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Enhancement of Vaginal Infection in Mice by Herpes Simplex Virus Type II with Progesterone (40156)

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Genital infection with Herpes Simplex Virus (HSV) is two to three times more common during pregnancy (1–3). Pregnancy must be considered as a possible predisposing factor in dissemination of primary (HSV) infection (4). Numerous investigators have shown that the pregnant mouse is more susceptible to poliomyelitis (5), encephalomyocarditis virus (6) and Herpes Simplex Type II (7).

Progesterone levels rise early in pregnancy and is maintained at a high level late in the pregnancy in the mouse (8), the rat (9) and the hamster (10). However, pregnant women differ in that they show little or no prepartum decline in peripheral progesterone levels (11).

This paper reports the role of progesterone as a significant factor in the enhancement of (HSV) II infection in mice.

Materials and methods. Animals. Six week old female and 6- to 8-week old, 6-day pregnant ICR mice were obtained from Flow Laboratories.

Virus and infection. HSV II, Savage strain isolated in 1973 and typed by the CDC, kindly supplied by Dr. Gary Cohen of the University of Pennsylvania, was used in all experiments. Three groups of mice were used: (a) pregnant mice were given 0.05 ml of a suspension containing 5×10^6 $TCID_{50}$ HSV II intravaginally in one dose on day 7 of pregnancy using an 18 gauge polyethylene catheter, (b) nonpregnant, progesterone-treated mice received 6 days of treatment with subcutaneous progesterone prior to intravaginal infection with HSV II using the same dose as the pregnant animals, (c) control, nonpregnant groups of mice did not receive progesterone.

Progesterone administration and hormone assays. Progesterone in oil (Lilly and Co., lot #OGx35A) was used in all experiments. Nonpregnant mice received 0.1 ml. daily subcutaneously which contained 2 mg of progesterone, starting 6 days prior to intravaginal infection with herpes virus. The radioimmune assay for progesterone (12) and the fluorometric assay for corticosterone (13) have been previously reported.

Viral assay. Animals were sacrificed on day 4, 6, 8 and 10 postinfection. Vagina, blood, brain, and liver were harvested for viral assay. Blood was also collected on day 4, 6, 8, and 10 postinfection from different animals for progesterone and corticosterone assays.

By using fine iris scissors, the vagina was freed from the surrounding subcutaneous tissue and posterior rectum. The vagina was traced to its insertion into the uterus and dissected free at that point. The vulva was then sharply severed from the vagina. This left the vagina free of any surrounding structures.

Organs were washed once with MEM and minced with fine scissors. A final 10% suspension w/v was made using MEM with 2% FCS supplemented with penicillin (100 units/ml), streptomycin (5 μg/ml) and amphotericin B (0.5 μ/ml). These specimens were stored at −60° until assayed for virus content. Virus titer is expressed as $TCID_{50}$/g tissue. All virus assays were performed in WI-38 tube cultures using serial tenfold dilutions. Tubes were read on day 7 postinoculation.

Results. Figure 1 compares mortality rates in pregnant, control nonpregnant, and nonpregnant progesterone treated mice infected with HSV II. Injections with subcutaneous progesterone alone caused no deaths. Nonpregnant untreated mice had a mortality rate of 10% while pregnant mice and progesterone treated mice showed a similar mortality rate of 88%. This 78% difference in mortality rate between untreated nonpregnant mice and progesterone treated or pregnant mice is highly significant ($P < 0.001$ using the chi square test).

