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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
EMERGENCE, NATURAL HISTORY, AND VARIATION
OF CANINE, MINK, AND FELINE PARVOVIRUSES

Colin R. Parrish

James A. Baker Institute
New York State College of Veterinary Medicine
Cornell University
Ithaca, New York 14853

I. Introduction
   Feline Panleukopenia Virus, Mink Enteritis Virus, and Canine Parvovirus

II. Historical Background
   A. Feline Panleukopenia Virus
   B. Mink Enteritis Virus
   C. Canine Parvovirus

III. Diseases
   A. Older Animals
   B. Fetal or Neonatal Infections
   C. Immunity
   D. Natural Host Ranges

IV. Antigenic Relationships
   A. Polyclonal Sera
   B. Monoclonal Antibodies: Antigenic Structure and Variation among Virus Types

V. Antigenic Variation and Genome Organization
   A. MEV Variation
   B. Natural Variation of CPV Strains
   C. Mutants of Viruses
   D. Genome Organization

VI. Other Properties of FPV, MEV, and CPV
   A. Hemagglutination Dependence on pH
   B. Experimental Host Range Properties

VII. Genetic Analysis and Variation among the Viruses
   A. Restriction Mapping
   B. DNA Sequence Analysis

VIII. Genetic Analysis of Antigenic, Host Range, and Other Functions within the
      VP-1/VP-2 Genes
       Mapping CPV-Specific Properties

IX. Virus Sequence Variation and Phylogenetic Relationships

X. Conclusions

References
I. Introduction

During 1978 a new parvovirus of dogs (canine parvovirus (CPV)) was recognized simultaneously as the cause of new diseases of dogs throughout the world. Within 2 years the virus had spread into and infected virtually every population of domestic and wild dogs which has been examined. The diseases were new syndromes for dogs, and serological testing has shown that the virus was not present in dogs before the mid-1970s. The sudden appearance of CPV has raised a number of important questions about the mutability and variation of the parvoviruses, and about its relationships to the closely related viruses of cats and minks. At a fundamental level the mechanisms which define the host range of the viruses were in need of examination. This chapter examines the emergence of CPV, the evidence concerning the previous emergence of mink enteritis virus (MEV) as the cause of a new disease in minks in the 1940s, and the mechanisms which determine the host ranges and other specific properties of the viruses of cats, minks, and dogs.

The host range of animal viruses is one of their most fundamental characteristics, as it determines the range of susceptible animals. Most viruses are restricted in host range and infect only a defined spectrum of susceptible species. The emergence of new viruses and the acquisition of new natural host ranges are unusual events. However, such new viruses have the potential to cause pandemics in their new nonimmune hosts. The emergence of viruses from other animal reservoirs is clearly involved in the evolution of new pandemic strains of influenza in humans (Webster et al., 1982; Palese and Young, 1982) and could be the origin of human immunodeficiency virus. It is likely that such events have occurred in the past to give rise to the present diversity of animal viruses.

**Feline Panleukopenia Virus, Mink Enteritis Virus, and Canine Parvovirus**

The viruses are classified as the feline parvovirus subgroup of the genus *Parvovirus*, within the family Paroviridae (Siegl et al., 1985). Feline panleukopenia virus (FPV), MEV, and CPV are classified as "host range variants." In addition to the viruses of cats, minks, and dogs, similar viruses naturally infect many species within the families Felidae, Canidae, Procyonidae, Mustelidae, and possibly the Viveridae. Here, canine parvovirus is referred to as CPV, except when it is necessary to distinguish between the two antigenic forms of the virus,
which are referred to as CPV type 2 (CPV-2) and CPV type 2a (CPV-2a) (see Section V,B).

II. HISTORICAL BACKGROUND

A. Feline Panleukopenia Virus

A disease similar to that caused by FPV has been known in both large and small cats for many years. The agent was shown to be a filterable virus during the 1920s (Verge and Cristoforoni, 1928) and was the subject of considerable study during the 1930s and 1940s (Hindle and Findlay, 1932; Lawrence and Syverton, 1938; Lawrence et al., 1940, 1943; Hammon and Enders, 1939a,b; Kikuth et al., 1940; Torres, 1941; Lucas and Riser, 1945). In these studies it was determined that a number of diseases, which had been variously described as feline distemper, spontaneous agranulocytosis, feline infectious enteritis, or malignant panleukopenia, were all caused by the same agent (Hammon and Enders, 1939a). Similar diseases in common raccoons (Procyon lotor) and Arctic (blue) foxes (Alopex lagopus) were known before 1940, the viruses involved being considered the same as FPV (Waller, 1940; Phillips, 1943; Goss, 1948).

B. Mink Enteritis Virus

In 1947 a new disease was observed among minks in the area of Fort William, Ontario, Canada (Schofield, 1949; reviewed by Burger and Gorham, 1970). Initially named Fort William disease and later called mink viral enteritis, the agent was described as MEV. The relationships between mink viral enteritis and FPV were recognized early, due to the close similarities between the clinical signs and the characteristic pathology of the two diseases (Wills, 1952), and the antigenic similarity between the viruses was demonstrated by cross-protection studies during the early 1950s (Wills, 1952; Wills and Belcher, 1956). Early studies indicated that the experimental inoculation of minks with tissues from FPV-infected cats gave a similar disease to that caused by MEV (MacPherson, 1956), although in preliminary studies inoculations of kittens with MEV-containing tissues did not show any clinical disease. More recent studies have examined the in vivo host ranges and virulence of the viruses in more detail (see Section II,D).

The origin of MEV in Ontario has not been determined. Within 10–12 years the disease had been observed among farmed mink
throughout the United States, Europe, and Scandinavia (Knox, 1960; Tuomi and Kangas, 1963; Kull, 1966). Studies of MEV were hampered until the mid-1960s by the inability to grow the virus in vitro and by a lack of sensitive or specific serological tests. It is therefore not known whether MEV was a long-existing virus which had been present in minks many years before 1947 or whether it had only recently acquired the host range for minks at that time. FPV and MEV were both isolated in tissue culture during the 1960s (Johnson, 1964, 1965; Lust et al., 1965; King and Croghan, 1965). The delay in isolating the viruses was probably due to the fact that they replicate only in dividing cells and cause a slow and nondescript cytopathic effect in infected cell cultures. However, the identification of characteristic Feulgen test-positive intranuclear inclusion bodies and fluorescent antibody staining methods for identifying infected cells allowed the viruses and antibody responses to be analyzed and titrated (Johnson, 1965, 1967c; Lust et al., 1965; Gorham et al., 1966; Scott et al., 1970).

During the 1960s and 1970s studies of FPV and MEV revealed them to be paroviruses and to be closely related biochemically and serologically (Johnson, 1967a; Johnson et al., 1974; Flagstad, 1975, 1977). Effective vaccines were developed which controlled the diseases in cats and minks (Wills and Belcher, 1956; Pridham and Wills, 1959, 1960; Burger et al., 1963; Gorham et al., 1965; King and Gutekunst, 1970; Davis et al., 1970).

C. Canine Parvovirus

During 1978 and 1979 outbreaks of previously unrecognized disease syndromes were observed in dogs in a number of countries (Eugster et al., 1978; Appel et al., 1978, 1979a; Burtonboy et al., 1979; Gagnon and Povey, 1979; Horner et al., 1979; Johnson and Spradbrow, 1979). The diseases were characterized by death due to acute or chronic nonsuppurative myocarditis in pups 3–16 weeks of age or pyrexia accompanied by vomiting and/or diarrhea in older animals.

The enteritis and pathological signs observed were similar to those of FPV in cats or MEV in minks, with necrosis of the intestinal crypt epithelium, eventual loss of the villus structure in the small intestine, and necrosis and depletion of the lymphocytes in various lymphoid tissues. Within a few months of the first observation of the diseases, a parovirus resembling FPV was isolated or observed by electron microscopy in samples from dogs with myocardial or enteric forms of the disease. The ability of the virus from a case of myocarditis to cause enteritis was also demonstrated (Robinson et al., 1979a, 1980b). The close relationship of this virus to FPV was recognized early (Appel et
PARVOVIRUS EMERGENCE AND VARIATION

al., 1978, 1979a; Johnson and Spradbrow, 1979), and effective vaccines were developed within a short period (Eugster, 1980; Carmichael et al., 1981, 1983).

The global spread of CPV has been examined by the analysis of stored sera collected from dogs during the 1970s. The earliest CPV-positive sera reported were collected in Greece during 1974, and the reactivities of the three positive sera of 28 dogs examined from that year were confirmed by virus neutralization assays (Koptopoulos et al., 1986). Positive sera were collected in Belgium and The Netherlands in late 1976 and during 1977 (Schwers et al., 1979; Osterhaus et al., 1980). The virus spread rapidly around the world during 1978, the first positive sera reported being collected in Australia during May 1978, in the United States during June 1978, in Denmark between January and June 1978, in New Zealand between July and October 1978, and in Japan during July 1978 (Walker et al., 1980; Carmichael et al., 1980; Have and Andersen, 1982; Jones et al., 1982; Azetaka et al., 1981; Mohri et al., 1982).

Studies of sera collected from 1184 wild coyotes (Canis latrans) in three regions of the United States between 1972 and 1983 showed the first positive sera to be present during 1979, and by 1981 the majority of the animals in all areas were seropositive (Thomas et al., 1984). The first positive sera from a population of wild wolves in Alaska were collected during 1980 (Zarnke and Ballard, 1987).

The rapid global spread of the virus was most likely a consequence of the high titers of virus shed in the feces of infected dogs and the resistance of these parvoviruses to inactivation in the environment, which would have allowed its transport, probably on inanimate objects, even into countries with strict quarantine procedures for dogs (Bouillant and Hanson, 1965; Carmichael et al., 1981; Pollock, 1982; Meunier et al., 1985a).

III. DISEASES

The pathogenesis of diseases in animals of various ages is influenced primarily by the requirement of autonomous parvoviruses for actively dividing cells (Margolis and Kilham, 1965; Tennant et al., 1969; Tattersall, 1972). It is also likely that this requirement determines many of the differences seen between infections of fetal, neonatal, or older animals. However, among the dividing cells in any animal, not all are permissive for virus replication. For example, the lymphoid and intestinal epithelial tissues, which contain rapidly dividing cell populations, are targets for virus replication by FPV, MEV,
and CPV, but appear to be resistant to infections by many parvo-
viruses in other species, such as the minute virus of mice (MVM) and
H1 virus in hamsters, indicating that developmentally regulated prop-
erties of the different dividing cell populations can restrict parvovirus
replication at the cellular level (reviewed by Cotmore and Tattersall,
1987). Although the relationships between cell replication rates and
the susceptibility of tissues to parvovirus infection and replication in
dogs, minks, or cats have not been examined in detail, it is clear that
there are differences between the infections of animals at different
ages.

A. Older Animals

In animals older than 6 weeks at the time of infection, the patho-
genoses of infections of cats with FPV, minks with MEV, or dogs with
CPV are similar, and the diseases in all species are therefore described
together. Serological studies indicate that many infections by CPV in
dogs (and probably by FPV and MEV in their respective hosts) are
mild or subclinical (Smith et al., 1980; Meunier et al., 1980; Parrish
et al., 1982a). Signs seen in affected animals include pyrexia, diarrhea,
and vomiting, along with leukopenia and/or a relative lymphopenia.
The incidence of leukopenia or lymphopenia varies among the differ-
ent viruses, panleukopenia being a striking feature of FPV infections
of cats, but is uncommon in CPV infections, although a relative lym-
phopenia is often observed in the latter case (FPV: Hammon and En-
ders, 1939a,b; Lawrence et al., 1940; Rohovsky and Griesemer, 1967;
Larsen et al., 1976; Ichijo et al., 1976; Carlson et al., 1977; MEV: Rey-
nolds, 1969; CPV: Robinson et al., 1980b; Carmichael et al., 1981; Pol-
lock, 1982; Macartney et al., 1984a). Whether this difference reflects
differences in the virus or the host is not known. Death of severely
affected animals is likely to be a consequence of the extensive destruc-
tion of the gut epithelium, with dehydration and endotoxic shock con-
tributing to the disease.

Although the prominent clinical sign observed after infection is
most likely to be enteritis, the virus replicates in many organs of the
infected host. The course of infection is rapid, with little virus being
recovered from tissues or feces after 10–14 days postinfection (FPV:
Csiza et al., 1971a; CPV: Macartney et al., 1984b). Natural infection is
most likely via the oronasal route (FPV; Csiza et al., 1971a; MEV:
Myers et al., 1959; Reynolds, 1970; CPV: Appel et al., 1979b; Carman
and Povey, 1982; Pollock, 1982; Macartney et al., 1984a), although
animals can be infected by most parenteral routes. After oral or
oronasal inoculation initial virus replication occurs in the tonsils and
the regional and mesenteric lymph nodes, and shortly thereafter in the thymus and the spleen (FPV: Csiza et al., 1971a; Carlson et al., 1977, 1978; CPV: Macartney et al., 1984b; Meunier et al., 1985a; Carman and Povey, 1985b). Between 4 and 6 days after oral inoculation, infected cells are present in the intestine, and after day 5 postinoculation virus is excreted in large amounts in the feces; in the case of CPV between $10^7$ and $10^9$ infectious units may be excreted per gram of feces (Car-michael et al., 1981; Meunier et al., 1985a,b; Carman and Povey, 1985a).

