ice-salt mixture at −5°C for 2 minutes. At the end of 2 weeks the immersion was repeated without any apparent effect on the animals which were carefully observed for an hour following the second exposure. All of them survived.

In order to be certain that the size of the animals had nothing to do with their lack of response to this treatment we repeated this portion of the experiment using 10 male guinea pigs all of which weighed between 200 and 250 g (the size of those used by Karady). Again the second exposure was entirely without effect in producing any signs of shock. After another lapse of 2 weeks their hind limbs were again immersed in the ice-salt mixture. Following this third exposure they were carefully observed for a period of one hour and once more failed to show any anaphylactic response. All animals survived.

Finally, another group of 7 male guinea pigs (weighing 200 to 250 g) were subjected to heat by immersing their hind limbs in water at 56°C for 2 minutes. Three weeks later they were again subjected to the same treatment, but no signs of anaphylaxis were observed in any of them during the hour following the re-exposure, and all survived.

Summary. We have been unable to confirm the report of Karady that guinea pigs can be sensitized anaphylactically to heat or cold by heating or chilling their limbs. We were also unable to confirm Karady's report that guinea pigs can be sensitized to heated or chilled guinea pig serum.

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Uterine and Extrauterine Localizations of Experimental Fibroids Induced in Guinea Pig by Prolonged Administration of Estrogens.*

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The occurrence of uterine fibroids was reported in a number of guinea pigs treated for several months with estrogens.[1] *Extrauterine*

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abdominal fibroids also were induced in guinea pigs injected subcutaneously with estrogens in the course of 4 months.[2] Fibroids appear even if quantities as small as 5γ of the monobenzoic ester of estradiol per injection or a total of 400γ are given in the course of 3 months.† All estrogens, free or esterified, natural or synthetic, proved to be tumorigenic.[3] Tablets of estradiol introduced beneath the skin may induce fibroids as early as in 3 weeks.[4] There is an enormous variability as to the localization of uterine and extrauterine fibroids, although when a sufficient number of animals is observed, some typical localizations can be established. A survey of these localizations of experimental fibroids is given in the present paper (Figs. 1-16) based on the results of the autopsy of several hundred females to which the following 10 estrogens were administered: estrone, estriol, estradiol, 4 esters of the latter (monobenzoate, monocaprylate, dipropionate, 17-benzoate-3-n-butyrate), stilbestrol, dipropionate of stilbestrol, hexestrol.

Uterine tumors are mostly subserous (Figs. 3, 4, 14-C) and can become pedunculated (Fig. 2). Very often they are to be found in the mesometrium (Figs. 6, 8); intramural fibroids and those of the submucosa are rare (Fig. 9). The whole surface of the uterine stem and part of the uterine horns may be covered by an extensive fibroid (Fig. 1). The angle between the horns is often the site of a tumor. There may be also a chain of small tumors on the ventral surface of the uterus (Fig. 5).

Subserous fibroids may be present also on the vagina and very rarely in the vaginal submucosa (Fig. 10). In the *castrate* guinea pig an almost constant site of fibroids is near the upper ends of the tubes (Figs. 4, 5); these "apical" tumors probably originate in the mesosalpinx and can attain an enormous size (Figs. 7, 14-C), especially when joining with neighboring tumors of the spleen (Fig. 4) or the abdominal wall. The apical tumor can descend also into the pelvis (Fig. 14-C). Sometimes enormous tumoral masses en-

