The Influence of Norethynodrel on the Hypophysis[1] (34739)

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Even though norethynodrel constitutes 98.5% of the widely used contraceptive pill Enovid, no conclusive study of its effect on pituitary structure has been carried out. With respect to the rat, Lakshman and Nelson (1) reported that norethynodrel does not alter pituitary microanatomy but Holmes and Mandl (2) observed an increase in the relative volume of chromophobes in the absence of general cytological alteration. Blaquier (3) reported a reduction in basophils. Similar disagreement exists regarding the influence of norethynodrel on pituitary weight, no change (1, 3–5) and an increase (2) having been reported. However, the extensive evidence that norethynodrel stimulates the secretion of prolactin (6–10) and, depending on the dose level, either elevates the gland content of luteinizing hormone (LH) (11, 12) or reduces the content of total gonadotropin (5) indicates that significant alteration in pituitary cytology is to be expected. Determination of the pituitary cytological response to norethynodrel is of special interest because it has both progestational and estrogenic (4) properties and little is known about the combined action of naturally occurring progestins and estrogens on pituitary cells.

The recent development of an immunochemical staining method (13), in which peroxidase-labeled antibody is utilized, makes possible identification of pituitary cell types (14) with an accuracy not heretofore possible. Relying primaily on this procedure, a study was made of the cytological response of the hypophysis to norethynodrel.

Methods. Young, adult female Sprague-Dawley rats were distributed between 3 experiments (Table I). In Expt. I the effect of a high dose of norethynodrel (1.5 mg/100 g of body wt/day) was observed in intact rats. In Expt. II, a lower dose of norethynodrel (0.375 mg/100 g body wt/day) was studied in rats ovariectomized 29 days prior to the first injection, with treatment being continued for 7 days. At this level an increase in pituitary prolactin content is demonstrable by means of bioassay (9). Experiment III was designed to reveal the minimal dose of norethynodrel that will alter pituitary cytology. For this purpose different groups of rats, ovariectomized 27 days prior to initiation of therapy, received daily doses of norethynodrel ranging from 5 to 150 μg for 9 days. Norethynodrel was administered subcutaneously as a suspension in Upjohn vehicle 98 (carboxymethylcellulose, polysorbate, and propylparaben) diluted 1:2 with 0.9% saline. In Expts. I and II the daily dose was divided between a morning and afternoon injection; in Expt. III, it was given in one daily injection. Controls received equivalent volumes of the suspension medium.

At termination of the experiments the rats were decapitated while under sodium amytal anesthesia. Hypophyses were fixed in Bouin's fluid, embedded in paraffin, and sectioned frontally at 3 μ. For differential cell counts 10 sections from 3 equally spaced zones were placed on a slide and stained with aldehyde fuchsin and Masson. Similar slides were first stained immunochemically for prolactin cells and then counterstained with the Masson procedure. Single sections from these zones were placed on other slides for immunochemical staining alone.

For immunochemical staining, rabbit antisera to the following hormones were used: human growth hormone (anti-HGH); rat prolactin (anti-RP); porcine corticotropin (anti-PC), this preparation having been ob-

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TABLE I. The Effect of Norethynodrel on Organ Weights.[a]