In all species, infection of the lymphoid tissues (e.g., the thymus, lymph nodes, and spleen) results in lymphocytolysis, cellular depletion, and, subsequently, tissue regeneration in surviving animals. Virus replication and cell destruction in lymphoid tissues occur mostly in areas of dividing cells, including germinal centers of the lymph nodes and the thymus cortex (Fig. 1) (FPV: Hammon and Enders, 1939a,b; Lawrence et al., 1940; Kikuth et al., 1940; Carlson et al., 1977, 1978; MEV: Reynolds, 1970; Krunajevic, 1970; CPV: Cooper et al., 1979; Robinson et al., 1980b; Macartney et al., 1984a,b; Carman and Povey, 1985a,b).

The bone marrow can be affected severely, with a marked decrease in cellularity and decreases in cells of the myeloid, erythroid, and megakaryocytic series (FPV: Hammon and Enders, 1939a,b; Lawrence et al., 1940; CPV: Robinson et al., 1980b; Boosingher et al., 1982; Macartney et al., 1984a; Carman and Povey, 1985b). Although endotoxemia could play a role in the development of severe bone marrow lesions, both virus and infected cells are present in the bone marrow of CPV-infected dogs (Macartney et al., 1984b; O'Sullivan et al., 1984; Meunier et al., 1985a).

In the gut the virus replicates in and destroys the rapidly dividing cells in the intestinal crypts. The subsequent loss of newly formed epithelium results in a flattened attenuated epithelium, with shortening of the intestinal villi (Figs. 2 and 3) (FPV: Rohovsky and Griese-mer, 1967; Larsen et al., 1976; Okaniwa et al., 1976; MEV: Landsverk and Nordstoga, 1978; CPV: Cooper et al., 1979; Yasoshima et al., 1982; Macartney et al., 1984c). These changes might lead to a loss of osmotic regulation, resulting in diarrhea, often with blood and mucus, as well as vomiting. Animals might become dehydrated and/or pyrexic, possibly because of endotoxin uptake from the gut.

The rate of intestinal epithelial cell turnover affects the severity of the lesions, probably by determining the number of cells able to replicate virus. This effect was demonstrated during infections of germ-free cats (Rohovsky and Grieseimer, 1967; Carlson et al., 1977), or after the treatment of cats with acid enemas which increased cell replication and
FIG. 1. Sections of (A) thymus or (B) mesenteric lymph node from dogs 5 days after parenteral inoculation with CPV and staining with a fluorescent antibody against CPV. The distribution of antigen in the thymus cortex and surrounding the lymph node germinal centers is shown. Infected cells contain intranuclear antigen, while many cells containing only cytoplasmic antigen most likely contain phagocyted proteins.
Lesions in the small intestine of a dog after inoculation with CPV. (A) The observed patterns of intestinal lesions. (B) Duodenal mucosa with lesions of pattern B. Villi are stunted and occasionally fused (arrow). The crypts are dilated and lined with attenuated epithelial cells. Neutrophils (n) are present in the mucus overlying the affected mucosa. (C) Jejunal mucosa with lesions of pattern D: regenerating villi. The villi are short and covered with cuboidal epithelium, and the crypts are elongated and lined with cuboidal epithelial cells. From Macartney et al. (1984b) with permission.
subsequent FPV replication in the colon (Shindel et al., 1978). A correlation was observed between the viral titers in serum and feces and the severity of the disease observed in dogs inoculated with CPV (Meunier et al., 1985b). Intestinal parasites have been suggested to play a role in the natural pathogenesis of CPV infections (Chalifoux et al., 1981; Pollock, 1982).

B. Fetal or Neonatal Infections

1. Feline Ataxia

In 1967 FPV was identified as the cause of an uncharacterized feline cerebellar ataxia, which had previously been thought to be a genetically inherited disease (Johnson et al., 1967; Kilham et al., 1967). When kittens were infected either in utero or shortly after birth, viral
Fig. 3. Scanning electron micrographs of (A) the intestinal epithelium of the jejunum of a normal dog and (B) a dog killed 6 days after oral inoculation with CPV. In the latter case severely affected areas have completely lost the surface epithelium. Numerous crypt openings (c) are seen in the specimen from the control dog. From Macartney et al. (1984c) with permission.
replication in the cells of the external germinal epithelium of the developing cerebellum resulted in cerebellar hypoplasia (Csiza et al., 1971a; Kilham et al., 1971). While some kittens developed a widespread infection, leading to fetal death (Kilham et al., 1967), most viable kittens subsequently suffered from ataxia (Kilham et al., 1967; Csiza et al., 1971a,b). Although ferrets are probably not a normal host for FPV (Parrish et al., 1987; Veijalainen, 1986), ferrets inoculated with FPV prenatally or shortly after birth by the intracranial or other parenteral routes develop similar cerebellar lesions (Kilham et al., 1967; Duenwald et al., 1971).

2. Canine Myocarditis

Initial reports of CPV disease described outbreaks of acute myocarditis in puppies, mostly between 3 and 8 weeks old, although some animals were up to 16 weeks of age at the time of death (Kelly and Atwell, 1979; Huxtable et al., 1979; Robinson et al., 1979a,b, 1980a; Hayes et al., 1979; Jesyk et al., 1979; Carpenter et al., 1980). Mortality in litters varied between 20% and 100%, and disease onset in apparently normal puppies was rapid, with pups dying of acute heart failure characterized by cardiac arrhythmia, dyspnea, and pulmonary edema (Robinson et al., 1979b, 1980a; Hayes et al., 1979; Jesyk et al., 1979; Parrish et al., 1982a). Electrocardiographically, affected pups showed a variety of subclinical abnormalities, and death appeared to result from progressive muscle necrosis, leading to ventricular fibrillation (Robinson et al., 1979b; Carpenter et al., 1980).

The primary pathological lesion observed was multifocal necrosis of the myocardium, often with a mononuclear cell infiltrate. Myocardial cells often contained intranuclear Feulgen test-positive amphophilic inclusion bodies. Lungs were diffusely edematous, with peribronchial and perivascular edema, most likely secondary to the heart failure (Robinson et al., 1980a; Jesyk et al., 1979; Carpenter et al., 1980).

In a prospective study of CPV infections in a dog colony (Parrish et al., 1982a) and in experimental infections of pups (Meunier et al., 1984), it was shown that pups that developed myocarditis were infected either in utero or shortly after birth, often several weeks before the acute deaths due to heart failure, suggesting a chronic progression of the heart muscle damage. The age dependence of susceptibility of the myocarditis is probably related to the active cell division of the myocardial cells, which appears to occur only in pups under 15 days of age (Bishop, 1972).

It is of interest that there are no reports of cerebellar hypoplasia in dogs or myocarditis in cats infected with CPV or FPV, respectively. Although virus-infected cells have been observed within the heart
muscle cells of neonatal kittens after experimental FPV infections (Csiza et al., 1971b), disease such as that in the puppies infected with CPV has not been described. It is not known whether the difference in the pathogeneses in neonatal cats and dogs is a consequence of differences in the susceptibility of the host cells and tissues or to some other difference in virus tissue tropism.

Occasional neonatal infections give rise to a generalized infection, lesions being observed in many different tissues (Lenghaus and Studdert, 1982; Johnson and Castro, 1984). In utero infections in cats or Arctic foxes by FPV or blue fox parvovirus (BFPV), respectively, could result in fetal death and resorption, abortion, or neonatal death (Kilham et al., 1967, 1971; Veijalainen and Smeds, 1988). However, natural infections of dogs by CPV did not appear to affect reproduction (Meunier et al., 1980).

C. Immunity

The functional immunity against these viruses appears to be mediated through serum antibodies. Pups or kittens which acquire maternal immunity from their dams are protected against parvovirus infection until their serum antibody titers decline to low levels [Brun et al., 1979 (FPV); Parrish et al., 1982a; Pollock and Carmichael, 1982; Ishibashi et al., 1983]. In addition, after parenteral administration of anti-CPV antibodies to dogs, the animals were completely protected against oral challenge, and no virus replication was seen in the intestinal epithelium 4–6 days after infection (Meunier et al., 1985a).

Whether secretory antibodies play any role in immunity to or recovery from disease is unclear, although it has been shown that levels of coproantibody correlated inversely with the severity of the disease in dogs naturally infected with CPV (Rice et al., 1982), and the levels and classes of antibody in the jejunum collected by cannulation after either CPV infection or vaccination suggested that the antibody was being specifically secreted (Nara et al., 1983).

D. Natural Host Ranges

The natural host ranges of these viruses have not been well defined. Little of the available information has been independently confirmed, and some of the results appear contradictory or unexpected. Since natural infections by contaminating viruses are an ever-present risk in experimental studies, and the viruses are not readily characterized without monoclonal antibody reagents or restriction enzyme mapping of viral DNAs, the host ranges that have been described should be
regarded as tentative in many cases until further confirmations are available.

Early studies were limited by the fact that virus could then be detected only by animal infections, so that virus or viral replication was only detected if it caused clinical disease. More recent studies have enabled the virus replication to be examined in more detail.

The natural hosts of FPV, MEV, and CPV are, by definition, the cat, mink, and dog, respectively (Siegl et al., 1985). Viruses isolated from or observed in other species have not been systematically classified and are therefore named either after a virus from a related host species or after the host animal from which they were isolated; for example, raccoon parvovirus (RPV), BFPV, and raccoon dog parvoviruses (RD) have all been described. The genetic and biological relationships among the viruses from the various hosts, their natural host ranges, and pathogenic potentials are poorly understood.

All hosts are members of five families (i.e., Felidae, Canidae, Procyonidae, Mustelidae, and Viverridae) within the order Carnivora, although within the various families only certain genera or species have been reported to be susceptible. A recently described phylogeny of the Carnivora based on thermal stability of DNA/DNA hybrids is shown in Fig. 4 (Wayne et al., 1989).

Virtually all large and small cats (i.e., several genera of the family Felidae) appear to be susceptible to FPV (Hindle and Findlay, 1932; Goss, 1948; Johnson, 1964). Within the family Procyonidae common raccoons (P. lotor) have been known to be susceptible to FPV since before 1940 (Waller, 1940; Goss, 1948; Nettles et al., 1980) and are also highly susceptible to both virus replication and disease after inoculation with MEV or FPV (Barker et al., 1983). Raccoons are not susceptible to CPV (Appel and Parrish, 1982). Ringtail coatis (family Procyonidae, Nasua nasua) are also reported to be naturally susceptible to FPV (Johnson and Halliwell, 1968). Civets (family Viverridae) are affected by a disease similar to that caused by FPV in cats (Nain et al., 1964).

Among the family Mustelidae only MEV has been described. Domesticated ferrets (Mustela putorius) appear to be susceptible to infection by FPV when inoculated in utero or by the intraperitoneal route 1–2 days after birth, and can develop cerebellar hypoplasia (Kilham et al., 1967; Duenwald et al., 1971). No such effect was seen when ferrets were 3 days of age or older (Duenwald et al., 1971; Parrish et al., 1987), and no serological evidence for natural infections by other similar parvoviruses has been reported (Veijalainen, 1986). Serological evidence for infection of wild striped skunks (Mephitis mephitis) has been reported, but a virus has not been isolated or characterized. CPV, FPV,
The hosts of the FPV subgroup of parvoviruses include members of the families Canidae [represented, for example, by the dog, black-backed jackal (Canis mesomelas), and Arctic fox], Mustelidae (e.g., mink), Procyonidae (e.g., raccoon), Viverridae (e.g., civet), and Felidae (e.g., domestic and other cats). From Wayne et al. (1989) with permission.

or MEV isolates did not replicate in skunks after experimental inoculation, and the animals developed only low or undetectable antibody responses (Barker et al., 1983).

Farmed American minks (Mustela vison) are susceptible to experimental infection by both FPV and MEV. The viruses replicate in a number of organs and are shed in the feces of many animals. Although early reports suggested that FPV isolates could cause clinical disease in mink (MacPherson, 1956), more recent studies, in which virus replication was more carefully monitored by titration, showed that only actual isolates from minks caused significant clinical disease in minks, while most infections by FPV or RPV were subclinical. In the
same studies CPV isolates replicated to only low titers in minks after experimental parenteral inoculation (Barker et al., 1983; Parrish et al., 1987). It therefore appears that the principal difference between FPV and MEV in minks is manifested by a difference in virulence for minks, rather than host range.

The susceptibility of the members of the family Canidae has not been clearly defined, although it appears that, besides domestic dogs, most, if not all, members of the genus *Canis* are susceptible to CPV (coyote: Evermann et al., 1980; Thomas et al., 1984; gray wolf: Mech et al., 1986; Zarnke and Ballard, 1987). Other Canidae that are naturally susceptible to CPV include the maned wolf (*Chrysocyon brachyurus*) (Fletcher et al., 1979), the bushdog (*Speothos venaticus*) (Mann et al., 1980; Janssen et al., 1982), the crab-eating fox (*Cerdocyon thous*) (Mann et al., 1980), and the raccoon dog (*Nyctereutes procyonoides*) (Neuvonen et al., 1982; Veijalainen, 1986).

Among the foxes, which are all members of the family Canidae, the red fox (*Vulpes vulpes*) and Arctic fox (*A. lagopus*) appear to be susceptible to infection by a virus distinct from CPV, which might be FPV or a virus closely related to it (Phillips, 1943; Neuvonen et al., 1982; Barker et al., 1983; Veijalainen, 1986; Veijalainen and Smeds, 1988). In a serological survey of wild red foxes in Ontario, Canada, 79.4% had antibodies to a virus related to CPV or FPV (Barker et al., 1983). On experimental oral inoculation with CPV, MEV, or FPV isolates, red foxes did not develop clinical disease and all viruses were shed only in low titers, but the animals developed strongest serological responses against FPV (Barker et al., 1983).

