The Interaction of Clomiphene, Estradiol, and Progesterone in the Control of Rat Uterine Glycogen Metabolism[1]

WILLIAM L. POTEAT[2] AND WALTER J. BO
Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103

ABSTRACT Uterine glycogen accumulation was studied in ovariectomized rats treated with all combinations of clomiphene citrate (0.25 mg/kg) estradiol (1.0 μg) and progesterone (5.0 mg). The rats were given three consecutive daily dosages and killed 24 hours after the final dosage. Based on biochemical data, either estradiol or clomiphene increased uterine glycogen concentration and total glycogen, but progesterone did not. Progesterone significantly suppressed both the estradiol and clomiphene-induced glycogen increases. Based on the histochemical results, progesterone also suppressed the estradiol and clomiphene-induced glycogen responses, but the tissue affected differed. Clomiphene markedly increased luminal epithelial glycogen whereas estradiol induced primarily myometrial glycogenesis. Progesterone completely suppressed the clomiphene-induced epithelial effect and partially suppressed the estradiol-induced myometrial effect. Clomiphene also suppressed the estradiol-induced myometrial response. The results indicate that progesterone does have a significant interaction with clomiphene in the control of uterine morphology and biochemistry. The results also stress the importance of correlated histochemical and biochemical studies in the study of clomiphene-induced uterine glycogenesis.

Clomiphene citrate, a non-steroidal fertility agent (Kistner, '68), has estrogenic and antiestrogenic effects on the female reproductive system (Holtkamp et al., '60; Roy et al., '64; Greenblatt, '68; Sankaran and Prasad, '72). The drug's uterotrophic effects may be involved in its postcoital contraceptive action in the rat (Emmens, '70; '73). Clomiphene increased uterine glycogen when administered to ovariectomized rats and suppressed estradiol-induced uterine glycogenesis (Wood et al., '68; Mohla and Prasad, '68). A striking difference in glycogen localization after clomiphene and estradiol treatment has been reported (Poteat and Bo, '71). Glycogen was observed in the luminal epithelium of clomiphene-treated ovariectomized rats but was not seen in these cells after estradiol treatment (Poteat and Bo, '71).

Although the interaction between clomiphene and estradiol is well documented (e.g., Roy et al., '64; Greenblatt, '68; Sankaran and Prasad, '72) little information concerning the interaction of clomiphene and progesterone is available. Clomiphene has no progestational effects and does not interfere with the action of progesterone on the uterus (Greenblatt, '68).

The purpose of this study was to investigate the interaction of clomiphene and progesterone in the control of uterine glycogen metabolism and to further investigate the interaction between clomiphene and estradiol in order to clarify conflicting histochemical and biochemical data in the literature. Clomiphene has been reported to be as potent as estradiol in stimulating uterine glycogenesis (Wood et al., '68) but to be weakly estrogenic in this regard (Mohla and Prasad, '68). Poteat and Bo ('71) found the drug's glycogenic potency to depend on the uterine tissue being studied.

MATERIALS AND METHODS

Virgin Holtzman rats weighing 200-220 g were housed four/cage at a controlled temperature (72°F) and allowed free access to food

Accepted January 3, '77.
[1] Supported by USPHS Grant AM 08029-6.
[2] Present address for reprint requests: Department of Anatomy, University of South Carolina School of Medicine, Columbia, South Carolina 29208.

and water. The lights were on for 14 hours (0630-2030 hours).

The rats were bilaterally ovariectomized under ether anesthesia, rested ten days, and divided into eight experimental groups: Group 1: 0.5 ml 5% acacia by gavage (control), Group 2: clomiphene citrate [3] (0.25 mg/kg body weight by gavage in acacia vehicle), Group 3: estradiol dipropionate [4] (1.0 μg/rat, sc in cottonseed oil), Group 4: progesterone [5] (Lipo-Lutin®, 5.0 mg/rat, sc), Group 5: clomiphene + estradiol, Group 6: clomiphene + progesterone, Group 7: estradiol + progesterone, and Group 8: clomiphene + progesterone + estradiol. Groups 5 to 8 received the same doses of each compound as Groups 2 to 4. The rats were treated once/day for three convecutive mornings between 0730 and 0930 and killed by cervical dislocation 24 hours after the final treatment. At autopsy the uterine horns were removed, trimmed of mesentery, lightly blotted, weighed and cut into several pieces. Three pieces from each horn (ovarian, middle and cervical) were fixed in cold picric acid formalin (9 parts 80% alcohol, 1 part 10% formalin, saturated with picric acid) and processed for histology and histochemistry as described previously (Poteat and Bo, '71). Another piece was weighed, oven-dried 24 hours at 100°C, and reweighed. The remaining pieces of each horn were weighed separately and digested in hot 30% KOH for replicate glycogen determinations by a modification of the indirect anthrone procedure of Seifter et al. ('50), using the anthrone reagent as outlined by Carroll et al. ('56), and coprecipitating the glycogen with saturated sodium sulfate as recommended by van Handel ('65). A glycogen standard was used and the results expressed as mg glycogen/g tissue (wet and dry) and total glycogen (μg/uterus).

Histochemical results were based on glycogen grading of the individual slides, read without knowledge of treatment. Myometrium and luminal epithelium were graded differently, and comparisons between grades for these two areas are not valid. The myometrium was graded on a 0-5 scale representing increased glycogen deposition in the outer and inner layers. Number and size of the granules were the criteria. The grade for the luminal epithelium was based on a 0-4 scale representing increased number of cells containing glycogen relative to the total number of cells in the cross section.

