PREVENTION OF HORMONE ACTION BY LOCAL APPLICATION OF ACTINOMYCIN D

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Previous work in this laboratory has suggested that growth hormone may exert its anabolic effect on liver by initial activation of RNA synthesis. Observations on the incorporation of labeled precursors revealed that after hormone administration the nuclear and microsomal fractions of RNA were increased significantly.[1] Short-pulse labeling experiments indicated that the stimulation was localized initially in the nuclear fractions of RNA.[2] On prolongation of the time of labeled precursor incorporation an increased specific activity appeared also in the microsome fraction. Nuclear RNA fractions from liver of normal and growth hormone-treated rats have been subfractionated into three components according to the method of Sibatani *et al.*[3] Sucrose gradient centrifugation studies on these fractions revealed maximum stimulation in a fraction associated with nucleolar RNA.[4] This fraction of RNA is a potent stimulator of amino acid incorporation into proteins in a cell-free system of *E. coli* of the type described by Matthaei and Nirenberg.[5] The growth hormone stimulation of RNA synthesis is inhibited by actinomycin D.[4] These results have prompted the suggestion that in exerting its hormonal effect, the primary action of growth hormone is the specific stimulation of messenger RNA synthesis and that this is a controlling prerequisite for secondary biologic effects such as cytoplasmic growth and cell multiplication. Evidence is now presented to suggest that the secondary cellular effects of two additional hormones, estradiol-17β and chorionic gonadotrophin, depend on initial stimulation of messenger RNA synthesis. The work supports the hypothesis advanced by Hamilton in a recent communication[6] with respect to the action of estrogens.

Materials and Methods.—Inhibition of estradiol action on vaginal mucosa: Female rats of the AIIMS colony (derived from the Wistar strain) were ovariectomized at age 60–90 days, and the vaginal smears were followed daily to ascertain complete anestrus. After a priming dose of estradiol, the animals were allowed to revert to anestrus and were then used in the experiments. All animals were injected subcutaneously with 0.6 μg estradiol-17β daily for four days, and the vaginal smears were recorded starting with the first day of injection. In the experimental groups, cotton plugs saturated with actinomycin D solution (0.5–5.0 μg) were inserted in the vagina and replaced twice daily. The control group underwent the same

procedure with 0.9% saline-saturated plugs. On the fourth day after initiating treatment the animals were sacrificed, the uteri were weighed on a torsion balance, and the vaginae fixed in Bouin's fixative for histologic preparation and study.

Inhibition of gonadotrophin action on the testis: Immature males ranging from 20 to 35 days of age[7] were utilized to test the effect of intratesticular actinomycin D on the response of the testis to systemically administered chorionic gonadotrophin. Each animal served as its own control. During the ten-day period of gonadotrophin administration (62.5 IU daily), the left testis was injected daily with 0.05 μg or 0.1 μg actinomycin D in a volume of 0.05 ml saline. The 27-gauge hypodermic needle was inserted through the scrotum and tunica albuginea, and the dosage delivered from a 0.25 ml tuberculin syringe. The right testis was treated similarly with an equal volume of saline. To test the specificity of the actinomycin D effect, an identical procedure was followed with streptomycin and puromycin at dosages as high as 10 or 100 times that employed for actinomycin D. After ten days of treatment the animals were sacrificed, the testes were weighed separately on a torsion balance, and testis and epididymis were immersed in Bouin's fixative for routine histologic preparation.

Results and Discussion.—Estradiol administration to ovariectomized rats causes proliferation of the endometrium as well as growth and keratinization of the vaginal mucosa. The hormone action begins with a marked increase in RNA content and RNA synthesis rate in the uterus.[6, 8] According to these reports, the latter event precedes the active phase of protein synthesis. In the work now reported, the vaginal mucosa has been selected as the target organ of estradiol action. The inhibition of RNA synthesis has been achieved by local administration of actinomycin D, which has been adequately demonstrated to inhibit DNA-dependent RNA biosynthesis[9–11] in a variety of bacterial and mammalian cells. Actinomycin D (0.5 μg), applied locally by means of cotton plugs, inhibited completely the development of vaginal cornification (Table 1). The vaginal smear remained of the castrate type throughout the period of observation, and the histologic preparations showed no evidence of cornification. Estrogen-treated animals with saline-saturated vaginal plugs developed cornified vaginal smears on the second or third day of treatment. That the actinomycin D blockade was limited to the vagina is revealed by the uterotropic effect noted in treated animals (Table 1). These observations suggest that the secondary biologic effect of estradiol in causing proliferation and cornification of the vaginal epithelium can be completely blocked as a result of the inhibitory effect of actinomycin D on RNA synthesis in the target tissue.

