Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

CHAPTER 16

THE RODENT PARVOVIRUSES

Peter Tattersall
and
Susan F. Cotmore

Departments of Laboratory Medicine
and Human Genetics
Yale University School of Medicine
New Haven, Connecticut

I. THE VIRUS GROUP

The parvoviruses are a large family of physically similar viruses infecting animals as diverse as man and moth (1,2). Those which naturally infect vertebrates are divided into two subgroups, the adeno-associated viruses (AAV) and the autonomous parvoviruses. Members of the AAV subgroup, now also known as the Dependoviruses, are totally dependent upon either adenovirus or herpesvirus coinfection for their own replication. At present there is no published evidence of a member of this subgroup naturally infecting rodents. There is no *a priori* reason, however for believing that such defective agents do not exist, and investigators studying rodent adenovirus or herpesvirus infections *in vivo* or in cell culture should be aware of the potential problems that such contaminants would present.

Viral and Mycoplasmal Infections
of Laboratory Rodents

It is our intention here to review the properties and potential influence on experimental systems of those members of the autonomous parvovirus subgroup known to infect rodents. To date, over 30 distinct strains of autonomous parvovirus have been isolated and shown to fall into 13 separate accepted serotypes (3). For our purposes, here we define serotypes on the basis of equivalent cross-neutralization of infectivity or inhibition of hemagglutination, although it is quite probable that these tests may not be examining exactly the same set of antigenic determinants. Of the 13 serotypes of vertebrate autonomous parvoviruses, only three are known to be enzootic in rodent populations under natural conditions. These are represented by Kilham's Rat Virus (RV), Toolan's H-1 virus and Crawford's Minute Virus of Mice (MVM). These have been related to, or distinguished from, other parvovirus isolates of possible rodent origin by the reciprocal hemagglutination inhibition (HAI) studies summarized in TABLE I. Those virus strains showing equivalent reciprocal inhibition and no cross-reaction with other isolates can be grouped as members of the same serotype, such as RV, X14 and H-3. The reason for significant one-way cross reactions between different isolates such as H-3 and H-1 with TVX is not at present understood, and awaits extended reciprocal analyses (with monoclonal antibodies for instance) for further explanation. A number of additional parvovirus isolates, listed in TABLE II, have been related to a particular rodent serotype by cross-neutralization and HA1. For these there is less extensive data to relate them to other serotypes in this subgroup. In many cases, these isolates represent distinct different strains of each serotype, as they can be distinguished from one another by differences in virulence, pathogenicity, target cell specificity or hemagglutination spectrum (10,20,26,28,29,30,31,32).

II. PROPERTIES OF RODENT PARVOVIRUSES

Increasing knowledge of the physicochemical properties and replication of parvoviruses has shed light on some hitherto rather enigmatic observations, and also has predictive value in assessing the potential impact of parvovirus infection on experimental systems. Therefore a summary of these properties is pertinent here. In this, we are fortunate in that much of the detailed information

TABLE I. Rodent Parvovirus Inter-relationships

Anti-serum to	Target HA antigen								
	RV	X14	H3	TVX	H1	HT	RTV	LuIII	MVM
RV	160	80	80	–[a]	–	–	–	–	–
X14	80	160	160	–	–	–	–	–	–
H3	640	1,280	1,280	80	–	–	–	–	–
TVX,	–	–	–	1,280	–	NT	–	–	–
H1	–	–	–	320	20,480	>20	–	–	–
HT	–	–	–	NT	>20	>20	–	NT[b]	NT
RTV	–	–	–	–	–	–	2,560	80	–
LuIII	–	–	–	–	–	NT	–	2,560	–
MVM	–	–	–	–	–	NT	–	–	10,240

[a] = Not Detectable at 1:20.
[b] = Not Tested.
Table adapted from Siegl (4).

available at present has been derived from rodent parvovirus systems, notably MVM and H-1. More extensive treatment of these properties and the references to the original work from which the information is abstracted can be found in a recent review of the subject (reference 2).

A. Physicochemical Properties and Genome Structure

Fractionation of extracts of parvovirus-infected cells or tissues in cesium chloride density gradients generally yields three classes of virus particle; "empty" capsids which band at 1.32 g/ml, DNA containing "full" particles banding between 1.41 and 1.46 g/ml, and a heterogeneous

TABLE II. RODENT PARVOVIRUS ISOLATES

Isolate	Source	Year	Reference
RV serotype:			
RV12	sarcoma-bearing rats	1959	Kilham & Olivier (5)
RV13			Kilham (6)
H-3 (D)[a]	cortisone-treated rats (prob)[b]	1960	Dalldorf (7)
Krisini[c]	carcinogen-treated rat	1962	Zhdanov & Merekalova (8)
X-14	carcinogen-treated rat	1963	Payne et al. (9)
L-S	rat chloroleukemic tumor	1963	Lum & Schreiner (10)
RV171	Moloney leukemia virus stock	1964	Kilham & Moloney (11)
RV308[d]	congenitally infected rat	1966	Kilham & Margolis (12)
HER1	cyclophosphamide treated rat	1967	ElDadah et al. (13)
HHP	uncertain origin[e]	1968	Margolis et al. (14)
Kirk	human Detroit-6 cell culture	1971	Mirkovic et al. (15)
TRV	transplantable rat tumor	1977	Campbell et al. (16)
RV-Yale	diseased rat tissues	1983	Coleman et al. (17)
H-1 serotype:			
H-1 (T)	cortisone-treated rat (prob)[b]	1960	Toolan et al. (18)
H-1 (M)	DNA inoculated RETC[f]	1965	Moore & Nicastri (19)
CR	primary RETC	1969	Kilham & Margolis (20)
BR	primary RETC	1969	Kilham & Margolis (20)
KA	cortisone-treated rat	1969	Kilham & Margolis (20)

(continued)

Table II. (continued)

Isolate	Source	Year	Reference
MVM serotype:			
MVM (CR)	mouse adenovirus stock	1966	Crawford (21)
890	normal mouse kidney	1970	Parker et al. (22)
MVM(i)	transplantable mouse lymphoma	1976	Bonnard et al. (23)
CZ-7	contaminated calf serum	1980	Nettleton & Rweyemamu (24)

[a] - also called OLV (osteolytic virus).
[b] - see text for further discussion.
[c] - also called Zhdanov virus.
[d] - originally called SpRV (12) and subsequently SPRV (25) and SREV (26), but most frequently RV308 (27).
[e] - isolation not published, see also Margolis and Kilham (26).
[f] - Rat embryo tissue culture.

intermediate species of defective particles containing deleted forms of the viral genome. Some properties of empty and full particles, as determined for MVM, are listed in TABLE III. All of the infectivity of such a preparation resides with the DNA-containing full virions, predominantly with a form banding at 1.41 g/ml. This species of virion usually contains VP3 as its major structural polypeptide, whereas virions banding denser, at 1.46 g/ml, contain predominantly the VP2 polypeptide with little detectable VP3. Recent work has shown that VP1 and VP2 are primary translation products of viral-specific messenger RNA and that VP3 is derived by the cleavage, only in full virions, of approximately 30 amino acids from the N-terminus of each VP2 molecule in the assembled virion. This maturation cleavage apparently can occur either in the nucleus late in infection or early in the infectious process inititated by VP2-containing virions. Although consistent observations

TABLE III. Characteristics of Parvoviral Particles *

Property	Full Virions	Empty Capsids
Particle diameter	18-22 mu	18-22 mu
Buoyant density in CsCl	1.46-1.41	1.32
S_{20w}	110S	70S
Molecular weight	5.6×10^6	4.2×10^6
Particles per hemagglutin unit	$1-2 \times 10^8$	2×10^8
particle: infectivity ratio	200-400:1	n.d.
absorption E_{260}/E_{280}	1.38	0.67
extinction coefficient $E^{1\%}_{280}$	71.2	17.8
protein	73.7%	100%
DNA	26.3%	n.d.
RNA, carbohydrate	n.d.	n.d.
Structural proteins:		
VP1 mw	83,300	83,300
% total protein	15-18	15-18
VP2 mw	64,300	64,300
% total protein	variable	82-85
VP3 mw	61,400	n.d.
% total protein	variable	<1
Genome:		
molecules per virion	1	-
size	5.1 kilobases	-
structure	single-stranded, linear non-permuted	-
-strand: +strand	100:1	-

* date presented were determined for MVM (1,2)
n.d. = none detected

record the denser virions with a high VP2 content and the lighter virion with a high VP3 content, there is evidence that these two sets of properties are not necessarily associated.

The capsid is assembled as an isometric structure comprising a total of about 60 molecules of structural protein arranged in an icosahedral shell which appears to have 32 capsomers (Figure 1). The infectious virion is a similar structure containing the viral genome, and comprises the majority of particles shown in Figure 1. The virion is non-enveloped and does not appear to contain any essential lipid, carbohydrate or RNA. Its simple, robust structure affords it a degree of stability which is one of the major

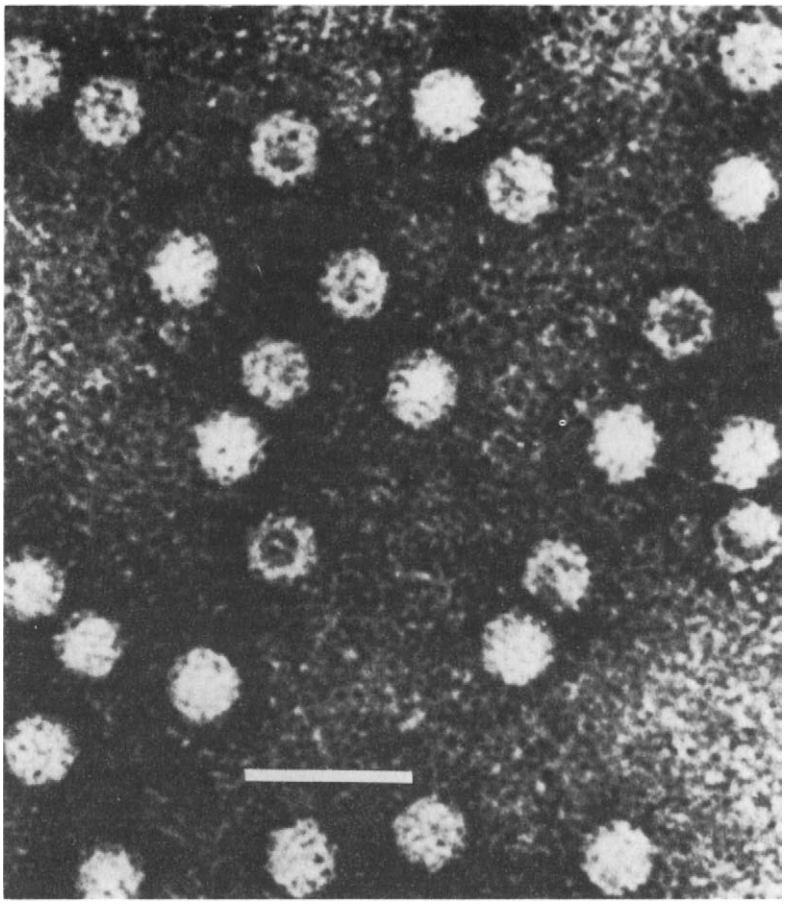


Figure 1. Electron micrograph of purified MVM(p) virions, stained with 3% phosphotungstic acid and photographed at an instrument magnification of 80,000X. The horizontal bar represents 50 mμ.

factors in the ability of these viruses to establish enzootic infections and persist as contaminants in animal care environments. Infectivity is remarkably stable to desiccation, heating and even moderate levels of chaotropic agents, such as urea and sodium dodecyl sulfate. Comparative peptide mapping of the viral polypeptides first demonstrated the precursor-product relationship of VP2 and VP3 also established that the entire sequence of the major polypeptide was contained within the minor polypeptide VP1. It is not clear yet how these polypeptide species are arranged within the capsid but the preponderance of basic amino acids in the VP1-specific region of the minor polypeptide suggests that this region may be located internally and complexed with the viral DNA in full virions. The full virions do not contain any histones, but whether the DNA inside mammalian parvovirus virions is bound with polyamines remains a possibility since this has been demonstrated for the densoviruses, the insect virus subgroup of parvoviruses.