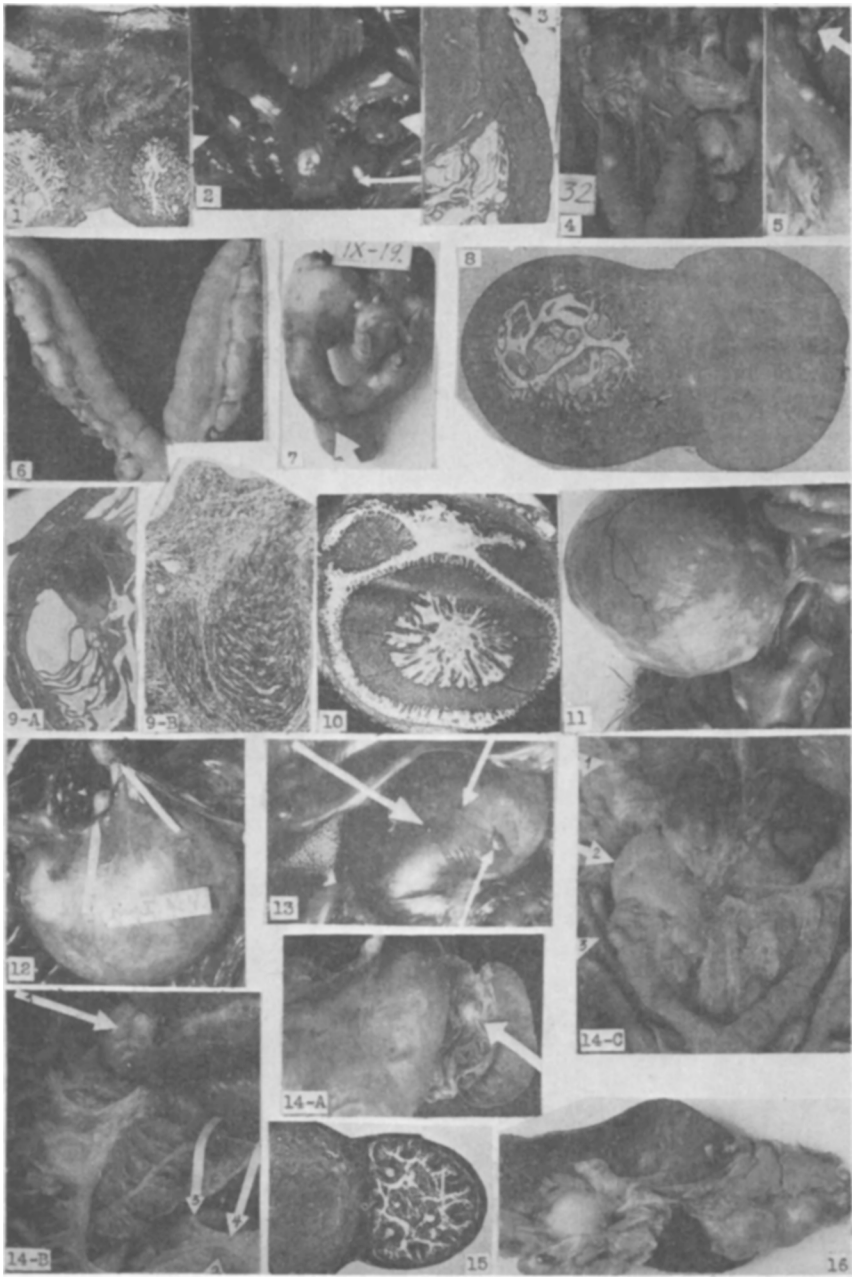
1 Nelson, W. O., *Anat. Rec.* 1937, **68**, 99; *Endocrinology*, 1939, **24**, 50; Moricard, R., and Cauchoix, J., *C. R. Soc. Biol.* (Paris), 1938, **129**, 556.

2 Lipschütz, A., and Iglesias, R., *C. R. Soc. Biol.* (Paris), 1938, **129**, 519; Iglesias, R., *Public. Med. Exp.* (Chile), 1938.

† Lately we have found that fibroids can be induced by as little as 2γ per injection or a total of only 80γ of estradiol in the course of 3 months when the monocaprylic ester is used (Lipschütz, Vargas, Baeza-Rosales and Baeza Herrera, to be published soon).

3 See Lipschütz, Vargas, Koref, Jedlicky, Bellolio, Murillo, Rodríguez, Chaume, Szabó, Ruz, *C. R. Soc. Biol.* (Paris), 1938-1939, **129**, 131; *The Lancet*, 1939-1940; *Public. Med. Exp.* (Chile), 1939-1940; *Rev. Chil. Hig. y Med. Prevent.*, in press.

4 Lipschütz, A., and Vargas, L., Jr., *The Lancet*, 1939, **1**, 1313.



EXPLANATION OF FIGURES.

In each figure explanation three data are given: the first indicates the quantity per injection, the second the total amount and the third the duration of treatments.

FIG. 1. Estradiol-monobenzoate 40γ (total 2.1 mg) 125 days. Tumor on the

dorsal face of the uterus. No sharp limit between tumor and myometrium. Adenomatous condition of endometrium. × 5.

FIG. 2. Estradiol-monobenzoate 20γ (total 5.8 mg) 183 days. Three pedunculated tumors of uterus.

FIG. 3. Estradiol-monobenzoate 20γ (total 2.6 mg) 310 days. Large subserous tumor with deformation of myometrium. × 5.

FIG. 4. Estradiol-monocaprylate 10γ (total 0.4 mg) 90 days. Subserous uterine tumor. Apical tumors on both horns; the left one with ramifications covering left kidney and adhering to spleen, pancreas and abdominal wall.

FIG. 5. Estradiol-monocaprylate 10γ (total 0.4 mg) 108 days. Chain of subserous tumors on the right horn; the chain corresponds to the muscular elevation present in the normal uterus. Arrow points to small apical tumor.

FIG. 6. Estradiol-monocaprylate 10γ (total 0.4 mg) 91 days. Mesometrial tumors.

