

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<sup>6</sup> one never observes cornification in this species even when great quantities are given for 10 months or more (unpublished results).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 Again, we never observed great hypophyseal hypertrophy in the guinea pig treated with estrogens (unpublished results of Lipschütz and Vargas).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,<sup>7</sup> can be elicited with total amounts ranging from less than 0.4 to 3.0 mg of estradiol when given as the monacrylate (40 injections of 10 to 80  $\gamma$ ).

*Summary.* A quantity of the monacrylate of  $\alpha$ -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).

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### 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.<sup>1</sup> Two hundred  $\gamma$  of free estradiol<sup>2, 3</sup> are less active than 5  $\gamma$  of estradiol given as monobenzoate.<sup>4</sup> The monacrylic ester of

<sup>6</sup> Lipschütz, A., Vargas, L., Jr., Jedlicky, A., and Bellolio, P., *Am. J. Cancer*, 1940, **89**, 185.

<sup>7</sup> McEuen, C. S., Selye, H., and Collip, J. B., *Lancet*, 1936, **1**, 775; Zondek, B., *Lancet*, 1936, **1**, 776.

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estradiol was shown in our first set of experiments to be even more active than the monobenzoic one.<sup>5</sup> The results of a more extensive comparative study of the different esters are given in this paper.

As seen from Table I most animals show some fibrous reaction even with 5  $\gamma$  per injection; there may be fibrous strands in the mesentery and a tumoral seed on the spleen, the stomach and the parts of abdominal wall surrounding the spleen. However, there is only a small number of animals with individual tumors of a certain size. We applied in our experiments a system of classification<sup>6</sup> which greatly facilitated our work, though the system is a rather arbitrary one (Figs. 1 and 2). The *total tumoral effect* represents the sum of 4 numerical values relating to the size of tumors of the uterus (subserous, mesometrial), "apical" tumors, tumors of the

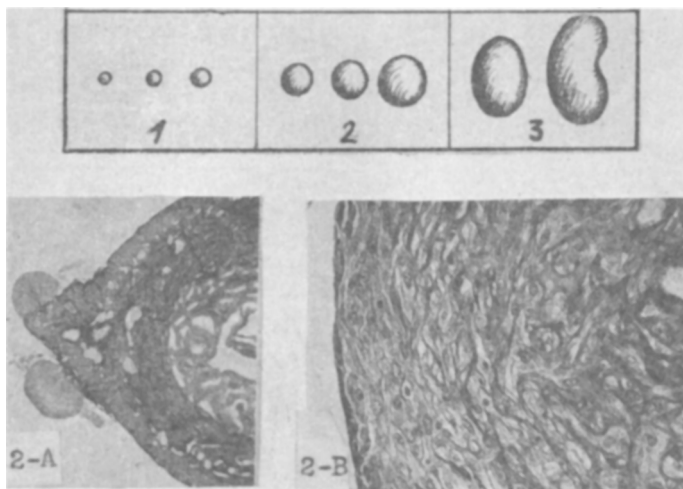


Fig. 1. Diagram of classification of tumors according to size. *Natural size.*

Fig. 2. Two subserous uterine fibroids induced by 40 injections of 5  $\gamma$  of monobenzoate in the course of 3 months (VI. 96). A.  $\times 10$ . Size of individual tumors somewhat less than class 1. Fibroids at both sides of ventral ridge of myometrium. Adenomatous polyps in the uterine cavity. B.  $\times 200$ . Spindle shaped cells at the periphery; superficial cells flattened. Sclerotization in the depth of the fibroid.—Van Gieson.

<sup>1</sup> Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol. (Paris)*, 1939, **130**, 9.

<sup>2</sup> Lipschütz, A., Rodríguez, A., and Vargas, L., Jr., *C. R. Soc. Biol. (Paris)*, 1939, **130**, 939.

<sup>3</sup> Rodríguez, F., *Public. Med. Exp. (Chile)*, 1940.

<sup>4</sup> Bellolio, P., *Public. Med. Exp. (Chile)*, 1939.

<sup>5</sup> Jedlicky, A., Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol. (Paris)*, 1939, **130**, 1466. The 17 benzoate-3-n-butyrate of estradiol also proved to be tumorigenic but the number of experiments was too small to allow quantitative interpretation.

<sup>6</sup> Lipschütz, A., and Vargas, L., Jr., *C. R. Soc. Biol. (Paris)*, 1939, **131**, 27.

digestive tract and the abdominal wall, and splenic tumors (Figs. 3 and 4). The tumors of each of these 4 regions are arranged in 4 classes characterized by the values 0.5, 1, 2 and 3. The size of tumors of the last 3 classes is as indicated in Fig. 1. With 5  $\gamma$  of estradiol per injection given as the monobenzoic or dipropionic ester only 8 out of 28 animals showed tumors of size 1 or over. The average total effect was 1. In each of these 2 lots there was only one animal with a total effect of 3 or 3.5 (Fig. 3). On the other hand, with the same quantity of estradiol given as the monocaprylic ester, 3 out of 4 animals showed a considerable tumoral reaction and the average total effect was 5, instead of 1 with the 2 other esters. Similar comparative results were obtained with 10  $\gamma$  of esterified estradiol per injection (Table I).

We tried to explain the greater tumorigenic action of the monobenzoic ester as compared with that of free estradiol by the assumption<sup>1</sup> that by injecting the ester, which is slowly absorbed, thrice weekly a stable folliculinaemia might be maintained more easily than by injecting the free hormone which is rapidly absorbed and

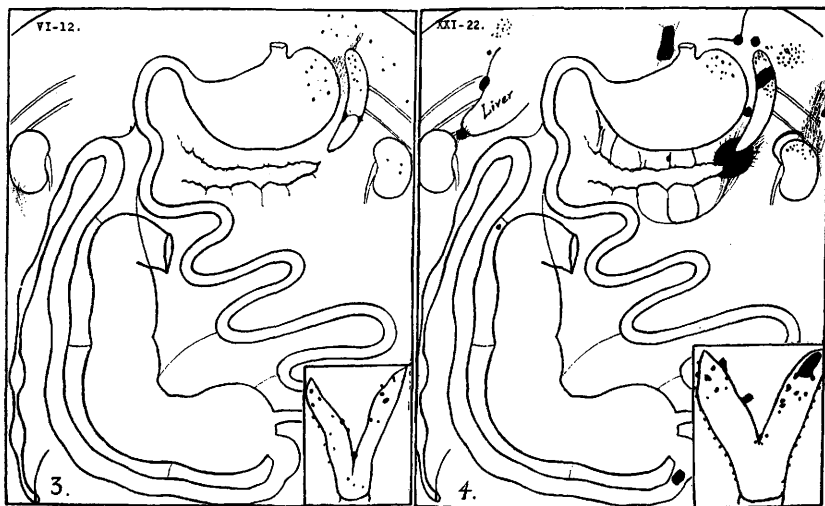


Fig. 3. Maximal reaction in animal receiving 40 injections of 5  $\gamma$  of dipropionate in the course of 3 months (VI. 12). Multiple small uterine fibroids, subserous and mesometrial (class 2, due to their multiplicity). Tumoral seed on the stomach, abdominal wall, left kidney (class 0.5). Tumoral seed and small tumors on the spleen (somewhat less than class 1). Fibrous strands around spleen and right kidney. Total tumoral effect 3.5.  $\times 0.4$ .

Fig. 4. Maximal reaction in animal receiving 39 injections of 5  $\gamma$  of monocaprylate in the course of 3 months (XXI. 22). Multiple uterine fibroids, subserous and mesometrial (class 3). Various tumors of different size of the digestive tract, liver and abdominal wall (class 3) and of the spleen (class 3). Tumoral seed on spleen, stomach, abdominal wall and left kidney. Cyst in thickened capsule of left kidney. Fibrous strands around upper pole. Total tumoral effect 9.  $\times 0.4$ .

TABLE I.  
Seventy Castrate Female Guinea Pigs, Treated with Subcutaneous Injections of Various Esters of Estradiol During 3 Months.

| Ester        | Quantity estradiol*<br>inj.<br>thrice weekly | No. of inj. | Total animals | No. with some conjunctive reaction | No. with tumors, class 1 or larger | Avg total tumoral effect | No. of animals with total tumoral effect of 3 or over |
|--------------|----------------------------------------------|-------------|---------------|------------------------------------|------------------------------------|--------------------------|-------------------------------------------------------|
| Monobenzoic  | 5                                            | 40          | 14            | 14                                 | 2                                  | 1                        | 1                                                     |
| Dipropionic  | 5                                            | 40          | 14            | 14                                 | 6                                  | 1                        | 1                                                     |
| Monocaprylic | 5                                            | 40          | 4             | 4                                  | 3                                  | 5                        | 3                                                     |
| Monobenzoic  | 10                                           | 40          | 15            | 12                                 | 4                                  | 1                        | 0                                                     |
| Dipropionic  | 10                                           | 40          | 14            | 14                                 | 4                                  | 1                        | 1                                                     |
| Monocaprylic | 10                                           | 39          | 9             | 9                                  | 9                                  | 6                        | 5                                                     |

\*The equivalent of estradiol is given, and not the weight of the esterified substance.

destroyed. Experiments with tablets of free estradiol introduced under the skin by which a continuous flow of hormone in the body is maintained<sup>7</sup> favor our assumption. But why then the difference in the tumorigenic action of the various esters? It has been shown by Miescher, Scholz and Tschopp<sup>8</sup> that when the total dose is given in 2 injections on 2 consecutive days, certain esters induce in the rat a greater and more lasting augmentation of the uterus than others. One may assume that in the work of Miescher, *et al.*, the prolonged action of certain esters is responsible for their greater hysterotropic effect. However, in our work with tumorigenesis in which the different estrogens are injected thrice weekly in the course of several months, this explanation seems insufficient. One may tentatively suggest that esterification is also a means of protecting the natural estrogen against inactivation and that the degree of protection differs according to the acid used. This suggestion is indeed speculative and needs experimental demonstration.

*Summary* When 40 subcutaneous injections of 5 to 10  $\gamma$  of an esterified estradiol (monobenzoic, dipropionic or monocaprylic ester) or a total of 200 to 400  $\gamma$  are given to castrate female guinea pigs in the course of 3 months, uterine and extrauterine fibroids will appear. The number of animals in which fibroids are induced is larger with the monocaprylic ester; the size of the fibroids also is much more remarkable with the monocaprylate.

<sup>7</sup> Lipschütz, A., and Vargas, L., Jr., *Lancet*, 1939, 1, 1313.

<sup>8</sup> Miescher, K., Scholz, C., and Tschopp, E., *Biochem. J.*, 1938, 32, 725.