The Effects of Phytoestrogens on Neonatal Rat Uterine Growth and Development (43861)

KEVIN L. MEDLOCK,*,[1] WILLIAM S. BRANHAM,* AND DANIEL M. SHEEHAN*,†

Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Department of Health and Human Services, Food and Drug Administration, Jefferson, Arkansas 72079-9502 and Departments of Biochemistry and Pharmacology and Toxicology,† University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205*

Abstract. **Phytoestrogens found in clover, alfalfa, and soybeans have caused reproductive toxicity in several mammalian species. Other estrogens, such as diethylstilbestrol (DES), are developmental toxicants, reducing uterine estrogen receptor (ER) concentration, altering uterine growth, and eliciting reproductive tract abnormalities in the rat. The present study examines the effects of the phytoestrogens coumestrol and equol on the developing rat uterus. Various doses of these compounds were injected sc on postnatal days (PND) 1–5 or 1–10 to ascertain their effects on uterine weight and ER levels, and on PND 10–14 to determine their effects on uterine weight and gland genesis. Coumestrol (PND 1–5) was about 10^{-3} as potent as DES in increasing uterine weight (wet or dry) while equol increased dry weight only, with a potency of 10^{-5} that of DES. Although the 10 and 100 μg doses of coumestrol (PND 1–5 or 1–10) initially increased uterine wet weight, by PND 20 uterine weights either equaled or fell significantly below controls. The 100-μg dose of coumestrol (PND 1–5 or 1–10) reduced ER levels at all ages, while the 10-μg dose was not as effective. Equol (PND 1–5 or 1–10) did not affect ER levels. Premature uterine gland genesis occurred by PND 9 for the PND 1–5 100-μg coumestrol dose. When given on PND 10–14 (the critical period of gland genesis), 10 μg and 100 μg of coumestrol and 10 μg DES greatly increased uterine weight, while no effect was elicited by equol. Although coumestrol and equol inhibited uterine gland genesis in a dose-dependent manner, neither abolished gland genesis as did 10 μg of DES or tamoxifen. These data demonstrate that coumestrol elicits uterine biochemical and morphological toxicity much like DES. Equol decreased uterine gland number without increasing uterine wet weight or luminal epithelial hypertrophy, which is inconsistent with either an estrogenic or antiestrogenic action in the uterus.** [P.S.E.B.M. 1995, Vol 208]

Phytoestrogens may act as estrogens or antiestrogens (1–5, 9, 13) while causing both direct and indirect toxicological effects (6–8, 10–12) on the reproductive tracts of mammals. Although the adverse effects of phytoestrogens on livestock have been studied since the mid 1940s (14), interest in the possible benefits and risks of phytoestrogens in humans has recently increased (12). Most investigations of the effects of phytoestrogens on the reproductive tract, however, have been conducted in the rodent (1–4, 7–11, 16–18), where estrogenic potency depends on the particular phytoestrogen and endpoint. For example, miroestrol is equipotent to diethylstilbestrol (DES) or 17β-estradiol (E_2) (4), coumestrol is 200 times less potent than E_2 (18), and equol is at least 10^3-fold less potent (17). Coumestrol-induced alterations in the reproductive tract of neonatally treated mice include persistent vaginal cornification, hemorrhagic ovarian follicles, and premature vaginal opening (8, 19). In mature mice, coumestrol caused decreased ovulation rates and an increase in embryo degeneration (11).

We have used the neonatal rat to determine estrogen effects on the ontogeny of various biochemical and

[1] To whom requests for reprints should be addressed at Division of the Reproductive and Developmental Toxicology, HFT-130, National Center for Toxicological Research, Jefferson, AR 72079-9502.

Received May 25, 1994. [P.S.E.B.M. 1995, Vol 208]
Accepted September 28, 1994.

0037-9727/95/2083-0307$10.50/0

morphological parameters. These studies provide a basis for a comparative assessment of developmental insult to the reproductive tract for both estrogens and antiestrogens (20–25). Neonatal (postnatal days [PND] 1–5) estrogen exposure causes a slightly premature appearance of uterine glands but ultimately results in reduced uterine gland numbers (20–21). During the period of rapid uterine gland development (PND 10–14) estrogens cause a dose-dependent delay in the appearance of uterine glands (20, 21). This delay in the onset of uterine gland development may be related to the maintenance of estrogen-induced luminal epithelium hypertrophy (25). Antiestrogens also cause substantial and long-lasting luminal epithelium hypertrophy with little uterine weight gain. However, antiestrogens are potent inhibitors of uterine gland genesis (21, 22). Both estrogens and antiestrogens cause a persistent reduction in uterine estrogen receptor (ER) levels (22, 26).

