DIRECT PROGESTATIONAL ACTION OF PROGESTERONE
AND CERTAIN RELATED STEROIDS ON THE
ENDOMETRIUM OF THE RHESUS MONKEY

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SUMMARY

Solid intrauterine implants of certain steroids were made in prepubertal and adolescent
ovariectomized and oestrone-primed rhesus monkeys. Implants of the following steroids
produced localized progestational changes in the endometrial glands: progesterone, deoxy-
corticosterone, ethisterone, testosterone, methyl testosterone and methyl dihydrotestosterone. Preg-
nenolone produced only minimal progestational effects. Pregnanediol showed no progestational
effect and failed to maintain the endometrium.

A comparison of the direct progestational action of a number of steroids on the endometrium
has been made in the four species so far investigated (mouse, rabbit, cat and monkey, each of
which represents a different mammalian order). Direct progestational action requires a fairly
close structural similarity to progesterone in all cases, but the degree of similarity required shows
a marked species difference.

It has been shown that solid implants of crystalline progesterone inserted in the tied-
off segment of the uterine horns can induce progestational changes in the glands of
the endometrium in both rabbits [Höhn & Robson, 1950] and cats [Robson & Sharaf,
1951a]. The progestational development of the glands is limited to the implanted
segment of the uterus. A direct progestational action of this type was also obtained
with deoxycorticosterone acetate (DCA), but not with several other steroids in the
cat, and with DCA, ethisterone, pregnenolone, 17-methyl testosterone and 17-
methyl dihydrotestosterone in the rabbit. In view of this species difference with
regard to direct progestational action of steroids other than progesterone, it seemed
desirable to test a selection of steroids including progesterone in a primate.

MATERIAL AND METHODS

Female rhesus monkeys (Macca mulatta), weighing from 1.5 to 3.3 kg at the start of
the experiments, were used. Since puberty in this species occurs at an average body
weight of 3 kg [Asdell, 1946], most of the animals were immature, but a few may
be presumed to have been pubertal or young adults; the body weights of the animals
used are recorded in table 1.

Each experimental animal was subjected to three operations under Nembutal
anaesthesia (30 mg/kg), given intraperitoneally and supplemented, where necessary,
with open ether. The first operation was a bilateral ovariectomy, and this was
followed by ten equal daily subcutaneous injections of oestrone in olive oil to produce
the total doses shown in table 1. The priming doses were at first graded according
to body weight, but in the later experiments were standardized at 100 μg/day,
irrespective of body weight. At the second operation, on the day following the last oestrone injection, a thick silk ligature was placed tightly about the body of the uterus to separate its cavity into an upper segment into which a steroid implant was inserted through an opening in the fundus, which was then closed with fine catgut sutures, and a lower segment serving as a control. At the sides of the uterus, the separating ligature was threaded through part of the myometrium to avoid occlusion of the ascending branch of the uterine artery and so preserve a uterine blood supply, since the ovarian artery had been cut between ligatures at ovariectomy. Implants were left in the uterus for 7–8 days, and during this period no further injections were given. At the third operation the body of the uterus, divided into implanted and control segments, was removed above a tight ligature encircling the cervix. The excised segments were fixed in 10% formol-saline, and several paraffin sections from each segment, cut at 6–8 µ and stained in haematoxylin and eosin, were prepared for microscopic examination.

Implants of steroid substances were generally prepared by heating the crystalline powder until it was just fused. In the case of DCA and pregnenolone acetate and in one experiment with progesterone, commercial pellets of compressed material or steroid with a vehicle were used.

RESULTS

As a guide to the interpretation of results, one ovariectomized oestrone-primed animal was given daily subcutaneous injections of 0.77 mg progesterone in olive oil for 8 days (total dose 6.16 mg). The endometrium of this animal was thicker than in any of the animals to which steroids were administered by uterine implantation, and the glands had the typical shape of the mid-secretory phase as described and illustrated by Corner [1923].

The results of uterine implants are shown in Table 1, and in Pl. 1, figs. 1–3. The responses are graded as follows: ++ indicates a marked progestational effect in the glands corresponding to that observed in the animal, referred to above, which received 6.16 mg progesterone systemically; + indicates a lesser effect, and ± a minimal response.

