AY-11483, A NEW TYPE OF ORALLY ACTIVE OESTROGEN

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Summary. The oral oestrogenic activity of AY-11483 has been compared to that of oestriol and mestranol. The compound shows significant activity in the vaginal cornification test in rats and mice; however, it has a relatively weak effect in the uterotrophic assay in rats, mice and rabbits. Furthermore, like oestriol, it exerts a weak effect on the endometrium in rabbits. Based on our findings, AY-11483 should be classified as an impeded oestrogen with possible clinical application.

INTRODUCTION

The physiological importance of oestrogens and their effect on fertility are well known. A new class of oestrogens was described by Huggins & Jensen (1955) which they called "impeded estrogens". The characteristics of this type of compound are: a shallow dose–response curve in the uterotrophic assay and the inhibition of the uterine growth-promoting effect of oestrone. Oestriol is the prototype of the impeded oestrogens and is known to exert a weak effect on the uterus, both in animal experiments (Merill, 1958; Puck & Hübner, 1956; Overbeek & de Visser, 1958) and in the human (Puck & Hübner, 1957; Borglin, 1959). Furthermore, Haskins, Moszkowski & Whitelock (1968) reported that oestriol had a weak oestrogenic effect on vaginal cytology in humans. Since there are several states of oestrogen deficiency (e.g. menopause), there is clearly a need in the clinic for potent, orally active oestrogens which would exert only a weak stimulating effect on the uterus.

A series of seventeen substituted oestradiol derivatives was synthesized and tested (Lefebvre, Marshall, Revesz, Banik & Deghenghi, 1967) in our laboratories, of which 3-acetoxy-17α-[3-furyl]-1,3,5(10)-7-oestratetraen-17-ol (AY-11483) was investigated in detail. The contraceptive effect of this compound in rats was reported in a previous communication (Banik, Revesz & Herr, 1969). The purpose of this paper is to describe some oestrogenic parameters of AY-11483 and to compare its oral potency to mestranol and oestriol.

MATERIALS AND METHODS

Oestrogenic potency was determined in the vaginal cornification and uterotrophic assays in Sprague-Dawley rats and Swiss albino mice; while the effect
on uterine weight and on the endometrium was tested in New Zealand albino rabbits.

All compounds were administered orally by gavage in 0.1 or 0.2 ml sesame oil. Control animals received the vehicle only.

Vaginal cornification test (Allen–Doisy test)
The method described by Allen & Doisy (1923) was used with a slight modification (Revesz & Chappel, 1966) in ovariectomized adult rats (275 to 325 g) and mice (25 to 30 g). The ED$_{50}$ was the total dose which induced vaginal cornification in 50% of the animals.

### Table 1
**ALLEN–DOISY TEST IN RATS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of rats</th>
<th>No. of doses p.o.</th>
<th>$ED_{50} \pm S.E.$ (µg/rat)</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mestranol</td>
<td>100</td>
<td>5</td>
<td>46.0 ± 8.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Oestriol</td>
<td>45</td>
<td>7</td>
<td>76.0 ± 17.3</td>
<td>0.61</td>
</tr>
<tr>
<td>AY-11483</td>
<td>25</td>
<td>5</td>
<td>2.4 ± 0.4</td>
<td>20.0</td>
</tr>
</tbody>
</table>

* Average body weight: 300 g.

### Table 2
**ALLEN–DOISY TEST IN MICE**

<table>
<thead>
<tr>
<th>Compound</th>
<th>No. of mice</th>
<th>No. of doses p.o.</th>
<th>$ED_{50} \pm S.E.$ (µg/mouse)</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mestranol</td>
<td>35</td>
<td>4</td>
<td>1.8 ± 0.2</td>
<td>1</td>
</tr>
<tr>
<td>Oestriol</td>
<td>20</td>
<td>4</td>
<td>9.6 ± 1.4</td>
<td>0.19</td>
</tr>
<tr>
<td>AY-11483</td>
<td>30</td>
<td>6</td>
<td>5.0 ± 2.0</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* Average body weight: 30 g.

Uterotrophic assay

**Rats and mice.** This test was carried out in intact 22- to 23-day-old rats weighing 34 to 42 g (Dorfman & Dorfman, 1954) and in mice weighing 11 to 15 g (Rubin, Dorfman, Black & Dorfman, 1951). Compounds were administered once daily for 3 consecutive days, the uteri were dissected out 24 hr after the last injection and weighed following the removal of the intraluminal fluid by blotting on filter paper.

**Rabbits.** Immature female rabbits weighing 900 to 1100 g were treated once every 2nd day over a period of 5 days (total of three doses). On the 6th day, the animals were killed, the uterine weights determined and results expressed as mg per 100 g of body weight.

