Transplacental Infection of Fetuses of Rabbits With Herpes Simplex Virus. (23758)

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There is increasing evidence of the role of the blood in dissemination of a virus from the portal of entry to the sites of infection in various organs(1) and in the fetuses. Belenky (2) reported the presence of vaccinia virus in the blood and placenta of pregnant animals following vaccination but was unable to detect the virus in the amniotic fluid, umbilical cord or in the organs and blood of the embryos. Berry and Slavin(3) were able to isolate herpes simplex virus from the blood of infected adult mice but were not able to isolate the virus from their embryos. Scheidegger(4) reported that the embryos of mice died when the adults were inoculated with psittacosis or ectromelia viruses. Swan(5), Gregg (6) and others have described cases of congenital malformations of children born to mothers who have been infected during pregnancy with rubella virus. Winsser, *et al.*(7) discovered a viremia in a newborn whose mother suffered paralytic poliomyelitis shortly before childbirth and Schaeffer, *et al.*(8) isolated the poliovirus from the aborted fetus of a mother who had the disease.

Our report describes studies initiated to determine if herpes simplex virus crosses the placental barrier of an infected, pregnant rabbit during the viremia stage and invades the fetal tissues.

Materials and methods. The HF strain of herpes simplex virus obtained from the Communicable Disease Center, Montgomery, Ala., and propagated in this laboratory on the chorioallantoic membranes (CAM) and in the yolk sacs of embryonated hen's eggs, in the brains of white Swiss mice and on the cornea of the rabbit was used as the infecting agent. Five young adult white doe rabbits were bred with bucks of the same strain. Fourteen days after breeding, the does were lightly anesthetized with ether and 0.1 ml of the virus suspension was massaged into the scarified cornea. One ml of blood was removed aseptically from the marginal ear veins of the rabbits at 24 hours and 48 hours and every 12 hours thereafter through 180 hours. Blood samples were also taken just prior to surgery. The presence of the virus in these samples was determined by the production of lesions on the CAM(1). Fifty-four hours following inoculation, the rabbits were anesthetized with ether and surgical procedures were used to remove one gravid horn of the bifurcate uterus. The other horn was left intact so that the rabbit might go to term and deliver. The newborn was then examined for any fetal anomalies that might be present. The fetuses from each rabbit were dissected from the excised uterine horns, washed thoroughly with sterile physiological saline and ground in sterile tissue grinders. This material was diluted 1:2 with buffered gelatin saline and 0.1 ml of this homogenate of fetuses from each rabbit was inoculated onto each of 8 CAM. The inoculated chick embryos were incubated for 72 hours. At this time the membranes were harvested and examined for typical herpes simplex plaques which would indicate the presence of the virus in fetal tissue.

Results. Viral isolations were accomplished from the blood specimens taken at 24 hours, 48 hours and at the time of surgery from all 5 rabbits. The virus was also demonstrated from blood specimens at varying times through 156 hours. All of the CAM inoculated with homogenates obtained from the fetuses of all 5 of the experimental rabbits showed the presence of typical herpetic plaques, indicating the presence of herpes simplex virus in the fetal tissue. Comparative titrations of the virus isolated from the fetuses were performed according to Scott(9) in the presence of specific immune serum and buffered gelatin saline in order to identify the

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TABLE I. Results of Neutralization Test on CAM Using Virus Recovered from Fetuses of Rabbits Infected with Herpes Simplex Virus.

Inoculum	Dilutions of the virus							
	10^{-0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Virus + antiserum	4+*	4+	3+	2+	2+	+	—	—
Virus + BGS	4+	4+	4+	3+	3+	3+	2+	+

* Plaque formation on CAM.

4+, membranes heavily infected; 3+, membranes contained 16-25 plaques; 2+, membranes contained 6-15 plaques; +, membranes contained 1-5 plaques; —, membranes contained no plaques.

CAM, chorioallantoic membranes; BGS, buffered gelatin saline.

isolated virus. Representative results (Table I) demonstrated that infectivity of the virus was decreased by the specific immune serum.

Discussion. Herpes simplex virus was recovered from the fetuses of the 5 experimentally infected rabbits by inoculation of fetal homogenate on the CAM of the developing chick embryo. The virus was proved to be herpes simplex by the neutralization of infectivity with specific immune serum.

The finding of herpes simplex virus in the blood of these experimentally infected animals confirmed previous results(1) and thus indicated that the fetuses were probably infected by the virus crossing the placental barrier.

Zuelzer and Stulberg(10) presented data indicative of a viremia stage in newborn infants who succumbed to overwhelming herpetic infections. With the results here reported, the role of a viremia stage in the infection of fetuses of a herpes simplex infected rabbit could prove to be a valuable lead in discovering other possible causes of congenital malformations in newborn infants.

These findings pose several questions: What role does this virus play in causing fetal anomalies? Can this virus serve as the primary infectious agent and remain in a latent state in the offsprings' tissues to manifest itself later in life? If the virus can thus invade fetal tissues early in life, what role does it play in failure of an animal to build up protective antibodies against future exposure to this virus? The answers to these and other questions are now being sought.

Summary. Herpes simplex virus was isolated from the fetuses of 5 rabbits which had been inoculated intracorneally with herpes simplex virus. The isolated virus was identified with specific immune serum.

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