3\,\pi r_1^2 r_2$ where r_1 is the minor radius [17]). Approximately 80% of the tumors produced by this method are dependent on hormones for induction and growth [18, 19].

Treatment with 4-acetoxy-A (injection). Animals with at least one tumor of 2 cm in diameter, were grouped so that the number of rats, the number of tumors and the total tumor volume in each group were as similar as possible at the beginning of the experiment. The animals were injected s.c. twice daily for 4 weeks with 4-acetoxy-A (5 mg/100 g b.w./day) in Steroid Suspending Vehicle (vehicle distributed by the Cancer Chemotherapy Program, National Cancer Institute, NIH). Control rats were injected on the same schedule with vehicle only (Table 1, No. 1). Vaginal smears were taken daily.

On the last day of treatment 3 h after the last injection of 4-acetoxy-A, the rats were anesthetized with Nembutal, the uterine veins ligated and the ovarian veins cannulated [20]. Blood was collected for 10 min or to 1 ml from the ovarian vein. Steroid secretion per 10 min was calculated from the concentration × blood flow (Table 2). As the 4-acetoxy-A treated rats were anestrous throughout treatment, control animals were cannulated on diestrus 1 of their cycle. Peripheral blood was collected by heart puncture from each animal after sampling ovarian vein blood.

Treatment with testosterone. To compare the effect of testosterone on rat mammary tumor regression, groups of rats were injected s.c. twice daily for 4 weeks with various doses of testosterone propionate in sesame oil (Fig. 1). Six rats with a total of 18 tumors received 50 μg/100 g/day, 3 rats with 16 tumors received 125 μg/100 g/day and 2 rats with 8 tumors received 250 μg/100 g/day. In addition, a

* Steroid Suspending Vehicle: 9 mg sodium chloride, 5 mg sodium carboxymethyl cellulose-7 LP., 0.004 ml polysorbate 80, 0.009 ml benzyl alcohol, 1 ml water. Klucel: 9 mg sodium chloride, 3 mg hydroxypropyl cellulose, 1 ml water.

Table 2. The effect of 4-acetoxy-A on steroid secretion rates and gonadotropin levels in DMBA-induced tumor bearing rats after 4 weeks of treatment

†Dose (mg/100 g/day)	Estrone (pg/10 min)	Estradiol (pg/10 min)	Progesterone (ng/10 min)	LH (ng/ml)	FSH (ng/ml)	Prolactin (ng/ml)
0	159 ± 38	*167 ± 30	‡15.5 ± 5.5	37.6 ± 0.6	131 ± 0.7	83 ± 3.3
50	83 ± 18	*47 ± 17	5.5 ± 2.0	32.0 ± 6.0	120 ± 12	71 ± 17.1

* Significantly different ($P < 0.05$).

† Five rats in each group were injected sc twice daily. Steroid hormones were measured in ovarian venous plasma collected 3 h after the last injection of 4-acetoxy-A. Blood from control animals was collected on diestrus I. Gonodotrophins were assayed in peripheral plasma taken immediately following the ovarian collection. Results reported as mean ± SE.

‡ Value from 4 rats.

group of 6 rats with 21 tumors received 5 mg/100 g/day testosterone.

Treatment with 4-acetoxy-A (silastic wafer plus injection). A silastic wafer for each rat was prepared by mixing 0.5 g elastomer (282, medical grade, Dow Corning), pulverized steroid(s) (where applicable, see below) and a drop of stannous octoate polymerizing catalyst, then allowing the mix to harden under a weighed glass plate to ensure uniform diameter and thickness. The silastic wafer was divided into 4 pieces and each was placed under the dorsal skin of the neck. The wafers were replaced once a week. After