Exp. no.	Group	Treatment	No. of rats	Ovari-ectomy	Daily dose nor. (mg)	Days treatment	Mean body wt (g)			Mean organ wt (mg)	
							At ovar.	At treat.	Final	Hyp.	Uterus
I	a	Norethynodrel	20	—	1.5[b]	27–34	—	156 ± 9[c]	174 ± 11	12.9 ± 2.1	712 ± 256
	b	Vehicle	19	—	—	27–34	—	159 ± 10	219 ± 13	11.8 ± 1.6	419 ± 146
										a vs. b, NS	
II	a	Norethynodrel	7	+	0.375[b]	7	179 ± 7	260 ± 13	236 ± 10	18.0 ± 1.8	—
	b	Vehicle	6	+	—	7	178 ± 4	259 ± 12	271 ± 16	15.7 ± 2.2	—
	c	Vehicle	7	—	—	7	181 ± 5	239 ± 11	248 ± 9	14.2 ± 1.6	—
										a vs. b, NS	
III	a	Norethynodrel	4	+	0.150	9	169 ± 4	256 ± 4	227 ± 7	13.1 ± 0.2	244 ± 35
	b	Norethynodrel	4	+	0.075	9	175 ± 6	265 ± 3	253 ± 2	12.3 ± 1.0	178 ± 19
	c	Norethynodrel	4	+	0.037	9	176 ± 5	268 ± 8	251 ± 15	12.1 ± 0.7	220 ± 32
	d	Norethynodrel	2	+	0.019	9	174 ± 1	234 ± 18	243 ± 19	11.4 ± 1.0	164 ± 28
	e	Norethynodrel	4	+	0.009	9	174 ± 3	234 ± 13	241 ± 14	13.3 ± 0.5	167 ± 50
	f	Norethynodrel	4	+	0.005	9	175 ± 3	222 ± 9	233 ± 9	12.2 ± 1.5	158 ± 35
	g	Vehicle	2	+	—	9	172 ± 7	231 ± 5	237 ± 11	12.1 ± 0.5	96 ± 6
										a vs. g, $p < .05$	
										e vs. g, NS	

[a] NS = not significant; nor. = norethynodrel; Hyp. = hypophysis; Student's *t* test.

[b] Per 100 g of body weight.

[c] Standard deviation.

tained from Parke, Davis and Co.; and human chorionic gonadotropin (anti-HCG).[2] In previous publications the specificity of these preparations has been evaluated for staining growth hormone and prolactin cells (15) and corticotropin cells (16, 17), and the cells correlated with those revealed by histologic staining. The cells delineated by anti-HCG will be designated as gonadotropin cells because most preparations of HCG contain other antigens including follicle-stimulating hormone (FSH) and the specificity of our antiserum for LH cells is not yet established. Nevertheless, the distribution of the cells revealed by this antiserum was similar to that of LH cells identified with immunofluorescence by Monroe and Midgley (18) using absorbed anti-HCG. Also, the antisera to HCG used by Monroe and Midgley and by us had similar immunologic characteristics (Midgley, personal communication). Nevertheless, since Nakane (19) holds that LH and FSH are secreted by the same cell type, the cells revealed with anti-HCG in this study will be designated gonadotropin cells.

Observations. At a dose level of 0.037 mg/day or higher norethynodrel either caused a loss or restricted the rate of increase in body weight (Table I). The uterotropic property of norethynodrel was manifested by an increase in uterine weight (Expt. III). In contrast, although there seemed to be a tendency for norethynodrel to increase pituitary weight in all experiments, the differences between glands from hormone- and vehicle-treated rats were significant only for one ovariectomized group which received norethynodrel in Expt. III. Thus, pituitary enlargement was a rare outcome of norethynodrel treatment.

Growth hormone cells. Ovariectomy had little effect on growth hormone cells although they appeared to become somewhat enlarged; they were more densely arranged due to reduction in size of the intervening prolactin cells as previously reported (15). Treatment with norethynodrel elicited only mild changes in growth hormone cells, somewhat reducing their size in some intact and ovariectomized rats (Expts. I and II). In ovariectomized, norethynodrel-treated rats, growth hormone cells were often more dispersed as a consequence of prolactin cell hypertrophy. In Expt. III, differential cell counts showed that the relative number of growth hormone cells was not significantly altered, being 35 ± standard deviation 3.6% for vehicle-treated, ovariectomized rats and 31.4 ± 3.5% for norethynodrel-treated ovariectomized animals.

Prolactin cells. Ovariectomy caused a marked reduction in size of prolactin cells as previously reported (15). Treatment of ovariectomized rats with norethynodrel at doses ranging from 0.037 mg/day for 9 days (Expt. III) to 0.375 mg/100 g of body weight/day for 7 days (Expt. II) induced a general and marked enlargement of prolactin cells (Fig. 1). At dose levels of 0.005 to 0.019 mg/day no response, or possible enlargement of only isolated cells was obtained. In Expt. II, the percentage of prolactin cells (stained immunochemically) increased for an average of 20.8 ± 4.5% in ovariectomized, vehicle-treated rats to 33.2 ± 1.9% after norethynodrel administration.

