Effect of Actinomycin D on the Induction of Uterine Alkaline Phosphatase of Growing Rats by 17β-Estradiol

LERNER and co-workers[1] and BIALY and PINCUS[2] showed that high doses of actinomycin D administered s.c. and i.p. inhibited various enzymatic activities of uteri which were stimulated with 17β-estradiol. The inhibition was attributed to a non-specific toxic effect of the drug on the animals. STEPLEWSKI[3] demonstrated that actinomycin D injected i.p. to ovariectomized rats blocked the uterine alkaline phosphatase activity induced by estradiol. MANSOUR[4] reported that the intrauterine application of actinomycin D depressed the induction of uterine alkaline phosphatase by 17β-estradiol. We have shown that the induced activity may or may not be inhibited depending upon the amount of actinomycin D administered by the intrauterine route[5].

In this study the actinomycin was administered s.c. to growing rats to observe the effect of the drug on the body and uterine weights and uterine alkaline phosphatase activity induced by 17β-estradiol.

Actinomycin D (Lyvoc-Cosmogen) was purchased from Merck, Sharp and Dohme. 17β-estradiol was purchased from Mann Research Co., New York, N. Y. Growing female rats of Sprague-Dawley strain weighing 35–40 g were divided in 4 groups of 10 animals each. Control animals, group 1, received s.c. 0.05 ml of sesame oil. Animals of group 2 received s.c. 10 μg of actinomycin D in 0.02 ml of water. Group 3 animals received s.c. 0.5 μg of 17β-estradiol in 0.05 ml of sesame oil every other day for a total of 1.5 μg. Animals of group 4 received s.c. 10 μg of actinomycin in 0.02 ml of water followed by 0.5 μg of estradiol in 0.05 ml of sesame oil 1 h later. The animals were killed by decapitation on the six day. The uterine alkaline phosphatase activity was determined as outlined in Sigma Technical Bulletin No. 104[6] and expressed as nmoles of *p*-nitrophenol formed per h/μg of DNA of the homogenate. A small piece of the uteri was fixed in Bouin's solution, sectioned and stained with hematoxylin and eosin. DNA was determined with the diphenylamine reaction[7].

The Table shows the effect of estradiol and actinomycin D on the body and uterine weights and uterine alkaline phosphatase activity. The average weight gain of the control and estradiol-treated animals was about 3 g/day/animal in contrast to 0.7 g/day/animal of the actinomycin-treated groups. Actinomycin D (10 μg/40 g body weight) had a suppressive influence on the body weights and uterine weights in comparison to the control and estradiol-treated animals. The uterine alkaline phosphatase activity was not affected. The results are in agreement with the reports of BIALY and PINCUS[2] that the systemic administration of actinomycin causes non-specific toxicity before any inhibition of induced uterine enzymatic activities is observed. It is known that actinomycin D does have effects unrelated to RNA synthesis[8,9].

Histological sections of the uteri showed intact endometrium and findings compatible with estrogen stimulation. Intrauterine application of actinomycin D (6 μg) caused a denudation of the endometrium[5]. SZEGO and LIPPE[10,11] reported that the suppressive influence of actinomycin on the uterus stimulated by estrogen was mediated by activation of adrenocortical hypersecretion. NICOLETTE and MUELLER[12] however, concluded that this antibiotic prevented most of the uterine response to estrogen in adrenalectomized rats. Although our results do not resolve this problem, it is conceivable that at low concentration the antibiotic acts as a toxic agent and incites hypersecretion of adrenocorticoids and at higher doses inhibits uterine metabolism directly.

Effect of 17β-estradiol and actinomycin D on the body weight, uterine weight and uterine alkaline phosphatase activity

	Body weight g	Uterine weight (mg)	Alkaline phosphatase (nmoles *p*-nitrophenol/ mg DNA/h)
Controls	57 ± 1.6	26 ± 2.1	4.2 ± 0.5
Actinomycin-treated	41 ± 1.3	20 ± 1.1	4.4 ± 0.5
17β-Estradiol	52 ± 0.8	111 ± 11.6	7.4 ± 0.5
17β-Estradiol, actinomycin-treated	39.9 ± 1.1	83 ± 8.6	7.1 ± 0.5

The mean value (± S.E.) for 10 animals in each group is presented.

Zusammenfassung. Es wurde Actinomycin D s.c. an junge Ratten verabreicht. Beobachtet wurde die Wirkung der Substanz auf Körpergewicht, Uterusgewicht sowie auf die alkalische Phosphataseaktivität des Uterus vor und nach 17β-Oestradiol Stimulation. Actinomycin D (10 μg/40 g/Ratte) verhinderte sowohl bei mit Oestradiol behandelten Tieren, wie auch bei den Kontrollen ein Ansteigen des Körper- oder Uterusgewichts. Der Einfluss auf die durch Oestradiol induzierte alkalische Phosphataseaktivität des Uterus blieb minimal und bedeutungslos.

V. BOTTE and S. S. KOIDE

The Population Council, Rockefeller University, New York (N. Y. 10021, USA) and Istituto di Zoologia e Anatomia Comparata, Università di Camerino (Macerta, Italy),
9 May 1968.

[1] L. J. LERNER, R. HILF, A. R. TURKHEIMER and J. MICHEL, J. Endocr. *33*, 531 (1965).
[2] E. BIALY and G. PINCUS, Endocrinology *78*, 236 (1966).
[3] Z. STEPLEWSKI, Bull. Acad. pol. Sci. *14*, 387 (1966).
[4] A. M. MANSOUR, Acta Endocr. *54*, 541 (1967).
[5] V. BOTTE, S. S. KOIDE and S. J. SEGAL, Endocrinology *82*, in press.
[6] Tech. Bull. No. 104, Sigma Chemical Co., St. Louis, Mo., USA (1963).
[7] Z. DISCHE, in *The Nucleic Acids* (Ed. E. CHARGRAFF and J. N. DAVIDSON; Academic Press, New York 1955), Vol. 1, p. 285.
[8] M. REVEL, H. H. HIATT and J. REVEL, Science *146*, 1311 (1964).
[9] J. LASZLO, D. S. MILLER, K. S. MCCARTY and P. HOCHSTEIN, Science *151*, 1007 (1966).
[10] B. M. LIPPE and C. M. SZEGO, Nature *207*, 272 (1965).
[11] C. M. SZEGO and B. M. LIPPE, Steroids, Suppl. II, 235 (1965).
[12] J. A. NICOLETTE and G. C. MUELLER, Endocrinology *79*, 1162 (1966).