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PRELIMINARY REPORT

EFFECT OF HORMONE TREATMENT ON GENITAL HERPETIC INFECTION IN MICE

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The possible association of genital herpetic infection with cervical cancer was first suggested by Naib et al. (1966), who noted an increased rate of cervical anaplasia in women with cytologically detected herpetic cervicitis. Since then, extensive retrospective and prospective epidemiological studies have been carried out on this problem (Nahmias et al., 1970, 1973, 1974; Rawls et al., 1973). Another approach to this problem is to establish genital herpetic infections in animals and examine the rate of development of cervical anaplasia in the infected animals. It has been possible to infect the genitalia of mice (Nahmias et al., 1967; Muñoz, 1973; Nishiura et al., 1974), hamsters (Burnstein, 1965), guinea pigs (Lucás et al., 1974) and monkeys (Nahmias et al., 1971; London et al., 1971; Kalter et al., 1972).

In a preliminary report we described the establishment of genital infection of female mice (the ICR strain) with type 1 and 2 herpes simplex virus (Nishiura et al., 1974). Histological examination of uterine tissues of mice 24 hr after intravaginal infection showed cytopathological changes characteristic of herpetic infection, that is the appearance of giant cells and formation of intranuclear inclusions in affected cells. These changes were most pronounced 3 to 4 days after infection, gradually disappearing in the next 10 days. Results of fluorescent antibody studies and viral infectivity titrations of infected uterine tissues coincided well with these pathological observations on the general course of virus infections.

We also tried to infect the uterus of monkeys with type 2 herpes virus, since this has been reported by others (Nahmias et al., 1971; London et al., 1971; Kalter et al., 1972). Unexpectedly one tufted capuchin monkey (Cebus apella) showed resistance to viral infection by the intravaginal route in two trials although no neutralizing antibody against the virus could be detected in her serum. Cytological examinations of vaginal smears taken at the time of each inoculation showed that at both times her uterus was in the proliferative phase. This finding prompted us to examine the possible relation between cyclic changes of the squamous epithelium of the uterus and the susceptibility of the cells to herpetic infection. This paper is a preliminary report of experiments on the effect of hormone treatment on genital herpetic infection in mice.

Six to 8 weeks old female mice (the ICR strain) were used. To arrest the physiological, functional and morphological states of the squamous cell layer of the uterus in the estrus

or diestrus stage, mice were treated with hormones: the hormones used were estrogen and gestagen. Two groups of 14 female mice were used. One group was given 5 intradermal injections in the back with 1 mg doses of estradiol (Schering Co.), and the other was similarly treated with 30 mg of progesterone (Teikokuzoki Co.). These injections were given 5, 3 and 1 days before, and 1 and 3 days after virus inoculation. To see the effects of hormones on epithelial cells of the uterus, vaginal smears were taken every day until the day of sacrifice and examined after Papanicolaou staining. Virus was inoculated by inserting a cotton pellet soaked in a solution of about 10^7 PFU/ml of type 2 herpes simplex virus into the vagina. The virus strain used was the Syn^- variant of UW 268. As described above, during normal infection pathological changes of the uterus were greatest 3 to 4 days after virus infection, so 7 mice each group were sacrificed on day 3 and other 7 on day 4 after virus inoculation. The uteri were excised and immediately immersed in Bouin's fixative and then histological preparations were made. More than ten frontal sections of each uterus were observed. The occurrence of viral infection was substantiated by demonstrating cytological changes characteristic of herpetic infection, i.e. syncytial cells with intranuclear inclusions.

None of the 14 mice injected with estrogen showed evidence of virus infection of their uterine tissues, whereas the cytopathological changes characteristic of infection were observed in the uteri of 12 of the 14 mice treated with progesterone, i.e. 6 of 7 mice sacrificed on day 3 and 6 of 7 mice sacrificed on day 4. Figures 1 and 2 show the appearance of the squamous cell layer of the uterine cervix of estrogen-treated mice. Keratinization of the superficial layer is prominent, especially in Fig. 1. On the other hand Figs. 3 and 4 show the cytopathological changes characteristic of herpetic infection in the same sites in mice treated with progesterone. Figure 3 shows

