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## Progestational Activity of 6α-Methyl-17α-Acetoxyprogesterone. (24789)

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It is difficult to demonstrate progestational activity of 17α-hydroxyprogesterone as measured by endometrial proliferation in the rabbit whether administration is by the subcutaneous or oral route(1). In contrast, 17α-acetoxyprogesterone (AP)* is 4 to 6 times as potent as progesterone by subcutaneous injection and 2 to 5 times as potent as ethinyltestosterone orally in producing proliferative changes in the rabbit endometrium (unpublished). When assayed by the effect on endometrial carbonic anhydrase, AP was 7.3 times progesterone, and on the glandular total muscosal area (G/M ratio) it was 3.3 times as active as progesterone when administered subcutaneously(2). This compound has also been shown to exhibit a significant amount of oral activity(2). In women, 17α-acetoxyprogesterone is more than twice as potent as ethinyltestosterone as a progestin (3). A new progestin, structurally related to AP, 6α-methyl-17α-acetoxyprogesterone (MAP)†(4) has been reported to be more active than AP in G/M ratio and endometrial carbonic anhydrase studies(2). This paper reports its activity by the McPhail assay(5) and in an ovulation inhibition test.

*Methods. McPhail rabbit endometrial proliferation test.* Intact immature female rabbits were injected subcutaneously once daily for 6 days with 5 μg of estradiol benzoate in 0.2 cc of CMC (0.9% NaCl, 1% carboxy methylcellulose, 0.4% polysorbate 80, .042% propylparaben in water). Progestins were given in 0.2 cc of CMC per day subcutaneously or 2 cc of cottonseed oil per day orally. All doses are expressed as total amount of compound administered over a 5-day period. To eliminate bias in analysis, slides of the uterine sections were coded and identified only after grading had been completed. Because of the low oral potency of progesterone, the standard compound used was AP. Potency ratios derived from these data are not precise because of the inescapable subjective nature of the measurements and difficulty in statistical analysis of a measurement of this type. *Rabbit ovulation inhibition test.* This test was a modification of the method described by Makepeace *et al.*(6). The test animal was the adult rabbit weighing 2-4 kg. Test compounds were administered subcutaneously in 0.2 cc of CMC; 24 hours later each rabbit was caged with a male and allowed to copulate. Approximately 24 hours after copulation, laparotomy was performed and the ovaries examined for ovulation points.

*Results. Endometrial proliferation.* Results of these tests comparing the potency of MAP and AP on the McPhail assay are

* *Prodox*, registered trade mark, Upjohn Co., Kalamazoo, Mich.

† *Provera*, registered trade mark, Upjohn Co., Kalamazoo, Mich.

TABLE I. Activities of 17-Acetoxyprogesterone and 6α-Methyl-17-acetoxyprogesterone following Subcutaneous and Oral Administration on the Mc-Phail Assay.

| Compound | Total dose | No. rabbits | Mean response | Range |
|---|---|---|---|---|
| | *Subcut. admin.* | | | |
| 17-Acetoxypro-gesterone | 25 μg | 1 | <.5 | |
| | 50 | 7 | 1.3 | .5–3 |
| | 100 | 9 | 2.6 | 2 –3 |
| | 150 | 2 | 2.5 | " |
| | 200 | 11 | 3.86 | 2.5–4 |
| 6-Methyl-17-ace-toxyproges-terone | 5 | 5 | 1.2 | 0 –4 |
| | 10 | 3 | 2.3 | 1 –4 |
| | 20 | 3 | 4 | 0 |
| | *Oral admin.* | | | |
| 17-Acetoxypro-gesterone | 2 mg | 4 | .6 | .5–1 |
| | 5 | 8 | 2 | .5–3 |
| | 10 | 9 | 3.3 | 2.5–4 |
| | 20 | 4 | 4 | 0 |
| 6-Methyl-17-ace-toxyproges-terone | 50 μg | 6 | 1.25 | .5–2 |
| | 100 | 8 | 2.69 | 2 –3.5 |
| | 200 | 4 | 3.6 | 3.5–4 |

shown in Table I. MAP at 200 μg orally elicited a nearly maximal endometrial response; the dose of AP necessary to produce a similar response was between 10 and 20 mg or approximately 75 times the equipotent dose of MAP. Likewise, at 50 μg, MAP produced a response, duplication of which required a dose of approximately 3 mg of AP. Ratio of activity at the lower dose is therefore approximately 1:60. We can estimate from these data that MAP has an oral potency 60 to 75 times that of AP. By the same type of analysis, MAP is 6 to 8 times AP when administered subcutaneously.

