STEROIDAL INFLUENCE ON MORPHOLOGY AND BEHAVIOUR IN GUINEA-PIGS*

W. D. FOOTE AND D. W. PETERSON†

Division of Animal Science, College of Agriculture, University of Nevada, Reno

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Genital masculinization of newborn females, resulting from treatment of the pregnant dam with various synthetic progestagens, has been reported in humans by Wilkins, Jones, Homaln & Stempfel (1958), Grumbach, Ducharme & Moloshok (1959), Bongiovanni & McPadden (1960) and Jones & Wilkins (1960), in rats by Ravesz, Chappel & Gandry (1960), and in guinea-pigs by Foote, Foote & Foote (1968). This androgenic effect of certain progestational steroids leads to the question of whether or not these progestins are capable, like androgen, of influencing other functions, such as differentiation of the nervous system. Such an influence would be demonstrated by failure to ovulate and the occurrence of male-type sexual behaviour at maturity. Several researchers have shown that androgen causes male sexual differentiation of the hypothalamus during a critical time in the fetus or neonatal animal (the stage depending upon the species). Following androgen influence during this critical period, adult female rats (or males with ovarian transplants) failed to show ovarian cyclical activity and ovulation (Harris, 1964; Gorski & Wagner, 1965; Gorski, 1966). Female guinea-pigs, similarly androgenized during development, showed male-type sexual behaviour in adulthood (Goy, 1966). Pertinent literature on sex determination was organized by Harris & Edwards (1970).

This experiment was designed to determine whether or not certain synthetic progestagens which cause fetal masculinization also cause hypothalamic sexual differentiation in guinea-pigs. The following hormones were tested by injecting 1 mg per animal daily from Day 18 to Day 60 of gestation: testosterone, progesterone, 19-nor-17α-ethynyltestosterone (Norlutin), and medroxyprogesterone acetate (MAP).

Within 1 day of birth, all young were sexed externally, and also internally by laparotomy. The genitalia of all males appeared to be essentially normal. Assessment of the degree of female masculinization was based upon penis-like clitoral development externally and upon the presence of Wolffian duct and prostatic tissue internally. The ovaries, uteri and vaginas appeared macroscopically normal in all cases. Animals identified both externally and internally as males were discarded. Animals identified as females at laparotomy were weaned 2 weeks after birth and checked twice daily for the onset of oestrus,

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† Present address: The University of Alabama Medical Center, Birmingham, Alabama 35233, U.S.A.
shown by vaginal opening and the presence of cornified epithelial cells in the vaginal smear. Females which reached puberty and had established oestrous cycles were placed with males. Females which failed to come into oestrus, were examined at laparotomy at 120 days of age to determine whether or not ovulation was occurring and were then placed with males. All females placed with males remained for 70 days or until they became pregnant. During this period, they were checked for oestrous twice daily. Females which conceived were allowed to wean their litters and were then killed. Those which did not become pregnant were killed 30 days after removal from the males. All animals were examined at autopsy for external and internal masculinization to determine the permanency of fetal masculinization. As Table 1 illustrates, the genitalia in the untreated females appeared to be normal in anatomy and function and these females also showed normal sexual behaviour. Progesterone-treated females had a record similar to that of the controls, though only one of these animals conceived. All testosterone-treated females showed external masculinization, and one had a structure resembling a unilateral Wolffian duct. Although most of the females in this group ovulated after maturity, none established oestrous cycles, none conceived, and two of four reaching maturity demonstrated male-type behaviour by mounting other females. Response to Norlutin treatment was similar to that for testosterone, except that Norlutin-treated females did establish oestrous cycles, and one conceived and littered. All newborn females in the MAP-treated group had more pronounced external masculinization than in the other groups. Most of these females also had Wolffian ducts and prostatic tissue. At maturity, all females in the MAP-treated group ovulated but only one conceived and littered. The one which conceived did not have internal masculinization. None in this group demonstrated male-type behaviour.

It is not known whether masculinization prevented conception physically or was related to physiological conditions incompatible with conception or early pregnancy. It was noted, however, that conception did occur in two animals

![Image](https://www.bioscientifica.com/)

**Table 1**

PROPORTION OF FEMALE GUINEA-PIGS MASCUCLINIZED FOLLOWING PRENATAL STEROID TREATMENT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Masculinization</th>
<th>Activities after maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neonatal</td>
<td>Mature</td>
</tr>
<tr>
<td></td>
<td>External</td>
<td>Internal</td>
</tr>
<tr>
<td>Untreated</td>
<td>0/11</td>
<td>0/11</td>
</tr>
<tr>
<td>Progesterone</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Testosterone</td>
<td>10/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Norlutin</td>
<td>8/9</td>
<td>0/9</td>
</tr>
<tr>
<td>MAP</td>
<td>14/14</td>
<td>14/14</td>
</tr>
</tbody>
</table>

MAP = medroxyprogesterone acetate.

* Treatment in utero by daily subcutaneous injections of the dam with 1 mg hormone on Days 18 to 60 of gestation.
with external masculinization only, one of which had been treated with Norlutin and one with MAP.

Both external and internal masculinization present at birth tended to be maintained to maturity, though slight regression was observed in a few cases.

These results confirm earlier findings (Foote et al., 1968) that MAP causes greater female fetal masculinization than the other natural or synthetic steroids tested, including testosterone. Although none of these hormones given prenatally prevented ovulation taking place at maturity, testosterone and Norlutin appeared to cause a male-orientated differentiation of the nervous system, as indicated by male-type behaviour at maturity.

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REFERENCES


