11922

# Zoological Specificity of the Tumoral Reaction Towards Estrogens in Rats.*

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While abdominal fibroids can be easily elicited in guinea pigs by a prolonged treatment with estrogens, they have never been reported in rats, mice and monkeys under similar experimental conditions. Two different possibilities may account for this zoological specificity of the tumoral reaction: first, the conjunctive tumorigenic threshold may be different according to the species; secondly, the reacting cellular type of the mesenchyme may be absent in certain species. Our quantitative experiments with abdominal fibroids in the guinea pig[1] offer the opportunity to study these two possibilities. We have, therefore, investigated the possibility of eliciting abdominal fibroids in rats by injecting a highly potent tumorigenic ester of estradiol in quantities many times those necessary to induce these tumors in the guinea pig.

Eleven castrate female rats (103 to 260 g) received thrice weekly subcutaneous injections of 10 $\gamma$ of $\alpha$-estradiol given as the 17-caprylic acid ester (14.6 $\gamma$ of the ester); 7 castrate (60 to 295 g) and 9 non-castrate (65 to 247 g) females received 80 $\gamma$ per injection. The animals were autopsied 90 to 100 days after the first injection. Uterine weights ranged between 160 and 1200 mg. Atypical proliferation of uterine epithelium with thickening, cornification and sometimes of precancerous developments were found even with 10 $\gamma$ per injection (Fig. 1). Hypophyseal hypertrophy also was considerable (Fig. 2). Hypophyseal weights of 17 to 44 mg were reached with 10 $\gamma$ per injection and of 20 to 192 mg with 80 $\gamma$ per injection. The average hypophyseal weight per 100 g body weight was 14 mg in the group receiving 10 $\gamma$ per injection, and 33 mg in those receiving 80 $\gamma$ per injection, as against 4 mg per 100 g body

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1 Lipschütz, A., Vargas, L., Jr., Baeza-Rosales, H., and Baeza-Herrera, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 76.

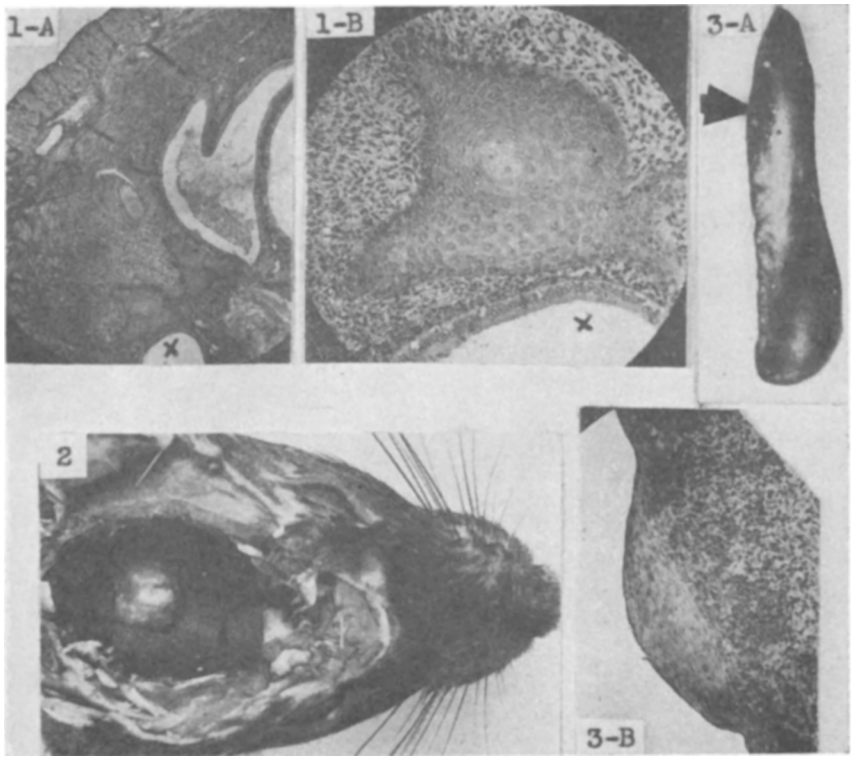


FIG. 1. Uterine horn of castrate rat (220 g) which received 38 injections of 10 γ of estradiol (in form of the 17-caprylate) in the course of 91 days. A. × 23. Cystic hyperplasia with beginning pyometra. Epithelial ingrowths into the submucosa. Note cornification in the lower right of the photograph. B. × 100. Part of the epithelial ingrowth, to show concentric disposition of cells; beginning of pearl formation in the center (XIV, R. 8).

FIG. 2. × 1.5. Enormous hypertrophy of the anterior lobe of the hypophysis in female rat, 38 injections of 80 γ, 91 days. Weight of animal 120 g; of hypophysis—66 mg (XIV, R. 23).

