ours were kept so for 48 hours or longer.‖

Summary. Normal rats placed in a low oxygen atmosphere did not elaborate in their plasma a factor capable of stimulating increased incorporation of Fe^{59} into red cells of normal or hypophysectomized recipients. However, when their liver was acutely damaged with carbon tetrachloride and they were then placed in low oxygen atmosphere, significant amounts of such a factor appeared in their plasma. This is interpreted as further evidence that the liver, under ordinary circumstances, has a significant part in the mechanisms that control red cell production.

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‖ Subsequent studies have shown 1) there is variation in susceptibility of groups of rats to the hepatotoxic effect of carbon tetrachloride. Only those with more severe damage show increased amounts of erythropoietic factor in their plasma at 48 hours. 2) increased levels of plasma erythropoietic factor have been found regularly in the plasma of hypoxic rats without liver damage at 24 hours. This has disappeared in every instance at the 48 hour interval.

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Uterotrophic Effect of Digoxin Administration in the Rat.* (23177)

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Le Winn(1) has described gynecomastia in men receiving prolonged digitalis medication for management of congestive heart failure. He suggested that digitalis steroids have an estrogen-like effect. A search of the literature has failed to reveal any description of estrogen-like stimulation in digitalized human or animal females. During the course of investigating the relationship between cardiotonic glycosides and protein metabolism, we observed that the uteri of mature ovariectomized female rats were trophically stimulated by the administration of digoxin. As a result, experiments were designed to further investigate this observation.

Material and method. Nineteen 68-day-old female Sprague-Dawley rats were bilaterally ovariectomized and divided into 4 groups following a 21-day post-operative period. The groups were treated as follows for

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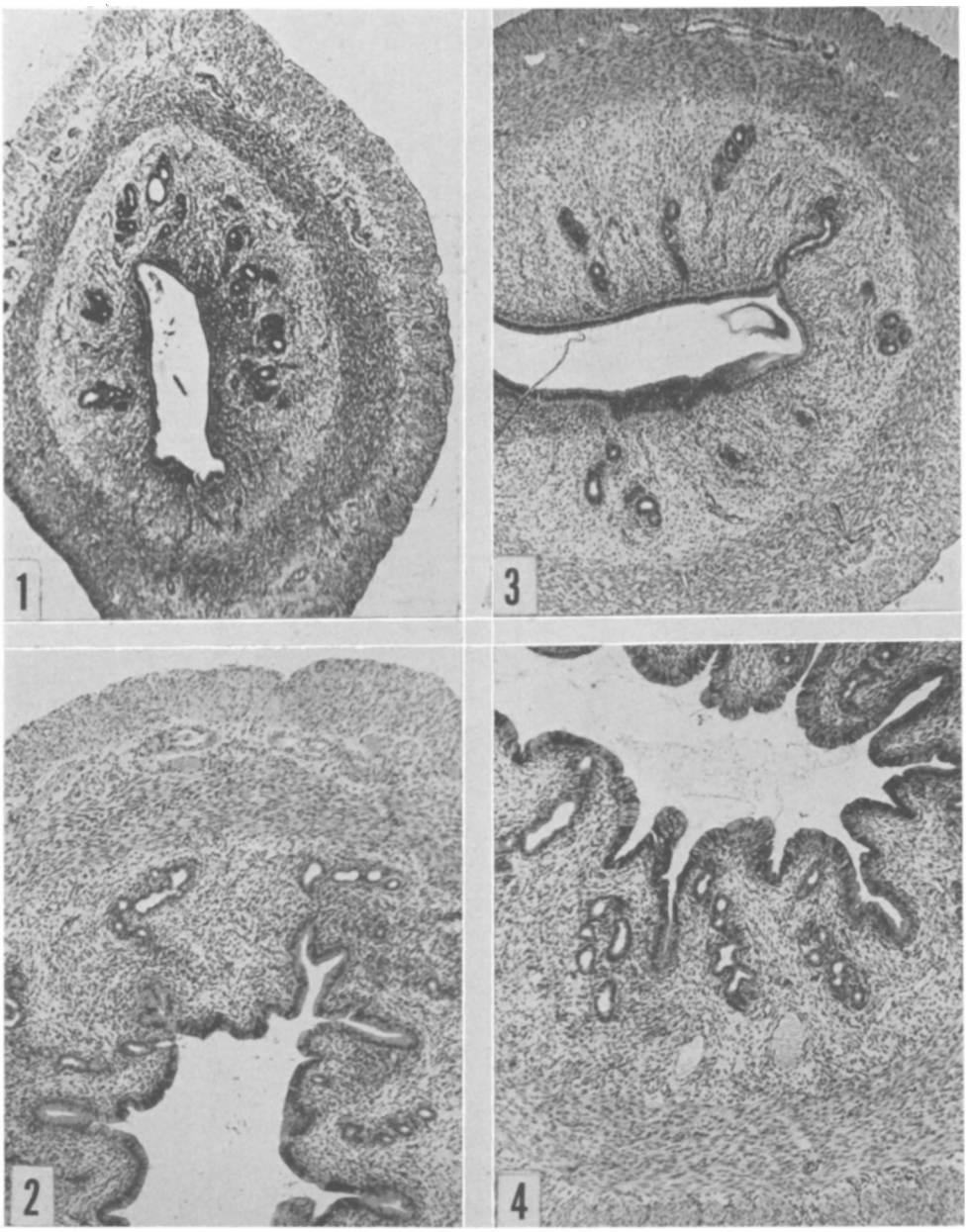

