Pseudohermaphrodite Rat: End Organ Insensitivity to Testosterone

Abstract. *The pseudohermaphrodite rat is characterized by lack of androgen-dependent differentiation. Treatment of these rats with testosterone failed to produce the expected changes in preputial and adrenal gland weights and in hexobarbital metabolism. This insensitivity of the end organ to testosterone could not be explained by defective formation of dihydrotestosterone.*

Stanley and Gumbreck (*1*) have described a form of male pseudohermaphroditism in the rat. This is an inherited defect passed by normal females to one half of male offspring. The pattern of inheritance is suggestive of X-linked recessive transmission; however, this cannot be rigorously tested because the affected pseudohermaphrodite males are sterile. These males are characterized by lack of androgen-dependent differentiation. There is a female phenotype with a short vagina, small phallus, and well-developed nipple lines. Testes are in the inguinal canals, but other parts of the male reproductive tract, such as the scrotum, prostate, seminal vesicles, and vas deferens, do not develop. The failure of the pseudohermaphrodite rat to masculinize could be explained either by low androgen production or by insensitivity of the end organ to testosterone. Although we demonstrated that the first of these possibilities obtains, the pseudohermaphrodite rat may secrete 25 percent as much testosterone as normal males; thus it seemed likely that even this quantity of testosterone could result in some androgen-dependent growth (*2*). We therefore attempted to determine whether the tissues of these rats were sensitive to androgens. We demonstrate that the liver microsomes, preputial glands, and adrenal glands do not respond to testosterone administration.

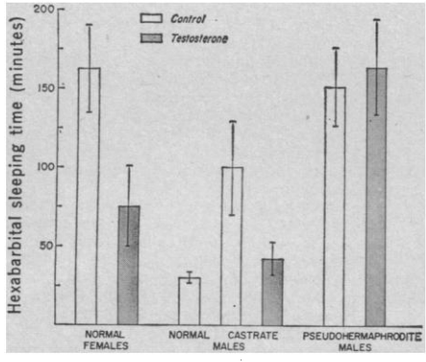

Fig. 1. Hexobarbital sleeping times in control rats and those treated with testosterone (1.0 mg/day for 3 weeks). The sleeping time represents the number of minutes required for a rat to right itself after receiving hexobarbital 125 mg per kilogram of body weight.

Liver microsomes from normal male rats metabolize barbiturates faster than those of females. This sex difference in microsomal enzyme activity is dependent on androgens and is manifest in the intact animal by longer hexobarbital sleeping times in females than in males (*3*). We therefore determined hexobarbital sleeping times in normal and pseudohermaphrodite rats before and during testosterone treatment (Fig. 1). Intact males have shorter sleeping times than females and castrate males. Testosterone treatment decreased the sleeping times of the females and castrate males. By constrast, the long sleeping times of the pseudohermaphrodite rats were unaffected by testosterone. This indicates that the livers of these rats are insensitive to testosterone stimulation of hepatic microsomal enzymes which metabolize hexobarbital.

The preputial glands are paired sebaceous organs in the pubic area of the rat. They are smaller in the female than the male and grow in response to testosterone treatment. By contrast, the adrenal glands of the female are larger than those of the male and decrease in size during testosterone administration. The preputial and adrenal gland weights of pseudohermaphrodite rats are comparable to those of females but do not change appropriately in response to testosterone (Table 1). Furthermore, androgen treatment does not promote citrate excretion, phallic growth, or hair coarsening in pseudohermaphrodite rats as it does in normal rats (*4*).

These observations suggest that the lack of androgen-dependent differentiation in the pseudohermaphrodite rat is due to genetic defect of tissue which results in insensitivity to testosterone. In this regard these rats are similar to patients with testicular feminization who have a female phenotype, inguinal testes, and end organ unresponsiveness to testosterone.

In androgen-dependent tissues, such as the prostate, seminal vesicles, and preputial glands, testosterone is reduced to dihydrotestosterone (5α-androstan-17β-ol-3-one), a potent androgen, which has been proposed as an intracellular active metabolite of testosterone (*5*). It has been reported that patients with testicular feminization were unable to convert testosterone to dihydrotestosterone both in vitro and in vivo (*6*). In accord with this, defective formation of dihydrotestosterone was suggested as the basis of the androgen insensitivity in testicular feminization. We therefore compared dihydrotestosterone formation in vitro in the normal and pseudohermaphrodite rat. Minced preputial glands were incubated with radioactive testosterone in Krebs-Ringer phosphate buffer, *p*H 7.4 under an atmosphere of O_2 and CO_2 (95 : 5). Each incubation flask contained 8 μM [4-^{14}C]-testosterone, 120 to 200 mg of tissue, 5 ml of buffer, 12 μM glucose-6-phosphate, 3 μM nicotinamide-adenine dinucleotide phosphate and two Kornberg units of glucose-6-phosphate dehydrogenase. Radioactive testosterone and its metabolites were isolated by reverse isotope dilution and crystallized to constant specific activity. Testosterone utilization by both normal tissue and tissue from pseudohermaphrodites was the same, and the following metabolites were isolated in equal quantities from both incubations: 5α-androstan-3α-ol-17-one; 5α-androstan-3α,17β-diol; 5α-androstan-3,17-dione; and androstan-4-en-3,17-dione. The rate of dihydrotestosterone formation ranged from 5 to 7 nmole/g per hour in incubations of both normal preputial glands and those from pseudohermaphrodites (*7*). These studies suggest that the unresponsiveness of pseudohermaphrodites to androgen is not due to reduced tissue ac-

Table 1. Effect of testosterone on preputial and adrenal glands of normal and pseudohermaphrodite rats. Testosterone in sesame oil at a dose of 1.0 mg per day for 62 days produced a significant change ($P < .01$) in female but not in pseudohermaphrodite organ weights. Results are expressed as the mean ± S.E. There were five or six animals per group.