### IV. Antigenic Relationships

#### A. Polyclonal Sera

Early studies of MEV and FPV indicated that antibodies formed to the two viruses protected animals against infection by CPV (Schofield, 1949; Wills, 1952; Wills and Belcher, 1956; Meyers et al., 1959). These studies depended on the production of clinical signs by the viruses as the indication of infection.

Cross-neutralization studies of MEV and FPV using *in vitro* culture revealed that antisera against the FPV and MEV strains examined gave similar neutralization titers against the alternative virus strain (Johnson, 1967a; Flagstad, 1975, 1977).

Several studies of the antigenic relationships between FPV or MEV and CPV were performed using polyclonal sera and revealed that the
viruses all gave similar titers in neutralization or hemagglutination inhibition tests, although there was an indication of higher titers in the homologous than in the heterologous reactions (Flower et al., 1980; Lenghaus and Studdert, 1980; Parrish et al., 1982b; Tratschin et al., 1982).

**B. Monoclonal Antibodies: Antigenic Structure and Variation among Virus Types**

The production of monoclonal antibodies (mAbs) against the various viruses has illuminated much of the complexity of the relationships among the various viruses.

Mouse mAbs against a purified 1978 CPV isolate revealed CPV-specific determinants, as well as determinants in common among CPV, FPV, RPV, and MEV (Parrish et al., 1982b; Burtonboy et al., 1982; Parrish and Carmichael, 1983; Surleraux et al., 1987). Six of eight rat mAbs made against a 1973 FPV isolate reacted with CPV, RPV, FPV, and some MEV isolates, while two reacted more strongly with the FPV and MEV isolates than with the CPV isolates (Fig. 5) (Parrish and Carmichael, 1983).

When the mAbs were used to examine the antigenic structure of the viral capsid, the results revealed a variety of overlapping epitopes (Parrish and Carmichael, 1983). The CPV-specific mAbs appeared to recognize a single antigenic determinant, since all CPV isolates gave similar reactivities (Fig. 5). Antigenically variant neutralization escape mutants of CPV selected with two CPV-specific mAbs lost reactivity to all five CPV-specific mAbs, as well as to a group of five virus type-common mAbs (Fig. 6). Selection of mutant viruses with three of these type-common mAbs revealed two types of variation. Selection with mAb 6 or 12 gave variants which lost reactivity only to the three virus type-common mAbs prepared against CPV (mAbs 6, 12, and 18). In contrast, selecting with mAb B resulted in viruses which had lost reactivity to the same group of five virus type-common mAbs that were selected against by the CPV-specific mAbs, as well as the five CPV-specific mAbs (Fig. 6) (Parrish and Carmichael, 1983).

The two FPV-specific mAbs (i.e., G and H) recognized two different epitopes, as they reacted differently with various MEV or RPV strains (Fig. 5). In addition these mAbs selected mutant viruses with different patterns of reactivity, and the escape mutants selected by one mAb did not lose reactivity to the other (Fig. 6). mAb G cross-reacted with CPV, although to a much lower titer than to FPV (Parrish and Carmichael, 1983).

The remaining virus type-common mAbs in this study (Parrish and
Fig. 5. Antigenic typing of FPV subgroup isolates. CPV (CPV-2 (a–d) and CPV-2a (e) strains), FPV, RPV, and MEV were titrated against mAbs, using the hemagglutination inhibition test. mAbs identified by numbers were prepared against CPV-a, and those identified by letters were prepared against FPV-b. Solid squares, at least 25% of the titer with the homologous virus; crosshatched squares, 5% to less than 25% of the titer with the homologous virus; open squares, less than 5% of the titer with the homologous virus, or no reaction. From Parrish and Carmichael (1983) with permission.

Carmichael, 1983) revealed a number of distinguishable epitopes when used to select variants. However, the patterns revealed by the analysis of naturally variant viruses (the MEV type 2 isolates, the CPV-102/10 mutant, and the CPV-2a variant) (Parrish et al., 1984, 1985; Parrish and Carmichael, 1983, 1986) suggest that these epitopes comprise only one or two distinct determinants (see Section V).

Competitive binding studies between 125I-labeled and unlabeled mAbs revealed the surface of the viruses to be comprised of two non-cross-competiting determinants, one recognized by mAbs 6, 12, and 18, and the other by the remaining mAbs in that panel (Fig. 7). The exten-
Fig. 6. Reactions of mAb-selected neutralization escape mutants of CPV-d or FPV-b in the hemagglutination inhibition assay or radioimmunoassay. X, mAb used for selection; solid squares, at least 25% of the titer with the original virus; crosshatched squares, 5% to less than 25% of the titer with the original virus; open squares, less than 5% of the titer with the original virus, or no detectable reaction. From Parrish and Carmichael (1983) with permission.

Sive cross-competition between mAbs was probably a consequence of the small size of the parvovirus capsid (i.e., about 257 Å from the initial crystallographic diffraction data of CPV crystals) (Luo et al., 1988)) and the number of protein subunits (most likely about 10 VP-1 and 60 VP-2) (Tattersall et al., 1976; Paradiso et al., 1982; Paradiso, 1983), suggesting that each epitope is repeated many times. The exposed area on each repeated subunit available for antibody binding would therefore be small compared to the size of an antibody molecule.

In the study described all mAbs both inhibited hemagglutination and neutralized the virus infectivity (Parrish et al., 1982b; Parrish and Carmichael, 1983). In other studies some mAbs were nonneutralizing or were neutralizing only when an anti-rat IgG second-stage antibody was added to the neutralization reaction (Burtonboy et al., 1982;
Fig. 7. Relationships between antigenic sites on CPV or FPV capsids revealed as binding of $^{125}$I-labeled mAbs in the presence of dilutions of unlabeled mAbs. X, $^{125}$I-labeled mAbs used in each set of tests; solid squares, efficient competition: less than 20% of the control CPM bound in the highest concentration of competing mAbs; hatched squares, partial blocking: 20–60% of the control counts per minute (CPM) bound in the highest concentration of competing mAbs; open squares, no competition: over 60% of the control CPM bound in the highest concentration of competing mAbs. From Parrish and Carmichael (1983) with permission.

Surleraux et al., 1987). Thirty-nine of 47 mAbs in the latter study were either directly or indirectly neutralizing, suggesting that the determinants were mostly exposed on the surface of the intact virion. Eight of the 47 mAbs were nonneutralizing under any of the conditions tested (Surleraux et al., 1987). Of the 47 mAbs 31 did not react in Western blots with proteins electrophoresed in sodium dodecyl sulfate–polyacrylamide gels and transferred to nitrocellulose sheets, and this group included the five CPV-specific mAbs in the study.

Of the 16 mAbs which reacted in Western blots, 12 reacted with both VP-2 and VP-3 in the expected molar ratio, three reacted only with VP-1, and one reacted only with VP-2. The mAb reactive only with VP-2 did not react with the blotted proteins after the digestion of full
virions with trypsin, suggesting that it reacted with an amino-terminal region of the protein affected by the trypsin-cleavable site in the VP-2 of DNA-containing virions (Surleraux et al., 1987; Clinton and Hayashi, 1976; Paradiso, 1981; Paradiso et al., 1984). The mAbs with the various reactivities in Western blot reactivities included neutralizing, indirectly neutralizing, and nonneutralizing antibodies.

The antigenic relationships between a parvovirus (RD) isolated from raccoon dogs and a parvovirus isolated from an Arctic fox (BFPV) were examined by mAb analysis (Veijalainen, 1988). Of 19 mouse mAbs against the RD, two were specific in the hemagglutination inhibition test for that virus; three reacted with RD and CPV to equal titers, but to lower titers with MEV isolates; and 14 reacted to equal titers with all virus types tested. Of 20 mouse mAbs prepared against BFPV, one reacted in the hemagglutination inhibition test only with BFPV and with an MEV isolate; five reacted with BFPV, FPV, and MEV isolates, but to lower titers with a 1982 isolate of CPV; and 15 reacted to equal titers with all virus types (Veijalainen, 1988). These results suggest that RD is similar or identical to CPV, but distinct from the FPV, MEV, and BFPV isolates. Conversely, BFPV appears to be closely related antigenically to FPV and MEV but distinct from CPV.

V. ANTIGENIC VARIATION AND GENOME ORGANIZATION

A. MEV Variation

When examined with mAb panels prepared against CPV or FPV, three antigenic types of MEV were observed among the MEV isolates collected in the United States and Europe during the 1960s, 1970s, and 1980s (Parrish and Carmichael, 1983). Antigenic type 1 MEV appeared to be similar to the FPV isolates examined, while MEV types 2 and 3 differed in their reactivities to various CPV, FPV, or MEV type-common mAbs. The type 3 MEV isolates reacted with only six of 16 FPV-reactive mAbs in the panel used, while the type 2 MEVs reacted with 12 of the 16 mAbs (see Fig. 5).

The MEV types 2 and 3 isolates could be readily distinguished from the type 1 viruses by digestion of viral replicative-form DNA with various restriction endonucleases (Parrish et al., 1984). The TaqI restriction patterns are shown in Fig. 8. The epidemiological significance of the MEV antigenic variation is not known. In cross-protection studies minks immunized with inactivated preparations of any of the three MEV types were protected against parenteral challenge by any of the alternative antigenic types (Parrish et al., 1984).
Fig. 8. Digests of replicative-form DNA from various MEV isolates with the Taql restriction enzyme. The DNA was electrophoresed in a 2% agarose gel and stained with ethidium bromide. Antigenic type 1 MEV isolates (strains a, i, g, e, and f) can be distinguished from the type 2 (strains b, h, c, d, and l) and type 3 (strains j and k) isolates. From Parrish et al. (1984) with permission.

B. Natural Variation of CPV Strains

Analysis of CPV isolates collected after 1978 revealed that viruses collected after 1980 did not react with several mAbs which reacted with the pre-1980 virus isolates (Parrish et al., 1985). Originally described as pre-1980 and post-1980, or old and new, antigenic types, these types were later designated CPV-2 and CPV-2a, respectively (Parrish et al., 1988b,c). A panel of nine mouse mAbs prepared against a 1984 isolate of CPV-2a contained three mAbs which reacted with CPV-2a isolates but not with CPV-2 isolates (Fig. 9). The CPV-2a and CPV-2 isolates also differed in the patterns of their DNA after digestion with the HphI restriction endonuclease (Fig. 10) (Parrish et al.,
### FIG. 9. Reactions of CPV isolates from 1978 to 1984 with mAbs made against either a 1978 isolate of CPV (CPV-a) or FPV-b (A) or against a 1984 CPV isolate (CPV-39). Shaded are those reactions of mAbs which distinguish CPV-2 (1978-1980) from CPV-2a (1981-1984) isolates. The reactions of FPV-c are also shown. From Parrish et al. (1985) with permission. Copyright 1985 by the AAAS.

Testing sera collected from wild (hence, nonvaccinated) coyotes in Utah, Texas, and Idaho (Thomas et al., 1984), using an Ouchterlony agar gel immunodiffusion test, showed that the same change in virus types occurred among the CPV viruses infecting these populations between 1980 and 1981 (Parrish et al., 1988b). This indicates that the replacement of CPV-2 with CPV-2a between 1979 and 1981 was most

| mAb | 1978 | 1979 | 1980 | 1981 | 1982 | 1983 | 1984 | FPV-c |
|-----|------|------|------|------|------|------|------|-------|
| A)  | b    | 18   | 21   | 22   | 43   | 44   | 25   | 26    | 14   | 31 | 15 | 39 |
| 7   | 512  | 256  | 512  | 1024 | 512  | 256  | 512  | 128  | 128  | 128 | 128 | 128 | 128 | 128 | <2 |
| 14  | 128  | 64   | 32   | 64   | 64   | 64   | 256  | 128  | 128  | 128 | 256  | 128  | 128  | 128  | 128  | <2 |
| 25  | 64   | 64   | 32   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | <2 |
| C   | 128  | 64   | 64   | 64   | 128  | 128  | <2   | <2   | <2   | <2   | <2   | <2   | <2   | 64   | 64   | 64   |
| D   | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | <2 |
| E   | 256  | 256  | 256  | 256  | 128  | 128  | 256  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | <2 |
| J   | 128  | 64   | 128  | 32   | 128  | 128  | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | 64   |
| 6   | 64   | 64   | 128  | 128  | 128  | 128  | 128  | 256  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  |
| 12  | 64   | 128  | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   |
| A   | 32   | 64   | 64   | 64   | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  |
| I   | 32   | 64   | 32   | 32   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   |
| 1   | 1024 | 512  | 1024 | 1024 | 512  | 512  | 512  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  |
| B   | 128  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  | 256  |
| 16  | 128  | 256  | 256  | 256  | 256  | 256  | 256  | 128  | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 128  |
| 15  | 64   | 256  | 128  | 256  | 256  | 256  | 128  | 256  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 64   |
| f   | 2048 | 1024 | 1024 | 2048 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 | 1024 |
| B)  | 1D1  | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | 4    |
| 7D6 | 4    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 4    |
| 7E2 | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | <2   | 2    |
| 2A9 | 64   | 64   | 32   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 2    |
| 2E2 | 16   | 32   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 2    |
| 2E12| 128  | 256  | 64   | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 32   |
| 3G6 | 64   | 64   | 32   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 64   | 32   |
| 4A12| 128  | 256  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 32   |
| 4E9 | 64   | 128  | 32   | 128  | 64   | 128  | 64   | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 128  | 64   |

Typing of CPV isolates from various countries with mAbs which distinguished CPV-2 and CPV-2a showed that the CPV-2a isolates among viruses collected in Japan and the United States during 1979 and 121 of 125 CPV isolates collected in Denmark during 1980 were of the CPV-2a type (Table I). By 1981 CPV-2a was the predominant virus in all countries (Parrish et al., 1988b).
FIG. 10. Digests of replicative-form DNA from CPV isolates from 1978 to 1984 with the HphI restriction enzyme, electrophoresed in a 2% agarose gel. Patterns show the difference in restriction digests between CPV-2 (1978–1980) and CPV-2a (1981–1984) isolates. Size standards (MW) are φ×174 DNA digested with HaeIII restriction enzyme. From Parrish et al. (1985) with permission. Copyright 1985 by the AAAS.

likely due to some natural advantage of the latter virus, not to any human intervention, such as vaccination.

