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.

1 This study was supported by a Canadian MRC grant, No. MA-7131, to M.W., to whom correspondence should be addressed.

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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.

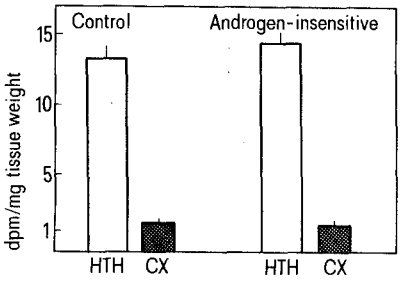
The androgen-insensitivity or testicular feminization syndrome is characterized by an inherited resistence to androgens. This syndrome was first described in humans[2] and later found in mice[3], rats[4], and cattle[5]. Affected individuals are genotypic males, but due to resistence to androgens secreted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

nuclei of androgen-insensitive and wild-type male rats. As can be seen, there are no group differences in the level or in the pattern of estradiol binding. Both androgen-insensitive and wild-type males show selective binding of estradiol to hypothalamic nuclei as compared with cortical nuclei. Pretreatment with 2 µg of unlabeled estradiol 30 min before an injection of radiolabeled hormone substantially reduced the level of binding in the hypothalamic nuclei. Following pretreatment, dpm/mg tissue weight measures ± SEM were as follows: androgen-insensitive rats: HTH 1.47 ± 0.18, cortex 1.55 ± 0.13; wild-type males: HTH 0.41 ± 0.02, cortex 0.50 ± 0.05. Thus, estrogen binding in neural tissue of androgen-insensitive rats is similar to wild-type males, showing both tissue specificity and finite binding capacity. These data indicate that androgen-insensitive rats possess a functional estrogen binding system in the brain. Similar findings also were reported in androgen-insensitive mice[10,11]. Given these data, then estrogen as a metabolite of androgen, could be mediating some of the behavioral and physiological responses found in individuals with androgen-insensitivity[14,19-22].

1 This research was supported by grants Nos HD-13957 and HD-14726 from the National Institute of Child Health and Human Development.

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## PRO EXPERIMENTIS

### Improved treadmill to avoid foot and tail injuries of small animals

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*Summary.* A treadmill was improved so as to eliminate foot and tail injuries in small test-animals. The improvement was inexpensive and easily fabricated by the researchers.

Small animals often incur foot and tail injuries on treadmills having a series of metal rods that function as the electrically shocking mechanism[1]. These injuries are caused by inserting the foot and tail between 2 rods or between a rod and the moving belt. When this space is filled, animal hair and excreta accumulate at the corner between the belt and the shocking mechanism, and then wet excreta causes problems of short-circuiting. To avoid these problems, an improved treadmill was developed in our laboratory. It has been used to exercise 5 rats simultaneously or 10 mice in pairs.

*Apparatus.* As shown in figure 1, bakelite plates, 65 mm wide (slightly narrower than the width of each compartment), 75 mm long, and 2 mm thick are attached to a metal shaft. The surface of each plate is waxed, lest it should be wetted by the urine of the animal, and is provided with an electrically charged grid.

Together with a pair of mirrors, a light-source and receiver are so arranged that 2 IR-beams run through each compartment. When each animal is exercising in the front part of the compartment, the arm of a solenoid is used to rotate the distant end of the plate upward. Therefore, excreta on the belt pass under the plate into a tray. Whenever one of the animals moves to the back of the compartment, it is detected through the interception of the light beam. The electric circuit to the solenoid is cut off by this interception, thereby releasing the solenoid arm and lowering the distant end of the plate until it loosely touches the belt. The animal receives an electric shock from the charged grid on the plate without inserting the tail and foot between the belt and the plate. The optimal angle of the plate against the belt is about 30°.

*Experiment.* Eighteen 7-9-week-old, experimentally naive rats were used. Each animal was placed in the compartment provided with the improved shocking devices mentioned above (I-test). They were first trained on the moving belt at a speed of 15 m/min for 3 min, then exercised at 25 m/min for 30 min. After a few days, they were tested in the same way as for the I-test on a treadmill equipped with the original shocking mechanism (O-test).

*Results and discussion.* In the I-test, no injuries occurred in the rats except that the nails of 2 animals were slightly injured. On the other hand, 5 rats were severely injured in the O-test, and 3 animals slightly injured their tails, feet or nails. All of the severe injuries were in animals that had accidentally inserted a foot or tail between charged rods, or between a rod and the moving belt. Thus, the improved treadmill not only saved the animals from foot and tail