In this report, we characterize the effects of coumestrol and equol on uterine weight gain, luminal epithelial cell height (LEH), gland genesis, and the short-term regulation of ER level in neonatal rats. As well, the persistence of these effects on uterine weight, ER levels, and glands is examined in older rats following neonatal dosing.

Materials and Methods

Animals. Offspring from date-mated Sprague-Dawley rats from the NCTR breeding colony were culled according to sex and the females randomly distributed to dams within 24 hr of birth. Groups of pups were injected sc, in the middorsal region, on PND 1–5 or 1–10 with various doses of coumestrol or equol in 10 μl of sesame oil. Control animals were untreated. The animals were sacrificed on PND 5, 10, 15, 20, and 25. The number of animals used at each time point and treatment group ranged from 21 at PND 5 to three at PND 25.

Chemicals. Coumestrol and equol were purchased from Spectrum Chemical Mfg. Corp. (Garden, CA). DES was obtained from Research Plus Steroid Laboratories, Inc. (Denville, NJ). Tamoxifen (TAM) was a gift from Stuart Pharmaceuticals (Wilmington, DE). [^{3}H]-E_2 (sp act 92–115 Ci/mmol) was purchased from Dupont NEN Products (Boston, MA). All other chemicals were laboratory grade.

Uterotropic Dose-Response. Pups were injected daily on PND 1–5 with 0.001, 0.01, 0.1, 10, or 100 μg DES; 0.01, 0.1, 1, 10, 50, 100, or 1000 μg coumestrol; or 1, 10, 100, or 1000 μg equol. On PND 5, the pups were weighed and killed by cervical dislocation after being lightly anesthetized with ether. The uteri were removed, weighed, placed on preweighed squares of aluminum foil, dried overnight at 70°C, and then reweighed.

ER Time Course. Pups were injected on PND 1–5 or 1–10 with 10 or 100 μg coumestrol or 100 μg equol. One hour before sacrifice, all animals were injected with 10 μg DES to maximize ER binding in the nuclear fraction and thus avoid confounding of results due to alpha-fetoprotein contamination (27). Following sacrifice, the uteri were pooled and then homogenized in cold TE buffer (10 m*M* Tris, 1.5 m*M* EDTA, pH 7.4) at a concentration of 30 mg tissue/ml of buffer. The homogenates were processed as described previously with nuclear ER levels being determined by the [^{3}H]-E_2 exchange assay (24).

Uterine Gland Genesis. *Time course study.* Pups were injected on PND 1–5 with 10 or 100 μg coumestrol or equol. They were sacrificed at PND 5, 9, 26, and 60 for comparison of effects with those reported previously for E_2, DES, and TAM (20–22). Uteri were carefully removed, stripped of connecting mesentery, and fixed in 10% neutral buffered formalin. They were processed using standard histological procedures, stained with hematoxylin and eosin, and sectioned at 4 μm. At least six sections per animal were scored for uterine glands and measurements of LEH (25).

Dose-response study. Control pups and those given 1, 10, or 100 μg of coumestrol; 10, 100, or 1000 μg of equol; or 10 μg of DES or tamoxifen were injected on PND 10–14. They were sacrificed on PND 14 and treated as described above.

Statistics. Statistical analyses were done using a two-way ANOVA and are presented as means ± SEM with a level of significance of $P < 0.05$ (significance was determined by Duncan's multiple range test).

Results

Following dosing on PND 1–5 and evaluation on PND 5 the uterine wet weight dose-response curves for DES and coumestrol were parallel (Fig. 1A). There was a slight but not significant uterine wet weight gain at the highest dose of equol. DES was about 4×10^2-fold more potent than coumestrol. Uterine dry weight dose-response curves for DES, coumestrol, and equol were all parallel in the ascending part of the curves (Fig. 1B). Equol induced a significant dry weight increase only at the highest dose with a potency about 10^{-5} of that for DES. For dry weight, DES was about 3×10^3-fold more potent than coumestrol. Thus, coumestrol was about 8-fold more potent for wet weight gain than dry weight gain relative to DES.

