# The Biological Profile of Progesterone 3-Cyclopentyl Enol Ether as Compared with That of Progesterone. (29947)

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Biological similarity between progesterone and its 3-cyclopentyl enol ether has been claimed on the basis of clinical reports that the latter is excreted as urinary pregnanediol and produces a normal secretory endometrium(1,2). Further support for this similarity has been presented by Caie and Klopper(3), who suggested that the orally administered enol ether is slowly hydrolyzed to free progesterone in the human body. If this hypothesis were correct, one might expect that the enol ether would have mimicked the biological effects of progesterone in laboratory animals. Since our early studies did not reveal biological similarity of the two compounds, we have extended our investigation. Evidence is presented which casts some doubt on the conversion theory and furthermore indicates that the amount of enol ether eventually converted to free progesterone and/or the rate of such a conversion, could barely have had any biological effect.

*Materials and methods.* The steroids tested were progesterone and its 3-cyclopentyl enol ether (PCPE). The chemical purity of the enol ether was determined by appropriate analytic methods(4). Progesterone and the chemically pure 3-enol ether were administered dissolved in sesame oil.

*Progestational activity.* Immature Dutch rabbits weighing 900-1100 g were used. Estradiol 17$\beta$ (5 $\mu$g/an/day) was injected subcutaneously for 6 consecutive days. From the 7th day the progestins were administered, once a day, for 5 consecutive days along with one-tenth the original dose of estrogen. PCPE was given either orally (15, 25, or 35 mg/an/day) or intraperitoneally (3 or 10 mg/an/day), whereas progesterone was injected subcutaneously (200 or 400 $\mu$g/an/day). On the 12th day the animals were sacrificed, a middle section of the left uterine horn was removed, examined histologically and rated according to McPhail.

*Pregnancy maintenance.* On the 9th day of pregnancy, 200-220 g Wistar rats were bilaterally ovariectomized under ether anesthesia. Sham-operation was likewise performed under the same condition. In both ovariectomized and sham-operated animals the presence of implantation sites was carefully checked at time of operation. Treatment started on the same day as ovariectomy and continued up to and including the 20th day of pregnancy. Orally administered PCPE (12 or 48 mg/an/day) was compared with subcutaneous progesterone (1 or 4 mg/an/day). On the 21st day the animals were sacrificed, the number of implantation sites and living fetuses was recorded, and the percentage of fetal survival was calculated.

*Determination of urinary pregnanediol.* Immature female rabbits weighing 900-1100 g were treated subcutaneously with 5 $\mu$g of estradiol 17$\beta$ for 10 consecutive days. Urine collection began on day 9 and lasted for 48 hours. On the morning of the 11th day, the amount of estrogen was reduced to 0.5 $\mu$g and treatment with the progestins began. PCPE and progesterone were given orally at the daily dose of 100 mg per animal in 2 equally divided amounts of 50 mg each, 8 hours apart. This treatment continued for 7 consecutive days. Urine collection started on the 6th day and continued for 48 hours. Glacial acetic acid was used as a preservative until urinary pregnanediol was determined according to the method of Klopper *et al*(5).

*Potentiation of barbiturate anesthesia.* Female albino rats weighing 45-50 g received

TABLE I. Progestational Activity of PCPE and Progesterone.

| Treatment | No. of rabbits | Total dose (mg) | Route | Avg McPhail index |
|---|---|---|---|---|
| Progesterone | 4 | 1 | s.c. | 2.2 |
| " | 6 | 2 | s.c. | 4 |
| PCPE | 3 | 15 | i.p. | 1.3 |
| | 3 | 50 | i.p. | 3.6 |
| | 4 | 75 | p.o. | 2 |
| | 4 | 125 | p.o. | 2.5 |
| | 4 | 175 | p.o. | 3.5 |

TABLE II. Pregnancy Maintenance in Ovariectomized Rats Following PCPE or Progesterone.

| Treatment | Total No. of mothers | Route of administration | Daily dose (mg) | Total No. of implantation sites | Total No. of living fetuses | % Fetal survival* |
|---|---|---|---|---|---|---|
| Ovariectomized | 6 | — | — | 0 | 0 | 0 |
| Sham-operated | 5 | — | — | 52 | 51 | 98.1 |
| Ovariectomized + progesterone | 9 | s.c. | 1 | 45 | 5 | 5.3 |
| *Idem* | 9 | s.c. | 4 | 76 | 36 | 38.5 |
| Ovariectomized + PCPE | 5 | p.o. | 12 | 29 | 0 | 0 |
| *Idem* | 5 | p.o. | 48 | 49 | 0 | 0 |

