EFFECT OF DIETHYLSTILBESTROL CONCENTRATION AND DURATION OF CONTACT WITH NURSING LEVANT VOLES, ON THE REPRODUCTION OF THEIR FEMALE OFFSPRING

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A 5-minute contact of nursing mothers of the Levant vole, Microtus guentheri, with paper impregnated with a DES suspension in soybean oil (125 μg/cm²) caused sterilization of 60% of the newborn female offspring. A 1-hour contact was sufficient to sterilize more than 90% of them. When the DES concentration was decreased to 1 μg/cm² only 44% of the newborn female offspring were sterile although their nursing mothers had been exposed to contact with the treated paper for a period of 2 hours. The concentration of 0.1 μg/cm² was totally ineffective in causing sterilization. Treatment of nesting material with DES offers a promising means of reproductive suppression in Levant voles.

KEY WORDS: Microtus guentheri; diethylstilbestrol (DES); chemosterilization; contact sterilization; sterility diagnosis; DES contact of mothers sterilizes female offspring.

This study constitutes part of a series of investigations concerning the contact effect of diethylstilbestrol dipropionate (DES) on the reproduction of the Levant vole, Microtus guentheri (4-9). Introduction of this synthetic estrogen into the bodies of pregnant females of various mammals (including the Levant vole) by injection, by ingestion of treated food, or by absorption through the skin, disturbs implantation or induces death of fetuses at early stages of development. Moreover, if the substance penetrates through the placenta into fetuses at later stages of development, or is ingested by pups through their mother's milk during the first few days of life, it disturbs gonadal development, thereby inducing sterility or a significant delay in the onset of sexual maturity (2,5,6,7). Our previous investigations showed that a 2-hour contact of a nursing mother (0 - 3 days after delivery) with filter paper impregnated with a suspension of DES in soybean oil (125 μg DES/cm²) causes sterilization of an overwhelming majority of the female offspring (9). That study had been restricted to female offspring, since even highly effective male sterilization would have had almost no practical importance in lowering the reproductive rate of voles or other polygamous rodents in nature (12). The purpose of the present study was to determine the minimum concentration of DES and the shortest period for the mother's contact with it, which would ensure the sterilizing effect. A detailed review of the publications dealing with the effect of DES...
on rodents and other mammals may be found in earlier papers of this series (4-7, 9). We failed to find any recent papers directly connected with this study.

During the present study, we tested the fertility of 798 young females out of 356 litters born in a laboratory population of Levant voles, at the Institute of Plant Protection, The Volcani Center. In 200 of these litters, the nursing females on the 1st - 3rd days after delivery were placed singly in plastic cylinders (diam 12.5 cm, height 15 cm), the bottoms of which were covered with filter paper impregnated with a suspension of DES in soybean oil (0.1, 1, 5, 15, 31, 62 and 125 μg DES and 5 μl oil per 1 cm²). The females were left in the cylinders for 2, 3, 5, 10, 15, 30, 60 or 120 min, and then returned to their cages to continue nursing. The effect of each DES concentration and duration of contact was tested on a minimum of ten female offspring. In some cases, data from additional females were collected (exact number of surviving females in each litter can be established only after weaning) and these were also included in the statistical processing.

The female pups of test and control groups were weaned and tested at the age of 2 weeks. The traditional method of testing fertility involves the raising of young female voles for 1.5 months until they reach sexual maturity, and then examining reproductive results when caged with fertile males for 2 months. However, we implemented a new method based on the observation that at the weaning age (2 weeks) every reproductively normal female of the Levant vole reacts to contact with DES by opening its vaginal orifice. This reaction may be observed after an exposure period of 2 - 3 days (6); in females which fail to develop normally and are subsequently sterile, the vaginal orifice remains closed. This observation was verified by conventional fertility testing (5) of 58 adult females, whose reaction to DES had been tested at 2 weeks of age. Out of the 30 females whose vaginal orifice remained closed after contact with the DES-treated paper, none became pregnant after a 2-month period of caging with proven males. At the same time, under identical test conditions, 27 out of the 28 control females whose vagina was open after contact with DES-treated paper (96.4%) became pregnant. These results point to the high reliability of the described method. Doubtful cases, in which it was difficult to determine whether the vaginal orifice was closed or not (for example, if the orifice was very small or if there was only a hollow), averaged only ~9% in test females and less than 5% in control females.

To test the fertility of weaned female offspring by this new method, each female was examined and the state of its vaginal orifice was recorded.

Two-hour contact of a nursing Levant vole with DES had a definite sterilizing effect, even if the solution was diluted to a very low concentration (1 μg DES/1 cm² paper) (Table 1). The percentage of sterilization increased with rising DES concentration: 100% sterilization was achieved with 125 μg/cm². At this DES concentration, even 5 min contact of a nursing vole was sufficient to sterilize 61% of its female offspring; when the contact lasted 3 min, one female out of 13 tested was sterile. The percentage of sterility increased with duration of contact, up to 95% when the vole was exposed for 1 h and 100% following 2 h of contact. However, no effective sterilization occurred at the lowest concentration tested (0.1 μg/ cm²), even after 2 h of contact.

Probit analysis on percent sterile females in different experimental and control groups, showed that sterility increases significantly (P<0.001) when either the concentration of DES or the duration of contact is increased. The pattern of the changes in percent fertility and percent sterility between groups conformed significantly to a probit model, both for changes in DES concentration at a constant duration of the contact, and for changes in duration at a constant concentration.

A quadratic response surface was fitted to percent sterility for the two independent variables, log DES concentration and log duration of contact, with a satisfactory fit (= 0.79).

The percentage of sterilized female offspring was not proportionate to the duration of their mother's contact with the DES-impreg.