Placental Transfer of Herpes Simplex Virus in Pregnant Rabbits.* (32197)

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(Introduced by Carl G. Harford)
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Herpes simplex virus is known to produce disseminated disease in the newborn that frequently ends in the death of the infant (1). Hepato-adrenal necrosis, the pathologic entity usually resulting from dissemination of this virus, has been associated with this disease since the original description by Hass in 1935(2). The source of infection in these newborns is often obscure, resulting in the question whether they are infected *in utero via* transplacental passage or if their infection is acquired during their delivery through the birth canal? The presence of intranuclear inclusions in the esophageal tissue of some of these infants suggested oral ingestion and infection as a portal of entry (3). However, the recent reports of Mitchell and McCall(4), Witzleben and Driscoll(1), and Sieber *et al*(5) would strongly suggest transplacental transmission in their patients. We were interested in investigating this problem in an experimental animal-host system to determine if herpes simplex virus crosses the placenta and what maternal reproductive tissues, fetal tissues, fetal membranes, and fetal fluids were infected. We were also interested in determining the incidence of fetal death, fetal injury, and the types and incidence of congenital anomalies, if any occurred.

Methods and materials. Animals. New Zealand female albino rabbits were purchased from a local commercial laboratory and after a period of observation in our laboratory were mated to know the exact time of conception.

Virus. Herpes simplex virus (strain J.F.) was kindly supplied by Dr. Margaret Smith. This was an isolate from the central nervous system of a fatal case of herpes simplex encephalitis. This virus was then adapted to HEp-2 and Minnesota esophageal epithelial (EE) cells. The virus inoculum was of the 6th and 7th EE, 10th HEp-2 passage.

Tissue culture. HEp-2 cells were kindly supplied by Dr. Carl Harford, and the Minnesota esophageal epithelial (EE) cells were kindly supplied by Dr. Robert Tankersly. These cells were grown in 10% calf serum in minimal essential Eagle's medium (MEM) and maintained in 2% calf serum in MEM. This medium contained 100 u/ml of penicillin 50 μg/ml streptomycin.

Antibody titer. Herpes simplex neutralizing antibody was determined, using routine methods in HEp-2 tissue culture cells(6).

Identification of virus. Virus recovered from all specimens was identified by neutralization in HEp-2 tissue culture cells using specific immune serum(6).

Procedure. New Zealand female albino rabbits in their 14-20th day of gestation were given either $10^{1.6}$ or $10^{6.6}$ $TCID_{50}$ of herpes simplex virus within 2-3 minutes *via* the marginal ear vein. A pre-inoculation serum and blood were obtained for determination of herpes simplex virus antibody and viral isolation. Bloods were obtained post-inoculation 5, 10, 15, 30, 45, 60, 90, and 120 minutes, then hourly for the first 6 hours and subsequently at 6-hour intervals to determine the duration of viremia. All animals were sacrificed under anesthesia within 51 hours post-inoculation. Maternal tissues and blood, fetal tissues, fetal membranes, and fetal fluids were obtained for virus isolation. The fetuses nearest the ovary and cervix in each uterine horn were the ones used for virus isolation when possible. A serum was obtained for herpes simplex virus antibody from the doe at termination of experiments.

Results. The 7 rabbits given $10^{1.6}$ $TCID_{50}$

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TABLE I. Virus Isolation from Rabbits Given Herpes Simplex.

Rabbit No.	Sacrificed post-inoculation hours	Virus inoculum $TCID_{50}$	Maternal tissues: Decidua	Uterine horn	Vagina	Cervix	Ovary	Uterine milk	Mammary glands	Fetal tissues, membranes and fluids: Fetal placenta	Embryo	Exocoelomic fluid	Amniotic fluid	Allantoic fluid	Yolk sac	Amnion	Fetal membranes	Perichaeental chorion
1	18	$10^{6.6}$	3/3*	2/2	0/1	0/1		1/1		3/3	2/4		1/1	1/1			1/3	
2	51	$10^{6.6}$	4/4	4/4	1/1	1/1	2/2	4/4	0/1	4/4	3/4		1/2				4/4	
3	49	$10^{6.6}$	3/4	4/4	0/1	1/1	2/2		0/1	2/4				Absorbed				
4	24	$10^{6.6}$	4/4	3/4	1/1	1/1	2/2	2/2	1/1	4/4	3/4			3/3		0/1	3/4	
5	26	$10^{6.6}$	4/4	0/4	0/1	0/1	2/2	1/1	0/1	4/4	2/4	2/2	2/3	2/2	1/1	1/3		1/1
6	49	$10^{6.6}$	3/3	2/4	1/1	1/1	2/2		0/1	3/3	2/3	1/1	1/2	1/1	2/2	2/2	1/1	3/3