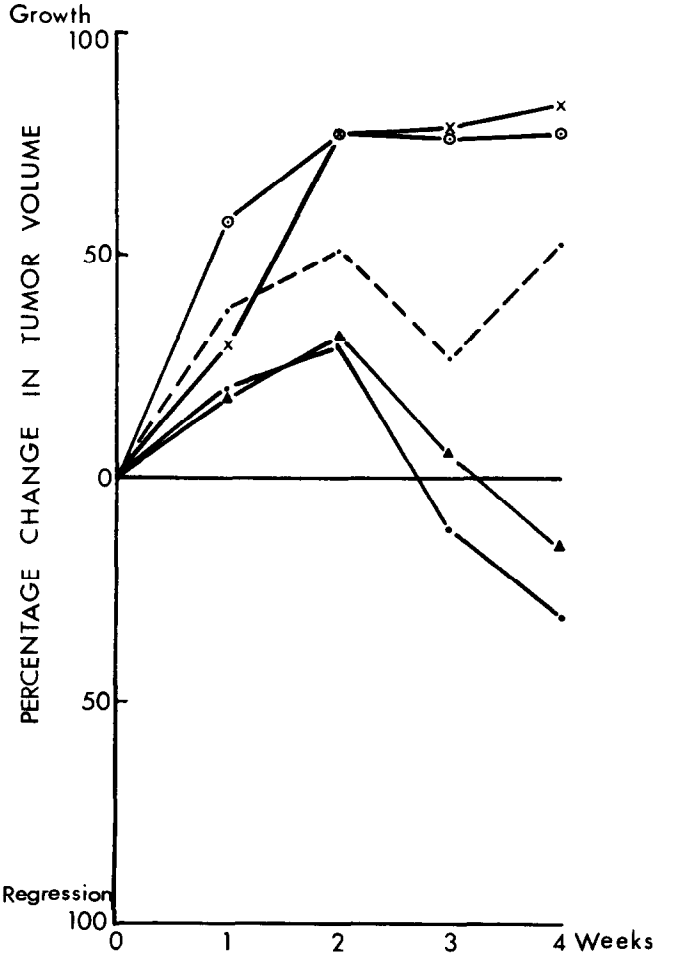

Fig. 1. The effect of testosterone propionate on the mean tumor volume of DMBA-induced rat mammary tumors. Groups of rats were administered testosterone propionate in sesame oil by s.c. injection twice daily. Six rats with 18 tumors received 20 μg/100 g/day (×——×); five rats with 14 tumors received 50 μg/100 g/day (⊙——⊙); 3 rats with 16 tumors received 125 μg/100 g/day (▲——▲); 2 rats with 8 tumors received 250 μg/100 g/day (●——●). Seven control rats with 24 tumors were treated with vehicle (-----) (sesame oil) only on the same schedule.

the wafers were removed, they were extracted with ether in a Soxhlet extractor for 3 h. The ether was then evaporated and the 4-acetoxy-A diluted with methanol and the concentration determined from the U.V. absorption. Two rats were treated with wafers prepared as above but with [6,7 ^{3}H]-4-acetoxy-androstenedione in addition. These wafers were removed and extracted as above and the amount of 4-acetoxy-A released quantitated from the radioactivity.

Rats with at least one tumor 2 cm in diameter were divided into 2 groups as described above. One group received wafers containing 4-acetoxy-A (150 mg) and in addition, were injected s.c. twice daily with 4-acetoxy-A (1.25 mg/100 g/day) in Steroid Suspending Vehicle–Klucel (5:1). The control group received wafers without 4-acetoxy-A and were injected with vehicle (Table 1, No. 2).

In another experiment (Table 1, No. 3) rats in group 1 received wafers of 4-acetoxy-A (150 mg) and twice daily injections of 4-acetoxy-A (2.5 mg/100 g/day). Rats in group 2 were treated as group 1 except that wafers contained 25 μg estradiol in addition to 4-acetoxy-A. After the second week, group 2 also received s.c. injections of estradiol (0.2 μg/rat/day) in sesame oil.

Body weight. All animals were weighed at the beginning of the experiments and each week thereafter. For comparison, a group of ovariectomized rats, a group of rats that was treated with DMBA but did not develop tumors and a group treated with testosterone, were also weighed weekly.