Based on the dose-response curves, phytoestrogen doses were chosen for ontogeny studies after neonatal dosing. Again, both 10 μg and 100 μg coumestrol doses elicited significant uterine weight gain on PND 5 (Fig. 2A). By PND 10, only the 100 μg coumestrol dose group still showed increased uterine weight. At PND 10, 20, and 25 for the 10 μg coumestrol group, and PND 20 and 25 for the 100 μg coumestrol and

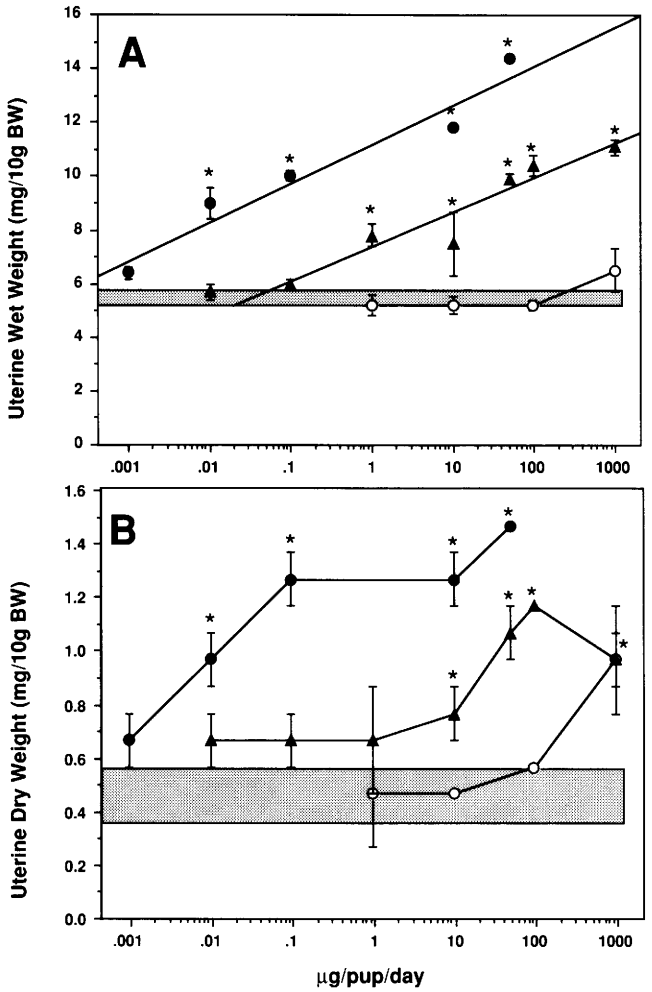

Figure 1. Uterine weight dose-response in rat pups either untreated or injected on PND 1–5 with DES (●), coumestrol (▲), or equol (○) and sacrificed on PND 5. (A) Uterine wet weight; (B) Uterine dry weight. Shaded area represents the untreated means ± SEM. The data are present as means ± SEM with $n \geq 7$. Asterisks indicate significant differences at $P \leq 0.05$.

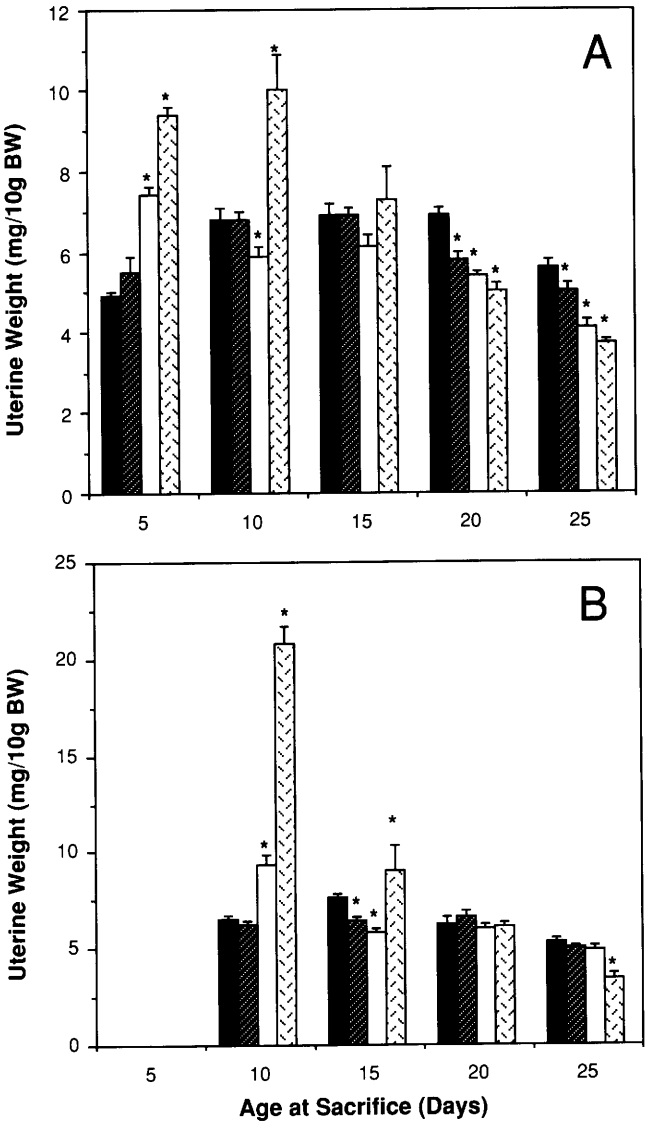

Figure 2. Uterine weight in untreated rat pups (■) or pups injected neonatally with 100 µg equol (▨), 10 µg coumestrol (▭), or 100 µg coumestrol (▭) and sacrificed on PND 5, 10, 15, 20, and 25. (A) Pups were injected on PND 1–5; (B) Pups were injected on PND 1–10. The data are presented as means ± SEM with a $n \geq 18$. *Significant difference at $P \leq 0.05$.