It is clear from these results that progesterone, DCA, ethisterone, testosterone, methyl testosterone and methyl dihydrotestosterone have direct progestational action on the glands of the endometrium. A progestational action on the uterine glands following systemic administration of some of these substances has been previously reported, e.g. for DCA [Zuckerman, 1941], testosterone propionate [Engle & Smith, 1939], and methyl testosterone [Hisaw, 1943].

Pregnenolone acetate produced only very slight and rather patchy progestational changes in the glands. This is possibly attributable to enzymatic conversion of pregnenolone to some related steroid within the endometrium, as demonstrated by Nissim [1952] with human placenta.

In the two experiments with pregnanediol not only did the implant fail to produce any progestational development of the uterine glands, but only the basal portions of the endometrium were retained in both implanted and control segments (Pl. 1, fig. 3). This loss of the more superficial endometrial layers is presumably due to oestrogen withdrawal bleeding. Unfortunately, no direct evidence of withdrawal bleeding can
Table 1. Effect of uterine implants of steroids in oestrone-primed ovariectomized rhesus monkeys
(Unless otherwise stated the residues of the implants were found in the implanted segment.)

<table>
<thead>
<tr>
<th>Animal wt.</th>
<th>Oestrone total given in 10 days (kg)</th>
<th>Steroid implanted</th>
<th>Dose (mg)</th>
<th>Im planted</th>
<th>Control</th>
<th>Wt. of implant residue (mg)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>600</td>
<td>Progesterone (‘Prolutron’ tablet, Schering Co.)</td>
<td>2</td>
<td>++</td>
<td>-</td>
<td>None found</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>600</td>
<td>Progesterone (fused)</td>
<td>2.0</td>
<td>++</td>
<td>-</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>500</td>
<td>Progesterone (fused)</td>
<td>17</td>
<td>++</td>
<td>-</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>2.4</td>
<td>600</td>
<td>DCA (compressed pellet)</td>
<td>15</td>
<td>++</td>
<td>-</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>Fregnenolone acetate (tablet)</td>
<td>20</td>
<td>±</td>
<td>-</td>
<td>None found</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>600</td>
<td>Fregnenolone acetate (tablet)</td>
<td>20</td>
<td>±</td>
<td>-</td>
<td>None found</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>700</td>
<td>Fregnenolone acetate (fused)</td>
<td>19</td>
<td>++</td>
<td>-</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>Ethisterone (fused)</td>
<td>17</td>
<td>++</td>
<td>±</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>Testosterone (fused)</td>
<td>20.5</td>
<td>++</td>
<td>±</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>Testosterone (fused)</td>
<td>21.6</td>
<td>++</td>
<td>-</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>Methyl testosterone (fused)</td>
<td>21.6</td>
<td>++</td>
<td>-</td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td>2.45</td>
<td>1000</td>
<td>Methyl dihydrotestosterone (fused)</td>
<td>26</td>
<td>++</td>
<td>-</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>2.15</td>
<td>1000</td>
<td>Methyl dihydrotestosterone (fused)</td>
<td>21</td>
<td>++</td>
<td>-</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1.9</td>
<td>1000</td>
<td>Methyl dihydrotestosterone (fused)</td>
<td>18</td>
<td>±</td>
<td>-</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>1000</td>
<td>Pregnanediol (fused)</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>Residue found outside uterus</td>
<td></td>
</tr>
<tr>
<td>2.3</td>
<td>1000</td>
<td>Pregnanediol (fused)</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>None found</td>
<td></td>
</tr>
</tbody>
</table>

Degree of progestational development of glands: ++, marked; +, less marked; ±, minimal; -, absent.
be furnished, as the animals were not examined for signs of bleeding during the implantation period.

In the experiments with steroids other than progesterone, the endometrium of both implanted and control segments was still maintained at the end of the 7-8 day period of implantation. This cannot, however, be taken as definite proof of the prevention of oestrogen withdrawal bleeding in these cases, since Zuckerman [1937] after daily administration of comparable doses of oestrone for 14 days observed withdrawal bleeding after intervals varying from 6 to 13 days.