RESULTS

Hormonal activity of 4-acetoxy-A

Seminal vesicle weights for groups of rats treated with doses of 6 and 12 mg 4-acetoxy-A were indistinguishable from control values. At doses of 24 and 48 mg there was a minimal increase in seminal vesicle weight. Slightly more weight change was seen in the ventral prostate. From the dose response curve obtained the androgenic activity of 4-acetoxy-A was calculated to be 1.1% that of testosterone.

Competition of 4-acetoxy-A for estrogen binding sites

4-Acetoxy-A exhibited no competition for estrogen binding sites even at the highest concentrations tested. Using the maximum dose level (10^{-5} M) the relative binding potency [21] of testosterone compared to estradiol was only 5% whereas for the antiestrogen it was 80% and more than 100% for DES.

Treatment of tumor-bearing rats with 4-acetoxy-A (injections)

Injections of 4-acetoxy-A were effective in bringing about regression of 74% of tumors to less than half the original size at the end of 4 weeks treatment (Table 1, No. 1). This compared with regression to a similar extent in 18% of tumors in the control group. The percentage of growing tumors and tumors with "variable" growth patterns were also greater in the control groups compared to treated (82% vs 27%, respectively).

The effect of 4-acetoxy-A on hormonal levels in tumor bearing rats

After 4 weeks of treatment, the mean concentration of estradiol in ovarian venous blood was significantly lower ($P < 0.05$) in 4-acetoxy-A treated animals than in controls (Table 2). The mean value for estrone appeared lower than controls, but this was not statistically significant. Progesterone values also tended to be low in treated animals but the difference was not significant, however there was a large variation in values among control animals.

Treatment of tumor-bearing rats with 4-acetoxy-A (silastic wafers and injections

Wafers were estimated to release 7 mg 4-acetoxy-A per day. Thus, the total amount of 4-acetoxy-A administered in this experiment from the wafer plus injections was 10.8 mg/300 g rat/day, less than given by injections alone. However, the wafer method was more effective in causing tumor regression than 4-acetoxy-A injections alone. All tumors responded to treatment by regressing to less than half their original size; 29% regressed completely and 35% regressed to less than 0.02 cc. In comparison, a total of 24% tumors regressed in the control group, none completely and 40% more than doubled in size (Table 1, experiment 2).

The effect of 4-acetoxy-A plus estradiol on mammary tumors

In order to gain further evidence that tumor regression observed with 4-acetoxy-A, was due to suppression of estradiol, one group of rats was treated with silastic wafers containing 4-acetoxy-A alone and one group with 4-acetoxy-A plus estradiol (25 μg) per wafer (Table 1, No. 3). There was marginal tumor regression during the first 2 weeks. From the second week these animals were injected with an additional 0.2 μg estradiol/rat/day in sesame oil. By the fourth week of treatment 24% of tumors had more than doubled in size, although 48% regressed (Table 1, No. 3). There was a significant increase ($P < 0.05$) in mean tumor volume of the estrogen treated rats compared to the group of rats treated with 4-acetoxy-A alone (Fig. 1). As observed previously, marked tumor regression occurred in all the rats receiving wafers and injections of only 4-acetoxy-A. In this experiment the injected dose was twice as great as in experiment 2 (Table 1). Thus in 4 weeks 64% (18/28) tumors regressed completely and 14% regressed to such an extent that they could no longer be measured accurately but could only be felt as a thickening of the skin. In total 96% of tumors responded to treatment by regressing to less than half their original size; only one tumor did not respond and it had a variable

growth pattern. In one animal 5 new tumors appeared during the first week of treatment; 4/5 regressed completely, one regressed to less than 0.02 cc.

Treatment of tumor-bearing rats with testosterone

Doses of 20 μg and 50 μg/100 g/day testosterone propionate (TP) did not cause mammary tumor regression but resulted in rather greater increase in tumor volume than controls. Treatment with 125 and 250 μg/100 g/day TP resulted in tumor growth during the first 2 weeks. This was followed by a period of tumor regression. However, the tumor volumes were not significantly different before and after treatment (Fig. 1). Treatment with 5 mg/100 g/day testosterone for 4 weeks was less effective than 4-acetoxy-A in causing mammary tumor regression. Thus, a smaller proportion of tumors regressed to less than 0.02 cc with testosterone (Table 1). Also the percentage decrease in mean tumor volume was less with testosterone than with 4-acetoxy-A (Fig. 2).