FIG. 1. Cross section of uterus of ovariectomized rat. ×35.
FIG. 2. Cross section of uterus of ovariectomized rat which received estrone. ×35.
FIG. 3. Cross section of uterus of ovariectomized rat which received digoxin. ×35.
FIG. 4. Cross section of uterus of ovariectomized rat which received both digoxin and estrone. ×35.

7 days: Group 1 (4 rats) with a mean weight of 239 g served as castrate controls; Group 2 (5 rats) with a mean weight of 262 g received a daily subcutaneous injection of digoxin† (2.0 mg/kg); Group 3 (5 rats) with

† Digoxin was generously supplied by Burroughs Wellcome Co. who have recently adopted the trademark "Lanoxin" to facilitate distinction between digoxin and digitoxin.

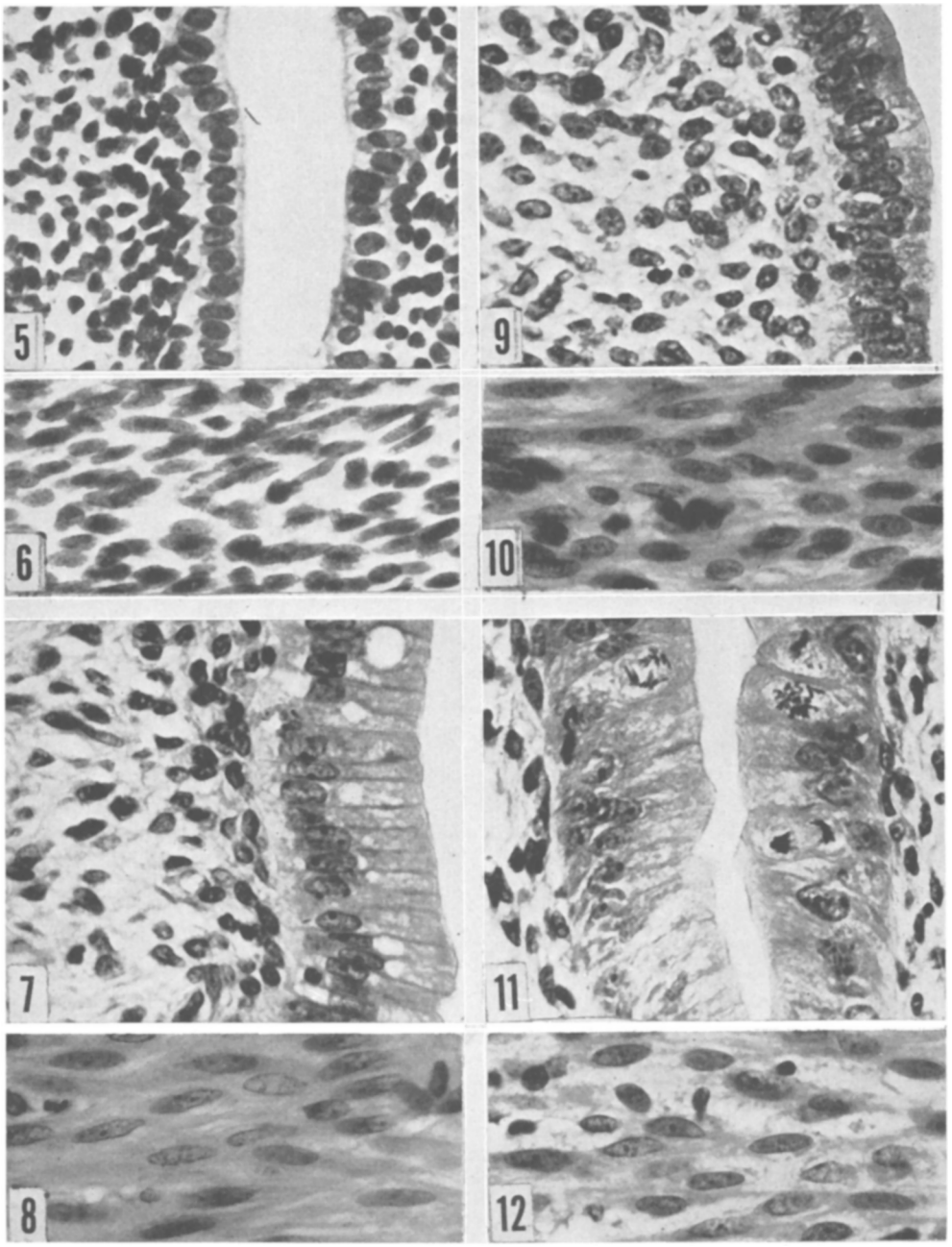

FIG. 5, 6. Epithelium, stroma and smooth muscle of ovariectomized rat uterus. ×344.
FIG. 7, 8. Epithelium, stroma and smooth muscle of estrone-treated ovariectomized rat uterus. ×344.
FIG. 9, 10. Epithelium, stroma and smooth muscle of digoxin-treated ovariectomized rat uterus. ×344.
FIG. 11, 12. Epithelium, stroma and smooth muscle of digoxin-estrone-treated ovariectomized rat uterus. ×344.

a mean weight of 244 g received 0.1 mg of estrone in oil subcutaneously on the first and fourth experimental days; Group 4 (5 rats) with a mean weight of 241 g received both digoxin and estrone in the above doses. Late in the 7th experimental day, all rats were sac-

rificed, their uteri removed, fixed in Bouin's solution, sectioned and stained with hematoxylin and eosin. A vaginal smear of each rat was made at this time.

Results. Castrate control. (Fig. 1, 5, 6). The uteri of these animals presented the typical well known reaction to bilateral ovariectomy. Grossly, the uteri were small and anemic. Histological sections showed a low epithelial lining, small endometrial glands, a compact stroma consisting of closely packed cells with intensely basophilic nuclei, and a myometrium composed of small muscle cells. The vaginal smears all showed the presence of the diestrus state.