The rodent parvovirus genome is a predominantly single-stranded DNA molecule with palindromic sequences at each end which are folded to form terminal hairpins. The rodent parvoviruses generally package one strand sense so that at least 99% of virions contain a molecule of opposite sense to the viral RNA transcripts expressed in infected cells. This technically makes the rodent parvoviruses negative-strand viruses. By convention, the genome is depicted with the 3' end of the negative-sense DNA molecule at the left and the 5' end at the right (Figure 2). Rodent parvovirus genomes are typically about 5.1 kilobases long, and have been completely sequenced for H-1(T), and both MVM(i), the immunosuppressive MVM strain, and MVM(p), the prototype strain derived by plaque purification from MVM(CR). The left hand hairpin, which is a primer for the copying of the rest of the molecule by DNA polymerases both *in vitro* and *in vivo* is 115 nucleotides long. The right-hand end contains around 200 nucleotides. The bulk of the DNA lying between these two structures is coding sequence. As shown in Figure 2, there are two long blocks of open reading frame separated by translational stop signals in all reading frames between 45 and 46 map units. The block of open reading frame lying in the right half of the genome encodes the capsid polypeptides, VP1 and VP2, with the entire sequence of VP2 lying in the C-terminal three quarters of the VP1 sequence. These polypeptides are translated prodominantly predominantly from the most abundant viral mRNA species, R3, which is transcribed off the major promoter lying at 38 map unit. Two cytoplasmic viral transcripts are produced off a promoter lying at 4 map units. One of these, R2, has 30% of the

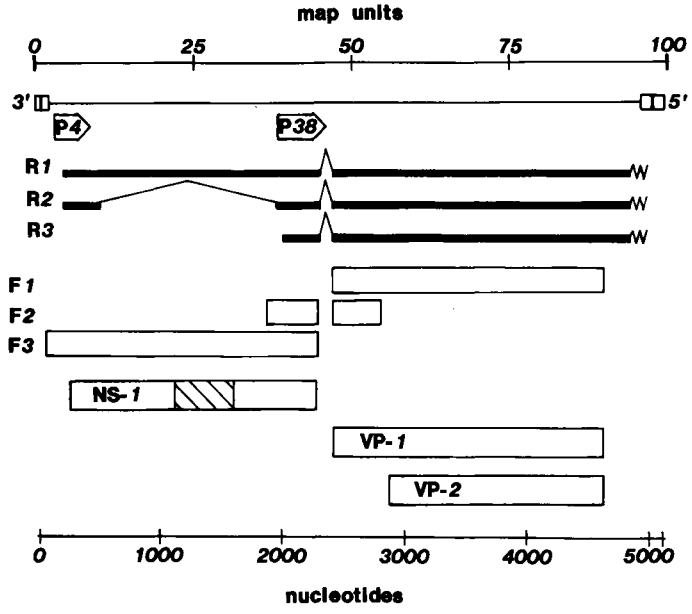


Figure 2. Physical and genetic map of MVM(p). The viral genome is displayed with the 3' end of virion single-stranded DNA, which is the negative strand with respect to transcription, on the left at zero map units. The boxes to the left and right hand ends represent the terminal palindromes, which occupy two map units at the 3' end and four map units at the 5' end. The arrows marked P4 and P38 denote the two transcriptional promoters, located at 4 and 38 map units respectively. The three major cytoplasmic transcripts R1, R2 and R3 are represented by thick black lines with the thin, wavy lines indicating their polyadenylated tails, and the thin, straight lines indicating the introns spliced out in the production of the mature messages. The significant runs of open reading frame present in transcribed sequences (i.e., in the positive, or complementary, strand) are shown as open blocks labelled F1, F2 and F3 (frame 1, frame 2 and frame 3) to denote their phasing with respect to nucleotide number 1. The regions known to encode the major non-structural protein, NS-1, and the structural polypeptides, VP-1 and VP-2 are dislayed below this, above the nucleotide scale. The cross-hatched area within the NS-1 sequence is the region of this protein, expressed in E. coli as a fusion polypeptide, against which the monospecific anti-NS-1 antibodies were raised for use in the experiment shown in Figure 3.

genome sequence spliced out, between 8 and 38 map units, and its translation products are not yet established. However the R1 transcript gives rise to the major non-structural protein NS-1, of 83,000 to 85,000 mw, which is encoded in the long block of open reading frame occupying the left half of the genome. All three of these cytoplasmic transcripts are also spliced in the region 44-46 map units. The NS-1 polypeptide is a nuclear phosphoprotein which may play a role in viral DNA replication, and is antigenically highly conserved across the autonomous parvovirus subgroup. Analysis of in vitro translation products of virus-specific mRNA has demonstrated a second, non-structural protein, NS-2, with a molecular weight between 24,000 and 26,000 encoded by MVM and H-1. This protein has yet to be assigned a coding region within the genome, or located within the infected cell.

B. Viral Replication

Several aspects of the virus life cycle are of paramount importance in understanding the pathology of parvovirus-induced disease and in predicting possible ways in which inadvertant introduction of these viruses might interfere with other experimental systems. First, it is important to note that, in common with most other non-enveloped viruses, productive infection is always lytic. This is to say that the only known way in which progeny virus can be released from one infected cell so as to infect a neighboring cell is by lysis and concomitant cell death. Second, not all virus-host cell interactions are productive, but some of these non-productive interactions can still be lytic. A number of different types of non-productive interactions have been investigated; in some cases one can learn much about particular virus-host relationships by studying systems in which the virus fails to grow.

Productive infection is initiated by adsorption of the virion to specific cell-surface receptors. The presence of functional receptors on the cell surface is apparently under developmental control, since some differentiated cell types lack them and are completely resistant to virus infection. Little is known about the mechanics of the early steps of replication except that endocytosis appears to take place through coated pits. Endocytosed virions appear to be transported intact to the nucleus, there the DNA is uncoated prior to the first step in viral DNA synthesis, the synthesis of the complementary stand. There do not appear to be any classical early functions expressed by the incoming

parvovirus genome - that is, there is no detectable viral gene expression which preceeds viral DNA replication. This is important since an early requirement for viral DNA replication, and one upon which all further viral functions depend, is a currently uncharacterized host factor which is expressed transiently during the S-phase of the mitotic cycle. Probably because of the lack of early gene expression, the virus is unable to stimulate cells resting in another stage of the cell cycle to progress through to S-phase and thus to competence as a host for viral replication. This requirement for the S-phase host factor coupled with the inability to stimulate resting cells has been established in cell culture systems, and is reflected in parvoviral disease of the whole animal as a preference for actively proliferating tissues as sites of attack. These two properties are probably the major aspects of viral growth which underlie the predominantly teratogenic potential of the autonomous parvoviruses.

When the requirement for transition through S-phase is met in a productive host, the early stages of viral DNA replication and viral transcription proceed concurrently, with subsequent translation giving rise first to predominantly the NS-1 polypeptide. This stage is followed by the synthesis of large amounts of viral capsid proteins. DNA replication proceeds through double-stranded replicative forms (RF) of monomer, dimer and tetramer size classes, which exhibit the interesting feature that dimers and tetramers are palindromic arrangements of unit length viral duplex DNA molecules. All of these intermediates have complex terminal structures involving hairpin ends or extended terminal palindromes, many of which have a protein of unknown origin covalently linked to their 5' ends. The individual structural polypeptides are synthesized in the cytoplasm and are then transported to the nucleus. In the nucleus, assembled capsids take part in the packaging of progeny DNA single-strands in a reaction requiring concomitant assymetric DNA replication. Eventually, a mixture of empty, precursor capsids and full virions is released from the cell following nuclear degeneration and rupture of the plasma membrane by mechanisms as yet undefined. As summarized in TABLE IV, infection of a productive host cell culture gives rise to wholesale cell death. However, for any cell types, continued culture allows for the outgrowth of colonies of virus-resistant cells. Resistant cells may have several different phenotypes with respect to the step of virus replication which is affected. One common phenotype is stable loss of expression of the virus receptor.

TABLE IV. Parvovirus-Host Cell Interactions *In Vitro*

Interaction	Viral Antigen	Viral DNA	Cell Killing	Consequence	Example
PRODUCTIVE	+	+	+	extensive cell death followed by regrowth of selected virus-resistant epigenetic cell variants of various phenotypes.	MVM(p) in mouse A9 fibroblasts
RESTRICTIVE	-	+	-	persistent carrier state; selection of *hr* virus mutants; cytopathic crisis, etc; coevolution of virus and cell variants.	MVM(i) in mouse A9 fibroblasts
ABORTIVE	+	-	+ or -	may or may not be cytotoxic; reverted to productive, lytic infection by oncogenic transformation; transformation rates may be drastically reduced in presence of parvovirus.	MVM(p) or MVM(i) in normal human fibroblasts
CRYPTIC	-	-	-	mitogenic stimulation activates virus, leading to progeny virus production and cell death.	MVM(i) in resting T-cells.

Another is the inability to support viral transcription. Yet other resistant cell types appear to be affected in their ability to carry out an early step in the penetration and transport of input virus to the nucleus.

Similarly, virus-host cell interactions have been characterized during the study of infection of non-productive cell types *in vitro*, and can usefully be divided into three categories; restrictive, abortive and cryptic (TABLE IV).

