phase, when the cell appears as a "true" polyploid (*10*).

Endoreduplication therefore appears to be a principal mechanism responsible for the doubling of the stemline chromosome number. However, whatever the mechanism responsible for chromosome doubling, the form and time course of the process when induced by x-rays appears to be the same for diploid and tetraploid cells.

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Congenital Anomalies Induced in Hamster Embryos with H-1 Virus

Abstract. *Intravenous injection of H-1 virus into pregnant hamsters early in gestation produces an embryocidal and teratogenic effect. The congenital malformations, the presence of inclusion bodies in the fetuses, and the fact that the maternal animals are not affected by this virus, are points of similarity to the teratogenic effects of rubella virus and cytomegalic-inclusion disease virus in man.*

This report describes congenital malformations and fetal deaths induced by the intravenous inoculation of H-1 virus (*1*, *2*) into pregnant hamsters. To our knowledge only four viruses have been reported to have an etiologic relation to fetal defects in mammals. These include two agents causing congenital malformations in man, the rubella virus (*3*) and the cytomegalic inclusion virus (*4*). The other two viruses, associated with the use of vaccines in domestic animals, are the attenuated hog cholera virus vaccine which has caused edema and limb malformations in newborn pigs (*5*) and a modified blue-tongue live-virus vaccine used in pregnant sheep (*6*). Sheep so inoculated produced stillborn lambs or lambs which showed symptoms of central nervous system disease. It is difficult to ascertain whether lesions in the newborn pigs or lambs were true congenital malformations due to direct action of a teratogenic virus or whether they represented anomalies due to secondary effects of the immunization procedure.

Table 1. Effects of intravenous injection of H-1 virus on embryonic mortality in the pregnant golden hamster.

Gestation (day)	Vaccine *	Mothers treated (No.)	Fetuses (No.)		
			Living	Abnormal	Dying or resorbed
6	a	6	8	6	81
6	b	8	54	42	45
6	c	3	21	11	5
7	a	5	14	11	46
7	b	1	3	3	10
7	c	1	9	2	3
8	a	1	0	0	10
10–12	a	6	38	‡	24

* a, Undiluted tissue culture virus (see text); b, 10^{-1} dilution; c, 10^{-2} dilution. † Malformed or stunted (see text). ‡ All dead or dying; none malformed.

The H-1 virus was originally described by Toolan (*1*) and as used in our experiments had been carried through an unknown number of hamster passages. Stock preparations were obtained from virus propagated in rat embryo tissue cultures. The tissue culture methods used (*7*, *8*) are generally similar to those described by Moore (*9*).

Syrian golden hamsters were bred in the early evening hours, and the females were left with the males overnight. The day after breeding was counted as the first day of gestation, and the pregnant animals were caged individually, and fed diets of Purina lab chow. Pregnant hamsters (Table 1) were anesthetized with Nembutal (6.5 mg/100 g) after which 1.5 ml of a suspension of H-1 virus in tissue culture fluid was injected directly into the lingual vein (*10*) over a period of 1 minute. Animals were injected on either the 6th, 7th, 8th, 10th, or 12th days of gestation with identical volumes of either undiluted, or of 10^{-1} or 10^{-2} dilutions of the virus suspensions. On either the 10th or 13th day of gestation, the hamsters were lightly anesthetized with ether, and cardiac blood was drawn into a syringe containing heparin. Sterile specimens of uterus, placenta, and the corresponding fetus were collected and frozen at −40°C until the virus was titrated. For histologic examination corresponding specimens of uterus and placenta from the same animals, as well as specimens of maternal liver, kidney, and spleen, were fixed in Bouin's fluid. The embryos and fetuses were separated under a dissecting microscope and placed in alcohol-formalin-acetic acid fixative. Specimens from all maternal, placental, and uterine tissues were sectioned and stained with hematoxylin and eosin. Certain fetal specimens were sectioned and stained in a similar manner.

The maternal animals were watched daily after the injection of the H-1 virus. No signs of disease were noted. Maternal weight gain from the day of injection until the day the animals were killed was normal for pregnant animals, and histologic studies of sections of maternal kidney, liver, and spleen revealed no evidence of disease or of inclusion bodies.

The undiluted virus had a marked effect on embryos (Table 1), causing embryonic death in 2 to 4 days. In many of the litters it was not possible to determine the time of death, although in those considered to be recently dead or dying there was neither fetal motion on stimulation nor detectable circulation. Resorbed fetuses had placentas of normal size. An exencephalic fetus from a mother which received 1.5 ml of undiluted virus on the 7th day of gestation is shown in Fig. 1. Four surviving littermates had marked dilatation of the pericardial cavity; the pericardial cavity of some of these animals was filled with blood. Figure 2 represents another unusual malformation, lateral herniation of the liver—a common finding in animals whose mothers were treated on the 7th and 8th days of gestation. Other malformations noted in addition to these were facial clefts, facial asym-

metry, microcephaly, ectopic hearts, and umbilical hernias.

