Table 2. Number of colonies per plate in stimulated cultures of peripheral blood and leukapheresis leukocytes collected and cultured simultaneously at 2×10^5 nucleated cells per plate for 14 days.

Source of inoculum	No. of subjects	Colonies				Positive colonies (%)
		Mean No.	Median No.	Mean No. in positive cultures	Median No. in positive cultures	
Peripheral blood	29	18	9	30	28	58
Leukapheresis leukocytes	29	23	11	34	21	65

six occasions no CFC were found in the peripheral blood when colonies were grown from LL, and on three occasions CFC were grown from PBL and not from LL. The inability to grow colonies on these occasions may have been due either to the absence of CFC or to difficulties with the culture technique. On nine occasions bone marrow was aspirated at the same time the leukapheresis was performed, and the median number of CFC in the bone marrow, PBL, and LL did not differ from the overall results in Table 1.

Bone marrow was aspirated from three patients prior to and after a series of leukaphereses; in two of these cases the number of CFC was higher on the second aspirate than it was on the first.

In conclusion, CFC have been demonstrated in the peripheral blood in a concentration of approximately 1 per 10,000 nucleated cells. The presence of CFC in the peripheral blood has also been demonstrated recently by others but in lower concentration (*10*), possibly because the investigators were not working in the linear portion of the inoculum response curve. The colonies consisted predominantly of granulocytic cells and appear to be similar to those colonies that were previously demonstrated in the bone marrow of mice (*1*) and man (*2*) and that are derived from committed stem cells. The CFC circulating in the peripheral blood can be collected in large quantities by leukapheresis with a blood-cell separator and in numbers similar to those found in the peripheral blood. Since the blood-cell separator collects 20 to 30 percent of the circulating leukocytes, of which 60 to 90 percent are large mononuclear cells (*11*), this would suggest that the CFC are present in the large mononuclear cell fraction of the peripheral leukocytes.

In a series of five leukaphereses, in which a total of 50 liters of whole blood can be processed, 5×10^{10} nucleated cells containing a total of 4×10^7 CFC (calculated from the median CFC of 17 in the LL) can be collected. If one assumes an average nucleated cell count in the bone marrow of 15×10^3, there would be 2.0×10^7 CFC (calculated from the median of 57 CFC in the marrow) in 500 ml of bone marrow. This suggests that the transfusion of sufficient quantities of peripheral leukocytes might be as effective as bone marrow in the granulocytic repopulation of patients with severe myelosuppression. Evidence of this has been shown in identical twins (*9*). Uncommitted stem cells capable of differentiation to all myeloid cell lines have not been demonstrated with this system. This does not preclude their presence in the peripheral blood and their potential value in marrow repopulation. For instance there is evidence, from circulation experiments in dogs (*8*) and in those patients who develop myeloid grafts after leukocyte transfusions from donors with chronic myelogenous leukemia (*7*), that these cells do exist in the peripheral circulation.

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Increasing the Multiplicity of Ribosomal RNA Genes in Drosophila melanogaster

Abstract. *In wild-type* Drosophila melanogaster *females there are about 250 ribosomal RNA genes in each nucleolus organizer region of the two X chromosomes. When this same nucleolus organizer region is present in flies in only a single dose, the number of ribosomal RNA genes increases to approximately 400. This increase is most easily explained by disproportionate replication of these genes.*

In the eucaryotes thus far studied the multiple copies of the 18*S* and 28*S* RNA genes are clustered at the nucleolus organizer (NO) locus (*1–3*). Experiments with both *Xenopus* and *Drosophila* have shown that the amount of 18*S* and 28*S* ribosomal RNA (rRNA) hybridizable per unit DNA is directly proportional to the dosage of the NO segment per genome (*2, 3*). However, in the course of investigating the redundancy of the rRNA genes in *Drosophila melanogaster*, I have discovered several cases which contradict this observation. When flies carry only one NO region (Fig. 1A), on their X chromosome, there are approximately 150 more rRNA genes per X as compared to the value when the same wild-type X chromosome is present as part of the usual two doses in X/X females. This striking increase occurs during the development of these individuals, probably involves more than one cell type, and cannot be due to gene recombination between homologous chromosomes.