Restrictive infection, which is perhaps the best characterized of these, is exemplified by studies on the prototype (p) and immunosuppressive (i) variants of MVM. These differ from one another by being restricted for growth in one another's productive host cell type, which are fibroblasts and T-lymphocytes, respectively. The two viruses are serologically indistinguishable and have been designated allotropic variants of the MVM serotype, to indicate their distinct target cell specificities. Somatic cell hybrids between these two cell types are productive hosts for both viruses. These results suggest that each restrictive host cell type lacks a factor necessary for the productive replication of the restricted virus strain, and that these factors are expressed as a function of the differentiated state of the host cell. Biochemical studies have demonstrated that these host factors operate at the level of initiation of viral transcription. Genetic mapping studies suggest that the viral components, or allotropic determinants, which determine what differentiated cell types each virus strain can grow in may be tissue-specific transcriptional enhancer sequences. The consequences of restrictive infection to the entire cell population are of interest in the context of the impact of viral contamination on experimental cell cultures. With all restrictive virus-cell combinations examined in culture, there is a small subpopulation of cells, continually being replenished during the growth of the culture, which are able to support fully productive, lytic virus replication. The remainder of the culture restricts virus replication at the level of transcription and infection has little or no effect on cell growth rate or viability. Infection of such cultures with a restricted virus can therefore result in a carrier culture, continually producing low levels of virus without apparent cytopathic effect, a condition which may persist for some time in culture. During passage of such cultures the low level of virus replication can give rise to a virus mutant, called an extended host range (*hr*) mutant, which has gained the ability to transcribe and replicate its genome in the formerly restrictive host cell type. The appearance of *hr*

mutants in a persistently-infected restrictive cell culture results in a cytopathic crisis, with concomitant massive virus production. As this interaction is now converted to a productive one, there is often a rapid selection of cellular variants which are resistant to the newly-arisen virus mutant. It appears that many of these cellular variants can be restrictive for the new virus and the whole cycle can begin over again. This cycle can apparently occur several times before the culture is overgrown by cell variants which have lost the virus receptor and are therefore resistant to infection by all the new variant viruses. At this point, spontaneous curing of virus from the culture may be observed. Throughout this process, the overall phenotypic characteristics of the cell population are changing. This situation would obviously be highly undesirable for many types of cell culture study.

In some types of non-productive virus-cell interaction, especially those across species barriers (for instance, the infection of normal human fibroblast cell lines with rodent parvoviruses), significant expression of viral nonstructural and capsid antigens has been observed to occur in the absence of detectable progeny virus production. These interactions are termed abortive infections. In abortive infections the virus fails to establish its own DNA replication program, and the infection may or may not result in cell death, depending upon the particular combination of virus strain and cell line. All cells in the culture appear to be able to sustain viral transcription and antigen expression, often to levels found in productively-infected cells. This type of interaction is of particular interest since transformation of the cell by viral agents, such as SV40, convert the abortive interaction to a fully productive one. Dual SV40 and parvovirus infections are not productive, however, implying that transient SV40 early gene expression is not sufficient for reversal of the abortive phenotype, and that the establishement of the fully transformed state is required for these cells to support a productive parvovirus growth cycle. Some mouse cell strains, both selected resistant mutants and apparently normal lines, possess this transformation-sensitive phenotype with respect to MVM infection.

A final distinct interaction between virus and host cell is a direct result of the requirement for the host to traverse the S-phase and the virus' inability to stimulate resting cells to do so. Consequently, the infection of naturally resting but otherwise productive host cells leads to a situation we denote cryptic infection. An example of this would be the infection of unstimulated splenic T-cells

with the immunosuppressive strain of MVM. In this case, activation of cryptically infected T-cells with concanavalin A or antigen leads to the active replication of the virus to produce progeny resulting in lysis of the activated cell. Infection may then spread among other susceptible, cycling cells in the population.

III. PREVALENCE AND TRANSMISSION

The sources of the several published isolations of RV summarized in TABLE II point overwhelmingly to its natural host being the rat. In addition to the isolation of these relatively well characterized strains, additional isolations of RV from normal rats have been reported by Robey et al. (33), who also documented a high percentage (up to 40%) of RV-specific HAI antibody-positive sera collected from both wild rats and laboratory rat strains. Similar evidence for the ubiquitous distribution of RV antibodies has been reported by Kilham and co-workers (5,34) for both feral (40-62%) and laboratory (89%) rat populations. In a study of an enzootic RV infection of a closed laboratory colony of rats Robinson et al. (35) showed that by seven months of age approximately two thirds of the population had circulating antibody, infection occurring between two months and seven months of age. Prior to this age, about half of the animals retained passive immunity to the virus acquired from their mothers during neonatal life.

The natural host species of the H-1 serotype, and indeed H-3, a member of the RV serotype, remain somewhat more enigmatic. H-1 and H-3 were originally isolated by the blind passage in newborn hamsters of subcellular fractions of the human tumor cell lines HEp1 and HEp3 respectively. These tumors had previously been maintained by serial tansplantation in immunosuppressed rats. Since, as mentioned above for antibodies to RV (H-3), H-1 antibodies have also been found to be widespread in both wild (up to 100%) and laboratory (80%) rats (34), it has been proposed that the original source of both viruses was indeed the rat. However, isolation of these two viruses following direct inoculation of human material into newborn hamsters has also been reported (36), a result which could be taken to support a human origin of H-1 and RV serotype viruses. Arguing against this conclusion are reports of the extremely low frequency (2.7%) of H-1 and RV antibodies in normal human populations (37), an incidence some thirty-fold lower than

that found in rat population. As the newborn hamster was the vehicle of isolation in all of these studies, it is of considerable significance that no published data are available for the frequency of H-1 or RV antibodies in laboratory or breeder hamster populations. Attempts to link rodent parvoviruses to human reproductive disorders also resulted in somewhat conflicting reports. Newman and coworkers (38) surveyed some 50 tissue specimens from spontaneous human abortions by injecting tissue homogenates into newborn hamsters without achieving a single virus isolation. However, Guiglielmino et al. (37) reported an increased incidence of rodent parvovirus antibodies in sera drawn from women with a history of stillbirth and repeated spontaneous abortion. In a survey of 350 such sera they found 13.71% positive for H-1 antibodies and 9.14% positive for RV antibodies, whereas the frequencies in 300 normal control sera for these two antibody specificities were 2.66% and 1.66%, respectively. These results are themselves at variance with the previous findings of Monif and coworkers (39), who reported a somewhat lower incidence (1.5%) of antibodies to H-1 among sera collected from 130 women with histories of spontaneous abortion or offspring with congenital abnormalities. Taken together, these data do not define a natural host for H-1 or RV beyond question, although the difference in antibody frequencies for both viruses between rat and man is suggestive that human immunity and the possible isolation of virus from human tissue may represent zoonoses. In support of this is the finding that H-1, at least, can replicate in and establish viremia in human volunteers inoculated intramuscularly with the virus (40). It is not known whether zoonoses of rodent parvoviruses occur frequently in human populations. Further epidemiological surveys comparing control groups with groups at risk, such as laboratory animal care personnel, will be necessary to answer this question.

There is no epidemiological evidence to suggest the natural infection of hamsters, mice or guinea pigs with either RV or H-1 parvovirus serotypes. It is clear, however, that the experimental infection of hamsters either transplacentally, directly *in utero*, or as neonates with both H-1 and RV results in a variety of congenital defects (6,20,41-49). The newborn mouse, however, only appears to develop clinical disease in response to RV following intracranial inoculation of large virus doses (13,50), whereas infection by subcutaneous or intraperitoneal routes appears to be assymptomatic (5,10,13). H-1 can also be transmitted to newborn mice by intracranial inoculation, causing clinical ataxia by destroying cells of the developing cerebellum (51).

The mouse is quite clearly the natural host of MVM. In a survey of mouse breeder colonies in the United States in 1970, Parker and his colleagues (52) showed that 38 out of 44 conventional colonies and three out of eight SPF colonies, but none of five germ-free colonies, contained mice positive for MVM-specific antibodies. The virus was found to be enzootic within affected colonies with an average of 74% of the mice seropositive, and their HAI titers often exceeding 1:100. In addition to the laboratory mice tested in this extensive study, some 390 sera from wild mice trapped in four different states were also tested. Of these, 20% were shown to contain MVM antibodies, with seropositive animals being found in all four states. These results attest to the widespread distribution of the virus in wild mouse populations as well as laboratory colonies.

As in the previously described study by Robinson et al. (35) of RV in a closed rat colony, Parker and coworkers analyzed the natural history of enzootic MVM infections in three mouse breeder colonies by examining the presence of antibody as a function of the animal's age. In the first six to eight weeks of life, the number of seropositive animals declined about two-fold to between 20% and 60%. In each colony, a sharp rise was noted between 8 and 12 weeks by which time over 90% of the animals in each colony had become seropositive. This high frequency of antibody-positive animals persisted up to 40 weeks of age. These data imply that young mice are protected in the first few weeks of life by maternally-derived antibody. When this passive protection diminishes by the end of the first month, the animals become susceptible and are infected by contact with contaminated fomites or other animals in the infectious phase of the disease. During the infectious phase, mice shed virus in feces and urine and may also transmit virus by the nasal-oral route (52). As described above, these viruses are extremely stable to desiccation. Therefore, shed virus would remain viable for long periods of time, ensuring that as young animals become susceptible they routinely become infected. In addition to their survey of wild and laboratory mice, Parker and colleagues (52) also tested sera from 1550 rats, 747 hamsters, 76 guinea pigs, 50 gerbils and 105 lemmings for MVM-specific antibodies. They found a high prevalence of low titer HAI activity in rats only, all other sera being negative. Kilham and Margolis have also reported a high incidence of MVM-specific HAI-positive rat sera (51). However, Parker's group demonstrated that the HAI activity they observed was removable by kaolin treatment, suggesting that it was a non-specific inhibitor. It is questionable, therefore,

whether there is really any reliable evidence for natural infection of rats with MVM. The overall evidence strongly points to MVM being exclusively a virus of mice. It is interesting, therefore, that MVM can be transmitted readily to hamsters and rats by experimental inoculation (51,53). In rats, MVM causes a subclinical infection whereas in newborn hamsters a lethal disease is induced involving extensive replication of virus in many tissues (51). Occasional hamsters surviving the effects of low doses of MVM sometimes developed the "mongoloid-like" deformities described below for RV and H-1 infections of hamsters, as well as peridontal disease. MVM can also establish transplacental infections in pregnant hamsters and rats inoculated parenterally with the virus.

In enzootically infected colonies, the major route of natural transmission for rodent parvoviruses appears to be horizontal, either by nasal-oral contact or through contaminated fomites. Since transplacental infection of the developing fetus has been demonstrated by experimental infection for many rodent parvoviruses in most rodent species, vertical transmission remains a possibility for spread of virus within colonies. The prevalence of antibody positivity among females of breeding age suggests that this is unlikely to be a major form of natural viral transmission. However, several instances of recovery of parvoviruses directly from primary embryo tissue cultures, notably the isolation of the CR and BR strains of H-1 (20), have been taken as examples of naturally-occurring transplacental infection.