Data was analyzed by Duncan's New Multiple Range Test and differences at $P < 0.05$ were considered significant. Differences among groups based on the histochemical evaluation of glycogen will be described as significant if a distinct difference was evident.

RESULTS

Uteri of the estradiol-treated rats were more distended by luminal fluid and were more hyperemic than those of the clomiphene or progesterone-treated rats. Both estradiol and clomiphene increased uterine size and vascularity more than progesterone did. Progesterone suppressed estradiol-induced luminal fluid accumulation, but clomiphene did not.

Results of the quantitative analysis of uterine weight (table 1) and glycogen (table 2) indicate that each compound increased all parameters over control values, but the relative increases were different for each. The single exception was progesterone-induced glycogen concentration, which was no different from control values ($P > 0.05$). Estradiol and clomiphene more than doubled the wet weight, but the dry weights were slightly less than doubled. Progesterone increased wet and dry weight, but significantly less than either estradiol or clomiphene did. The estradiol and clomiphene-induced weight increases were significantly reduced by concurrent progesterone treatment. The estradiol-induced weight increases were slightly reduced by clomiphene and significantly reduced in the group receiving all three compounds.

Estradiol and clomiphene each increased glycogen concentration and total glycogen, but progesterone did not. The estradiol-induced glycogen concentration was not significantly greater than that induced by clomiphene, but estradiol-induced total glycogen was greater. After concurrent clomiphene and estradiol treatment no significant difference in any glycogen parameter was detected (E + Cl vs E; E + Cl vs Cl). The difference in the relative responses of glycogen concentration and total glycogen in the combination treatment group compared to the two single treatment groups (E + Cl vs E or Cl) reflected the significant difference in the wet weight of the

[3] The clomiphene citrate, estradiol dipropionate and progesterone (Lipo-Lutin®) were supplied through the courtesy of the W. S. Merrell Co., Cincinnati, Ohio, Ciba Pharmaceutical Co., Summit, New Jersey, and Parke, Davis and Co., Detroit, Michigan, respectively.

[4] See footnote 3.

[5] See footnote 3.

combination group compared to the group that received estradiol alone.

Concurrent progesterone administration suppressed both glycogen concentration and total glycogen of the estradiol, clomiphene, and combined groups (E + Cl), but the effectiveness of progesterone in suppressing glycogenesis was different for each group.

TABLE 1

The combined and individual effects of clomiphene (Cl), *estradiol* (E) *and progesterone* (Pr) *on uterine weight and % water. Acacia* (A) *was given as the vehicle control. Results are expressed as mean ± standard error*

Treatment	Rats	Wet weight (mg)	Dry weight (mg)	% water
A	4	93.4± 7.7	19.7±1.6	78.9±0.2
Pr	5	135.8± 9.4	25.4±1.5	81.2±0.5
A vs Pr		P<0.01	P=NS	P<0.05
Cl	5	187.2± 3.9 [1]	34.8±1.5 [1]	81.4±0.4 [2]
Cl + Pr	5	143.8± 7.1 [2]	26.8±1.4 [3]	81.3±0.6 [3]
Cl vs Cl + Pr		P<0.01	P<0.05	P=NS
E	7	208.6±16.0 [1]	38.8±3.2 [1]	81.4±0.3 [2]
E + Pr	5	158.2± 3.0 [2]	29.7±0.8 [2]	81.2±0.5 [3]
E vs E + Pr		P<0.01	P<0.01	P=NS
E + Cl	5	175.4± 8.2 [2,4]	34.7±1.5 [1]	80.2±0.2
E + Cl + Pr	5	142.2±6.0 [2,6,7]	26.5±1.4 [3,5,6]	81.3±1.0 [3]
E + Cl vs E + Cl + Pr		P<0.05	P<0.05	P=NS

[1] P < 0.01 vs A or Pr.
[2] P < 0.01 vs A.
[3] P < 0.05 vs A.
[4] P < 0.05 vs E.
[5] P < 0.05 vs Cl.
[6] P < 0.01 vs E.
[7] P < 0.01 vs Cl.
All other comparisons are either not significantly different (NS) or invalid.

TABLE 2

The combined and individual effects of clomiphene Cl), *estradiol* (E), *and progesterone* (Pr) *on glycogen concentration, total glycogen, and localization. Acacia* (A) *was given as the vehicle control. Results are expressed as mean ± standard error and glycogen grade* (histochemistry)

Treatment	Rats	Glycogen concentration		Total glycogen	Glycogen grade		
						Myometrium	
		mg/g WW	mg/g DW	μg/uterus	Luminal epithelium	Inner layer	Outer layer
A	4	1.13±0.07	5.35±0.31	106.6±13.3	–	–	1+
Pr	5	1.01±0.05	5.36±0.26	135.4± 7.2	–	1+	1+/2+
A vs Pr		P=NS	P=NS	P=NS			
Cl	5	1.80±0.12 [1]	9.70±0.70 [1]	335.9±22.3 [1,2]	4+	1+/2+	3+
Cl + Pr	5	1.38±0.12 [3]	7.38±0.57 [5]	195.0± 6.8 [8]	–	1+	3+
Cl vs Cl + Pr		P<0.05	P<0.05	P<0.01			
E	7	1.96±0.12 [1]	10.59±0.64 [1]	408.4±42.4 [1]	1+	3+	5+
E + P	5	1.58±0.11 [4]	8.41±0.65 [1]	249.1±17.4 [1]	–	1+/2+	3+
E vsE + Pr		P<0.05	P<0.05	P<0.01			
E + Cl	5	2.10±0.17 [1]	10.58±0.89 [1]	362.4±14.8 [1]	4+	1+	3+
E + Cl + Pr	5	1.49±0.09 [3,7]	8.00±0.43 [4,7]	209.7± 5.3 [6,8]	–	1+	3+
E + Cl vs E + Cl + Pr		P<0.01	P<0.05	P<0.01			