Estrone-treated (Fig. 2, 7, 8). Estrone administration to castrate rats resulted in rapid recovery of their uteri. Grossly, these organs were large, hyperemic and moderately distended with fluid. In section, the endometrium was thrown up into longitudinal folds, the stroma was wide and edematous, the glands enlarged, and the myometrium well developed and repaired. The surface epithelium consisted of tall columnar cells with oval nuclei. Many of these cells showed vacuolar degeneration. Many mitoses were present and limited to the lining epithelium. The vaginal smears of these rats showed that they were in estrus by the presence only of cornified cells.

Digoxin-treated (Fig. 3, 9, 10). Digoxin administration to ovariectomized rats resulted in an unmistakable trophic response of the uteri. Grossly, these organs were large and hyperemic, but not distended with fluid. Microscopically, the endometrium was not folded, the lining being composed of a single layer of columnar epithelial cells, of which some portions were pseudostratified. These cells were considerably taller than those of the castrate controls but not heightened to the extent of those of the estrone-treated group. Their nuclei were long and oval occupying a central position in the cells. The stroma was edematous; the stromal nuclei were large and vesicular, each with a prominent nucleolus. The endometrial glands were enlarged. The smooth muscle cells of the muscularis were markedly increased in size and were completely recovered from the castrate condition. An occasional mitotic figure was observed in the lining epithelium and glands but none were seen in the stroma or muscle layers. The vaginal smears of 2 of these rats indicated that they were in late metestrus. These smears contained cornified cells, leucocytes and epithelial cells. Three rats had smears consisting only of epithelial cells, which is characteristic of proestrus.

