MICROBIOLOGICAL REVIEWS, Sept. 1980, p. 468-490
0146-0749/80/04-0468/23$02.00/0

Vol. 44, No. 3

Viruses of Guinea Pigs: Considerations for Biomedical Research

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INTRODUCTION

The guinea pig, *Cavia porcellus*, is commonly used in both biological and immunological research. The use of this animal species has been so widespread for so many years that the term *guinea pig* has become synonymous with *experimental animal*. This species has proven to be of considerable value in microbiological research for isolation of microbial organisms and studies of their pathogenesis, for studies of antibody production, and as a source of "complement." The guinea pig is an apparently healthy and hardy animal, subject to relatively few naturally occurring viral diseases (53).

Although several viruses have been isolated from this animal species, there have been relatively few reports concerned with viral diseases of guinea pigs. In this paper emphasis will be on those viruses of guinea pigs which are either endogenous or acquired, the methods by which they are recognized, and their pathogenicity after experimental infection. Endogenous viruses, in particular, potentially complicate research in both virology and immunology; hence, investigators must be aware of their presence and the pathogenesis of their infections in order to interpret data correctly.

Anatomic similarities between guinea pigs and humans have been noted. The structure of the guinea pig placenta (19), for example, closely resembles that of humans and permits the transplacental transmission of many viruses, including herpesviruses. Thus, guinea pigs provide an important animal model for studying human viral diseases, especially congenital infections with cytomegalovirus (CMV) (9, 28a, 48, 49).

VIRUS ISOLATIONS FROM GUINEA PIGS: HISTORICAL BACKGROUND

Several deoxyribonucleic acid (DNA)- and ribonucleic acid (RNA)-containing viruses have been isolated from guinea pigs (Table 1). Among the DNA viruses, herpesviruses are most common. In 1920 Jackson described an intracellular parasite present in the duct cells of guinea pig salivary gland tissues (47). However, in 1926

TABLE 1. *Naturally occurring virus infections of guinea pigs*

Nucleic acid contained	Virus group	Virus type	Name commonly used	Reference
DNA	Herpesvirus	Caviid herpesvirus type 1	GPCMV guinea pig salivary gland virus	Jackson (47) Cole and Kuttner (11)
		Caviid herpesvirus type 2	GPHLV	Hsiung and Kaplow (41)
		Caviid herpesvirus type 3	Guinea pig X-virus	Bia et al. (J. Virol., in press)
	Poxvirus		Guinea pig poxlike virus	Hampton et al. (29)
RNA	Paramyxovirus	Parainfluenza virus type 1	Sendai virus	Van Hoosier and Robinette (74)
		Parainfluenza virus type 2[a]		Van Hoosier and Robinette (74)
		Parainfluenza virus type 3[a]		Van Hoosier and Robinette (74)
		Parainfluenza virus type 5	Guinea pig parainfluenza virus 5	Hsiung et al. (36) Bia and Hsiung (unpublished data)
		Mumps virus[a]		Hsiung et al. (36)
		Pneumonia virus of mice[a]		Van Hoosier and Robinette (74)
	Retrovirus	GPRV	Guinea pig leukemia virus	Nadel et al. (59), Opler (63)
			Guinea pig C-type	Hsiung (33), Nayak and Murray (62)
			Guinea pig B-type	Dahlberg et al. (15)
			Guinea pig G-type	Hsiung (34)
	Reovirus	Reovirus type 3[a]		Van Hoosier and Robinette (74)
	Arenavirus	Lymphocytic choriomeningitis virus[a]	LCM	Van Hoosier and Robinette (74)
	Enterovirus	Murine encephalomyelitis virus[a]	GD-VII (Theiler virus)	Van Hoosier and Robinette (74)

[a] Antibody studies only.

Cole and Kuttner demonstrated that these intracellular inclusions were caused by a filterable agent (11, 50) or salivary gland virus which was later named guinea pig CMV (GPCMV). Nevertheless, in vitro cultivation of GPCMV was not accomplished until 1957, by Hartley et al. (30). In fact, GPCMV was the first among the CMVs to be recognized as a filterable agent that caused the distinctly swollen cells in salivary glands, from which the name of the virus group was derived. GPCMV has been classified by the International Committee on Nomenclature of Viruses as caviid herpesvirus type 1 (68).

In 1969, as part of a longitudinal survey of latent virus infection in laboratory animals, another herpesvirus of guinea pigs, caviid herpesvirus type 2, was isolated first from leukemic and then from nonleukemic strain 2 guinea pigs (41) and later from Hartley guinea pigs (42); subsequently, several laboratories reported similar findings (3, 14, 60, 65). Because the original isolation of the guinea pig herpes-like virus (GPHLV) was from leukemia-susceptible strain 2 guinea pigs (41), the natural history of this virus infection in guinea pigs gained considerable attention during the early 1970s (see Guinea Pig Herpes-Like Virus: Infection In Vivo). More recently, a third herpesvirus, caviid herpesvirus type 3, was isolated from inbred strain 2 guinea pigs (F. J. Bia, W. C. Summers, C. K. Y. Fong, and G. D. Hsiung, J. Virol., in press). Serologically, this new herpesvirus of guinea pigs showed no antigenic cross-reaction with either GPCMV or GPHLV by either the neutralization test or the immunoferritin electron microscopic technique. In addition, biological and molecular characterization demonstrated that this third herpesvirus is distinctly different from the two well-known herpesviruses of guinea pigs (Bia et al., in press).

With regard to other DNA viruses, there has been one report in the literature of pox-type virus particles observed in cell cultures derived from fibrovascular growth on the thigh muscles of guinea pigs in one colony (29). The source of virus infection in these guinea pigs was not discussed.

In the RNA virus group, evidence of parainfluenza virus infection in guinea pigs was first recognized by the presence of antibody titers to parainfluenza viruses (36), especially parainfluenza virus type 5 (SV_5) antibody, in guinea pig sera. Normal guinea pig sera and commercially available complement often contained parainfluenza virus type 5 antibody, which was a problem when guinea pigs were used for preparing hyperimmune type-specific antisera for the parainfluenza viruses (75). (For details, see below.) On one occasion parainfluenza virus type 1, or Sendai virus, was isolated from a guinea pig colony which was in proximity with mice (74). Recently, a parainfluenza virus isolate serologically identical to SV_5 was obtained from the salivary gland of a guinea pig (see Mixed Infections).

Other RNA viruses included a retrovirus which was originally observed in the leukemic cells of strain 2 guinea pigs with L_2C leukemia (59, 63). Since similar virus particles were not found in normal guinea pigs during early investigations, it was thought that these virus particles were actually the guinea pig leukemia virus. Not until 1972, when attempts to cultivate the so-called guinea pig leukemia virus were successful, did we demonstrate that a similar virus, later named guinea pig retrovirus (GPRV), was induced in cultured guinea pig cells which were maintained in a medium containing 5-bromo-2'-deoxyuridine (BUdR) (33). (Details will be discussed below.)

Antibodies to other RNA-containing viruses, including reovirus, arenavirus, and enterovirus, have been noted (74), and antibody-positive reactors have been found in this animal species. Occasionally, mixed infection with DNA- and RNA-containing viruses have been obtained from the same culture derived from the same animal; and these will be discussed in Mixed Infections.

GUINEA PIG HERPESVIRUSES: INFECTION IN VITRO

At least two well-characterized herpesviruses, GPCMV and GPHLV, are commonly harbored by guinea pigs; therefore, it is important for investigators to recognize their presence and to have some means for differentiating them. In the following paragraphs, the growth characteristics of these two guinea pig herpesviruses in cell cultures are compared with regard to cytopathology and ultrastructural development, as well as some recent studies on molecular virology.

Cytopathology and Growth Rate in Cell Culture

The growth rates of GPCMV and GPHLV in guinea pig embryo (GPE) and guinea pig kidney (GPK) cells were compared by Hsiung et al. (44) (Fig. 1). There was no evidence of cytopathic effect or significant numbers of intranuclear inclusions in GPK cells infected with GPCMV, although the virus persisted in the GPK cells for 8 to 10 days with low infectivity titers. As determined by both cytopathic effect and nuclear

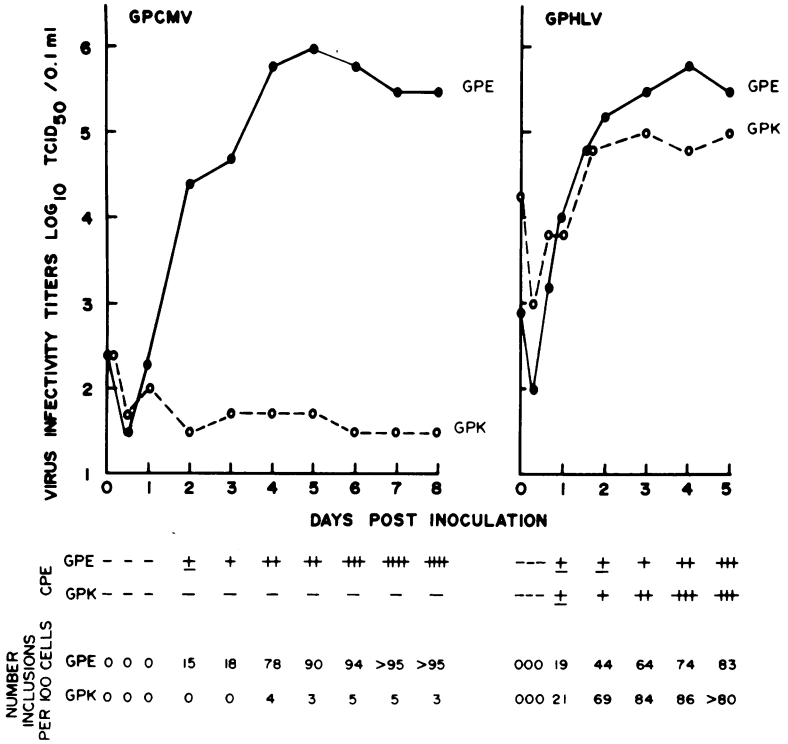

FIG. 1. *Growth curves of GPCMV and GPHLV in GPE and GPK cell cultures. (Note: the number of inclusion-bearing cells in GPCMV-infected GPK cells was very small.) Abbreviations: TCID50, 50% tissue culture infective dose; CPE, cytopathic effect. Reproduced from reference 44 with permission.*

inclusion counts, GPHLV was found to replicate somewhat more rapidly than GPCMV in GPE cells. Although both viruses show narrow host spectra, rabbit kidney cells are susceptible to GPHLV (42), but not to GPCMV (44).