FIG. 7. Estradiol-monobenzoate 80γ (total 2.4 mg) 85 days. Apical tumors on both sides. Partial necrosis of uterine horns (arrow).

FIG. 8. Estradiol 400γ (total 20.8 mg) 123 days. No sharp limit between myometrium and mesometrial tumor. Polyps in the uterine cavity. × 5.

FIG. 9. Estradiol 17-benzoate-3-n-butyrate 10γ (total 0.4 mg) 91 days. A—"Fibroids" of the uterine submucosa. Cystic glandular hyperplasia. × 5. B—Same preparation. × 45. No sharp limit between "tumor" and stroma of uterine mucosa.

FIG. 10. Estradiol-monobenzoate 20γ (total 2.6 mg) 310 days. Uterine cervix surrounded by vagina. Two vaginal fibroids, one pedunculated. × 5.

FIG. 11. Estradiol-monobenzoate 80γ (total 3.2 mg) 110 days. Cystic dilatation of right tube with thick fibrous masses in the wall of the cyst. Apical tumor on the left (upper half of left uterus cut away).

FIG. 12. Estradiol-monobenzoate 40γ (total 2.1 mg) 124 days. Tumor near cardias, and tumor of small curvature.

FIG. 13. Estradiol-monobenzoate 80γ (total 5.8 mg) 183 days. Subserous tumors of the stomach.

FIG. 14. Estradiol-monobenzoate 80γ (3.0 mg) 88 days. A—Tumor in the hilum of spleen (arrow). B—Mesenteric tumor (arrow 1). Fibrous strands in the mesentery (between arrows 2, 3 and 4, with small tumor of the intestine (arrow 3). C—Apical tumors, the right one enormous (arrow 2). Subserous uterine tumor (arrow 3). Tumor between diaphragm and abdominal wall (arrow 1).

FIG. 15. Estradiol-monobenzoate 80γ (total 3.9 mg) 122 days. Mesenteric tumor. × 5.

FIG. 16. Estradiol-monocaprylate 10γ (total 0.4 mg) 90 days. Tumors in the hilum of the spleen.

globe the upper third or more of the uterine horn (Fig. 7). There is often no sharp limit between the myometrium and the tumor (Figs. 1, 8). Sometimes the myometrium is deformed by the subserous tumor (Fig. 3). The fibroids of the submucosa are still less delimited (Fig. 9-B).

The subserous *extrauterine* tumors are to be found on most of the abdominal organs: on the stomach (Figs. 12, 13), spleen (Figs. 14-A, 16), pancreas, liver, kidney, urinary bladder; in the epiploon, in the mesentery from the pylorus to the rectum (Figs. 14-B, 15); on the abdominal wall (Figs. 4, 13) and the diaphragm (Fig. 14-C). Very frequently fibrous strands visible to the naked eye can be found on the abdominal wall, the mesentery and other parts of the abdominal cavity.

The uterine or extrauterine localizations have no connection with lymphatic glands.

There is no case with tumors in the thoracic cavity. Tumors present on the abdominal side of the diaphragm may infiltrate between muscle fibres, but the thoracic side of the diaphragm remains always smooth and free of fibrous nodules. No tumors are found at the site of injection and only very exceptionally at the site of subcutaneous implantation of a tablet of estradiol.

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Studies on Fluorescence Associated with Proteins.

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The relative fluorescence intensities of the proteins and their hydrolysates were determined by measuring the dilution required to reduce the fluorescence of a given amount of protein or protein hydrolysate to the same intensity as the fluorescence of a diluted standard solution of quinine bisulfate.

The proteins prepared and studied in this investigation were: casein, wheat gluten, gliadin, glutenin, blood fibrin, gelatin, ovalbumin and zein. Hair and wool were also compared to the above proteins.

When examined in ultraviolet light of wavelengths 3100-4100 Å the proteins give a uniform bluish-white fluorescence in the solid state and a somewhat more green fluorescence in solutions. The fluorescence of these proteins is more green in basic solution than in acid, but the color change is not sharp.

Fluorescence of proteins is destroyed by oxidation with strong nitric acid or by ashing. The small amount of protein ash is not fluorescent in the solid state nor in acid, basic or neutral solution.

Organic solvents do not extract the fluorescent material from the solid protein nor from the protein hydrolysates in acid or basic solution. Likewise, dialysis experiments failed to remove the fluorescent material from protein solutions but after hydrolysis with strong acid the fluorescent material is readily removed from proteins by dialysis.

Hydrolysis of proteins by proteolytic enzymes or alkali produced only a slight increase in the amount of fluorescence. However, hydrolysis with hydrochloric acid, sulfuric acid, perchloric acid or phosphoric acid produced large increases in the fluorescence of those