tive antagonism (figure). Atropine (0.14 μM), mepyramine (0.25 μM), LSD (3.1 μM) and caffeine (52 μM) which abolished the responses to acetylcholine, histamine, 5-HT and ATP respectively did not affect those to oxytocin. Pretreatment of the tissue with the ganglion blocker pentolinium (19 μM) failed to alter oxytocin responses.

On cooling at 4 °C for 96 h, the spontaneous activity of the tissue was markedly reduced. Nevertheless, in this preparation, contractile responses to oxytocin were still evident (n = 5).

In Mg^{++}-free Krebs-Henseleit solution, oxytocin (5.4–540 nM) produced dose-related contractions (n = 5) though the sensitivity was less as compared with those in normal Krebs-Henseleit solution. On replacing $MgSO_4$ (2.4 mM) in the Mg^{++} free solution, the sensitivity of fowl rectum to oxytocin was restored (n = 5). The comparative sensitivity of the rectal preparations to oxytocin in the absence and presence of Mg^{++} is presented in the table. On the other hand, the presence or absence of Mg^{++} in Krebs-Henseleit solution did not affect the responses to acetylcholine.

Oxytocin (0.18–0.54 μM), acetylcholine (5.5–16.5 μM) and calcium chloride (2.7–9 mM) exhibited a dose-related excitatory effect on fowl rectum bathed in Ca^{++}-free K^{+}-rich Krebs-Henseleit solution (n = 5). However, the magnitude of the responses to both oxytocin and acetylcholine was markedly reduced and the maximal response as obtained in normal Krebs-Henseleit solution could not be elicited even on increasing the concentration of the drugs. On depolarization, the autorhythmicity of the tissue was completely inhibited and the phasic nature of the oxytocin contraction was changed to a tonic one. Since the magnitude of responses was very small, it was difficult to study the antagonism with blockers.

Discussion. The selective susceptibility of the excitatory actions of oxytocin to α-thioglycerol in fowl rectum suggests the presence of specific oxytocin receptors in this part of the gastrointestinal tract. The presence of oxytocin receptors is further strengthened by the restoration of the sensitivity to oxytocin by readmission of Mg^{++} to Mg^{++}-free Krebs-Henseleit solution. Thus, the present finding is in agreement with those reports of observations on isolated uterine[14,15] and gastrointestinal smooth muscle preparations[16] wherein it has been suggested that Mg^{++} acts as a co-factor in the interactions of neurohypophyseal hormones with their respective receptors.

The persistence of contractions in the depolarized preparations (Ca^{++}-free, K^{+}-rich) indicated that apart from depolarization, oxytocin possibly produces contraction of the tissues by mobilizing the membrane bound Ca^{++} through its receptor-operated channels. Operation of such a mechanism in K^{+}-depolarized tissues is evident in cases where spasmogens like acetylcholine, carbachol, histamine and 5-hydroxytryptamine produce contractions through interaction with their specific receptors resulting in opening of the receptor-operated-channels and mobilizing loosely bound Ca^{++} that brings about excitation-contraction coupling[17].

The physiological implications of the sensitivity of the fowl rectal segment to low concentrations of oxytocin are yet to be elucidated. Although the present studies do not provide any experimental evidence with regards to arginine-oxytocin (the predominant posterior pituitary oxytocic principle in birds), it may be postulated that the fowl neurohypophyseal hormone might facilitate oviposition by contracting the rectal segment besides its primary excitatory action on the reproductive smooth muscles. This physiological role is further substantiated by the increased blood concentrations of the neurohypophyseal hormones at the time of oviposition[18].

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Treatment of pregnant rats with haloperidol delays the onset of sexual maturation in female offspring

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Summary. Treatment of pregnant rats with haloperidol (1 mg/kg/day) during the last week of gestation induces a significant delay in sexual maturation of female offspring.

Chronic exposure of fetal rats to the dopamine antagonist haloperidol (HP) is reported to modify the subsequent development and function of central dopaminergic neurons. Thus behaviour, brain dopamine levels, dopamine receptors and pituitary prolactin secretion are all affected by maternal treatment with HP[2,3]. These observations are of major clinical importance in view of the widespread use of neuroleptics during pregnancy and by nursing mothers[4,5]. Since dopamine is clearly implicated in reproductive control mechanisms[6,7] via well-described hypothalamic

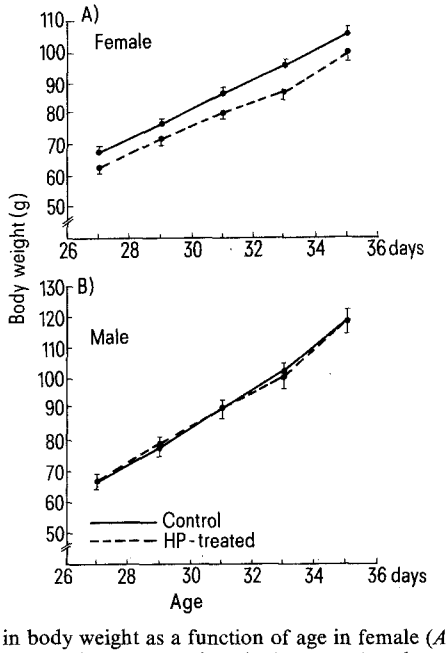
dopamine pathways[8] it appeared timely to investigate whether HP treatment might influence the onset of sexual maturation.