TABLE 1

INHIBITION OF ESTRADIOL-INDUCED VAGINAL CORNIFICATION BY LOCAL APPLICATION OF ACTINOMYCIN D

Treatment (Subcutaneous) daily	Treatment (Intravaginal) topical	Number of animals	No. with vaginal cornified within 72 hr	Average Uterine Weight Wet mg	Average Uterine Weight Dry mg
Nil	Nil	6	0	84	17
0.6 μg estradiol-17β	Saline only	6	6	204	49
0.6 μg estradiol-17β	0.5–5.0 μg* actinomycin D	9	0	195	47.5

* Cotton plugs saturated with 0.5, 2.0, or 5.0 μg actinomycin D in saline solution had the identical effect of inhibiting vaginal cornification without preventing uterine stimulation. Three castrate females were employed at each dose level.

Further observations were made utilizing as the hormone-target organ system, the effect of exogenous gonadotrophin (human chorionic gonadotrophin) on the development of the immature rat testis. In gonadotrophin-treated animals, intratesticular injection of doses as low as 0.05 μg of actinomycin D daily caused a significant inhibition of testicular growth as compared to the contralateral control testis (Table 2). Similar treatment with streptomycin or puromycin did not influence the response of the testis to gonadotrophin. A daily dose of 5.0 μg puromycin, 100 times the effective dose of actinomycin D, failed to cause a significant difference in weight between the treated and control testes.

TABLE 2

EFFECT OF INTRATESTICULAR INJECTION OF ACTINOMYCIN D, PUROMYCIN, OR STREPTOMYCIN ON TESTICULAR RESPONSE TO CHORIONIC GONADOTROPHIN*

Antibiotic†	Daily dose (μg)	Age at start (days)	Final Testis Weight: Right (saline) mg ± S.D.‡	Final Testis Weight: Left (antibiotic) mg ± S.D.	P value
Actinomycin D	0.05	35	830.8 ± 44.5	539.8 ± 63.1	$P < 0.02$
Actinomycin D	0.1	25	410.7 ± 30.8	227.2 ± 63.6	$P < 0.05$
Streptomycin	0.5	21	343.3 ± 7.3	348.4 ± 2.97	$P > 0.5$
Puromycin	0.5 or 1.0	25	406.5 ± 46.8	408.3 ± 62.5	$P > 0.5$

* Chorionic gonadotrophin was administered subcutaneously at a daily dose of 62.5 International Units.
† Six animals were employed in each group.
‡ Standard deviation was calculated by the formula: S.D. = $\sqrt{\frac{\Sigma d^2}{n}}$.

Cross sections of actinomycin D-treated testes revealed a mixed picture with large areas of seminiferous tubules in the immature state, nonpatent, small in diameter (Fig. 1). In other areas of the gland some tubules had enlarged and become patent, and complete spermatogenesis could be seen. Nevertheless, in keeping with the small weight of the gland, immaturity of the tubules was the characteristic histologic finding. The Leydig cells were nonaggregated and gave no evidence of maturation changes. The epididymis contained some spermatozoa, but the predominant feature was the presence of large numbers of sloughed immature germ cells, characteristic of testicular impairment. In contrast, the saline-treated testes from the same animals had responded normally to gonadotrophin treatment. Spermatogenic waves were complete in most tubules, and tubular growth and tortuosity was apparent (Fig. 2). The corresponding epididymis was filled with spermatozoa.

To confirm the specificity of the actinomycin D blockade, gonadotrophin-treated immature males received 0.05 μg actinomycin D in the left testis and considerably higher doses of puromycin in the right testis. In each case, testicular development proceeded in the puromycin-treated testis, while the actinomycin D-injected gland remained small and immature (Table 3).