Vaginal cytology

Daily vaginal smears indicated that the rats remained in diestrous throughout treatment with 4-Ac-A. This observation is consistent with reduced estradiol production (Table 2). Control animals cycled, although they tended to be slightly less regular than healthy animals.

Body weights

Mean body weights before and at the end of treatment are shown in Table 3. Tumor regression was not correlated with loss of weight. Animals treated with 4-acetoxy-A injections increased in weight by 16%, also rats treated with wafers increased in weight by 8%. Controls which had tumors, as well as DMBA-treated rats which did not develop tumors, maintained essentially a steady weight. Tumor-bearing rats treated with testosterone had a slight but not statistically significant increase in body weight. After ovariectomy, there was an increase in weight

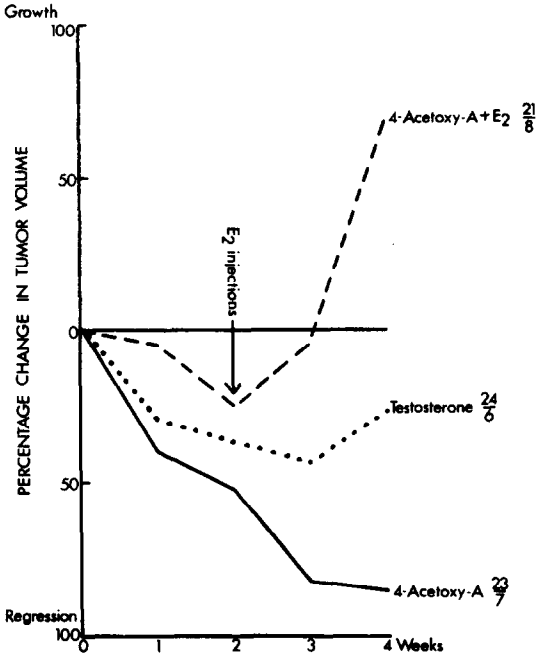

Fig. 2. The effect of 4-acetoxy-androstene-3,17-dione (4-acetoxy-A) and of 4-acetoxy-androstene-3,17-dione + estradiol (E_2) on mean tumor volume of DMBA-induced rat mammary tumors. 4-Acetoxy-A (——) was administered to 7 rats with 23 tumors by silastic wafer (150 mg/0.5 g silastic) and injection (2.5 mg/100 g rat/day) s.c. twice daily. In 8 rats with 21 tumors treated with estradiol, this steroid (25 μg) was incorporated into the wafer with 4-acetoxy-A (----) and after the second week injections of estradiol (0.2 μg/rat/day) were given. For comparison, a group of 6 rats with 24 tumors (· · · · ·) was treated with testosterone only (5 mg/100 g/day) as s.c. injections daily.

Table 3. Mean body weight (g) of rats with DMBA-induced mammary tumors: the effect of 4 weeks of treatment with 4-acetoxy-A

Rats	No. rats	Weight before treatment	Weight after treatment	Weight change (%)
1. No. tumors	9	308 ± 9	313 ± 9	+1.6
2. Ovx.	3	262 ± 6	303 ± 4	+15.6*
3. Controls	12	313 ± 7	316 ± 8	+1.0
4. 4-acetoxy-A (inj.)	11	315 ± 8	366 ± 11	+16.2*
5. 4-acetoxy-A (wafer)	4	299 ± 12	323 ± 13	+8.0*
6. 4-acetoxy-A + E_2	6	285 ± 8	283 ± 13	−0.7
7. Testosterone	6	300 ± 14	320 ± 14	+6.7

Mean ± S.E.
1. Rats were gavaged with DMBA but did not develop tumors, received no treatment.
2. Rats with tumors were ovariectomized, received no treatment.
3. Rats with tumors received vehicle injections s.c. twice daily.
4. Rats with tumors received 4-acetoxy-A (5 mg/100 g/day) injections s.c. twice daily.
5. Rats with tumors received 4-acetoxy-A (150 mg/0.5 g) wafer +2.5 mg/100 g injections s.c. twice daily.
6. As above but with 25 μg estradiol in wafer and 0.2 μg estradiol injected s.c. daily.
7. Rats with tumors received testosterone (5 mg/100 g/day) injections s.c. twice daily.
* Increase was significant ($P < 0.05$).

similar to that with 4-acetoxy-A treatment (Table 3). When rats were treated with wafers containing 4-acetoxy-A plus estradiol, there was no weight increase.