equol groups, uterine weights were significantly lower than controls. Ten days of treatment (PND 1–10) significantly increased uterine weight in the 10 and 100 µg coumestrol dose groups (Fig. 2B). However, by PND 15, the 10 µg equol and coumestrol groups had uterine weights significantly lower than controls, while rats given 100 µg coumestrol had uterine weights significantly greater than controls. By PND 20, no differences were seen in any group, whereas by PND 25 uterine weights in the 100 µg coumestrol group were significantly lower than controls.

There was no effect of 100 µg equol at ages up to PND 25 on uterine ER levels when given on PND 1–5 (Fig. 3A). After 5 days of coumestrol treatment (Fig. 3A), the 100 µg dose reduced the ER level to 60% of controls. By PND 10, ER levels decreased even further, to 15%–20% of controls. Subsequently, ER levels remained 40%–70% below controls (Fig. 3A). The 10 µg dose of coumestrol caused a significant ER reduction on PND 10, 15, and 25. After 10 days of exposure (PND 1–10), 100 µg equol reduced ER significantly, but at later ages, ER remained at control levels (Fig. 3B). By contrast, both coumestrol doses severely reduced the ER levels to 20%–40% of controls on PND 10 (Fig. 3B). At later ages, the ER level in the 10 µg coumestrol dose group progressively increased, until by PND 20–25 it was not different from controls. In rats given 100 µg coumestrol, the ER level remained at 20% of control on PND 15 after which it increased with time to a value about two thirds of controls.

Following PND 1–5 dosing, no uterine glands were observed at PND 5 in either the controls or any treated pups (Table I). Equol treatment had no effect on gland number at any age. However, at PND 9, there was a

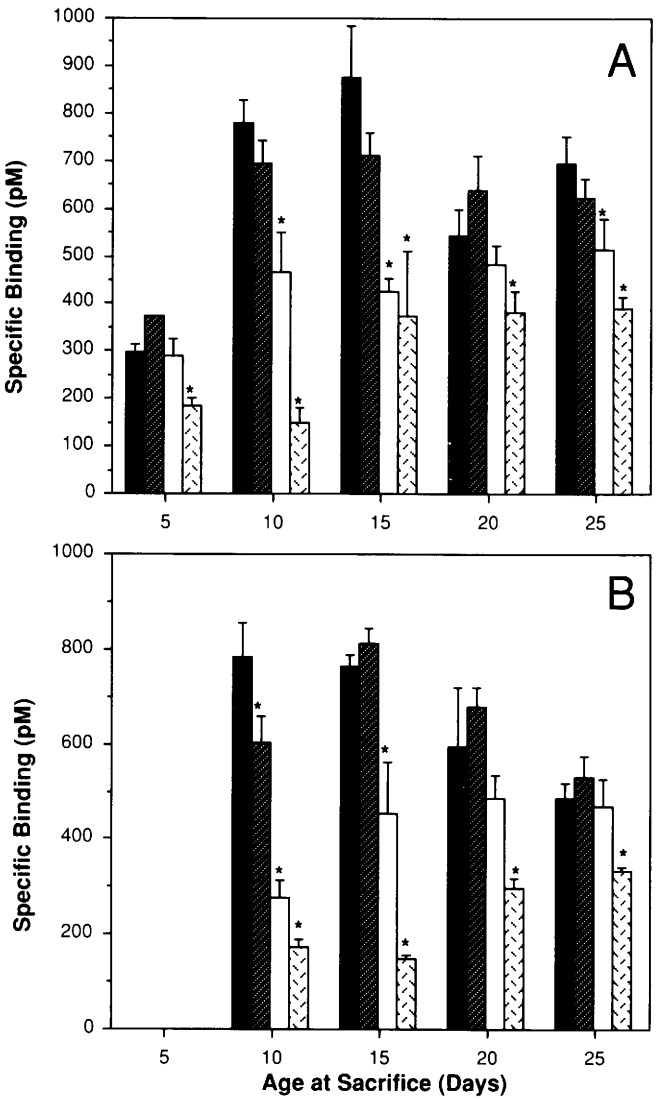

Figure 3. Uterine ER levels in nontreated rat pups (■) or pups injected neonatally with 100 μg equol (▨), 10 μg coumestrol (□), or 100 μg coumestrol (▨) and assayed on PND 5, 10, 15, 20, and 25. (A) Pups were injected on PND 1–5; (B) Pups were injected on PND 1–10. The data are presented as means ± SEM with a $n \geq 3$. *Significant difference at $P \leq 0.05$.

