

Urodynamic and morphologic changes in the lower portion of the urogenital tract after administration of estriol alone and in combination with phenylpropanolamine in sexually intact and spayed female dogs

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Objective—To compare the urodynamic and morphologic effects of the administration of estriol alone and in combination with phenylpropanolamine on the lower portion of the urogenital tract in female dogs.

Animals—3 sexually intact and 3 spayed female Beagles without urinary incontinence.

Procedure—Dogs received estriol (2 mg, PO) once daily for 7 days followed by estriol (2 mg, PO) and phenylpropanolamine (1.5 mg/kg, PO) once daily for 7 days. Urethral pressure profilometry, diuresis cystometry, and vaginourethrography were performed before treatment (day 0) and at days 7 and 14. The maximum urethral pressure (MUP) and closure pressure (MUCP), urethral functional and anatomic profile lengths, integrated pressure (IP), plateau, distance before MUP, maximum meatus pressure, threshold pressure, threshold volume, compliance, urethral length, and vaginal length and width were measured.

Results—Before treatment, no urodynamic differences were observed between the 2 groups; however, vaginal length and width were significantly shorter in spayed dogs. Compared with day 0 values, estriol treatment significantly increased MUP, MUCP, and IP values at day 7, but at day 14, this effect decreased despite phenylpropanolamine administration. No morphologic changes from baseline were detected after either treatment in any dog.

Conclusions and Clinical Relevance—Data suggest that estriol mainly acts on the urethral sphincter mechanism by increasing urethral resistance in sexually intact and spayed female dogs without urinary incontinence. Administration of estriol and phenylpropanolamine did not increase the urethral resistance more than estriol alone. The urodynamic effects of estriol in female dogs with urinary incontinence remain to be elucidated. (*Am J Vet Res* 2006;67:901–908)

Urethral sphincter mechanism incompetence is the most common cause of acquired urinary incontinence in spayed female dogs.¹ Among dogs, the prevalence of the disease in spayed females is higher than it

ABBREVIATIONS

USMI	Urethral sphincter mechanism incompetence
MUCP	Maximum urethral closure pressure
UFPL	Urethral functional profile length
MUP	Maximum urethral pressure

is in sexually intact females.^{2–5} Indeed, Thrusfield et al⁶ determined an incidence rate per animal-year of 1.7% in neutered dogs and 0.22% in sexually intact dogs; the relative risk for development of USMI was 7.8 times greater in neutered dogs. Among female dogs with USMI, 90% are spayed.^{2,4} Clinical signs of USMI, such as urine leakage during sleep or periods of excitement, may develop as long as 10 years after spaying.^{1,3,7} Results of urodynamic studies^{8–10} have indicated that MUCP and UFPL values in female dogs with USMI are significantly decreased, compared with findings in female dogs without urinary incontinence.

Urethral sphincter mechanism incompetence has been compared to postmenopausal urinary incontinence in women because both conditions are associated with a state of estrogen deficiency.⁷ In women, the lack of estrogens induces major changes in the lower portion of the urinary tract, including bladder and urethral mucosal atrophy, reduction of urethral and periurethral collagen content and urethral submucosal vascularity, and decreased sensitivity of the urethral smooth musculature to α -adrenergic stimulation.¹¹ These postmenopausal structural changes lead to functional urodynamic modifications such as decreases in MUCP^{12–17} and UFPL¹⁴ and increases in bladder and premicturition detrusor pressures.¹⁷

Structural and functional changes that develop in the urogenital tract of dogs after spaying have been sparsely studied in veterinary medicine. In dogs, vaginal length and volume are decreased in spayed females, compared with findings in sexually intact females.^{18,19} Reichler et al²⁰ detected a significant decrease in MUCP in spayed female dogs 10 months after sterilization. One might hypothesize that decreased urethral resistance in spayed dogs could be related to the lack of estrogens and could predispose these animals to the development of USMI. Therefore, estrogen replacement has been widely used in the treatment of stress incontinence in women or USMI in female dogs.^{2,21–31} The extensive use of estrogens in the treatment of these conditions was reinforced by identification of high-

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affinity estradiol receptors in the urethra, vagina, and pubococcygeal muscle and, to a lesser extent, in the bladder of humans, rabbits, and rats.³²⁻³⁵ In female dogs, estrogen receptors have been identified in the transitional epithelium and subjacent stroma of the proximal portion of the urethra, but were not detected in the kidneys, ureters, bladder, and distal portion of the urethra.³⁶

Estriol, a naturally occurring estrogen, is commonly used in humans and is currently the estrogenic substance of choice for treatment of female dogs. It differs from other estrogen compounds, such as estradiol and stilbestrol, because it occupies the nuclear-bound receptors for a relatively short period,³⁷ thereby decreasing the risk of associated adverse effects. Estriol has been used for decades in hormone replacement therapy for women and has not been associated with bone marrow depletion.³⁸ Long-term administration of estriol in female dogs has not been accompanied by adverse effects such as pyometra and medullar aplasia.³⁹

Unexpectedly, hormone replacement in postmenopausal women has led to controversial and contradictory results.²¹⁻²⁵ Whereas some studies^{24,26-29} have indicated improvement of the symptoms of urinary incontinence associated with urogenital atrophy and recurrent urinary tract infections after oral or intravaginal administration of estriol, other studies^{25,40-42} did not reveal any improvement of the urinary symptoms after estrogen treatment. Although increased urethral pressure was detected by Dessole et al²⁹ after intravaginal administration of estriol, other investigators detected minimal²³ or no changes^{22,43} in the values of urodynamic parameters with the exception of an increased ratio of pressure transmission to the proximal portion of the urethra.^{23,24,29,43}

In female dogs with USMI, the success rate for complete urinary continence with administration of estriol is approximately 65%.^{30,31} The urodynamic effects of estriol administration have been sparsely investigated in the veterinary literature. In 1 study,^{44,a} an increased UFPL and threshold bladder pressure were detected following estriol administration in Beagles.

On the basis of evidence that estrogens increase the sensitivity of the α -adrenergic receptors to the sympathetic stimulation,^{7,45-48} it appears reasonable to combine an α -adrenergic agent with estrogen to try to obtain a synergetic therapeutic effect. Results of several studies^{40,49-53} have indicated that administration of such drug combinations is superior to the administration of estriol alone, in terms of improving urinary symptoms attributable to stress incontinence in postmenopausal women. However, results are contradictory regarding the urodynamic effects of this type of combination treatment. In some studies,^{50-52,54} values of various urodynamic parameters increased after the combined administration, compared with effects after administration of estriol alone, whereas another study⁵² did not reveal any modification in values of the urodynamic parameters, although the symptoms in postmenopausal women with stress urinary incontinence were greatly improved.

Investigations of the effectiveness of estrogen combined with an α -adrenergic agent in female dogs are

almost nonexistent. Creed⁴⁷ identified an increased UFPL after administration of estrogens and phenylephrine in female dogs without urinary incontinence, whereas this effect was not detected in incontinent females. The purpose of the study reported here was to evaluate and compare the urodynamic and morphologic effects of the administration of estriol alone and in combination with phenylpropanolamine on the lower portion of the urogenital tract in sexually intact and spayed female Beagles that did not have urinary incontinence.

Materials and Methods

Dogs—Six adult (mean $\pm$ SD age, 11 $\pm$ 1 years old) female Beagles were included in the study. The dogs were allocated to 1 of 2 groups on the basis of their reproductive status; group 1 included 3 sexually intact females in anestrus (determined on the basis of visual examination of the vulva and cytologic examination of a vaginal smear⁵⁵), and group 2 included 3 ovariectomized females. All dogs were born and housed at the Small Animal Theriogenology facilities of the Department of Clinical Sciences of the College of Veterinary Medicine, University of Liège. Animal housing, care, and experimentation were in accordance with Belgian governmental regulations and with the NIH Guide for Care and Use of Laboratory Animals.⁵⁶ Complete physical examinations were performed on each dog each week. Dogs were housed in groups of 2 to 5 in indoor-outdoor runs (2.5 $\times$ 10 m), exposed to natural light, fed a commercial dry food once a day in amounts sufficient to maintain body weight, and provided with water ad libitum. The dogs weighed 12 to 17 kg (mean $\pm$ SD weight, 15 $\pm$ 0.5 kg). Prior to each experiment, each dog underwent a physical examination, which included measurements of heart rate, respiratory rate, and rectal temperature, and a urine sample was obtained via cystocentesis for urinalysis and bacteriologic culture. Urinalysis included determination of specific gravity and pH and detection of blood, protein, bilirubin, and glucose when present. A vaginal smear was also performed during the course of the treatments to identify potential cytologic changes induced by the estrogenic supplementation.

Study design—Dogs received estriol^b (2 mg) PO once a day for 7 days (treatment A), immediately followed by the combined administration PO of estriol^b (2 mg) and phenylpropanolamine hydrochloride^c in an immediate-release formulation (1.5 mg/kg, PO) once a day for 7 additional days (treatment B). Urodynamic and morphologic measurements were performed in each dog the day before the first estriol treatment (baseline data; day 0) and on days 7 and 14 within 2 hours of drug administration. Each animal was used as its own control, to decrease variations attributable to interindividual differences and increase the statistical power, which is important when experimental groups are composed of small numbers of animals.

Prior to data collection, food was withheld from the dogs for 12 hours; dogs were allowed to urinate prior to each urodynamic measurement. Anesthesia was induced by use of an IV bolus of propofol^d (5 mg/kg) and was maintained with a continuous IV infusion of propofol at a rate of approximately 30 mg/kg/h. All dogs were intubated and monitored with a pulse oximeter^e; oxygen supplementation was provided if oxygen saturation was < 85%. When dogs were in a light plane of anesthesia, they were placed in right lateral recumbency.⁵⁷ After cystocentesis and collection of a sample for preparation of a vaginal smear, 3 successive simultaneous urethral pressure profiles and diuresis cystometry were performed, as previously described.⁵⁸ After the urodynamic test-

ing, each dog remained anesthetized in right lateral recumbency and vaginourethrography was performed, as described.⁵⁹

Data interpretation—By use of the urodynamic test results and examination of vaginourethrograms (lateral views), specific variables were assessed (Figure 1). These included MUP, MUCP (ie, the difference between MUP and bladder pressure), IP (ie, the functional area under the UFP, calculated as [MUCP × UFPL]), UFPL (defined as the distance between the point in the urethra where intraurethral pressure exceeds bladder pressure and the point where either a pressure plateau is detected or where the intraurethral pressure is less than bladder pressure), urethral anatomical profile length (ie, the distance between the point in the urethra where intraurethral pressure exceeds bladder pressure and the point where the intraurethral pressure decreases to atmospheric pressure), plateau (the distance between the end of the UFPL and the point where the intraurethral pressure falls to atmospheric pressure), the distance before MUP (defined as the distance between the point in the urethra where intraurethral pressure exceeds the total bladder pressure and the point of maximal urethral pressure), maximum meatus pressure (defined as the maximal pressure at the level of the external urethral orifice), threshold pressure (ie, urinary bladder pressure at the time of micturition), threshold

volume (ie, fluid volume retrieved from the urinary bladder at the time of micturition reflex), compliance, urethral length (ie, distance between the bladder neck and the external urethral orifice), vaginal length, vaginal width, the length of the dorsal median paracervical fold (distance A), the distance between the dorsal median paracervical fold and the vestibulovaginal junction (distance B), and the distance between the vestibulovaginal junction and the external urethral orifice (distance C').

Compliance was calculated by use of the following equation:

$$\text{Compliance} = \frac{(\text{threshold volume} - V_o)}{(\text{threshold pressure} - P_o)}$$

where V_o and P_o are the urinary bladder volume and pressure at the start of cystometric evaluation, respectively. Definitions of urodynamic measurements were in accordance with those of the International Continence Society.⁶⁰

Statistical analysis—Data are expressed as mean ± SE. Differences in age and weight between groups were determined by use of a 1-way ANOVA. Two-way ANOVAs with interaction (treatment or group) were performed by use of statistical software.[†] Within-treatment comparisons (differences between successive times of testing), between-treat-

Table 1—Mean ± SE values of urodynamic and morphometric variables in 3 sexually intact and 3 spayed female dogs before (day 0) treatment, after treatment with estriol (2 mg, PO, q 24 h) for 7 days (day 7), and after the same treatment with estriol combined with phenylpropanolamine (1.5 mg/kg, PO, q 24 h) for another 7 days (day 14).

Variable	Day 0		Day 7		Day 14	
	Sexually intact	Spayed	Sexually intact	Spayed	Sexually intact	Spayed
MUP (cm H ₂ O)	23 ± 9	30 ± 9	65 ± 9*	46 ± 9	43 ± 9	46 ± 9
MUCP (cm H ₂ O)	19 ± 8	27 ± 8	60 ± 8*	38 ± 8	37 ± 8	41 ± 8
Integrated pressure (cm × cm H ₂ O)	100 ± 39	145 ± 39	255 ± 39*	234 ± 39	197 ± 39	214 ± 39
UFPL (mm)	79 ± 6	82 ± 6	86 ± 6	89 ± 6	87 ± 6	87 ± 6
UAPL (mm)	104 ± 6	100 ± 6	98 ± 6	100 ± 6	107 ± 6	93 ± 6
BMUP (mm)	52 ± 10	47 ± 10	62 ± 10	63 ± 10	63 ± 10	66 ± 10
MMP (cm H ₂ O)	23 ± 12	14 ± 12	23 ± 12	25 ± 12	13 ± 12	20 ± 12
Plateau (mm)	15 ± 3	8 ± 3	12 ± 3	7 ± 3	14 ± 3	9 ± 3
Threshold pressure (cm H ₂ O)	49 ± 7	33 ± 7	46 ± 7	50 ± 7	46 ± 7	48 ± 7
Threshold volume (mL)	401 ± 81	407 ± 81	406 ± 81	452 ± 81	457 ± 81	422 ± 81
Compliance (mL/cm H ₂ O)	10 ± 2	16 ± 2	10 ± 2	11 ± 2	11 ± 2	10 ± 2
Urethral length (cm)	11 ± 2	10 ± 1	10 ± 1	11 ± 1	11 ± 2	11 ± 2
Distance A (cm)	1 ± 0.2†	0.8 ± 0.3	1.2 ± 0.2	0.5 ± 0.3	1.3 ± 0.2	0.5 ± 0.3
Distance B (cm)	12 ± 1†	7 ± 2	12 ± 1	9 ± 2	12 ± 1	8 ± 2
Distance C' (cm)	1 ± 0.3	1 ± 0.4	2 ± 0.3	1.2 ± 0.4	1.3 ± 0.3	1.2 ± 0.4
Vaginal length (cm)	15 ± 2†	8 ± 2	16 ± 2	11 ± 2	15 ± 2	10 ± 2
Vaginal width (cm)	4 ± 0.5†	1 ± 0.6	3 ± 0.5	1.5 ± 0.7	3 ± 0.5	1.8 ± 0.6

*Value significantly ($P < 0.05$) different from baseline value (day 0) for that group. †Value significantly ($P < 0.05$) different from value for spayed females at day 0.
UAPL = Urethral anatomic profile length. BMUP = Distance between the point in the urethra where intraurethral pressure exceeds the total bladder pressure and the point of MUP. MMP = Maximum meatus pressure.

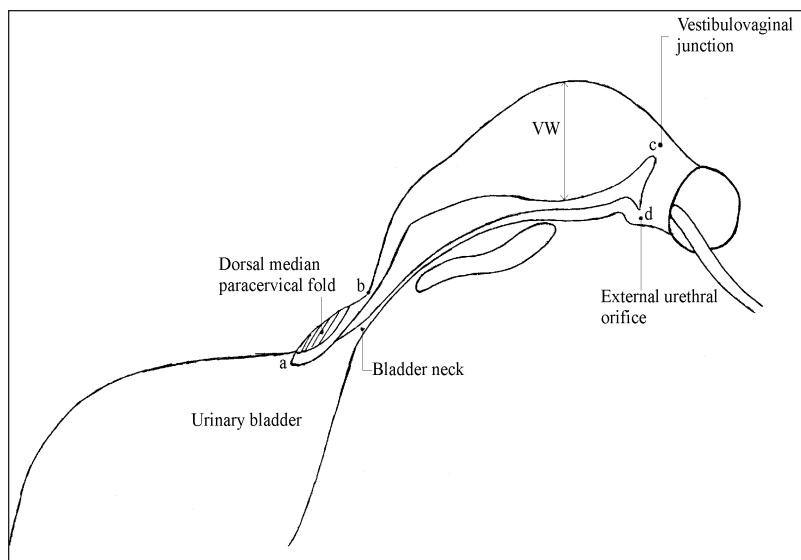


Figure 1—Drawing of the features of interest on a vaginourethrogram (lateral view) in a female dog. The urethral length is defined as the distance between the bladder neck and the external urethral orifice. The vaginal length is defined as the distance between the cervix and the external urethral orifice. Distance A is defined as the length of the dorsal median paracervical fold (a to b); distance B is defined as the distance between point b and the vestibulovaginal junction (c); distance C' is defined as the distance between point c and the external urethral orifice (d). VW = Vaginal width. (Adapted from Hamaide AJ, Verstegen JP, Snaps FR, et al. Influence of the estrus cycle on urodynamic and morphometric measurements of the lower portion of the urogenital tract in dogs. *Am J Vet Res* 2005;66:1075–1083. Reprinted with permission.)

ments comparisons, and between-groups comparisons were made. A value of $P < 0.05$ was considered significant.⁶¹

Results

Physical examination, urinalysis, and bacteriologic culture of urine revealed no signs of disease of the lower portion of the urinary tract in any of the dogs included in the study. Mean $\pm$ SE values of urodynamic and morphologic measurements were calculated (Table 1). At days 7 and 14, cytologic examination of vaginal smears from 2 sexually intact and 2 spayed female dogs had evidence of estrogenic maturation including the presence of RBCs, mitotic figures, and a few keratinized cells. Cytologically, vaginal smears of the remaining sexually intact and spayed females were compatible with smears from female dogs in the pro-estrous phase.

Urodynamic measurements—At day 0, no significant differences in the urodynamic variables were detected between sexually intact and spayed female dogs. Compared with findings at day 0, MUP, MUCP, and integrated pressure values were increased from baseline in both groups of female dogs after treatment A; however, these differences from baseline values were significant ($P = 0.004$, $P = 0.005$, and $P = 0.006$, respectively) only in sexually intact females. Compared with values obtained after treatment A, treatment B did not induce significant differences in MUP, MUCP, and IP values.

Radiographic measurements of the urethra and vagina—At day 0, vaginal length and width, as well as distances A and B, were significantly ($P = 0.008$, $P =$

0.006 , $P = 0.01$, and $P = 0.004$, respectively) shorter in spayed female dogs, compared with values in sexually intact female dogs. After treatment A (day 7), no significant differences in the values of the radiographic measurements were identified between the 2 groups. Similarly, after treatment B (day 14), no significant differences in the values of the radiographic measurements were identified between the 2 groups. At days 7 and 14, radiographic measurements did not differ from baseline values in either group.

Discussion

The first purpose of the present study was to evaluate the effects of administration of estriol on the function of the lower portion of the urogenital tract in sexually intact and spayed female dogs that did not have urinary incontinence. The dosage of estriol used was chosen on the basis of previous reports^{30,31} in which a starting dosage of 0.5 to 2 mg/dog, PO, once daily for 7 days was recommended. Because estriol undergoes metabolism via the entero-hepatic cycle, important variations in plasma concentrations are detected among dogs, which may explain the lack of relationship between the recommended dosage and the weight of the dog.^{30,31}

Although estriol supplementation has been associated with improvement or complete cure of clinical signs in women and female dogs with urethral sphincter deficiency,^{24,26–31} treatment-associated urodynamic changes in humans have been variable.^{23,29} In the present study, a significant increase in urethral resistance in female dogs was detected after 7 days of treatment with estriol, as indicated by significant increases in MUP, MUCP, and integrated pressure values (compared with pretreatment baseline values). An integrated pressure value defines the functional area under the urethral profile curve, which is a better measure of urethral sphincter competence than MUCP or UFPL alone because it more fully characterizes sphincter deficiency in humans and is a useful parameter in assessing urethral function.⁶² Although MUP, MUCP, and integrated pressure values were increased after estriol treatment in both groups of dogs in our study, these values were significantly increased from baseline only in the sexually intact females. We believe that the lack of significance associated with the increases in the spayed group could be related to the small number of dogs or to a reduced capacity of spayed animals to respond to the treatment. It must be also acknowledged that the plane of anesthesia was not standardized among dogs in terms of identical infusion rates. The goal was to achieve a similar light plane of anesthesia in each dog with persistence of eye blinking and jaw tone. Although good reproducibility of urodynamic measurements in individual female dogs by use of propofol

has been described,⁵⁸ interindividual variations attributable to the systemic effects of propofol could have influenced the urodynamic results.

In a previous study⁶³ of the influence of the endogenous hormones on the urinary tract in dogs, slight increases in MUP and MUCP values during proestrus, compared with values during anestrus, were detected, which could be explained by the effects of the increased estrogen plasma concentration associated with the proestrous phase on the lower portion of the urinary tract.

In the present study, the increased urethral resistance detected after estrogen administration could be explained by several mechanisms. In humans, dogs, and rabbits, estrogens stimulate α -adrenergic receptors of the urethra and increase their sensitivity to sympathetic stimulation,^{7,45-48,64,65} leading to increased smooth muscle contractility.⁶⁶ In the urethra of rabbits, Larsson et al⁶⁷ detected an increase in the number of α_2 -adrenergic receptors after estrogen supplementation, compared with the pretreatment value. In women, it is suggested that estrogens stimulate collagen production and increase collagen density in the urethral wall and supporting structures of the urethra.^{68,69} Blood flow in the sinusoidal plexus, which plays an important role in urethral function,^{70,71} is enhanced after estrogen administration.^{72,73} Estrogen replacement in women also induces maturation of the vaginal and urethral epithelium⁷⁴ with an associated increased thickness of the urethral wall, which potentially contributes to a better seal of the urethral lumen. In the present study, maturation changes were detected via cytologic examination of vaginal smears from all dogs during the 14 days of treatment, suggesting that the observed increased urethral resistance could be related to a thickening of the wall of the vagina and subsequently that of the urethra. In 1 sexually intact female dog, the findings indicative of proestrus after estriol supplementation could have been an early return to estrus. In female dogs, sterilization induces a decrease in smooth muscle mass and urethral connective tissue density,⁷⁵ which could be associated with the decrease in MUCP values detected after spaying.²⁰ Estrogen supplementation could therefore be beneficial in reversing these structural connective changes and enhance urethral resistance. However, in rats, administration of 17 β -estradiol does not modify the volume of the smooth musculature nor the innervation to the proximal portion of the urethra.⁷⁶

Interestingly, the significant increase in functional area detected after estriol supplementation was mainly a result of the modification of the urethral pressure profile curve in its distal portion, corresponding to the distal fourth of the urethra, which is rich in striated musculature. After ovariectomy in dogs, density of type I fibers from the striated urethral musculature decreases to a greater extent than type II fibers,⁷⁷ which could play a role in the weakness of the sphincter mechanism because type I fibers are supposed to be involved in the maintenance of urinary continence at rest. The administration of estrogens in adult female cats has induced motoneuron axonal growth, suggesting that sex steroid hormones can influence motoneurons⁷⁸ and possibly influence the number of muscular

fibers, via their innervation. This urodynamic observation suggests that estrogen action on the urethra could take place in all components of the urethral wall, including not only the smooth musculature, vascular plexus, connective tissue, and urothelium but also the striated musculature.

The values of the cystometric measurements were not significantly different after the administration of estriol (treatment A) in either the sexually intact or spayed female dogs. However, spayed dogs responded to some extent to the estriol supplementation, whereas values from the sexually intact dogs did not vary from baseline after treatment A. Although not significant, threshold pressure was increased and compliance values decreased from baseline after treatment A in the spayed dogs, whereas no difference was detected in the sexually intact dogs. These results could corroborate previous findings,^{44,a} but once again, investigation of a larger number of dogs would be necessary to draw definite conclusions.

The action of estrogens on the detrusor muscle is controversial. Although Levin et al⁷⁹ determined that there was an increased density of cholinergic receptors and an increased response to α -adrenergic, muscarinic cholinergic, and purinergic agonists after administration of estrogens in rabbits, other authors found a decrease in the density of muscarinic cholinergic receptors in the urinary bladder muscle^{80,81} and inhibition of the intracellular influx of calcium ions.⁸² Whereas the former effects could explain a possible decreased bladder compliance after estrogen supplementation, the latter effects could lead to a decreased detrusor tone and an increased bladder capacity,^{82,83} which could account for the improvement of the urinary signs attributable to bladder hyperreflexia that are observed in postmenopausal women taking estrogen supplements.^{22,84} As the actual structural changes occurring in the detrusor muscle in female dogs after sterilization are still unknown, it is difficult to speculate on the cystometric changes to be expected after estrogen replacement in sexually intact or spayed dogs.

The combined administration of estriol and phenylpropanolamine (treatment B) did increase urethral resistance, compared with baseline values, but did not induce changes other than those associated with administration of estriol alone. Furthermore, the urodynamic values decreased after 2 weeks of treatment, despite the continued administration of estriol and the addition of phenylpropanolamine. As the combined treatment was administered to female dogs that had initially received estriol alone for 7 days, it was not possible to differentiate the effect that was attributable to the administration of estriol from the effect that was attributable to the combined treatment. The values obtained after the combined treatment were therefore not compared with baseline values, but were compared with values obtained after the estriol supplementation alone, to analyze the effects of the phenylpropanolamine administration. It appears that phenylpropanolamine added to the estriol supplementation did not induce the changes detected in a previous study,⁸⁵ in which single daily administration of phenylpropanolamine was effective in increasing the urethral

resistance in continent female dogs. One limitation of the present study was that treatment of the dogs with phenylpropanolamine alone was not investigated; if that had been included, we would have been able to compare the effects of phenylpropanolamine administered alone and in combination with the estriol. However, on the basis of our previous experience, we could speculate that the administration of phenylpropanolamine alone would have resulted in a significant increase in urethral pressure, compared with the baseline value. We choose to add the administration of phenylpropanolamine to the treatment with estriol after 7 days of the latter on the basis of the fact that initial treatment with estrogen would stimulate α -adren-
 ergic receptors of the urethra and would increase their sensitivity to sympathetic stimulation.^{7,45-48,64,65} The reason why no additional increases in the values of the profilometric parameters were detected after the addition of phenylpropanolamine to the initial estriol treatment is unknown at this time. In a study⁵² in humans, no changes in urodynamic parameters were detected despite the addition of phenylpropanolamine, although clinical improvement of the symptoms was evident. Further studies to assess the clinical efficacy of the combination of phenylpropanolamine and estriol are therefore warranted. The fact that the values of the profilometric parameters were lower after 14 days of treatment could indicate that a certain degree of estrogenic receptor downregulation could have been present after 2 weeks of estriol supplementation. Indeed, Batra and Iosif⁸⁶ determined that after an initial increase in estrogenic receptor density in the urogenital tract of female rabbits during the first week of estrogen treatment, this density greatly decreased; this suggested that after the initial stimulation of synthesis, a prolonged estrogen treatment could result in receptor downregulation. This theory is reinforced by the decreased activity of peroxidase, a marker of estrogenic activity, after prolonged estrogen treatment in rabbits.⁸⁶

In the present study involving a limited number of animals, no urodynamic differences between the sexually intact and spayed female dogs were detected prior to treatment. Structural changes in the bladder wall that could possibly lead to modifications of bladder function, such as detrusor hyperreflexia, were not detected in the spayed dogs of our study. Also, modifications of urethral function were not detected in the spayed dogs, which is in disagreement with findings of Reichler et al,²⁰ who detected decreased urethral resistance in female dogs 10 months after ovariectomy. However, compared with sexually intact dogs, threshold pressure was decreased and compliance was increased (albeit not significantly) in spayed dogs. It is interesting to note that these findings are similar to those reported for old sexually intact female dogs without urinary incontinence⁶³ and in female dogs with USMI.⁸⁷ It must be acknowledged again that the number of dogs in of our study groups was low and that a significant difference in threshold pressure and compliance could not have been detected because of the low power of the study and the fact that the groups were comprised of different animals.

The second purpose of our study was to evaluate the effects of the administration of estriol on the mor-

phologic features of the lower portion of the urogenital tract in sexually intact and spayed female dogs without urinary incontinence. Prior to treatment, vaginal length and width were significantly smaller in spayed female dogs, compared with the values in sexually intact dogs, which is in agreement with previous findings.^{18,19} Administration of estriol, alone and in combination with phenylpropanolamine, did not modify the morphologic parameters of the urogenital tract in both groups of female dogs in the present study. This finding does not corroborate results of other studies in dogs^{18,59,88} that identified enlargement of the vagina under the influence of exogenous or endogenous estrogens. During the estrous cycle, morphologic changes are observed in the urogenital tract after 8 days of estrogenic impregnation.⁶³ This influence of the endogenous estrogens on the urogenital tract has been described previously⁶³; in that study, there was a significant increase in all vaginal measurements, as well as an increase in urethral length, during the proestrous phase. It must be noted that the urethral length was not modified by the estrogenic treatments of the present study. Previous studies^{79,81,82} in rabbits have revealed a significant increase in the weight of the urogenital tissues (including the bladder, which appeared large and distended) after 1 to 3 weeks of estrogen supplementation, with no further changes after 4 weeks of treatment. These changes were probably a result of mucosal hyperplasia. The fact that maturation changes were detected cytologically on the vaginal smears of all the female dogs in our study reinforced the idea that mucosal hyperplasia could have been present in the treated dogs. Therefore, we were assuming that the duration of the treatments in our study would have allowed us to determine morphologic changes in the urogenital tract. However, because of the short half-life of estriol, a longer treatment period is perhaps required to detect morphologic changes; in previous investigations,^{18,89} morphologic changes in the urogenital organs of dogs and rats were detected after 3 to 4 months of estrogen supplementation or cessation of estrogen supplementation. The findings of the present study question the hypothesis that the positive effects of estrogen supplementation on the resolution of signs of urinary incontinence in female dogs could be attributable to an increase in vaginal mass that is capable of modifying the bladder neck position or the urethral length.

Our data have indicated that there are significant differences in morphologic features of the lower portion of the genital tract (with no associated urodynamic differences) between sexually intact and spayed female dogs. In the present study, the administration of estriol at the recommended dosage for 7 days significantly increased the urethral resistance in sexually intact and spayed female dogs without urinary incontinence. This effect diminished over the subsequent 7-day period, despite the continuation of estriol administration and the addition of phenylpropanolamine to the treatment protocol. Significant changes in bladder function or in the morphologic parameters of the lower portion of the urogenital tract were not detectable after 14 days of estrogen supplementation. These results suggest that estriol mainly acts on the urethral sphincter mechanism and has minimal

effects on the morphologic parameters of the urogenital tract. However, as Beagles are not predisposed to USMI,⁵ further studies involving large numbers of clinical cases are required to draw definite conclusions. Further investigations are also needed to better characterize the structural changes that develop in the lower portion of the urinary tract of female dogs after sterilization and the consequent related changes induced by estrogen replacement in spayed female dogs with urinary incontinence.

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