DISCUSSION

Since 4-hydroxy-A had been shown to be a good aromatase inhibitor *in vitro* and to control estrogen-dependent processes *in vivo* including growth of hormone dependent tumors [2], we are comparing its properties to several derivatives including the corresponding 4-acetoxy compound. 4-Acetoxy-A is an effective inhibitor of estrogen biosynthesis *in vitro* [11]. Although it was less effective than 4-OH-A in causing complete regression of mammary tumors in rats when injected twice daily in a suspending vehicle, greater efficacy was obtained with 4-acetoxy-A when administered from silastic wafers. Similarly in previous reproductive studies we noted that when administered from silastic wafer the efficacy of 4-acetoxy-A improves [11]. If 4-acetoxy-A acts as an aromatase inhibitor *in vivo*, it would probably depend on competing with the natural substrate, 4-androstene-3,17-dione or testosterone, at the enzyme site. Hence, the wafer would be expected to provide a more constant supply of drug to the enzyme. 4-Acetoxy-A has the advantage of being rather more stable than 4-hydroxy-A and less affected by increases in temperature.

To understand more about the mechanism of action of 4-acetoxy-A, plasma samples from rats treated with 4-acetoxy-A for one month were analyzed for various hormones and the values compared to those of samples from similar rats which had been treated with vehicle for 1 month. Blood samples were taken from control rats during diestrus when estradiol levels are low. We found ovarian estradiol secretion in the treated rats was 28% of the mean control value. Reduced secretion was also observed at the time of the proestrous estrogen surge in normal cycling rats after 2 days of treatment with 4-acetoxy-A or 3 h of treatment with 4-hydroxy-A [2, 11]. The mean estrone secretion of treated rats was 52% of the controls but this was not statistically significant with the sample size used. Similarly, no decrease was found in estrone secretion with 4-OH-A treatment during proestrous [2]. One possibility for the lesser effect of the aromatase inhibitors on secretion of this weaker estrogen might be some inhibition of 17β-oxidoreductase as well as aromatase, thereby affecting the ratio of estrone to estradiol. A similar lack of effect on estrone conversion has also been observed by us when testing inhibitors *in vitro* with [^{3}H]-androstenedione as substrate. Progesterone values were not statistically different, although concentrations of this steroid tended to be lower in 4-acetoxy-A treated animals than in vehicle treated controls. However, in other studies (unpublished) reduced progesterone secretion has not been observed. Whether there is an effect of 4-acetoxy-A on earlier biosynthetic pathways is being investigated.

Although estradiol values were reduced by 4-acetoxy-A treatment, LH levels were not increased as would occur after ovariectomy. Levels of LH and FSH in the normal range could be advantageous in treating breast cancer since gonadotrophins appear to stimulate aromatase activity and might counteract the effectiveness of the inhibitor. We have found marked increase in ovarian aromatase from PMSG stimulated rats [22]. We are currently investigating whether aromatase inhibitors directly effect gonadotropins.

The mean prolactin level of rats treated with 4-acetoxy-A was slightly but not significantly less than that of control animals. These data indicate that tumor regression with 4-acetoxy-A may result primarily from reduced estrogen rather than from reduced prolactin secretion. Although it has been suggested that tumor growth in DMBA-induced rat mammary tumors is mainly prolactin dependent [23, 24], most studies have been carried out with high doses or stimulated levels of prolactin. Recently other studies indicate that estrogen may be required for prolactin to exert its effect [25–28].