Treatment	Preputial (mg)	Adrenal (mg per 100 g of body weight
	Male	
None	130 ± 9	11 ± 1.3
Castrate	62 ± 8	17 ± 2.0
	Female	
None	61 ± 5	26 ± 3.0
Testosterone	170 ± 16	14 ± 2.0
	Pseudohermaphrodite	
None	71 ± 5	25 ± 3.0
Testosterone	87 ± 8	24 ± 2.0

tivity of 5α-steroid reductase as has been suggested in testicular feminization (*6*).

The observation of normal dihydrotestosterone formation by pseudohermaphrodites does not necessarily diminish the potential importance of this androgen as an active intracellular product of testosterone. It is possible that the androgen insensitivity of both pseudohermaphrodite rats and patients with testicular feminization is due to defective nuclear uptake and binding of dihydrotestosterone. That dihydrotestosterone is an ineffective androgen in both these conditions supports this supposition (*8*).

Thus several tissues of the pseudohermaphrodite rat are insensitive to testosterone. This end organ unresponsiveness can explain the lack of androgen-dependent differentiation in these rats. This insensitivity is present not only in end organs such as the preputial gland and phallus, which are commonly thought of as androgen responsive tissues, but also in other organs such as the liver and adrenal gland where testosterone also exerts direct and indirect effects. Elucidation of this abnormality should provide clues to the mechanism of action of testosterone. Furthermore, the pseudohermaphrodite rat provides a model for the study of an inherited condition which is the rodent counterpart of a syndrome in man—testicular feminization.

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Rat Thoracic Duct Lymphocytes: Types that Participate in Inflammation

Abstract. *Newly formed small lymphocytes with a short life-span in the blood are the only cells from thoracic duct lymph which accumulate in acutely inflamed tissue. This conclusion is drawn from studies in which rats with induced peritoneal exudates were injected intravenously with radioactively labeled thoracic duct cells. Radioactivity, originally vested in newly formed donor small lymphocytes, was found later in a small number of similar exudate cells. Small lymphocytes generated 10 days or more before the thoracic ducts were cannulated failed to localize in peritoneal exudates, although the cells moved in large numbers from the blood into lymph nodes.*

Cells with the dimensions and structural features of small lymphocytes migrate from the blood into inflamed tissue, but the origin of these cells and their relationship to blood monocytes and macrophages are still debated (*1*, *2*). Studies in which tritiated thymidine (^{3}H-thymidine) is used indicate that many of the small lymphocyte-like cells in inflammatory exudates are the progeny of recently divided precursors (*2*). Two sources of these lymphocytes have been suggested, namely, bone marrow and lymphoid tissue. Our investigation evaluates the contribution made by the circulating pool of lymphocytes to the evolution of an acute inflammatory exudate. We conclude that only a small portion of the lymphocytes which enter blood by way of the thoracic duct have the ability to move into the inflamed peritoneal cavity. By exploiting the fact that rat thoracic duct cells vary with respect to their life-span in circulation (*3*) and the intensity to which they become radioactively labeled, we could identify the migrant cells as newly formed small lymphocytes with a short life-span in circulation.

Thoracic duct cells were labeled radioactively by injecting adult Lewis rats intraperitoneally every 8 hours for 10 days with 0.34 μc of ^{3}H-thymidine (3 c/mmole per gram of body weight). Cells issuing from thoracic duct fistulae during 12-hour collection periods were washed once in nonradioactive medium (*4*) and injected intravenously into syngeneic recipients. To minimize reutilization of radioactive material released from labeled donor cells, we infused the animals at 1 ml/hour with Ringer solution that contained 50 μg of nonradioactive thymidine per milliliter.

Sterile peritoneal exudates were induced in prospective recipients of lymphocytes by injecting the animals intraperitoneally with 3 ml of 12 percent casein per 100 g of body weight. The exudates, harvested 3 days later, contained about 75 percent macrophages, 15 percent small lymphocyte-like cells, and varying numbers of polymorphonuclear leukocytes, eosinophiles, and mast cells.

In the first experiment, thoracic duct cells were collected between 12 and 24 hours after the donors were given the last of a series of injections of ^{3}H-thymidine. Autoradiographs prepared with Ilford K5 emulsion and exposed for 5 weeks showed that the majority of large lymphocytes and about one-third of the small lymphocytes were radioactively labeled.

Table 1 shows the distribution of the labeled cells in tissues of individual rats 24 and 48 hours after intravenous injection. Of the labeled cells found in lymph nodes, one was a large lymphocyte; the rest were typical small lymphocytes. The majority of labeled large lymphocytes became localized in splenic red pulp or the intestinal wall; many small lymphocytes were localized on splenic white pulp or the cortex of lymph nodes (*5*). The lymph nodes and blood contained heavily labeled, and lightly labeled, small lymphocytes in approximately the same proportion as were present in the donor inoculum. By comparison, peritoneal exudates induced in the same animals contained a special subpopulation of heavily labeled small lymphocytes. Autoradiographs showed that 36.4 percent of the radioactive donor cells were heavily labeled small lymphocytes with 20 or more grains. Yet all but 2 of the 19 labeled cells found in the exudates were heavily labeled small lymphocytes of this type. Seventeen of these labeled exudate cells were typical small lymphocytes, and two were small lymphocytes with a relatively large cuff of faintly basophilic cytoplasm. The possibility that labeled blood-borne cells were added to the exudate during the trauma of harvest was excluded by enumerating erythrocytes in the exudate. Lymphocytes accompanying these erythrocytes could have ac-