FIG. 3. Spleen of male rat (280 g), 28 injections of 80 γ, 62 days. A. × 1.5. Excrescences of whitish color on the surface. B. × 100. Fibrous structure of splenic excrescence (XIV, D. 24).

weight in the normal or castrate female.[2] Body weight which first increased, decreased later in all animals.† *None of the 27 females showed uterine or extragenital fibroids or fibrous strands.*

The quantity of the monocaprylate given in the above experiments was 5 to 40 times that necessary to elicit fibroids in the guinea pig (10 to 80 γ, compared with 2 γ). When calculated per 100 g of body

[2] Lipschütz, A., *Arch. Portugu. Soc. Biol.*, 1936, **5**, 179; Lipschütz, A., and Villagrán, G., *C. R. Soc. Biol.* (Paris), 1936, **131**, 203.

† The average weight of hypophysis per 100 g body weight has been calculated according to maximal body weights reached.

weight the quantities injected in the rat were from 15 to 120 times that used in the guinea pig. As seen from the above statements various severe effects (decrease of body weight, atypical growth of uterine epithelium, and great increase in size of hypophysis) were produced in the rat but no fibroids were elicited.

Similar experiments were made with 30 non-castrate males (33 to 330 g) which received 2 to 80 γ per injection thrice weekly in the course of 81 to 102 days. Hypophyseal weights 3 times that of normal males were obtained with 2 to 4 γ per injection (15 to 35 mg); the average hypophyseal weight per 100 g body weight was 8 mg compared with 2.5 mg in the normal male.[3] The corresponding averages with 8 to 10 γ per injection were 15 mg, and 34 mg with 80 γ. In the latter group there were hypophyseal weights of 110 to 140 mg in 4 out of 10 males. *No fibroids or fibrous strands were found.* On the contrary, with 80 γ of esterified estradiol and especially of the monocaprylate most male guinea pigs show fibrous strands and sometimes also fibroids.[3]

One may be inclined to conclude from these results that in the rat the cellular substratum which would be able to react towards estrogens with development of fibroids is lacking. However, there is one observation which seems contrary to this.

In a single male which died after having been treated for only 62 days (80 γ per injection) small white excrescences were found on the surface of the spleen (Fig. 3). Their microscopical structure was fibrous and similar, though not quite identical, to that of the "tumoral seed" of the guinea pig treated with estrogens.[4, 1] Though the tumoral seed cannot be as yet identified with fibroids, extensive work of Lipschütz and associates with guinea pigs has shown beyond any doubt that these small excrescences are to be considered as the sign of a beginning tumorigenic action[5] especially in the male guinea pig which has a lesser tumoral sensitivity than the female. The occurrence of similar structures in the rat, though in only one out of 58 animals treated, would be in favor of the idea that the difference between guinea pig and rat is only a question of threshold.

Whether or not this explanation is justified cannot as yet be said with certainty. It is not likely that differences of threshold can account for the differential behavior of the uterine mucosa and of

[3] Koref, O., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol* (Paris), 1939, **130**, 303; Jedlicky, A., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol.*, (Paris), 1939, **130**, 1464.

[4] Lipschütz, A., Iglesias, R., and Vargas, L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 788.

[5] Vargas, L., Jr., and Lipschütz, A., *C. R. Soc. Biol.* (Paris), 1938, **129**, 810.

the anterior lobe of the hypophysis in guinea pig and rat. Though atypical proliferation of the endometrium starts in the guinea pig even with minute quantities of esterified estradiol[6] one never observes cornification in this species even when great quantities are given for 10 months or more (unpublished results). On the contrary, in the rat cornification of the uterine mucosa occurs under these experimental conditions. Again, we never observed great hypophyseal hypertrophy in the guinea pig treated with estrogens (unpublished results of Lipschütz and Vargas). However, as shown by the present experiments, enormous hypophyseal enlargement in the rat, described by former workers as tumoral,[7] can be elicited with total amounts ranging from less than 0.4 to 3.0 mg of estradiol when given as the monocaprylate (40 injections of 10 to 80 γ).

*Summary.* A quantity of the monocaprylate of α-estradiol up to 40 times that which is able to induce abdominal fibroids in the *guinea pig*, is insufficient to elicit similar tumors in the *rat*. The same or smaller quantity induces in the rat a highly pronounced atypical proliferation of epithelial tissues (endometrium, anterior lobe of the hypophysis).

## 11923

## Comparative Conjunctive Tumorigenic Action of Three Different Esters of Estradiol.*

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Our previous work has shown that the conjunctive tumorigenic action of estradiol in the guinea pig is greatly enhanced by esterification.[1] Two hundred γ of free estradiol[2, 3] are less active than 5 γ of estradiol given as monobenzoate.[4] The monocaprylic ester of

[6] Lipschütz, A., Vargas, L., Jr., Jedlicky, A., and Bellolio, P., *Am. J. Cancer*, 1940, **39**, 185.

[7] McEuen, C. S., Selye, H., and Collip, J. B., *Lancet*, 1936, **1**, 775; Zondek, B., *Lancet*, 1936, **1**, 776.

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