It appears to involve a mechanism whereby the rRNA gene cluster of a standard wild-type X chromosome undergoes disproportionate replication. This phenomenon may be related to the magnification phenomenon described by Ritossa (*4*).

When X/X wild-type females [from the wild-type stock Ore-R M (*5*)] are mated to males that have their X and Y chromosomes attached (symbolized here as $\overline{XY}$, Fig. 1B), X/$\overline{XY}$ females and X/0 males are produced (*6, 7*). DNA's from both parents and progeny of this cross were isolated, and the percentage of the DNA complementary to rRNA was determined as indicated (Table 1). In experiment 1 (Table 1) the X chromosome of the wild-type female contains approximately 255 rRNA genes. When this same X was combined with an $\overline{XY}$ chromosome in X/$\overline{XY}$ females, the number of rRNA genes was the sum of the two chromosomes. However, when present as a single dose in X/0 males, this X then contained 430 rRNA genes, or an increase of approximately 175 rRNA genes per X. This increase in the number of rRNA genes per X in X/0 males is not the result of erratic variability in the DNA-RNA hybridization technique or the result of genetic contamination of the *Drosophila* stocks for the following reasons. (i) A second independent isolation and hybridization of DNA from the wild-type females used in experiment 1 (Table 1) gave a value for the percentage of DNA hydridized of 0.462 ± 0.020 as compared to 0.445 ± 0.022, confirming the reproducibility of the measurements obtained by the hybridization technique. Moreover, two separate ways of calculating the number of rRNA genes per X are in close agreement. Dividing the percentage of DNA hybridized of X/X females by 2 gives a value of 255 rRNA genes per X; whereas subtracting the percentage of DNA hybridized of $\overline{XY}$ males from the percentage of DNA hybridized of X/$\overline{XY}$ females gives 275 rRNA genes per X. The additivity of the number of rRNA genes in the X and $\overline{XY}$ chromosomes serves as a useful independent check on the reproducibility of the extraction and hybridization of the DNA. (ii) Brain squashes from six larvae of X/0 and X/$\overline{XY}$ progeny were also made and the metaphase nuclei were examined. In all cases the male larvae contained a single X and three pairs of autosomes, and the female larvae contained one X, one $\overline{XY}$, and three pairs of autosomes.