Ultrastructural Development in Infected Cells

Certain distinct differences are apparent in guinea pig cells infected with either GPCMV or GPHLV when examined by electron microscope (Fig. 2). In GPCMV-infected cells, numerous tubular structures are seen within the nuclear inclusions (24, 57), but are not found in the GPHLV-infected guinea pig cells. On the other hand, clusters of enveloped virus particles enclosed in large vacuoles are often seen in GPHLV-infected nuclei (27), but rarely found in GPCMV-infected GPE cells. Only mature enveloped virus particles are found extracellularly when cells are infected with GPHLV, but both dense bodies and virus particles are commonly seen with GPCMV-infected GPE cells; the vast majority of extracellular particles consist of dense bodies (24).

Molecular and Biochemical Analysis

Properties of guinea pig herpes-like virus deoxyribonucleic acid. In early studies Nayak reported that GPHLV had a DNA buoyant density of 1.716 g/cm^3 (60). More recently, Huang showed two major populations of GPHLV DNA prepared from noncloned-virus-infected culture (unpublished data). Approximately 80% of viral DNA, with a density of 1.716 g/cm^3, contained only a portion of the viral genome (Fig. 3, samples A_1, A_2, and B_1), and 20% of the DNA, with density of 1.705 g/cm^3, contained the whole genome sequence (Fig. 3A_3 and B_2). These results suggest defectiveness of GPHLV grown in GPE cells. In contrast, 80% of viral DNA obtained from cells infected with cloned virus at low multiplicity (0.1 to 0.2) was intact (Fig. 4, clone 1, fraction C, and clone 2, fraction C), whereas the other 20% was defective, with great repetition (Fig. 4, clone 1, fractions A and B, clone 2, fraction B). These repetitive sequences were detected even at low multiplicity of infection immediately after cloning.

Lack of genetic relatedness between guinea pig cytomegalovirus and guinea pig

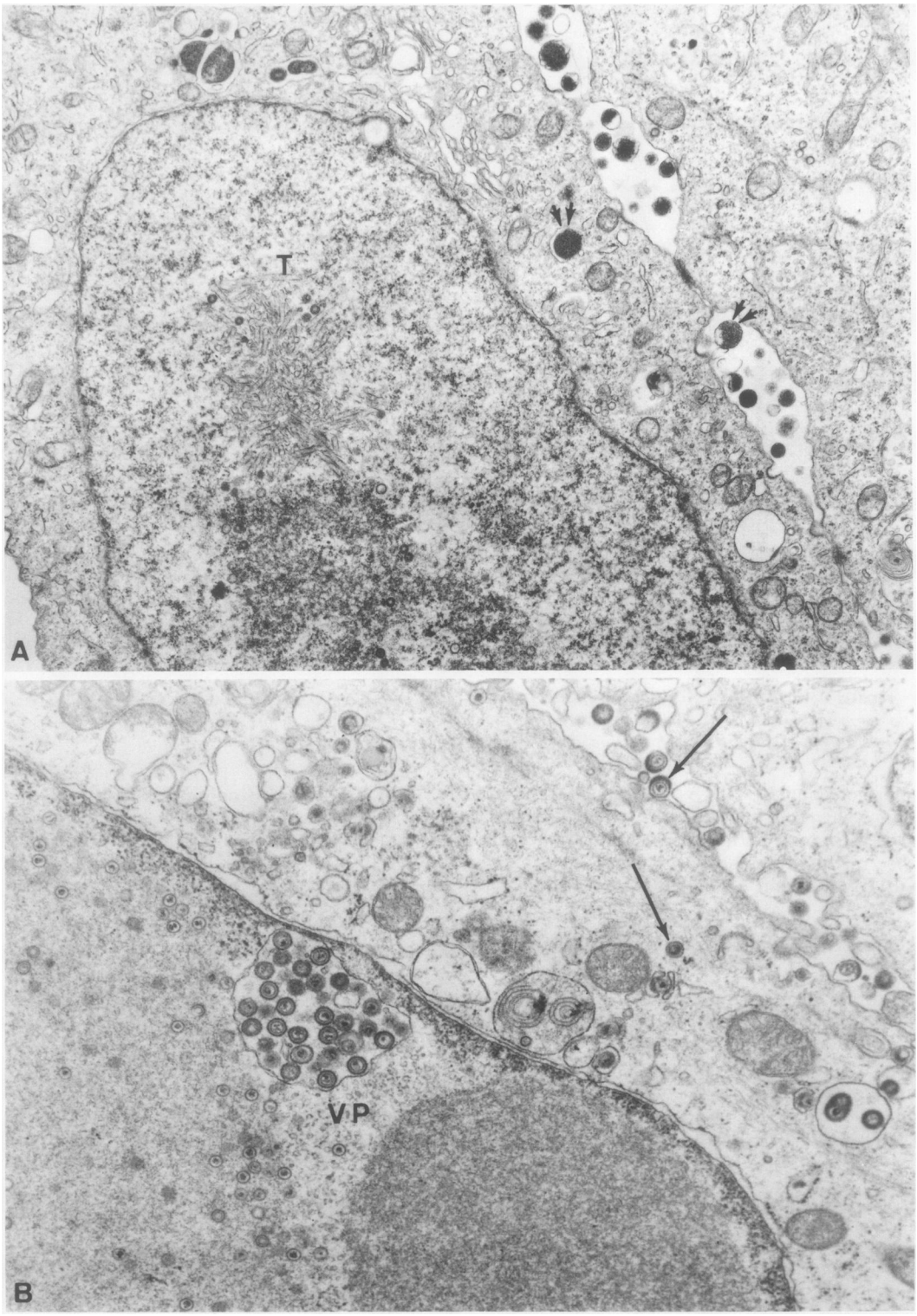

FIG. 2. *Electron micrographs of guinea pig herpesvirus-infected cells. (A) GPCMV-infected GPE cell; note intranuclear nucleocapsids, tubules (T), intracytoplasmic and extracellular dense bodies (double arrows), and virions (×13,400). (B) GPHLV-infected GPK cell; note intranuclear nucleocapsids, viral package (VP) containing enveloped virions, and intracytoplasmic and extracellular virus particles (single arrows) (×16,800). (Modified from reference 44.)*

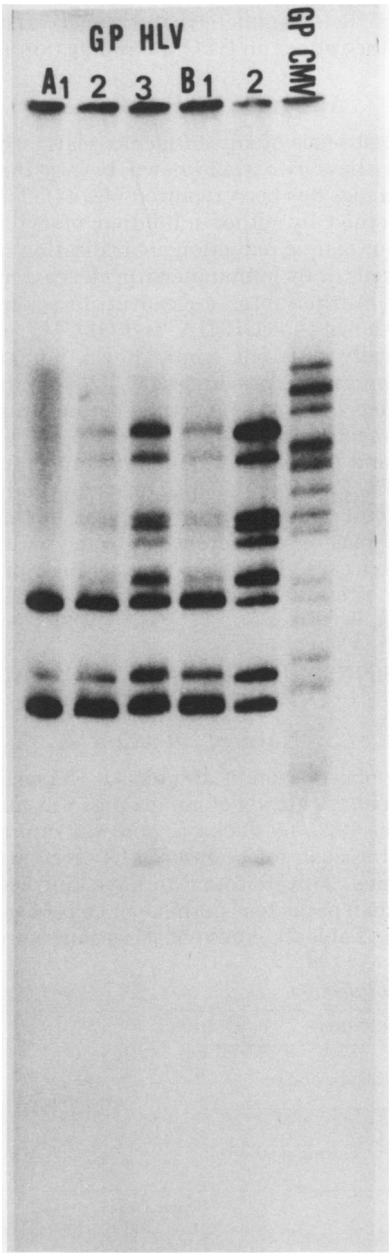

FIG. 3. *Restriction endonuclease analysis of GPHLV and GPCMV DNA. Purified* ^{32}*P-labeled GPHLV and GPCMV DNAs (approximately* 2×10^4 *cpm per gel) were digested with restriction enzyme XbaI at 37°C for 2 h and then subjected to 1% slab gel electrophoresis. Kodak X-omat R film was used for the autoradiograph. DNA samples A and B of GPHLV were obtained from two separate experiments.* A_1, A_2, *and* B_1 *had a density of 1.716;* A_3 *and* B_2 *had a density of 1.705. (Courtesy of E. S. Huang.)*