* Calculated according to the formula:

$$\frac{\text{Total No. of living fetuses in group}}{\text{Total No. of mothers in group} \times \frac{\text{Total No. of implantation sites in sham-operated group}}{\text{Total No. of mothers in sham-operated group}}} \times 100.$$

one administration of the test compounds. PCPE or progesterone were administered either orally or intraperitoneally at the various doses indicated in Table IV. The test compounds were given either alone or were followed by administration of the barbiturate. In the latter case, an aqueous solution of hexobarbital sodium was administered intraperitoneally at a subhypnotic dose of 25 mg/kg, 60 minutes after steroid administration. The animals were considered anesthetized when loss of righting reflex occurred. The righting reflex was considered to be lost when the animal, placed on its back, did not regain its normal posture within 120 seconds.

*Results. Progestational activity.* Table I indicates that the progestational activity of intraperitoneally administered PCPE was 3-4 times greater than that following its oral administration. Orally and intraperitoneally administered PCPE was respectively, 1/80th and 1/20th as effective as subcutaneously injected progesterone.

TABLE III. Determination of Urinary Pregnanediol.

| Treatment | Daily dose* (mg) | Route | Urinary pregnanediol levels (mg/24 hr) Pre-treatment | Post-treatment |
|---|---|---|---|---|
| Progesterone | 100 | p.o. | .017 | 2.28 |
| " | 100 | p.o. | .026 | 1.60 |
| " | 100 | p.o. | .023 | 1.88 |
| PCPE | 100 | p.o. | .015 | .58 |
| " | 100 | p.o. | .020 | .55 |
| " | 100 | p.o. | .017 | .59 |

* Given in 2 equally divided amounts of 50 mg each, 8 hr apart.

*Pregnancy maintenance.* The data in Table II show that oral doses of PCPE 10-20 times those of subcutaneous progesterone were unable to maintain pregnancy in ovariectomized rats. Larger doses were not technically feasible.

*Determination of urinary pregnanediol.* The data in Table III show that 3-4 times as much urinary pregnanediol was recovered following oral administraiton of progesterone than of a comparable dose of PCPE.

*Potentiation of barbiturate anesthesia.* The data in Table IV show that when administration of the steroids was followed by intraperitoneal administration of the barbiturate, doses of progesterone which did not produce any anesthetic effect if given alone did potentiate the anesthetic effect of a subhypnotic dose of the barbiturate. PCPE at doses up to 40 times the effective dose of progesterone, failed to potentiate barbiturate anesthesia whether administered intraperitoneally or orally.

*Discussion.* There is evidence(6) that, with the exception of bypassing the gastrointestinal tract, the pathway of absorption of intraperitoneally and orally administered steroids is identical. The observation that progesterone 3-cyclopentyl enol ether (PCPE) possesses greater progestational activity following intraperitoneal than oral administration would therefore suggest that PCPE is partially transformed (hydrolyzed?) to a less active compound(s) in the gut.

Indirect evidence that free progesterone might be formed during these transformation processes was obtained by determining the

TABLE IV. The Potentiation of Barbiturate Anesthesia by Progesterone as Compared with Its 3-Cyclopentyl Enol Ether.

| Treatment | Route of administration | Dose/animal (mg) | No. of animals losing righting reflex | |
|---|---|---|---|---|
| | | | Without barbiturate | With barbiturate* |
| Sesame oil | p.o. | — | 0/40 | 0/40 |
| " " | i.p. | — | 0/40 | 0/40 |
| Progesterone | p.o. | .625 | 0/10 | 0/10 |
| " | p.o. | 1.250 | 0/10 | 4/10 |
| " | p.o. | 2.500 | 0/10 | 10/10 |
| Progesterone-3-cyclopentyl enol ether | p.o. | 25.000 | 0/10 | 0/10 |
| Progesterone | i.p. | .625 | 0/10 | 4/10 |
| " | i.p. | 1.250 | 0/10 | 6/10 |
| " | i.p. | 2.500 | 0/10 | 10/10 |
| Progesterone-3-cyclopentyl enol ether | i.p. | 25.000 | 0/10 | 0/10 |

* Hexobarbital sodium was given intraperitoneally at subhypnotic dose of 25 mg/kg one hr after steroid administration.