[1] P < 0.01 vs A or Pr.
[2] P < 0.05 vs E.
[3] P < 0.05 vs Pr.
[4] P < 0.05 vs A; P < 0.01 vs Pr.
[5] P < 0.05 vs A or Pr.
[6] P < 0.01 vs E or Cl.
[7] P < 0.01 vs E.
[8] P < 0.05 vs A.
All other comparisons are either not significantly different (NS) or invalid.

Progesterone suppressed clomiphene-induced glycogenesis more than it did the estradiol-induced effect.

Histochemical analysis clarified and allowed more accurate interpretation of the quantitative data (table 2). No glycogen was observed in the luminal epithelium of the control rats (fig. 1) and only a little was seen in the outer longitudinal layer of the myometrium (fig. 14). Estradiol caused marked glycogen deposition in the outer longitudinal layer of the myometrium (fig. 16), but only a few luminal epithelial cells contained glycogen (fig. 2). Clomiphene induced marked glycogen deposition in the luminal epithelium (fig. 3) but only a moderate myometrial increase (fig. 15). Although clomiphene induced glycogen deposition in nearly every luminal epithelial cell, no glycogen was observed in glandular epithelium (fig. 4). Clomiphene increased myometrial glycogen, but significantly less than estradiol did (figs. 15, 16). Progesterone appeared to increase myometrial glycogen slightly, but the difference between its effect and the control response was not distinct. Progesterone caused no epithelial glycogen accumulation. The outer longitudinal layer of all groups contained more glycogen than the inner circular layer.

Although there was little difference in the quantitative analysis of glycogen among the clomiphene, estradiol, and clomiphene + estradiol groups, the epithelial and myometrial responses were quite different. Clomiphene-induced glycogen deposition was primarily epithelial, whereas the estradiol-induced response was primarily myometrial. The combination treatment caused a histochemical appearance similar to clomiphene-treated rats; a moderate amount of myometrial glycogen and the maximum amount of epithelial glycogen were observed (fig. 4).

Progesterone suppressed both the clomiphene and estradiol-induced glycogen concentrations and total glycogen, but the tissue affected was different. Progesterone significantly suppressed the estradiol-induced myometrial response and the clomiphene-induced epithelial response. There was a complete absence of observable glycogen in the luminal epithelium of the clomiphene + progesterone group (figs. 3 vs 5). Progesterone did not appear to reduce the clomiphene-induced myometrial glycogen response. After treatment with all three compounds myometrial glycogen was significantly reduced compared to the estradiol group, and luminal epithelial glycogen was absent compared to either the clomiphene or clomiphene + estradiol group (figs. 4 vs 6).

The morphology of the luminal epithelium was characteristic for each treatment. The height of the epithelium was low (control and progesterone), intermediate (estradiol), or high (clomiphene) (figs. 7-9). In combined treatment groups the epithelium was low in all groups receiving progesterone, but high in the clomiphene + estradiol group. Nuclei of the luminal epithelium were centrally located in progesterone-treated animals (fig. 10), but primarily basally located in the estradiol group (fig. 8) and scattered in the clomiphene group (fig. 9). Vacuolated spaces were observed above and below the nuclei in progesterone-treated animals. Nuclei were also central in all progesterone combination groups (Cl + Pr, E + Pr, and Cl + E + Pr). The surface area and general morphology of the lumen were also characteristic for each individual treatment. The luminal surface area of clomiphene and progesterone-treated rats appeared to be slightly greater than controls (figs. 11 vs 12), but the luminal epithelium of clomiphene-treated rats appeared quite thick owing to the very high columnar epithelium. The luminal surface area of estradiol-treated rats was extensive due to a convoluted, folded morphology, and mitotic figures were abundant (fig. 13).

Progesterone suppressed the characteristic epithelial changes induced by both clomiphene and estradiol; the surface area, height and nuclear location of all progesterone combination groups resembled the progesterone group (figs. 6, 10). Epithelial morphology of the clomiphene + estradiol group very closely resembled that of the clomiphene group (figs. 4 vs 12).

DISCUSSION

The primary significance of these results is the important interaction which was found between clomiphene and progesterone in the control of several morphologic and chemical uterine parameters. Progesterone's antiestrogenic activity is well established (Courrier, '50; Edgren et al., '67; Terenius, '70; Rochefort et al., '72; Tachi et al., '72). Chatterjee et al. ('74) presented evidence that progesterone may interfere with clomiphene's effect on the hypothalamic-pituitary axis. Progesterone clearly suppressed clomiphene-

induced increases in wet and dry weight, glycogen concentration, total glycogen, and luminal epithelial glycogen deposition. The other significant results of this study were those demonstrating the tissue specificity of the glycogen response to estradiol and clomiphene, the unmasking of clomiphene's antiestrogenic effect through the use of a correlated histochemical and biochemical study, and the different effects of clomiphene and estradiol on uterine morphology.