CPV isolates from Japan which were characterized by mAbs as CPV-2 or CPV-2a were examined for their abilities to hemagglutinate at 37°C or 4°C. The CPV-2 isolates were found to hemagglutinate only at 4°C, while most of the CPV-2a isolates were able to hemagglutinate at both 4°C and 37°C, although there were some virus isolates which were antigenically characterized as CPV-2a isolates, but which only
### TABLE I

**Antigenic Types of CPV Isolates Collected in Various Countries between 1978 and 1984 and Tested with Monoclonal Antibodies**

| Year of virus isolation | 1978 | 1979 | 1980 | 1981 | 1982 | 1983 | 1984 |
|-------------------------|------|------|------|------|------|------|------|
| Country of origin       | 2b   | 2a   | 2    | 2a   | 2    | 2a   | 2    |
| United States           | 4    | 0    | 5    | 1    | 5    | 6    | 2    |
| Denmark                 | 0    | 0    | 1    | 2    | 0    | 7    | 0    |
| France                  | 1    | 0    | 2    | 0    | 1    | 0    | 1    |
| Belgium                 | 0    | 3    | 1    | 1    | 5    | 0    | 6    |
| Japan                   | 2    | 0    | 2    | 0    | 1    | 0    | 1    |
| Australia               | 2    | 0    | 2    | 0    | 1    | 0    | 2    |

*From Parrish et al. (1988b) with permission.*

C. Mutants of Viruses

Few mutants of the FPV subgroup viruses have been described or characterized (Rivera and Sundquist, 1984; Parrish and Carmichael, 1986; Parrish et al., 1988a). An isolate of MEV was reported that did not hemagglutinate pig, rhesus monkey, or horse erythrocytes at pH 6.8 at 4°C (Rivera and Sundquist, 1984). As the pH used in this study was near the upper range of the conditions reported by others for the hemagglutination of FPV (Carmichael et al., 1980; Moraillon and Moraillon, 1982; Veijalainen, 1988; Parrish et al., 1988c), it is not clear whether that mutant was truly nonhemagglutinating.

A nonhemagglutinating strain of CPV was derived after 12–22 passages of a 1978 isolate of the virus in the NLFK feline kidney cell line. The nonhemagglutinating virus showed no antigenic differences from wild-type CPV-2 when examined with a total of 82 mAbs prepared against CPV-2, CPV-2a, or FPV, and contained only two specific nucleotide and predicted amino acid sequence differences within the VP-1/VP-2 gene, at nucleotides 1631 and 2185 in the VP-1 gene (around 77 and 88 map units (m.u.) (Parrish et al., 1988a,c). Of these two differences the sequence at nucleotide 2185 was also present in two FPV isolates, FPV-Carl and FPV-a (Carlson et al., 1985; Parrish et
TABLE II
RELATIONSHIPS BETWEEN TEMPERATURE DEPENDENCE OF HEMAGGLUTINATION AND THE ANTIGENIC TYPE WITH CPV-2- OR CPV-2a-SPECIFIC mAbs

| Year of isolation | Number of isolates | 4°C/37°C Dependence | Antigenic CPV type |
|-------------------|--------------------|---------------------|-------------------|
| 1978              | 1                  | ≥64                 | +                 | 2     |
| 1979              | 3                  | ≥64                 | +                 | 2     |
|                   | 1                  | 2                   | -                 | 2a    |
| 1980              | 4                  | ≥64                 | +                 | 2     |
|                   | 2                  | 8                   | -                 | 2     |
|                   | 1                  | 2                   | -                 | 2a    |
| 1981              | 2                  | 8                   | -                 | 2     |
|                   | 1                  | 2                   | -                 | 2a    |
| 1982              | 1                  | ≥64                 | +                 | 2     |
|                   | 5                  | 2                   | -                 | 2a    |
| 1983              | 3                  | 1–2                 | -                 | 2a    |
| 1984              | 7                  | 2–8                 | -                 | 2a    |

* Adapted from Senda et al. (1988) with permission.

The ratio between the hemagglutination titer of the virus isolate when assayed at 4°C or 37°C.

Antigenic type determined by testing with CPV-2- or CPV-2a-specific monoclonal antibodies in the hemagglutination inhibition test.

It is known that FPV-a hemagglutinates under standard conditions, indicating that the functional difference in the nonhemagglutinated mutant is most likely due to the sequence at nucleotide 1631, suggesting that an amino acid affects the structure of the viral ligand which binds to the erythrocyte virus-binding receptor. Whether the nonhemagglutinating virus exhibits other differences from the wild-type virus is not known.

Another mutant of CPV-2 was derived during passage of a 1978 isolate of CPV-2 102 times in primary canine kidney cells and an additional 10 times in the NLFK feline kidney-derived cell line. During the passages in NLFK cells, a mutant (CPV-102/10) was selected which no longer replicated in dogs or in dog cells and which was variant antigenically when tested in the hemagglutination inhibition test with mAbs prepared against CPV-2 or FPV (Parrish and Carmichael, 1986). The mutation in CPV-102/10 was identified by recombination mapping, in which restriction fragments prepared from replicative-form DNA of each virus were religated to form the entire genome and then transfected into NLFK cells. By examination of seven recombi-
nant viruses prepared between CPV-102/10 and a wild-type CPV strain, the antigenic and host range differences of CPV-102/10 were mapped to a 438-base pair 64–73 m.u. BglII–PvuII fragment of the viral genome (Fig. 11) (Parrish and Carmichael, 1986). Within this region there were two CPV-102/10-specific nucleotide and amino acid sequence differences at adjacent codons (i.e., nucleotides 1400 and 1403 in the VP-1/VP-2 gene sequence) (Parrish et al., 1988c).

Fig. 11. (a) Analysis of recombinants (Rec.) between a wild-type CPV-2 isolate (CPV-d) and the antigenic and host range mutant CPV-102/10. Recombinants were formed by ligating genome fragments from these two viruses at various restriction sites (i.e., TaqI, PstI, BglII, and PvuII). Viruses produced after transfection of cell cultures were examined for recombinant genome type. (b) Antigenic and host range types of CPV-d and CPV-102/10 and the recombinants between them. Antigenic types are represented by reactions with mAb 14 (control) or mAb 8 (which reacts with CPV-d, but not with CPV-102/10) in the hemagglutination inhibition assay, while host ranges are represented by NLFK (feline) or A72 (canine) cell culture supernatant hemagglutination titers 4 days after inoculation with 0.02 pfu of virus per cell. The antigenic type and host range of the mutant virus map between the BglII and PvuII sites (64–73 m.u.). From Parrish and Carmichael (1986) with permission.
D. Genome Organization

The genome organization appears to be similar to that described for several other related autonomous parvoviruses, including H1, MVM, and bovine parvovirus (Rhode and Paradiso, 1983; Astell et al., 1983; Chen et al., 1986). The proposed genome organizations of MVM and CPV are shown in Fig. 12. The sequence contains an open reading frame DNA from a promoter at 4 m.u. (i.e., p4), which encodes the NS-1 protein which has been extensively characterized for H1 and MVM (Cotmore et al., 1983; Paradiso, 1984; Cotmore and Tattersall, 1986, 1988).

An 83-kDa protein was precipitated from in vitro translated CPV mRNA, using rabbit antisera prepared against bacterially expressed fusion proteins from the middle portion of the NS-1 gene of MVM. However, no translation product was precipitated by antiserum against a sequence from the amino terminus of the MVM NS-1 protein, which is contained in both the 83-kDa NS-1 and 25-kDa NS-2 proteins of MVM (Cotmore and Tattersall, 1986). The carboxy-terminal two-thirds of the FPV NS-1 gene sequence was expressed in Escherichia coli as a fusion with the tryptophan LE gene product and the proteins used to produce antibodies in rabbits. These antibodies recognized proteins of 84, 60, 53, and 49 kDa, the most abundant product being the 60-kDa form (Carlson et al., 1987). The relationships of the various proteins to each other and to the 83-kDa protein precipitated from the in vitro translated CPV mRNA have not been determined, but the smaller proteins most likely represent degraded forms of the 83-kDa NS-1 protein.

It is not clear whether CPV encodes a protein equivalent to the 25-kDa NS-2 protein of MVM and H1, which is translated from the MVM R2 transcript. In MVM this transcript contains 84 amino acids from the amino terminus of NS-1 in the third open reading frame, with a carboxy-terminal portion derived from 40–46 m.u. of the MVM sequence after mRNA splicing. This portion is encoded from open reading frame 2 and is followed by a second minor splice (i.e., 46–48 m.u.) to give a further short sequence encoding carboxy-terminal domains in open reading frame 2 (Morgan and Ward, 1986; Cotmore and Tattersall, 1986, 1987). An equivalent transcript or product in CPV or FPV has not been identified. The presence of a stop codon upstream from the second (minor) splice and the low homology between the translated open reading frames in this region between the sequences of MVM and CPV or FPV suggests that the latter viruses have different patterns of transcription and translation of the NS proteins from those seen for MVM and H1 (Cotmore and Tattersall, 1986).

The genomic 5' 60% of the parvovirus genome encodes the VP-1 and
Fig. 12. Coding strategy of the minute virus of mice (MVM) genome (A) and that proposed for the CPV genome (B). The cytoplasmic transcripts are aligned with the viral DNA strand (orientation 3' to 5'), showing the promoters at 4 and 38–40 m.u. Major blocks of open reading frame are shown for MVM in each of the three reading frames (F1, F2, and F3) and the sequences which encode NS-1, NS-2 (which has not been identified in CPV), and the capsid proteins VP-1 and VP-2. (A) The open reading frames used to encode the various parts of each virus are shown as numbers in the transcript map. From Cotmore and Tattersall (1987) and Reed et al. (1988) with permission.
VP-2 from mRNAs transcribed from a promoter around 40 m.u. (Fig. 12) (Carlson et al., 1985). The two proteins are most likely translated from differently spliced forms of the R3 transcript, as described in detail for MVM (Morgan and Ward, 1986), although it is possible that R1 and R2 transcripts can also be translated to form VP-1 or VP-2 (Cotmore and Tattersall, 1987). Messages from the CPV p40 promoter are able to direct the translation of both VP-1 and VP-2 when cloned in a bovine papillomavirus expression vector (Mazzara et al., 1987).

Alternative splices predicted to generate VP-1 and VP-2 messages are most likely derived from two different splice donors. One, prior to the methionine codon thought to be the initiation codon of VP-1, would give rise to an mRNA in which the first AUG used would be that initiating VP-2 (Rhode, 1985, Carlson et al., 1985; Morgan and Ward, 1986). The alternatively spliced mRNA would most likely use a predicted splice donor after the predicted ATG initiating VP-1, giving 10 codons prior to the splice, then the mRNA could be spliced to the same acceptor as for the VP-2 message. The outcome of the alternative splices would encode an additional 153 amino acids in VP-1, to give VP-1 and VP-2 of predicted sizes of 79,845 and 64,661 Da, respectively, for FPV (Carlson et al., 1985) and similarly sized proteins for CPV (Rhode, 1985; Reed et al., 1988). Whether these mRNA splicing reactions actually occur in CPV and FPV has not yet been determined.

The genomes of CPV, FPV, MEV, and RPV isolates contain various directly repeated units of unknown significance in the genomic 5' (largely noncoding) region. Some of the rearrangements could have arisen during passage of the viruses in tissue culture, although some of the variable sequences are present in wild-type low-passage isolates of CPV (Fig. 13) (Rhode, 1985; Carlson et al., 1985; Parrish et al., 1988c). No correlation between the form of the repeat units and any biological function has been described, but all viruses examined in one study had at least one copy of each repeat, and the DNA sequences were conserved, suggesting that the sequences play some role in the virus life cycle (Cotmore and Tattersall, 1987; Parrish et al., 1988c).

VI. OTHER PROPERTIES OF FPV, MEV, AND CPV

A. Hemagglutination Dependence on pH

All virus types hemagglutinate erythrocytes from a variety of species at 4°C in buffers with pH values below 7.0 (Johnson, 1971; Goto et al., 1974; Goto, 1975; Mochizuki et al., 1978; Senda et al., 1988). FPV and MEV isolates hemagglutinated pig or rhesus monkey erythrocytes
only at pH values below 7.0, and the pH optimum for most strains is below pH 6.6 (Carmichael et al., 1980; Moraillon and Moraillon, 1982). In contrast, CPV isolates hemagglutinated with approximately equal titers in buffers with pH values up to 8.0 (Carmichael et al., 1980; Parrish et al., 1988c). RD showed a hemagglutination dependence on pH similar to that of CPV isolates, while the BFPV was similar to FPV or MEV isolates, supporting the mAb antigenic typing results of these viruses (Veijalainen, 1988).