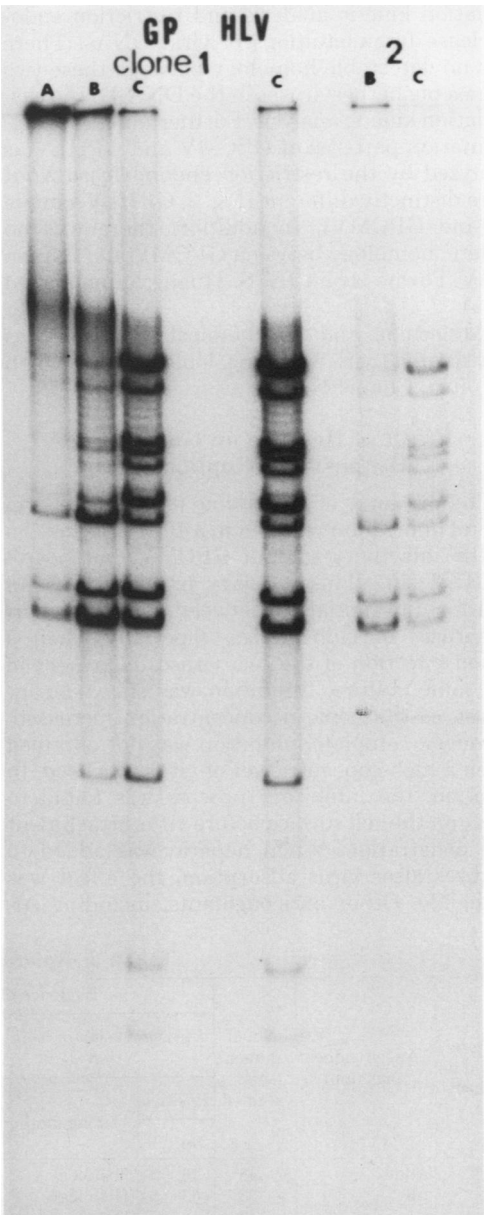

FIG. 4. *Restriction fragment patterns of GPHLV DNAs obtained from two purified cloned virus stocks. DNAs used in gels for fractions A and B of clone 1 and the gel for fraction B of clone 2 each had a density of 1.716, and DNAs used in the gels for fraction C of clone 1 and fraction C of clone 2 each had a density of 1.705; center C is a duplication of fraction C of clone 1. (Courtesy of E. S. Huang.)*

herpes-like virus. The relatedness of GPCMV and GPHLV was examined by DNA-DNA reassociation kinetic analysis and restriction endonuclease fragmentations of viral DNAs. There was no detectable homology between these two guinea pig herpesviruses in the DNA-DNA reassociation kinetic analysis. Furthermore, the fragmentation patterns of GPCMV and GPHLV as analyzed by the restriction endonuclease *Xba*I were distinctly different (Fig. 3, GPHLV sample B_2 and GPCMV). In addition, there was no genetic homology between GPCMV and human CMV Towne strain (E. S. Huang, unpublished data).

(Molecular and Biochemical Analysis was contributed by E. S. Huang, University of North Carolina, Chapel Hill.)

Effect of Heparin on Guinea Pig Herpesvirus Replication

The presence of as little as 0.1 U of heparin per ml of medium resulted in a 10-fold reduction in the infectivity titer of GPCMV, but not of GPHLV (10). This property provides a useful tool for differentiating between these two herpesviruses of guinea pigs, especially when a mixed infection of the two viruses is present in the same culture. Inhibition was shown to increase as the heparin concentration increased. However, complete inhibition was not obtained when a high concentration of virus was used. In addition, the inhibitory process was found to occur on the cell surface before virus attachment and penetration. When heparin was added to cultures after virus adsorption, the effect was negligible. Other anticoagulants, including Alsever solution (sodium citrate, 3.8% in saline) and ethylenediaminetetraacetic acid, exerted no inhibitory effect on GPCMV replication (10).

Antigenic Distinctiveness

The absence of any antigenic relationship between these two well-known herpesviruses of guinea pigs has been reported (42, 44). This was determined by either inhibition of cytopathic effect or plaque reduction neutralization tests in GPE cells or by immunoferritin electron microscopy. Furthermore, cross-neutralization tests have shown that GPHLV and GPCMV are antigenically different from known human and other animal herpesviruses (30, 42, 44), although there has been one report indicating a one-way cross-reaction between human herpes simplex virus and GPHLV by the complement fixation test (65). Rabbits are better hosts for production of specific antiserum to each virus type, since guinea pigs may be latently infected with other herpesviruses. However, guinea pigs produce higher titers of antibody to GPCMV than do rabbits.

GUINEA PIG CYTOMEGALOVIRUS: INFECTION IN VIVO

Natural Infection

The observation of an unusual change in the submaxillary glands of guinea pigs was made as early as 1920, by Jackson, and was interpreted as a protozoan infection (47). Of 48 guinea pigs examined, 26 were found to have "intracellular protozoan parasites" in the duct cells of salivary glands (Table 2). These observations were con-

TABLE 2. *Natural GPCMV infection*

Date reported or studied	Age of guinea pigs (mo)	No. of guinea pigs studied	Evidence of GPCMV infection						Reference
			Virus inclusion or isolation			Antibody studies			
			Positive		Testing method	Positive		Testing method[a]	
			No.	%		No.	%		
1920	Adult	48	26	54	Histology	ND[b]			Jackson (47)
1926	>6	75	63	84	Histology	ND			Cole and Kuttner (11) and Kuttner (50)
	<1	43	3	7	Histology	ND			
1930	Adult	19	6	32	Histology	ND			Andrews (2)
1957	5	39	3	8	Histology	NK[c]	58	NT (CPE)	Hartley (30)
1959	1-8	NK	NK	33	Histology	NK	38	CF	Smith (71)
1974	Adult	50	7	14	Histology	ND			Doisi and Georgescu (18)
1975-1979	2-4 (Hartley)	204	6	2	Virus isolation	15	25	NT (CPE or PFU)	Hsiung et al. (37), Choi and Hsiung (9), Griffith and Hsiung (28a)
1976-1979	2-4 (strain 2)	133	0	0	Virus isolation	0	0	NT (CPE)	Bia et al. (4)

[a] NT, neutralization test either by inhibition of cytopathic effect (CPE) or by plaque reduction (PFU) (neutralizing antibody titers of 1:5 or greater were considered positive); CF, complement fixation test.

[b] ND, Not done.

[c] NK, Not known.

firmed by Cole and Kuttner 6 years later, at which time they noted that 84% of full-grown guinea pigs showed swollen epithelial cells in the ducts of submaxillary glands but that only 7% of guinea pigs under 1 month old showed these changes (11, 50). The latter investigators further demonstrated that a filterable virus, the salivary gland virus or CMV, was responsible for the atypical cells with nuclear inclusions seen in salivary gland duct cells of guinea pigs. Ever since these early observations, typical intranuclear and intracytoplasmic inclusions within the duct cells of the salivary glands (Fig. 5A and B) have been considered reliable indications of an animal's being infected (2, 30, 71). Under electron microscopic examination, the intranuclear inclusions contained immature virus particles and intracytoplasmic inclusions contained numerous mature virus particles (Fig. 5C). More recently, we have found that animals showed maximum numbers of inclusions at 3 to 4 weeks after primary infection with salivary gland-passaged virus (23). Thereafter, the numbers of inclusions decreased significantly, although virus infectivity titers persisted for 30 weeks. During 1975 to 1979 a longitudinal study was undertaken in order to learn the incidence of naturally occurring GPCMV infection among commercially available animals (Table 2). GPCMV was isolated from the salivary glands of 6 animals in a total of 204 Hartley strain guinea pigs examined during the 5 years of surveillance, although low titers of GPCMV-neutralizing antibody were observed in 25% of the animals tested. The percentages of antibody-positive animals obtained from different sources varied from shipment to shipment, with as few as 8% at one time and as many as 50% at another time. Occasionally, guinea pigs in certain shipments showed no evidence of prior GPCMV infection. During the same time period a total of 133 strain 2 guinea pigs were tested; none showed antibody to GPCMV before inoculation, and they were highly susceptible to infection (4).

Experimental Infection

Viremia during acute primary infection. Guinea pigs without preexisting antibody show virus in their blood 2 to 14 days after intraperitoneal inoculation with GPCMV; occasionally viruria is detected as well. The viremic stage is brief but reproducible (37). Infectious virus is also recovered from the spleen, kidneys, and lungs of infected animals during the first 2 weeks of infection (Table 3). Virus recovery from the salivary gland and pancreas commences later than that from other tissues during primary infection and is followed within 14 days post-inoculation by detectable titers of neutralizing antibody.

Viruria during chronic persistent infection. Once guinea pigs have been infected with GPCMV, chronic persistent infection is readily established. In animals sacrificed from 3 to 10 weeks after inoculation, high levels of infectious virus have been consistently recovered from the salivary glands and pancreas despite the presence of high levels of circulating neutralizing antibody. It was apparent from the experiments of Hsiung et al. (37) that as antibody titers increased, virus was generally not recovered from tissues other than the pancreas and salivary glands 11 weeks post-inoculation (Table 3). However, virus has been recovered from the urine of some animals, especially inbred strain 2 guinea pigs, as late as 16 weeks post-inoculation (4). Furthermore, female strain 2 guinea pigs appear to excrete GPCMV in urine more often than do male guinea pigs (4).

Mode of transmission. (i) Transplacental transmission. Transplacental transmission of GPCMV has been demonstrated in guinea pigs during acute primary maternal infection, i.e., 5 to 24 days post-inoculation (Table 4). In studies by Hsiung and co-workers, infectious virus was recovered from 27 of 44 placental tissues and 9 of 37 fetal tissues, including brain, lungs, and kidneys, tested after initiation of maternal infection and independent of stage of gestation (9, 28a). Infectious virus was also isolated from cervical swabs of the infected mothers (28a), No virus was detectable in the tissues of 43 fetuses taken from female guinea pigs which were infected for 40 or more days (Table 4). Animals in the latter groups showed significant levels of circulating antibody, concurrent with infectious virus in the maternal salivary glands, typical of chronic persistent infection. Newborn guinea pigs whose mothers had been inoculated more than 30 days before delivery of the neonates, when tested at birth, showed levels of antibody comparable to maternal antibody levels, which declined significantly 1 to 2 months after birth (28a). Transplacental transmission of GPCMV was also reported recently by other investigators (48, 49).