Materials and methods. 9 pregnant Sprague-Dawley rats weighing approximately 240 g were obtained from Canadian Breeding Farm and Laboratories (St. Constant, Quebec) and maintained in light- (lights on 07.00–19.00 h) and temperature- (21 °C) controlled rooms. The animals were housed in individual plastic cages and given free access to food and water. Beginning on day 15 of gestation, 3 rats were injected daily with HP (0.2 ml, 0.08% tartaric acid solution; s.c.; 1 mg/kg b.wt) until parturition. The offspring of the HP-treated rats were pooled and fostered to untreated mothers at birth. The litters of the remaining 3 dams were similarly pooled and served as controls. The pups were weighed every 2 days until weaning (day 21 after birth). At this time male and female littermates were caged separately but under identical conditions. Body weights of male pups were recorded every 2 days from day 27 of life to day 35. In addition, females were checked for vaginal opening (V.O.). On the day of V.O., body weights, and the weights of the ovaries and uteri were determined. Oviducts were inspected under binocular magnification for the presence of ova. Sample means were compared using Student's 2-tailed t-test. The growth curves of the female pups were compared by analysis of variance (AOV) of linear regression models containing quantitative (age) and qualitative (treatment) independent variables[9] using the Minitab computing system. 2-way AOV was used for comparison of the body weights of the male pups. A value with $p<0.05$ was considered to denote a significant difference.

Results. Litters of the treated groups were slightly smaller and body weight values showed that the HP-treated offspring were lighter on the day following birth: males plus females of the treated group ($n=28$) weighed 6.0 ± 0.1 g whereas control animals ($n=35$) weighed 6.7 ± 0.1 g ($p<0.01$; $\pm$ SEM). This difference persisted in the females (see fig. A for ages 27–35 days), but not in the males. AOV of the regression models for the female control and HP-treated groups indicated an effect of treatment ($F(1,128)=27.5$, $p<0.01$ for the y intercepts) although the growth rates did not differ ($F(1,127=0.3$, $p>0.05$ for the slopes). Thus, at day 33 of life when only a few rats had shown V.O., treated animals ($n=10$) weighed 87.0 ± 2.3 g compared to 96.0 ± 1.7 g for the control group ($n=14$; $p<0.01$). Growth curves of the male pups showed that there were no differences between the HP-treated and untreated groups (fig. B; AOV, $F(1,160)=0.04$ for the treatment, $(F(4,160)=0.1$ for the interaction of treatment with age, $p>0.05$ in both cases).

Prenatal HP exposure was found to delay sexual maturation in female offspring. The table shows that V.O. occurred significantly later ($p<0.01$) in rats that had been exposed to HP prenatally. Furthermore, HP-treated offspring were significantly heavier ($p<0.05$) at V.O. There were no differences in ovarian and uterine weights on the day of V.O. (table). All rats, treated and untreated, were observed to have ova or fresh corpora lutea at V.O. There was no effect of treatment on the number of ova (results not shown).

Discussion. Postnatally, the developing nervous system appears to be susceptible to the influence of neuroleptic drugs such as HP[4]. The recent work of Plach et al.[2,3] indicates that such effects can also be exerted in utero. This work has largely addressed the problem from a behavioural or psychopharmacological standpoint and the available evidence clearly points to a derangement in central dopamine systems as a possible explanation for the effects of HP. Our results show that HP also delays female puberty in the offspring of HP-treated rats. The effect could be mediated through inhibition of prolactin secretion in the female offspring. Prolactin plays a key role in the sexual maturation of female rats[7] and Plach et al[3]. have shown that the inhibitory effect of dopamine on prolactin secretion is enhanced in HP-treated immature rats. Alternatively, the dopamine receptors described by Wuttke et al.[7], which may influence the release of hypothalamic gonadotrophin-releasing hormone (GnRH), could also be inhibitory to the progress of first ovulation via an increase in sensitivity. However, an important observation is that the body weights of treated females, but not males, were significantly below those of control animals (fig. A). Controversy surrounds the notion that puberty occurs at a critical body size[10] and our results imply that the delay in puberty was not completely attributable to lower body weights. The table shows that the HP-exposed offspring were heavier on the day of V.O. Note, however, that no effect of HP was observed on weight gain in male rats (fig. B). This female-selective effect on body weight is not without precedent since



Increases in body weight as a function of age in female (*A* control, $n=18$; HP-treated, $n=11$) and male (*B* control and HP-treated, $n=17$) rats after prenatal exposure to haloperidol (HP). Values are means $\pm$ SEM.