The mechanism by which progesterone suppressed the clomiphene-induced uterine responses is not evident from the present data. Since others have found that clomiphene occupies the same receptors as estradiol (Eisenfeld and Axelrod, '67; Wyss et al., '68; DiPietro et al., '69; Terenius, '71) and that progesterone modifies estradiol-induced uterotrophic effects by various mechanisms (Terenius, '70; Terenius and Ljungkvist, '72; Rochefort et al., '72), any of several antagonistic influences may be operating in the clomiphene:progesterone interaction. Interactions between synthetic and natural estrogens, antiestrogens, and progesterone are complex. In the present study a synthetic antiestrogen, clomiphene, which has some estrogenic potency, suppressed some uterotrophic effects of estradiol. Conversely, a natural antiestrogen, progesterone, suppressed not only the estrogenic effects of estradiol or clomiphene but also the unusual response of the luminal epithelium to clomiphene. The luminal epithelial morphology and glycogen content of every rat receiving progesterone either alone or in combination with clomiphene or estradiol was very similar. Perhaps, as Tachi et al. ('72) showed, progesterone excludes the entry not only of estradiol but also of clomiphene into the luminal epithelial cells.

Clomiphene's marked stimulation of luminal epithelial glycogen was an effect not caused by estradiol. Other investigators, using dosages of estradiol up to 10 μg/rat, have observed only a few cells containing glycogen (Bo and Atkinson, '52; Walaas, '52; Rosenbaum and Goolsby, '57; Bo, '59). Glycogen is not present in these cells at any stage of the normal estrous cycle nor during any of the preimplantation days of pregnancy (Bo and Smith, '66; Christie, '66; Takano, '72). Therefore, the clomiphene effect is a nonphysiologic response of the luminal epithelium to drug stimulation. Progesterone's complete suppression of this response has not been previously reported. Progesterone is known to play a significant role in the control of other luminal epithelial chemical constituents such as lipid (Alden, '47; Boshier and Holloway, '72), carbonic anhydrase (Makler and Morris, '71) and iodide (Brown-Grant, '65, '66; Rogers et al., '69). The present data indicate that progesterone plays a significant role as a glycogenolytic agent in the luminal epithelium of the ovariectomized rat. This is contradictory to its glycogenic effect in the glandular epithelium of the primate uterus (Vesterdal-Jorgensen, '50). A satisfactory explanation for this species difference is not apparent.

To explain clomiphene's luminal epithelial effect one must assume that the drug's effect is not through stimulation of known estrogen receptors as others have discussed (Wyss et al., '68; Terenius and Ljungkvist, '72; Rochefort et al., '72; Sankaran and Prasad, '72), or that clomiphene stimulates these receptors differently, from estradiol. We have no data to support either suggestion. Further questions arise as to whether the epithelial glycogen effect of clomiphene is the result of increased synthesis or decreased degradation of the polysaccharide in these cells. Furthermore, the luminal epithelium, glandular epithelium, and myometrium responded differently to the drug and hormone treatments. This may be an indication of the presence or absence of different receptors for glycogen metabolism in each tissue. Enzyme histochemistry should elucidate some of these questions.

Another difference in the uterotrophic effects of clomiphene and estradiol was their alteration of uterine morphology. As reflected by the wet weight increases, both compounds appeared to increase the thickness of the endometrium and myometrium. The luminal epithelium, however, responded quite differently to the drug and hormone. Clomiphene primarily caused hypertrophy of the luminal epithelium whereas estradiol caused some hypertrophy associated with marked hyperplasia. This was particularly evident in the greater luminal surface area of the estradiol-treated rats. Progesterone clearly suppressed both the effects, making luminal epithelial morphology a very sensitive index of uterotrophic responsiveness, as others have concluded (Terenius and Ljungkvist, '72).

The demonstration of a marked difference

in the histochemical and biochemical analyses of uterine glycogen after clomiphene and estradiol treatment was another significant result. Because of the difference in tissue sensitivity to the drug and hormone, it is of utmost importance to analyze glycogen both qualitatively and quantitatively. Based on biochemical data, one is led to believe that clomiphene is as potent as estradiol. Wood et al. ('68) made the same observation but with a different time and dose protocol. Clomiphene is usually reported to have weak uterotrophic effects (Roy et al., '64; Wood et al., '68; Wyss et al., '68; Sankaran and Prasad, '72). However, these data show that clomiphene is weakly estrogenic only in its effect on myometrial glycogen deposition, since its effect on glycogen concentration and total glycogen was as great as that of estradiol. Furthermore, clomiphene's effect on luminal epithelial glycogen at all doses and times tested was far greater than estradiol's (Poteat and Bo, '71; Poteat, '71). Clomiphene's antiestrogenic effect may be masked in biochemical analysis, as Wood et al. ('68) speculated, but the histochemical analysis unmasked the antiestrogenic effect of clomiphene on myometrial glycogen. Therefore, total uterine glycogen induced by clomiphene and estradiol was similar, but the affected tissue was quite different. The tissue variability of glycogen accumulation shows that both biochemical and histochemical data are necessary to make accurate interpretations of the effects of glycogenic agents. Statements concerning the estrogenic potency of clomiphene must consider time, dosage, parameter and tissue. Clomiphene's antiestrogenic effect was also detected in wet weight analysis, as has been reported (Roy et al., '64; Mohla and Prasad, '68; Wood et al., '68). Studies using the condition of progesterone-sustained delayed implantation as an experimental model for uterine glycogen metabolism (Mohla and Prasad, '68, '69) should take into account the ability of progesterone to suppress clomiphene-induced glycogen and weight parameters.