significant increase in glands at the 100 μg coumestrol dose (Table I). By PND 26, the gland numbers for all treatments were essentially the same as the controls (Table I). While gland numbers increased from PND 26 to PND 60, there were no significant differences between any of the groups. LEH was elevated only on PND 5 in the high-dose coumestrol group.

After PND 10–14 treatment, only the 10 and 100 μg doses of coumestrol significantly increased uterine weight (Fig. 4A). While the antiestrogen TAM increased uterine weight slightly but significantly at the 10 μg dose, the same dose of DES tripled uterine weight (Fig. 4A). Both coumestrol and equol caused a reduction in gland number, and the equol effect was dose dependent (Fig. 4B). Coumestrol appeared to be 10–100 times more potent than equol with respect to inhibition of gland genesis, although the maximum reduction (to 25% of controls) was the same for both compounds (Fig. 4B). Both the 10 μg TAM and DES doses dramatically reduced gland genesis. With the exception of TAM, the effect of all treatments on LEH was very similar to that seen for uterine weight (Fig. 4 A and C). The 10 μg TAM dose increased LEH as much as the 10 μg DES dose. No dose of equol increased LEH (Fig. 4C). The 10 and 100 μg doses of coumestrol increased LEH to double the control value, although both were significantly lower than TAM or DES (Fig. 4C).

Discussion

The data reported here demonstrate that the phytoestrogen coumestrol is estrogenic when examined at several ages during the postnatal development of the rat, using uterine growth and morphological and biochemical endpoints. By contrast, equol demonstrated characteristics inconsistent with any estrogen or antiestrogen previously studied in this system (20–25).

In immature rats, the uterotrophic potency of coumestrol and equol compared with E_2 or DES is 0.5% and 0.1%, respectively (17, 18). We have examined a variety of estrogens and antiestrogens with respect to uterine weight gain following treatment on PND 1–5 (20–24). Our wet weight data are in general agreement with the estimated potency of coumestrol but not equol in immature animals. If the difference in uterotrophic potency between coumestrol and equol is the same in neonates as in immature rats, then at the 100 μg/pup dose, we would have expected an average uterine weight of 9 mg instead of the actual value of 5 mg. We can estimate equol potency for uterine growth by using dry weight data, which suggests a potency that is 30-fold less than coumestrol or about 10^{-5}-fold less than DES. The former should be compared with the 5-fold lower potency of equol in immature rats (17, 18). These findings demonstrate an age-dependent alteration in relative equol potency. Pharmacokinetic differences between the neonatal and the immature rat (i.e., changes in metabolism and/or in excretion pathways) might explain this finding.

Comparison of parallel segments of the dose-response curves for wet and dry weight for coumestrol and DES shows that coumestrol is about 8-fold less potent than DES in causing dry weight gain compared with wet weight gain. Thus, wet and dry uterine weights give different estimates of relative potency for both coumestrol and equol.

Following PND 1–5 treatment, we observed a small but significant decrease in uterine weight on PND 20 and 25 with both coumestrol and equol. A similar effect has been previously reported for other neonatally administered estrogens (20). For this delayed estrogenic effect, equol appears to be about 10-

Table I. Effect of Treatment on PND 1–5 with Equol or Coumestrol on Uterine Glands and LEH

Treatment	5		9		26		60	
	Glands/ section	LEH	Glands/ section	LEH	Glands/ section	LEH	Glands/ section	LEH
Control	0.00	12.56 ± 0.82	0.00	23.11 ± 0.39	4.44 ± 0.21	15.52 ± 1.48	6.39 ± 0.42	18.29 ± 1.82
100 μg equol	0.00	14.43 ± 0.31	0.00	23.86 ± 0.40	3.61 ± 0.34	15.67 ± 0.46	7.35 ± 0.74	21.89 ± 4.00
100 μg coumestrol	0.00	30.52 ± 1.36[a]	1.67 ± 0.34[a]	24.24 ± 1.00	3.87 ± 0.49	12.36 ± 0.44	5.75 ± 0.92	21.86 ± 6.48

[a] Significantly greater than control.

fold less potent than coumestrol (compare 10 μg coumestrol with 100 μg equol; Fig. 2A). The decrease in uterine weight may be due to an alteration in the hypothalamo-hypophyseal-ovarian axis resulting in lowered estrogen secretion (28). Alternatively, the uterine weight reduction might be due to reduced responsiveness to estrogens since earlier work has shown that rats treated with DES on PND 1–5, ovariectomized and then implanted with an E_2 implant for a week gave only a 70% uterine weight response compared with the control response. This implies a persistent impairment of uterine responsiveness by the neonatal DES treatment.