(ii) Contact infection. Several experiments were undertaken by Choi and Hsiung to determine the degree to which GPCMV is spread by animal-to-animal contact (9). Among uninoculated guinea pigs that were housed with inoculated animals of the same sex, 4 of 13 contactees exhibited rises in antibody titer 2 to 3 months after contact, and infectious virus was recovered from the salivary gland of 1. When uninoculated guinea pigs were housed in pairs with inoculated

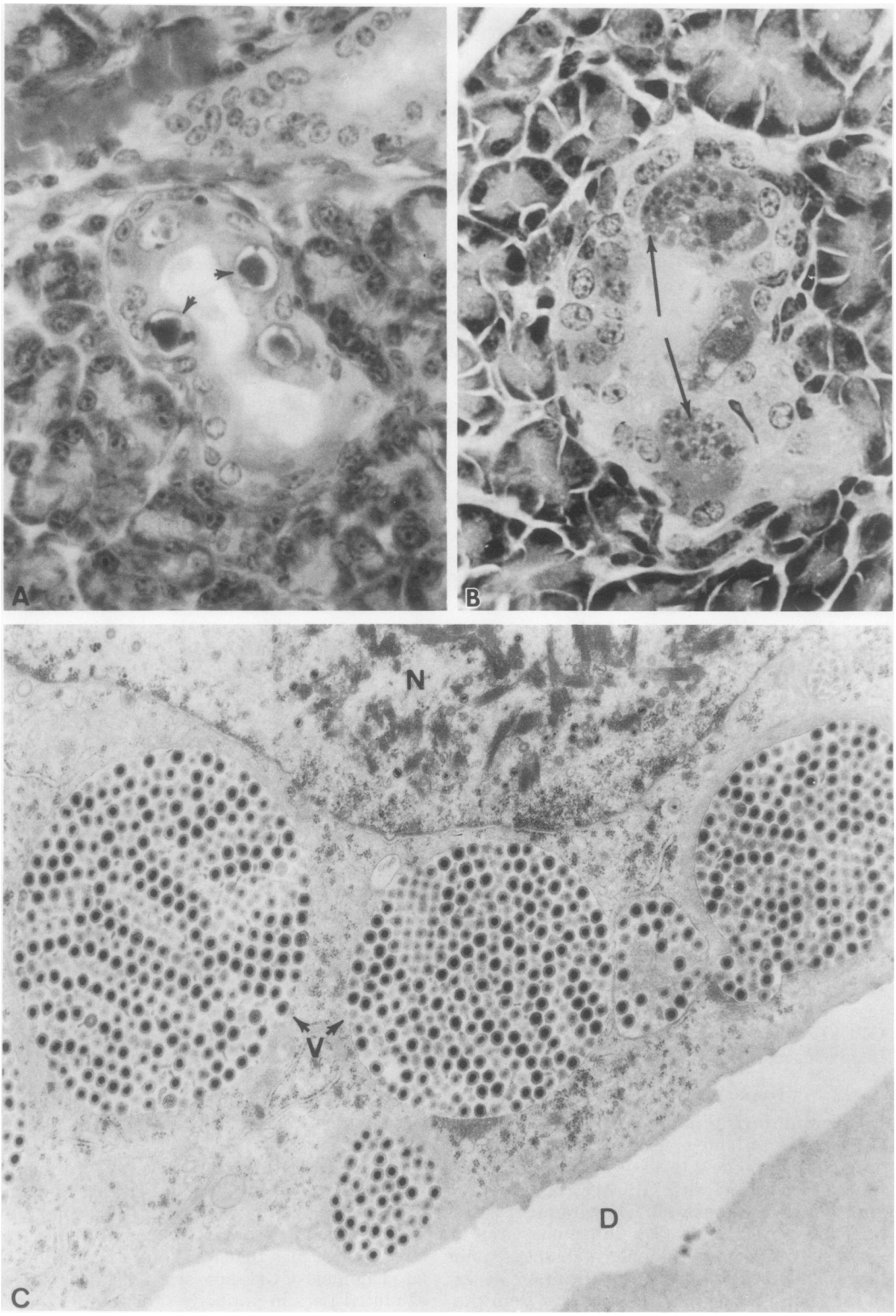

FIG. 5. *Intranuclear and intracytoplasmic inclusions induced by GPCMV in the duct cells of guinea pig salivary glands. (A and B) Hematoxylin-eosin-stained preparations (×400) of intranuclear inclusions (short arrows) and intracytoplasmic inclusions (long arrows) (modified from reference 72). (C) Electron micrograph (×13,400), showing viral nucleocapsids in the nucleus (N) and vacuoles (V) containing numerous mature virions in the cytoplasm (modified from reference 23).*

TABLE 3. *Distribution of GPCMV in the blood, urine, and various tissues during acute and chronic infection after intraperitoneal inoculation of Hartley guinea pigs with tissue culture-passaged virus stock*[a]

Wk post-inoculation	No. of animals showing GPCMV infection/no. examined in:						
	Blood	Urine	Kidney	Lung	Spleen	Pancreas	Salivary gland
1-2	18/23	2/16	10/14	10/11	9/14	6/11	16/23
3-10	1/12	0/2	1/7	1/5	2/7	3/3	12/12
11-37	0/16	0/11	0/15	0/13	0/15	4/9	11/16

[a] Modified from reference 37.

TABLE 4. *Transplacental transmission of GPCMV in experimentally infected guinea pigs*[a]

Virus inoculation	No. of days after virus inoculation	Mothers			Fetuses		
		No. studied	No. showing GPCMV in salivary gland	Antibody titer range[b] at sacrifice	No. studied	GPCMV in placenta[c]	GPCMV in fetal tissues[c]
During last 20 days of gestation	5-10	7	4	5-40	23	16/23	5/17
During first 10 days of gestation	15-24	6	6	10-20	23	11/21	4/20
On the day of conception or 5 to 10 days before conception	40-70	6	6	40-160	16	0	0
50 to 60 days before conception	90-150	8	7	80-640	27	0	0

[a] Modified from reference 9.
[b] Reciprocal dilutions of serum.
[c] Number that showed virus/number studied; fetal tissues include brain, lungs, and kidneys.

animals of the opposite sex, rises in antibody were observed in seven of seven contactees, and infectious virus was recovered from the salivary glands of five of the seven. Since five of the seven females became pregnant, sexual contact in the latter experiment can be assumed. This suggests that sexual contact is a more efficient means of spreading GPCMV than is environmental contact.

GUINEA PIG HERPES-LIKE VIRUS: INFECTION IN VIVO

Natural Occurrence

Inbred versus random-bred strains and age variations. Although GPHLV was initially isolated from a spontaneously degenerated kidney cell culture derived from a leukemic strain 2 guinea pig (41), subsequent study revealed that nonleukemic strain 2 guinea pigs also harbor the same virus in their blood and various organs (42). It should be noted that isolations of GPHLV from blood (leukocytes) or tissues of infected animals were accomplished only by cultivation of tissue cells or cocultivation with susceptible cell cultures (6, 42, 72). Infectious virus could not be isolated from cell-free tissue extracts obtained from infected animals or from infected tissues exposed to freezing and thawing before virus isolation attempts (42).

Guinea pigs, particularly older animals from inbred strains, show widespread infection with GPHLV. Hsiung found that inbred strain 2 and strain 13 guinea pigs, 6 to 12 months old, obtained from several different sources, consistently showed a high percentage of GPHLV infection, whereas relatively few random-bred Hartley strain guinea pigs exhibited natural GPHLV infection (Table 5) (34). However, later

TABLE 5. *Natural GPHLV infection in different guinea pig strains*[a]

Guinea pig strain	Age at time tested (mo)	No. studied	GPHLV isolation[b]	
			No.	%
Strain 2 (inbred)	<6	41	9	22
	6-12	35	28	80
Strain 13 (inbred)	<6	6	0	0
	6-12	6	6	100
Hartley (random bred)	<6	474	21	4
	6-12	17	3	17

[a] Reproduced from reference 34 with permission.
[b] Virus isolations were made from leukocytes or spleen tissues by cocultivation.

studies showed that hybrids derived from mating strain 2 with Hartley guinea pigs showed an infection rate comparable to that observed in the inbred strain 2 animals (Hsiung, unpublished data). Detailed study revealed that in strain 2 and hybrid guinea pigs tested at monthly intervals, GPHLV infection was evident in these guinea pigs by age 5 to 6 months or older (38). Natural GPHLV infection was present in almost all inbred strain 2 and strain 13 guinea pigs by age 10 to 12 months; virus was distributed widely in the blood and various tissues, but spleens always showed the highest virus content. In one instance, virus was recovered from the fetal lung tissues of a naturally infected strain 2 guinea pig, suggesting that transplacental transmission of this virus had occurred during natural infection (51).

Experimental Infection

Pathogenicity and latency. Hsiung and coworkers (6, 42, 51, 72) found that in experimentally infected Hartley guinea pigs, GPHLV could be recovered from a wide variety of tissues, including leukocytes, bone marrow, spleen, liver, lungs, kidneys, salivary glands, brain, etc. However, the highest titers of virus were always recovered from the spleen, regardless of route of inoculation. Figure 6B shows the distribution of GPHLV in the blood, spleen, and other tissues of guinea pigs during acute and chronic infection after intraperitoneal inoculation (72). Virus titers increased rapidly during the first 2 weeks of infection and reached the highest titer at week 3. Thereafter, significant virus titers persisted throughout the animal's life. No disease attributable to the virus was found, nor were any virus-induced intracellular inclusions noted in the infected tissues. As indicated above, recovery of GPHLV from infected guinea pigs requires cultivation or cocultivation of infected tissue cells (6, 42, 72). Neutralizing antibody, however, was detectable at about 2 weeks after inoculation and persisted at low levels throughout the test period of 12 to 18 months. Once GPHLV infection has been established, the virus can be recovered from the blood of the infected guinea pig throughout its lifetime.

Lam and Hsiung showed that experimental infection of rabbits with GPHLV revealed prompt antibody response regardless of route of inoculation (52). Virus persistence in rabbit tissues was difficult to demonstrate, although infectious virus could be recovered readily before the development of antibody. Rats and mice inoculated with GPHLV produced minimal, if any, antibody response, and no virus could be

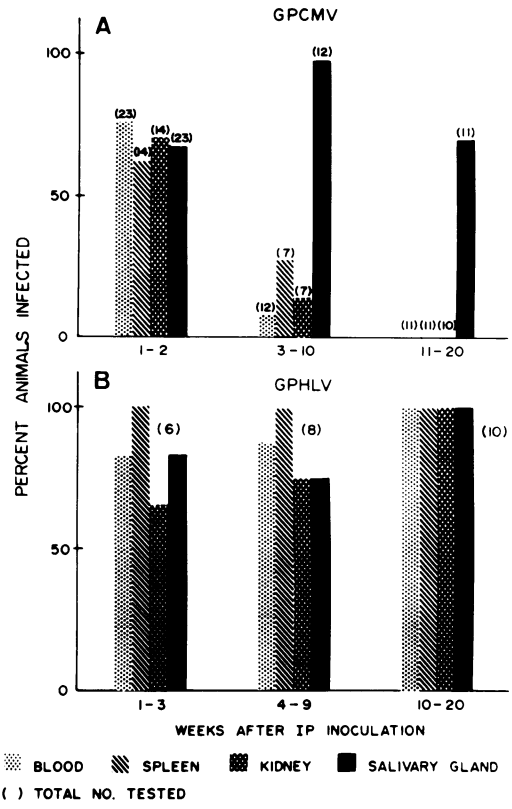

FIG. 6. *Distribution of GPCMV and GPHLV in different organs of infected guinea pigs 1 to 20 weeks after intraperitoneal (IP) inoculation. (Modified from reference 72.)*

recovered from the inoculated animals. Studies on experimentally infected hamsters have indicated that these animals are also nonpermissive hosts for GPHLV infection (Michalski and Hsiung, unpublished data).