Studies are being directed toward further characterization of drug-hormone interactions in the control of epithelial metabolism. Such studies should lead to a better understanding of the biologic action of clomiphene and its postcoital contraceptive action. Possible significance of the interaction of progesterone and clomiphene in the control of ovulation should also be investigated.

LITERATURE CITED

Alden, R. H. 1947 Implantation of the rat egg. II. Alteration in osmiophilic epithelial lipids of the rat uterus under normal and experimental conditions. Anat. Rec., *97:* 1-19.

Bo, W. J., and W. B. Atkinson 1952 Histochemical studies on glycogen deposition in the uterus of the rat. I. In intact cyclic animals and in castrates treated with ovarian hormones. Anat. Rec., *113:* 91-100.

Bo, W. J. 1959 Distribution of phosphorylase in the uteri of cyclic and hormone treated rats. J. Histochem. Cytochem., *7:* 403-408.

Bo, W. J., and M. S. Smith 1966 A histochemical study on the glycogen synthesizing enzyme in the myometrium of the pregnant and pseudopregnant rat. J. Reprod. Fertil., *12:* 237-242.

Boshier, D. P., and H. Holloway 1973 Effects of ovarian steroid hormones on histochemically demonstrable lipids in the rat uterine epithelium. J. Endocrinol., *56:* 59-67.

Brown-Grant, K. 1965 The metabolism of iodide by the thyroid gland and by the uterus during early pregnancy in the rat. J. Physiol. (London), *176:* 73-90.

——— 1966 Concentration of radio iodide by the uterus of the rat and the relationship to blastocyst implantation. J. Physiol., *184:* 418-432.

Carroll, N. V., R. W. Longley and J. H. Roe 1956 The determination of glycogen in liver and muscle by use of anthrone reagent. J. Biol. Chem., *220:* 583-593.

Chatterjee, A., T. Gupta and K. Sengupta 1974 Possible mode of action of clomiphene I. Reversal of clomiphene-induced interruption of decidual growth and gestation by prolactin or progesterone in rats. Acta endocrinol., *75:* 173-180.

Christie, G. J. 1966 Implantation of the rat embryo: glycogen and alkaline phosphatases. J. Reprod. Fertil., *12:* 279-294.

Courrier, R. 1950 Interactions between estrogens and progesterone. Vit. Horm., *8:* 179-214.

Di Pietro, D. L., F. J. Sanders and D. A. Goss 1969 Effect of cis and trans isomers of clomiphene citrate on uterine hexokinase activity. Endocrinology, *84:* 1404-1408.

Edgren, R. A., R. C. Jones and D. A. L. Peterson 1967 A biological classification of progestational agents. Fertil. Steril., *18:* 238-256.

Eisenfeld, A. J., and J. Axelrod 1967 Evidence for estradiol binding sites in the hypothalamus-effect of drugs. Biochem. Pharmacol., *16:* 1781-1785.

Emmens, C. W. 1970 Antifertility agents. Ann. Rev. Pharmacol., *10:* 237-254.

——— 1973 Inhibitors of implantation. J. Reprod. Fert, (Suppl.),, *18:* 177-184.

Greenblatt, R. B. 1968 Experimental studies using clomiphene citrate. In: Progress in Infertility. S. J. Behrman and R. W. Kistner, eds. Little, Brown and Co., Boston, pp. 455-466.

Holtkamp, D. E., J. G. Greslin, C. A. Root and L. J. Lerner 1960 Gonadotrophin inhibiting and anti-fecundity effects of chloramiphene. Proc. Soc. Exp. Biol. Med., *105:* 197-200.

Kistner, R. W. 1968 Induction of ovulation with clomiphene citrate. In: Progress in Infertility. S. J. Behrman and R. W. Kistner, eds. Little, Brown and Co., Boston, pp. 407-453.

Makler, A., and J. M. Morris 1971 Effect of postcoital

estrogen on uterine carbonic anhydrase. Fertil. Steril., *22:* 204-208.

Mohla, S., and M. R. N. Prasad 1968 Inhibition of estrogen induced glycogen synthesis in the rat by clomiphene. Steroids, *11:* 571-583.

——— 1969 Oestrogen-anti-oestrogen interaction: effect of U11100A, MRL-41 (clomiphene) and U11555A on oestrogen induced uterine glycogen and protein synthesis in the rat during delayed implantation. Acta Endocrinol., *62:* 489-497.

Poteat, W. L. 1971 A correlated histochemical and biochemical study of the effect of clomiphene citrate and estradiol dipropionate on rat uterine glycogen accumulation. J. S. C. Med. Assoc., *66:* 491.

Poteat, W. L., and W. J. Bo 1971 Histochemical study of estrogenic and antiestrogenic effects of clomiphene citrate on glycogen synthesis by myometrium and luminal epithelium of rat uterus. Anat. Rec., *169:* 717-726.

Rochefort, H., F. Ligon and F. Capony 1972 Effect of antiestrogens on uterine estradiol receptors. Gynec. Invest., *3:* 43-62.

Rogers, A. W., P. H. John and K. Brown-Grant 1969 Early effects of progesterone on the uterus of the ovariectomized rat. J. Anat., *106:* 182-183.