Treatment of intact rats with an exceedingly high dose of norethynodrel (Expt. I) led to striking cellular and nuclear hypertrophy of prolactin cells as observed in sections stained histologically or with a combination of histological and immunochemical staining. These cells were also distinguished by an enlarged, dense Golgi region and a peripheral striated zone which was interpreted to be indicative of expanded rough endoplasmic reticulum. Such cells would usually be classified as large chromophobes because they were extensively degranulated. However, their reactivity with anti-RP showed that they were prolactin cells. Almost no mitotic figures were found in the prolactin cells of any experimental group.

[2] Appreciation is extended to the following individuals for providing the hormone antisera indicated: Dr. A. R. Midgley, Jr., anti-RP for which purified rat prolactin prepared by S. Ellis was used, and anti-HCG; Dr. R. F. Knopf, anti-HGH for which Raben lots 12 and 14 of human growth hormone were used; and Dr. S. Pek, anti-PC for which partially purified porcine corticotropin obtained from Parke, Davis and Co. was used.

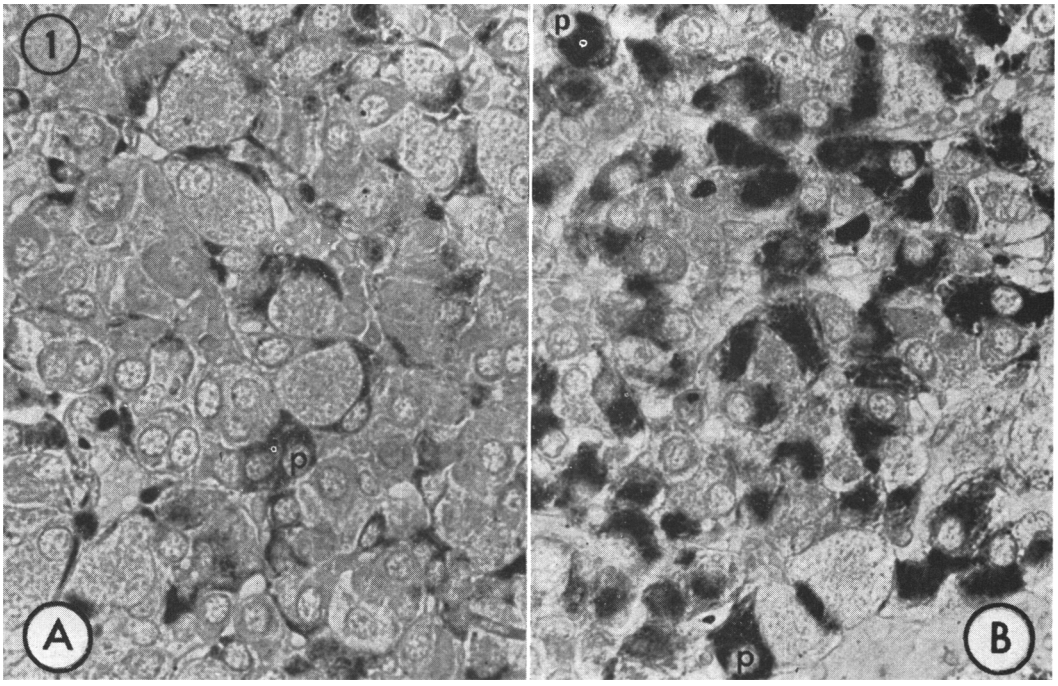

FIG. 1. Both hypophyses illustrated were stained with anti-RP for prolactin cells: (A) Pars distalis from a rat ovariectomized 36 days previously. Prolactin cells (P) are exceedingly small; × 600. (B) Pars distalis of a rat ovariectomized 36 days previously and treated over the last 7 days with a daily dose of 0.375 mg/100 g of body wt of norethynodrel. Prolactin cells (P) are greatly enlarged; ×600.

Gonadotropin cells. Treatment of intact rats with a high dose of norethynodrel for 27–34 (Expt. I) days resulted in a profound reduction in size and staining capacity of gonadotropin cells (Fig. 2A and C). They were enlarged after ovariectomy (Fig. 2B), but in ovariectomized rats treated with the 0.375 mg/100 g of body wt dose (Expt. II) gonadotropin cells were reduced in size (Fig. 2D), in some animals the change being great. Certain cells, especially those which were vacuolated due to ovariectomy, seemed to resist the action of norethynodrel. With doses in range of 0.037 to 0.075 mg (Expt. III) noticeable but mild reduction in size of gonadotropin cells occurred.