Effect on the endometrium in immature rabbits

In the classical Clauberg assay (Clauberg, 1930), immature female rabbits were primed with different oestrogens given perorally (p.o.) every 2nd day over a period of 5 days (total of three doses), followed by a standard daily dose.
AT-ll4:83, a new type of orally active oestrogen

of 0·5 mg s.c. of progesterone for 5 days. The animals were killed on the follow-
ing day and sections of the endometrium were evaluated for progestational
effect according to the McPhail (1934) grading system (from 0 to +4).

RESULTS

Vaginal cornification test

Table 1 summarizes the results obtained in rats. AY-11483 was approxi-
mately twenty times more potent than mestranol and thirty times more potent
than oestriol.

Results obtained in mice are presented in Table 2. In this species, AY-11483
was nearly three times less potent than mestranol and about two times more
potent than oestriol.

Uterotrophic test

Rats and mice. Text-figures 1 and 2 show the uterotrophic effect in rats and mice.
In both species, mestranol gave a steep dose–response curve which reached a plateau at higher doses. On the other hand, AY-11483 and oestriol gave shallow dose–response curves. The latter two compounds were nearly equipotent but both were much weaker than mestranol. The relative potency of AY-11483 and oestriol to mestranol could not be calculated since these curves were not parallel to that of mestranol. The two assays indicate that both oestriol and AY-11483 have a weak uterotrophic effect in rats and mice.

Rabbits. Text-figure 3 gives the results of the uterotrophic effect in rabbits. A total dose of 60 µg of mestranol caused an approximate ten-fold increase in uterine weight over the controls. AY-11483 at a total dose of 240 µg was about equivalent in its uterotrophic effect to a 60-µg total dose of mestranol. A total dose of 480 µg of oestriol caused an approximate seven-fold increase in uterine weight over the controls. These results indicate that AY-11483 has a much weaker effect on uterine weight than mestranol but is more potent than oestriol in this species.

**Effect on the endometrium in immature rabbits**

The results summarized in Text-fig. 4 show that progesterone induced +3.9 proliferation of the endometrium when priming was effected with 30 µg of mestranol. By contrast, a total priming dose of 120 µg of oestriol or AY-11483 was required to produce the same degree of endometrial proliferation. These findings suggest that both oestriol and AY-11483 are nearly equipotent and

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**Text-fig. 3.** Effect of three different oestrogens on uterine weight in immature rabbits. ●, Mestranol; ×, oestriol; △, AY-11843; —, control.
AY-11483, a new type of orally active oestrogen

each of them exerts a much weaker oestrogenic effect on the endometrium than mestranol.

![Graph showing the effect of three different oestrogens on endometrium in immature rabbits](https://example.com/graph)

**DISCUSSION**

Although the oestrogenicity of a substance can be determined by a number of bioassays (Emmens, 1962), the vaginal cornification test of Allen & Doisy (1923) in ovariectomized rats and mice is perhaps the most specific assay for oestrogenicity. According to Astwood (1965), an oestrogen is defined as a substance which produces a cornified vaginal smear in the spayed animal. By contrast, the uterotrophic assay in immature rats or mice is less specific for oestrogenicity since androgens, progestins and even desoxycorticosterone (Velardo, 1959) can induce uterine growth in such animals. The results obtained in the Allen–Doisy test suggest that mestranol and oestriol are more potent in mice than in rats, while AY-11483 is comparatively more potent in rats than in mice. It is interesting to note that the Allen–Doisy values in rats and mice do not always run parallel. In view of the fact that AY-11483 was more potent than mestranol in the Allen–Doisy test in rats, one would expect that it would also be more potent in promoting uterine growth in the same species. In fact, AY-11483 was much less potent than mestranol; its uterotrophic effect was comparable to that of oestriol. These results suggest that there was a separation of activity with AY-11483 with regard to the vaginal cornification and the uterotrophic effects. This separation of activity could also be demonstrated in mice.

Furthermore, results obtained from the uterotrophic and endometrial proliferation assays in rabbits suggest that AY-11483 has less effect on the uterine weight and the endometrium than mestranol. However, when compared to oestriol, AY-11483 was more uterotrophic but was equipotent on the endometrial response.
Since AY-11483 gave a shallow dose–response curve like oestriol in the uterotrophic assay in rats and mice, this compound could also be classified as an “impeded estrogen” (Huggins & Jensen, 1955). Like oestriol, AY-11483 also has anti-oestrogenic action (Revesz, 1969, unpublished data) which is another property of an impeded estrogen.

The present experiments indicate that AY-11483 is a potent orally active oestrogen in the vaginal cornification test in rats and mice with weak effect on the uterine weight in rats, mice and rabbits and endometrium in rabbits. In view of the strong oestrogenicity in the vaginal cornification assay and the weak effect on the uterus, AY-11483 might be a useful drug in the menopause—in particular where fibroids are present—when stimulation of the uterus is undesirable. Furthermore, the weak effect of the compound on the myometrium and endometrium might be an advantage over the conventional combination of oestrogens with progestins for contraception.

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REFERENCES