Rosenbaum, R. M., and C. M. Goolsby 1957 The histochemical demonstration of hormonally controlled intracellular glycogen in the endometrium of the rat. J. Histochem. Cytochem., *5:* 33-46.

Roy, S., R. B. Greenblatt and V B. Mahesh 1964 Effects of clomiphene on the physiology of reproduction in the rat. II. Its oestrogenic and antioestrogenic actions. Acta Endocrinol., *47:* 657-668.

Sankaran, M. S., and M. R. N. Prasad 1972 A critique on the evaluation and mode of action of antiestrogens. Gynec. Invest., *3:* 142-147.

Seifter, S., S. Dayton, B. Novic and E. Muntwyler 1950 The estimation of glycogen with the anthrone reagent. Arch. Biochem., *25:* 191-200.

Tachi, C., S. Tachi and H. R. Lindner 1972 Modification by progesterone of oestradiol-induced cell proliferation RNA synthesis and oestradiol distribution in the rat uterus. J. Reprod. Fertil., *31:* 59-76.

Takano, S. 1972 Histochemical studies on carbohydrate metabolism in the endometrium during early pregnancy and delayed implantation of the rat. Kobe J. Med. Sci., *18:* 179-197.

Terenius, L. 1970 Two modes of interaction between oestrogen and anti-oestrogen. Acta Endocrinol., *64:* 47-58.

——— 1971 Structure-activity relationships of anti-oestrogens with regard to interaction with 17β-oestradiol in the mouse uterus and vagina. Acta Endocrinol., *66:* 431-447.

Terenius, L., and I. Ljungkvist 1972 Aspects on the mode of action of antiestrogens and antiprogestogens. Gynec. Invest., *3:* 96-107.

van Handel, E. 1965 Estimation of glycogen in small amounts of tissue. Analytical Biochem., *11:* 256-265.

Vesterdal-Jorgensen, J. 1950 The cytology of uterine epithelia. In: Modern Trends in Obstetrics and Gynecology. K. Bowes, ed. Harper and Brothers, New York, pp. 486-505.

Walass, O. 1952 Effects of oestrogens on the glycogen content of the rat uterus. Acta Endocrinol., *10:* 175-192.

Wood, J. R., T. R. Wrenn and J Bitman 1968 Estrogenic and antiestrogenic effect of clomiphene on rat uterus and vagina using glycogen as a parameter. Endocrinology, *82:* 69-74.

Wyss, R. H., R. Karsznia, W. L. Heinrichs and W. L. Herrmann 1968 Inhibitions of uterine receptor binding of estradiol by antiestrogens (clomiphene and CL-868). J. Clin. Endocrinol. Metab., *28:* 1824-1828.

PLATE 1

EXPLANATION OF FIGURES

1 Endometrium from a control rat treated with vehicle only. No glycogen is present in the luminal epithelium. PAS. × 326.

2 Endometrium from a rat treated with estradiol. A few luminal epithelial cells have glycogen in the apical region. PAS. × 310.

3 Endometrium from a rat treated with clomiphene. Note the high columnar luminal epithelium with heavy glycogen deposition. PAS. × 310.

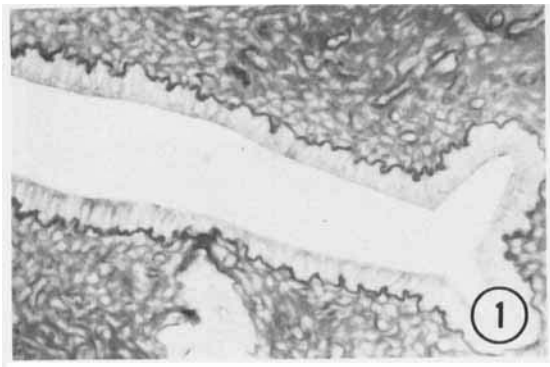
4 Endometrium from a rat treated with clomiphene and estradiol. Note the glycogen in every luminal epithelial cell and its absence in the glandular epithelium. PAS. × 77.

5 Endometrium from a rat treated with clomiphene and progesterone. Diastase-resistant PAS-positive material is seen in the apical cytoplasm of the luminal epithelial cells. PAS. × 310.

6 Endometrium from a rat treated with clomiphene, estradiol and progesterone. Note the low columnar luminal epithelium and the absence of glycogen in these cells. PAS. × 77.

7 Endometrium from a control rat treated with vehicle only. Note the low luminal epithelial cells with the nuclei in basal position. H and E. × 310.

8 Endometrium from a rat treated with estradiol. Note the moderate height of the luminal epithelium and the mitotic figure present in one of the cells. H and E. × 310.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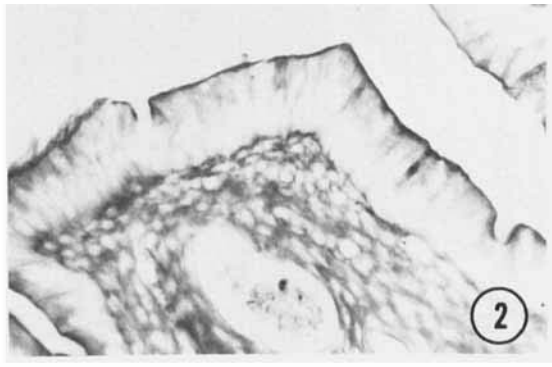
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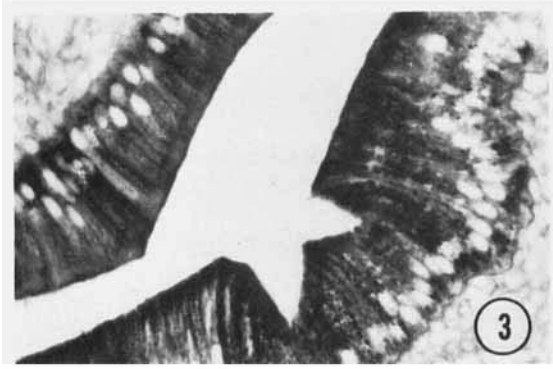
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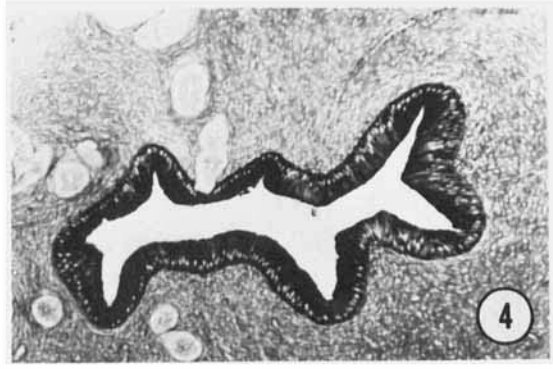
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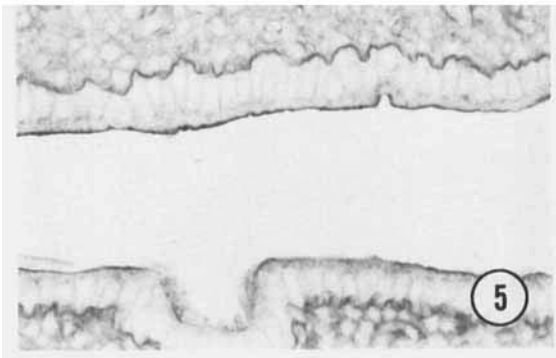
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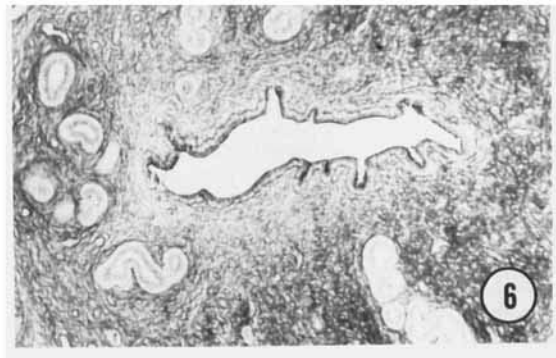
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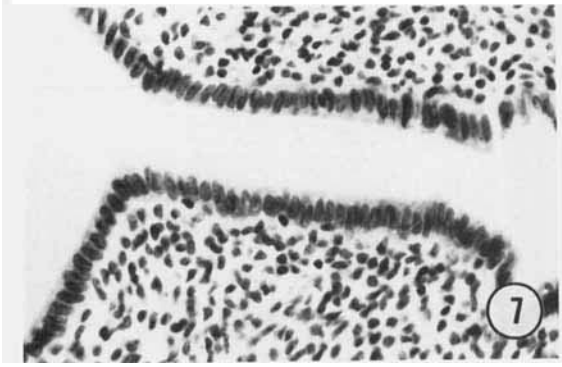
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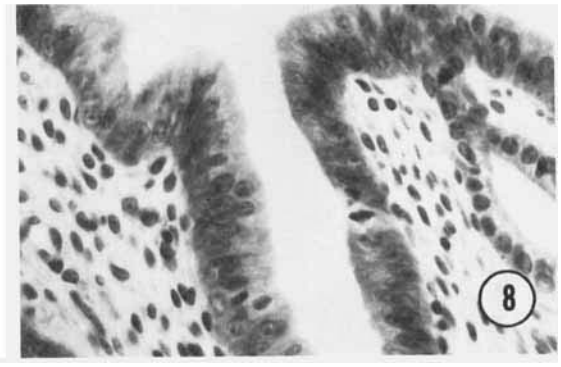
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PLATE 2

EXPLANATION OF FIGURES

9 Endometrium from a rat treated with clomiphene. Note the very tall luminal epithelium. H and E. × 310.

10 Endometrium from a rat treated with clomiphene and progesterone. Note the low luminal epithelial cells with apical and basal vacuoles around centrally located nuclei. H and E. × 310.

11 Uterus from a control rat treated with vehicle only. Note the small lumen. H and E. × 77.

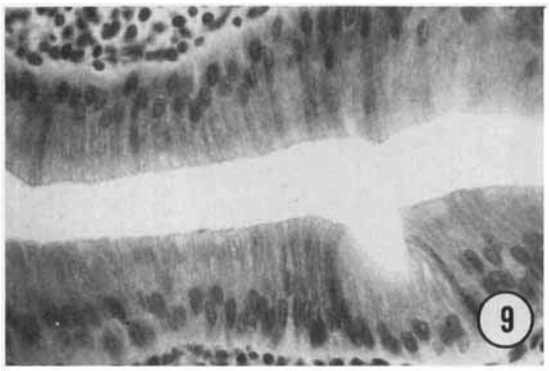
12 Endometrium from a rat treated with clomiphene. Note the small lumen with very high luminal epithelium. H and E. × 77.

13 Endometrium from a rat treated with estradiol. Note the large lumen with a convoluted, villous appearance and the moderate height of the luminal epithelium. H and E. × 77.