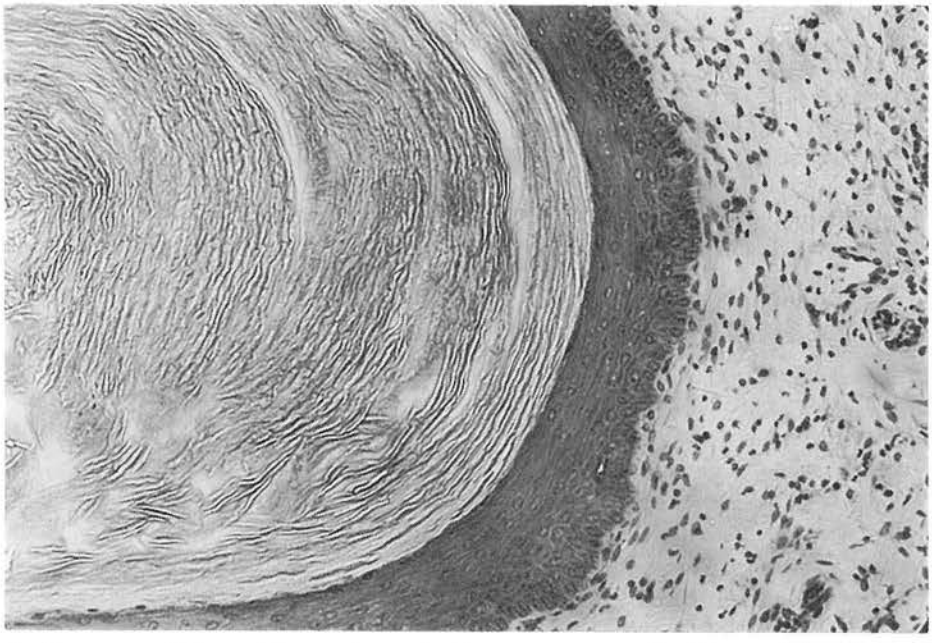

FIGURE 1. Squamous cell layer of the uterine cervix of an estrogen-treated and herpes-infected mouse. Keratinization and desquamation of the superficial layer are marked. ×160.

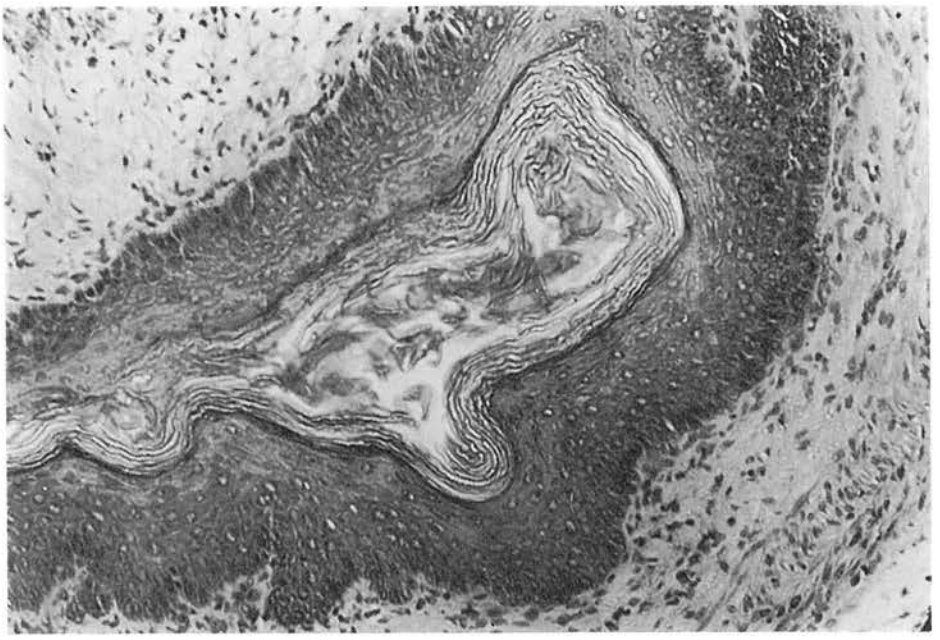

FIGURE 2. Squamous cell layer of the uterine cervix of an estrogen-treated and herpes-infected mouse. Keratinization and desquamation of the superficial layer are moderate. ×160.

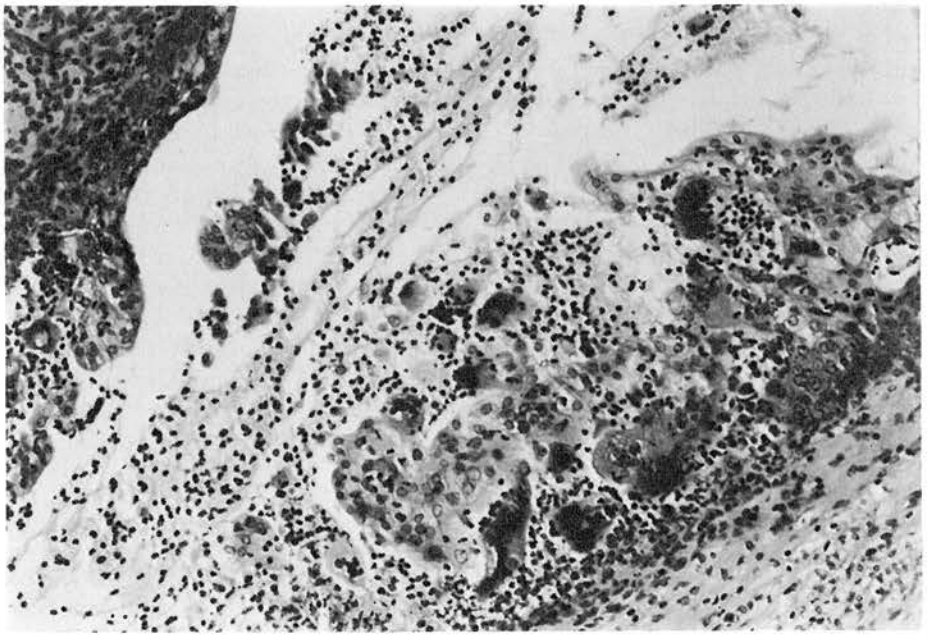

FIGURE 3. Squamous cell layer of the uterine cervix of a progesterone-treated and herpes-infected mouse. Many syncytial cells are seen in the right hand half and sloughing of the infected squamous cell layer is evident in the left. ×160.

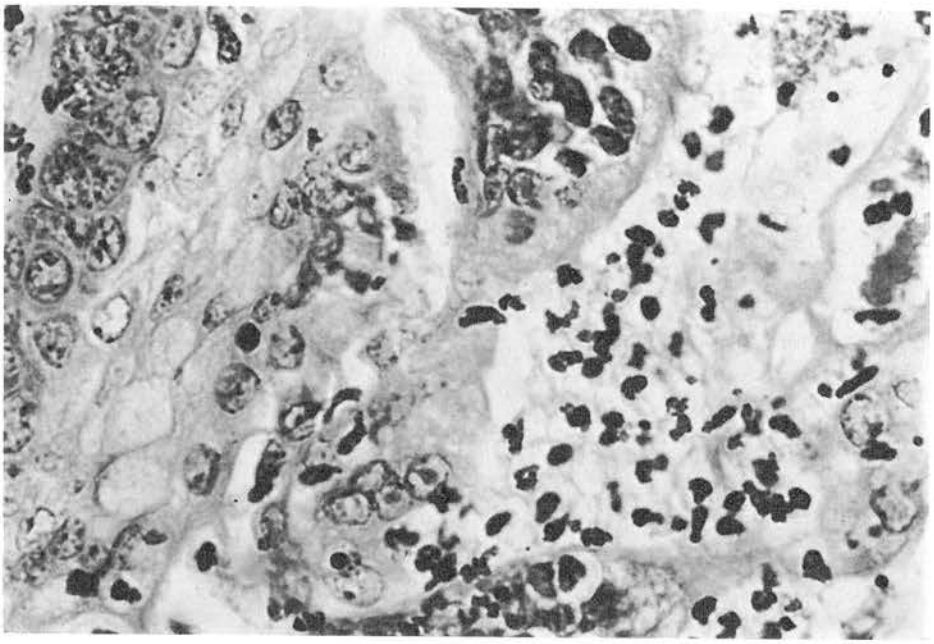

FIGURE 4. Squamous cell layer of the uterine cervix of a progesterone-treated and herpes-infected mouse. In the large syncytial cell mass seen in the middle, many nuclei with a full inclusion and some nuclei with shrunken inclusions with a halo are seen. ×640.

many syncytial cells, and sloughing of the squamous cell layer is evident in upper left of the picture. The middle third of the preparation seen in Fig. 4 shows a syncytial cell mass with one constriction (central part). It contains many nuclei with a full inclusion (upper middle part) and some nuclei with a halo (lower middle part).

Thus, this experiment showed a marked difference in the rates of virus infection in mice treated with estrogen and progesterone. No definite evidence of infection was observed in estrogen-treated mice, but this does not exclude the possible presence of small infectious foci, which could not be detected by histological examination.

In another experiment we examined the frequency of death after virus inoculation in groups treated with estrogen and progesterone: the frequency was much greater in the progestrone-treated groups although few mice in the estrogen treated groups also died.

The most likely explanation why no definite cytological changes of viral infection were seen in the estrogen-treated mice is that the superficial stratified layer formed at the estrus stage prevents virus particles from infecting the deeper squamous cells which are functionally and physiologically active and which are susceptible to virus infection. Further large scale experiments are now in progress on this subject and results will be published elsewhere.

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