**B. Experimental Host Range Properties**

Isolates of CPV, FPV, and MEV all replicate in feline kidney cells and many other feline cell lines in culture (Johnson, 1965; Appel et al., 1979b; Tratschin et al., 1982; Parrish et al., 1988c). CPV isolates replicate in canine cells in culture, but FPV and MEV isolates do not (Tratschin et al., 1982; Parrish et al., 1988c). Studies with RD and BFPV in tissue culture show these viruses to differ and to have host ranges similar to those for CPV and FPV, respectively (Veijalainen, 1988).

The experimental host ranges of the FPV subgroup viruses have not been well defined, and the many viruses and hosts make definitive
analysis of the various host ranges difficult. In early studies feline tissues containing FPV were inoculated into dogs, ferrets, ground squirrels (Citellus richardsonii), mongooses, rhesus monkeys, hamsters, chick embryos, rabbits, guinea pigs, mice, and rats without producing any signs of disease (Hindle and Findlay, 1932; Hammon and Enders, 1939a; Lawrence et al., 1940; Kikuth et al., 1940). FPV isolates inoculated into minks in a number of studies caused little clinical disease (Myers et al., 1959; Barker et al., 1983; Parrish et al., 1987). An FPV or RPV isolate was passaged repeatedly through minks in one study, but only one animal in each series of RPV- or FPV-inoculated minks showed any signs of clinical disease, in contrast to the MEV isolates which caused clinical disease in many of the inoculated minks (Parrish et al., 1987). Inoculation of an MEV isolate into a single cat resulted in clinical disease (Higashihara et al., 1981).

Inoculation of FPV isolates into dogs gave a limited replication in a number of lymphoid tissues, but the viruses could not be propagated repeatedly in this host (Parrish et al., 1988c). CPV isolates inoculated into cats showed little or no replication (Goto et al., 1984b; C. R. Parrish, unpublished observations).

VII. GENETIC ANALYSIS AND VARIATION AMONG THE VIRUSES

A. Restriction Mapping

Comparisons of the restriction enzyme cleavage maps of CPV, FPV, and MEV replicative-form DNAs revealed a number of differences in restriction sites (McMaster et al., 1981; Tratschin et al., 1982). Most of the variable sites mapped to the right-hand side of the genome, within the VP-1/VP-2 gene(s) (Fig. 14). The restriction maps of various wild-type and vaccine strains of FPV, MEV, and CPV were compared, using seven restriction enzymes (Tratschin et al., 1982). A Swiss isolate of CPV (Ka/BE, discovered in 1979) differed from the French V1 strain of MEV in 11 of 79 restriction enzyme sites examined, allowing unambiguous identification of the CPV isolates. Low-passage MEV and FPV isolates appeared to be similar in this analysis, differing in two or fewer restriction sites (Fig. 14) (Tratschin et al., 1982).

Two or three HinfI sites in two of the vaccine strains of FPV were similar to CPV-specific sites in the CPV isolates (Fig. 14) (Tratschin et al., 1982). Although this finding suggested a possible origin for CPV in the FPV vaccine viruses (Siegl, 1984), other studies comparing VP-1/VP-2 gene sequences of various CPV isolates with the two FPV vaccine strains indicated that these FPV vaccine strains were not progenitors of CPV (Parrish et al., 1988c; see Section IX).
Fig. 14. (a) Restriction enzyme maps of MEV and CPV virus replicative-form (RF) DNAs, aligned with the viral genomic (−) DNA shown as the 3′–5′ orientation. Vertical lines indicate sites present in both CPV and MEV, open circles show sites present only in MEV, and closed circles indicate sites present only in CPV (Swiss isolate). (b) Comparative restriction enzyme digestion maps of the RF DNAs of various CPV, FPV, and MEV isolates, along with those of six FPV vaccine strains. The map of the MEV isolate is used for comparison. Additional restriction sites are shown above the line, while sites absent from a virus strain are shown below it. Sites common to all viruses are not shown. Live attenuated FPV vaccine strains examined were from the following manufacturers' vaccines—strain A, Iffa-Merieux; strain B, Norden; strain C, Frieosythe/Wellcome; strain E, Phillips Roxanne; and strain F, Dellen—as well as one strain (strain D) derived from MEV from Connaught. Hf, HinfI; He, HaeIII; Hh, HphI; Al, AluI; MbII, MboII; Hc, HincII; Mbi, MboI. Comparisons of the DNA sequences of strains E and F are also shown in Fig. 17. From Tratschin et al. (1982) with permission.
An almost complete DNA sequence of one tissue culture-passaged vaccine strain of CPV isolated in 1978 has been reported (Reed et al., 1988). Partial nucleotide sequences of a tissue culture-passaged vaccine strain of FPV (about 22–97 m.u.) (Carlson et al., 1985) and a wild-type 1978 isolate of CPV (about 33–95 m.u.) (Rhode, 1985), as well as the VP-1 and VP-2 gene sequences of eight further isolates of CPV-2, CPV-2a, FPV, RPV, or MEV have also been reported (Parrish et al., 1988c).

VIII. Genetic Analysis of Antigenic, Host Range, and Other Functions within the VP-1/VP-2 Genes

Mapping CPV-Specific Properties

Recombinants between a CPV-2 isolate and an FPV isolate were prepared from purified viral replicative-form DNA, using methods described above for analysis of the CPV-102/10 mutant (Parrish and Carmichael, 1986; Parrish et al., 1988c). Recombinant genomes prepared at the PstI, BglII, and PvulII sites (at 59, 64, and 73 m.u. in the viral genome, respectively) were transfected into cell cultures, and recombinant viruses recovered were examined for the CPV-specific antigenic epitope, the pH dependence of hemagglutination, and the canine host range (Fig. 15 and Table III). These analyses showed that the CPV-specific pH dependence of hemagglutination and a sequence difference determining the CPV-specific antigenic epitope both mapped within the 300-base pair 59–64 m.u. region. Within that region there were only two CPV- or FPV-specific sequence differences—an asparagine–lysine change coded for by nucleotide 780 and an alanine–valine difference coded for by nucleotide 809—and therefore one or both of those differences gave rise to the differences in antigenic type and in the pH dependence of hemagglutination (Parrish et al., 1988c).

The host range differences between CPV and FPV proved genetically more complex, and only those properties that allow CPV to replicate in dogs have been defined. When the 59–73 m.u. region of FPV was replaced by the same region of CPV, the resulting virus replicated in dogs to a titer as high as that for the wild-type virus, identifying this region as being responsible for the canine host range of CPV (Fig. 15 and Table III). This region contained the two sequence differences associated with the CPV-specific epitope and pH dependence of hemagglutination in the 59–64 m.u. region, as well as one further CPV-specific coding change at 71 m.u. (asparagine–aspartic acid at nu-
Fig. 15. Analysis of recombinants between CPV-d and FPV-b prepared from replicative-form viral DNA and transfected into NLFK cells. Viruses recovered were confirmed by restriction enzyme analysis and DNA sequencing. (A) CPV, FPV, and five types of recombinants (Rec.) between these viruses prepared using the PstI, BglII, and PvuII sites at 59, 64, and 73 m.u., respectively, in the virus genome. (B) Hemagglutination inhibition titers of recombinant viruses with CPV-specific mAb 14. (C) The pH dependence of hemagglutination of the parental viruses and of the recombinants. Hemagglutination assays were performed in buffers with pH values of 6.2 and 7.0. From Parrish et al. (1988c) with permission.

cleotide 1468 in the VP-1/VP-2 gene) and a virus-specific noncoding difference at nucleotide 1200 (Fig. 16).

The requirements for and the relative importance of the various sequence differences within that region are not known. However, the region between 64 and 73 m.u. does not itself contain the host range function since Rec 1, which contained the 0–64 m.u. region from FPV and the 64–100 m.u. region from CPV, was not able to replicate in dogs (Fig. 15 and Table III). It appears that differences in both the 59–64 and 64–73 m.u. regions are required to endow the canine host range (Parrish et al., 1988c).

A similar region within the capsid proteins genes is also important for determining the host range differences between two strains of MVM which differ in their host ranges for various differentiated cells: MVM(p), replicating in fibroblast-derived cells, and MVM(i), replicating in lymphocyte-derived cells (Engers et al., 1981; Spalholz and Tattersall, 1983; Tattersall and Bratton, 1983; Kimsey et al., 1986). This
| Virus     | Thymus  | Mesenteric lymph node | Ileum   |
|-----------|---------|-----------------------|---------|
| CPV-d     | 1.2 × 10^6 | 1.6 × 10^3            | 7.0 × 10^4 |
|           | 3.0 × 10^4 | 1.7 × 10^3            | 2.2 × 10^4 |
| FPV-b     | 1.4 × 10^3 | <10                   | <10     |
|           | 8.0 × 10   | <10                   | <10     |
| Rec 1a    | 10       | 1.0 × 10^2            | 3.0 × 10 |
|           | 10       | <10                   | <10     |
| Rec 2     | <10      | <10                   | <10     |
|           | <10      | <10                   | <10     |
| Rec 3a    | 2.2 × 10^5 | 1.9 × 10^4            | 1.2 × 10^3 |
|           | 2.8 × 10^4 | 2.4 × 10^4            | 4.0 × 10^2 |
| Rec 3b    | 1.7 × 10^4 | 2.2 × 10^4            | 1.1 × 10^3 |
| Rec 4     |          |                       |         |
| Experiment 1 | 9.1 × 10^4 | <10                   | <10     |
|           | <10      | 5.0 × 10               | <10     |
| Experiment 2 | 2.7 × 10^6 | <10                   | 10      |
|           | <10      | <10                   | <10     |
| Experiment 3 | <10      | <10                   | <10     |
|           | <10      | <10                   | <10     |
| Rec 5a    | 1.3 × 10^4 | 2.8 × 10^6            | 3.1 × 10^3 |
|           | 2.7 × 10^6 | 2.0 × 10^3            | 4.1 × 10^2 |
|           | 8.0 × 10^6 | >4.0 × 10^7           | 4.0 × 10^2 |
|           | 2.2 × 10^6 | <10                   | <10     |
| Rec 5b    | 1.4 × 10^6 | 2.2 × 10^6            | 2.6 × 10^5 |
|           | 6.0 × 10^4 | 4.0 × 10^3            | 1.4 × 10^5 |

* Virus titers in various tissues of dogs 4 days after intramuscular inoculation with FPV, CPV, or recombinants (Rec). The dogs were inoculated with preparations of Rec 4 from tissue culture (experiments 1 and 3), while the dogs in experiment 2 were inoculated with virus recovered from the thymus of a dog inoculated with Rec 4 in experiment 1. From Parrish *et al.* (1988c) with permission.
difference in the host range mapped between 68 and 73 m.u. in these viruses and involved residues similar to those affecting the host range defined in this region for CPV or FPV (Figs. 11 and 15) (Antionetti et al., 1988; Gardiner and Tattersall, 1988a,b).

Nothing is known about the specific function by which the CPV-specific differences in the VP-1/VP-2 gene determine the canine host range. However, between MVM(i) and MVM(p) the functional difference within the VP-1/VP-2 gene does not affect virus binding to cell receptors, but influences early events in the replication of the virus, in either mRNA transcription or DNA replication (Spalholz and Tattersall, 1983; Antionetti et al., 1988; Gardiner and Tattersall, 1988a).

IX. Virus Sequence Variation and Phylogenetic Relationships

The VP-1/VP-2 gene sequences of a total of 10 isolates have been compared: two CPV-2, two CPV-2a, two MEV (types 1 and 2), one RPV, and three FPV isolates. An FPV and a CPV sequence were obtained from published sequences (Carlson et al., 1985; Rhode, 1985). Others were obtained from M13 phage clones of viral replicative-form DNA, which were sequenced using a series of primers complementary to various positions within the VP-1/VP-2 gene (Parrish et al., 1988c). The distribution of the variant positions within the VP-1/VP-2 gene is shown in Fig. 16. Although the differences in nucleotide sequences were evenly distributed through the genes, the coding changes were less evenly distributed. Changes that were FPV or CPV specific or that were specific for CPV-2a viruses tended to cluster into three regions.
within the gene: around 60 m.u., between 70 and 77 m.u., and around 85 m.u., near the carboxy terminus (Fig. 16) (Parrish et al., 1988c).

The differences in nucleotide sequences within the VP-1/VP-2 gene were used to prepare a network of the phylogenetic relationships between the viruses, assuming maximum parsimony (Fig. 17) (Fitch, 1977). Among the FPV, MEV, and RPV viruses there was a certain amount of apparently random sequence variation, but the viruses isolated from the different hosts could not be readily distinguished. However, the CPV isolates formed a separate cluster, which subdivided into the CPV-2 and the CPV-2a types that had previously been distinguished antigenically and by restriction enzyme analysis (Parrish et al., 1985). It is apparent from this analysis that CPV-2 and CPV-2a most likely derived from some common ancestor virus prior to 1978 and that CPV-2a was not simply a linear descendent of CPV-2 (Parrish et al., 1988c).