Digoxin-estrone-treated (Fig. 4, 11, 12). The combined administration of both digoxin and estrone resulted in a response similar to, but much more intense than, that observed following estrone treatment alone. Grossly, these uteri were larger than any of the others, hyperemic, and enormously distended with fluid. Histologically, the endometrium was folded longitudinally and the lining epithelial cells were as tall as, and sometimes taller than, similar cells of the estrone-treated uteri. Their nuclei were large and located near the base of the cells. Some of these nuclei were karyorrhectic. The endometrial glands were enlarged. The stroma was edematous; the stromal nuclei were either vesicular or fusiform in character. The stromal blood vessels were considerably enlarged. The myometrium was recovered from the atrophic castrate condition. Mitoses were more numerous and prominent in the epithelium and glands of these uteri than in either the estrone-treated or digoxin-treated uteri. Mitoses were not observed in the stroma or myometrium. The vaginal smears of these rats were typically estrus, being composed of cornified cells only.

Discussion. The hypertrophic and hyperplastic responses of the castrate uterus to estrogen stimulation are well known. These are characterized by cell division; an edematous wide stroma; enlarged columnar epithelial cells; and a well developed, repaired myometrium. According to the criteria of Hooker(2), the most consistent and conspicuous change in the castrate mouse uterus treated with progesterone was vesiculation of the stromal nuclei and prominence of their nucleoli. He also noted the absence of interstitial edema, the lack of significant change

in the epithelium and myometrium, and the high incidence of stromal mitoses.

Classification of the uterotrophic responses noted in the present study is difficult. The digoxin-treated uteri showed definite vesiculation of the stromal nuclei and prominent stromal nucleoli, but these were the only characteristics of progestational stimulation. On the other hand, the edematous stroma without mitoses, the hypertrophic surface epithelial cells and the repaired muscle cells may be used to classify the effect of digoxin as an estrogen-like response. The appearance of the vaginal smears of the digoxin-treated rats, some showing proestrus and some late metestrus, strongly suggests estrogenic stimulation, however small this stimulation may be. The magnitude of the changes evoked in uteri which received both digoxin and estrone suggests that the uterotrophic influence of digoxin may have been added to or superimposed upon the estrone effect.

The ultimate fate of digitalis glycosides is still disputed. Because of the structural similarity between cardiotonic glycosides and steroid hormones, one may speculate that a metabolite of digoxin, utilizing the cyclopentanoperhydrophenanthrene radical common to both groups of compounds, has estrogen-like qualities. Such a possibility as well as the roles of the pituitary, adrenals and liver are being investigated.

Summary. Digoxin administration to mature ovariectomized rats resulted in uterotrophic response evidenced by heightening of epithelium and enlargement of epithelial nuclei, edema of the stroma with vesiculation of stromal nuclei and prominence of stromal nucleoli, and recovery of the myometrium. The response of castrate uteri to estrone was intensified by the concurrent administration of digoxin.

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Production of Gastric Ulcers in Dogs on Protein Depletion Regime.* (23178)

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In higher mammals production of hemoglobin takes precedence over formation of most body proteins. Advantage was taken of that fact in 1939 when we were able to demonstrate that in the dog, hemoglobin production could be limited by protein intake(1). By "forced hemoglobin production" in dogs concomitantly maintained on a low protein intake circulating and reserve proteins were rapidly depleted. In recent studies in which a similar technic of protein reduction was used in studies of anaphylaxis(2) and homo-transplants† we have been impressed with the high incidence of gastric ulcer and particularly where a perforation was the immediate cause of death. These lesions varied quite considerably as to severity and location. In some instances there were patchy and in others quite extensive denudement of the gastric mucosa. In a few, deep indurating ulcers involved not only the mucosa but the muscularis. Very frequently complete perforation of the stomach occurred with appearance of "punched out" lesions frequently seen at human autopsies. Some of these changes can be seen in Fig. 1, 2 and 3. Most frequently the lesion was seen on the greater curvature or above the pyloric sphincter and uncommonly in the duodenum.

Methods. The procedure can be described briefly as follows: The dogs were bled ¼ of estimated circulating blood volume (72 ml/kg) on each of 3 successive days. The same

* Carried out under contract with Division of Biology and Medicine, U. S. Atomic Energy Com.

† In preparation.