Transplacental transmission. Since GPHLV was most commonly isolated from inbred guinea pigs, the question arose as to whether or not the virus was capable of passing through the placenta and being transmitted to the offspring of experimentally infected animals. Lam and Hsiung (51) inoculated female Hartley guinea pigs with GPHLV at various times during gestation, and their fetuses were examined for virus infection. Representative examples of GPHLV transplacental transmission after intraperitoneal inoculations are shown in Table 6. All virus isolations were made by cocultivation of infected tissues with GPK cells (51). The isolation of virus from the fetuses seemed to be related to the titer of virus the pregnant guinea pigs received and the duration of infection in the

TABLE 6. *Distribution of GPHLV in maternal and fetal tissues after experimental infection of Hartley guinea pigs*[a]

Guinea pig no.	Dose inoculated (log 50% tissue culture infective doses)	Days post-inoculation	Virus isolation by cocultivation[b]										Estimated fetal age (days)
			Maternal tissue						Fetal tissue				
			WBC	Sp	K	SG	Th	Pl	Lu	Sp	Th	Cord	
LA 23	5.3	88	+	+	+	−	+	+	−	+	+	ND[c]	50
LA 27	5.3	141	+	+	+	−	−	ND	Whole embryo			+	15
LA 94	5.3	119	+	+	+	+	+	+	+	−	−	+	50
LA 53	6.3	43	+	+	+	−	+	+	+	+	−	ND	60–70
LA 56	6.3	51	+	+	+	−	−	+	+	−	−	ND	60–70

[a] Modified from reference 51.

[b] Tissues: WBC, leukocytes; Sp, spleen, K, kidney; SG, salivary gland; Th, thymus; Pl, placenta; Lu, lung; Cord, umbilical cord. Symbols: +, virus isolated; −, virus not isolated.

[c] ND, Not done.

animals. It was noted that when animals received 5.3 log 50% tissue culture infective doses of virus suspension, their fetuses showed virus only when the mothers were infected for 88 days or longer. When animals received 6.3 log 50% tissue culture infective doses of virus suspension, infectious virus was isolated from their fetuses 43 to 51 days post-inoculation. Thus, animals receiving a smaller dose of virus required a longer interval for the virus to get established in the fetuses, whereas in animals receiving a larger dose of virus, infection occurred in the fetuses after a shorter period of time. This contrasts with GPCMV transplacental transmission, in which infectious virus was not recovered in the fetuses when maternal infection was longer than 40 days (Table 4). In the latter case, antibody appeared to play a role in the control of fetal infection (28a).

Oncogenicity: cell transformation and induction of tumors. Fong and Hsiung found that GPHLV strains isolated from both leukemic and nonleukemic guinea pigs were capable of transforming hamster embryo cells in culture (25). The virus strains isolated from leukemic strain 2 guinea pigs showed a somewhat higher transforming capacity than that of the virus isolates obtained from nonleukemic Hartley strain guinea pigs. Infectious GPHLV was recovered from the transformed hamster cells by cocultivation of several cell lines tested; however, the number of infected cells was small. Michalski et al. found that inoculation of hamsters with one of these transformed cell lines which had undergone repeated passages in cultures led to the development of tumors characterized as angioid sarcomas and fibrosarcomas (56). However, inoculation of GPHLV directly into hamsters did not induce tumor production. More recently, malignant transformation of rat embryo cells by GPHLV has been reported (65).

GUINEA PIG HERPESVIRUSES: ANIMAL MODELS FOR HUMAN HERPESVIRUS INFECTION

Comparison of Guinea Pig Herpes-Like Virus and Guinea Pig Cytomegalovirus In Vivo Pathogenicity

The pathogenesis and distribution of GPHLV are significantly different from those of GPCMV infection (Fig. 6). After experimental infection of guinea pigs with GPCMV, viremia is very brief, but the virus is consistently isolated from the salivary gland 2 weeks post-inoculation and thereafter (4, 12, 37). GPCMV is rarely found in the blood 4 weeks after primary acute infection. However in GPHLV-infected guinea pigs, virus is recovered in the blood immediately after inoculation and persists thereafter in the leukocytes and all other tissues that have been tested, including spleen, kidneys, and salivary glands, although recovery of GPHLV from tissues requires cocultivation techniques (6, 42, 72). Whereas intranuclear inclusions and virus particles have been observed in the duct cells of salivary gland tissue from guinea pigs that have received GPCMV-infected salivary gland tissue suspension (Fig. 5A and B), no evidence of GPHLV inclusions has been found in any tissues of GPHLV-infected animals. Guinea pigs inoculated with GPCMV promptly produce high titers of specific neutralizing antibody to the homologous virus; those inoculated with GPHLV develop lifelong latent infection of leukocytes accompanied by minimal levels of neu-

tralizing antibody to the virus. Although we have been able to isolate GPCMV from urine of GPCMV-infected guinea pigs, to date there have been no isolations of GPHLV from the same source (4).

Similarities Between Guinea Pig Herpes-Like Virus and Human Epstein-Barr Virus

One of the most interesting features of GPHLV is its interaction with host cells in vivo and in vitro. Leukocytes taken from infected guinea pigs show no intracellular viral antigen by immunofluorescence. Intranuclear inclusions and virus particles are not found by either light or electron microscopy (40). Nevertheless, the viral genome is present in these leukocytes, since virus-induced cytopathic effect and viral inclusions have been demonstrated after cocultivation with susceptible cells and herpesvirus particles were found after cultivation of the leukocytes. Guinea pig leukocytes carrying GPHLV resemble human leukocytes carrying Epstein-Barr herpesvirus, both showing the absence of viral antigens in vivo and their prompt appearance after in vitro cultivation. Table 7 compares some of the similarities between these two herpesviruses (35). In each instance there was an association with B lymphocytes and the neoplastic disease, but, as yet, neither virus has been shown definitively to be the causative agent in the development of the neoplastic disease in its respective host. Although GPHLV can be isolated from leukocytes by cocultivation with GPK or GPE cell monolayers, lymphoblastoid cell lines carrying GPHLV genomes have not been established (40). It is possible that GPHLV is a more lytic virus than Epstein-Barr virus when cultured in vitro.

TABLE 7. *Similarities between GPHLV and human Epstein-Barr herpesvirus (EBV)*[a]

Characteristic of infection	Virus	
	GPHLV	EBV
Natural host	Guinea pig	Human
Nature of infection	Latent	Latent
Primary site	Leukocyte	Leukocyte
Transforming capacity in vitro		
Heterologous species	+	+
Homologous species	−	+
Oncogenic potential in vivo		
Disease associated	L_2C leukemia	Burkitt's lymphoma
Cell type associated with disease (or proliferation)	B-lymphocytes	B-lymphocytes

[a] Reproduced from reference 35, with permission.

TABLE 8. *Similarities between GPCMV and human CMV*[a]

Manifestation of infection	Virus	
	GPCMV	Human CMV
Infection in vitro		
Nuclear inclusions	+	+
Mature virus particles and dense bodies	+	+
Infection in vivo		
Acute infection: viremia	+	+
Chronic infection: viruria	+	+
Congenital infection and transplacental transmission	+	+
Histopathology: intranuclear and intracytoplasmic inclusions in salivary glands	+	+

[a] Summary taken from references 4, 9, 23, 24, 28a, 48, and 49.

Comparison of Guinea Pig Cytomegalovirus and Human Cytomegalovirus

In many respects the pathogenesis of GPCMV infection in guinea pigs closely simulates that of CMV in humans. Since CMVs of humans and animals are relatively species specific, guinea pigs infected with GPCMV provide an excellent animal model for human CMV infection. Table 8 lists the similarities between GPCMV and human CMV infections, in their respective hosts. In ultrastructural studies on development of GPCMV both in vivo (23) and in vitro (24), surprising similarities have been noted between GPCMV in guinea pig cells and human CMV in infected human cells.

A most important finding has been the demonstration of transplacental transmission of GPCMV in guinea pigs (9, 28a, 48, 49). In contrast to the mouse system, the guinea pig possesses a placenta with a single trophoblast layer, similar in structure to the human placenta (19), which may allow the passage of virus from mother to fetuses. It has been found that transplacental transmission of GPCMV occurs in guinea pigs regardless of the time of gestation at infection, but it depends upon the duration of maternal infection (9, 28a). More recently, GPCMV congenital infection with brain damage has been demonstrated in newborn guinea pigs from which infectious virus has not been isolated (28a). Thus, GPCMV-infected guinea pig fetuses have provided an excellent model for studies of human congenital CMV infection.

PARAMYXOVIRUSES

Naturally Occurring Infection

Guinea pig sera chosen at random have dis-

played an irregular but broad spectrum of previously acquired antibodies to several parainfluenza viruses (Tables 9 and 10). For example, Hsiung et al. found that random samples of lyophilized complement, obtained from various commercial sources of pooled guinea pig sera, showed significant levels of antibody to parainfluenza type 5 (DA) virus and mumps virus in all lots tested and low levels of antibody to parainfluenza types 1 and 3 in certain lots (36). Among the various individual guinea pig sera tested, the results varied from shipment to shipment. In general, most of the guinea pigs showed parainfluenza type 5 antibody titers when they reached age 4 to 5 months. Interestingly, some of them also had hemagglutination inhibition antibody to mumps virus and to a lesser degree to parainfluenza type 2 and parainfluenza type 3, but only occasionally to parainfluenza type 1. There was no difference in antibody titers in sera obtained from different strains of guinea pigs.