X. Conclusions

The emergence of MEV in minks during the 1940s and the recent emergence of CPV in dogs during the 1970s are unusual events, which raise a number of questions about the evolution of viruses and the ways in which host ranges of the viruses are determined and restricted. By examining these phenomena, we hope to derive lessons about the natural history of viruses and the interactions of these viruses with their hosts.

Fig. 17. An unrooted network showing the phylogenetic relationships between virus isolates based on the nucleotide sequences of the VP-1/VP-2 gene between nucleotide 203 and the termination codon at nucleotide 2254. Isolates are feline panleukopenia virus (FPV-a, -b, and -Carl), mink enteritis virus (MEV-a and -d), raccoon parvovirus (RPV-a), canine parvovirus type 2 (CPV-d and -b), and CPV type 2a (CPV-15 and -31). FPV-Carl and FPV-a represent the Dellen Laboratories and Phillips Roxanne vaccine strains of FPV, respectively. From Parrish et al. (1988c) with permission.
The emergence of MEV appears to be an example not of altered host range, but was most likely due to the recognition of a long-existing virus which might have altered in virulence for minks. It is also possible that the disease was recognized at that time due to some change in the farming practices or possibly by a change in minks due to genetic selection. The differences in virulence for minks observed after inoculation of MEV or FPV suggests that there are subtle differences between FPV and MEV which have yet to be defined.

The emergence of CPV probably represents a different type of event. It is clear from the retrospective serological studies that CPV is a new virus of dogs and that it was first present in domestic dogs in Europe only during the mid-1970s. The widespread outbreaks and global spread of the virus did not occur until 1978. It is possible that this was due to some change in the virus at that time which gave it an epidemiological advantage. Genetic mapping studies indicate that only three or four sequence differences between the FPV and CPV-2 isolates within the VP-1/VP-2 gene determine all of the specific properties of CPV that have been defined: the pH dependence of hemagglutination, the CPV-specific epitope, and the host range for canine cells and dogs. Whether these changes are all that would be required to convert FPV into a successful canine pathogen is not known, but it is clear that CPV could have been derived from FPV or some closely related virus by only a few specific changes. How this occurred and which virus was the ancestor are unknown and are subjects for future studies.

These parvoviruses, therefore, present a unique opportunity for understanding the natural evolution and variation of viruses and for determining the ways in which viruses can gain new host range and other functions. In this case the viruses apparently emerged by two different mechanisms as pathogens of the members of two families (i.e., Mustelidae and Canidae) among the Carnivora which had previously been resistant to infection or disease. Further examination of the viruses and further definition of the specific genetic properties involved in the changes will allow the details of these unusual events and their implications for virus evolution and host range stability to be understood.

**References**

Antonietti, J.-P., Sahli, R., Beard, P., and Hirt, B. (1988). Characterization of the cell type-specific determinant in the genome of minute virus of mice. *J. Virol.* 62, 552-557.

Appel, M. J. G., and Parrish, C. R. (1982). Raccoons are not susceptible to canine parvovirus. *J. Am. Vet. Med. Assoc.* 181, 489.

Appel, M. J. G., Cooper, B. J., Greisen, H., and Carmichael, L. E. (1978). Status report: Canine viral enteritis. *J. Am. Vet. Med. Assoc.* 173, 1516-1518.
Astell, C., Thompson, M., Merchlinsky, M., and Ward, D. C. (1983). The complete DNA sequence of minute virus of mice, an autonomous parvovirus. *Nucleic Acids Res.* **11**, 999–1018.

Azetaka, M., Hirasawa, T., Konishi, S., and Ogata, M. (1981). Studies of canine parvovirus isolation, experimental infection and serologic survey. *Jpn. J. Vet. Sci.* **43**, 243–255.

Barker, I. K., Povey, R. C., and Voigt, D. R. (1983). Response of mink, skunk, red fox and raccoon to inoculation with mink virus enteritis, feline panleukopenia and canine parvovirus and prevalence of antibody to parvovirus in wild carnivores in Ontario. *Can. J. Comp. Med.* **47**, 188–197.

Bishop, S. P. (1972). Effects of aortic stenosis on myocardial cell growth and hyperplasia and ultrastructure in neonatal dogs. *Adv. Stud. Card. Struct. Metab.* **3**, 637–655.

Boosinger, T. R., Rebar, A. H., DeNicola, D. B., and Boon, G. D. (1982). Bone marrow alterations associated with canine parvoviral enteritis. *Vet. Pathol.* **19**, 558–561.

Bouillant, A., and Hanson, R. P. (1965). Epizootiology of mink enteritis: I. Stability of the virus in feces exposed to natural environmental factors. *Can. J. Comp. Med. Vet. Sci.* **29**, 125–128.

Brun, A., Chappuis, G., Precausta, P., and Terre, J. (1979). Immunization against panleukopenia: Early development of immunity. *Comp. Immunol. Microbiol. Infect. Dis.* **1**, 335–339.

Burtonboy, G., Coignoul, F., Delferriere, N., and Pastoret, P.-P. (1979). Canine hemorrhagic enteritis: Detection of viral particles by electron microscopy. *Arch. Virol.* **61**, 1–11.

Burtonboy, G., Bazin, H., and Delferriere, N. (1982). Rat hybridoma antibodies against canine parvovirus. *Arch. Virol.* **71**, 291–302.

Carlson, J. H., and Scott, F. W. (1977). Feline panleukopenia. II. The relationship of intestinal mucosal cell proliferation to viral infection and development of lesions. *Vet. Pathol.* **14**, 173–181.

Carlson, J. H., Scott, F. W., and Duncan, J. R. (1977). Feline panleukopenia. III. Pathogenesis in germfree and specific pathogen-free cats. *Vet. Pathol.* **14**, 79–88.

Carlson, J. H., Scott, F. W., and Duncan, J. R. (1978). Feline panleukopenia. III. Development of lesions in the lymphoid tissues. *Vet. Pathol.* **15**, 383–392.

Carlson, J., Rushlow, K., Maxwell, I., Maxwell, F., Winston, S., and Hahn, W. (1985). Cloning and sequence of DNA encoding structural proteins of the autonomous parvovirus feline panleukopenia virus. *J. Virol.* **55**, 574–582.

Carlson, J. O., Lynde-Maas, M. K., and Zheng-da, S. (1987). A non-structural protein of feline panleukopenia virus: Expression in *Escherichia coli* and detection of multiple forms in infected cells. *J. Virol.* **61**, 621–624.

Carman, S., and Povey, C. (1982). Successful experimental challenge of dogs with canine parvovirus-2. *Can. J. Comp. Med.* **46**, 33–38.
Carman, P. S., and Povey, R. C. (1985a). Pathogenesis of canine parvovirus-2 in dogs: Haematology, serology and virus recovery. *Res. Vet. Sci.* 38, 134–140.

Carman, P. S., and Povey, R. C. (1985b). Pathogenesis of canine parvovirus-2 in dogs: Histopathology and antigen identification in tissues. *Res. Vet. Sci.* 38, 141–150.

Carmichael, L. E., Joubert, J. C., and Pollock, R. V. H. (1980). Hemagglutination by canine parvovirus: Serologic studies and diagnostic applications. *Am. J. Vet. Res.* 40, 784–791.

Carmichael, L. E., Joubert, J. C., and Pollock, R. V. H. (1981). A modified live canine parvovirus strain with novel plaque characteristics. I. Viral attenuation and dog response. *Cornell Vet.* 71, 408–427.

Carmichael, L. E., Joubert, J. C., and Pollock, R. V. H. (1983). A modified canine parvovirus vaccine 2. Immune response. *Cornell Vet.* 73, 13–29.

Carpenter, J. L., Roberts, R. M., Harpster, N. K., and King, N. W. (1980). Intestinal and cardiopulmonary forms of parvovirus infection in a litter of pups. *J. Am. Vet. Med. Assoc.* 176, 1269–1273.

Chalifoux, A., Elazhary, Y., and Frechette, J. L. (1981). Parvovirus enteritis: Possible role of endo parasites. *Med. Vet. Quebec* 11, 66–70.

Chen, K. C., Shull, B. C., Moses, E. A., Lederman, M., Stout, E. R., and Bates, R. C. (1986). Complete nucleotide sequence and genome organization of bovine parvovirus. *J. Virol.* 60, 1085–1097.

Clinton, G., and Hayashi, M. (1976). The parvovirus MVM: A comparison of heavy and light particle infectivity and their density conversion in vitro. *Virology* 74, 57–63.

Cooper, B. J., Carmichael, L. E., Appel, M. J. G., and Greisen, H. (1979). Canine viral enteritis. II. Morphologic lesions in naturally occurring parvovirus infection. *Cornell Vet.* 69, 134–144.

Cotmore, S. F., and Tattersall, P. (1986). Organization of the nonstructural genes of the parvovirus minute virus of mice. *J. Virol.* 58, 724–732.

Cotmore, S. F., and Tattersall, P. (1987). The autonomously replicating parvoviruses of vertebrates. *Adv. Virus Res.* 33, 91–174.

Cotmore, S. F., and Tattersall, P. (1988). The NS-1 polypeptide of minute virus of mice is covalently attached to the 5' termini of duplex replicative-form DNA and progeny single strands. *J. Virol.* 62, 851–860.

Cotmore, S. F., Sturzenbecker, L. J., and Tattersall, P. (1983). The autonomous parvovirus minute virus of mice encodes two nonstructural proteins in addition to its capsid polypeptides. *Virology* 129, 333–343.

Csiza, C. K., Scott, F. W., de Lahunata, A., and Gillespie, J. H. (1971a). Pathogenesis of feline panleukopenia virus in susceptible newborn kittens. I. Clinical signs, hematology, serology, and virology. *Infect. Immun.* 3, 833–837.

Csiza, C. K., de Lahunata, A., Scott, F. W., and Gillespie, J. H. (1971b). Pathogenesis of feline panleukopenia virus in susceptible newborn kittens. II. Pathology and immunofluorescence. *Infect. Immun.* 3, 838–846.

Davis, E. V., Gregory, G. G., and Beckenhauer, W. H. (1970). Infectious feline panleukopenia (developmental report of a tissue culture origin formalin-inactivated vaccine). *VM/SAC, Vet. Med. Small Anim. Clin.* 65, 237–242.

Duenwald, J. C., Holland, J. M., Gorham, J. R., and Ott, R. L. (1971). Feline panleukopenia: Experimental cerebellar hypoplasia produced in neonatal ferrets with live virus vaccine. *Res. Vet. Sci.* 12, 394–396.

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.
Eugster, A. K. (1980). Studies on canine parvovirus infection: Development of an inactivated vaccine. *Am. J. Vet. Res.* 41, 2020–2024.

Eugster, A. K., Bendele, R. A., and Jones, L. P. (1978). Parvovirus infection in dogs. *J. Am. Vet. Med. Assoc.* 173, 1340–1341.

Evermann, J. F., Foreyt, W., Maag-Miller, L., Leathers, C. W., McKeirnan, A. J., and LeaMaster, B. (1980). Acute hemorrhagic enteritis associated with canine coronavirus and parvovirus infections in a captive coyote population. *J. Am. Vet. Med. Assoc.* 177, 784–786.

Fitch, W. M. (1977). On the problem of discovering the most parsimonious tree. *Am. Nat.* 111, 223–257.

Flagstad, A. (1977). Feline panleukopenia virus and mink enteritis virus. A serological study. *Acta Vet. Scand.* 18, 1–9.

Fletcher, K. C., Eugster, A. K., Schmidt, R. E., and Hubbard, G. B. (1979). Parvovirus infection in maned wolves. *J. Am. Vet. Med. Assoc.* 175, 559–566.

Flower, R. L. P., Wilcox, G. E., and Robinson, W. F. (1980). Antigenic differences between canine parvovirus and feline panleukopenia virus. *Vet. Rec.* 107, 254–256.

Gagnon, A. N., and Povey, R. C. (1979). A possible parvovirus associated with an epidemic gastroenteritis in Canada. *Vet. Rec.* 104, 263–264.

Gardiner, E. M., and Tattersall, P. (1988a). Evidence that developmentally regulated control of gene expression by a parvoviral allotropic determinant is particle mediated. *J. Virol.* 62, 1713–1722.

Gardiner, E. M., and Tattersall, P. (1988b). Mapping of the fibrotropic and lymphotropic host range determinants of the parvovirus minute virus of mice. *J. Virol.* 62, 2605–2613.

Gorham, J. R., Hartsough, G. R., Burger, D., Lust, S., and Sato, N. (1965). The preliminary use of attenuated feline panleukopenia virus to protect cats against panleukopenia and mink against virus enteritis. *Cornell Vet.* 55, 559–566.

Gorham, J. R., Hartsough, G. R., Sato, N., and Lust, S. (1966). Studies of cell culture adapted feline panleukopenia virus—Virus neutralization and antigenic extinction. *VM/SAC, Vet. Med. Small Anim. Clin.* 61, 35–40.

Goss, L. J. (1948). Species susceptibility to the viruses of Carre and feline enteritis. *Am. J. Vet. Res.* 9, 65–68.

Goto, H. (1975). Feline panleukopenia in Japan. II. Hemagglutinability of the isolated virus. *Jpn. J. Vet. Sci.* 37, 431–438.

Goto, H., Yachida, S., Shiahata, T., and Shimizu, K. (1974). Feline panleukopenia in Japan. I. Isolation and characterization of the virus. *Jpn. J. Vet. Sci.* 36, 203–211.

