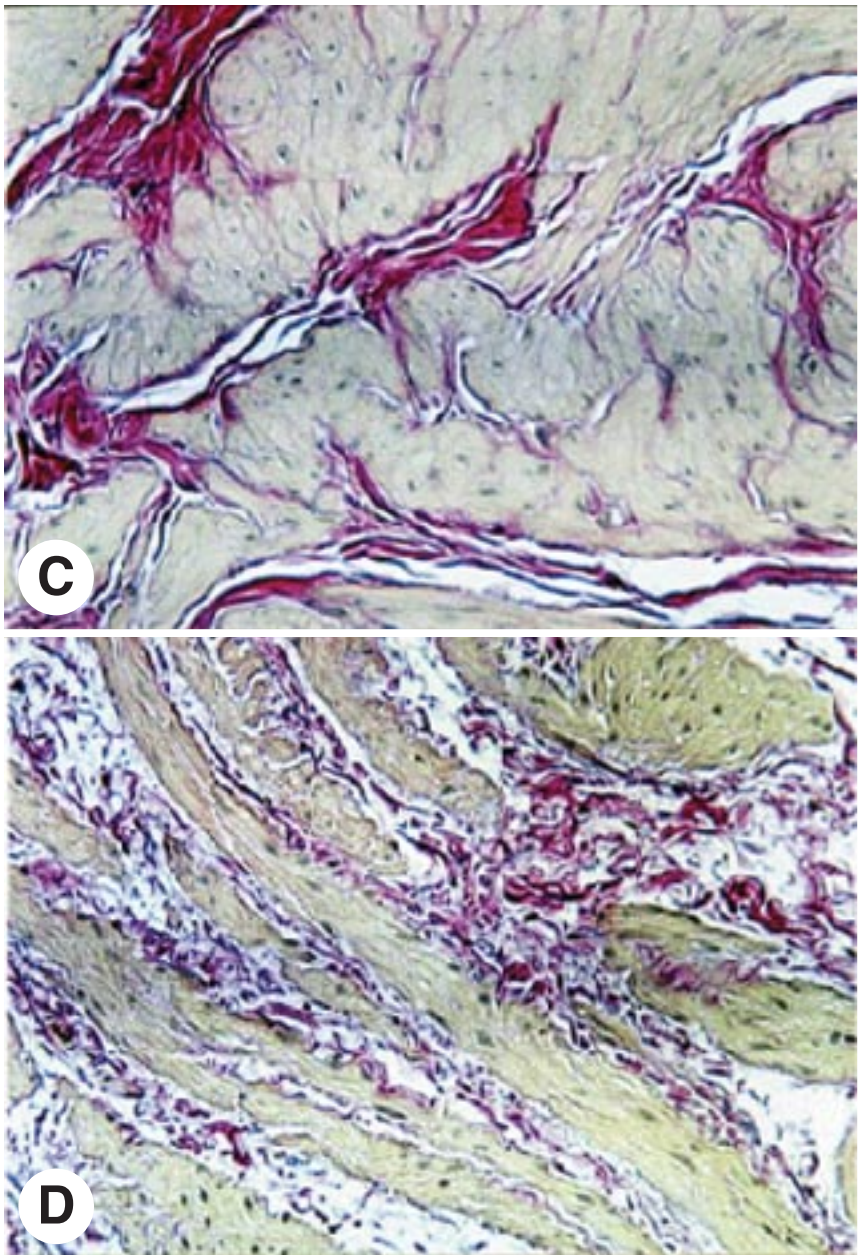

Figs. 2C and D.
Legends, see the previous page.

the duration of estrogen replacement, estrogen was introduced for 4 or 8 weeks to 2 different groups for any consequence stemming from longer duration of treatment.

We observed plasma E2 levels lower than 15 pg/mL in Group IV. The fertility period of rats lasts approximately until the age of 15 months, at which time the estrous cycle ceases (Handa et al., 1987; Shulman et al., 1987; Albert et al., 1991). According to Albert et al. (1991), rats with normal body weights maintain the plasma E2 level slightly below the mean of 15 pg/mL throughout the estrous cycle. In the present study, we gave no estrogen replacement to Groups I and IV. The plasma E2 levels obtained from 16-month-old rats in Group I exceeded 15 pg/mL, slightly higher than expected at such an age. In Group IV, as expected, 18-month-old rats' levels were slightly lower than 15 pg/mL. Groups II and III received estrogen replacement, and their plasma E2 levels were significantly increased to more than 30 pg/mL and 40 pg/mL, respectively. Therefore, our E2-replacement method

provided adequate levels for studying its effects on rat bladders.

In a comparison to normal control rats, ovariectomized rats had a decreased bladder weight and increased body weight (Longhurst et al., 1992). Also in our observations, we found that bladder weight was significantly heavier in Group III than Groups I and IV, while there were no significant differences in Group II compared to the other groups. However, Group II had a higher tendency of mean bladder weight than Groups I and IV. Thus, the bladder tended to gain weight when the E2 level was increased, which suggests that E2 replacement after menopause increases bladder weight. The bladder had been thought to gain weight in muscle content and epithelium thickness (Rocha et al., 2002). We observed no significant difference in mean body weight among groups, but estrogen-replaced rats lost some weight. The mechanism for how estrogen protects the bladder against collagen formation is not yet known. Some evidence suggests it has a cardioprotective action preferably through nitric oxide from endothelial release: estrogen replacement may prevent the proliferation of fibroblasts and collagen deposition in the blood vessels of the heart (Blacher et al., 2000). Whether a similar mechanism exists in the bladder is a subject of further study.

The number of blood vessels influences intraurethral pressure (Versi and Cardozo, 1986). Estrogen replacement therapy was reported to increase the number of periurethral and bladder vessels (Madeiro et al., 2001). In ovariectomized rats, however, the number of bladder vessels was decreased (Rocha et al., 2002). We observed no significant differences in the number of bladder vessels among groups, but only a little increase in Groups II and III. Estrogen acts directly on the number of bladder vessels in the urethra and periurethral tissue. This action could explain the increase in the number of bladder vessels due to estrogen replacement. Even with estrogen replacement, however, the increase in the number of bladder blood vessels was slight in our experiments: the increase in muscle content and cell cycle activity might have acted together.

Estrogen treatment inhibits tissue weight gain and cardiac output, and so blood flow of the urinary tract is also increased by estrogen (Batra et al., 1985, 1986). In this study, there was a significant difference in blood flow between E2-replaced groups and the control and placebo groups. Estrogen increases blood flow as previously reported (Batra et al., 1986) as well as in the present study. The slight increase in bladder blood flow we observed may be due to the increase in bladder weight and overall blood volume (not measured in the present study). According to the literature, the compliance functions of the bladder and urinary pressure are also improved with estrogen. These improved functions also help improve circulation (Fleischmann et al., 2002). Our estrogen replacement therapy for mature rats resulted in an increase in bladder muscle content and blood circulation, similarly as reported (Rocha et al., 2002).

In medical trials conducted since the 1960s predominantly in the United States, menopausal women have been given progesterone concurrently with estrogen to protect against increased risks of cancer. Studies in Japan have also shown estrogen useful in the treatment of stress incontinence (Suzuki et al., 1997). Since the present study has shown the benefits and advance effects of estrogen replacement therapy in rats, it should be advocated as a treatment in general practice.

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