When estradiol (25 μg) was incorporated into the wafer containing 4-acetoxy-A during the first two weeks of treatment, some tumor regression occurred although to a less extent than with 4-acetoxy-A alone. Possibly this dose of estradiol was insufficient or the release rate was inadequate to counteract the effect of 4-acetoxy-A on tumor regression. During the following 2 weeks when 0.2 μg/rat estradiol injections were also given, there was significant increase in tumor volume and number.

Androgens are well known to cause mammary tumor regression [22] so to determine whether the activity of 4-acetoxy-A *in vivo* might be due to its androgenicity rather than aromatase inhibition, we compared the effect of various doses of testosterone propionate. At the total dose of 4-acetoxy-A administered (15 mg/300 g rat/day, Table 1) the androgenic activity would be equivalent to 150 μg testosterone/rat. No tumor regression occurred with doses of 150 μg/rat/day TP or less, at 750 μg/rat there was minimal regression (Fig. 1), suggesting the 4-acetoxy-A probably does not act primarily by the same mechanism as testosterone to cause mammary tumor regression in the rat.

Tumor regression did not correlate with weight loss, in fact body weight increased with 4-acetoxy-A injections to about the same extent as occurred after ovariectomy. Increased weight following ovariectomy has been shown to be related to lack of estrogen [30]. A similar cause may be the reason for the increase in weight with 4-acetoxy-A treatment. Also, when estradiol was given in addition to 4-acetoxy-A, there was no change in weight.

These results taken together suggest that tumor regression by 4-acetoxy-A was probably due to reduced estradiol production. Since the compound has little androgenicity (1.1% of testosterone) and

does not interact with estrogen receptors, its mechanism of action *in vivo* seems likely to be mainly aromatase inhibition.

Acknowledgements—We thank Nancy De Mauro and Daniel Murray for their dedicated assistance; also David Watson, Charlene Franz and Carol Roberson for performing the radioimmunoassays.