We were unable to find reports in the literature of isolation of parainfluenza viruses from guinea pigs, except for one report of parainfluenza type 1 (Sendai) virus isolated from guinea pigs when they were housed with virus-contaminated mice (74). More recently, a parainfluenza virus strain serologically identical to SV_5 has been isolated from the salivary gland of a Hartley guinea pig simultaneously infected with GPCMV (F. J. Bia, unpublished data). Since most guinea pigs have demonstrated significant preexisting levels of antibody to parainfluenza type 5 virus, it is possible that the chances of isolating parainfluenza type 5 virus are limited. In a study on comparison of cell susceptibilities to parainfluenza virus infection, it was noted that GPE cells were more susceptible to parainfluenza type 5 than all the other parainfluenza virus types tested (M. L. Landry, unpublished data). SV_5, a strain of parainfluenza type 5 virus, was originally isolated from monkey kidney cell cultures (46). It was not clear whether the monkeys actually contracted parainfluenza type 5 virus from humans or other animal species, including guinea pigs, after they were kept in captivity (32).

Antibody Response After Experimental Infection

In studies by Hsiung et al., guinea pigs showing no detectable preexisting antibody to parainfluenza type 5 virus often showed a high incidence of heterotypic antibody rise when inoculated with one of the other parainfluenza virus types (36). It was possible that a number of these animals had been infected previously with parainfluenza type 5, but did not possess antibodies at a level measurable by the techniques used. In the early 1960s it was found that antisera prepared in guinea pigs against parainfluenza virus type 1, 2, or 3 occasionally contained a common antibody, i.e., to parainfluenza type 5 virus. This common antibody in antisera prepared in this animal species has caused considerable confusion in identification of the parainfluenza viruses isolated from clinical specimens (75). In addition, guinea pigs and hamsters possessing antibody to parainfluenza type 5 virus were found to be resistant to experimental infection with this virus (7).

In studies of the antigenic properties of the DA strain of parainfluenza type 5 in guinea pigs, hamsters, and rabbits, it was noted that both homologous and heterologous antibody responses were demonstrated after immunization with the virus (36). The extent of heterologous antibody rise was found to be dependent upon the degree of susceptibility of a specific animal species and upon the presence or absence of preexisting antibody to members of the parainfluenza-mumps virus group (36). Parainfluenza type 5 (DA) virus and mumps virus could conceivably be considered antigenic variants of the same virus on the basis of data obtained from sera taken from immunized guinea pigs (Fig. 7). In contrast, no antigenic relationship could be demonstrated between parainfluenza type 5 and mumps viruses when hamsters were used for

TABLE 9. *Hemagglutination inhibition and neutralizing antibody titers to parainfluenza viruses in pooled sera (complement) of normal, healthy guinea pigs*[a]

Source of pooled sera	Total no. of pools tested	Hemagglutination inhibition antibody titer[b] to:					
		Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Parainfluenza 5	Mumps	Newcastle disease virus
Lab A	2	20	—	—	80 (20)	40	—
Lab B	4	—	—	40	40 (10)	40	—
Lab C	2	20	—	20	320 (40)	80	—

[a] Modified from reference 36.

[b] Reciprocal serum dilution. —, Titer less than 1:10. Numbers in parentheses are neutralizing antibody titers.

TABLE 10. *Hemagglutination inhibition antibody titers to parainfluenza viruses in single sera of normal, healthy guinea pigs*[a]

Source of individual animals	Total no. of single sera tested	% with hemagglutination inhibition antibody titer greater than 1:10					
		Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Parainfluenza 5	Mumps	Newcastle disease virus
Lab A	240	0	21	16	84	71	0
Lab B	140	5	30	10	70	48	0

[a] Modified from reference 36.

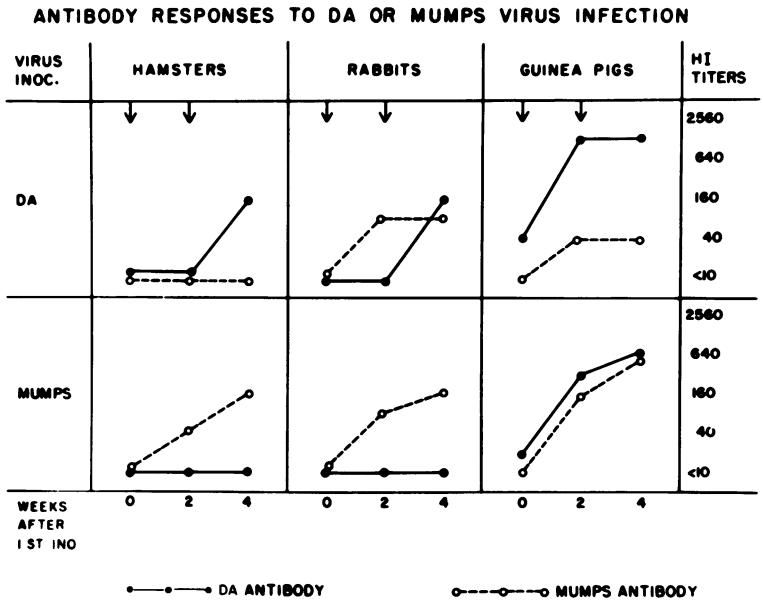

FIG. 7. *Antibody responses to parainfluenza type 5 (DA) or mumps virus in hamsters, rabbits, and guinea pigs as measured by hemagglutination inhibition (HI) test. Abbreviations: ino, inoc, inoculation. (Reproduced from reference 36, with permission.)*

immunization (36). Similarly, a one-way antigenic crossing between parainfluenza type 5 (SV5) virus and parainfluenza type 2 (CA) virus was demonstrated in antisera prepared in guinea pigs (8), whereas no antigenic relationship could be demonstrated between parainfluenza type 5 (SA) virus and parainfluenza type 2 (CA) virus when antisera prepared in hamsters were used (70). Since DA, SV_5, and SA are serologically identical, the name parainfluenza virus type 5 has been used (32). Thus, investigators should be aware of the possibility that prior infection with parainfluenza viruses, especially type 5, may have occurred in any guinea pig used for antiserum production.

RETROVIRUSES OF GUINEA PIGS

Nomenclature for the Guinea Pig Retroviruses

Since the original observation of virus-like particles in tissues and cells of L_2C leukemic guinea pigs that were absent in nonleukemic guinea pigs, the name "guinea pig leukemia virus" has been used in many of the early reports describing the virus particles observed (22, 59, 63). In 1972, Hsiung first demonstrated that a similar virus particle could be induced in cell culture, as had previously been shown with murine C-type virus; therefore, the name "guinea pig C-type virus" was used (33).

Morphogenically, the GPRV particles, especially those induced by BUdR, show some similarity to the B-type viruses of the murine species. Thus, the guinea pig virus has been classified with the murine B-type virus rather than the C type (15, 69). However, no interspecies-specific antigen has been demonstrated with the GPRV. Furthermore, the reverse transcriptase of the guinea pig retrovirus is magnesium dependent (55). Since GPRV is serologically distinct from the murine B-type virus as well as possessing several unique morphological char-

acteristics of its own, Hsiung has suggested that GPRV be placed in a separate category and be designated G type, representing guinea pig virus of the Retroviridae family (34). For the convenience of the present discussion, we shall use the term *GPRV*.

Observation of Virus Particles in Cells of L_2C Leukemic Guinea Pigs and Placental, Fetal, and Nerve Tissues of Normal Guinea Pigs

Electron microscopic studies by Fong and Hsiung (26, 27) revealed two morphologically distinct types of virus particles in various tissues of guinea pigs with L_2C leukemia, namely, intracisternal A-type particles 90 to 100 nm in diameter with electron-lucent centers and extracellular, "mature" virus-like particles approximately 90 to 110 nm in diameter with electron-dense cores. The former were usually formed by budding into the endoplasmic reticulum. The mature virus-like particles were always seen in the intercellular space, and a great number were found in the plasmas of leukemic guinea pigs (26, 27). In addition, a small number of intracytoplasmic A-type virus particles 80 nm diameter were also found in leukemic cells.

After an extensive search for virus particles in normal guinea pig tissues, intracisternal A-type particles were observed by Hsiung et al. in placental cells and in gonads of both male and female fetuses (39). Similar particles had been seen previously in the gonad cells of fetal guinea pigs (1, 5) as well as in germinal centers of normal guinea pigs (54). Virus particles were most prevalent in fetal tissues at approximately 30 to 40 days of gestation, decreasing in frequency in older fetuses and rarely found after birth. The intracytoplasmic A-type virus particles have been found in the spiral ganglion and trigeminal ganglion of normal guinea pigs (13, 73). These intracytoplasmic A-type virus particles observed in the ganglia of guinea pigs resemble the intracytoplasmic A-type particles observed in BUdR-induced virus particles in cultured guinea pig cells. Table 11 summarizes the distributions of the different types of GPRV under different conditions.

Induction of Guinea Pig Retrovirus in Cultured Cells

Since GPRV particles are seldom observed in normal adult guinea pig tissues, attempts have been made to induce the virus in cultured guinea pig cells. Hsiung et al. found that after exposure to BUdR (40 μg/ml of culture medium), both intracytoplasmic and extracellular GPRV particles were present in all primary cells derived from normal guinea pigs regardless of tissue origin or guinea pig strain (39) (Table 12). The same types of virus particles have also been observed in passaged normal and transformed

TABLE 11. *Schematic drawing illustrating the distribution of GPRV in tissues and cultured cells from leukemic and normal guinea pigs*[a]

DISTRIBUTION OF GUINEA PIG ONCORNAVIRUS IN VIVO AND IN VITRO

	INTRACELLULAR		BUDDING AT CELL MEMBRANE	EXTRACELLULAR	
	INTRACISTERNAL A	INTRACYTOPLASMIC A		ENVELOPED A	"MATURE PARTICLES"
LEUKEMIC GUINEA PIG:					
TISSUE	+++	+	−	RARE	+
PLASMA, SERUM	*	*	*	+	+++
NORMAL GUINEA PIG:					
FETAL TISSUE	+++	−	−	−	−
TRIGEMINAL GANGLION	−	+	−	RARE	−
PLASMA, SERUM and AMNIOTIC FLUID	*	*	*	−	RARE
CULTURED GUINEA PIG CELLS:					
WITH BrdU	−	++	+	+	+++
WITHOUT BrdU	−	−	−	−	−

[a] Symbols: +++, large numbers of single particles or groups, frequently found; ++, single particles and occasional clusters of particles; +, single particles, infrequently seen; −, not seen; *, absence of cells. BrdU, BUdR (Reproduced from reference 27, with permission).

TABLE 12. *Induction of GPRV in cultured guinea pig cells*[a]

Type of culture	Culture designation	Guinea pig strain	Tissue origin	Observation of GPRV in cell culture	
				Without BUdR	With BUdR
Primary cell	A	2	Adult spleen, kidney	–	++
	B	13	Adult spleen, kidney	–	++
	C	Hartley	Adult spleen, kidney	–	++
	AD023	2-Hartley hybrid	30-day-gestation fetus	–	+++
Passaged cell	106	2	7,12-Dimethylbenz[*a*]-transformed GPE cells	+	++
	74	2	3-Methylcholanthrene-transformed GPE cells	+	++
	LgpS	2-Hartley hybrid	Leukemic spleen cell line	–	++

[a] Reproduced from reference 34, with permission.

[b] –, No virus particles; +, a few virus particles; ++, numerous virus particles; +++, large aggregates of virus particles.

cell lines (21) derived from various guinea pig tissues after exposure to BUdR induction. Similar findings have been reported subsequently by many laboratories (15, 62, 67). Morphologically and biochemically, these extracellular virus particles induced in cell cultures derived from normal guinea pigs are similar to the virus-like particles in plasmas obtained from leukemic guinea pigs. However, repeated experiments to induce disease in guinea pigs with GPRV either derived from cultured cells or obtained from the plasmas of leukemic guinea pigs have been unsuccessful. As yet, there is no biological method for assaying the infectivity of GPRV, despite numerous studies by various investigators (26, 55, 62, 64).

Biochemical Studies of Guinea Pig Retrovirus

More recently, investigators have focused on the in vivo and in vitro expression of GPRV and the biochemical and antigenic characterization of its virions. The proviral DNA sequences of the BUdR-induced GPRV have been present in a relatively constant amount in all guinea pig cell DNAs examined so far, indicating that the GPRV is an endogenous virus system (55, 61). GPRV messenger RNA is synthesized in BUdR-treated normal guinea pig cultured cells and in the spleen and lymphoblasts of leukemic guinea pigs, but not in untreated normal spleen, liver, or GPE cells (16, 17). The messenger RNA synthesized appears to represent the entire GPRV genome.

Virus particles from the BUdR-treated guinea pig embryo cells have RNA that is at least 90% homologous with the virus particles obtained from plasma of leukemic guinea pigs and resemble the latter insofar as possessing a similar density (1.16 to 1.18 g/cm^3) in sucrose gradients and a reverse transcriptase requirement for Mg^{2+} rather than Mn^{2+} (69). The guinea pig retrovirus does not possess a group-specific antigen and is not antigenically related to murine, hamster, rat, feline leukemia, RD-114, woolly monkey, or Mason-Pfizer monkey retroviruses (58, 64).

MIXED INFECTIONS

Mixed Infections In Vitro

Guinea pig herpes-like virus and guinea pig retrovirus. Cultured GPK or guinea pig spleen cells, especially those derived from old inbred strain 2 guinea pigs, often show spontaneous herpesvirus cytopathic effects 5 to 7 days after cells are cultured. If BUdR is added to maintain such cultured cells, both GPHLV and GPRV can be observed simultaneously by electron microscopy (Fig. 8) (38). In cultures with mixed infection, progeny virus particles of both GPHLV and GPRV can be identified on the basis of their distinctive morphologies and differences in size (27, 28). Application of immunoferritin electron microscopy techniques to the doubly infected cells, using antiserum specific to each virus, reveals a small number of virus particles which exhibit retrovirus morphology but react with antiserum to GPHLV as shown by ferritin tagging to the surface of the retrovirus particles (27, 28). Since GPRV or GPHLV particles derived from singly infected cells did not react with heterologous antiserum, Fong and Hsiung first reported that a pseudotype of GPRV (GPHLV) was produced in cultured guinea pig cells doubly infected with both GPRV and GPHLV (27). Although pseudotype virus particles had been reported in mixed experimental infections of cultures with herpes simplex

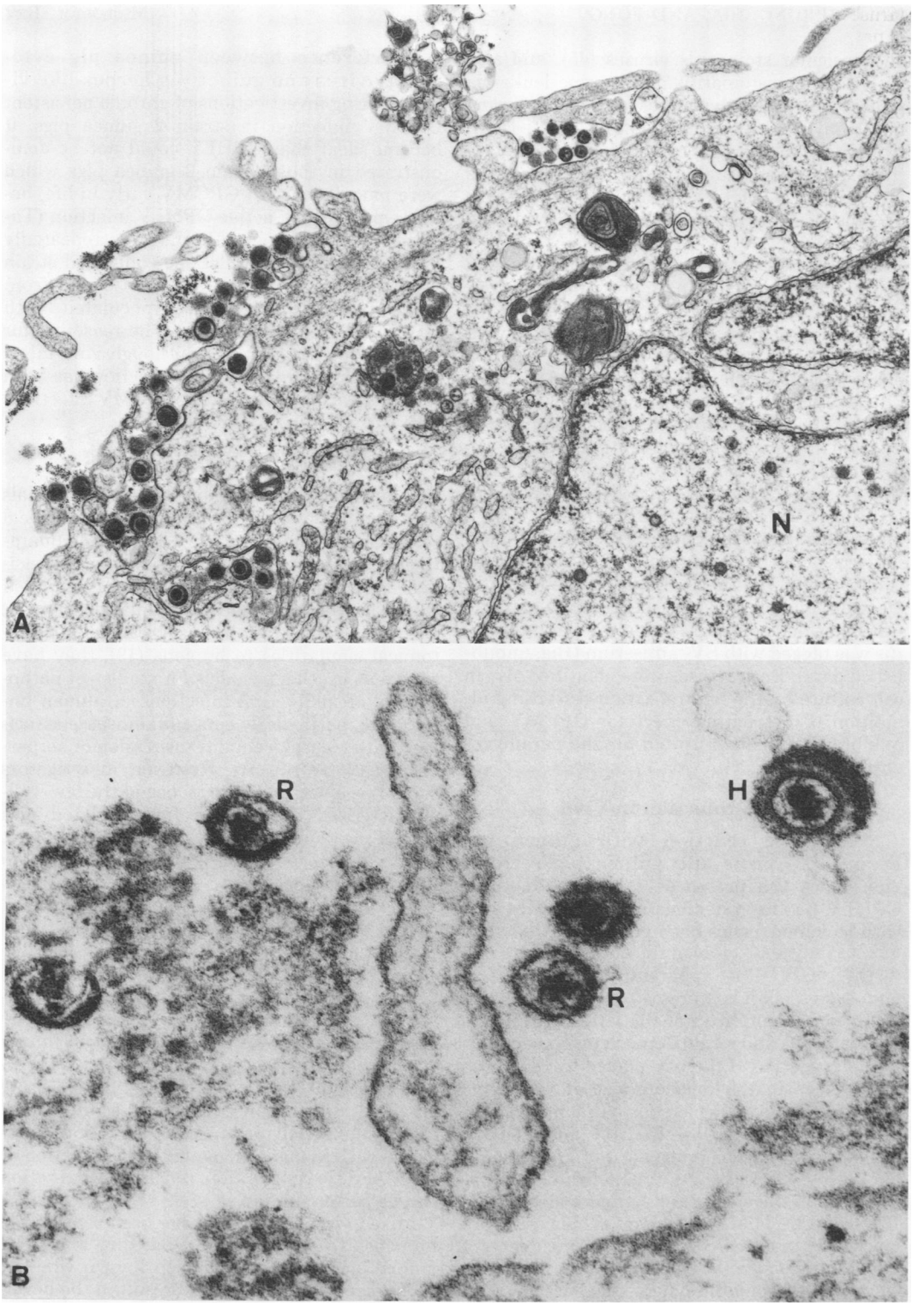

FIG. 8. *Electron micrographs showing mixed infections with GPHLV and GPRV. (A) At low magnification (×19,680), nucleocapsids of GPHLV in the nucleus (N) and extracellular virus particles of both GPHLV and GPRV at the cell surface. (B) At higher magnification (×100,800), GPHLV (H) and GPRV (R). (B is modified from reference 35.)*

and vesicular stomatitis viruses (45), and also with vesicular stomatitis and murine leukemia viruses (76), pseudotype virus particles occurring in natural host cells experimentally infected with two endogenous viruses had not been seen before these studies. The ability of the pseudotype virus GPRV (GPHLV) to infect guinea pig cells or cells of other animal species has not been determined.

Guinea pig cytomegalovirus and guinea pig paramyxovirus. In an attempt to recover infectious virus from the salivary glands of a guinea pig which was previously inoculated with GPCMV, we observed an unusual cytopathic effect in a GPE cell culture inoculated with the salivary gland suspension. Upon addition of a 0.5% guinea pig erythrocyte suspension into the infected monolayer, hemadsorption of the erythrocytes was observed. The hemadsorbing virus was subsequently isolated and identified serologically as SV_5, a strain of parainfluenza type 5 virus. This mixed infection, i.e., GPCMV, a herpesvirus, and the paramyxovirus of guinea pigs, is illustrated in Fig. 9. Using immunoferritin electron microscopy, the herpesvirus particle was identified as GPCMV and the paramyxovirus was tagged with SV_5 antiserum (Bia, unpublished data). Both viruses were identified also in cell culture by the neutralization test, using inhibition of cytopathic effect for GPCMV and inhibition of hemadsorption for the paramyxovirus isolate.

Mixed Infection In Vivo

Synergistic reaction with guinea pig herpes-like virus and guinea pig retrovirus. Since the presence of both GPRV and GPHLV has been demonstrated in guinea pigs with leukemia, it has been postulated that both viruses may play a role in the oncogenesis of this disease. GPRV is apparently present in all guinea pigs, but it is expressed only under certain conditions. Expression of the latent GPHLV is generally age and strain dependent. Experimental investigation of the role played by these two virus types in the development of neoplastic disease in guinea pigs has shown that inoculation of GPHLV or mixtures of GPRV and GPHLV has led to the development of self-limited lymphoproliferative changes characterized by hyperplasia in the spleen and lymph nodes (Table 13) (34, 43). However, when guinea pigs are inoculated with GPRV alone, the incidence of hyperplasia is significantly lower. It is not known whether a comparable synergistic reaction could occur naturally when the two virus infections take place at specific times, such as during pregnancy or in the neonatal period.

Interference between guinea pig cytomegalovirus and guinea pig herpes-like virus. During investigations of chronic persistent GPCMV infection in strain 2 guinea pigs, it became clear that GPHLV could not be demonstrated in those strain 2 guinea pigs which were inoculated with GPCMV early in life, before emergence of active GPHLV infection (Table 14) (4). Although GPHLV was repeatedly isolated from the parallel age-controlled strain 2 guinea pigs, GPHLV was not isolated from 43 animals experimentally inoculated with GPCMV at an early age (4). The reason(s) for these findings is not clear, although viral interference resulting from CMV infection has been reported with human or murine CMV infections as discussed (4).

CONCLUDING REMARKS

Guinea pigs are common laboratory animals and have been used extensively for biomedical research over many years. They are comparatively clean animals, with a relatively low incidence of endogenous virus infection as compared with other animals, especially simian species (31, 46). Since guinea pigs have several distinct anatomical similarities to humans (19), they have served as excellent models for studies of pathogenesis of many viral infections of human importance, particularly congenital disease associated with transplacental transmission of herpesviruses (9, 48, 49, 51). However, investigators should be aware of the presence of the few well-characterized viruses (Fig. 10) associated with guinea pigs and should understand the impact of the presence of these viruses in order to interpret data properly.

The study of endogenous guinea pig viruses, on the other hand, especially those of the herpesvirus group, has proven to be of considerable significance in understanding the pathogenesis of comparable latent viruses in humans. Although endogenous viruses are not ordinarily associated with overt disease, they can, under specific conditions, be activated to produce characteristic disease syndromes. Thus, using GPCMV infection in guinea pigs as an animal model, the serious medical complications associated with human congenital CMV infection or the CMV syndrome after renal transplantation can be better studied.

Mixed virus infections have been reported in humans as well as in plants, bacteria, and other animal species. It is not even known whether such mixed infections would result in the development of pseudotypes in vivo. They could conceivably result in a beneficial effect or lead to irreparable damage to the host. Experimental

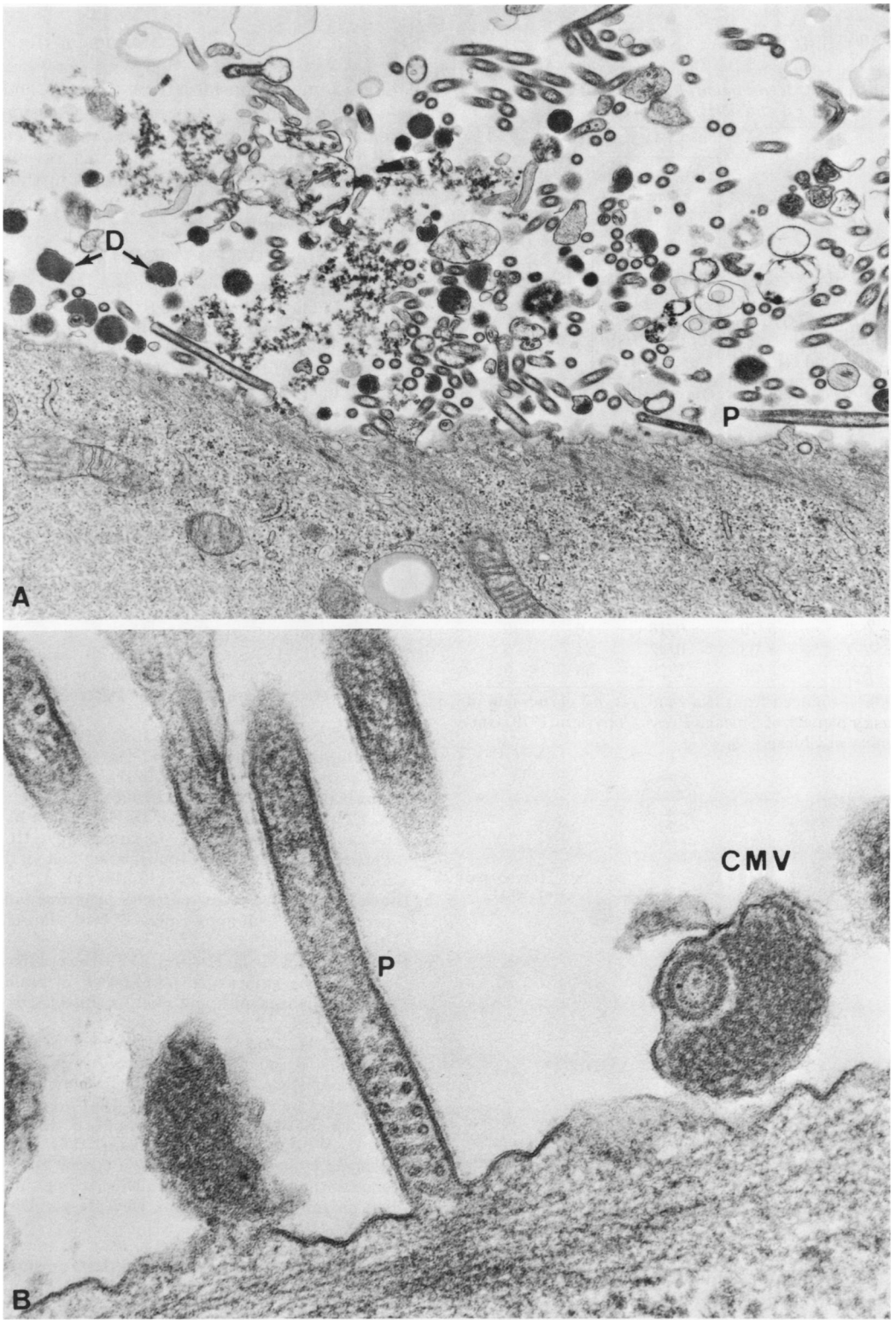

FIG. 9. *Electron micrographs showing mixed infections with GPCMV and a paramyxovirus. (A) At low magnification (×16,320), both GPCMV dense bodies (D) and virions, as well as many filamentous forms of paramyxovirus (P), are seen on the cell surface. (B) At higher magnification (×100,800), a budding of paramyxovirus (P) and a GPCMV virion (CMV) are shown.*

TABLE 13. *Experimental infection of guinea pigs with GPHLV and GPRV*[a]

Material inoculated	Lymphoid hyperplasia			
	No. positive/no. examined		% Positive	
	Spleen	Lymph node	Spleen	Lymph node
GPRV alone	2/11	2/11	18	18
GPHLV alone	15/21	15/20	71	75
GPHLV and GPRV	14/20	21/22	67	95
Ultraviolet-irradiated GPHLV	0/15	0/15	0	0

[a] Reproduced from reference 34, with permission.

TABLE 14. *Effect of experimental GPCMV infection on natural GPHLV infection in strain 2 guinea pigs*[a]

Age of guinea pigs (mo)	No. of GPHLV isolated/no. tested		% of animals with GPHLV isolated	
	Uninoculated controls	GPCMV-inoculated	Uninoculated controls	GPCMV-inoculated
0–2	5/79	0/16	6	0
2–4	8/33	0/42	24	0
4–6	23/40	1/29	58	3

[a] Reproduced from Bia et al. (4), by permission of the University of Chicago Press. Copyright 1979, University of Chicago.

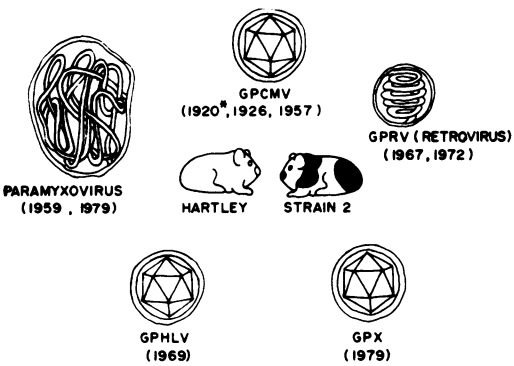

FIG. 10. *Schematic diagram illustrating the few well-known viruses which are commonly isolated from guinea pigs. GPX is a newly characterized herpesvirus of guinea pigs (Bia et al., in press).*

development of pseudotype viruses and research into their pathogenesis should provide insight into their potential for acting in either fashion. Pseudotype viruses might also provide information regarding their potential role in instances of human disease that are associated with either mixed viral infection or viruses that, by themselves, cannot be identified as sole causative agents.

There are many considerations that can and should be borne in mind when one is assessing the significance of endogenous viruses of guinea pigs in biomedical research, and this review would serve its purpose if it stimulates further thoughts on the pathogenesis of natural viral infections and viral latency.

ACKNOWLEDGMENTS

Portions of the work described were carried out under U. S. Public Health Service (USPHS) research grant HD10609 from the National Institute of Child Health and Human Development, USPHS research training grant AI 07018 from the National Institute of Allergy and Infectious Diseases, and Veterans Administration Research Fund.

We are grateful to Kari Hastings, Mary Wright, Ruth Donahue, and Barbara Nunes for their help in the preparation of this manuscript. The data for molecular and biochemical analysis of GPCMV and GPHLV DNAs were kindly supplied by E. S. Huang, University of North Carolina, Chapel Hill.

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