The mode and extent of horizontal transmission appears to depend upon the strain as well as the serotype of parvovirus involved. For instance, Lipton et al. (54) reported excretion of RV in feces, but not in urine, for up to twelve days after infection with the HER strain, and that inoculated rats were infectious for contact cohorts for fifteen to twenty days. In contrast, Novotny and Hetrick (55) found RV present in urine of suckling rats inoculated parenterally as newborns with high doses of RV-13. These authors also found that RV-13 was capable of vertical transmission in that litters of infected dams developed disease when nursed by normal mothers, whereas normal litters nursed on infected mothers remained well (55). However, Kilham and Margolis (25) showed that RV strain SpRV may be excreted briefly in the milk of rats inoculated with virus in late pregnancy. Dams infected on the first day postpartum showed RV in their milk within 24 hours and continued to shed virus in their milk for up to twelve days after infection, and up to five days after detectable seroconversion (25). Parker and his

colleagues (52) showed that transmission of MVM between infected and susceptible animals can occur by direct contact or by limited nasal-oral contact as well as by urinary or fecal contamination. However, transmission does not appear to occur efficiently by airborne dissemination, even across a space as little as eight inches. Susceptible mice in contact with infected animals or their fomites seroconverted within three weeks of exposure, but susceptible mice experiencing only limited contact took a week or longer to achieve comparable antibody responses (52). The same study reported that the tissue culture-derived Crawford strain of MVM was not excreted in urine or feces and was not transmitted to susceptible animals kept in close contact with mice infected with the virus by intraperitoneal inoculation. However, a later study by Smith (56) demonstrated efficient transmission of MVM (Crawford strain) between cagemates under similar conditions. The Parker report (52) did not contain sufficient details of their experimental infections with Crawford strain MVM to identify the probable basis for this discrepancy, but factors such as initial infecting dose of virus and mouse strain might affect the ability of experimentally inoculated virus to be transmitted.

The widespread enzootic distribution of parvoviruses and their requirement for dividing cells leads to their frequent occurrence as contaminants of tumor cell lines and tumor virus stocks which have been passaged in animals. This was most dramatically demonstrated by the study of MVM by Parker and colleagues (52) who found that 44% of spontaneous tumors from various sources regularly transplanted in mice were contaminated with the virus. A further study (57) which screened transplantable tumors for eleven different agents showed that MVM, with an incidence of 32%, was second only to lactic dehydrogenase virus in frequency of isolation. This incidence is very high and leads one to wonder how many experiments in cancer chemotherapy and immunology have been influenced by the unsuspected presence of MVM. The isolation of both MVM(i) and the TRV strain of RV as immunosuppressive agents present in transplantable tumors (16,23) underlines the profound effects such contamination might have on the interpretation of both *in vivo* and *in vitro* experiments with infected tumors or material derived from them.

IV. PATHOGENESIS

A. Overt Disease

Numerous studies on the pathogenicity of rodent parvoviruses have shown that, in general, they cause fetal and neonatal abnormalities by destroying specific cell populations which are rapidly proliferating during the normal course of development (20,46,47,48,58,59,60). These same tissues are usually mitotically quiescent and therefore resistant in the mature animal and, consequently, few of the viruses cause overt disease in the adult. Animals are particularly sensitive to parvovirus infection in the first few days of life, and intracerebral inoculation of neonatal animals, especially hamsters, with these agents can cause runting and a characteristic "mongoloid-like" deformity (41,42). The craniofacial and periodontal lesions which generate the deformity appear to be due to selective viral attack on developing skeletal and dental tissues (61,62,63, 64). Unlike Down's Syndrome in man, which it resembles, this condition is not associated with chromosomal abnormalities (65) nor is it hereditary, since with careful husbandry such animals can be raised to breeding age and produce normal offspring (64). Perhaps the most characteristic result of intracerebral parvovirus infection of neonates is cerebellar hypoplasia (67), often leading to chronic ataxia (66,67). Once, again, this condition was shown to be due to viral depletion of a rapidly proliferating cell population, in this case involving cells of the cerebellar granular cortex (47).

Many parvoviruses will cross the placenta and establish infections of the fetus. A number of factors affect the outcome of fetal infections, such as route of inoculation, virus strain and dose, the species of pregnant host and the mice of infection during gestation. In some cases inoculation with a large dose of virus early during gestation will give rise to a generalized and devastating lethal infection involving much of the mesodermal tissue of the embryo and resulting in resorption or mummification (19,41,47,65,67,70). Infection at late times during gestation may yield viable offspring which, in addition to the runting and cerebellar hypoplasia mentioned earlier, may also exhibit hemorrhagic encephalopathy (13,26,71) or neonatal hepatitis and enteritis (12,72). Again, involvement of cell populations with high mitotic activity at, or subsequent to, the time of infection is a consistent feature of these diseases (47,48). Factors such as host species,

virus strain and route of inoculation affect the course of disease mainly by determining whether the virus reaches the developing fetus. The effects of the time of infection during gestation suggest that different cell types in different embryonic tissues pass through a state of sensitivity and are sequentially, but transiently, "at risk" during fetal development. This "risk period" corresponds well with the time of rapid proliferation of that cell type during the process of organogenesis. The requirement for S-phase transition for productive infection discussed above has been proposed as the basis for the specific tissue tropisms observed during parvovirus teratogenesis, and to explain the resistance of the adult animal as compared to the fetus or neonate (47,59). In support of this idea, several studies have shown that resistant adults can be rendered sensitive to disease by inducing some tissue to undergo an abnormal proliferative response. Thus partial hepatectomy prior to H-1 infection will render the normally resistant adult rat susceptible to viral hepatitis where the sites of viral attack are the regenerating margins of the liver (73). Likewise, susceptibility to similar viral disease can be brought about by inducing mitotic activity in the adult liver by carbon tetrachloride damage or by infection with the parasite Cysticerus fasciolaris (48,73). Osteolytic parvovirus strains will also infect healing bone fractures, causing defective callus formation in normally resistant adult hamsters (45,75).

Although proliferative activity appears to be a prerequisite for target organs, it is clear that not all tissues which turn over rapidly are necessarily subject to parvoviral attack (46). While most adult tissues are mitotically quiescent compared to the fetus and neonate, many, such as gut epithelium and the hematopoetic system, contain large numbers of cycling cells. One might expect these cells, which are essential for the host organism's well-being and survival, to be targets for parvovirus attack in the adult. The sparing of these tissues by the majority of parvovirus strains is underlined by the existence of a small subset of parvoviruses which frequently cause fatal disease in adult animals by bringing about the extensive destruction of gut epithelium and, in some cases, cells of the reticuloendothelial system. The notable example of this type of pathogenic behaviour has for a long time resided outside the rodent parvovirus subgroup, with strains of the feline panleukopenia/mink enteritis/canine parvovirus serotype and the Aleutian Disease virus of mink, (2,76). However, the recent isolation of a strain of RV which cause fatal disease in young adult rats (17) shows that this

property may occur within serotypes naturally infecting rodent species. In vitro studies provide evidence for strain-dependent variations in tissue tropism and the potential for changes to occur by mutation in the spectrum of differentiated cell types a particular virus strain can infect (31,32,77). Therefore, a simple explanation for the emergence of virus strains pathogenic for the adult host is that such viruses have mutated to include, within their repertoire of productive host cell types, some which normally proliferate rapidly in the mature animal. Conversely one might predict that evolutionary pressure toward an enzootic life style in crowded populations, such as wild or laboratory rodent colonies afford has led to the elimination of these targets from the repertoires of the commonly studied strains of rodent parvoviruses.

As described in an earlier section, in vitro studies on target cell specificity have provided significant support for the hypothesis that lytic virus growth is modulated by developmentally-regulated components operating in the host at the cellular level. Mohanty and Bachman (78) have reported that the actively dividing cells of the early mouse embryo are resistant to killing by MVM. Murine embryonal carcinoma cells, the stem cells of teratocarcinoma, are resistant to MVM(p) as are many of their differentiated derivatives (79,80). However, when these cells are induced to differentiate in vitro, they give rise to at least one differentiated cell type, resembling a fibroblast, which supports productive MVM(p) replication. Taken with the analysis of the reciprocal restrictions of MVM(p) and MVM(i) replication in each other's productive host cell type described earlier, these studies demonstrate that cell cycling, although necessary, is not sufficient for the lytic, productive replication of individual parvovirus strains, and that the differentiated state of the host cell is of paramount importance.

In some studies of experimental parvovirus pathogenesis, especially in the 1960's when the viruses were being assessed for their ability to cause tumors, several investigators reported instances of tissue hyperplasia, such as odontomas and cementomas, resulting from parvovirus infection (30,61,62,63,81). When further characterized, these were found invariably to be benign. This appears a somewhat enigmatic observation, since no evidence has been found in cell culture studies for any type of cellular transformation elicited by these viruses. In addition most animal studies have revealed that these viruses exhibit an oncosuppressive rather than tumor-indicative behaviour. One possible explanation for these hyperplasias is that they might be

caused by viral ablation of a distinct cell type which normally exerts a controlling influence on the extent of growth in the hyperplastic tissue, either by direct contact or by the production of humoral factors. Studies on viral target-cell biology in such situations may shed light on some growth-control processes in normal development.

B. Tumor Association and Oncosuppression

The repeated isolation of parvoviruses from tumors or carcinogen-treated animals was originally taken to suggest that these viruses might have some causal association with neoplastic disease. Indeed, many later attempts to isolate H-1 and RV from non-malignant tissues by the original procedures proved unsuccessful (5,82). Since that time, however, many studies on the long term influence of parvovirus infection on the host animal have shown the opposite conclusion to pertain. That is, these viruses interfere with, and in some cases appreciably suppress, tumor formation in their hosts. This remarkable property is of obvious concern when unintentional introduction of a parvovirus occurs in cancer-related research in both animal and cell culture systems. In one long-term animal study reported by Toolan (83), involving large numbers of hamsters, those which had survived H-1 infection at birth without pathological syndrome had a five-fold lower spontaneous tumor rate than their uninfected siblings. Furthermore, the tumor rate among survivors exhibiting the "mongoloid-like" craniofacial lesions associated with neonatal H-1 infection, was five-fold lower still. In several studies, parvovirus infection has been shown to suppress tumor formation by a number of transforming viruses and chemical carcinogens. For instance, RV was shown to suppress leukemia induction by Moloney leukemia virus in rats (84), and H-1 infection of hamsters was shown to suppress tumor formation by adenovirus (85) and dimethybenzanthracene (86).

Elucidating the mechanism by which parvoviruses suppress tumor induction in whole animal systems is vastly complicated. A more feasible approach would be to study in vitro analogs in which the effect of parvoviral replication on the process of cell transformation could be examined in the absence of major extrinsic factors such as hormonal and immune responses. Initial attempts to study the interaction between a transforming virus and an autonomous parvovirus in cell culture exploited the finding that human embryo lung fibroblasts would not support lytic growth of H-1 unless the cells were coinfected with human adenovirus type 12 (Ad12)

(87). It was shown that in the absence of adenovirus, H-1 could enter the cell and expressed capsid antigen, but did not form infectious progeny virions (88). Coinfection with Ad12 resulted in the maturation of H-1, which occurred prior to the induction of cellular thymidine kinase by the helper virus (89) and resulted in a overall reduction in Ad12 replication (88). Since human fibroblasts are permissive for lytic adenovirus replication, any influence of H-1 on the transforming potential of the helper adenovirus could not be assessed, and no information was obtained as to whether the helper function was expressed early or late during the adenovirus life cycle.

Recently, Mousset and Rommelaere described an in vitro system which promises to be of great value in the study of parvovirus-oncogene interactions (90), and which illustrates the types of potential interference exerted by these viruses in in vitro cell transformation studies. They described the isolation of a Balb/c 3T3 mouse fibroblast variant which is resistant to cell killing by MVM(p) by virtue of an intracellular block to viral replication. Although this cell line is identical to its MVM-sensitive parent in its susceptibility to SV40 transformation, coinfection or superinfection of these mutant cells with MVM drastically reduces the ability of SV40 to successfully transform them. Furthermore they demonstrated that these mutant cells, once transformed by SV40 in the absence of MVM, were now capable of supporting a productive infection with MVM. This implies either that an SV40 early gene product can directly complement the cellular defect for MVM replication, or that transformation has activated, or reactivated, a cellular gene whose product is missing in the mutant and is required for MVM growth. A potential role for a transforming gene in parvovirus replication can therefore now be studied in vitro and it is to be hoped that such studies will reveal the mechanism by which parvoviral tumor suppression operates in the whole animal.

C. Persistence, Latency and Activation.

Several reports in the literature have suggested that a frequent, or perhaps inevitable, consequence of parvovirus infection is the establishment of a longterm persistent or latent infection in the host animal. Before discussing this aspect of parvoviral biology it is important to note that all of these studies were done with animals drawn from enzootically infected colonies and therefore, the evidence derived from them is rather circumstantial. To date, no

study has been published in which viral persistence or latency have been examined in animals experimentally infected under conditions where shedding and re-infection is eliminated. One should view the following evidence with this caveat in mind. Firstly, most of the strains of rodent parvoviruses listed in TABLE II were derived from enzootically infected animals which exhibited moderate to high levels of antiviral antibody prior to some experimental manipulation which led to the isolation of virus (5,6,8,9, 10,11,13,16,20,23). Another consistent observation is that infected animals sustain appreciable levels of circulating virus-specific antibody for long periods after virus infection, perhaps for life (33,35,52). Since the period after experimental infection during which viral antigen can be demonstrated in host tissues by conventional techniques is relatively short (56), generally under two weeks, such sustained antibody titers imply continual, low-level stimulation of the immune system. While this may occur in many enzootic situations by successive rounds of reinfection from the contaminated environment, it may also be due to a continuous low level of virus production in the host animal. Perhaps the most convincing evidence for parvoviral persistence is the demonstration that infectious virus can be isolated from tissues of animals with significant antibody titers. The reports of successful isolation of MVM and RV from the tissues of immune hosts have involved animals from enzootically infected colonies, thus involving a fully transmissible agent, and are subject to the caveat of continuous reinfection mentioned above. Nevertheless, the consistent recovery of infectious virus from tissues of highly immune animals speaks against this alterative explanation. Parker and his colleagues (52) used the mouse antibody production (MAP) test to demonstrate directly the presence of MVM in kidney extracts of adult immune mice and in the blood of such animals after blind passage of extracts in virus-free mouse embryo tissue culture. As might be expected, attempts to recover virus from mice under 35 days of age were unsuccessful, independent of the (passive) immune status of these mice. However, virus could be isolated with high frequency from the kidneys of 40 to 45 day-old mice in the same colony, even though the majority of these had HAI titers between 1:80 and 1:320. In similar studies of a Sprague-Dawley rat colony endemically infected with RV, Robey et al. (33) were able to isolate virus from at least three out of the five tissues obtained from each of the five immune adult rats that they tested. In this case, they used blind passage of tissue extracts in a rat nephroma cell line known to be free of RV, and most positive tissue abstracts yielded virus

within two or three passages. Virus was not consistently isolated from any particular tissue, but interestingly animals with the highest HAI titers (1:320 to 1:1280) had the highest frequency of positive tissues. They were unable to detect directly any virus in gradient fractions of concentrated tissue extracts from such animals, but this was probably due to the insensitivity of the assay (HA) they employed. Although the evidence cited above strongly suggests that parvoviral infections may persist in the whole animal, there are no reports in the literature which establish persistence as an experimentally reproducible phenomenon. Likewise there have been no demonstrations of parvoviral latency as evidenced, for example, by the presence of sequestered intracellular viral genomes in the absence of infectious virus particles. However, despite the lack of definitive proof for or against, both persistence and latency are likely to be part of the normal infectious process of parvoviruses. Perhaps the most important aspect of potential latency in the context of this article is the ability of certain experimental manipulations to activate virus infection. As can be seen in TABLE II, immunosuppression and tumor induction (or carcinogen treatment) figure largely in the activation of what is presumably latent virus (7,8,9,11,13,18,20). For instance, in one study the administration of a single, nonlethal dose of cyclophosphamide to clinically normal rats resulted in central nervous system lesions from which the HER strain of RV was subsequently isolated (13). On the other hand, two of the original isolations of RV were made from metastasizing sarcomas of the liver associated with encysted Cysticercus fasciolaris (5). Various other RV strains, such as X14 and Krisini virus, were recovered only after carcinogen treatment (8,9). Whether immunosuppression and tumorigenesis have some common feature which leads to the facilitation of virus isolation from treated animals is not known. The fact that cell-free tissue extracts can be shown to contain infectious virus upon continued blind passage in susceptible cell culture suggests that virus production proceeds in latently infected animals, at least to a limited extent. Persumably it is this extracellular virus which infects the exogenous tumor cells during the initial contamination of transplantable tumors, an event commonly observed with these viruses (52,57). Essentially nothing is known about the cell types producing this virus, the frequency of affected cells, their locations or the latent form of the viral genome. Likewise, the manner in which the

progeny virus escapes inactivation by the high circulating levels of apparently neutralizing antibody found in such latently infected animals remains to be elucidated.

V. IMPLICATIONS FOR RESEARCH

In the preceding sections we have described what is known of various aspects of the natural history of parvovirus infection and, where possible, we have used that information to predict scenarios in which the presence of a parvovirus might affect the outcome of an experimental procedure. These are summarized in TABLE V and for the most part are self-explanatory when considered in conjunction with the preceding text. Some of these scenarios are imaginary. That is to say, there are no published reports, to our knowledge, which substantiate them in detail and they are merely predictive of potential problems. For others, however, there are well documented accounts of such interference actually occurring. For instance, the isolation of MVM(i) is a good example of the effect of parvoviral contamination of a transplantable tumor on subsequent immunological experiments. Initially Bonnard and Herberman reported that a factor produced by the EL-4 (G-) lymphoma markedly suppressed the generation of cytotoxic T-lymphocytes in allogeneic mixed lymphocyte cultures (91). On further investigation, this factor proved to be an infectious agent specifically neutralized by reference MVM antiserum (23). However, Crawford's original MVM isolate (21) had a slight enhancing effect on cell mediated cytotoxicity in equivalent tests, indicating that the immunosuppressive virus was a variant of MVM with a virus-cell interaction different from that of the prototype laboratory strain (23). Subsequently this virus was isolated and has been compared extensively with the prototype virus. This allotropic variant of MVM, known as MVM(i), is capable of lytic growth in cytotoxic T cell clones, will abrogate cytotoxic T cell responses, reduce T cell mitogenic responses and interfere dramaticaly with helper dependent B cell responses *in vitro* (92,93,94). Moreover, under appropriate conditions MVM(i) is highly cytopathic *in vitro* for the original tumor cell line EL-4 (G-) from which it was isolated (32), suggesting that during passage *in vivo*, the developing transplanted tumor was probably protected by the imune response of the mouse either to the contaminating MVM or to MVM experienced previously in an enzootically infected

TABLE V. Potential Interference by Parvoviruses with Whole Animal or Tissue Culture Experiments

1. Tumorigenesis Studies.		
In vivo	a)	Contamination of transplantable tumors and tumor virus stocks
	b)	Reduction in transplantable tumor take (by direct oncolysis or modulation of immune response to tumor cells).
	c)	Selection of new transplantable tumor phenotypes
	d)	Reduction in viral or chemical tumorigenesis
In vitro	a)	Reduction in viral- or oncogene-mediated cell transformation by conversion of abortive to productive parvovirus infection
	b)	Contamination of tumor virus stocks
2. Immunological Studies.		
In vivo	a)	Modulation of lymphocyte mitogenic responses
	b)	Cryptic infection of lymphocytes
	c)	Interference with humoral antibody spectrum
In vitro	a)	Abrogation of cytotoxic T cell responses
	b)	Reduction in T-cell mitogenic stimulation
	c)	Abrogation of helper-dependent B-cell responses
3. Infectious Disease Studies/Cell Biology Studies.		
In vivo	a)	Disease modification by interviral interactions
	b)	Immunosuppression of experimental host
In vitro	a)	Primary tissue culture degeneration
	b)	Carrier culture establishment
	c)	Selection of new cell line phenotypes

colony. Alternatively, during prolonged co-cultivation the contaminating MVM may have selected from the original EL-4 cells a subpopulation restrictive for growth of the virus, but which reverted with high enough frequency to maintain a carrier culture. At what point the virus was picked up by the tumor in its passage history is not clear, but its isolation in this way exemplifies the caution with which *in vitro* results with *in vivo*-derived material should be treated. These results also emphasize the importance of knowing the parvovirus status of both tumor cell lines and animal colonies. A similar result has been reported for the isolation of an immunosuppressive variant of RV from a contaminated rat mammary adenocarcinoma (16).

In addition to the effects MVM(i) has on *in vitro* immunological responses, it is of interest to note here that infection of the whole animal with either MVM(i) or MVM(p) leads to a depression of both splenic T cell and splenic B cell mitogenic stimulation indices (95). In addition MVM(i) can be shown to infect splenic lymphocytes in a cryptic fashion, being activated to replicate by ConA stimulation (96). MVM(i) also interferes markedly with the ability of the infected animal to develop a normal humoral antibody response to antigenic determinants on its own viral capsid, but does not impair antibody response to the major non-structural protein (97).

The potential influence of persistent or latent parvovirus infection on tumorigenicity studies is predominantly inferred from the oncosuppression studies described in an earlier section, and from the frequent isolation of virus from immune animals by tumor passage or following exposure to carcinogens. The potential for contaminating primary cell lines and virus stocks is obvious and it is surprising that more examples of this are not reported. Fortunately, there is a potent, but unidentified, inhibitor of parvovirus growth found frequently in some commercial preparations of calf or fetal calf serum, common cell culture additives (98,99) and it may be that this inhibitor often protects cell cultures from contamination.

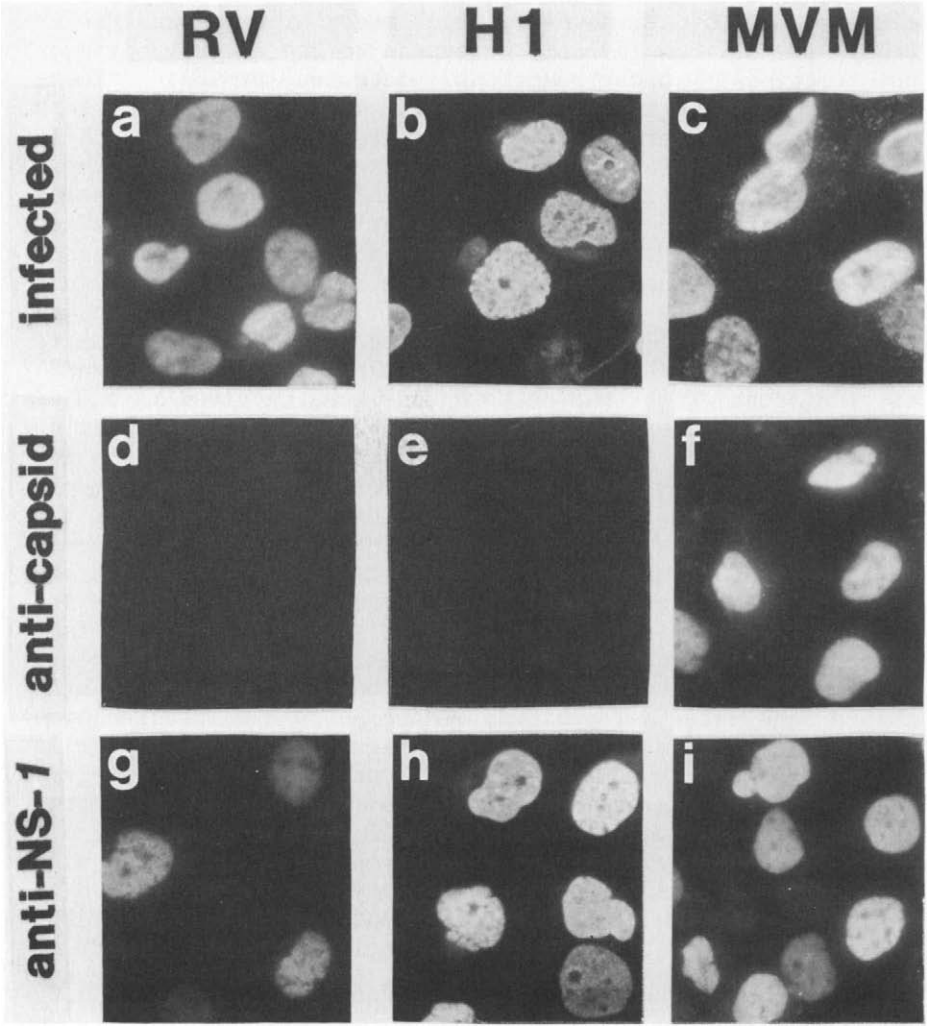
Finally, latent parvovirus infection could potentially be a problem in rodent breeding colonies. The porcine parvovirus, which is closely related to the rodent parvoviruses, causes widespread infertility and infectious abortion in pigs (58). Although, in theory, the rodent virus could produce similar effects, we are not aware of any published evidence which indicates that this problem ever occurs.

VI. DIAGNOSIS AND PREVENTION

It is clear from preceding sections that the rodent parvoviruses are eminently capable of establishing enzootic infections in laboratory colonies in which virus infection is almost universally asymptomatic in the absence of experimental manipulation. There are, therefore, no reliable procedures for clinical or morphological diagnosis of rodent parvovirus infection. The consequences of introducing even one infected animal are potentially very serious since many of the virus strains spread very rapidly and the only effective means of control is the destruction of the affected colony followed by repopulation with virus free stock (100). Serological testing offers the most effective means of parvoviral diagnosis, supported when possible, by virus isolation. Five types of assay have been used routinely for parvovirus serology; hemagglutination inhibition (HAI), complement fixation (CF), plaque or end point CPE neutralization tests (NT), flourescent antibody tests (FA) and enzyme-linked immunoassay (EIA). It is not our purpose here to evaluate critically the relative merits of these tests as this is the subject of other chapters in this volume (101,102), but a few comments are relevant to the overall aims of this article. Of these assays, HAI, CF and NT will reliably distinguish between rodent parvovirus serotypes whereas, for reasons discussed below, FA and EIA (when infected cells are used as the target) will efficiently demonstrate the presence of parvovirus antibody without discriminating between individual serotypes. Probably the most widely used serotype-specific assay is HAI. It is relatively insensitive, compared with NT, and subject to the presence of non-specific inhibitors present in many sera. Some of these non-specific inhibitors are resistant to removal by convenient, routine means such as kaolin extraction or neuraminidase digestion. It is, however, much cheaper and more rapid than NT. For laboratories which have access to high titer stocks of the three rodent parvovirus serotypes and contained tissue culture facilities in which to work with them, NT is the assay of choice. Several variants of this test are available, among which the EIA version described by Smith (103) is probably the most convenient. Plaque neutralization employing standard plaque assay conditions with a predetermined virus dilution incubated with dilutions of serum for one hour at 37°C prior to assay on cell monolayers has been adapted successfully to multi-well dish format in our laboratory (104). We usually use the SV40-transformed human fibroblast

cell line, 324K (32), for convenience, since it will plaque many strains of MVM, XRV and H-1. This assay is both time consuming and expensive, and individual assays must be carried out to screen for each viral serotype. Routine screening for parvovirus infections of unknown serotype can be adequately achieved by FA or EIA, although assignation of positive sera to a particular serotype must then be made by HAI, CF or NT. The antigenic distinction between MVM, RV and H-1 and the possible significance of one way cross-reactions have been discussed in a previous section and elsewhere (105). However, the study of Cross and Parker (106) is of considerable relevance to the understanding of unidirectional cross-reactions detected by FA. These authors explored the antigenic relationships between members of the three serotypes using antibodies raised to each in rats. They found that while these serotypes did not cross-react when analyzed by reciprocal HAI, CR or CPE neutralization tests, the anti-RV and anti-H-1 antisera stained MVM-infected rat cells as efficiently as the homologous anti-MVM antiserum. However, the anti-MVM antiserum did not stain RV or H-1 infected cells. To explain this, it has been suggested (105) that, during the infection of rat cells *in vitro*, all three viruses elicit a common antigen, which is different from the capsid antigens involved in hemagglutination, interaction with complement- fixing antibody or neutralization. Furthermore, it was presumed that, in the rat *in vivo*, this common antigen is expressed in immunogenic amounts following inoculation of infectious RV and H-1 but not when the inoculated virus is MVM. Subsequent experiments revealed that these common antigenic determinants are not carried on the viral capsid but are expressed on the major viral non-structural protein NS-1 (107), a nuclear phosphoprotein which is only synthesized in cells which are actively replicating the virus. We have dissected the anti-capsid and anti-NS-1 antibody responses in MVM infected mice in the experiment described in Figure 3. Here 324K cells separately infected with MVM, RV or H-1 have been stained with sera from several mouse sources. Firstly, MVM-infected mouse serum efficiently stains all three infected cell types. Secondly, sera from mice repeatedly immunized with purified, non-infectious MVM empty capsids stain MVM-infected cells equivalently to the infected mouse serum, but show no discernable staining with cells infected with RV or H-1, compared to preimmune serum. However, when a mouse serum monospecific for antigenic determinants carried on the MVM NS-1 protein is used, a typical distinct nuclear

staining is seen which is almost equivalent for cells infected with each of the three virus serotypes. Interestingly, the infected mouse serum and the anti-capsid serum also stain cytoplasmic structures, but only in MVM infected cells. These structures are not stained in these cells by the anti-NS-1 antibody and we believe them to be viral particles accumulated in endocytotic vesicles. Thus, there is little if any cross-reaction by FA between the capsids of MVM, RV and H-1, and the strong cross-reactions detected by



this technique reside almost entirely with the major non-structural polypeptide synthesized in infected cells. Antibodies to these non-structural proteins will be elicited only if the immunized animal is actively infected by the virus, and thus the rats inoculated with RV or H-1 by Cross and Parker (106) made such antibodies while those inoculated with MVM did not. Recent studies have shown that these NS-1-specific common antigenic determinants exist across an even broader spectrum of autonomous parvoviruses than just those infecting rodents. Strong cross-reactions have been detected between the MVM NS-1 polypeptide and those encoded by LuIII, FPV/CPV and PPV (97). Obviously, such a broadly cross-reacting technique as FA, although unable to identify specific serotypes, would theoretically detect infection by some parvoviruses for which no HAI, CF or NT test exists. This opens up the possibility of detecting hitherto unsuspected agents in rodent, and other populations. Indeed there are indications that agent(s) unrelated to MVM, RV or H-1 by HAI but detectable by FA cross-reaction are circulating in commercial populations of both mice and rats (108).

Since the only method for effective control of parvovirus infections is to destroy the colony and repopulate, it obviously pays an investigator to avoid scrupulously the introduction of these agents into clean areas. We have listed in TABLE VI the properties of parvoviruses which we feel are most relevent to their potential introduction to a clean colony. Any cages, bedding, instruments or other paraphernalia which have been in contact with infected animals must be considered as a potential means of transmitting infection, and adequate sterilization procedures should be employed. Depending upon the physical properties of the contaminated equipment, autoclaving, hypochlorite

Figure 3. Rapidly dividing 324K cells (32) seeded on glass slides were infected at 15-30 pfu per cell with RV (a,d,and g), H-1 (b,e, and h) or MVM (c,f, and i). At 24 hours after infection the cells were fixed with 2.5% buffered paraformaldehyde, permeablized with 0.1% Triton X100 and stained with a 1:40 dilution of serum:- from an MVM-infected Balb/CByJ mouse (a,b, and c), or serum from Balb/CByJ mice hyperimmunized with either highly purified, non-infectious MVM(p) empty capsids (d,e, and f) or a 20 kilodalton segment of the NS-1 polypeptide produced in E. coli by recombinant DNA techniques (g,h, and i). The location of the bacterially- expressed NS-1 segment within the NS-1 gene is indicated in Figure 2.

TABLE VI. Properties of Rodent Parvovirus Relevant to their Potential Introduction into Animal Facilities

1. Exceptional Stability
2. Commonly Enzootic
3. Predominantly Asymptomatic
4. Possible Persistent or Latent Infection of Whole Animal
5. Age-dependent Antibody Distribution
6. Frequent Tumor and Virus Stock Contaminants

soaking or UV irradiation should be used, in that order of preference. Likewise, one should be aware of the possibilities for physical transmission of virus by human vectors, such as central service employees or colleagues moving between enzootically infected colonies or colonies of unknown status, and the clean area. Adequate precautions should be taken therefore while handling animals, such as the wearing and frequent changing of disposable gloves.

The high frequency of enzootic infection in breeder colonies and the possibility of a persistent phase following acute infection means that all rodents or rodent-derived products coming into a clean facility are suscept. If one does not have reliable evidence to the contrary, it is reasonable to assume that any incoming animals or animal-derived materials are carrying virus. Strict quarantine rules should be observed for animals received from sources where the parvovirus status of the colony is not constantly monitored and reported. It is of considerable importance to realize that the age distribution of animals positive for anti-parvovirus antibody, discussed in a previous section, means that weanling animals are likely to be uniformly seronegative on arrival, although they may already be infected and seroconvert over the next few weeks. At this stage, they will almost certainly be infectious for other animals and should be kept isolated for several weeks before final serology is performed and negative animals are introduced into a clean area. Similarly, transplantable tumors, tissue culture cell lines and virus stocks to be introduced into a clean area should be tested for parvovirus by MAP test (22)

in isolated animals. This approach has the advantage that the resulting sera can be screened for a variety of undesirable contaminants. This particular precaution is extremely important for successfully excluding parvoviruses from an animal facility, since they are such widespread contaminants of tumor lines and tumor virus stocks (57). Finally, it is of course necessary to have an adequate program for the control of feral rodents in and around the facility, since the incidence of parvovirus infection in wild populations is appreciable.

VII. FUTURE RESEARCH

Much remains to be learned about the natural history of parvovirus infection. We are still ignorant of the details of many aspects of viral replication at the cellular level, including the nature of the requirement for host S-phase transition, a phenomenon which shapes the biology of these viruses at every level. There is still no definitive evidence for parvoviral persistence or latency, although there is strong circumstantial evidence to suggest that such states may exist, and we know nothing about the mechanism by which immunosuppression or carcinogenesis can promote virus recovery. Moreover we know very little, and need to know a lot more, about the way in which these viruses can suppress the onset or course of neoplastic disease.

Although we are learning about target cell specificity from allotropic virus variants, we know very little, if anything, about the viruses in the field. Field strains appear to be more pantropic than the frequently studied laboratory strains. It may be that this is an artifact introduced during the isolation of laboratory strains. When virus strains are isolated in cell culture we may be selecting for an allotropic subset of a complex field strain or merely selecting host range mutants which have arisen *in vitro*. It is clear, therefore, that we currently understand somewhat less than we need to know, and far less than there is to know.

ACKNOWLEDGEMENTS

We thank Dr. Abigail Smith for many useful discussions and for critically reading the manuscript. The authors were supported by NIH grant number CA29303 from the National Cancer Institute.

REFERENCES

1. Ward, D.C. and Tattersall, P. (eds). Replication of Mammalian Parvoviruses. Cold Spring Harbor Press, New York, 1978.
2. Berns, K.I. (ed). The Parvoviruses. Plenum Press, New York, 1984.
3. Siegl, G., Bates, R.C., Berns, K.I., Carter, B.J., Kelly, D.C., Kurstak, E. and Tattersall, P. (1984). Characteristics and Taxonomy of Parvoviridae. Intervirology 23: 61-73.
4. Siegl, G. (1976). The Parvoviruses. In: "Virology Monographs," (S. Gard and G. Hallauer, eds.), Vol. 15, Springer Verlag, Berlin, 1976.
5. Kilham, L. and Olivier, L.J. (1959). A latent virus of rats isolated in tissue culture. Virology 7: 428-437.
6. Kilham, L. (1961). Rat Virus (RV) infections in hamsters. Proc. Soc. Exptl. Biol. Med. 106: 825-829.
7. Dalldorf, G. (1960). Viruses and human cancer. Bull. N.Y. Acad. Med. 36: 759-803.
8. Zdhanov, V.M. and Merekalova, Z.I. (1962). Isolation of a virus from connective tissue of carcinogen-treated rats. Vop. Virus. 7: 339-342.
9. Payne, F.E., Shallaberger, C.J. and Schmidt, R.W. (1963). A virus from mammary tissue of rats treated with X-rays or methylcholanthrene. Proc. Am. Assoc. Cancer Res. 4: 51.
10. Lum, G.S. and Schreiner, A.W. (1963). Study of a virus isolated from a chloroleukemia Wistar rat. Cancer Res. 23: 1742-1747.
11. Kilham, L. and Moloney, J.B. (1964). Association of Rat Virus and Moloney luekemia virus in tissues of inoculated rats. J. Nat. Cancer Inst. 32: 523-531.

12. Kilham, L., and Margolis, G. (1966). Spontaneous hepatitis and cerebellar "hypopolasia" in suckling rats due to congenital infections with Rat Virus. Am. J. Path. 49: 457-475.
13. ElDadah, A.H., Nathanson, N., Smith, K.O., Squire, R.A., Santos, G.W. and Melby, E.C. (1967). Viral hemorrhagic encephalopathy of rats. Science 156: 392-394.
14. Margolis, G., Kilham, L. and Ruffolo, P.R. (1968). Rat virus disease, an experimental model of neonatal hepatitis. Exptl. Mol. Pathol. 8: 1-20.
15. Mirkovic, R.R., Adamova, V., Boucher, D.W. and Melnick, J.L. (1971). Identification of the Kirk "Hepatitis: Virus as a member of the parvovirus (picodnavirus) group. Proc. Soc. Exptl. Biol. Med. 138: 626-631.
16. Campbell, D.A., Staal, S.P., Manders, E.K., Bonnard, G.D., Oldham, R.K., Salzman, L.A. and Herberman, R.B. (1977). Inhibition of in vitro lymphoproliferative responses by in vivo passaged rat 13762 mammary adenocarcinoma cells. II. Evidence that Kilham Rat Virus is responsible for the inhibitory effect. Cell. Immunol. 33: 378-391.
17. Coleman, G.L., Jacoby, R.J., Bhatt, P.N., Smith, A.L. and Jonas, A.M. (1983). Naturally occurring lethal parvovirus infection of juvenile and young-adult rats. Vet. Path. 20: 49-56.
18. Toolan, H.W., Dalldorf, G., Barclay, M., Chandra, S. and Moore, A.E. (1960). An unidentified filterable agent isolated from transplanted human tumors. Proc. Natl. Acad. Sci. 46: 1256-1259.
19. Moore, A.E. and Nicastra, A.D. (1965). Lethal infection and pathological findings in AxC rats inoculated with H virus and RV. J. Nat. Cancer Inst. 35: 937-947.
20. Kilham, L. and Margolis, G. (1969). Transplacental infection of rats and hamsters induced by oral and parenteral inoculations of H-1 and Rat Viruses (RV). Teratology 2: 111-124.
21. Crawford, L.V. (1966). A minute virus of mice. Virology; 26: 602-612.
22. Parker, J.C., Cross, S.S., Collins, M.J., and Rowe, W.P. (1970). Minute Virus of Mice. I. Procedures for quantitation and detection. J. Nat. Cancer, Inst. 45: 297-303.
23. Bonnard, G.D., Manders, E.K., Campbell, D.A., Herberman, R.B. and Collins, M.J. (1976). Immunosuppressive activity of a subline of the mouse EL-4

lymphoma. Evidence for minute virus of mice causing the inhibition. J. Exp. Med. 143: 187-205.
24. Nettleton, P.F. and Rweyemamu, M.M. (1980). The association of calf serum with the contamination of BHK 21 clone 13 suspension cells by a parvovirus serologically related to the Minute Virus of Mice (MVM). Arch. Virol. 634: 359-374.
25. Kilham, L. and Margolis, G. (1974). Transmission of Rat Virus in milk of rats. J. Inf. Dis. 129: 737-740.
26. Margolis, G. and Kilham, L. (1970). Parvovirus infections, vascular endothelium and hemorrhagic encephalopathy. Lab. Invest. 22: 478-488.
27. Kilham, L., Buckler, C.E. Ferm, V.H. and Baron, S. (1968). Production of interferon during Rat Virus infection. Proc. Soc. Exptl. Biol. Med. 129: 274-278.
28. Toolan, H.W. (1967). Agglutination of the H-viruses with various types of red blood cells. Proc. Soc. Exptl. Biol. Med. 124: 144-146.
29. Toolan, H.W. (1968). The Picodnaviruses: H, RV, and AAV. Int. Rev. Exptl. Path. 6: 135-180.
30. Toolan, H.W. (1983). Degeneration of lens and overgrowth of Harderian glands in hamsters neonatally infected with parvovirus MVM-i. Proc. Soc. Exptl. Biol. Med. 172: 351-356.
31. Mitra, S., Snyder, C.E., Bates, R.C. and Banerjee, P.T. (1982). Comparative physicochemical and biological properties of two strains of Kilham Rat Virus, a non-defective parvovirus. J. Gen. Virol. 61: 43-54.
32. Tattersall, P. and Bratton, J. (1983). Reciprocal productive and restrictive virus-cell interactions of immunosuppressive and prototype strains of Minute Virus of Mice. J. Virol. 46: 944-955.
33. Robey, R.E., Woodman, D.R. and Hetrick, F.M. (1968). Studies on the natural infection of rats with the Kilham Rat Virus. Am. J. Epidemiol. 88: 139-143.
34. Kilham, L. (1965). Viruses of laboratory and wild rats. Nat. Cancer Inst. Monograph 20: 117-140.
35. Robinson, G.W., Nathanson, N. and Hodous, J. (1971). Sero-epidemiological study of Rat Virus infection in a closed laboratory colony. Am. J. Epidemiol. 94: 91-100.
36. Toolan, H.W., Buttle, G.A.H., and Kay, H.E.M. (1962). Isolation of the H-1 and H-3 viruses directly from human embryos. Proc. Am. Assoc. Cancer Res. 3: 368.

37. Guglielmino, S., Tempera, G., Pappalardo, G. and Castro, A. (1978). H-1 and X-14 parvovirus antibodies in women with abortions and still-births. Acta. Virol. 22: 426-428.
38. Newman, S.J., McCallin, P.F. and Sever, J.L. (1970). Attempts to isolate H-1 virus from spontaneous human abortions: a negative report. Teratology 3: 279-281.
39. Monif, G.R.G., Sever, J.L. and Cochran, W.D. (1965). The H-1 and the RV viruses and pregnancy: serological study of certain groups of pregnant women. J. Pediatrics 67: 253-256.
40. Toolan, H.W., Saunders, E.L., Southam, C.M., Moore, A.E. and Levin, A.G. (1965). H-1 virus viremia in human. Proc. Soc. Exptl. Biol. Med. 119: 711-715.
41. Toolan, H.W. (1960). Experimental production of mongoloid hamsters. Science 131: 1446-1448.
42. Kilham, L. (1961). Mongolism associated with rat virus (RV) infection in hamsters. Virology 13: 141-143.
43. Ferm, V.H. and Kilham, L. (1964). Congential anomalies induced in hamster embryos with H-1 virus. Science; 145: 510-511.
44. Ferm, V.H. and Kilham, L. (1965). Histopathologic basis of the teratogenic effects of H-1 virus on hamsters embryos. J. Embryol. Exptl. Morph. 13: 151-158.
45. Baer, P.N., Garrington, G.E. and Kilham, L. (1971). Effect of age and H-1 virus on healing fractures in hamsters. J. Gerontol. 26: 373-377.
46. Lipton, H.L. and Johnson, R.T. (1972). The pathogenesis of Rat Virus infections in the newborn hamster. Lab. Invest. 27: 508-513.
47. Kilham, L. and Margolis, G. (1975). Problems of human concern arising from animal models of intrauterine and neonatal infections due to viruses: a review. I. Introduction and Virologic Studies. Prog. Med. Virol. 20: 113-143.
48. Margolis, G. and Kilham, L. (1975). Problems of human concern arising from animal models of intrauterine and neonatal infections due to viruses: a review. II. Pathologic Studies. Prog. Med. Virol. 20: 144-179.
49. Toolan, H.W. (1978). Susceptibility of the Syrian hamster to virus infection. Fed. Proc. 37: 2065-2068.
50. Matsuo, Y. and Spencer, H.J. (1969). Studies on the infectivity of Rat Virus (RV) in BALB/c mice. Proc. Soc. Exptl. Biol. Med. 130: 294-299.

51. Kilham, L. and Margolis, G. (1970). Pathogenicity of minute virus of mice (MVM) for rats, mice and hamsters. Proc. Soc. Exptl. Biol. Med. 133: 1447-1452.
52. Parker, J.C., Collins, M.J., Cross, S.S. and Rowe, W.P. (1970). Minute Virus of Mice. II. Prevalence, epidemiology, and occurrence as a contamination of transplanted tumors. J. Nat. Cancer Inst. 45: 305-310.
53. Kilham, L. and Margolis, G. (1971). Fetal infection of hamster, rats and mice induced with minute virus of mice (MVM). Teratology 4: 43-62.
54. Lipton, H.G., Nathanson, N. and Hodous, J. (1973). Enteric transmission of parvoviruses: pathogenesis of rat virus infection in adult rats. Am. J. Epidemiol. 6: 443-446.
55. Novotny, J.F. and Hetrick, F.M. (1970). Pathogenesis and transmission of Kilham Rat Virus in rats. Inf. Immunol. 2: 298-303.
56. Smith, A.L. (1983). Response of weanling random-bred mice to inoculation with Minute Virus of Mice. Lab. Animal Science 33: 37-39.
57. Collins, M.J. and Parker, J.C. (1972). Murine virus contaminants of leukemia virus and transplantable tumors. J. Nat. Cancer Inst. 49: 1139-1143.
58. Siegl, G. (1984). Biology and pathogenicity of autonomous parvoviruses. In: "The Parvoviruses" (K.I. Berns, ed.), pp. 297-362, Plenum Press, New York.
59. Margolis, G. and Kilham, L. (1965). Rat Virus, an agent with an affinity for the dividing cell. In: "Slow, Latent and Temperate Virus Infections", (D.C. Gadjusek, C.J., Gibbs, and M. Alpers, eds.), NINDB Monogr. 2, pp. 361-367. U.S. Department of Health, Education and Welfare.
60. Kilham, L. and Ferm, V.H. (1964). Rat Virus (RV) infections of pregnant, fetal and newborn rats. Proc. Soc. Exptl. Biol. Med. 117: 874-879.
61. Baer, P.N. and Kilham, L. (1962). Rat Virus and periodontal disease. I. The periodontium in the mongoloid hamster. Oral. Surg., Oral Med., and Oral Path. 15: 756-763.
62. Baer, P.M. and Kilham, L. (1962). Rat Virus and periodontal disease. II. Onset and effect of age at time of inoculation. Oral. Surg., Oral Med., and Oral. Path. 15: 1302-1311.
63. Baer, P.N. and Kilham, L. (1964). Rat Virus and periodontal disease. IV. The aged hamster. Oral. Surg. 18: 803-811.

64. Ferm, V.H. and Kilham, L. (1965). Skeletal studies of virus-induced dwarfism. Growth 29: 7-16.
65. Galton, M. and Kilham, L. (1966). Chromosomes of "Mongoloid" hamsters. Proc. Soc. Exptl. Biol. Med. 122: 18-22.
66. Toolan, H.W. (1978). Maternal role in susceptibility of embryonic and newborn hamsters to H-1 parvovirus. In: "Replication of Mammalian Parvoviruses: (D.C. Ward and P. Tattersall, eds.), pp. 161-176. Cold Spring Harbor Press.
67. Kilham, L. (1961). Rat Virus (RV) infections in the hamster. Proc. Soc. Exptl. Biol. Med. 106: 825-829.
68. Kilham, L. and Margolis, G. (1964). Cerebellar ataxia in hamsters inoculated with Rat Virus. Science 143: 1047-1048.
69. Kilham, L. and Margolis, G. (1966). Viral etiology of spontaneous ataxia of cats. Am. J. Pathol. 48: 991-1011.
70. Soike, K.F., Iatropoulis, M. and Siegl, G. (1976). Infection of newborn and fetal hamsters induced by inoculation of LuIII parvovirus. Arch. Virol. 51: 235-241.
71. Margolis, G. and Kilham, L. (1972). Rat Virus infection of megakaryocytes: a factor in hemorrhagic encephalopathy? Exptl. Molec. Path. 16: 326-340.
72. Margolis, G., Kilham, L. and Ruffolo, P.R. (1968). Rat virus disease as an experimental model of neonatal hepatitis. Exptl. Molec. Pathol. 8: 1-20.
73. Ruffolo, P.R., Margolis, G. and Kilham, L. (1966). The induction of hepatitis by prior partial hepatectomy in resistant adult rats injected with H-1 virus. Am. J. Pathol. 49: 795-824.
74. Kilham, L., Margolis, G. and Colby, E.D. (1970). Enhanced proliferation of H-1 virus in livers of rats infected with Cysticercus fasciolaris. J. Inf. Dis. 121: 648-652.
75. Engler, W.O., Baer, P.N. and Kilham, L. (1966). Effects of Rat Virus on healing osseous wounds. Arch. Path. 82: 93-98.
76. Porter, D.C., and Cho, H.J. (1980). Aleutian Disease of Mink: a model for persistent infection. In: "Comprehensive Virology" Vol. 16 (H. Fraenkel-Conrat and R.R. Wagner, eds.), pp. 233-256, Plenum Press, New York.
77. Ron, D., Tattersall, P. and Tal, J. (1984). Formation of a host range mutant of the lymphotropic strain of Minute Virus of Mice during persistent infection of mouse L cells. J. Virol. 52: 63-69.

78. Mohanty, S.B. and Bachmann, P.A. (1974). Susceptibility of fertilized mouse eggs to Minute Virus of Mice. Inf. Immun. 9: 762-763.
79. Miller, R.A., Ward, D.C. and Ruddle, F.H. (1977). Embryonal carcinoma cells (and their somatic cell hybrids) are resistant to infection by the murine parvovirus MVM, which does infect other teratocarcinoma-derived cell lines J. Cell. Physiol. 91: 393-402.
80. Tattersall, P. (1978). Susceptibility to Minute Virus of Mice as a function of host-cell differentiation. In: "Replication of Mammalian Parvoviruses" (D.C. Ward and P. Tattersall, eds.), pp. 131-149. Cold Spring Harbor Press.
81. Baer, P.N. and Kilham, L. (1974). Dental defects in hamsters infected with minute virus of mice. Oral. Surg. 37: 385-389.
82. Toolan, H.W. (1961). Studies on a viral agent associated with human tissues. Proc. Am. Assoc. Cancer Res. 3: 273.
83. Toolan, H.W. (1967). Lack of oncogenic effect of the H-viruses. Nature; 214: 1036.
84. Bergs, V.V. (1969). Rat virus-mediated suppression of leukemia induction by Moloney virus in rats. Cancer Research 29: 1669-1672.
85. Toolan, H.W. and Ledinko, N. (1968). Inhibition by H-1 virus of the incidence of tumors produced by adenovirus 12 in hamsters. Virology 35: 475-478.
86. Toolan, H.W., Rhode, S.L. and Gierthy, J.F. (1982). Inhibition of 7,12-Dimethylbenz(a) anthracene-induced tumors in Syrian hamsters by prior infection with H-1 parvovirus. Cancer Research 42: 2552-2555.
87. Ledinko, N. and Toolan, H.W. (1968). Human adenovirus type 12 as a "helper" for growth of H-1 virus. J. Virol. 2: 155-156.
88. Ledinko, N., Hopkins, S. and Toolan, H. (1969). Relationship between potentiation of H-1 growth by human adenovirus 12 and inhibition of the "helper" adenovirus by H-1. J. Gen. Virol. 5: 19-31.
89. Ledinko, N. and Toolan, H. (1970). Relationship between induction of thymidine kinase and potentiation of growth of H-1 virus by human adenovirus 12. J. Gen Virol. 7: 263-266.
90. Mousset, S. and Rommelaere, J. (1982). Minute virus of mice inhibits cell transformation by simian virus 40. Nature 300: 537-539.

91. Bonnard, G.D., and Herberman, R.B. (1975). Suppression of the generation of cytotoxic lymphoblasts by murine lymphoma cells. Fed. Proc. (FASEB) 34: 1002.
92. McMaster, G.K., Beard, P., Engers, H.D. and Hirt, B. (1981). Characterization of an immunosuppressive parvovirus related to the minute virus of mice. J. Virol. 38: 317-326.
93. Engers, H.D., Louis, J.A., Zubler, R.H. and Hirt, B. (1981). Inhibition of T cell-mediated functions by MVM(i), a parvovirus closely related to Minute Virus of Mice. J. Immunol. 127: 2280-2285.
94. Molitor, T., Smith, A.L., and Tattersall, P., unpublished observations.
95. Smith, A.L., Bottomly, K., Winograd, D.F., Tattersall, P.J., Barthold, S.W., Saldamarco, S., and Brownstein, D.G. Splenocyte proliferative responses and production of soluble mediators are altered in BALB/cByJ mice infected with mouse hepatitis virus, minute virus of mice or sendai virus. Manuscript in preparation.
96. Molitor, T. and Tattersall, P., unpublished observations.
97. Cotmore, S.F. and Tattersall, P., unpublished observations.
98. Siegl, G. and Kronauer, G. (1980). A plaque assay for feline panleukopenia virus. J. Gen. Virol. 46: 211-218.
99. Tokuhisa, S., Inaba, Y., Sato, K., Miura, Y., Akashi, H., Satoda, K. and Matumoto, M. (1981). Inhibitors of bovine parvovirus, coronavirus and rotavirus in precolostral and fetal bovine sera. Vet. Microbiol. 6: 143.
100. Jacoby, R.O., Bhatt, P.N. and Jonas, A.M. (1979). Viral Diseases. In: "The Laboratory Rat" (H.J. Baker, J.R. Linsey and S.H. Weisbroth, eds.), Vol. 1, pp. 271-306, Academic Press, N.Y.
101. Smith, A.L. Methods with potential for application to rodent virus isolation and identification. Chapter 35, this volume.
102. Smith, A.L. Serological tests for antibody detection to rodent viruses. Chapter 34, this volume.
103. Smith, A.L. (1985). An enzyme immunoassay for identification and quantification of infectious murine parvovirus in cultured cells. J. Virol. Methods, 11: 321-327.
104. Tattersall, P. and Bratton, J., unpublished observations.

105. Ward, D.C. and Tattersall, P.J. (1982). Minute Virus of Mice. In: "The Mouse in Biomedial Research" (H.L. Foster, J.D. Small and J.G. Fox, eds.), Vol. II, pp. 313-334, Academic Press.
106. Cross, S.S. and Parker, J.C. (1971). Some antigenic relationships of the murine parvoviruses: Minute Virus of Mice, Rat Virus and H-1 Virus. Proc. Soc. Exptl. Biol. Med. 139: 105-108.
107. Cotmore, S.F., Sturzenbecker, L.J. and Tattersall, P. (1983). The autonomous parvovirus MVM encodes two nonstructural proteins in addition to its capsid polypeptides. Virology 129: 333-343.
108. Smith, A.L., personal communication.