tion, might be due to disproportionate gene replication (*4*). The striking increase in rRNA genes reported here occurs during the ontogeny of a single generation and is consistent with this view. Furthermore, my results suggest that the extent of disproportionate replication of the rRNA genes in an X chromosome could be inversely proportional to the number of rRNA genes in the opposite homolog. This would allow conditions of low rRNA gene redundancy to revert rapidly and yet would also maintain the wild-type level of 18*S* and 28*S* rRNA genes at a relatively constant multiplicity.

The phenomenon of magnification is confined exclusively to males carrying mutations of the rRNA genes in both sex chromosomes (*4*). The significance of the system described above is that disproportionate rRNA gene replication occurs in standard wild-type X chromosomes. Moreover, the fact that this occurs in both X/0 males and X_{-NO}/X females suggests that more than a single cell type is involved, and thus the phenomenon may be analogous to the amplification of the rRNA genes in the oocyte of many organisms [see (*12*)]. Finally, it appears that in *Drosophila* disproportionate gene replication is associated with a mechanism capable of sensing a deficiency in the number of rRNA genes.

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5. Ore-R M and Ore-R S stocks are wild-type lines established from two different populations. These stocks as well as all others described in this report are maintained in the collection of Dr. Jack Schultz, Institute for Cancer Research, Philadelphia, Pa.
6. $\overline{XY}$ males are maintained by mating them to females that possess one $\overline{XY}$ chromosome and an X chromosome (derived from the Ore-R S stock) carrying the mutant gene, y^2 (*7*) which causes the body color of the fly to be yellow as in X/0 males. This stock has the genetic symbol $Y^sX \cdot Y^L$, $In(1)EN/y^2$ (*7*). For simplicity it is referred to as the A01-1 or $\overline{XY}/X$ stock. $\overline{XY}$ males mated to $\overline{XY}/X$ females give rise to a progeny of $\overline{XY}$ sons that are genetically identical to their fathers and $\overline{XY}/X$ daughters that are genetically identical to their mothers. X/0 males are also produced but they are sterile, lacking a Y chromosome for fertility. $\overline{XY}/\overline{XY}$ females usually do not survive (less than 1 in 2000) in this particular stock, and those that do are frequently sterile. Thus, the only viable and fertile flies produced in a stock of $\overline{XY}$ males and $\overline{XY}/X$ females are $\overline{XY}$ males and $\overline{XY}/X$ females that are genetically identical to their parents. The $\overline{XY}$ males of this stock were used in experiments 1 and 3.
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8. $\overline{XY'}$ males were derived from the $\overline{XY}$ males described above (*5*) in 1966 and maintained as a separate line by mating them to females that have their two X chromosomes attached ($\overline{XX}$, attached X). The progeny of the mating of $\overline{XY'}$ males to $\overline{XX}$ females are $\overline{XY'}$ sons and $\overline{XX}$ daughters that have genotypes identical to their fathers and mothers, respectively. The $\overline{XY'}/\overline{XX}$ genotype is lethal. This stock is described by the symbol, $Y^sX \cdot Y^L$, $In(1)EN/C(1)RM$, w^{18h} (*7*).
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Genital Herpesvirus Hominis Type 2 Infection: An Experimental Model in Cebus Monkeys

Abstract. *Genital infection with herpesvirus hominis type 2 was established in ten female cebus monkeys. Clinical and laboratory findings in the cebus mimic closely those observed in humans, thus providing an experimental model which may be used in the study of the possible role of genital herpetic infection in cervical cancer and in perinatal and chronic neurological diseases.*

The possible role of genital herpesvirus hominis (HVH) type 2 infection in fetal and neonatal diseases, cervical cancer, and chronic neurological disease (*1–3*) stimulated the search for an experimental model among nonhuman primates. Reports by other workers on the ability to establish an HVH infection in primates has varied according to the species tested and the method of inoculation used. In some species (for example, rhesus monkeys) it has been very difficult to establish an infection, whereas in others, such as owl monkeys, a fatal generalized disease has been produced after conjunctival inoculation with HVH type 1 (*2*). Attempts to infect any primate species with HVH by the genital route, or specifically HVH type 2 by any route, have not been previously recorded.

Genital infection with a type 2 strain (CUR) recovered from a female patient was attempted in three primate species: squirrel monkeys (*Saimiri sciurea*), rhesus monkeys (*Macaca mulata*), and cebus monkeys (*Cebus albifrons*). The virus used had been isolated and passed three or four times in primary tissue cultures of rabbit kidney. A method for genital inoculation of virus similar to that previously found successful with mice (*4*) was used in the inoculation of the monkeys. Cotton pellets were soaked in undiluted virus [titer, $10^{5.5}$ to $10^{6.5}$ tissue culture infected dose (50 percent effective) per milliliter] and implanted deep in the vaginal vault. The establishment of a successful genital herpetic infection in inoculated monkeys was followed by several methods: (i) the clinical development of vesicular or ulcerative lesions; (ii) isolation of virus after swabbing of the vagina and cervix three or more days after inoculation; (iii) detection of virus in cervico-vaginal cells by direct immunofluorescent techniques (*5*); and (iv) demonstration of neutralizing antibodies against HVH in serum obtained from convalescent subjects one or more weeks after genital inoculation (*6*).