REFERENCES

1. Schwarzel W. C., Kruggel W. and Brodie H. J.: Studies of the mechanism of estrogen biosynthesis. VIII. The development of inhibitors of the enzyme system in human placenta. *Endocrinology* **92** (1973) 866–880.
2. Brodie A. M. H., Schwarzel W. C., Shaikh A. A. and Brodie H. J.: The effect of an aromatase inhibitor, 4-hydroxy-4-androstene-3,17-dione, on estrogen-dependent processes in reproduction and breast cancer. *Endocrinology* **100** (1977) 1684–1695.
3. Longcope C., Pratt J. H., Schneider S. H. and Fineberg S. E.: Aromatization of androgens by muscle and adipose tissue *in vivo*. *J. Clin. Endocr. Metab.* **46** (1978) 146–152.
4. Hemsell D. L., Grodin M. M., Brenner P. F., Sitteri P. K. and MacDonald P. C.: Plasma precursors of estrogen II. Correlation of the extent of conversion of plasma androstenedione to estrone with age. *J. clin. Endoc. Metab.* **38** (1974) 476–479.
5. Miller W. R. and Forrest A. P. M.: Oestradiol synthesis by a human breast carcinoma. *Lancet* **2** (1974) 866–868.
6. de Thibault de Boesinghe L., Lacroix E., Eechaute W. and Leusen I.: Oestrogen synthesis by human breast carcinomas. *Lancet* **2** (1974) 1268.
7. Li K., Chandra D. P., Foo T., Adams G. B. and McDonald D.: Steroid metabolism by human mammary carcinoma *Steroids* **28** (1976) 561–574.
8. Schindler A. E.: Endometrium-karzinom und Extraglandulare Ostrogenbiosynthese. *Gerburtsh.u. Frauenheilk.* **37** (1977) 242–251).
9. Lipton A. and Santen R. J.: Medical andrenalectomy using aminoglutethimide and dexamethasone in advanced breast cancer. *Cancer* **33** (1974) 503–512.
10. Levin J. M., Goldman A. S., Rosato F. E. and Rosato E. E.: Therapy of dimethylbenzanthracene-induced mammary carcinomas in the rat by selective inhibition of steroidogensis. *Cancer* **38** (1976) 56–61.
11. Brodie A. M. H., Wu J-T., Marsh D. A. and Brodie H. J.: Aromatase inhibitors III. Studies on the antifertility effect of 4-acetoxy-4-androstene-3,17-dione. *Biol. Reprod.* **18** (1978) 365–370.
12. Joshi H. S. and Labhsetwar A. P.: The pattern of ovarian secretion of oestradiol and oestrone during pregnancy and the post-partum period in the hamster. *J. Reprod. Fert.* **31** (1972) 299–302.
13. Labhsetwar A. P., Joshi H. S. and Watson D. J.: Temporal relationship between estradiol, estrone and progesterone secretion in the ovarian venous blood and LH in the peripheral plasma of cyclic Hamsters. *Biol. Reprod.* **8** (1972) 321–326.
14. Thorneycroft J. H. and Stone S. C.: Radioimmunoassay of serum progesterone in women receiving oral-contraceptive steroids. *Contraception* **5** (1972) 129–149.
15. Odell W. G., Rayford T. L. and Ross G. T.: Simplified, partially automated method for radioimmunoassay of human thyroid stimulating, growth, luteinizing, and follicle stimulating hormones. *J. Lab. Clin. Med.* **70** (1977) 973–980.
16. McGuire W. L. and DeLaGaza M.: Improved sensitivity in the measurement of estrogen receptor in human breast cancer. *J. clin. Endocr. Metab.* **37** (1973) 986–989.
17. DeSombre E. R. and Arbogast L. Y.: Effect of the antiestrogen C1628 in the growth of rat mammary tumors. *Cancer Res.* **34** (1974) 1971–1976.
18. Huggins C., Briziarelli G. and Sutton H.: Rapid induction of mammary carcinoma in the rat and the influence of hormones on tumors. *J. Exp. Med.* **109** (1959) 25–42.
19. Huggins C., Grand L. C. and Brillantes F. P.: Mammary cancer induced by a single feeding of polynuclear hydrocarbons and its extinction. *Nature* **189** (1961) 204–207.
20. Shaikh A. A.: Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. *Biol. Reprod.* **5** (1971) 297–307.
21. Korenman S. G.: Radio-ligand binding assay of specific estrogens using soluble uterine macromolecules. *J. Clin. Endocr. Metab.* **28** (1968) 127–130.
22. Brodie A. M. H., Schwarzel W. C. and Brodie H. J.: Studies in the mechanism of estrogen biosynthesis in the rat ovary—I. *J. steroid Biochem.* **7** (1976) 787–793.
23. Pearson O. H., Llerena O., Llerena A., Molina A. and Butler T.: Prolactin-dependent rat mammary cancer—a model for man? *Trans. Assoc. Am. Physicians* **82** (1969) 225–238.
24. Nagasawa H. and Yanai R.: Effects of prolactin on growth hormone on growth of carcinogin-induced mammary tumors of adreno-ovariectomized rats. *Intern. J. Cancer* **6** (1970) 488–495.
25. Sinha D., Cooper D. and Dao T.: The nature of estrogen and prolactin effect on mammary tumorgenesis. *Cancer Res.* **33** (1973) 411–414.
26. Leung B. S. and Sasaki G. H.: On the mechanism of prolactin and estrogen action in 7,12-Dimethylbenz(a) anthracene-induced mammary carcinoma in the rat II. *In vivo* tumor responses and estrogen receptor. *Endocrinology* **97** (1975) 564–572.
27. Leung B. S., Sasaki G. H. and Leung J. S.: Estrogen-prolactin dependency in 7,12-dimethylbenz(a)anthracene-induced tumors. *Cancer Res.* **35** 621–627 (1975).
28. Clemens J. A., Welsch C. W. and Meites J.: Effects of hypothalamic lesions on incidence and growth of tumors in carcinogen-treated rats. *Proc. Soc. Exptl. Biol. Med.* **127** (1968) 969–972.
29. Segaloff A.: Hormones and breast cancer. *Recent Prog Hormone Res.* **22** (1966) 351–379.
30. Dubuc P. U. Effects of estradiol implants on body weight regulation in castrated and intact female rats. *Endocrinology* **95** (1974) 1733–1736.