level of urinary metabolites following oral administration of either free progesterone or PCPE to immature female rabbits. Urinary pregnanediol determinations showed that the level of this metabolite significantly increases following oral administration of either compound. As previously observed in man(7,3), 3-4 times as much pregnanediol was recovered following administration of progesterone than of a comparable dose of PCPE. Caie and Klopper(3) suggest that PCPE is slowly hydrolyzed to free progesterone in the body and that this phenomenon is responsible for the low level of urinary pregnanediol found in the first 24-48 hours following its oral administration. If this hypothesis were valid, one would expect that these hydrolytic processes would take place in parts of the body other than the intestine. Furthermore, if the slow conversion of PCPE to progesterone did occur in sites other than the intestine, the prolonged administration of the enol ether should have led to an accumulation of this substance in the body. As a result of this accumulation phenomenon, one would have expected a higher level of urinary pregnanediol than that following oral administration of progesterone. This hypothesis was not confirmed by our results, since only 1/3 to 1/4 as much pregnanediol could be recovered from the urine following prolonged oral administration of PCPE as could be recovered following a comparable dose of progesterone. It seems evident, therefore, that the eventual transformation of PCPE to progesterone could only occur in the gut. This assumption is further substantiated by the observation that oral doses of PCPE 10-20 times those of subcutaneously injected progesterone were unable to maintain pregnancy in ovariectomized rats. In fact, if conversion of PCPE to progesterone had occurred in parts of the body other than the intestine, in consideration of the large doses administered, enough progesterone should have been formed to be able to maintain pregnancy. It should also be pointed out that pregnanediol may be derived from steroids other than progesterone(8) and thus its presence in the urine following administration of PCPE does not guarantee that progesterone was an intermediate in its formation.

Our final experiments on hexobarbital potentiation actually cast doubt that any transformation to free progesterone occurred in the gut or elsewhere. There is evidence(9) that progesterone potentiates barbiturate anesthesia by decreasing the hepatic degradation of barbiturates. It will be noted that small doses of progesterone (0.625-1.25 mg) were able to interfere with the hepatic degradation of barbiturates, whereas doses of PCPE up to 40 times those of progesterone were ineffective. This would indicate that (a) PCPE is either not transformed to progesterone, and/or (b) the rate of such a conversion is not of such a magnitude as to have any biological effect. A conversion of 5% would have been detected by the method used. This would also suggest that hepatic enzymes other than those involved in progesterone metabolism might be responsible

for the metabolism of PCPE. If this is true, our experiments raise the questions as to whether the substance excreted in the urine and characterized as "pregnanediol" by the Klopper method is actually 5$\beta$-pregnan-3$\alpha$, 20 $\alpha$-diol or alternatively, whether the "pregnanediol" measured originates from PCPE or arises from endogenous sources under the influence of PCPE. To answer these points, the metabolism of isotopically labeled PCPE must be studied in animals as well as in humans.

*Summary.* Progesterone and its 3-cyclopentyl enol ether (PCPE) have been compared in a battery of biological tests. Orally or intraperitoneally administered PCPE was respectively 1/80th and 1/20th as active as subcutaneously injected progesterone in the Clauberg test. Oral doses of PCPE, 10-20 times those of subcutaneous progesterone were unable to maintain pregnancy in ovariectomized rats. Three to four times as much "pregnanediol" was detected in the urine of rabbits following oral administration of progesterone than a comparable dose of PCPE. Orally or intraperitoneally administered PCPE at doses 20-40 times those of effective doses of progesterone failed to potentiate the anesthetic effect of a subhypnotic dose of sodium hexobarbital. These data demonstrate that PCPE and progesterone have a markedly different biological profile and cast serious doubt on the possibility that biologically active amounts of PCPE are converted to and/or metabolized in the same way as progesterone, as claimed by others. Our data strongly support the view that if any transformation of PCPE to progesterone occurs, the likeliest site of this conversion is the gut.

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## An Inexpensive and Simple Method for Preparing Chromosome Spreads.*† (29948)

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The extent of recent literature in cytogenetics conceals the fact that relatively few laboratories perform chromosome analyses as a routine procedure, and even fewer analyze populations of a size desirable for extraction of statistically valid information. A major deterrent is probably cost of time and money; and a minor one, possibly, the feeling that tissue culture techniques require highly specialized equipment and skill. In fact, the basic technique is quite straightforward and fully amenable, without loss of reliability, to simplification and economy. The method de-

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