The coumestrol data imply that coumestrol has a longer half-life than equol or DES. The pattern of uterine weight gain and loss for the 100 μg coumestrol dose differed from that reported earlier for 10 μg DES in which there was a 3-fold increase over controls on PND 5 which dropped by approximately 50% on PND 10 and was significantly lower than controls on PND 15 (20). The 100 μg coumestrol effect was more persistent than DES in that normalized uterine weight at PND 10 was the same as on PND 5. Perhaps coumestrol is removed more slowly than DES due to metabolic differences, retention in fat depots, or other pharmacokinetic differences.

PND 1–10 treatment was less sensitive than PND 1–5 in eliciting later uterine weight reduction. This apparent lowered responsiveness of the PND 1–10 dosing schedule compared with PND 1–5 may be due to the fact that on PND 25, examination is 20 days postdosing in the 5-day regimen, while it is 15 days postdosing for the PND 1–10 schedule.

The effect of equol on PND 1–5 in subsequently lowering uterine weight on PND 20 and 25 is not reflected in lowered ER levels. Coumestrol, however, elicited dose-dependent, persistent decreases in uterine ER. This decrease is not due to the uterine weight loss, per se, since the same weight of control or treated tissue (30 mg/ml) is used in the ER assay. On PND 1–10, coumestrol again induced a dose-dependent, persistent decrease in ER, while a late uterine weight decrease was seen only on PND 25 in the high-dose coumestrol group. Thus, ER reduction appears to be a

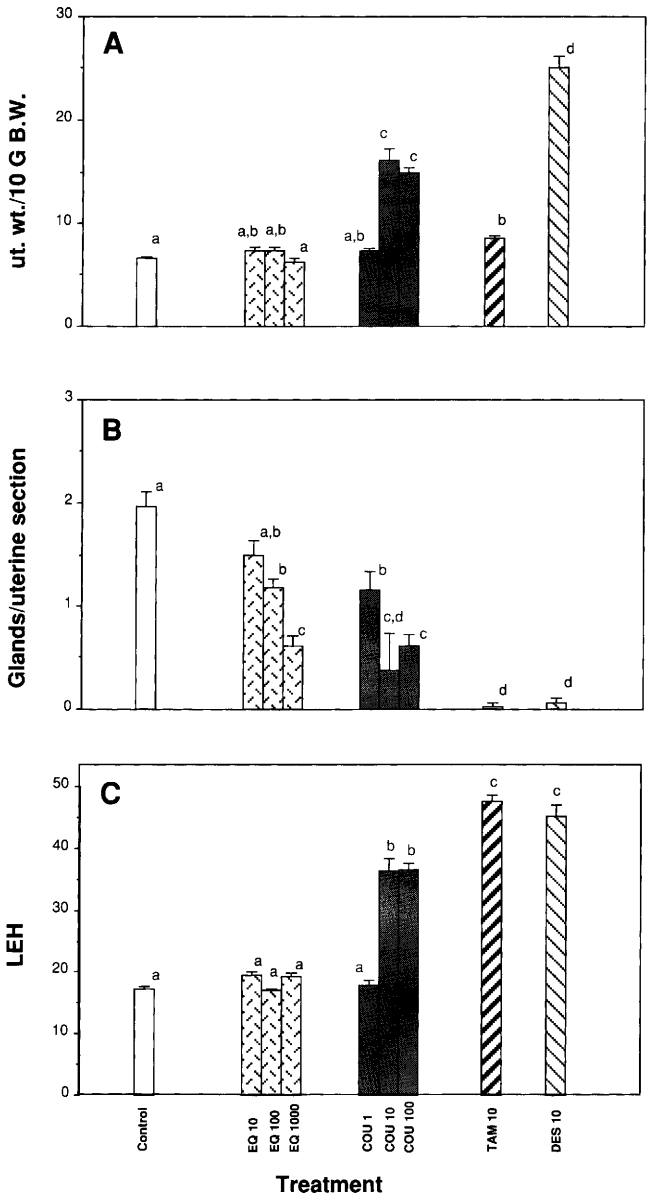

Figure 4. Dose-response for normalized uterine weight (A), gland number (B), and luminal epithelial cell height (LEH) (C) in rat pups injected on PND 10–14 and sacrificed on PND 14. Treatments included untreated controls; 10, 100, or 1000 μg/pup of equol, 1, 10, or 100 μg/pup of coumestrol or 10 μg/pup of tamoxifen or DES. The data are presented as means ± SEM with a $n \geq 7$. Values with different letters are statistically different from each other at $P \leq 0.05$.

more sensitive measure of early coumestrol exposure than is uterine weight reduction.

Coumestrol given on PND 1–5 induced an increase in uterine LEH and premature gland genesis, but had no effect on later gland levels. Equol was without effect. Potent estrogens such as E_2, DES, and ethynylestradiol induce premature gland genesis but long-term gland numbers are lowered (21, 25). In this study, controls showed the same ontogenic pattern of LEH and gland number as previously reported (25).

Dosing with estrogens and antiestrogens during the critical period of gland genesis (PND 10–14) has a more profound effect on inhibition of gland development than dosing on PND 1–5, a period prior to gland appearance (22, 25). However, antiestrogens, such as TAM, and estrogens, such as DES, have distinctly different properties during this period of development. While DES greatly increases uterine weight, TAM only slightly increases it. Both inhibit gland appearance and increase LEH. These effects were replicated when TAM and DES were used as positive controls in this study. Coumestrol behaved like an estrogen, increasing uterine weight and LEH, and inhibiting gland appearance. Equol, however, had no effect on uterine weight or LEH, but inhibited gland appearance in a dose-dependent manner. Equol appears about 100-fold less potent than coumestrol for this endpoint. Thus equol shows a different pattern of effects on the developing uterus during PND 10–14 than either estrogens or antiestrogens. This finding is consistent with the earlier described effect of equol following treatment on PND 1–5 in increasing uterine dry weight but not wet weight, and having a persistent effect on uterine weight out to PND 25, without lowering ER levels during the same developmental period. Coumestrol, on the other hand, behaves like DES in the types of effects observed. These effects could be explained by different levels of sensitivity to estrogens for different endpoints.

It is unclear why equol shows a pattern of effects during development that are different from estrogens or antiestrogens. Earlier studies on equol suggested that it is an estrogen, albeit a weak one (17). Possibilities for such a different pattern for equol include dissociation of noncausally linked estrogen-regulated events (i.e., wet weight from dry weight [29]), an additional mechanism of toxicity beyond its weak estrogenic activity, an additional site of action (i.e., at a different cell type, tissue, or receptor) or pharmacokinetic and/or metabolic differences leading to altered pharmacodynamics.

This study has demonstrated the developmental potency and toxicity of the phytoestrogens, coumestrol, and equol. Coumestrol exhibits properties found with estrogens generally, while equol possesses a pattern of developmental outcomes that is not shared by either estrogens or antiestrogens. The findings with equol require more detailed studies of its pharmacological and toxicological properties to understand its mechanism of action.

The authors gratefully acknowledge the contributions of Mary Lee Leamons for her technical assistance in conducting the ER assay; Loetta Bradford and Diann Smith for manuscript preparation; and the General Services Branch, National Center for Toxicological Research, for preparation of the figures. The authors also express appreciation to Vera Campbell, Hazel Carpenter, Howard Durrett, Charles Law, Marsha Law, Vicki Thompson, and Alice Crumpton for their care and attention in animals maintenance and hormone injections.

