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Chapter 9

Viral Diseases of the Digestive System

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I. INTRODUCTION

At a symposium on the viruses of laboratory rodents held in 1965, Parker et al. (1966) described the results of antibody tests for a number of virus infections in 34 colonies of mice throughout the United States. In these, reovirus 3 infection occurred in 28 (82%) and murine hepatitis in 25 (74%). Although there was at that time no routine serologic test for
epidemic diarrhea of infant mice, it is common knowledge that virtually every colony of conventional mice suffered that infection to a greater or lesser extent.

In recent years, with the advent of measures such as cesarean derivation, barrier-sustained breeding and maintenance procedures, routine serologic surveillance, laminar flow hoods or rooms, and filter-top cages, the problems associated with these diseases have become somewhat less critical. Nevertheless, they are at times and under certain circumstances still troublesome.

All three infections may cause serious, debilitating, and sometimes fatal diarrheal disease in nursing and weanling mice. Thus, the economic impact on commercial mouse colonies can be severe. Furthermore, newborn mice, pooled from many dams and then redistributed to them at random, are used for the isolation and identification of certain viruses in diagnostic and epidemiological studies, for example arboviruses (Shope, 1980), coronaviruses (Mcintosh et al., 1967), and reoviruses (Stanley, 1977). Clearly, indigenous infection with related or identical agents in only a few such infants could confound and compromise the validity of observations following the inoculation of test materials. It should be pointed out, too, that Collins and Parker (1972) demonstrated both reovirus 3 and murine hepatitis virus as contaminants in some murine leukemia and transplantable tumor specimens.

Whereas it is mainly from these standpoints that the diseases in question derive importance, they should not be dismissed without considering their intrinsic value as models for elucidating the pathogenesis and control of related infections in man and other animals as well as their utility for the molecular biologist.

II. EPIZOOTIC (EPIDEMIC) DIARRHEA OF INFANT MICE (EDIM), MOUSE ROTAVIRUS ENTERITIS

A. Historical Background

Cheever and Mueller (1947, 1948), Pappenheimer and Enders (1947), and Pappenheimer and Cheever (1948) were the first to describe the pathological changes and epidemiology of EDIM. Runner and Palm (1953) and Cheever (1956) also contributed to knowledge concerning the epidemiology and etiology of the disease. Thereafter Kraft (1957, 1958, 1961, 1962b, 1966) reported on studies regarding the etiology, mode of transmission, carrier state, immune response, pathogenesis, and control of the disease. Serologic studies were also undertaken by Blackwell et al. (1966).

Adams and Kraft (1963, 1967) first demonstrated the agent in electron micrographs of infected infant mouse intestinal epithelium. Banfield et al. (1968) enlarged on those findings, comparing the virions to those of the reoviruses. Particles of similar morphology were subsequently observed in the diarrheal feces of many species, primarily in the young: cattle (Mebus et al., 1969), man (Flewett et al., 1974), horse (Flewett et al., 1975), pig (Rodger et al., 1975), sheep (Snodgrass et al., 1976), rabbit (Bryden et al., 1976), deer (Tzipori et al., 1976), goat (Scott et al., 1978), and dog (England and Poston, 1980). In addition, one virus, SA-11, was isolated from a nondiarrheal monkey, and another, OA (offal agent), was recovered from intestinal washings of sheep in an abattoir (Els and Lecatsas, 1972).

Much and Zajac (1972) purified EDIM virus from diarrheal infant mice and further characterized it.

Based on its morphology and other known characters, EDIM virus has been placed into the genus Rotavirus in the family Reoviridae (Matthews, 1979). During the past decade, knowledge of the rotaviruses as agents of diarrheal disease of young mammals has burgeoned, especially with regard to those affecting children, calves, and piglets. Reviews concerning the genus have been published by Wyatt et al. (1978), McNulty (1978, 1979), Flewett and Woode (1978), Andrewes et al. (1978), and Holmes (1979). Additional comments may be found in the American Veterinary Medical Association Panel Report of the Colloquium on Selected Diarrheal Diseases of the Young (Anonymous, 1978).

B. Properties of the Virus

1. Classification

The classification of EDIM virus in the genus Rotavirus, family Reoviridae, derives from its morphology and mode of replication as seen in electron micrographs (Adams and Kraft, 1967; Banfield et al., 1968), which were later shown to resemble those observed in ultrathin sections in human intestinal material containing “reovirus-like” particles (Bishop et al., 1973). The term rotavirus (L. rota, wheel) was proposed by Flewett et al. (1974) because of its morphology as viewed in negative-contrast electron micrographs. It has become widely used in preference to duovirus, which is synonymous (Davidson et al., 1975).

2. Strains of EDIM Virus

There is no evidence that antigenic or pathogenetic variants of EDIM virus exist. Analysis of RNA segment and structural polypeptide variation, however, as has been accomplished for other rotaviruses (Kalica et al., 1978; Derbyshire and Woode, 1978; Rodger and Holmes, 1979), may reveal differences among strains. Further, several serotypes have
been described among the human rotaviruses by means of the serum neutralization test (Beards et al., 1980). This technique may also prove useful in future studies of EDIM virus strains.

3. Physical Properties

a. Morphology, Size, and Composition. Kraft (1962b), using Millipore filters, determined the size of the infective EDIM virion to be between 33 and 100 nm. In electron micrographs, Adams and Kraft (1967) recognized two principal particle types: a spherical one, 75–80 nm in diameter, with two double membranes surrounding a nucleoid or core, and another, likewise spherical but less frequently seen, 65 nm in diameter with only one double membrane. Tubular structures as well as coreless (electron-lucent) spherical particles were also occasionally encountered. Banfield et al. (1968) described the tubular structures more extensively and demonstrated them within nuclei as well as in the cytoplasm.

Holmes et al. (1975) studied EDIM virus by negative-contrast electron microscopy as well as in ultrathin sections of mouse intestines and reached similar conclusions as to the size and morphology of the virions. Much and Zajac (1972) determined the mean diameter of 100 virions of purified EDIM virus to be 54.4 ± 2 nm. On the other hand, Melnick (1979) gives the diameter of the Rotavirus genus as 70 nm. Possibly Much and Zajac (1972) measured only incomplete particles, those lacking the outer capsid layer.

Specific details for EDIM virus with regard to RNA molecular weight, number of capsid polypeptides, number and shape of capsomers, etc., are lacking in the literature. However, all rotaviruses are regarded as possessing essentially spherical capsids, 60–80 nm in diameter, with icosahedral symmetry (Fig. 1). The genome consists of 10–12 molecules of double-stranded RNA with a total molecular weight of about $15 \times 10^6$.

b. Effect of Heat. As a filtrate of intestinal suspension, infectivity of EDIM virus is retained at 4°C for 1 hr, whereas

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Fig. 1. Electron micrograph of purified rotavirus. Uranyl acetate negative stain. Pseudoreplica technique. Scale bar = 100 nm. (Courtesy of Erskine Palmer, Center for Disease Control, Atlanta.)
about 50% is lost at 4°C for 24 hr or at 37°C for 1 hr. Although some infectivity (>0.05%) remains at either 56°C or 60°C for 30 min, it is abolished at 70°C for 15 min (Cheever and Mueller, 1947; Kraft, 1957, 1962b). Much and Zajac (1972) found that the purified virus is unstable at both 4°C and −24°C for 2 weeks but found that at −70°C infectivity is retained for at least 4 weeks.

c. Effect of Chemicals. In an intestinal filtrate, EDIM virus titer is not significantly reduced when held in ether or 0.1% sodium deoxycholate at 4°C for 24 hr (Kraft, 1962b). According to Much and Zajac (1972), the purified virus is stable in 20% ether, 5% chloroform, or 0.1% sodium deoxycholate at 4°C for 1 hr and, as is true for other rotaviruses, is resistant to pancreatin.

Ward and Ashley (1980a,b) examined the effects of the anionic detergent sodium dodecyl sulfate and of the chelating agent ethylenediaminetetraacetate on purified simian (SA-11) virus and determined that low concentrations and mild temperature conditions readily inactivated the agent. Both chemicals modified the viral capsid to prevent adsorption of the inactivated virions to cells. Indeed, this study was the outgrowth of a need to determine the survival of enteric viruses in wastewater. It had been determined that wastewater sludge reduced the heat necessary for simian rotavirus inactivation. Ionic detergents in the sludge were identified as the active components. Nonionic detergents did not destabilize the virus; further, these compounds protected the virus from the destabilizing effect of sodium dodecyl sulfate. Destabilization by both cationic and anionic detergents was found to be dependent on the pH of the medium.

d. Effect of pH. A systematic study of the stability of EDIM virus to extremes of pH has not been reported. Much and Zajac (1972) treated virus preparations during purification procedures at pH 9.0 without apparent loss of titer.

e. Antigenic Determinants. EDIM virus shares a common antigen with other rotaviruses that is demonstrable by a variety of serologic tests. This determinant is associated with the inner capsid of the virion (Mathan et al., 1977; Woode et al., 1976). Based on the neutralization test, on the other hand, EDIM virus appears to be type specific, neutralization of infectivity being associated with the intact (complete) virion (Thouless et al., 1977b).

Kraft (1958) was unable to demonstrate an EDIM virus hemagglutinin using guinea pig, adult and embryonic chicken, adult and embryonic mouse, rabbit, hamster, human 0, and sheep erythrocytes. However, Fauvel et al. (1978), studying calf and human rotaviruses by means of osmotic shock to release cell-bound virus from BS-C-1 tissue culture cells, prepared a hemagglutinin for human 0, guinea pig, and bovine red cells that is apparently associated with the intact virion. They also succeeded in this regard with the simian SA-11 rotavirus.

4. Relationship to Other Viruses

Kraft (1966) determined that EDIM virus is antigenically distinct from reovirus 3. Hyperimmune sera prepared in mice against EDIM virus by Blackwell et al. (1966) did not react with pneumonia virus of mice, mouse encephalomyelitis virus, reovirus 3, newborn mouse pneumonitis (K) virus, polyomavirus, or murine (mouse) hepatitis virus.

Using gel electrophoresis, Smith and Tzipori (1979) examined the RNA of rotaviruses from six animal species. Reproducible differences in RNA migration patterns were found between all isolates (from calf, pig, mouse, deer, foal, and dog-adapted human isolates). Pig, mouse, and foal viruses each yielded 11 bands; calf, 9; deer, 10; and dog-adapted human isolates 12 bands.

5. Growth and Distribution in Mice

Replication of EDIM virus occurs in epithelial cells of the villi of the small intestine. In electron micrographs, Adams and Kraft (1967), Banfield et al. (1968), and Holmes et al. (1975) demonstrated replication by budding in distended cisternae of the endoplasmic reticulum. The significance of intracytoplasmic and intranuclear tubular structures encountered is not clear.

Using direct immunofluorescence, Wilsnack et al. (1969) found that EDIM virus antigen in infant mice is limited to the cytoplasm of the villous epithelium from the duodenum to the colon and is detectable within 48 hr after peroral inoculation. Stainable virus was also seen in the intestines of contact infant mice without necessarily causing clinical signs to appear. The virus could not be stained in the stomach or liver of mice infected either naturally or experimentally.

Kraft (1958) studied the distribution of infectious EDIM virus in 3-day-old mice following peroral inoculation. At 3 hr, virus was detected in the stomach and small and large intestines (the cecum was not tested) and could not be recovered from the lungs, liver, spleen, kidney, or blood. At 24 hr, all those tissues were positive except for the kidney. The bladder and urine, as well as the brain, were also devoid of the agent, but at 30 hr, these too were positive.

At 72 hr, the liver, spleen, kidney, and intestines yielded virus, but the brain was negative. The blood, stomach, lungs, bladder, and urine were not tested at that interval. At 6 days, blood, liver, and intestines, the only tissues examined, still contained virus.

Ingested virus from a diarrheal litter brings about an active infection in the nursing dam that previously had been non-diarrheal herself and had had only nondiarrheal litters (Kraft,
1958). Blood, liver, spleen, and feces all contain infectious virus in the dam 1 week after her litter is given virus perorally as an intestinal filtrate. Later, Kraft (1961) determined that adult male mice can also be intestinal carriers for at least 17 days after a single peroral exposure.

With regard to the mechanism of cell penetration, Holmes et al. (1976) proposed that lactase of the villous brush border of the intestine may be the receptor that uncoats the rotavirus virion by attacking the glycosylated polypeptides of the outer capsid. In addition, pancreatic enzymes within the lumen may also be instrumental in the infectious process (see Section II,B,6).

The question of cofactors that might enhance pathogenicity or of interfering substances, in addition to antibodies, that might inhibit EDIM virus replication is open. Kraft (1958) found increased numbers of Clostridium tertium in the intestine of diarrheal animals, and Pappenheimer and Enders (1947) remarked on the persistent presence of "coccoid bodies" in the intestines of diarrheal animals as seen by light microscopy. In both instances, these may be considered opportunistic organisms. Their effect on the severity of the clinical disease is unknown, however.

One report (LaBonnardiere and deVaurieux, 1979) concerns the question of interferon production by bovine rotavirus and EDIM virus. Orally instilled bovine rotavirus, which is non-pathogenic for infant mice, was shown to delay the onset of EDIM virus-induced diarrhea when the latter agent was given orally 48 hr after the bovine agent. Only intact infectious bovine virus was capable of eliciting this effect. Interferon could not be found in the mouse small intestine at various times after instillation of the bovine agent after EDIM virus infection; therefore the nature of the interference is not clear. It may be of significance for possible immunization of mice with active virus that some bovine rotavirus replication could be visualized in mouse intestinal cells by means of immunofluorescence, although detectable antigen was not evident from utilization of the ELISA test.

6. Growth in Tissue and Organ Culture

The use of standard methods to grow EDIM virus in tissue culture has resulted for the most part in failure.

Habermann (1959) made 10 serial passages of EDIM virus in Chang liver cells but reported results only after the second passage, when supernatant fluid produced diarrhea in infant mice. Kraft (1958) was unable to grow the agent in monkey kidney epithelial cells, mouse embryo, two strains of mouse fibroblasts, HeLa cells, human intestine (Henle), and human conjunctiva. Thouless et al. (1977b) cultivated EDIM virus as well as other rotaviruses in LC MK2 cells but could not serially subculture them. Viral multiplication was detectable only by means of immunofluorescence. On the other hand, Rubenstein et al. (1971) grew the agent for three passages in organ cultures of embryonic mouse ileum, cecum, and colon but not in esophagus or duodenum cultures. Subculture in mouse embryo fibroblasts was unsuccessful.

It may be of some importance for successful cultivation of EDIM virus that treatment of tissue culture passage material with pancreatin enhances the virus yield of porcine rotavirus (Thiel et al., 1978). Further, Babiuk et al. (1977) and Almeida et al. (1978) state that calf rotavirus production is markedly enhanced when passaged in the presence of trypsin. Clark et al. (1979) produced high-titer calf rotavirus in a variety of continuous cell lines, also by utilizing trypsin, and Matsuno et al. (1977) were successful in producing plaques with bovine rotavirus in monkey kidney cell monolayers when trypsin was incorporated in the overlay medium. Estes and Graham (1979) found that simian virus titers were enhanced by both trypsin and elastase in Vero as well as MA104 cells. Ramia and Sattar (1980) studied additional proteolytic enzymes utilizing plaque formation by simian virus SA-11 in MA104 cells as the endpoint. Elastase, α-chymotrypsin, subtilase, pronase, and pancreatin were as effective as trypsin, whereas pepsin, papain, and thermolysin were ineffective.

Another approach with human rotavirus has been to incorporate the agent into cells by means of low-speed centrifugation of the two together (Banatvala et al., 1975; Bryden et al., 1977; Schoub et al., 1979; Wyatt et al., 1980).

It remains to be seen if similar treatments will lead to successful cultivation of EDIM virus in tissue culture.

7. Growth in Fertile Hens' Eggs

Chick embryos appeared unaffected when the chorioallantois was inoculated. The agent did survive up to 5 days, but an increase in infectious titer could not be demonstrated (Kraft, 1958).

C. Pathogenesis

Moon (1978) has discoursed on pathogenetic mechanisms in diarrheal diseases, comparing EDIM, calf rotavirus infection, transmissible gastroenteritis coronavirus infection of pigs (TGE), and feline panleukopenia as to the predilection of each virus for particular regions of enteric epithelium. The character of each disease appears to be reflected not only in the particular group of cells but also in the numbers of host cells involved.

1. The Clinical Disease

Lucid descriptions of the natural clinical disease are given by Cheever and Mueller (1947), Seamer (1967), and McClure et al. (1978). The experimental disease does not differ significantly (Kraft, 1957, 1962b). Overt illness is confined to pre-
weanling mice. The first signs usually appear at 7–8 days of age. In mild cases diarrhea is manifested by a minimal amount of pasty fecal material about the perineum. In severe cases the amount is copious, the entire infant becoming soiled. Rectal impaction may occur at about 12–16 days of age, and death can ensue if the impacted mass is not removed spontaneously or deliberately. Death does not seem to be the result of the virus infection per se, but rather as a consequence of protracted obstipation.

In pure EDIM virus infections, mice continue to nurse throughout their illness (in the absence of impaction). They may be slightly stunted (Fig. 2), but those with mild cases soon attain the weight of their nondiarrheal peers.

Especially when diarrhea is mild, morbidity in the natural disease may be difficult to ascertain, but one could quite correctly assume that, if diarrheal signs are observed in a few litters, it is likely that all infants in a colony will sooner or later be affected. Concerning mortality, Cheever and Mueller (1947) state that there was both 100% recovery and 100% lethality in their outbreaks. Based on present knowledge, the first is suggestive of EDIM virus infection and the latter of another disease, perhaps murine (mouse) hepatitis.

At no time do adult mice exhibit signs of illness ascribable to EDIM virus infection.

2. Pathology

a. Gross Pathology. In the experimentally induced disease, the absence of external clinical signs cannot be relied upon for a negative diagnosis (Kraft, 1957). Necropsy of each animal is essential. In this way, the appearance of the colonic contents can be observed, which in normal infant mice are burnt orange in color and semisolid but formed in consistency. The colon is not distended.

In diarrheal mice, however, even in those with no external soiling, the colonic contents are fluid or mucoid, bright lemon yellow to amber, or gray-green, with no formed feces in evidence. Gas is often seen in the colon and cecum, which are distended. The stomach, too, is distended with curdled milk (except in terminal cases with anal impaction). All other organs appear normal.

Gross change is not seen in the intestines of adults exposed perorally. It is noteworthy that in germ-free infant mice, experimental EDIM virus infection appears in the gross as it does in conventional mice (Kraft, 1966).

Findings in the spontaneous disease are identical.

b. Microscopic Pathology. Pappenheimer and Enders (1947) noted that the only changes were in the small intestine, where there was a slight increase in the cellularity of the stroma of some of the villi. Frank inflammation was lacking. They, together with Pappenheimer and Cheever (1948), described fuchsinophilic intracytoplasmic inclusions that were always related to clinical EDIM and were found only in the villous epithelial cells of the small intestine. They described degenerating, inclusion-bearing cells as well. Inclusions were seen only in the early stages of the disease. Correspondence between these inclusions and any of the ultrastructures later
The histologic picture has been confirmed by others (Adams and Kraft, 1967; McClure et al., 1978). There is no inflammatory reaction in the intestines. Inclusions may indeed be found in enterocytes near the tips of villi, especially in the jejunum, and infrequently in the sloughed cells seen in the lumen. Epithelial cells near and at the tips of villi are frequently vacuolated.

Acres and Babiuk (1978) have pointed out that in all species studied, the rotaviruses cause diarrhea by attacking and destroying the columnar epithelium of the small intestine. Middleton (1978) considers that cell migration from the crypts is speeded in response to diarrhea and that the infected cells are relatively immature. Enzyme levels, high thymidine kinase and low sucrase, are similar in such cells to those of normal crypt cells and thus support this view.

The microscopic appearance belies the severity of the gross and clinical findings of EDIM. In this regard, D-xylose absorption has been studied in calves in which 60–90% reduction occurred during the acute illness with the calf rotavirus (Woode et al., 1978). Aberrations in sodium transport have also been investigated in other animals and in humans (Middleton, 1978), but such studies have not been carried out in mice using the mouse agent.

3. Transmission

Cheever and Mueller (1947) and Kraft (1957) demonstrated that the agent is transmissible perorally. Kraft (1957) showed further that dissemination in a mouse colony is mediated principally by the airborne route.

Vertical transmission of the agent has not been demonstrated. On the other hand, the fact that viremia can occur in the adult suggests that transplacental transmission should be investigated.

Fomites, arthropods, or humans acting as passive vectors cannot be excluded as initiators of an epizootic, but they are probably not as important as the mice themselves. Since adult mice can become carriers for some days after a single exposure to the virus, these are likely to be instrumental in bringing about an epizootic.

4. Immune Response

Kraft (1961) examined the neutralization test in EDIM and found that antibodies were not always formed following infection, and when they were present, they tended to be low in titer. Sera from adult mice having had lifelong contact with the agent (in the form of consistently diarrheal litters) were more apt to neutralize the virus than those of mice exposed for the first time as adults. On the other hand, hyperimmunization of mice has resulted in the production of significant serum titers of both complement-fixing and neutralizing antibodies (Blackwell et al., 1966).

Antibody response measured by other serologic tests has not been investigated for EDIM, nor have immune substances in lactal secretions been measured directly. Suggestive of their presence, however, are data showing that infants of primiparae who themselves had been diarrheal in infancy resisted about 10 ID₅₀ more of EDIM virus than did offspring of previously non diarrheal dams (Kraft, 1961). Antirotaviral immunoglobulins have been found in both colostrum and milk in man (Yolken et al., 1978c; Simhon and Mata, 1978; Thouless et al., 1977a), bovines (Acres and Babiuk, 1978), and lambs (Snodgrass and Wells, 1976). Indeed, local immunity afforded by lactal immune substances is regarded as crucial for protection against any intestinal infection in the young (Welliver and Ogra, 1978; Snodgrass and Wells, 1976).

D. Epizootiology

1. Host Range

a. Species Affected. There is no evidence that species other than the mouse (Mus musculus) are susceptible to EDIM virus infection. On the other hand, other rotaviruses cross species barriers. In the future, this may also prove true for the mouse agent, EDIM.

b. Age and Sex Susceptibility. As indicated, mice of all ages can be infected, but overt disease is restricted to animals up to about 12–13 days of age at the time of first exposure. There seems to be no predilection for a particular sex.

c. Influence of Parity (Birth Order). The observation that first litters are more apt to show copious and protracted soiling than those of later parity has been reported by Cheever and Mueller (1947, 1948) and by Runner and Palm (1953). Kraft (1962b) reported similar findings in a mixed infection in a conventional colony. It is not clear if the cause for this resides in the immune response of the dam, cofactors such as opportunistic microbiota that can influence the disease picture as secondary factors, or genetics of the host and/or viral agent.

d. Strains of Mice. Cheever and Mueller (1948) studied the disease in four strains of mice: Harvard, Schwentker, CFW, and C (National Cancer Institute). Using percentage of animals weaned as the endpoint, the CFW strain was the most susceptible.

2. Prevalence and Distribution

EDIM Virus is probably worldwide in distribution. Whether it occurs in wild mouse populations is unknown. Its prevalence...
is difficult to estimate, since serologic tests have not been readily available, and it is not customary to sacrifice animals for the purpose of examining the appearance of their intestinal tract or for electron microscopic visualization of fecal contents.

3. Latent and Chronic Infection

As has been shown experimentally, latent carriers can exist. The carrier rate in a diarrheal colony is not known, nor is the frequency of viral shedding under colony conditions.

4. Seasonal Periodicity

Cheever and Mueller (1948) examined seasonal variations in the weaning percentage in their mouse strains and found that there was a significant effect in only the CFW mice. They experienced the lowest weaning rate in the late fall and winter. Runner and Palm (1953), studying C3H mice, indicated that there was a higher incidence of diarrhea in December/January than in September/November, although their figures are not vastly different: 59% and 44%, respectively.

E. Diagnosis

1. Serology

A number of serologic tests have been developed for the rotaviruses. The references which follow are cited as examples and are not intended to be all-inclusive. Furthermore, the techniques have been applied mainly to rotaviruses other than EDIM virus: neutralization (Kraft, 1961; Blackwell et al., 1966), complement fixation (Wilsnack et al., 1969; Kapikian et al., 1976; Thouless et al., 1977b), direct immunofluorescent staining or precipitin (Wilsnack et al., 1969; Spence et al., 1975; Foster et al., 1975; Peterson et al., 1976), immune electron microscopy (Kapikian et al., 1974; Bridger and Woode, 1975), immunoelectroosmophoresis (Tuvevsson and Johnsson, 1976; Middleton et al., 1976), enzyme-linked immunosorbent assay (ELISA) (Scherrer and Bernard, 1977; Ellens et al., 1978; Yolken et al., 1978a, b, c), radioimmunoadsay (Acres and Babiuk, 1978; Kalica et al., 1977; Middleton et al., 1977), immunodiffusion (Woode et al., 1976), hemagglutination inhibition (Fauvel et al., 1978), enzyme-linked fluorescence assay (ELISA) (Yolken and Stopa, 1979), an unlabeled soluble enzyme peroxidase-antiperoxidase method (Graham and Estes, 1979), plaque reduction test (Estes and Graham, 1980), serologic trapping on antibody-coated electron microscope grids (Nicolaieff et al., 1980), a solid phase system (SPACE, solid phase aggregation of coupled erythrocytes) for detection of rotaviruses in feces (Bradbume et al., 1979), and immune electron microscopy with serum in agar diffusion (Lamontagne et al., 1980).

Large-scale serologic surveys by any of these techniques for detection of EDIM virus infection have not appeared in the literature. Impetus for developing a routine serologic test for EDIM may be afforded by the report of Ghose et al. (1978) that the ELISA technique has proved more useful than the complement fixation test for extensive epidemiologic studies of human rotavirus infection.

More recently, Sheridan and Aurelian (1981) have described an ELISA test for EDIM virus which should prove beneficial for both practical (serologic) purposes and for investigations of the antigenic structure of the virion.

2. Virus Isolation and Visualization

In the absence of a reliable tissue culture system, EDIM virus isolation is generally impractical. Bacteria-free filtrates of intestinal suspensions can, of course, be given to diarrheafree animals (gnotobiotes or axenics), but this is expensive and inefficient. On the other hand, such filtrates may be concentrated by ultracentrifugation and examined in the electron microscope for characteristic virus particles (Bryden and Davies, 1975; Flewett, 1978). The particles may also be identified by immune electron microscopy.

3. Necropsy of Mice; Sentinel Animals

A practical approach to a presumptive diagnosis would be to kill selected animals in order to examine the appearance of the colonic contents. The inclusion of sentinel dams with litters in a breeding colony should be considered. These might be sacrificed at intervals to determine the presence of EDIM in the colony. Together with the clinical history of the mouse colony, this practice may provide a fairly reliable, although not pathognomonic, indication of the presence of EDIM. Histopathologic examination could also be of value.

F. Control and Prevention

Based on experimental results, Kraft et al. (1964) proposed the use of air-filter devices, essentially dust caps for each cage, for the practical control of airborne transmission of EDIM in a commercial mouse colony. It was subsequently shown (Kraft, 1966) that 43% of first litters and 79% of all other litter parities were weaned from cages without filters, whereas in cages provided with filters, weaning percentages were 96 and 99%, respectively, during the same observation period. It should be pointed out that both EDIM and mouse hepatitis virus (LIVIM) were present simultaneously in that colony. Although the filter devices did not eliminate the disease(s), they probably reduced the pathogenic microbial load in the immediate environment of the susceptible animals, but the precise mechanism by which
this control method succeeds to the extent that it does is obscure.

Since that time (1964), various devices based on a filter cage or filter-top design have been utilized. Some of these are described by Simmons and Brick (1970). Woods et al. (1974) evaluated them from the viewpoint of environmental factors within the cages. They found that dry bulb temperature differentials, comparing environments inside and outside the cage, were not significantly different (about 2°C) between filtered and unfiltered cages, but that dew point differentials were significantly greater in the filtered cages (about 5°C) than in unfiltered cages (about 3°C). However, they concluded that a suitable cage size for a particular species and number of animals could compensate for the higher wet bulb readings under filters to maintain acceptable conditions for the animals. Currently, several types of filter covers or bonnets are available commercially.

Vaccination as a method of control has not been attempted. Judging from the lack of reports to the contrary, caesarian derivation together with barrier maintenance apparently eliminates and controls the infection.

Kunstý (1962) attempted to control EDIM by decontamination of air by the use of triethylene glycol but was unable to do so. The use of antibiotics, too, is without value, although there seems to be amelioration of signs for a short period as secondary organisms are temporarily reduced in number.

As indicated by Kraft (1966), it may be relatively easy to establish a colony of mice free from EDIM virus infection. This may be accomplished by eliminating those breeding pairs whose first litter is diarrheal. Filter devices are required for this, and they and the animals must be handled with the aid of a transfer or laminar flow hood using sterile techniques during observation and handling of the animals. The method is suitable for small colonies, but it is impractical for commerce where caesarian derivation and barrier maintenance are the methods of choice.

III. REOVIRUS 3 INFECTION
(HEPATOENCEPHALOMYELITIS, ECHO 10 VIRUS INFECTION)

A. Historical Background

Reovirus 3 was first isolated from the feces of an Australian child manifesting a cough, fever, vomiting, hypertrophic tonsils, and bilateral bronchopneumonia. It was named hepatoencephalomyelitis virus by Stanley et al. (1953), who originally recovered the agent. Sabin (1959) proposed the name reovirus for a group of agents associated with the respiratory and enteric tracts of humans. They were found to be ether resistant, about 70 nm in size by membrane filtration but of unknown shape, and caused distinctive cytopathic effects in monkey kidney tissue cultures. One of the ECHO group of viruses, ECHO 10, now synonymous with reovirus, became a member of the new group on that basis. (ECHO is the acronym for enteric cytopathic human orphan, agents isolated in tissue culture from asymptomatic humans—so-called viruses in search of disease.) Stanley (1961) then demonstrated that the hepatoencephalomyelitis virus was serologically identical to reovirus 3.

Additional strains have since been recovered from humans, other mammals, marsupials, birds, insects, and reptiles (for review, see Stanley, 1974), and from mollusks (Meyers, 1979; Meyers and Hirai, 1980). Reoviruses have been divided into three serotypes on the basis of hemagglutination inhibition and neutralization tests (Sabin, 1959; Rosen, 1960).

Reovirus 3 was established as an indigenous murine virus by Hartley et al. (1961) and by Cook (1963). Reviews concerning the biological and clinical aspects of disease caused by this agent have been prepared (Stanley, 1974, 1977).

When Gomatos et al. (1962) and Gomatos and Tamm (1962) discovered that reoviruses possess double-stranded RNA, a unique characteristic among viruses, molecular biologists were inspired to study them in minute detail. Currently, knowledge of reovirus replication on biochemical and biophysical planes is as extensive as that available for any other virus and is, for the most part, outside the scope of this chapter. Interested readers are therefore referred to reviews by Shatkin (1969) and Joklik (1974) for extended literature coverage and detailed discussion and to Andrewes et al. (1978) for a condensed overview.

B. Properties of the Virus

1. Classification

Reovirus 3 belongs to the genus Orthoreovirus, or Reovirus, in the family Reoviridae (Melnick, 1979).

2. Strains of Virus

Wild-type strains of reovirus 3 include: Dearing, the prototype strain (Sabin, 1959), isolated from a child with diarrhea; Abney (Rosen, 1960), isolated from a child with a febrile upper respiratory infection; CAN 230, from a case of Burkitt’s lymphoma (Bell et al., 1964); and several strains obtained from naturally infected cattle (Rosen, 1960). Mutant, temperature-sensitive (ts) strains have been developed in the laboratory (Fields and Joklik, 1969) and have been used for studying the synthesis of viral RNA and peptides (Cross and Fields, 1972; Fields et al., 1972) as well as for examining problems of pathogenesis. A neurotropic strain was also de-
3. Physical Properties of the Virus

a. Morphology, Size, and Composition. The reovirus 3 virion (Fig. 3) has a mean diameter of about 60–76 nm and is icosahedral in shape, with 5:3:2 symmetry. Particles have a core, an inner layer or shell containing a number of capsomers 50 Å in diameter, and an outer capsid composed of 92 capsomers 90 Å in diameter and 40 Å apart (Vasquez and Toumier, 1962, 1964; Jordan and Mayor, 1962; Luftig et al., 1972). As reviewed by Joklik (1970), the genome consists of 10 discrete segments of double-stranded RNA that can be grouped into three classes on the basis of their molecular weight. The polypeptides encoded by each of the RNA segments have been characterized (McCrae and Joklik, 1978).

Genetic maps of reovirus 1, 2, and 3 have been constructed by means of polyacrylamide gel electrophoresis of genome RNAs of recombinants between the three types (Sharpe et al., 1978). Further, the genome segments that contain the ts lesion of several mutants of reovirus 3 have been determined by similar genetic mapping procedures (Ramig et al., 1978).

b. Effect of Heat. Stanley et al. (1953) and Cook (1963) stated that the virus survives heating for 2 hr at 56°C or 30 min at 60°C. A variant recovered from L cells by Gomatos et al. (1962) was found to have a half-life at 37°C of 157 min, at 45°C of 33 min, and at 56°C of 1.6 min.

Temperature-sensitive mutants have been alluded to above (Sections B,2 and B,3,a).

c. Effect of Chemicals. Stanley et al. (1953) found the virus to be relatively resistant to hydrogen peroxide, 1% phenol, 3% formalin, and 20% lysol but sensitive to 70% ethanol for 1 hr at room temperature. It was sensitive to 3%

Fig. 3. Electron micrograph of purified reovirus 3. Uranyl acetate negative stain. Pseudoreplica technique. Scale bar = 100 nm. (Courtesy of Erskine Palmer, Center for Disease Control, Atlanta.)
formalin at 56°C. Ether treatment was ineffective. Rozee and Leers (1967) determined that although chloroform does not affect infectivity, it does destroy the hemagglutinin. Wallis et al. (1964) found that Mg	extsuperscript{2+} enhances the titer of reovirus at 50°C. The infective titer increased four to eight times by heating for 5-15 min in 2 \text{M} MgCl_2. Other divalent cations and NaCl were ineffective. Hemagglutinin was not affected. It is thought that the high temperature and Mg	extsuperscript{2+} caused activation of reovirus particles that were inactive in the original preparations.

As with rotavirus, Ward and Ashley (1978) found that reovirus was sensitive to anionic detergents in wastewater sludge, i.e., these chemicals decreased the temperature needed to inactivate the virus. Cationic detergents were more active than anionic, and nonionic detergents were inactive in decreasing reovirus thermal stability.

Mutagens (nitrous acid, nitrosoguanidine, and proflavin) have been applied to reovirus 3 (Fields and Joklik, 1969). The resulting mutants are of interest not only to the molecular biologist but to the clinical virologist as well, since some of them produce altered disease pictures (Fields and Raine, 1972). For example, when inoculated into rats, wild-type virus produced a necrotizing encephalitis, whereas a mutant gave rise to a slowly progressive communicating hydrocephalus.

The effects of enzymes are described below (see Section III,B,5).

d. Effect of pH. In phosphate-citrate buffers, Stanley et al. (1953) ascertained that the virus is stable between pH 2.2 and 8.0.

e. Antigenic Determinants. Sabin (1959) demonstrated that the mammalian reoviruses known at that time could be divided into three serologic groups by neutralization tests. They could also be differentiated by hemagglutination inhibition (Rosen, 1960), Hull et al. (1956) having discovered that ECHO 10 virus possessed a hemagglutinin for human O erythrocytes. Later, Gomatos and Tamm (1962) reported that reovirus 3, but not reovirus 1 or 2, agglutinated ox erythrocytes. The reovirus 3 hemagglutinin was inhibited by nonspecific substances such as normal mouse, rabbit, or rat serum, and by Vibrio cholerae filtrate.

Weiner et al. (1978) were able to show that the SI RNA segment, which is associated with type specificity, encodes the polypeptide that determines the hemagglutinating properties of the virion.

Complement-fixing antigens were prepared by Stanley et al. (1953, 1954) and by J. C. Parker et al. (1965, 1966). These are group specific.

Leers et al. (1968) determined that reovirions display at least one type-specific and one to two group-specific antigens when studied by immunodiffusion. With the more sensitive immunoelectrophoresis technique, however, these authors encountered two type-specific and four group-specific precipitin lines.

4. Growth in Mice

The agent is regarded as pantropic in mice. In neonates, Stanley et al. (1953) observed that within 48 hr after intraperitoneal inoculation, virus could be recovered from the liver. Subsequently, it was found in the central nervous system in very high titer. Both cellular and plasma viremia are present. Kundin et al. (1966) used immunofluorescence to locate reovirus 3 antigen in suckling mice dying after subcutaneous inoculation. