# Biological Profile of Quingestanol Acetate (33977)

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The 3-enol ethers of $\Delta^4$-3-ketosteroids were introduced by Ercoli and Gardi in 1960 (2) and were shown to possess unusual biological properties (3, 9). One of these compounds, 17$\alpha$-ethynyl-19-nor-testosterone acetate 3-cyclopentyl enol ether[3] belongs to the norandrostane series of progestational steroids and was found to be a potent antiestrual and contraceptive agent in rats (5) and as effective as its parent ketone, 17$\alpha$-ethynyl-19-nor-testosterone acetate (ENTA) in suppressing pituitary gonadotropin hypersecretion (4). The present paper presents data on other biological properties of this orally active progestogen.

*Materials and Methods.* The steroids tested were: (I) 17$\alpha$-ethynyl-19-nor-testosterone (ENT); (II) 17$\alpha$-ethynyl-19-nor-testosterone-17$\beta$ acetate (ENTA); (III) 17$\alpha$-ethynyl-19-nor-testosterone-17$\beta$ acetate-3-cyclopentyl enol ether (ENTACP); (IV) 17$\alpha$-methyltestosterone (MT); (V) 17$\alpha$-ethynylestradiol (EE); (VI) 6$\alpha$-methyl-17$\alpha$-acetoxy progesterone (MAP); and (VII) progesterone.

All compounds administered were dissolved in sesame oil. Rats used throughout the experiments were Sprague-Dawley derivatives from Hemlock Hollow Farm, Wayne, N. J.

1. *Progestational activity.* The test was performed according to the method of Clauberg (1) as modified by McPhail (7). New Zealand immature female rabbits were injected subcutaneously once daily with estradiol-17$\beta$ (5 $\mu$g/animal/day) for 6 consecutive days. Beginning on the seventh day, ENTA or ENTACP were administered orally once daily for 5 consecutive days along with 1/10 original amount of estrogen. Autopsy was performed on day 12. Midsections of each uterine horn were removed, sectioned, and the degree of progestational activity was determined according to the McPhail scale (7).

2. *Pregnancy maintenance.* Female rats (200–250 g body wt) were caged overnight with fertile males. The following morning vaginal smears were examined for the presence of spermatozoa (day 1 of pregnancy). On day 9 the animals were bilaterally ovariectomized under ether anesthesia. Control animals were sham operated. Oral treatment with ENTA or ENTACP (1 or 4 mg/animal/day) with or without the concomitant administration of estradiol-17$\beta$ subcutaneously (1$\mu$g/animal/day) was begun immediately after ovariectomy and continued throughout day 20 of pregnancy. Laparotomy was performed on day 22 (13).

3. *Androgenic activity. A. Daily administration.* Male albino rats, 35–45 g body weight, were castrated under ether anesthesia. Oral treatment with the test compounds started on the day of castration and lasted for 7 consecutive days. Autopsy was performed on the morning of day 8 and the wet weights of ventral prostate and seminal vesicles (without coagulating gland and devoid of fluid) were determined to the nearest 0.1 mg.

*B. Single administration.* Experimental conditions were similar to those described in (A) except that a single oral dose of the test compounds was administered on the day of castration and autopsy and weight of the accessory organs determined at 1, 2, 3, and 7 days after administration.

4. *Masculinization of female fetuses.* A modification of the methods of Revesz *et al.* (10) and Sholer and De Wachter (11) was used. The test compounds were administered either orally or subcutaneously to pregnant rats from days 15 to and including 21 of pregnancy. On the day of birth the pups were weighed and their sex was determined by the presence of ovaries or testes. Degree of masculinization of female fetuses was deter-

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mined on the basis of ano–genital distance. If delivery was delayed, the fetuses were removed by cesarean section on day 23. For each compound and dose level the following parameters were considered: (a) total number of masculinized fetuses, (b) total number of mothers which delivered them, and (c) the number of masculinized females delivered by each mother. Ano–genital distances of $<2.0$ mm were considered normal for females, between 2.1 and 3.0 mm pseudohermaphroditic and $>3.1$ mm normal for males.

5. *Uterotrophic activity.* Immature female rats, 30–35 g body weight, received the test compounds once daily by oral route for 3 consecutive days. Autopsy was performed on day 4 and the wet weight of the uterus (after pressing out the interauterine fluid) determined to the nearest 0.1 mg.

6. *Effect on adrenal and adrenocortical function.* Male rats, 200–250 g body weight, were given the test compounds by oral route once daily for 14 consecutive days. Twenty-four hr after the last treatment, the animals were injected subcutaneously with 1 unit of Zn-ACTH and anesthetized with pentobarbital sodium 25 min later. Blood samples for corticosterone determination were obtained by heart puncture 60 min after ACTH injection. Plasma corticosterone levels were determined by the method of Van Der Vies *et al.* (14). The animals were thereafter killed with overdose of pentobarbital and the weight of the adrenals was determined.

7. *Effect on organs and body weights.* Male rats, 170–180 g body weight, were given the test compounds once daily by oral route at doses of 5 or 25 mg/kg for 14 consecutive days. Autopsy was performed on the day after the last administration and adrenals, hypophysis, thyroid, and thymus weights were determined. Initial and final body weights were also recorded.

8. *Effect on pregnancy.* Mature female rats were caged with fertile males between 1 and 3 a.m. in a reverse lighted room (dark from 6 a.m. to 6 p.m.; light from 6 p.m. to 6 a.m.) Copulation was determined either by the presence of the copulation plug and/or that of spermatozoa in the vaginal smear (day 0 of pregnancy). The test compounds were administered orally at doses of 0.5 or 5 mg/animal on postcoital days 1 to 4, 4, or 4 to 11. Autopsy was performed on day 12 after mating and the uteri were examined for presence of blastocyts and/or implantation sites.

TABLE I. Progestational Activity.

| Treatment | No. of animals | Total dose (mg/animal) | Route | McPhail score |
|---|---|---|---|---|
| Controls | 10 | — | — | 0 |
| ENTA | 8 | 0.5 | po | 0.06 |
| | 8 | 2.0 | po | 2.14 |
| ENTACP | 7 | 0.5 | po | 0.64 |
| | 8 | 2.0 | po | 3.37 |

9. *Storage in body fat.* Mature castrated male rats, 200–250 g body weight, were given a single oral dose of 5 mg of the test compounds on the morning of the third postoperative day. The animals were killed 24 hr later. Perirenal fat was excised, weighed, immediately transferred to a 7-ml Tenbroeck grinder containing approximately 4 ml of methylene chloride and processed according to the method of Meli *et al.* (8). Steroid content of the sample was determined fluorimetrically according to the method of Steinetz *et al.* (12).

*Results. 1. Progestational activity.* The data are shown in Table I. Statistical analysis of the results indicate that ENTACP is about 2 times as effective as its parent ketone, ENTA, in producing secretory changes of the endometrium in the estrogen-primed immature female rabbit.

*2. Pregnancy maintenance.* The data are shown in Table II. With the exception of one mother which received a daily dose of 4 mg of ENTACP and 1 $\mu$g of estradiol-17$\beta$ and which had 9 living fetuses and 3 resorptions, pregnancy was not maintained with any of the other treatments. However after 22 days, resorption sites were still visible in the ENTACP-treated animals, whereas none were visible in the ENTA groups.

*3. Androgenic activity. A. Daily administration.* The data are shown in Table III. Statistical analysis of the results indicates that all compounds tested significantly stimu-

TABLE II. Pregnancy Maintenance.

| Treatment | No. of animals | Daily dose (mg) | Estradiol-17β (μg) | No. of ip sites | No. of resorptions | No. of living fetuses |
|---|---|---|---|---|---|---|
| Controls | 5 | — | — | 52 | 1 | 51 |
| ENTACP | 5 | 1.0 | — | 36 | 36 | 0 |
| | 5 | 1.0 | 1.0 | 11 | 11 | 0 |
| | 3 | 4.0 | — | 32 | 32 | 0 |
| | 5 | 4.0 | 1.0 | 61 | 52 | 9 |
| ENTA | 5 | 1.0 | — | 0 | 0 | 0 |
| | 5 | 1.0 | 1.0 | 0 | 0 | 0 |
| | 5 | 4.0 | — | 0 | 0 | 0 |
| | 5 | 4.0 | 1.0 | 0 | 0 | 0 |

lated the accessory organs. Potency ratios are based on the calculated arithmetic average of potency estimates obtained with the two accessory organs for each compound. ENT is 5.7 times as effective as ENTA. ENTACP is approximately 1.5 times as effective as ENT and about ⅓ as effective as MT.

*B. Single administration.* The data are shown in Fig. 1. ENT, ENTA, and ENTACP were less effective than MT in stimulating the accessory organs. The rate of the decline in weight of the accessory organs following the single oral administration of ENT, ENTA, and ENTACP is faster as compared with that of MT indicating that none of the 19-nor-testosterone derivatives possesses prolonged biological activity. In fact, by day 7 the weights of the accessory organs of ENT-, ENTA-, or ENTACP-treated animals were only slightly different from those of controls.

4. *Masculinization of female fetuses.* The data are shown in Table IV. With the exception of progesterone, all of the other progestational agents caused masculinization of the female fetuses. A parallel, dose-dependent effect was observed in all of the 3 parameters under consideration following the oral administration of ENT, ENTA, ENTACP, and MAP. The daily dose of 0.9 mg of ENT, ENTA, and ENTACP exhibited similar masculinizing properties, whereas MAP was more effective. As far as total number of masculinized fetuses, our data for ENT, MAP, and progesterone are in agreement with those of Scholer and DeWachter (11). ENTACP did

TABLE III. Androgenic Activity.

| Treatment | No. of animals | Daily dose (μg/animal) | Body wt (mg/100 g) | |
|---|---|---|---|---|
| | | | Seminal vesicles | Ventral prostate |
| Controls | 20 | — | 6.9 ± 0.5 | 20.2 ± 2.2 |
| ENT | 10 | 200 | 14.4 ± 0.8 | 34.2 ± 2.4 |
| | 10 | 400 | 14.6 ± 0.9 | 36.9 ± 2.6 |
| | 10 | 800 | 20.9 ± 1.4 | 42.9 ± 3.8 |
| ENTA | 10 | 200 | 8.8 ± 0.6 | 24.2 ± 1.6 |
| | 10 | 400 | 10.5 ± 0.7 | 26.5 ± 1.6 |
| | 10 | 800 | 13.2 ± 0.5 | 30.8 ± 2.2 |
| ENTACP | 20 | 200 | 12.1 ± 1.0 | 33.5 ± 1.7 |
| | 20 | 400 | 16.8 ± 0.6 | 42.8 ± 1.8 |
| | 20 | 800 | 25.1 ± 1.6 | 57.6 ± 2.5 |
| MT | 10 | 200 | 14.0 ± 1.1 | 59.3 ± 3.2 |
| | 10 | 400 | 19.4 ± 1.5 | 72.8 ± 6.0 |
| | 10 | 800 | 24.7 ± 2.3 | 84.5 ± 5.4 |

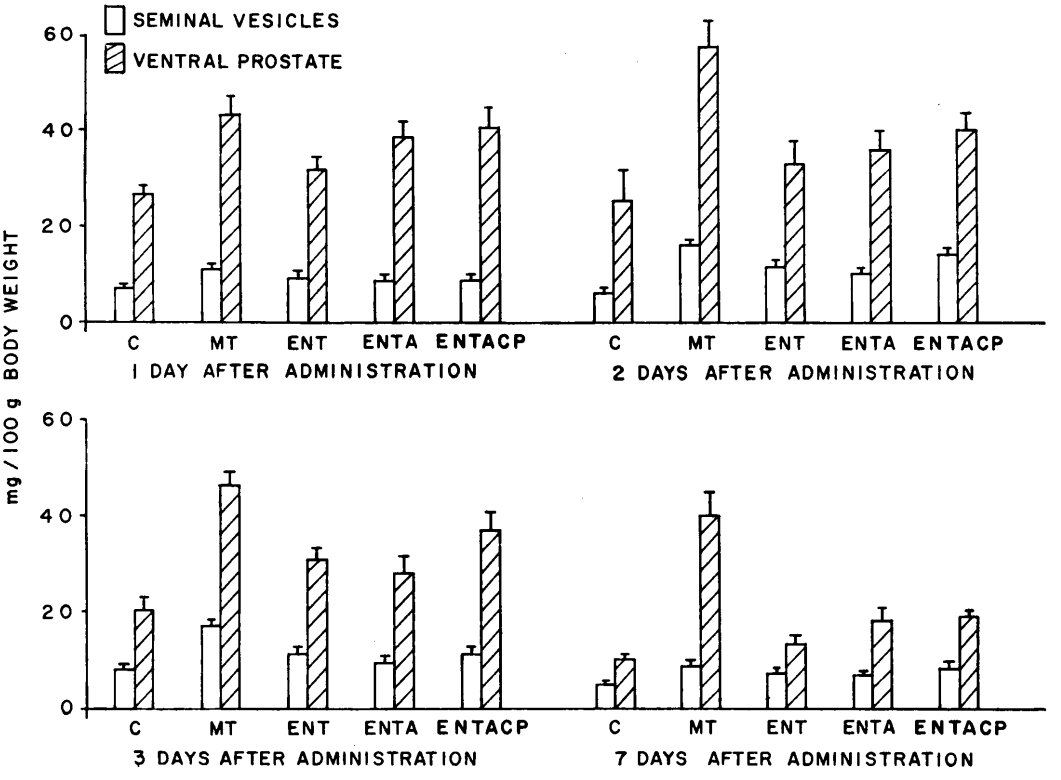


FIG. 1. Rate of accessory organ weight decline following single oral administration.

not delay delivery or interfere with parturition. Resorption of fetuses occurred more frequently in rats treated with ENTACP than with the other compounds tested.

5. *Uterotrophic activity.* The data are shown in Table V. Statistical analysis of the results indicates that all of the compounds tested significantly stimulated uterine growth. ENTA was 2.3 times as effective as ENT. Both compounds, however, behaved like impeded estrogens (shallow dose–response curve slope). ENTACP, on the other hand, behaved like an estrogen (steep dose–response curve comparable to that of EE). ENTACP was approximately 2 times as effective as ENTA and 1/200 as effective as EE.

6. *Effect on adrenal and adrenocortical function.* The data are shown in Table VI. Both ENT and ENTACP significantly increased adrenal weight as compared to controls, the latter being more effective than the former. Neither compound affected the adrenal response to ACTH. However when data were calculated to take into consideration the differences in adrenal weight, both ENT and ENTACP had significantly reduced the capacity of the adrenal cortical tissue present to respond to ACTH.

7. *Effect on body and organ weights. A. Body weight.* The data are shown in Table VII. Compared to controls, a significant reduction in body weight gain occurred in all tested groups which was proportional to the dose administered; ENTACP $\cong$ ENT $>$ ENTA.

*B. Organ weights. 1. Weights expressed on an absolute value basis.* The data are shown in Table VII. With the exception of the lower dose of ENTA, there was a significant increase in adrenal weight with all compounds at all doses. A significant increase in the weight of the hypophysis was observed at the lower dose of ENTA and both doses of ENTACP. A significant increase in thyroid weight was noted only at the higher dose of ENTA. With the exception of the lower dose

TABLE IV. Masculinizing Properties of Some Progestogens.

| Treatment | Daily dose (mg) | Route | No. of mothers | No. of impl. sites | No. of resorptions | No. of fetuses[a] | | | | | Av wt of fetuses (g) | Ano–genital distance (mm) | | | | | | No. of mothers with following no. of masculinized fetuses | | | | | | Remarks[b] | Fetal masculinization (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Spontaneous delivery | | | Cesarean delivery | | | 2.0 | | 2.1–3.0 | | 3.1 | | | | | | | | | |
| | | | | | | L | D | E | L | D | | Ov | Test | Ov | Test | Ov | Test | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Controls | — | — | 30 | 406 | 21 | 371 | 12 | 2 | — | — | 5.4 | 208 | — | 1 | — | — | 174 | 1 | — | — | — | — | — | | 0 |
| P | 10 | sc | 10 | 129 | 2 | — | — | — | 119 | 8 | 5.8 | 67 | — | — | — | — | 60 | — | — | — | — | — | — | | 0 |
| | 30 | sc | 10 | 140 | 6 | — | — | — | 134 | — | 5.4 | 67 | — | — | — | — | 67 | — | — | — | — | — | — | | 0 |
| ENTA | 0.1 | po | 10 | 137 | 20 | 114 | 3 | — | — | — | 5.3 | 64 | — | — | — | — | 53 | — | — | — | — | — | — | | 0 |
| | 0.3 | po | 10 | 142 | 11 | 127 | 4 | — | — | — | 5.4 | 68 | — | 5 | — | — | 58 | 3 | 1 | — | — | — | — | | 3.8 |
| | 0.9 | po | 10 | 132 | 7 | 91 | 15 | 3 | 16 | — | 5.1 | 48 | — | 15 | — | — | 57 | 3 | 2 | — | 2 | — | — | 2 d | 12.1 |
| ENT | 0.1 | po | 10 | 132 | 9 | 106 | 4 | — | 10 | 3 | 5.3 | 65 | — | 1 | — | — | 57 | 1 | — | — | — | — | — | | 0.8 |
| | 0.3 | po | 10 | 130 | 7 | 91 | 7 | — | 23 | 2 | 5.2 | 51 | — | 4 | — | — | 68 | 2 | 1 | — | — | — | — | | 3.2 |
| | 0.9 | po | 10 | 125 | 21 | 72 | 11 | — | 21 | — | 5.1 | 41 | — | 19 | — | — | 44 | 4 | 3 | — | 1 | 1 | — | | 18.3 |
| MAP | 0.1 | po | 10 | 134 | 4 | 103 | 21 | 1 | — | 5 | 5.2 | 60 | — | — | — | — | 56 | — | — | — | — | — | — | 13 d | 0 |
| | 0.3 | po | 10 | 142 | 14 | 103 | 13 | 1 | 11 | — | 5.4 | 53 | — | 14 | — | — | 60 | 6 | 1 | 2 | — | — | — | | 10.1 |
| | 0.9 | po | 10 | 125 | 6 | 82 | 16 | 1 | 14 | 6 | 4.9 | 31 | — | 36 | — | — | 49 | — | 3 | 2 | 2 | 2 | 1 | 2 d | 30.8 |
| ENTACP | 0.1 | po | 9 | 124 | 7 | 103 | 14 | — | — | — | 5.4 | 39 | — | 10 | — | — | 67 | 3 | 2 | 1 | — | — | — | 1 d | 8.6 |
| | 0.3 | po | 6 | 91 | 22 | 69 | — | — | — | — | 5.1 | 14 | — | 9 | — | — | 46 | 2 | — | 1 | 1 | — | — | 1 m | 13.0 |
| | 0.9 | po | 9 | 113 | 18 | 76 | 10 | 9 | — | — | 5.5 | 23 | — | 14 | — | — | 42 | 1 | 2 | 1 | — | — | 1 | 7 d<br>2 m | 17.7 |

[a] L = living; D = dead; E = eaten.

[b] d = dead pup(s) not measured; m = mother(s) had complete resorption.

TABLE V. Uterotropic Activity.

| Treatment | No. of animals | Daily dose ($\mu$g/animal) | Uterine wt (mg) |
|---|---|---|---|
| Controls | 10 | — | 13.8 ± 0.9 |
| ENT | 10 | 20.00 | 26.2 ± 1.7 |
| | 10 | 60.00 | 34.3 ± 3.8 |
| | 10 | 180.00 | 39.4 ± 2.4 |
| ENTA | 10 | 20.00 | 28.3 ± 2.6 |
| | 10 | 60.00 | 45.3 ± 2.3 |
| | 10 | 180.00 | 42.0 ± 2.3 |
| ENTACP | 10 | 20.00 | 25.3 ± 1.4 |
| | 10 | 60.00 | 52.7 ± 3.1 |
| | 10 | 180.00 | 60.8 ± 2.4 |
| EE | 20 | 0.05 | 46.5 ± 2.5 |
| | 20 | 0.15 | 63.8 ± 2.1 |
| | 20 | 0.45 | 75.4 ± 1.4 |

of ENTA, the weight of the thymus was significantly decreased, this effect being proportional to the dose administered. ENTACP > ENT > ENTA.

2. *Weights expressed in mg/100 g of body weight.* The data are shown in Table VII. With the exception of the lower dose of ENTA the weight of the adrenals was significantly increased. A significant increase in the weight of the hypophysis was observed at all doses and with all compounds. Thyroid weight was significantly increased at the higher dose of ENT and both doses of ENTACP. With the exception of the lower dose of ENTA, the weight of the thymus was significantly decreased (ENTACP > ENT > ENTA). This effect was proportional to the dose given for both ENTA and ENTACP but not ENT.

8. *Effect on pregnancy.* The data are shown in Table VIII. The oral administration of ENTA or ENTACP at the daily dose of 0.5 mg/animal from day 1 to 11 after mating did not prevent pregnancy.

Both ENTA and ENTACP at the oral daily dose of 5 mg/animal administered during the preimplantation period interfered with the nidation processes. When administered on the day of implantation (day 4 after mating) or for the following 7 days, approximately 50% of the ENTACP-treated animals had implantations, all of which were in the process of being reabsorbed. When administered only on day 4 of pregnancy, ENTA did not interfere with nidation but when treatment was extended up to and including day 11 of pregnancy implantation was prevented in all of the animals.

9. *Storage in body fat.* Oral administration of ENTA or ENTACP was not followed by storage of steroid material in body fat: At 24 hr after single oral administration of 5 mg/animal of ENTA or ENTACP, the average steroid concentration in groups of two castrated male rats was 0 $\mu$g of steroid/total amount of fat/animal.

*Discussion.* Most of the biological properties of 17$\alpha$-ethynyl-19-nor-testosterone-17$\beta$ acetate (ENTA) were greatly potentiated by 3-enol etherification with cyclopentyl alcohol. The ENTACP is approximately twice as active as its parent ketone (ENTA) in bringing about secretory changes of the endometrium in estrogen primed immature rabbits. ENTA did not maintain pregnancy in ovariectomized rats whereas treatment with ENTACP resulted in a partial maintenance of pregnancy in one animal. Furthermore, unlike

TABLE VI. Influence of ENT and ENTACP on Adrenal Weight and Plasma Corticosteroid Levels after ACTH Injection.

| Treatment | No. of animals | Daily dose (mg/animal) | Route | Adrenal wt (mg/100 g of body wt) | Plasma free corticosteroids after ACTH ($\mu$g/100 ml) | Plasma free corticosteroids after ACTH: Activity in relation to adrenal wt (%) |
|---|---|---|---|---|---|---|
| Controls | 17 | — | — | 13.4 ± 0.49 | 43.1 ± 1.4 | 100 |
| ENT | 6 | 5.0 | po | 19.9 ± 1.50 | 37.0 ± 3.0 | 58 |
| Controls | 6 | — | — | 14.1 ± 0.55 | 44.0 ± 2.1 | 100 |
| ENTACP | 6 | 5.0 | po | 26.1 ± 0.95 | 41.0 ± 1.6 | 50 |

TABLE VII. Effects of Oral Administration (14 days) of ENT, ENTA, or ENTACP on Organ and Body Weights in the Male Rat.

| Treatment | Daily dose (mg) | No. of rats (I/F) | Body wt change (g) | Organ wt (absolute and relative values) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Adrenals | | Hypophysis | | Thyroid | | Thymus | |
| | | | | (mg) | (mg/100 g) | (mg) | (mg/100 g) | (mg) | (mg/100 g) | (mg) | (mg/100 g) |
| Controls | — | 20/20 | 79 ± 2.3 | 33.6 ± 0.9 | 13.4 ± 0.5 | 8.4 ± 0.2 | 3.4 ± .09 | 17.8 ± 0.5 | 7.1 ± 0.3 | 385.3 ± 16.4 | 154.4 ± 8.1 |
| ENT | 5 | 20/20 | 39 ± 5.9[a] | 37.6 ± 1.6[a] | 18.2 ± 1.3[a] | 8.6 ± 0.2 | 4.1 ± 0.1[a] | 17.0 ± 0.8 | 8.0 ± 0.4 | 265.5 ± 21.3[a] | 121.6 ± 7.7[a] |
| | 25 | 20/20 | 18 ± 1.9[a] | 39.7 ± 1.4[a] | 20.4 ± 0.7[a] | 8.5 ± 0.1 | 4.3 ± 0.1[a] | 16.6 ± 0.6 | 8.5 ± 0.3[a] | 243.8 ± 14.3[a] | 124.4 ± 6.8[a] |
| ENTA | 5 | 20/20 | 62 ± 3.3[a] | 36.1 ± 1.4[a] | 15.0 ± 0.8 | 9.9 ± 0.2[a] | 4.1 ± 0.1[a] | 18.6 ± 0.6 | 7.7 ± 0.2 | 349.5 ± 18.8 | 144.2 ± 7.3 |
| | 25 | 20/20 | 38 ± 3.5[a] | 38.9 ± 1.3[a] | 18.1 ± 0.6[a] | 8.9 ± 0.3 | 4.2 ± 0.1[a] | 15.6 ± 0.4[a] | 7.2 ± 0.2 | 281.0 ± 10.7[a] | 131.3 ± 5.0[a] |
| ENTACP | 5 | 20/20 | 31 ± 2.7[a] | 39.9 ± 1.3[a] | 19.2 ± 0.5[a] | 9.4 ± 0.2[a] | 4.5 ± 0.1[a] | 18.7 ± 0.7 | 8.9 ± 0.3[a] | 251.3 ± 10.6[a] | 120.7 ± 6.4[a] |
| | 25 | 20/20 | 15 ± 3.2[a] | 40.1 ± 1.1[a] | 21.3 ± 0.4[a] | 9.2 ± 0.2[a] | 4.9 ± 0.1[a] | 16.3 ± 0.6 | 8.7 ± 0.3[a] | 120.2 ± 6.8[a] | 63.7 ± 3.4[a] |

[a] Significant $p < 0.05$.

ENTA, ENTACP-treated animals had still visible resorption sites at the time of autopsy. These findings suggest that pregnancy continued for a longer period in ENTACP than ENTA-treated animals. This effect might be attributable to the greater estrogenicity and androgenicity of ENTACP as compared with ENTA. Such properties have been suggested by Stucki (13) as being possibly necessary for pregnancy maintenance.

Despite structural similarities and common biological properties, the uterine growth-stimulating activity of ENT or ENTA was entirely different from that of ENTACP when dose–response curves were compared. In this test ENTACP behaved like a true estrogen (steep dose–response curve slope similar to that of EE) whereas ENT or ENTA acted like an impeded estrogen (*i.e.*, estriol), an androgen or a progestogen (shallow slope).

Similarly, differences in parallelism between ENT or ENTA (shallow slope) and ENTACP (steep slope similar to that of MT) were observed when seminal vesicles or ventral prostate growth-stimulating activities were compared. Although significantly more androgenic in this test, ENTACP produced the same degree of masculinization of female fetuses as ENT or ENTA. The three 19-nortestosterone derivatives were significantly less masculinizing than MAP which does not stimulate the growth of seminal vesicles or ventral prostate in immature castrated rats (6). These findings are in agreement with other data in the literature which likewise indicate that there is no direct relationship between androgenic and masculinizing properties (10, 11). Possibly, to more meaningfully apply laboratory data to humans, one should, in addition to the total number of masculinized fetuses, also consider the number of mothers delivering these pseudohermaphrodites as well as the number of masculinized fetuses delivered by each mother. Consideration of these parameters might aid the selection of synthetic progestogens to be used in threatened or habitual abortion.

On both an absolute and relative weight basis, ENTACP treatment produced the classical estrogenic effects of increasing adrenal

TABLE VIII. Fertility Studies.

| Treatment | Daily dose (mg/animal, po) | Treatment (days, pc) | (no.) Preg. rats (I/F) | ip sites | Living fetuses | Resorptions |
|---|---|---|---|---|---|---|
| Controls | — | — | 5/5 | 55 | 55 | 0 |
| ENTACP | 0.5 | 1,2,3,4 | 4/3 | 22 | 21 | 1 |
| | 0.5 | 4 | 5/5 | 50 | 50 | 0 |
| | 0.5 | 4–11 | 5/5 | 52 | 51 | 1 |
| | 5.0 | 1,2,3,4 | 5/0 | 0 | 0 | 0 |
| | 5.0 | 4 | 4/2 | 23 | 0 | 23 |
| | 5.0 | 4–11 | 5/3 | 33 | 0 | 33 |
| ENTA | 0.5 | 1,2,3,4 | 5/4 | 44 | 44 | 0 |
| | 0.5 | 4 | 5/5 | 45 | 44 | 1 |
| | 0.5 | 4–11 | 5/4 | 38 | 38 | 0 |
| | 5.0 | 1,2,3,4 | 5/0 | 0 | 0 | 0 |
| | 5.0 | 4 | 4/4 | 41 | 41 | 0 |
| | 5.0 | 4–11 | 5/0 | 0 | 0 | 0 |

weight while decreasing thymus weight and body weight gain. A significant increase in pituitary weight could be demonstrated only when the data were expressed on a relative basis. The ENT was as effective as ENTACP whereas ENTA was less active.

Adrenal response to ACTH (as measured by the total amount of plasma free corticosteroids following ACTH injections) was unaffected by either ENTA or ENTACP. However when the data were recalculated to take into account the differences in adrenal weight between treated and control animals, the two compounds had either reduced the capacity of the adrenocortical tissue present to respond to ACTH or the increase in adrenal size was not due to an increase in corticosteroid-producing tissue.

In spite of its significantly greater progestational, estrogenic and androgenic properties as compared with its parent ketone (ENTA), ENTACP has been reported to be as effective as the former in inhibiting pituitary gonadotropin hypersecretion in parabiotic rats (4). This effect is probably responsible for inhibition of ovulation and consequent contraceptive activity as demonstrated by Falconi and Ercoli (5) since in our experiments, post coital administration of a daily dose of 0.5 mg of either compound up to day 11 did not prevent implantation. This dose of ENTACP inhibited pregnancy when administered prior to copulation. A larger dose of either compound seems necessary to prevent pregnancy when administered after mating. The ENTACP is more effective than ENTA insofar as it interferes with pregnancy even when administered on day 4 after mating. Also, when treatment was begun at a late stage of pregnancy (day 15), ENTACP, unlike ENTA, caused partial to complete resorption of fetuses in some animals.

The postcoital antifertility properties demonstrated by ENTA or ENTACP could possibly be attributed to their ability to alter body estrogen levels. Such alterations could interfere with tubal transport, suppress the preimplantation estrogen surge on day 3 or antagonize the normal luteal function which is necessary for the successful continuation of pregnancy.

Unlike methyltestosterone-3-cyclopentyl enol ether (8), oral administration of ENTACP is not followed by storage of steroid material in body fat or prolongation of biological activity. It would, therefore, appear that other causes must be responsible for the greater biological activity brought about by 3-enol-etherification of ENTA.

*Summary.* Like other 3-enol ethers of $\Delta^4$-3-ketosteroids, 17$\alpha$-ethynyl-19-nor-testosterone acetate-3-cyclopentyl enol ether (EN

TACP) possesses unusual biological properties. ENTACP is approximately twice as active as its parent ketone (ENTA) in producing endometrial proliferation in the immature estrogen-primed rabbit. In stimulating uterine growth ENTACP behaves like a true estrogen whereas ENTA or 17$\alpha$-ethynyl-19-nor-testosterone (ENT) act as impeded estrogens, androgens, or progestogens, yielding a shallow dose–response curve. While ENTACP was significantly more androgenic than ENTA or ENT, the three compounds were equipotent in producing masculinization of female fetuses. Although adrenal hypertrophy was noted, neither ENT or ENTACP affected the corticoidogenic response of the adrenals to ACTH. However, when steroid output was corrected for differences in adrenal weight, both compounds either reduced the capacity of the adrenal cortical tissue to respond to ACTH or the increase in adrenal size was not due to an increase in corticosteroid producing tissue. When administered postcoitally, ENTACP was slightly more effective than ENTA in interfering with pregnancy. This may have been related to the inherent estrogenicity of each compound. Unlike methyltestosterone-3-cyclopentyl enol ether, orally administered ENTACP is not stored in body fat and does not have prolonged biological activity.

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Received Feb. 19, 1969. P.S.E.B.M., 1969, Vol. 131.