1. Kitts WD, Newsome FE, Runeckles VC. The estrogenic and antiestrogenic effects of coumestrol and zearalanol on the immature rat uterus. Can J Anim Sci **63**:823–824, 1983.
2. Perel E, Lindner HR. Dissociation of uterotrophic action from implantation-inducing activity in two non-steroidal oestrogens (coumestrol and genistein). J Reprod Fertil **21**:171–175, 1970.
3. Noteboom WD, Gorski J. Estrogenic effect of genistein and coumestrol diacetate. Endocrinology **73**:736–739, 1963.
4. Jones HEH, Pope GS. A study of the action of miroestrol and other oestrogens on the reproductive tract of the immature female mouse. J Endocrinol **20**:229–235, 1960.
5. Barnes S, Grubbs C, Setchell KDR, Carlson J. Soybeans inhibit mammary tumors in models of breast cancer. In: Pariza NE, et al., Eds. Mutagens and Carcinogens in the Diet. New York: Wiley-Liss, pp239–253, 1990.
6. Findlay JK, Buckmaster JM, Chamley WA, Cumming IA, Hearnshaw H, Goding JR. Release of luteinizing hormone by oestradiol-17β and a gonadotrophin-releasing hormone in ewes affected with clover disease. Neuroendocrinology **11**:57–66, 1973.
7. Hughes CL Jr. Effect of phytoestrogens on GnRH-induced luteinizing hormone secretion in ovariectomized rats. Reprod Toxicol **1**:179–181, 1988.
8. Burroughs CD, Mills KT, Bern HA. Long-term genital tract changes in female mice treated neonatally with coumestrol. Reprod Toxicol **4**:127–135, 1990.
9. Whitten PL, Russell E, Naftolin F. Effects of a normal, human-concentration, phytoestrogen diet on rat uterine growth. Steroids **57**:98–106, 1992.
10. East J. The effect of genistein on the fertility of mice. J Endocrinol **13**:94–100, 1955.
11. Fredricks GR, Kincaid RL, Bondioli KR, Wright RW Jr. Ovulation rates and embryo degeneracy in female mice fed the phytoestrogen, coumestrol. Proc Soc Exp Bio Med **167**:237–241, 1981.
12. Setchell KDR, Adlercreutz H. Mammalian lignans and phytoestrogens. Recent studies on their formation, metabolism and biological role in health and disease. In: Rowland IR, Ed. Role of the Gut Flora in Toxicity and Cancer. London: Academic Press, pp315–345, 1988.
13. Bradbury RB, White DE. Estrogens and related substances in plants. Vitam Horm **12**:207–233, 1954.
14. Braden AWH, Thain RI, Shutt DA. Comparison of plasma phytoestrogen levels in sheep and cattle after feeding on fresh clover. Aust J Agric Res **22**:663–670, 1971.
15. Kaldas RS, Hughes CL Jr. Reproductive and general metabolic effects of phytoestrogens in mammals. Reprod Toxicol **3**:81–89, 1989.
16. Farmakalidis E, Hathcock JN, Murphy PA. Oestrogenic po-

tency of genistin and daidzin in mice. Food Chem Toxic **23**:741–745, 1985.
17. Tang BY, Adams NR. Effect of equol on oestrogen receptors and on synthesis of DNA and protein in the immature rat uterus. J Endocrinol **85**:291–297, 1980.
18. Newsome FE, Kitts WD. Action of phytoestrogens coumestrol and genistein on cytosolic and nuclear oestradiol-17β receptors in immature rat uterus. Anim Reprod Sci **3**:233–245, 1980.
19. Burroughs CD, Bern HA, Stokstad ELR. Prolonged vaginal cornification and other changes in mice treated neonatally with coumestrol, a plant estrogen. J Toxicol Environ Health **15**:51–61, 1985.
20. Medlock KL, Sheehan DM, Nelson CJ, Branham WS. Effects of postnatal DES treatment on uterine growth, development, and estrogen receptor levels. J Steroid Biochem **29**:527–532, 1988.
21. Branham WS, Zehr DR, Chen JJ, Sheehan DM. Uterine abnormalities in rats exposed neonatally to diethylstilbestrol, ethynylestradiol, or clomiphene citrate. Toxicology **51**:201–212, 1988.
22. Branham WS, Sheehan DM, Zehr DR, Medlock KL, Nelson CJ, Ridlon E. Inhibition of rat uterine gland genesis by tamoxifen. Endocrinology **117**:2238–2248, 1985.
23. Sheehan DM, Branham WS, Medlock KL, Shanmugasundaram ERB. Estrogenic activity of zearalenone and zearalanol in the neonatal rat uterus. Teratology **29**:383–392, 1984.
24. Medlock KL, Lyttle CR, Kelepouris N, Newman ED, Sheehan DM. Estradiol down-regulation of the rat uterine estrogen receptor. Proc Soc Exp Biol Med **196**:293–300, 1991.
25. Branham WS, Sheehan DM, Zehr DR, Ridlon E, Nelson CJ. The postnatal ontogeny of rat uterine glands and age-related effects of 17β-estradiol. Endocrinology **117**:2229–2237, 1985.
26. Medlock KL, Branham WS, Sheehan DM. Long-term effects of postnatal exposure to diethylstilbestrol on uterine estrogen receptor and growth. J Steroid Biochem Molec Biol **42**:23–28, 1992.
27. Sheehan DM, Young M. Diethylstilbestrol and estradiol binding to serum albumin and pregnancy plasma of rat and human. Endocrinology **104**:1442–1446, 1979.
28. Bern HA, Talamantes CL Jr. Neonatal mouse models and their relation to disease in the human female. In: Herbst AL, Bern HA, Eds. Developmental Effects of Diethylstilbestrol (DES) in Pregnancy. New York: Thieme-Stratton, pp129–147, 1981.
29. Lan NC, Katzenellenbogen BS. Temporal relationships between hormone receptor binding and biological responses in the uterus. Studies with short- and long-acting derivatives of estriol. Endocrinology **98**:220–227, 1976.