* No. of virus positive specimens/No. of specimens tested.

of herpes simplex virus failed to have a demonstrable viremia, even 5 minutes post-inoculation. None of the rabbits developed antibodies against the inoculated virus. A total of 56 specimens of decidua, uterine horn, vagina, pericervical vagina, and uterine milk were taken from these 7 rabbits. Virus was isolated from 8 of 25 decidua and 2 of 18 uterine horn specimens. Only 1 of 143 specimens of fetal tissues, fetal fluids, or fetal membranes was positive for virus. The single positive fetal viral isolate was from a placenta where the maternal decidua also yielded virus.

The 6 pregnant rabbits given $10^{6.6}$ $TCID_{50}$ of herpes simplex virus had a viremia lasting 60-120 minutes. Virus was still present in the blood of rabbit no. 1 at 18 hours and rabbit no. 4 at 24 hours (Table I). After inoculation none of these rabbits developed antibodies against the inoculated virus during the period of observation. There were 48 fetuses or implantation sites in the uterine horns of these 6 rabbits (Table II). Fourteen (29.1%) of the fetuses were dead or had resorbed at the time the uterine horns were examined. All 8 fetuses in Rabbit no. 3 were dead or resorbed. All 11 fetuses in Rabbit no. 2, and 3 of the 9 fetuses in Rabbit no. 4 were hemorrhagic though still viable. The maternal reproductive system tissues were found to be infected with herpes simplex virus as seen in Table I. Only 1 of the 22 maternal deciduae failed to yield virus. Other maternal tissues, such as the uterine horn, ovary, and the uterine milk were usually infected with virus. In only 3 of the 6 rabbits was the vaginal tissue infected. Of the 6 mammary glands tested, virus was recovered from only one. Herpes simplex virus was recovered from 20 of the 22 fetal placentas tested but only 13 of 19 fetuses tested. Virus was usually recovered from the surrounding fetal fluids and fetal membranes.

Discussion. The duration of viremia produced by intravenous administration of herpes simplex virus appears to be related to the concentration of virus given. In these experiments, the total volume of fluid administered to the rabbits was similar whether $10^{1.6}$ or $10^{6.6}$ $TCID_{50}$ of herpes simplex virus

TABLE II. Incidence of Fetal Damage in Pregnant Rabbits Given Herpes Simplex Virus Intravenously.

Rabbit No.	Fetuses or implantation sites		Dead or resorbed fetuses		Fetuses tested for virus	
	Right horn	Left horn	Right horn	Left horn	Right horn	Left horn
1	2	2	0	0	2	2
2	4	7	0	2	2	2
3	7	1	7	1		
4	6	3	0	0	2	2
5	6	3	1	0	2	2
6	2	5	1	2	1	2
Total	48		14		19	

was given. Viremia was not detectable when the lower concentration of virus was given while it persisted for 60-120 minutes when the higher concentration was administered. In 2 of the 6 rabbits receiving the higher virus concentration, herpes sixplex was isolated from their blood 18 and 24 hours after inoculation. Biegeleisen and Scott(7) inoculated the HF strain of herpes simplex by corneal scarification and had a detectable viremia that persisted 132 hours with the initial specimen taken 24 hours after inoculation. Ferm and Low(8) detected only a "trace" of herpes simplex virus in the maternal blood of pregnant hamsters 48 hours after intravenous inoculation of virus.