We cannot estimate the frequency of malformations since many of the embryos were already dead and too disintegrated for study when the mothers were killed. From those litters which received 10^{-1} dilution of tissue culture virus most of the survivors were abnormal. The occurrence of a widespread viral infection of the fetuses was indicated by the numbers of intranuclear inclusion bodies in all of the mesenchymal tissues of the fetus, especially those in the region of the somatic mesoderm on the 13th day of gestation. There was an apparent reduction in the amount of skeletal muscle tissue. Other inclusion bodies, typical of H-1 virus infections (*9*), were noted in the smooth muscle cells of the wall of the aorta, the endodermal cells of the wall of the developing gut, the heart, the notochord, the lung, and in cartilage cells in the ribs and vertebral bodies.

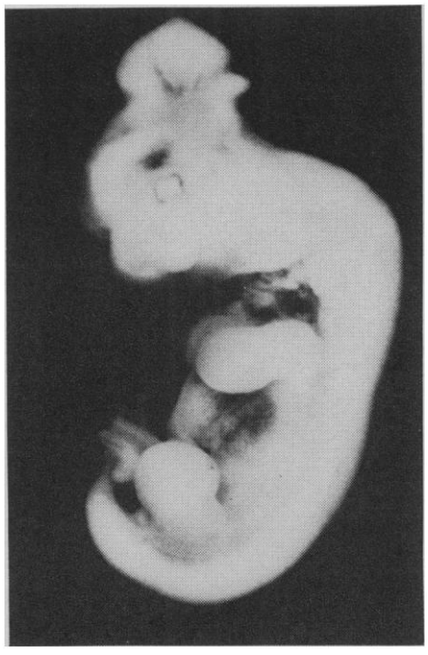

Fig. 1. Eleven-day-old hamster fetus whose mother received H-1 virus on the 7th day of gestation. Early exencephaly (× 7).

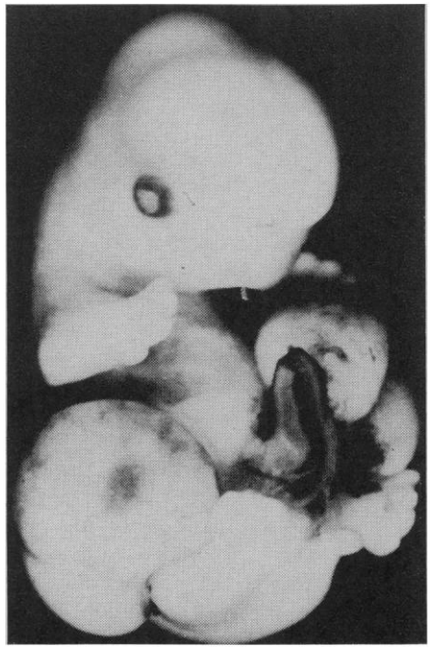

Fig. 2. Thirteen-day-old hamster fetus. H-1 virus (10^{-1}) on the 6th day of gestation. Marked bilateral abdominal herniation of liver tissue (× 5).

The H-1 virus recovered from placental and uterine tissues of a hamster inoculated on the 10th day of gestation and killed 3 days later had a titer of 10^{-3}. Virus was recovered in dilutions of 10^{-1} in similar tissues obtained from animals killed at 7 days.

In a pregnant hamster inoculated on the 7th day of gestation and killed 4 days later, H-1 virus was demonstrable in the placenta at a titer of 10^{-6} and, in the fetus, in a titer of 10^{-2}. While titers of recovered virus were not particularly high they did show, taken in conjunction with the finding of numerous inclusion bodies, that viral invasion of the fetus did take place.

Our results are not dissimilar to those associated with malformations following infection with rubella during human pregnancy. Selzer (*11*) has reported finding inclusion bodies and isolated rubella virus in tissues of a 20-mm human embryo from a mother in whom onset of clinical rubella occurred 10 days before abortion. Another parallel is afforded by cytomegalic inclusion disease. This condition is characterized by the presence of characteristic cytoplasmic inclusions and a persistent chronic viruria in human abortuses and newborns (*3*). Fetal infection with this virus undoubtedly occurs in the uterus.

Previous work in this laboratory showed that—like H-1 virus—a strain of rat virus was capable of penetrating the placenta. It did not, however, exert any embryocidal or teratogenic effect (*8*). Mumps virus used under similar experimental conditions (*12*) failed to penetrate the placenta in detectable amounts, and was without effect on the embryo. Herpes simplex virus was tried in an additional set of hamster experiments. It also failed to reach the embryo after intravenous injection of the mother, although the hamster fetus was susceptible when this agent was injected directly into the uterus (*13*). Thus, in our experience with various agents, H-1 virus has been the only one to penetrate the placenta and proliferate in the fetuses with production of congenital anomalies.

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Preferential Segregation of Chromosomes from a Trivalent in Haplopappus gracilis

Abstract. *Crosses between plants of* Haplopappus gracilis (n = *2*) *and a race with three pairs of chromosomes (tribivalens) gave a highly fertile five-chromosome hybrid. In both races the chromosomes with the satellites appear homologous, but the other two tribivalens chromosomes pair with the A chromosome (without a satellite) of* H. gracilis. *Disjunction from the resulting trivalent is preferential: the A chromosome goes to one pole and the two tribivalens chromosomes to the other.*

Haplopappus gracilis (Nutt.) Gray is a small, annual composite found in arid and semi-arid habitats of the southwestern United States and northern Mexico. Interest in this species derives from its low chromosome number of $n = 2$ (*1*) and its consequent value as a tool in certain radiation, physiological, and cytogenetic studies. Cytological study of the species has shown that