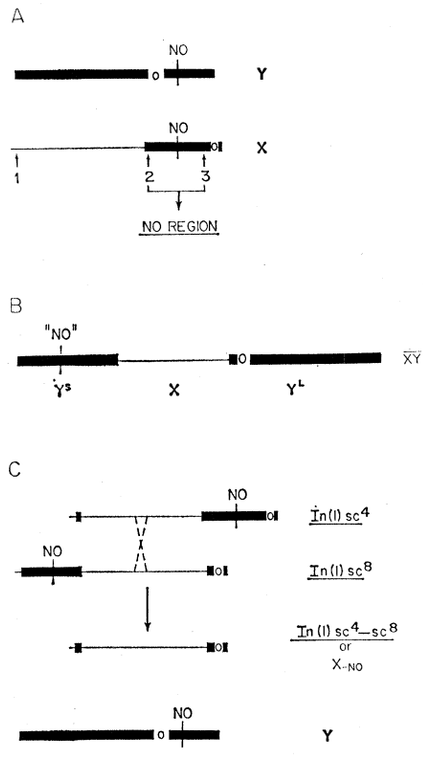

Fig. 1. Various rearrangements of the sex chromosomes of *Drosophila melanogaster* (*7*). (A) In addition to three sets of autosomes the male carries one X and one Y chromosome while the female contains two X's. The X and Y each contain one nucleolus organizer (NO) as indicated. The heavily darkened part of the X indicates the hetrochromatic region of this chromosome while the thin line represents the euchromatic portion; the Y is entirely heterochromatic. The open circle (○) designates the centromere. (B) A chromosome with the X and Y attached together can be synthesized whereby the short arm of the Y (Y^s) is linked to the left arm of an inverted X chromosome, and the long arm of the Y (Y^L) to the right arm of this X. This chromosome has the genetic symbol $Y^sX \cdot Y^L$, *In(1)EN* (*7*) but for convenience is abbreviated here as $\overline{XY}$. It contains one (or possibly two) NO clusters in the left heterochromatic arm (*7*). (C) An X chromosome can also be synthesized which lacks its NO and most of the surrounding heterochromatin. A female is obtained which contains one X chromosome designated *In(1)sc*^{4} which contains an inversion between points 1 and 2 (see A) while the other X is inverted between points 1 and 3 and is designated as *In(1)sc*^{8}. With appropriate markers on each X, such a female is mated to a male and the recombinant chromosome, *In(1)sc*^{4}-*sc*^{8} or X_{-NO}, is obtained in a male which contributes the NO of its Y for viability. The X_{-NO} chromosome lacks the NO region which is defined as that chromosome segment between points 2 and 3 of the inversions.

Therefore, the parents and progeny in experiment 1 (Table 1) do indeed possess the indicated genotypes free of any extra contaminating chromosomes. Also, since X/0 males are characteristically sterile because they lack a Y chromosome, several thousand X/0 males produced in experiment 1 (Table 1) were randomly selected and tested for their infertility. In all cases such X/0 males were sterile.

These experiments have also been repeated with a wild-type stock [Ore-R S stock (*5*)] whose X chromosome is of separate origin and an attached XY chromosome that has been carried in a stock separate from the one used in experiment 1 for several years—symbolized here as $\overline{XY}'$ (*8*). The results are summarized in experiment 2 (Table 1). Again, the X chromosome is additive with the $\overline{XY}'$, but an increase of approximately 130 rRNA genes per X occurs when this X is present as a single dose in X/0 males as compared to X/X females. The genotype of these X/0 males was verified by testing their sterility; cytological examination of X/0 male and X/$\overline{XY}'$ female larvae also confirmed their genotypes.

The use of yet another X chromosome derived from the X used in experiment 2 (Table 1) but maintained in X/$\overline{XY}$ females as a separate line [stock A01-1 (6, 7)] for several years also demonstrates the increase in rRNA genes per X when it is present as a single dose in X/0 males. In this experiment $\overline{XY}$ males were mated to X/$\overline{XY}$ females, and the progeny of $\overline{XY}$ males, X/$\overline{XY}$ females, and X/0 males (*6, 7*) were separated and their DNA was isolated and hybridized to rRNA. The results are given in experiment 3 (Table 1). The X chromosome in X/$\overline{XY}$ females contains about 103 rRNA genes, but in X/0 males it has 248 such genes. This represents an increase of approximately 145 rRNA genes per X. The number of rRNA genes in this particular X is rather low compared to X chromosomes used in experiments 1 and 2 (Table 1). The precision of the hybridization technique (as discussed above) is such that these differences are significant and indicate, in agreement with a previous report (*9*), that different stocks may have widely different optima for rRNA gene redundancy. However, in this case as well, the rRNA gene content of the X is increased significantly when in the X/0 condition.

These three experiments demonstrate that a wild-type X chromosome can

increase its content of rRNA genes when present as a single dose in X/0 males relative to the number of rRNA genes per X in the X/X or X/$\overline{XY}$ female parent. Since this increase in rRNA genes occurs in X/0 males, it cannot be explained by gene recombination between homologs. However, an explanation involving sister chromatid exchange remains a possibility.