The effect of prenatal haloperidol treatment upon age, body weight, and ovarian and uterine weights at vaginal opening in rats

Group	n	Age days)	Body weight (g)	Ovarian weight (mg)[a]	Uterine weight (mg)[a]
Control	18	34 ± 0.4	106 ± 1.8	49 ± 2.8	139 ± 6.6
HP-treated	11	38 ± 0.8[b]	114 ± 3.3[c]	48 ± 4.5[d]	120 ± 12.1[d]

[a] Ovarian and uterine weights expressed per 100 g body weight; [b] $p<0.01$; [c] $p<0.05$; [d] not significantly different from control value. All values are means $\pm$ SEM.

Mirmiran et al.[11] have described such an influence of chlorimipramine in developing rats.

The present findings have an important bearing upon the therapeutic use of HP in man. They raise the possibility of long-term changes in somatic growth and/or reproductive functioning following prenatal exposure or transference of HP via breast milk.

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Estrogen binds to hypothalamic nuclei of androgen-insensitive *(tfm)* rats[1]

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Summary. Androgen-insensitive *(tfm)* rats possess a nuclear-estrogen binding system in the brain that is similar to that of wild-type control males. In these mutant rats, radiolabeled estradiol was bound predominantly to hypothalamic nuclei and this binding was of limited capacity.

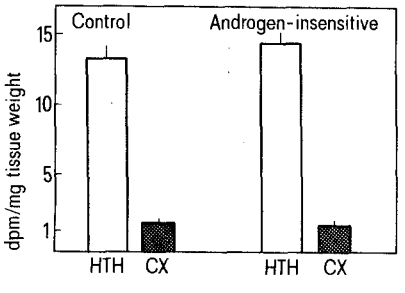
The androgen-insensitivity or testicular feminization syndrome is characterized by an inherited resistence to androgens. This syndrome was first described in humans[2] and later found in mice[3], rats[4], and cattle[5]. Affected individuals are genotypic males, but due to resistence to androgens secreted by their testes, they develop a feminine external phenotype.

The androgen-insensitivity is thought to be caused by a deficiency in cytoplasmic androgen-receptors which results in a defective nuclear uptake of the hormone[6–8]. Indeed, androgen binding is significantly reduced in neural cytosol from mutant rats[9], from mutant mice[10,11] and in skin fibroblasts from affected humans[8,12]. Even though these androgen-insensitive individuals have an obvious reduction in the number of receptors, they do show some physiological functions when given androgens. In androgen-insensitive rats, testosterone treatment has been shown to activate sexual behavior[13,14] and to inhibit the secretion of gonadotropins[9]. It is possible that these responses are not mediated by the androgen, but are mediated by estrogen derived intracellularly from that steroid[15]. If this is true, then it becomes critical to determine whether the mutation has any effects upon the neural estrogen binding system which physiological, biochemical and behavioral studies have shown to be predominantly hypothalamic[16].

In the present study we find that androgen-insensitive rats possess saturable nuclear-estrogen binding sites in the hypothalamus.

Materials and methods. Adult androgen-insensitive rats of the Stanley-Gumbreck strain (n = 12) and their male wild-type littermates (King-Holtzman, n = 11) were obtained from the International Foundation for the Study of Rat Genetics and Rodent Control (Introgen), Oklahoma City, Oklahoma. The rats were castrated under ether anesthesia. 4 days later they were injected i.v. with 40 µCi of [6,7, ^{3}H]-estradiol (sp. act. 47.9 Ci/mmole, New England Nuclear) in 200 µl of 15% ethanol. Some rats received an i.v. injection of 2 µg of unlabeled estradiol (Steraloids) dissolved in 200 µl of 15% ethanol 30 min prior to the injection of radiolabeled hormone. 1 h after the injection of radioactivity, the rats were sacrificed, the brains removed, placed on ice and the hypothalamic and parietal cortical samples were dissected and weighed. The procedure for the brain dissection and nuclear binding assay were carried out as previously described[17]. Briefly, the samples were homogenized in a solution containing 0.32 M sucrose, 1 mM potassium phosphate, pH 6.5, 3 mM $MgCl_2$, 0.25% Triton X-100 (v/v), and centrifuged at 850 × g for 10 min. The pellet was resuspended in the above solution prepared without the Triton X-100 and centrifuged again at 850 × g. The pellet was resuspended in a small volume of the 2nd solution and layered on a dense sucrose. After mixing, the sample was centrifuged at 24,000 rpm in a SW-50.1 rotor for 45 min. The resultant pellet, designated the nuclear sample[18], was extracted 5 times with 3 ml of toluene-based scintillation fluor.

Results and discussion. The figure shows the level of radioactivity extracted from the hypothalamic and cortical



Radioactivity levels in the nuclei of hypothalamic (HTH) and cortical (CX) tissues from castrated androgen-insensitive and littermate wild-type male rats 1 h after the i.v. administration of 40 µCi of ^{3}H-estradiol.