Corticotropin cells. Baker *et al.* (17) have reported previously that ovariectomy causes little change in corticotropin cells. The influence of norethynodrel on corticotropin cells in ovariectomized rats was studied in Expt. II with only mild and erratic changes being observed. In only 2 of 7 ovariectomized, norethynodrel-treated rats were corticotropin cells clearly enlarged over those in all ovariectomized animals that received vehicle.

Discussion. The general enlargement of prolactin cells elicited by norethynodrel correlates well with the many evidences of heightened prolactin secretion (6–10) that appear under such treatment. Such pituitary responses are generally regarded as being dependent on stimulation by estrogen. Early norethynodrel preparations possessed 3 to 7% the estrogenic activity of estrone as determined by biological assay (4) and were contaminated with mestranol. The preparations used in this study were free of such contamination as indicated by the absence of aromatic content (J. K. McGowan, personal communication). Thus, the changes elicited in prolactin cells were probably due to estrogenicity inherent either in the norethynodrel molecule itself or in the metabolite of it (20). Furthermore, accelerated secretion by prolactin cells probably accounts in part for the

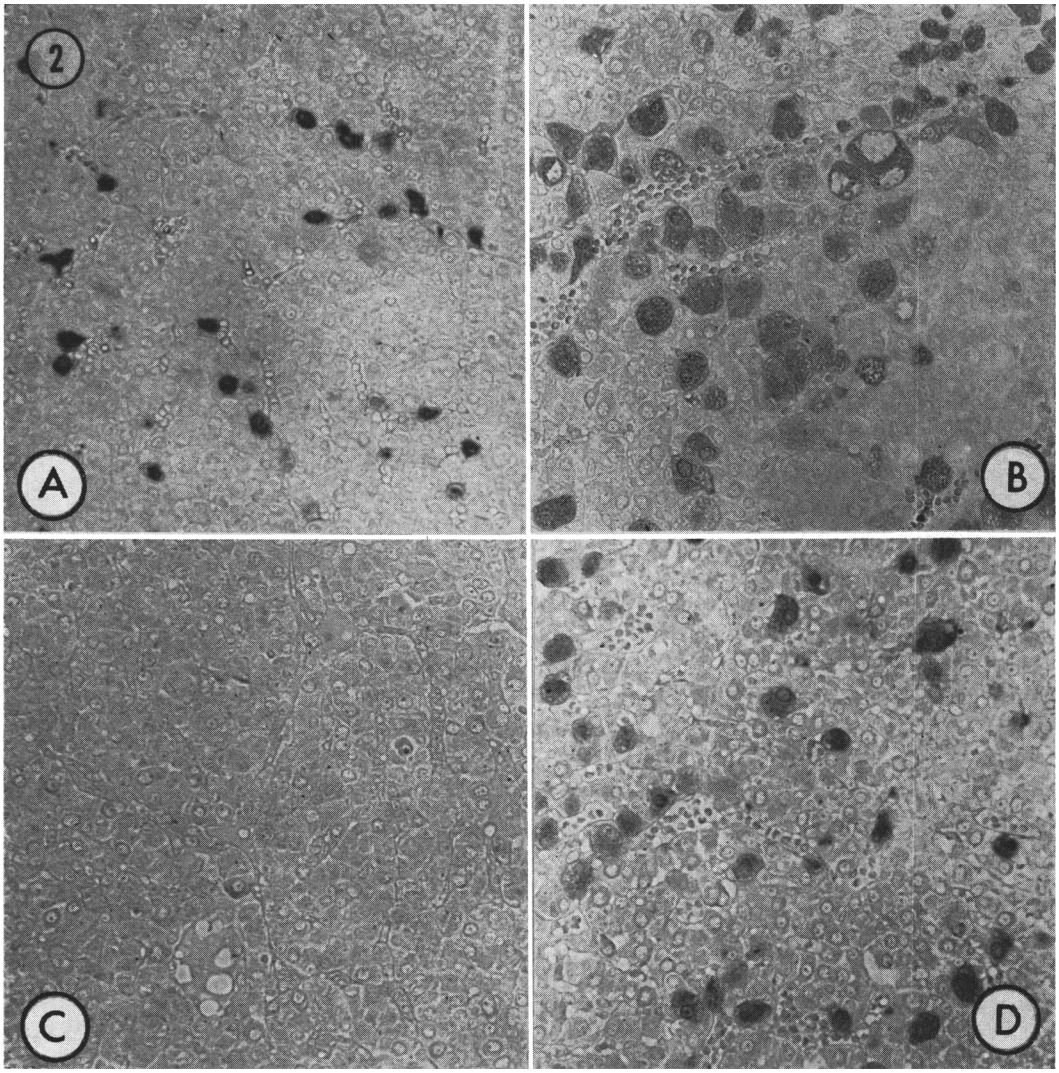

FIG. 2. All hypophyses illustrated were stained with anti-HCG for gonadotropin cells; ×250. (A) Vehicle-treated intact rat. Gonadotropin cells appear black. (B) Vehicle-treated rat, 36 days after ovariectomy. Gonadotropin cells are enlarged. (C) Intact rat treated daily with 1.5 mg of norethynodrel for 27 days. Gonadotropin cells are no longer recognizable. (D) Rat was ovariectomized 36 days previously and treated over the last 7 days with 0.375 mg of norethynodrel/100 g of body wt/day. Gonadotropin cells are smaller than in the ovariectomized, vehicle-treated animal (Fig. 1B).

capacity of norethynodrel to increase the incidence of mammary carcinoma in strains of mice that normally have either a high or low incidence of this neoplasm (21). The enlarged chromophobes observed by Holmes and Mandl (2) in norethynodrel-treated rats were undoubtedly prolactin cells which could not be identified as such with their methods.