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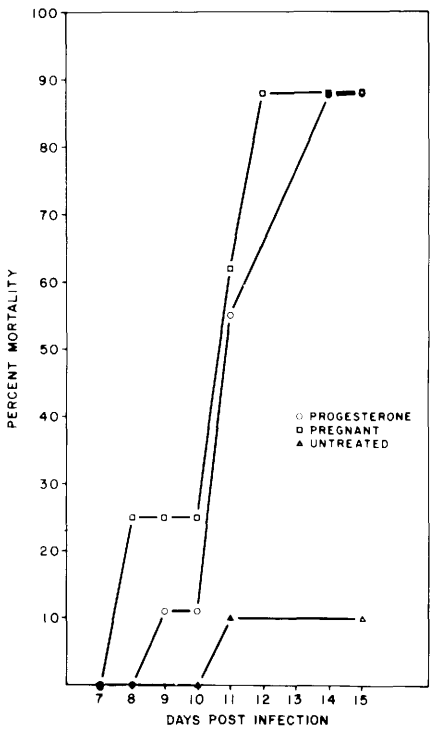

FIG. 1. Mortality in pregnant, control nonpregnant and nonpregnant progesterone treated mice infected with HSV II (5×10^6 $TCID_{50}$) intravaginally.

Figure 2 shows the levels of HSV II obtained in vaginal specimens of pregnant and nonpregnant progesterone treated mice infected vaginally with HSV II. The titers on day 4, 6, 8 and 10 post infection are similar in the pregnant and progesterone treated groups ranging from 1×10^4 $TCID_{50}$ to 5×10^2 $TCID_{50}$/g of tissue. Control nonpregnant mice had a titer of 2×10^2 $TCID_{50}$/g of tissue on day 4 and 6 postinfection. No virus was isolated on day 8 or 10 postinfection in these nonpregnant mice. The viral titers in brain tissue of the progesterone treated and pregnant groups of mice reach similar levels of 10^4 $TCID_{50}$/g of tissue by day 8 postinfection. This high titer of HSV II in the brain tissue of these mice correlated well with a subsequent rapid progression to the death of these animals. No virus was recovered from blood or liver.

Figure 3 reports the plasma levels of progesterone in the three groups. Levels of progesterone in control nonpregnant adult female mice ranged from 0.26 to 1.1 ng/ml. Pregnancy raises these levels as much as 10–70 times. The progesterone treated nonpregnant group had levels 80–100 times greater than those seen in the untreated nonpregnant group.

Table I gives data on corticosterone levels in pregnant, control nonpregnant and nonpregnant progesterone treated mice. The corticosterone levels in pregnant mice after day 10 of pregnancy (4 days postinfection) were markedly elevated over control levels. Specimens obtained from progesterone treated mice showed only slight elevations.

Discussion. Elevation of progesterone levels, either owing to pregnancy or to artificial administration, enhanced HSV infection in mice. The levels of progesterone found in the control and pregnant animals agreed with those reported by Murr *et al.* (8). Although there was a wide range in the progesterone levels in the pregnant and nonpregnant progesterone treated groups the viral titers obtained in vaginal and brain specimens were similar. It appears that elevation of proges-

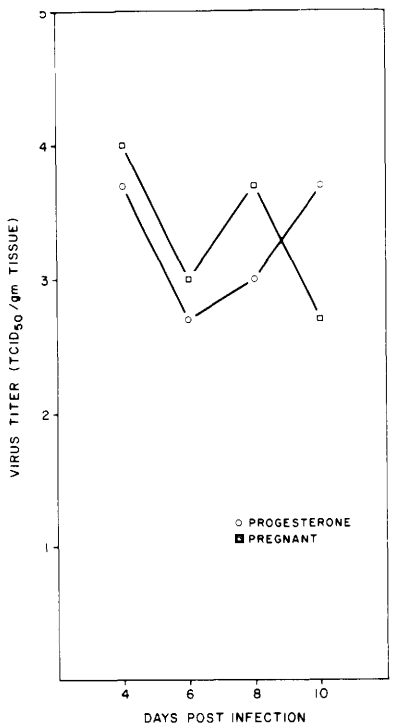

FIG. 2. Virus titer in vaginal tissue from pregnant and nonpregnant progesterone treated mice infected intravaginally with HSV II.