*Ovulation inhibition.* The ovulation-inhibiting activities of MAP, AP and progesterone are shown in Table II. Progesterone inhibited ovulation in 100% of the animals at 1 mg per animal, confirming results of Pincus (7). Under similar conditions, MAP completely inhibited ovulation at 50 μg, while AP allowed some of the animals to ovulate even at doses as high as 500 μg. In order to obtain direct comparisons of activities, a graph was prepared to show at which doses the compounds were equally effective. An expression, $ED_{50}$, was chosen for this purpose, *i.e.*, dose at which each compound was effective in preventing ovulation in 50% of the animals.

AP had an $ED_{50}$ of 100 μg; MAP, 20-33 μg; and progesterone, 750 μg. Arbitrarily assigning a value of 1 to the $ED_{50}$ of progesterone, we see that AP is 7 times and MAP is 20-30 times as potent as progesterone by this test.

*Discussion.* The foregoing results show that the presence of a methyl group at C-6 of 17α-acetoxyprogesterone increases its biological activity on the tests reported here and confirm earlier reports on the high endometrium-stimulating activities of MAP(4). This increase in progestational activity associated with presence of a 6α-methyl group is similar to but more marked than the increase in glycogenic and antiinflammatory activities of prednisolone and hydrocortisone when a 6α-methyl group is present(8).

In addition to the quantitative differences, MAP has been found to be qualitatively dif-

TABLE II. Comparison of Activities of Progesterone, 17-Acetoxyprogesterone and 6-Methyl-17-acetoxyprogesterone on Inhibition of Ovulation.

| Compound | Dose (mg) | No. rabbits tested | No. ovulating | % inhibition of ovulation | $ED_{50}$ (μg) |
|---|---|---|---|---|---|
| CMC | | 5 | 5 | 0 | |
| Progesterone | 1 | 4 | 0 | 100 | 750 |
| | .5 | 4 | 4 | 0 | |
| | .2 | 4 | 4 | 0 | |
| 17-Acetoxyprogesterone | .5 | 10 | 2 | 80 | 100 |
| | .2 | 7 | 2 | 71 | |
| | .1 | 4 | 2 | 50 | |
| | .05 | 4 | 3 | 25 | |
| | .025 | 5 | 5 | 0 | |
| 6-Methyl-17-acetoxyprogesterone | .2 | 1 | 0 | 100 | 20–33 |
| | .1 | 3 | 0 | 100 | |
| | .05 | 5 | 0 | 100 | |
| | .025 | 6 | 5 | 17 | |
| | .0125 | 4 | 3 | 25 | |

ferent from AP in its effect on maintaining pregnancy in ovariectomized rats and also by displaying other gonadal hormonal activities(9).

Oral activity was potentiated to a greater extent than parenteral activity when measured by endometrial proliferation. This has also been reported to occur when carbonic anhydrase or G/M ratio is used as an end point (2), but not to the same degree as when endometrial proliferation is used. This marked increase in oral potency is similar to that of AP over progesterone(2,10,11). This indicates that either intestinal absorption or metabolism of progesterone is markedly altered by addition of the 17α-acetoxy group and this increase of oral activity was further altered by addition of the 6α-methyl group.

*Summary.* 1) 6α-methyl-17α-acetoxyprogesterone is a highly active progestin based on its ability to promote a secretory endometrium and to prevent ovulation in rabbits. 2) Subcutaneously, 6α-methyl-17α-acetoxyprogesterone is 6 to 8 times and orally 60 to 75 times as active as 17α-acetoxyprogesterone in the McPhail assay. The ability of the 6-methyl compound to inhibit ovulation in rabbits is at least 20 times as great as that of progesterone subcutaneously, while 17α-acetoxyprogesterone is 7 times as active as progesterone.

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## Pressure-Volume Changes in Human Forearm Veins after Atropine Administration.* (24790)

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Atropine has been used clinically and experimentally to produce cardiac acceleration (1). Recent studies suggest that the drug may have additional major effects on the cardiovascular system(2,3). Berry and coworkers reported a fall in central venous pressure and stroke volume after atropine administration(3). These observations were interpreted to indicate pooling of blood in the veins. Such an interpretation implies reduction in venous tone since venous pressure falls after the drug is given. The experiments reported here were done to examine the tone of the forearm veins after atropine administration.

*Methods.* The subjects were 18 healthy male medical students. All studies were done with subjects supine. Venous pressure was measured in the antecubital vein of the dependent left arm with a Statham 0 to 5 cm Hg strain gauge. Venous tone was measured in the right forearm by the plethysmographic method previously described(4,5). Observations were made intermittently for at least 30 minutes before and after injection of 2.0 mg of atropine sulfate into an ankle vein. In

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