In comparing the structural effects elicited in the hypophysis by norethynodrel with those that follow treatment with other estrogens, two differences stand out. First, norethynodrel is much less potent in stimulating prolactin cells. While a minimal daily dose of 37 μg of norethynodrel (Expt. III) was required to produce a generalized effect on prolactin cells, only 2 μg of diethylstilbestrol given daily for 10 days increases the relative number of acidophils in adult ovariectomized rats (22), and 0.055 μg of estradiol/day for

10 days has a similar effect in immature rats (23). It is now clear that of the acidophil cell class, prolactin cells rather than growth hormone cells are stimulated by estrogen. Second, no mitotic figures were observed in the hypophyses of norethynodrel-treated rats while other estrogens accelerate mitotic activity in acidophils (22), this response occurring predominantly in prolactin cells. The incapacity of norethynodrel to induce hyperplasia probably accounts for its insignificant effect on pituitary weight as compared with other estrogens (22, 24, 25).

With respect to gonadotropin cells, correlation of cell structure with alteration in LH content under the influence of norethynodrel is less clear. At high dosage (0.5 mg daily for 7 days) (11) and low dosage (0.020 mg daily for 10 days) (12) the LH content of the hypophysis has been reported to be increased in intact rats. Within this range of doses our experiments dealt only with treatment of ovariectomized rats. In ovariectomized rats norethynodrel reduced the size of the enlarged gonadotropin cells and, in this instance, probably lowered the already elevated LH content of the gland. Thus, our observations tend to support those of Saunders (5) who found a reduction in pituitary gonadotropin content in adult ovariectomized rats at daily dose levels of 0.1 mg or higher.

In conclusion, these structural responses indicate that in addition to suppressing the gonadotropin-secreting cells of the rat hypophysis, norethynodrel also stimulates the prolactin cells.

Summary. With daily doses ranging from 0.037 mg for 9 days to 1.5 mg/100 g of body wt for 27 days, norethynodrel caused enlargement of prolactin cells as indentified by immunochemical staining. This reponse was especially prominent in ovariectomized rats. Hyperplasia of prolactin cells was not induced by norethynodrel which probably accounts for the absence of pituitary enlargement. The enlarged gonadotropin cells, presumed to be LH cells, that occur in ovariectomized rats were reduced in size by administration of norethynodrel. Norethynodrel induced little change in growth hormone and corticotropin cells.

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