Goto, H., Hirano, T., Uchida, E., Watanabe, K., Shinagawa, M., Ichijo, S., and Shimizu, K. (1984a). Comparative studies of physicochemical and biological properties between canine parvovirus and feline panleukopenia virus. *Jpn. J. Vet. Sci.* 46, 519–526.

Goto, H., Uchida, S., Ichijo, S., Shimizu, K., Morohoshi, Y., and Nakano, K. (1984b). Experimental infection of canine parvovirus in specific pathogen-free cats. *Jpn. J. Vet. Sci.* 46, 729–731.

Hammon, W. D., and Enders, J. F. (1939a). A virus disease of cats, principally characterized by aleucocytosis, enteric lesions and the presence of intranuclear inclusion bodies. *J. Exp. Med.* 69, 327–353.

Hammon, W. D., and Enders, J. F. (1939b). Further studies on the blood and the hematopoietic tissues in malignant panleukopenia of cats. *J. Exp. Med.* 70, 557–564.

Havec, P., and Andersen, A. B. (1982). Parvovirus-diarrhoe hos hunde i Danmark. Forkomst, samt laboratorie-diagnostic belyst en underjogelsae af virusudskillelse med feses. *Proc. Nord. Vet. Kongr., 14th* pp. 148–151 (in Norwegian).

Hayes, M. A., Russell, R. G., Mueller, R. W., and Lewis, R. J. (1979). Myocarditis in young dogs associated with a parvovirus-like agent. *Can. Vet. J.* 20, 126.
Higashihara, T., Izawa, H., Onuma, M., Kodama, H., and Mikami, T. (1981). Mink enteritis in Japan. I. Isolation and characterization of the causative virus and its pathogenicity in cat. Jpn. J. Vet. Sci. 43, 841–851.

Hindle, E., and Findlay, G. M. (1932). Studies on feline distemper. J. Comp. Pathol. Ther. 45, 11–26.

Horner, G. W., Hunter, R., and Chisholm, E. G. (1979). Isolation of parvovirus from dogs with enteritis. N.Z. Vet. J. 27, 280.

Huxtable, C. R. R., McHowell, J., Robinson, W. F., Wilcox, G. E., and Pass, D. A. (1979). Sudden death associated with a suspected viral myocarditis. Aust. Vet. J. 55, 37–38.

Ichijo, S., Osame, S., Konishi, T., and Ogata, H. (1976). Clinical and hematological findings and myelograms on feline panleukopenia. Jpn. J. Vet. Sci. 38, 197–205.

Ishibashi, K., Maede, Y., Oshugi, T., Onuma, M., and Mikami, T. (1983). Serotherapy for dogs infected with canine parvovirus. Jpn. J. Vet. Sci. 45, 59–66.

Janssen, D. L., Bartz, C. R., Bush, M., Marchwicki, R. H., Grates, S. J., and Montali, R. J. (1982). Parvovirus enteritis in vaccinated juvenile bush dogs. J. Am. Vet. Med. Assoc. 181, 1225–1227.

Jesyk, P. F., Haskins, M. E., and Jones, C. L. (1979). Myocarditis of probable viral origin in pups of weaning age. J. Am. Vet. Med. Assoc. 174, 1204–1207.

Johnson, R. H. (1964). Isolation of a virus from a condition simulating feline panleukopenia in a leopard. Vet. Rec. 76, 1008–1012.

Johnson, R. H. (1965). Feline panleukopenia. I. Identification of a virus associated with the syndrome. Res. Vet. Sci. 6, 466–471.

Johnson, R. H. (1967a). Feline panleukopenia virus—In vitro comparison of strains with a mink enteritis virus. J. Small Anim. Pract. 8, 319–324.

Johnson, R. H. (1967b). Feline panleukopenia virus. IV. Methods for obtaining reproducible in vitro results. Res. Vet. Sci. 8, 256–264.

Johnson, R. H. (1971). Serologic procedures for the study of feline panleukopenia. J. Am. Vet. Med. Assoc. 158, 876–884.

Johnson, B. J., and Castro, A. E. (1984). Isolation of canine parvovirus from a dog brain with severe necrotizing vasculitis and encephalomalacia. J. Am. Vet. Med. Assoc. 184, 1398–1399.

Johnson, R. H., and Halliwell, R. E. W. (1968). Natural susceptibility to feline panleukopenia of the coatimundi. Vet. Rec. 82, 582.

Johnson, R. H., and Spradbrow, P. B. (1979). Isolation from dogs with severe enteritis of a parvovirus related to feline panleukopenia virus. Aust. Vet. J. 55, 151.

Johnson, R. H., Margolis, G., and Kilham, L. (1967). Identity of feline ataxia virus with panleukopenia virus. Nature (London) 214, 175–177.

Johnson, R. H., Siegl, G., and Gautschi, M. (1974). Characteristics of feline panleukopenia virus strains enabling definitive classification as parvoviruses. Arch. Ges. Virusforsch. 46, 315–324.

Jones, B. R., Robinson, A. J., Fray, L. M., and Lee, E. A. (1982). A longitudinal serological survey of parvovirus infection in dogs. N.Z. Vet. J. 30, 19–20.

Kelly, W. R., and Atwell, R. B. (1979). Diffuse subacute myocarditis of possible viral aetiology: Cause of sudden deaths in pups. Aust. Vet. J. 55, 36–37.

Kikuth, V. W., Gonnert, R., and Schweickert, M. (1940). Infectiose aleukozytose der katzen. Zentralbl. Bakteriol., Parastienkd. Infektionskr. 146, 1–17 (in German).

Kilham, L., Margolis, G., and Colby, E. D. (1967). Congenital infections of cats and ferrets by feline panleukopenia virus manifested by cerebellar hypoplasia. Lab. Invest. 17, 465–480.

Kilham, L., Margolis, G., and Colby, E. D. (1971). Cerebellar ataxia and its congenital transmission in cats by feline panleukopenia virus. J. Am. Vet. Med. Assoc. 158, 888–901.
Kimsey, P. B., Engers, H. D., Hirt, B., and Jongeneel, C. V. (1986). Pathogenicity of fibroblast- and lymphocyte-specific variants of minute virus of mice. *J. Virol.* 59, 8–13.

King, D. A., and Croghan, D. L. (1965). Immunofluorescence of feline panleukopenia virus in cell culture: Determination of immunological status of felines by serum neutralization. *Can. J. Comp. Med.* 29, 85–89.

King, D. A., and Gutekunst, D. E. (1970). A new mink enteritis vaccine for immunization against feline panleukopenia. *VM/SAC, Vet. Med. Small Anim. Clin.* 70, 377–383.

Knox, B. (1960). Outbreaks of virus enteritis in mink in Denmark. *Nord. Veterinaermed.* 12, 166–168.

Koptopoulos, G., Papadopoulos, O., Papanastopoulos, M., and Cornwell, H. J. C. (1986). Presence of antibody cross-reacting with canine parvovirus in the sera of dogs from Greece. *Vet. Rec.* 118, 332–333.

Krunajevic, T. (1970). Experimental virus enteritis in mink. A pathologic-anatomical and electron microscopic study. *Acta Vet. Scand.* 11 (Suppl. 30), 1–88.

Kull, K.-E. (1966). Virusenterit: Sverige. *Vara Palsdjur* 8, 331 (in Swedish).

Landsverk, T., and Nordstoga, K. (1978). Virus enteritis of mink, a scanning electron microscopic study. *Acta Vet. Scand.* 19, 569–573.

Larsen, S., Flagstad, A., and Aalbak, B. (1976). Experimental feline panleukopenia in the conventional cat. *Vet. Pathol.* 13, 216–240.

Lawrence, J. S., and Syverton, J. T. (1938). Spontaneous agranulocytosis in the cat. *Proc. Soc. Exp. Biol. Med.* 38, 914–918.

Lawrence, J. S., Syverton, J. T., Shaw, J. S., and Smith, F. P. (1940). Infectious feline agranulocytosis. *Am. J. Pathol.* 16, 333–354.

Lawrence, J. S., Syverton, J. T., Ackart, R. J., Adams, W. S., Ervin, D. M., Haskins, A. L., Saunders, R. H., Stringfellow, M. B., and Wetrich, R. M. (1943). The virus of infectious feline agranulocytosis. II. Immunological relationships to other viruses. *J. Exp. Med.* 77, 57–64.

Lenghaus, C., and Studdert, M. J. (1980). Relationships of canine panleukopaenia (enteritis) and myocarditis parvoviruses to feline panleukopaenia virus. *Aust. Vet. J.* 58, 152–153.

Lenghaus, C., and Studdert, M. J. (1982). Generalized parvovirus disease in neonatal pups. *J. Am. Vet. Med. Assoc.* 181, 41–45.

Lucas, A. M., and Riser, W. H. (1945). Intranuclear inclusion in panleukopenia in cats. *Am. J. Pathol.* 21, 435–465.

Luo, M., Tsao, J., Rossmann, M. G., Basak, S., and Compans, R. W. (1988). Preliminary X-ray crystallographic analysis of canine parvovirus crystals. *J. Mol. Biol.* 200, 209–211.

Lust, S. J., Gorham, J. R., and Sato, N. (1965). Occurrence of intranuclear inclusions in cell cultures infected with infectious feline enteritis virus. *Am. J. Vet. Res.* 26, 1163–1166.

Macartney, L., McCandlish, I. A. P., Thompson, H., and Cornwell, H. J. C. (1984a). Canine parvovirus enteritis. 1: Clinical, haematological and pathological features of experimental infection. *Vet. Rec.* 115, 201–210.

Macartney, L., McCandlish, I.A.P., Thompson, H., and Cornwell, H. J. C. (1984b). Canine parvovirus enteritis. 2: Pathogenesis. *Vet. Rec.* 115, 453–460.

Macartney, L., McCandlish, I. A. P., Thompson, H., and Cornwell, H. J. C. (1984c). Canine parvovirus enteritis. 3: Scanning electron microscopical features of experimental infection. *Vet. Rec.* 115, 533–537.
MacPherson, L. W. (1956). Feline enteritis virus—Its transmission to mink under natural and experimental conditions. *Can. J. Comp. Med.* 20, 197–202.

Mann, P. C., Bush, M., Appel, M. J. G., Beehler, B. A., and Montali, R. J. (1980). Canine parvovirus infection in South American canids. *J. Am. Vet. Med. Assoc.* 177, 779–783.

Margolis, G., and Kilham, L. (1965). Rat virus, an agent with an affinity for dividing cells. *Monogr. Inst. Neurol. Dis. Blindness* 2, 361–367.

Mazzara, G. P., Destree, A. T., Williams, H. W., Sue, J. M., Belanger, L. M., Panicali, D., and Parrish, C. R. (1987). Successful vaccination of dogs with empty capsids derived from canine parvovirus—bovine papillomavirus chimeric plasmids. In “Vaccines ’87” (R. M. Chanock, R. A. Lerner, F. Brown, H. Ginsberg, eds.), pp. 419–424. Cold Spring Harbor Lab., Cold Spring Harbor, New York.

McMaster, G. K., Tratschin, J.-D., and Siegl, G. (1981). Comparison of canine parvovirus with mink enteritis virus by restriction site mapping. *J. Virol.* 38, 368–371.

Mech, L. D., Goyal, S. M., Bota, C. N., and Seal, U. S. (1986). Canine parvovirus infection in wolves (*Canis lupus*) from Minnesota. *J. Wildl. Dis.* 22, 104–106.

Meunier, P. C., Glickman, L. T., Appel, M. J. G., and Shin, S. J. (1980). Canine parvovirus in a commercial kennel: Epidemiologic and pathologic findings. *Cornell Vet.* 71, 96–110.

Meunier, P. C., Cooper, B. J., Appel, M. J. G., and Slauson, D. O. (1984). Experimental viral myocarditis—Parvovirus infection of neonatal pups. *Vet. Pathol.* 21, 509–515.

Meunier, P. C., Cooper, B. J., Appel, M. J. G., Lanieu, M. E., and Slauson, D. O. (1985a). Pathogenesis of canine parvovirus enteritis: Sequential virus distribution and passive immunization studies. *Vet. Pathol.* 22, 617–624.

Meunier, P. C., Cooper, B. J., Appel, M. J. G., and Slauson, D. O. (1985b). Pathogenesis of canine parvovirus enteritis: The importance of viremia. *Vet. Pathol.* 22, 60–71.

Mochizuki, M., Konishi, S., and Ogata, M. (1978). Studies on feline panleukopenia. II. Antigenicities of the virus. *Jpn. J. Vet. Sci.* 40, 375–383.

Mohri, S., Handa, S., Wada, T., and Tokiyoshi, S. (1982). Sero-epidemiologic survey on canine parvovirus infection. *Jpn. J. Vet. Sci.* 44, 543–545.

Moraillon, A., and Moraillon, R. (1982). Distinction des parvovirus félin et canins par hemagglutination. *Rec. Med. Vet.* 158, 799–804 (in French).

Morgan, W. R., and Ward, D. C. (1986). Three splicing patterns are used to excise the small intron common to all minute virus of mice RNAs. *J. Virol.* 60, 1170–1174.

Myers, W. L., Alberts, J. O., and Brandley, C. A. (1959). Certain characteristics of the virus of infectious enteritis of mink and observations on pathogenesis of the disease—Preliminary report. *Can. J. Comp. Med.* 23, 282–287.

Nain, K. P. D., Padmainhbaiyer, R., and Venugapolar, A. (1964). An outbreak of gastroenteritis in civet cats. *Indian Vet. J.* 41, 763–765.