The lower concentration of virus administered failed to cross the placenta of the rabbit and infect the fetuses. The higher concentration of virus did cross the placenta. This resulted in the infection of many of the fetuses and probably was the cause of death and resorption of about 30% of these fetuses within 2 days following inoculation of virus. Another 30% of the fetuses were hemorrhagic, and many of them may have died had we not terminated the experiments. No congenital anomalies were seen grossly in any of the surviving or dead fetuses. Previously Biegeliesen *et al*(9,10) demonstrated placental transfer of herpes simplex virus to rabbit fetuses by chorioallantoic membrane (CAM) isolation and fluorescent antibody demonstration in the fetuses. The localization of virus in the fetuses and incidence of fetal death and resorption was not stated nor were maternal tissues, fetal fluids, or fetal membranes examined for virus isolation.

Ferm and Low(8) failed to produce any increase in the rate of fetal resorption, nor were there any malformed fetuses, when herpes virus was given intravenously to pregnant hamsters during the first 8 days of gestation. They did detect small amounts of virus in hamster fetuses 24-48 hours after intravenous inoculation on the 13th day of gestation. Histologic lesions indicative of herpes simplex virus were present in the placenta of these hamsters. However, this did not interrupt the gestation of the 8 animals injected on the 13th day of gestation that were allowed to litter. All of the fetuses were normal when delivered and for the first 3 weeks of life.

The concentration of virus administered to produce the viremia is important in establishing transplacental infection with herpes simplex virus infection in the rabbit. Platt (11), using various concentrations of herpes simplex virus, has also shown that high concentrations of virus are required to produce generalized infection in the guinea pig. Platt observed that the perineal areas of the male guinea pig were more readily involved than those of the female in a generalized herpes simplex infection.

In the herpes simplex virus-rabbit host system, it is possible to produce a viremia and transplacental transfer of the virus if a large concentration of virus is given. This results in fetal death and fetal injury but without evidence of any gross congenital malformations. Virus was isolated from the fetal placentas, fetal membranes, and fetal fluids as well as the fetuses. Similarly, herpes simplex virus was isolated from the cervix in four (66.6%) and the vagina in three (50%) of the 6 rabbits given a high concen-

tration of virus. Virus in these tissues could potentially infect any animal passing through the birth canal. However, the mammary gland and its milk revealed virus in only one of the 5 rabbits tested and would suggest infrequent virus transmission postnatally by this route.

Present reports of severe herpes infections in newborns in man(1,4,5) suggest transplacental passage of virus within the last few days prior to delivery. These experiments demonstrate transplacental transfer can occur in rabbits, though only "traces" of virus crossed the placenta of hamsters(8). It is possible the anatomical differences of the placentas of these three animals may also be a factor in transplacental transmission of herpes virus(12). Previously, it had been thought infection with herpes virus was usually acquired from the maternal cervix or vagina at the time of delivery or from herpes infection of the lips or mouth of the mother or nursery personnel.

Summary. Two different concentrations of herpes simplex virus were given intravenously to pregnant rabbits. Low concentrations of virus failed to cross the placenta and disappeared from the blood rapidly. A higher concentration of virus produced a viremia of 60-120 minutes and caused the death and resorption of 30% of the fetuses. Virus was recovered from both maternal tissues as well as fetuses, fetal membranes, and fetal fluids.

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Influence of Glucose and Ammonium Ions on Endogenous Respiration.* (32198)

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The rate of endogenous respiration has been reported(1) to be repressed, unchanged, or increased during the oxidation of exogenous substrates by washed suspensions of microorganisms. The influence of the exogenous substrate appears to depend upon its nature, the past history of the cells employed in a particular test, and test conditions. Most studies have dealt with washed cells suspended in a buffer solution but recently Gronlund and Campbell(2) compared the influence of growth conditions on the endogenous respiration of *Pseudomonas aeruginosa* with that of cells suspended in a buffer solution or in the buffer to which glucose and/or ammonium ions were added. Marked suppression of endogenous $C^{14}O_2$ production by growth-labeled cells was noted in the growth medium and to a lesser extent in the presence of glucose in a Tris (hydroxymethyl) aminomethane buffer. In the present study all tests were carried out in a buffered salt solution of the same composition as employed for the growth medium and to which glucose and/or ammonium sulfate could be added in the same concentrations as employed in

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