early cryptozoite stage, is quickly lost in mouse skin and retained for a considerable period in chick skin. It seems unlikely that in the present experiments the inactivation would be in the nature of either an inflammatory or an immune reaction against the introduced foreign protein, for this skin had already been separated from its blood supply, and presumably therefore from all reactive responses of a chemotactic, immunologic or hormonal nature, for some minutes before introduction of the sporozoites occurred. It is tempting to consider tentatively that the failure of the mouse to become infected when the sporozoite of *P. gallinaceum* is introduced into its skin by the bite of the infected mosquito is due to either (a) the presence in the skin of a toxic anti-metabolite, or (b) the absence of a metabolite that is essential for the progressive existence of the introduced organism. Studies are under way in the attempt to elucidate these points.

Summary. When *Aedes aegypti* mosquitoes infected with *Plasmodium gallinaceum* were allowed to deposit the sporozoites of this organism while probing into the excised skin of the chick and the mouse, the infections resulting from the intraperitoneal implantations into clean chicks of these probed pieces of skin were in the ratio of 24 from the chick skin to one from the mouse skin. It appears that there is something deleterious to the survival of the introduced plasmodium in the skin of the mouse that is not dependent upon its living state.

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Ovarian Histology After Treatment of Rats with Norethynodrel.* (30228)

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Earlier microscopic studies(1,2,3) of the ovary after treatment of rats with 17α-ethinyl-17-hydroxy-$\Delta^{5(10)}$-estren-3-one (norethynodrel) revealed that corpora lutea are reduced in number or totally absent in some cases(1,2). Follicles are fewer, although some are cystic and larger than normal(1). Ovarian weight is reduced if treatment with norethynodrel is sufficiently prolonged. This report will present evidence that norethynodrel (a) stimulates the corpora lutea through mediation of pituitary secretion of luteotropin (prolactin) and (b) may cause involution of the interstitial tissue.

Methods. Young, adult female rats of the Sprague-Dawley strain were used. Their initial weight was 170-190 g. Norethynodrel was suspended in a medium consisting of Upjohn sterile vehicle 98 (carboxymethylcellulose, polysorbate and polyparaben) diluted 1:2 with 0.9% NaCl. The compound was injected subcutaneously in divided doses twice daily except in experiments involving hypophysectomized animals, when injections were made once daily. All control animals received an amount of vehicle equal to that given to norethynodrel-treated rats.

All rats were killed 24 hours after the last injection. The ovaries were removed while the rats were under sodium amytal anesthesia. After being weighed, the ovaries were fixed in either Bouin's fluid or 10% formalin combined 9:1 with 0.2 M phosphate buffer at pH 7.0. Sections of the formalin-fixed glands were stained with oil red O and hematoxylin. In some experiments other sections were stained by the Schultz(4) reaction for

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TABLE I. Summary of Experimental Conditions.

Treatment	No. of rats	Days treated	Dose, mg/100 g body wt
I. *Duration of treatment*			
Norethynodrel	9	2–4	1.5
Vehicle	9		
Norethynodrel	9	5	1.5
Vehicle	9		
Norethynodrel	5	7	1.5
Vehicle	5		
Norethynodrel	4	13	1.5
Vehicle	3		
Norethynodrel	14	27–34	1.5
Vehicle	14		
II. *Route of administration*			
Norethynodrel in food	9	7	1.0*
" –s.t.	10		1.5
" –s.c.	10		1.5
Vehicle–s.t.	5		
" –s.c.	10		
III. *Influence of hypophysectomy*			
Nonhyp.–norethynodrel	13	10	.658
" –vehicle	12		
Hyp.–norethynodrel	12		.658
" –vehicle	12		
IV. *Variation in dosage*			
Vehicle	12	10	
Norethynodrel	10		.011
"	10		.022
"	10		.045
"	15		.09
"	5		.188
"	5		.375
"	5		.75

Hyp. = hypophysectomized. s.t. = stomach tube. s.c. = subcutaneous.

* This dosage is lower than for the other groups because norethynodrel caused a reduction in food intake.

cholesterol and its esters. Sections of the Bouin-fixed ovaries were stained with hematoxylin and eosin.

Four experiments were carried out to study the influence of the following conditions on the response of the ovary to norethynodrel: (I) duration of treatment, (II) route of administration, (III) hypophysectomy, and (IV) variation in dosage. Grouping of the animals and other conditions of the experiments are given in Table I. In Exp. III, 17-27 days elapsed after hypophysectomy before initiation of treatment. Hypophysectomized animals retaining pituitary fragments, as determined grossly or by microscopic examination of the pituitary area, were eliminated from the experiment. Since norethynodrel at the initial daily dosage of 1.5 mg† caused hypophysectomized rats (Exp. III) to lose weight, 2 one-half reductions were necessitated during the treatment period; the average daily dose was .658 mg.

Observations. Norethynodrel caused a reduction in weight of the ovary at daily doses of .09 mg or more, administered for 10 days. Because of the retarded gain in body weight which also resulted from this treatment(1,3, 5), significant variation in the ratio of ovarian weight/body weight was not observed. In hypophysectomized rats (Exp. III), norethynodrel did not affect ovarian weight.

Striking alteration in the histology of corpora lutea was observed. At the 1.5 mg dose, administered for 2 days, depletion of sudanophilic lipid occurred in some but not all corpora. At 5 days, depletion of large droplets had occurred from almost all corpora. Large lipid droplets were retained in some cells of only those corpora which were most involuted. Accompanying the loss in sudanophilic lipid was a disappearance of the Schultz reaction for cholesterol and its esters which was given regularly by most corpora in the controls. The depletion of sudanophilic lipid was still present after 28-34 days of treatment. In spite of the profound loss of large lipid droplets, fine droplets, which were visible only under high magnification, remained in many cells. After 13 or more days of treatment, hypertrophy of the corpora (Fig. 1 A and B) and of the individual parenchymal cells was observed. In most corpora of the controls the parenchymal cell cytoplasm in H & E stained preparations was variable in density and often vacuolated (Fig. 1 C), but in norethynodrel-treated rats it was uniformly dense and less vacuolated (Fig. 1 D). Of particular importance is the fact that all corpora in an ovary responded to norethynodrel and generally all parenchymal cells were affected. The only exceptions were some cells of extremely involuted corpora.

The minimal daily dose which induced general lipid depletion from all corpora when administered for 10 days was .75 mg (Exp.

† All dosages are expressed in relation to 100 g body weight (b.w.)/day.

IV) (Fig. 2 A and B). Variable depletion from some corpora was observed at the .375 and .188 mg levels and was present in 4 out of 5 rats at .09 mg. No response occurred in hypophysectomized rats (Exp. III). One mg given daily in the food, or 1.5 mg by stomach tube was as effective in the induction of lipid depletion as 1.5 mg administered by subcutaneous injection.

Norethynodrel caused an involution of in-

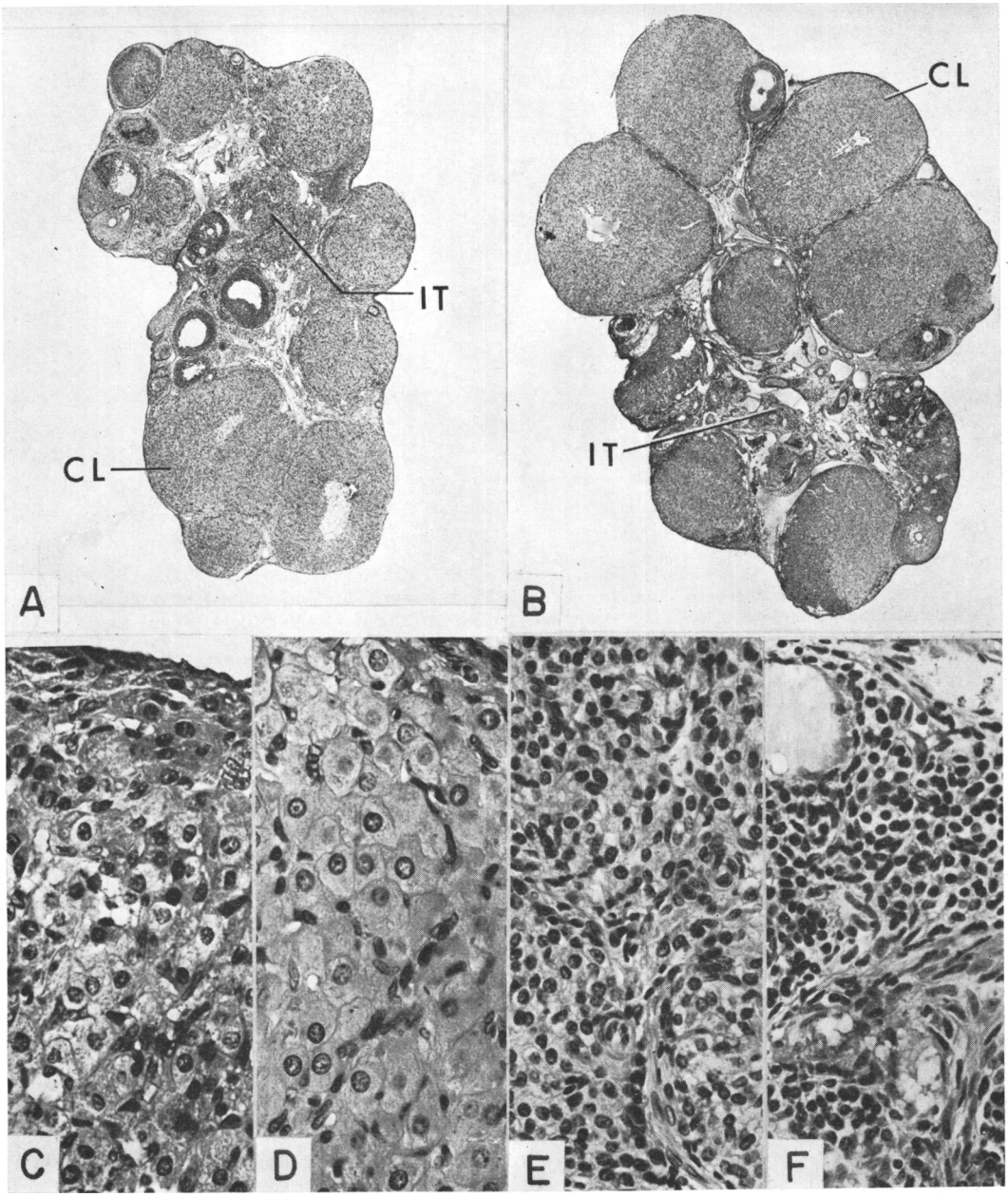

FIG. 1. Ovaries for all photographs were fixed in Bouin's fluid and stained with hematoxylin and eosin. A and B × 13. C-F × 300. A. Vehicle-treated control. B. Norethynodrel treatment at 1.5 mg for 27 days. Corpora lutea (CL) are enlarged and interstitial tissue (IT) is involuted. C. Corpus luteum, vehicle-treated control. D. Corpus luteum, same ovary as for B. Parenchymal cells are enlarged and cytoplasm is denser. E. Interstitial tissue, vehicle-treated control. F. Interstitial tissue, same ovary as for B. Parenchymal cells are reduced in size.

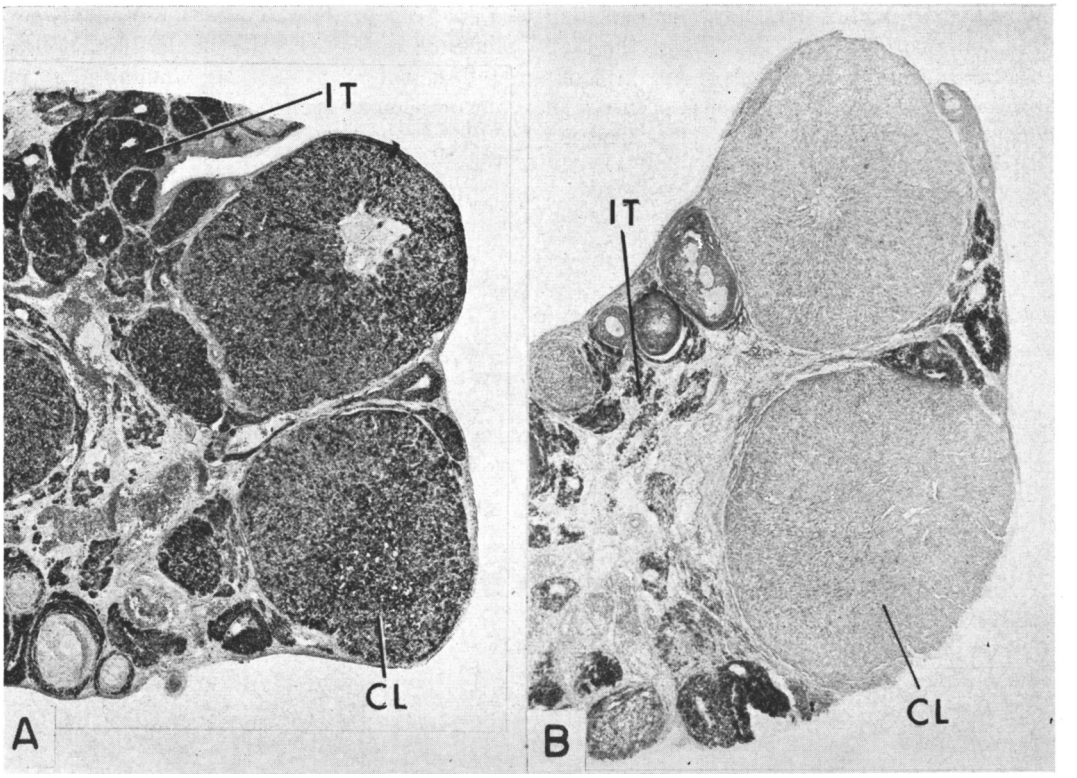

FIG. 2. Ovaries for both photographs were fixed in formalin and stained with oil red O and hematoxylin. × 25. A. Vehicle-treated control. The cells of interstitial tissue (IT) and corpus luteum (CL) contain much lipid. B. Norethynodrel at .75 mg/day for 10 days. Interstitial tissue is involuted and contains less lipid. Corpora lutea have lost much of their lipid.

terstitial tissue (Fig. 1 E and F) accompanied by loss of sudanophilic lipid (Fig. 2 A and B) and cholesterol and its esters as revealed by the Schultz reaction. After only 3 days of treatment at the 1.5 mg dose the reduction in size of the interstitial cells was variable and the loss of lipid only moderate. With prolongation of the period of treatment both effects became more pronounced. From 13 days on, involution of interstitial tissue was intense. Norethynodrel administered orally was as effective in the elicitation of these effects as when given by the subcutaneous route (Table I, Exp. II). The involution of interstitial tissue resulting from hypophysectomy was not enhanced further by the injection of norethynodrel (Exp. III). Indeed, norethynodrel had no perceptible effect on the ovary in the absence of the hypophysis. When administered for 10 days, norethynodrel caused marked involution of interstitial tissue accompanied by loss of lipid in all animals at all dose levels down to and including .375 mg (Exp. IV). At lower doses, the response was usually less clearcut and more likely not to be evident in all animals.

The changes induced in follicles by norethynodrel were less consistent. At dose levels of .09 mg and above, large follicles usually appeared to be more numerous, with a greater proportion of them undergoing atresia than was the case in the vehicle-treated controls. Early atresia was detected by the accumulation of sudanophilic lipid in the membrana granulosa as well as by other cytologic changes.

Discussion. The depletion of lipid from the corpora lutea and interstitial tissue under the influence of norethynodrel carries contrasting biological significance. In the case of corpora lutea, lipid depletion was accompanied by cellular hypertrophy and, therefore, may be regarded as a manifestation of stimulation and hyperactivity. On the other

hand, lipid depletion from the interstitial tissue was accompanied by cellular involution and, consequently, suggests hypoactivity following withdrawal of stimulation.

Corpora lutea in the cyclic rat of most strains are generally considered to be nonfunctional. Secretory activity by the corpora of rats requires stimulation by hypophyseal luteotropin, a hormone which is identical to prolactin(6). Since norethynodrel had no effect on the corpora of hypophysectomized rats, one may assume that its stimulatory effect on the corpora of nonhypophysectomized rats was mediated by the release of prolactin from the hypophysis. The validity of this assumption is supported by the extreme degree of mammary development which follows treatment of rats with norethynodrel (7,8), inasmuch as the action of prolactin is essential for this response. Depletion of cholesterol from corpora lutea is also indicative of prolactin stimulation(6). It may be assumed that the progestins secreted by the corpora when under the influence of norethynodrel will buttress the progestational action of the compound itself(10) in the induction of peripheral biological effects.

A partial block of the secretion of LH may be one explanation for the contraceptive action of norethynodrel(1,3). Nevertheless, conclusive evidence that norethynodrel alters the pituitary content or secretion of LH has not yet been secured from bioassay. The involution of the interstitial tissue observed in this study constitutes additional evidence that norethynodrel suppresses the release of LH by the hypophysis since interstitial cells are dependent on LH stimulation for maintenance of their structural integrity(6,11).

Norethynodrel possesses estrogenic activity equivalent to 3-7% that of estrone(10), which may account in part for its capacity to stimulate secretion of prolactin. With respect to this property norethynodrel resembles other estrogens(6). Finally, in the nontreated cyclic rat, only some corpora are said to possess the capacity to respond to prolactin, a definite decline in responsivity being observed in late diestrus(12). Norethynodrel, however, seemed to stimulate all corpora, even to a limited degree those which had undergone considerable involution, regardless of the stage of the estrous cycle.

Summary. Norethynodrel caused enlargement of the corpora lutea with cellular hypertrophy and depletion of sudanophilic lipid and cholesterol. These changes appeared to be mediated by the hypophysis through the accelerated secretion of prolactin. On the other hand, ovarian interstitial tissue underwent involution with depletion of sudanophilic lipid and cholesterol. These effects were probably mediated by suppressed secretion of LH by the hypophysis.

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