TABLE 3

COMPARATIVE EFFECT OF ACTINOMYCIN D AND PUROMYCIN ON TESTICULAR RESPONSE TO CHORIONIC GONADOTROPHIN

Actinomycin D μg	Puromycin μg	Final Testis Weight: Left (actinomycin D) mg ± S.D.	Final Testis Weight: Right (puromycin) mg ± S.D.	Significance
0.05	0.5	334.7 ± 47.7	507 ± 27.2	$P < 0.01$
0.05	2.0	226.8 ± 17.9	462 ± 25.8	$P < 0.01$

See footnotes, Table 2.

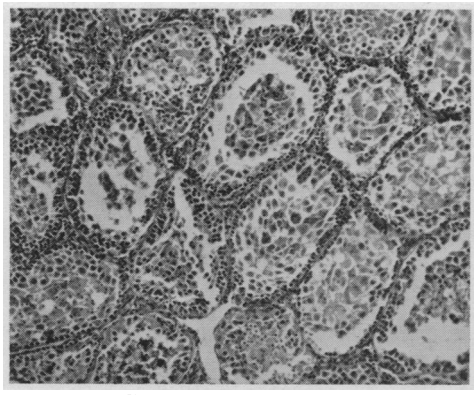

FIG. 1.—Cross section of testis from 45-day-old rat which received 62.5 IU chorionic gonadotrophin for ten days systemically and had concurrent daily intratesticular injections of 0.05 μg actinomycin D. Note immature tubules, incomplete spermatogenesis, and no evidence of gonadotrophin stimulation.

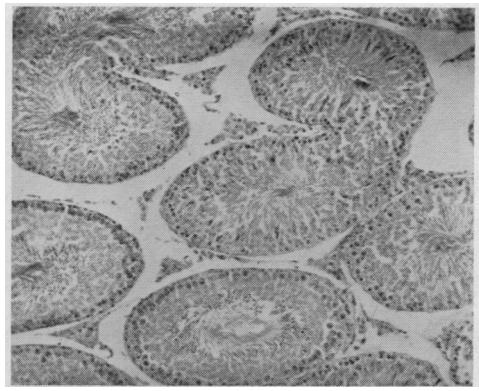

FIG. 2.—Cross section of contralateral testis of animal described in Fig. 1. This gonad received intratesticular injections of saline during the period of gonadotrophin treatment. Note complete spermatogenesis, tubular growth, and indication of normal response to gonadotrophin.

Finally, a group of four 20-day-old males (littermates) was treated with 0.05 μg actinomycin D daily for ten days in the left testis and the equivalent volume of saline in the right testis. It could be expected that puberal levels of endogenous gonadotrophin release and the initiation of puberal changes in the testes would not occur during the treatment period. The animals received no exogenous gonadotrophin. At the completion of the experiment the weight of the saline-treated testes averaged 109 ± 11 mg, and the actinomycin D-treated glands were essentially the same in average weight, 111 ± 9.1 mg. This indicates that the quiescent gonad is not significantly affected by local injection of actinomycin D. Consideration of the total experimental series would suggest that the ability of the testis to respond to gonadotrophin stimulation is blocked.

In view of the recognized interference by actinomycin D in the formation of polyribonucleotides, these results indicate the necessity of initial stimulation of RNA synthesis in order to achieve target organ changes in response to estradiol or chorionic gonadotrophin stimulation. Since similar findings have been reported with respect to the action of growth hormone,[4] thyroxin,[12] and androgen,[13] the possibility is suggested that the unifying mechanism in the action of a variety of stimulatory hormones, irrespective of their chemical nature, is the triggering of messenger RNA synthesis, specific for initiating the secondary biologic events characteristic of the target organ concerned.

Summary.—Estradiol-induced vaginal cornification in ovariectomized rats can be prevented by the intravaginal application of actinomycin D. The uterotrophic effect of the systemically administered estrogen is not affected in these animals. Intratesticular injection of actinomycin D prevents the immature rat testis from responding to exogenous gonadotrophin administration, while puromycin and streptomycin do not cause a similar blockade of hormone stimulation. It is suggested that a variety of stimulatory hormones, irrespective of their chemical nature, are characterized by the ability to influence the synthesis of messenger RNA as a prerequisite for the secondary biologic events characteristic of the particular target organ.

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