Three separate attempts to infect the genitalia of two female squirrel monkeys were unsuccessful. Similarly, attempts to infect five female rhesus monkeys also proved unsuccessful. We were able, however, to establish genital herpetic infection in ten female cebus monkeys. In all of these animals, the virus was isolated from cervico-vaginal swabs from 3 to 16 days after inoculation; convalescent subjects all had neutralizing antibodies in the serum (serums obtained before inoculation failed to show antibodies at a titer of 1:4). In three of five animals in which immunofluorescent studies were performed, HVH was detectable in cervico-vaginal cells 3 to 16 days after inoculation. All monkeys had inflamed vaginas and cervices, and seven had herpetic-like vesicles or ulcers on the

vulvas. In addition, four of the monkeys developed perineal and finger lesions from which HVH could be recovered.

In seven monkeys, attempts at genital reinfection with the same HVH type 2 strain were made 6 to 12 weeks after the initial inoculation. In three of these animals, in spite of significant titers of neutralizing type 2 antibodies in the serum, we could demonstrate reinfection by virological techniques. These monkeys had several discrete herpetic vesicles on the labia.

The findings obtained in the cebus female monkey genitally infected with HVH type 2 mimic closely laboratory and clinical observations of genital herpetic infections in women (*1*), in that incubation periods, clinical appearance of lesions, duration of virus recovery, and development of serum neutralizing antibodies of the two groups are similar. The successful establishment of this model in cebus monkeys now permits clarification of the effect of maternal genital herpetic infection on the fetus and newborn, the immunological responses to this infection, and the effectiveness of antiviral drugs in its therapy. In addition, long-term observations of monkeys infected with HVH type 2 may help to elucidate the possible role of genital herpetic infection in chronic neurological disease and in the development of cervical cancer.

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Herpesvirus Antigens on Cell Membranes Detected by Centrifugation of Membrane-Antibody Complexes

Abstract. *Herpesviruses specify new glycoproteins that bind to cell membranes and also appear in the envelope of the virion. Incubation of purified smooth membranes from infected cells with antiviral antibody results in an increase in the density of the membranes as determined by flotation in sucrose density gradients. The magnitude of this increase depends on the amount of antibody used; densities as high as 1.16 grams per cubic centimeter have been obtained (the density of the untreated membranes is 1.08 grams per cubic centimeter). Antiviral antibody does not increase the density of uninfected cell membranes nor do saline or normal rabbit serum change the densities of infected or uninfected cell membranes. Viral antigens—presumably the glycoproteins specified by the virus—are probably on the surface of the infected cell membranes and bind to them strongly enough to withstand the hydrodynamic forces applied to them in the sucrose gradient.*

The interactions of mammalian cells among themselves become altered after infection with herpes simplex virus. Thus, cells that normally seem to repel each other show varying degrees of clumping and fusion, depending on the virus mutant which infected them (*1*). In accord with the hypothesis that the alteration in the social behavior of infected cells is mediated by changes in the surface membrane, it was shown that infected cells acquire on their surfaces new determinant antigens similar to or identical with those on the surface of the herpes virion (*2*). Subsequently we purified cytoplasmic membranes from infected cells and reported that these membranes contain new glycoproteins absent from uninfected cells (*3*) and that virus strains differing with respect to their effects on the social behavior of infected cells specify different membrane glycoproteins (*4*).

We now report that antiviral antibody will bind to and consequently increase the density of purified smooth membranes from infected cells. The difference in density between the membranes that react with antibody and those that do not allows the separation of infected and uninfected cell membranes from an artificial mixture. The technique is based on the observation that purified smooth membranes have a buoyant density of approximately 1.08 g/cm^3 in sucrose solutions and will float to that density when placed on the bottom of a sucrose density gradient and subjected to high speed centrifugation. However, membranes which have reacted with antibody will have a much higher ratio of protein to lipid and hence band at a higher density than those which do not react with the antibody.

We infected human epidermoid carcinoma No. 2 (HEp-2) cells with herpes simplex strain MP and labeled the cells with [^{3}H]glucosamine from 4 to 20 hours after infection. Uninfected cells were labeled for a similar interval with [^{14}C]glucosamine. In this interval glucosamine is incorporated largely into membrane glycoproteins as glucosamine (68 percent) and galactosamine (32

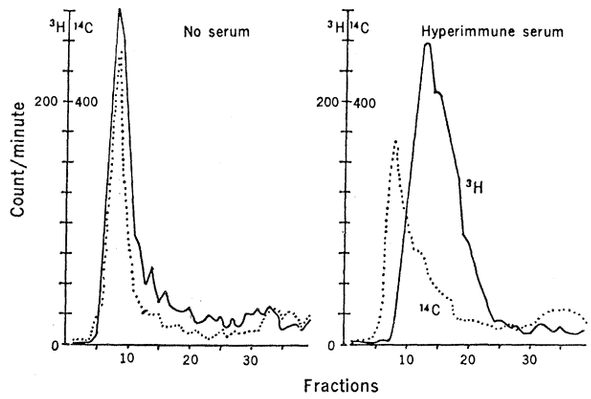

Fig. 1. Flotation of mixtures of infected and uninfected cell membranes in sucrose density gradients after incubation for 4 hours with buffered saline or hyperimmune serum. The incubation mixtures were made 50 percent (by weight) with respect to sucrose, overlaid with linear gradients of 10 to 45 percent (by weight) sucrose, and then with 3 ml of saline and centrifuged for 20 hours at 25,000 rev/min (Spinco SW27 rotor). The top of the tube is at the left. Solid line, infected cell membranes labeled with [^{3}H]glucosamine; dotted line, uninfected cell membranes labeled with[^{14}C]glucosamine.