Ovarian atrophy resulting from urethane injection of neonatal mice

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Summary. Treatment of neonatal mice with urethane (0.5 or 0.75 mg/g body wt, i.p.) caused a lack of follicular development beyond the primordial stage. An effect of the thymus on ovarian development is inferred.

During analysis of the results of an experiment investigating the interplay of urethane and caging stress, it was noted that ovarian dysgenesis was a constant occurrence in animals treated with urethane. The carcinogenic effects of urethane are well known (Nettleship & Henshaw, 1943; Salaman & Roe, 1953; Berenblum & Trainin, 1960; Toth, Della Porta & Shubik, 1961; Pietra, Rappaport & Shubik, 1961; Della Porta, Capitano & De Castillici, 1963; Della Porta & Terracini, 1969). When injected during the neonatal period urethane induces thymic lymphosarcomas. Although this type of tumour occurs with the highest frequency, other types occur later in life (Vesselinovitch, Mihailovich & Pietra, 1967; Berenblum, Chen & Trainin, 1968; Kommineni, Greenblatt, Mihailovich & Vesselinovitch, 1970). Apart from the induction of ovarian tumours (Vesselinovitch *et al.*, 1967), changes in the ovaries resulting from urethane injections have not been reported and are therefore discussed in this paper.

Methods

Mice of the C57BL/6Jl strain (Jackson Laboratory, Bar Harbor, Maine) were housed for breeding in one thermostatically controlled soundproof room in standard plastic mouse boxes, 27.94 × 17.78 × 10.16 cm, at 25.5°C with 12 h light and 12 h dark. All mice received commercial rodent pellets and water *ad libitum*.

The offspring were randomly divided into 2 groups. In Group 1, 247 males and 211 females were injected intraperitoneally with 0.5 mg urethane/g body weight (5% solution) on Days 7, 10, 13, 16, 19 and 22 after birth. Animals were weaned at 22 days of age and killed at 180 days of age. Twenty additional mice (10 males and 10 females) of the same age and strain were killed to provide organ weights for comparison, but they were not handled or injected as were the experimental animals.

The mice in Group 2 (137 males and 129 females) were injected with 0.75 mg urethane/g body weight (7.5% solution) on Days 12, 14, 16, 18 and 20 of age, weaned at 22 days of age and killed at 35 weeks.

Tissues were fixed in 10% formalin and freed of fat and connective tissue. Ovaries were weighed on a torsion spring balance (± 0.01 mg accuracy), and the testes and uteri on a Mettler type balance (± 1 mg accuracy). Paired organs were weighed together. Organs were embedded in paraffin wax, sectioned at 5 μ m, and stained with haematoxylin and eosin for microscopic study.

Vaginal smears from 14 urethane-treated and 5 untreated mice were taken in the afternoon for 12 consecutive days when mice reached 60, 140 and 165 days of age to determine characteristics of the oestrous cycle.

Thymus glands were examined for cancer, weighed, fixed and studied histologically.

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Results and discussion

Ovaries from all mice treated with urethane at 7 days of age lacked follicles and corpora lutea. The parenchyma consisted of hypertrophied interstitial cells interspersed with cords of epithelial cells (Pl. 1, Figs 1 and 2). Small primordial follicles were seen in Group 2 mice which received urethane at 12 days of age (Pl. 1, Fig. 3), but the interstitial cells were similarly hypertrophied (Pl. 1, Fig. 4). The mean weight of ovaries from urethane-treated mice was 2.7 ± 0.157 (S.E.M.) mg ($N = 173$) compared with 5.5 ± 0.374 mg ($N = 8$) for untreated sexually mature females of this strain. The difference between the mean ovary weights of females treated from Day 7 or from Day 12 of age was not significant (Group 1, 2.71 ± 0.129 mg, $N = 96$; Group 2, 2.82 ± 0.316 mg, $N = 77$). Urethane had no effect on the weight and histology of the testes.

Leucocytes were found in the vaginal smears for the 12 days of study, indicating that oestrus was not occurring in most females; the remaining few had irregular oestrous cycles.

Uterine weight was not influenced by urethane treatment in mice without cancer, being 54.3 ± 1.38 mg ($N = 155$) and 55.3 ± 4.25 mg ($N = 8$) in treated (Groups 1 and 2) and untreated females, respectively. A plot of the weights of 92 uteri from treated females in Group 1 against their respective ovary weights was linear with a positive correlation ($r = 0.2765$, $t = 2.7298$, $P < 0.01$).

A considerable number (43%) of the mice treated with urethane developed thymic lymphosarcoma. As would be expected, mean thymus weight from mice with thymic lymphosarcoma was higher than that of mice without cancer. Urethane-treated animals without cancer had heavier thymus glands than did non-treated mice (males: treated, 30.99 ± 0.72 mg, $N = 124$; untreated, 25.31 ± 1.92 mg, $N = 9$; females: treated, 40.03 ± 1.19 mg, $N = 97$; untreated, 33.43 ± 1.81 mg, $N = 10$).

In these experiments urethane had a profound effect on the ovary. Urethane does not have the same effect in adult mice, but the critical age for urethane-induced ovarian dysgenesis is not known. Neonatal thymectomy of mice also causes atrophied ovaries, absence of corpora lutea and replacement of parenchyma by hypertrophied interstitial cells (Nishizuka & Sakakura, 1969). Mice of the strain homozygous for the mutation 'nude' (*nu/nu*) are hairless, lack a thymus, have retarded growth, a life-span of 3–5 months and decreased fertility associated with irregular oestrous cycles and small ovaries (Flanagan, 1966). Eaton, Outzen, Custer & Johnson (1975) attributed ovarian dysgenesis among infertile nude mice to ill health, as mice maintained in a germ-free environment have a slightly higher fertility rate than conventionally kept mice.

Neonatal thymectomy, genetic absence of the thymus and urethane-inflicted damage to the thymus (present study) all cause infertility and suggest that the thymus is involved in the development of the ovary. Further evidence of a thymus-ovarian interaction is the occurrence of thymic cysts in mice injected with oestradiol (Ebbesen & Nielsen, 1972).

Ovarian atrophy may be caused by disruption of hormonal control. While uterine weights were not affected by urethane injections (in mice without cancer), ovarian weights were. However, a slight but statistically significant positive correlation was observed between the two, indicating that in spite of the greatly reduced size of the ovaries they might still be hormonally effective. Further evidence suggesting a hormonal control are studies demonstrating that anaesthetizing doses of urethane block the secretion and depress plasma concentration of luteinizing hormone (Wuttke, 1973; Blake, 1974). Urethane is also known to increase blood concentrations of vasopressin and oxytocin, thus demonstrating an effect on the neurohypophysis (Dyball, 1974). Secretions of ACTH and corticosterone are also elevated following urethane injection (Ondo & Kitay, 1973) and these in turn might affect ovarian development or immunological function. Another possibility is a virus infection of the ovaries after thymectomy or urethane administration (Eaton *et al.*, 1975), since thymectomy in rats does not cause sterility (Zbuzkova & Kincl, 1970). Thymectomy, urethane administration and genetic absence of the thymus cause depression of the immunological systems which would increase the possibility of a latent virus becoming active.

Sections of ovaries of urethane-treated mice.

Fig. 1. Mouse injected (0.5 mg urethane/g body weight) at 7 days of age; note the lack of follicles and corpora lutea. $\times 70$.

Fig. 2. Higher power view of ovary in Fig. 1, showing that the parenchyma consists of hypertrophied interstitial cells interspersed with cords of epithelial cells. $\times 430$.

Fig. 3. Mouse injected (0.75 mg urethane/g body weight) at 12 days of age, showing primordial follicles. $\times 430$.

Fig. 4. Mouse injected (0.75 mg urethane/g body weight) at 12 days of age; the interstitial cells are hypertrophied. $\times 430$.

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