DISCUSSION

In table 2 the direct progestational activity of various steroids on the endometrium of rhesus monkeys is compared with results so far published for other species. It should be noted that the results tabulated for the mouse were based not on the response of the endometrial glands, but on changes of the stromal nuclei in the atrophic endometrium of unprimed ovariectomized animals. It is clear that in the animals so far tested the list of direct-action progestogens is different for each species. However, no tests of direct progestational action in two species belonging to the same order of mammals are as yet available. Further studies on closely related species may well reveal greater agreement concerning the direct progestational action of the various steroids. It may therefore be speculated that progesterone, ethisterone, DCA, and certain androgens probably act as direct progestogens in the human uterus as in the rhesus monkey.

The degree of approximation to the chemical structure of progesterone required for direct progestational action on endometrial glands varies in the different orders of mammals.

Table 2. Direct endometrial progestogenic action of steroids in various species

(The data for women [first column], are included for comparison and refer to experiments in which no differentiation between direct and indirect progestogenic action was attempted. (For criteria of response see table 1; n.t. = not tested.))

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Human</th>
<th>Rhesus monkey</th>
<th>Cat</th>
<th>Rabbit</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethisterone</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>DCA</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pregnenolone</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Testosterone</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Testosterone propionate</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Methyl testosterone</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Methyl dihydrotestosterone</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Pregnatrienone</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>+</td>
<td>n.t.</td>
</tr>
<tr>
<td>Pregnanediol</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Androsterone</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>-</td>
<td>n.t.</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>.</td>
<td>n.t.</td>
<td>n.t.</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Authors: Human: Wenner & Joel [1939], ethisterone; Köhler & München [1961], DCA; Greenblatt [1942], testosterone propionate.
Cat: Robson & Sharaf [1951a].
Rabbit: Höhn & Robson [1950]; Robson & Sharaf [1951b]; Höhn [1952].
Mouse: Hooker & Forbes [1949].

HÖHN, E. O. 'Direct progestational action of progesterone and certain related steroids on the endometrium of the rhesus monkey', p. 361, line 3: for 'progesterone' read 'pregnanediol'
mammals of which representative species have been tested. In the monkey, as indicated in Table 2, the widest departures from the structure of progesterone are compatible with progestational action; in the rabbit the range is nearly as wide; in the cat it is very restricted; and in the mouse the requirement for the response of the nuclei of the stromal cells is apparently completely specific.

With regard to the correlation between chemical structure and direct progestational action, such action in the rabbit apparently requires:

(1) a steroid nucleus,
(2) a sidechain of at least 1 carbon at C-17,
(3) the presence of a C-3 ketone group or alternatively unsaturation at C-5 [Höhn & Robson, 1950; Höhn, 1952]. The steroids tested in the rhesus monkey were selected on the basis that similar requirements for direct progestational action might apply in this species. However, the results obtained show that in the rhesus monkey a sidechain at C-17 is not required, since testosterone was found to act directly. The very feeble activity of pregnenolone suggests that in the monkey the ketone group at C-3 is essential for direct action even in the presence of unsaturation involving C-5. Since methyl dihydrotestosterone showed direct progestational activity, it can be concluded that unsaturation involving C-5 is not essential.

I wish to express my gratitude to the authorities of the Suffield Experimental Station, Defence Research Board of Canada, where most of the experiments were carried out, and particularly to Dr M. K. McPhail, Chief, Section of Physiology, for making monkeys, technical assistance and working facilities available to me; to Dr K. W. Thompson of Organon, Inc., through whose co-operation methyl dihydrotestosterone, synthesized for this purpose, was made available to me; and to the following firms for supplying some of the steroids used: Ciba Company, Canada; Parke Davis, Canada; Schering Company and Roche Organon, Canada.

REFERENCES

EXPLANATION OF PLATE
(All sections stained by haematoxylin and eosin; x 55)
Fig. 1. Photomicrograph of a typical control segment showing a proliferative endometrium.
Fig. 2. Photomicrograph of an implanted segment, showing a typical progestational response (uterus implanted with 2·9 mg of fused progesterone).
Fig. 3. Photomicrograph showing erosion of all but the basal endometrial layer (uterus implanted with 17 mg of pregnanediol).