It was also of interest to determine whether a wild-type X would increase its content of 18*S* and 28*S* rRNA genes when opposite an X chromosome that lacks its NO region (X_{-NO}, Fig. 1C). Wild-type X/X females were mated to X_{-NO}/Y males (*10*), and the progeny, X_{-NO}/X females and X/Y males, recovered. The percentage of DNA hybridizable to 18*S* and 28*S* rRNA was determined on both parents and progeny, and the results are summarized in experiment 4 (Table 1). The rRNA genes of the X chromosome from X/X females are additive with those of the Y from X_{-NO}/Y males. However, in X_{-NO}/X females the X chromosome has increased its content of rRNA genes per X from about 255 to 395, an increase of approximately 140 rRNA genes per X relative to the X/X female.

These results indicate that, when the NO region (Fig. 1A) of the wild-type X chromosome occurs as a single dose in either X/0 males or X_{-NO}/X females there is a dramatic increase of approximately 150 rRNA genes per X relative to the number of such genes per X in the X/X female parent. I propose that this increase occurs as the result of disproportionate replication of the rRNA genes at some point in the course of the developmental cycle.

In contrast to the above, males whose Y chromosome has been exposed for many years to an X_{-NO} chromosome (in X_{-NO}/Y males) have half the number of rRNA genes as X/X females or X/Y males (*3*; experiment 4). This could be interpreted to mean that the rRNA genes in the Y chromosome are incapable of disproportionate replication. However, the number of rRNA genes in the Y before exposure to the X_{-NO} is not known, and the possibility remains that there has been disproportionate replication to the present level as the result of exposure to the X_{-NO} chromosome.

One obvious question is whether the X_{-NO}/X females can pass their increased rRNA gene number to successive generations. There is experimental evidence (*11*) that when the X_{-NO}/X females of experiment 4 (Table 1) were again mated to X_{-NO}/Y males (*10*) the resulting progeny of X_{-NO}/X daughters gave a value of the percentage of DNA hybridized per X nearly equal to that of their mothers while X/Y sons showed a percentage of DNA hybridized per X approximately equal to that of the standard wild-type X chromosome in X/X females (Ore-R M stock; *5*). Thus, the increased rRNA gene number persisted in those individuals of subsequent generations in which only a sing'e *NO region* was present and did not persist in those individuals of subsequent generations in which the wild-type NO of a Y chromosome was present.

Flies partially deficient in rRNA genes have much smaller bristles and develop more slowly than the wild type, a phenotype called bobbed, *bb* (*7*). Such *bb* mutants have the characteristic of reverting after a few generations to the wild-type phenotype, with a concomitant increase in the number of rRNA genes (*3, 4, 7*). It was suggested that this phenomenon, termed magnifica-

Table 1. Hybridization of ^{3}H-labeled 18*S* and 28*S* RNA to DNA of various genotypes. Females from the Ore-R M stock, Ore-R S stock, or A01-1 stock were mated to males (described in the text), and their progeny was separated according to genotype. Methods of maintaining *Drosophila melanogaster* stocks, purification of ^{3}H-labeled 18*S* and 28*S* RNA (45,000 count/min per microgram) and DNA-RNA hybridization have been described (*13*), except that, in the present experiments, hybridization time was shortened to 3 hours. The reaction was complete after 2 hours and remained unchanged for at least 12 hours. Filters containing 50 to 65 μg of DNA from adult flies of the appropriate genotypes were hybridized with 8.8, 13.2, 17.6, 22.0, and 26.4 μg of 18*S* and 28*S* [^{3}H]RNA. The plateau value was calculated in the following manner. The percentage of DNA hybridized at 13.2, 17.6, 22.0, and 26.4 μg of [^{3}H]rRNA from two or three determinations was averaged, and the standard error (S.E.) was calculated from these 8 or 12 values, respectively. Flat saturation plateaus were achieved over this range [see (*13*)]. As a typical example of the number of counts per minute per filter, filters containing 65 μg of DNA from X/X female or X/0 male DNA (experiment 1) gave, on the average, approximately 13,000 and 12,000 count/min per filter, respectively. In order to compare the percentage of DNA hybridized in X/0 males or X/$\overline{XY}$ females to that in the wild type, a correction factor must be introduced to compensate for the different DNA contents of these genotypes. The X or Y chromosome accounts for 10 percent of the DNA per genome. Therefore, since the concentration of 18*S* and 28*S* RNA genes per unit DNA in X/0 males will be enriched 10 percent relative to the wild-type genotype, the percentage of DNA hybridized in such males must be multiplied by 0.9. Conversely, the percentage of DNA hybridized in X/$\overline{XY}$ females must be multiplied by 1.1 to compensate for the 10 percent dilution of 18*S* and 28*S* RNA genes per unit DNA in this genotype. The number of rRNA genes was calculated with the assumption that the molecular weight of the diploid genome of *Drosophila melanogaster* is 2.4×10^{11} and the molecular weight of 18*S* and 28*S* RNA is 2.1×10^6 (*13*). Figures in parentheses represent the number of determinations.

	Genotype	DNA hybridized ± S.E.* (%)	Corrected DNA hybridized (%)	DNA hybridized per X (%)	18*S* + 28*S* RNA genes per X (No.)	Net increase of rRNA genes in X/0 or X_{-NO}/X genotypes (No.)
	Experiment 1 (Ore-R M, X/X ♀ × $\overline{XY}$ ♂)					
P_1	$\overline{XY}$ ♂	0.263 ± 0.022 (3)	0.263			
	X/X ♀	0.445 ± 0.022 (2)	0.445	0.223	255	
F_1	X/$\overline{XY}$ ♀	0.458 ± 0.010 (2)	0.504	0.241	275	
	X/0 ♂	0.418 ± 0.020 (2)	0.376	0.376	430	175
	Experiment 2 (Ore-R S, X/X ♀ × $\overline{XY}'$ ♂)					
P_1	$\overline{XY}'$ ♂	0.211 ± 0.011 (2)	0.211			
	X/X ♀	0.450 ± 0.016 (2)	0.450	0.225	256	
F_1	X/$\overline{XY}'$ ♀	0.366 ± 0.018 (2)	0.403	0.192	219	
	X/0 ♂	0.375 ± 0.030 (3)	0.338	0.336	386	130
	Experiment 3 (A01-1, $\overline{XY}$/X ♀ × $\overline{XY}$ ♂)					
P_1	$\overline{XY}$ ♂	0.263 ± 0.022 (3)	0.263			
	X/$\overline{XY}$ ♀	0.321 ± 0.014 (2)	0.353	0.090	103	
F_1	$\overline{XY}$ ♂	0.263 ± 0.022 (3)	0.263			
	X/$\overline{XY}$ ♀	0.321 ± 0.014 (2)	0.353	0.090	103	
	X/0 ♂	0.241 ± 0.019 (3)	0.217	0.217	248	145
	Experiment 4 ($Ore-R\ M$, X/X ♀ × X_{-NO}/Y ♂)					
P_1	X_{-NO}/Y ♂	0.217 ± 0.015 (2)	0.217			
	X/X ♀	0.445 ± 0.022 (2)	0.445	0.223	255	
F_1	X/Y ♂	0.467 ± 0.018 (2)	0.467	0.250	286	
	X_{-NO}/X ♀	0.346 ± 0.018 (2)	0.346	0.346	395	140

* The value for the percentage of DNA hybridized in $\overline{XY}$ males in experiments 1 and 3 and in X/X females in experiments 1 and 4 is taken from the same set of determinations.

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, $Y^s X \cdot Y^L$, $In(1)EN/C(1)RM$, w^{18h} (*7*).
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10. X_{-NO}/Y males are maintained in a stock whereby they are mated to a female that possesses a Y and an attached X which consists of two X chromosomes tandemly linked together, both of which lack the *NO region* (Fig. 1). This stock is designated as $In(1)sc^{4L}sc^{8R}$, $y\ sc^4sc^8\ cv\ v\ B/C(1)DX$, $y\ f/B^sY$ (*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