Nara, P. L., Winters, K., Rice, J. B., Olsen, R. G., and Krakowka, S. (1983). Systemic and local intestinal antibody response in dogs given both infective and inactivated canine parvovirus. *Am. J. Vet. Res.* 44, 1989–1995.

Nettles, V. F., Pearson, J. E., Gustafson, G. A., and Blue, J. L. (1980). Parvovirus infection in translocated raccoons. *J. Am. Vet. Med. Assoc.* 177, 787–789.

Neuvonen, E., Veijalainen, P., and Kangas, J. (1982). Canine parvovirus infection in housed raccoon dogs and foxes in Finland. *Vet. Rec.* 110, 448–449.

Okaniwa, A., Yasoshima, H., Kojima, A., and Doi, K. (1976). Fine structure of epithelial cells of Lieberkuhn’s crypts in feline panleukopenia. *Natl. Inst. Health Q. (Tokyo)* 16, 167–175.
Osterhaus, A. D. M. E., Drost, G. A., Wirahadiredja, R. M. S., and van den Ingh, T. S. G. A. M. (1980). Canine viral enteritis: Prevalence of parvo-, corona- and rotavirus infections in dogs in The Netherlands. Vet. Q. 2, 181–190.

O'Sullivan, G., Durham, P. J. K., Smith, J. R., and Campbell, R. S. F. (1984). Experimentally induced severe canine parvoviral enteritis. Aust. Vet. J. 61, 1–4.

Parese, P., and Young, J. F. (1982). Variation of influenza A, B, and C viruses. Science 215, 1468–1474.

Paradiso, P. R. (1981). Infectious process of the parvovirus H-I: Correlation of protein content, particle density and viral infectivity. J. Virol. 39, 800–807.

Paradiso, P. R. (1983). Analysis of the protein–protein interactions in the parvovirus H-1 capsid. J. Virol. 46, 94–102.

Paradiso, P. R. (1984). Identification of multiple forms of the noncapsid parvovirus protein NCVP1 in H-1 parvovirus infected cells. J. Virol. 52, 82–87.

Paradiso, P. R., Rhode, S. L., and Singer, I. I. (1982). Canine parvovirus: A biochemical and ultrastructural characterization. J. Gen. Virol. 62, 113–126.

Paradiso, P. R., Williams, K. R., and Costantino, R. L. (1984). Mapping of the amino terminus of the H-1 major capsid protein. J. Virol. 52, 77–81.

Parrish, C. R., and Carmichael, L. E. (1983). Antigenic structure and variation of canine parvovirus type-2, feline panleukopenia virus and mink enteritis virus. Virology 129, 401–414.

Parrish, C. R., and Carmichael, L. E. (1986). Characterization and recombination mapping of an antigenic and hostrange mutation of canine parvovirus. Virology 148, 121–132.

Parrish, C. R., Oliver, R. E., and McNiven, R. (1982a). Canine parvovirus infections in a colony of dogs. Vet. Microbiol. 7, 317–324.

Parrish, C. R., Carmichael, L. E., and Antczak, D. F. (1982b). Antigenic relationships between canine parvovirus type 2, feline panleukopenia virus and mink enteritis virus using conventional antisera and monoclonal antibodies. Arch. Virol. 72, 267–278.

Parrish, C. R., Gorham, J. R., Schwartz, T. M., and Carmichael, L. E. (1984). Characterization of antigenic variation among mink enteritis virus isolates. Am. J. Vet. Res. 45, 2591–2599.

Parrish, C. R., O’Connell, P. H., Evermann, J. F., and Carmichael, L. E. (1985). Natural variation of canine parvovirus. Science 230, 1046–1048.

Parrish, C. R., Leathers, C. W., Pearson, R., and Gorham, J. R. (1987). Comparisons of feline panleukopenia virus, canine parvovirus, raccoon parvovirus, and mink enteritis virus and their pathogenicity for mink and ferrets. Am. J. Vet. Res. 48, 1429–1435.

Parrish, C. R., Burtonboy, G., and Carmichael, L. E. (1988a). Characterization of a non-hemagglutinating mutant of canine parvovirus. Virology 163, 230–232.

Parrish, C. R., Have, P., Foreyt, W. J., Evermann, J. F., Senda, M., and Carmichael, L. E. (1988b). The global spread and replacement of canine parvovirus strains. J. Gen. Virol. 69, 1111–1116.

Parrish, C. R., Aquadro, C. F., and Carmichael, L. E. (1988c). Canine host range and a specific epitope map along with variant sequences in the capsid protein gene of canine parvovirus and related feline, mink and raccoon parvoviruses. Virology 166, 293–307.

Phillips, C. E. (1943). Haemorrhagic enteritis in the Arctic blue fox caused by the virus of feline enteritis. Can. J. Comp. Med. 7, 33–35.

Pollock, R. V. H. (1982). Experimental canine parvovirus infection in dogs. Cornell Vet. 72, 103–119.

Pollock, R. V. H., and Carmichael, L. E. (1982). Maternally derived immunity to canine...
parvovirus infection: Transfer, decline and interference with vaccination. *J. Am. Vet. Med. Assoc.* 180, 37-42.

Pridham, T. J., and Wills, C. G. (1959). The prevention of virus enteritis in newly weaned mink kits by the use of a homologous tissue vaccine. *J. Am. Vet. Med. Assoc.* 135, 279-282.

Pridham, T. J., and Wills, C. G. (1960). Variations in the effectiveness of commercial infectious feline enteritis vaccines in preventing virus enteritis of mink. *Can. Vet. J.* 1, 51-56.

Reed, A. P., Jones, E. V., and Miller, T. J. (1988). Nucleotide sequence and genome organization of canine parvovirus. *J. Virol.* 62, 266-276.

Reynolds, H. A. (1969). Some clinical and hematological features of virus enteritis of mink. *Can. J. Comp. Med.* 33, 155-159.

Reynolds, H. A. (1970). Pathological changes in virus enteritis of mink. *Can. J. Comp. Med.* 34, 155-163.

Rhode, S. L. (1985). Nucleotide sequence of the coat protein gene of canine parvovirus. *J. Virol.* 54, 630-633.

Rhode, S. L., and Paradiso, P. R. (1983). Parvovirus genome: Nucleotide sequence of H-1 and mapping of its genes by hybrid-arrested translation. *J. Virol.* 45, 173-184.

Rice, J. B., Winters, K. A., Krakowka, S., and Olsen, R. G. (1982). Comparison of systemic and local immunity in dogs with canine parvovirus gastroenteritis. *Infect. Immun.* 38, 1003-1009.

Rivera, E., and Sundquist, B. (1984). A non-haemagglutinating isolate of mink enteritis virus. *Vet. Microbiol.* 9, 345-353.

Robinson, W. F., Wilcox, G. E., Flower, R. L. P., and Smith, J. R. (1979a). Evidence for a parvovirus as the aetiologi agent in myocarditis of puppies. *Aust. Vet. J.* 55, 294-295.

Robinson, W. F., Huxtable, C. R. R., Pass, D. A., and Howell, J. M. (1979b). Clinical and electrocardiographic findings in suspected viral myocarditis in pups. *Aust. Vet. J.* 55, 351-355.

Robinson, W. F., Huxtable, C. R., and Pass, D. A. (1980a). Canine parvovirus myocarditis: A morphological description of the natural disease. *Vet. Pathol.* 17, 282-293.

Robinson, W. F., Wilcox, G. E., and Flower, R. L. P. (1980b). Canine parvoviral disease: Experimental reproduction of the enteric form with a parvovirus isolated from a case of myocarditis. *Vet. Pathol.* 17, 589-599.

Rohovsky, M. W., and Griesemer, R. A. (1967). Experimental feline infectious enteritis in the germfree cat. *Pathol. Vet.* 4, 391-410.

Schofield, F. W. (1949). Virus enteritis in mink. *North Am. Vet.* 30, 651-654.

Schers, A., Pastoret, P.-P., Burtonboy, G., and Thiry, E. (1979). Fréquence en Belgique de l’infection à parvovirus chez le chien, avant et après l’observation des premiers cas cliniques. *Ann. Vet. Med.* 123, 561-566 (in French).

Scott, F. W., Csiza, C. K., and Gillespie, J. H. (1970). Feline viruses. V. Serum neutralization test for feline panleukopenia. *Cornell Vet.* 60, 183-191.

Senda, M., Hirayama, N., Itoh, O., and Yamamoto, H. (1988). Canine parvovirus: Strain difference in haemagglutination activity and antigenicity. *J. Gen. Virol.* 69, 349-354.

Shindel, N. M., van Kruiningen, H. J., and Scott, F. W. (1978). The colitis of feline panleukopenia. *J. Am. Anim. Hosp. Assoc.* 14, 738-747.

Siegl, G. (1984). Panine parvovirus: Origin and significance of a “new” viral pathogen. In “The Parvoviruses” (K. I. Berns, ed.), pp. 383-388. Plenum, New York.

Siegl, G., Bates, R. C., Berns, K. I., Carter, B. J., Kelly, D. C., Kurstak, E., and Tattersall, P. (1985). Characteristics and taxonomy of Paroviridae. *Intervirology* 23, 61-73.
Smith, J. R., Farmer, T. S., and Johnson, R. H. (1980). Serological observations on the epidemiology of parvovirus enteritis of dogs. *Aust. Vet. J.* **56**, 149–150.

Spalholz, B. A., and Tattersall, P. (1983). Interaction of minute virus of mice with differentiated cells: Strain-dependent target cell specificity is mediated by intracellular factors. *J. Virol.* **46**, 937–943.

Surleraux, M., Bodeus, M., and Burtonboy, G. (1987). Study of canine parvovirus polypeptides by immunoblot analysis. *Arch. Virol.* **95**, 271–281.

Tattersall, P. (1972). Replication of the parvovirus minute virus of mice. I. dependence of virus multiplication and plaque formation on cell growth. *J. Virol.* **10**, 586–590.

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.

Tattersall, P., Cawte, P. J., Shatkin, A. J., and Ward, D. C. (1976). Three structural polypeptides coded for by minute virus of mice, a parvovirus. *J. Virol.* **20**, 273–289.

Tennant, R. W., Layman, K. R., and Hand, R. E. (1969). Effect of cell physiological state on infection by rat virus. *Virology* **11**, 872–878.

Thomas, N. J., Foreyt, W. J., Evermann, J. F., Windberg, L. A., and Knowlton, F. F. (1984). Seroprevalence of canine parvovirus in wild coyotes from Texas, Utah and Idaho (1972 to 1983). *J. Am. Vet. Med. Assoc.* **185**, 1283–1287.

Torres, E. (1941). Infectious feline gastroenteritis in wild cats. *North Am. Vet.* **22**, 297.

Tratschin, J.-D., McMaster, G. K., Kronauer, G., and Siegl, G. (1982). Canine parvovirus: Relationship to wild-type and vaccine strains of feline panleukopenia virus and mink enteritis virus. *J. Gen. Virol.* **61**, 33–41.

Tuomi, J., and Kangas, J. (1963). A fluorescent antibody technique for studies of mink virus enteritis. *Arch. Gesamte Virusforsch.* **13**, 432–434.

Veijalainen, P. (1986). A serological survey of enteric parvovirus infections in Finnish fur-bearing animals. *Acta Vet. Scand.* **27**, 159–171.

Veijalainen, P. (1988). Characterization of biological and antigenic properties of raccoon dog and blue fox parvoviruses: A monoclonal antibody study. *Vet. Microbiol.* **16**, 219–230.

Veijalainen, P. M.-L., and Smeds, E. (1988). Pathogenesis of blue fox parvovirus on blue fox kits and pregnant vixens. *Am. J. Vet. Res.* **49**, 1941–1944.

Verge, J., and Cristoferoni, N. (1928). La gastro enterite infectieuse des chats; est-elle due a un virus filterable? *C. R. Soc. Biol.* **99**, 312.

Walker, S. T., Feilen, C. P., Sabine, M., Love, D. N., and Jones, R. F. (1980). A serological survey of canine parvovirus infection in New South Wales, Australia. *Vet. Rec.* **106**, 324–325.

Waller, E. F. (1940). Infectious gastroenteritis in raccoons (Procyon lotor). *J. Am. Vet. Med. Assoc.* **96**, 266–268.

Wayne, R. K., Benveniste, R. E., Janczewski, D. N., and O'Brien, S. J. (1989). Molecular and biochemical evolution of the Carnivora. In "Carnivore Behavior, Ecology and Evolution" (J. L. Gittleman, ed.), pp. 465–494. Cornell Univ. Press, Ithaca, New York.

Webster, R. G., Laver, W. G., Air, G. M., and Schild, G. C. (1982). Molecular mechanisms of variation in influenza viruses. *Nature (London)* **296**, 115–121.

Wills, C. G. (1952). Notes on infectious enteritis of mink and its relationship to canine enteritis. *Can. J. Comp. Med.* **16**, 419–420.

Wills, G., and Belcher, J. (1965). The prevention of virus enteritis of mink with commercial feline panleukopenia vaccine. *J. Am. Vet. Med. Assoc.* **128**, 559–560.

Yasoshima, A., Doi, K., Kojima, A., and Okaniwa, A. (1982). Electron microscopic findings on epithelial cells of Lieberkuhn's crypts in canine parvovirus infection. *Jpn. J. Vet. Sci.* **44**, 81–88.

Zarnke, R. L., and Ballard, W. B. (1987). Serological survey for selected microbial pathogens of wolves in Alaska, 1975–1982. *J. Wildl. Dis.* **23**, 77–85.