14 Myometrium from a control rat treated with vehicle only. Note the few glycogen granules scattered in the outer longitudinal layer. PAS. × 310.

15 Myometrium from a rat treated with clomiphene and estradiol. Note the many small glycogen granules in the outer longitudinal layer. PAS. × 310.

16 Outer longitudinal layer of the myometrium from a rat treated with estradiol. Note the numerous large and small glycogen granules. PAS. × 310.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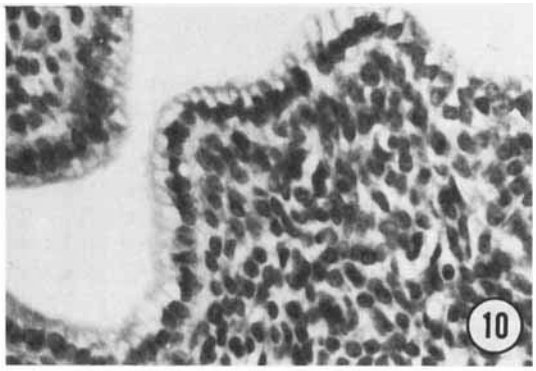
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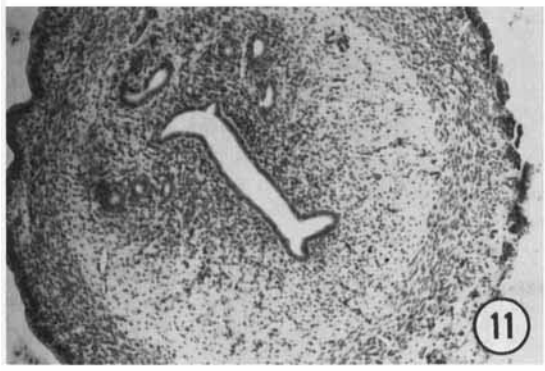
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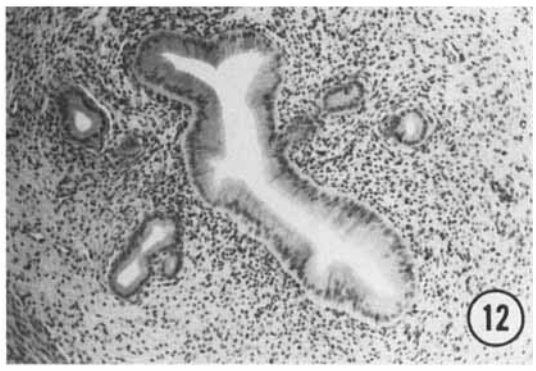
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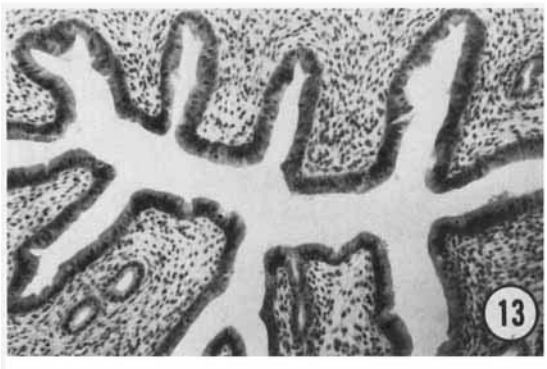
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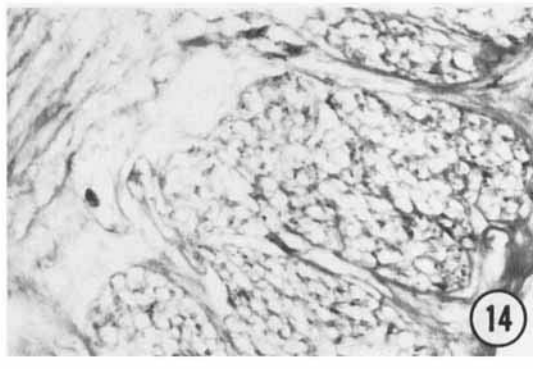
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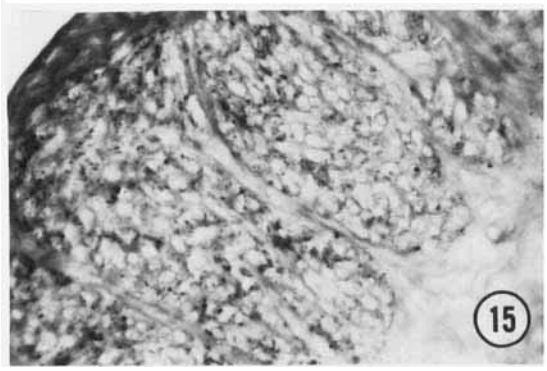
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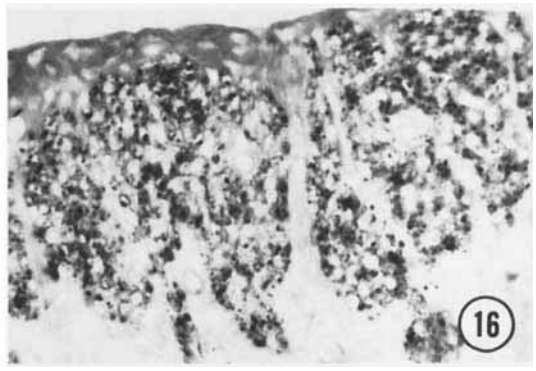
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