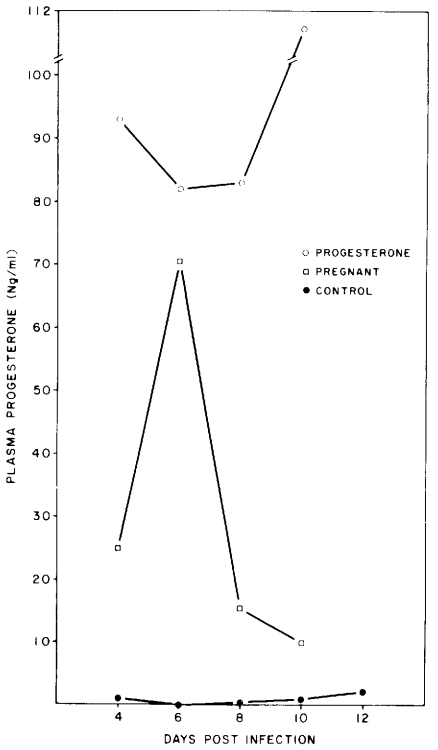

FIG. 3. Plasma progesterone ng/ml in pregnant, control nonpregnant and nonpregnant progesterone treated mice infected vaginally with HSV II at a dose of 5×10^6 $TCID_{50}$.

TABLE I. CORTICOSTERONE LEVELS (μg/100 ml) IN PREGNANT, CONTROL NONPREGNANT AND NONPREGNANT PROGESTERONE TREATED MICE INFECTED VAGINALLY WITH HSV II (EACH GROUP REPRESENTS THREE OR MORE ANIMALS).

Days postinfection	4	6	8	10
Pregnant	47.0	75.2	99.0	94.6
Control nonpregnant	12.0	—[a]	5.4	18.4
Nonpregnant progesterone treated	19.0	20.1	17.8	41.1

[a] Quantity of serum not sufficient.

terone above a certain critical level is needed for viral multiplication. The slight elevation in corticosterone in the progesterone treated group is difficult to explain. Hormonal effects of pregnancy may explain the increased susceptibility of the pregnant women to influenza (14, 15), poliomyelitis (16, 17), hepatitis (18), and varicella pneumonia (19).

Overall *et al.* (7) clearly demonstrated that pregnant mice are more susceptible than nonpregnant mice to intravaginal HSV II infection. He used 10^6 pfu of HSV II in Swiss-Webster mice and obtained 94% mortality in pregnant mice but only 50% mortality in nonpregnant mice. Using ICR mice we demonstrated a greater difference, 90% mortality in the pregnant group versus 10% in the nonpregnant controls with a dose of 5×10^6 $TCID_{50}$ of HSV II. Our experiments agree with Overall *et al.* (7) as to the pathogenesis of vaginal HSV II in mice. There is a local replication of the virus producing a vaginitis in the initial stage of infection. Direct extension to the CNS then occurs. The decreased mortality in our nonpregnant mice and our increased mortality in our pregnant mice could reflect the different strains used as well as to a slightly higher dose of virus used by the authors. Another explanation may be due to the age of mice used in these experiments. We obtained results using matched nonpregnant and pregnant mice. The age of mice used by Overall *et al.* (7) is not stated. No explanation of the enhanced susceptibility to HSV II of pregnant mice has been reported.

Numerous investigators have established the important role of CMI in the control of herpes virus infections (20–23). Evidence suggests that maternal cell-mediated immunity is lowered in pregnancy (24–27). Progesterone may be an important factor responsible for this depressed maternal cell-mediated immunity (28). Depression of CMI could explain our results showing enhanced susceptibility to HSV II infection of female mice treated with progesterone, but on the other hand, a direct effect on the vaginal wall is also possible.

Summary. Nonpregnant, pregnant and progesterone treated mice were infected intravaginally with HSV II. Mortality rates were 10%, 88% and 88% respectively. Progesterone levels were 80–100 times greater in the treated mice and 10–70 times greater in the pregnant mice than controls. We were able to simulate the increased susceptibility of pregnant mice by administration of progesterone to nonpregnant female mice.

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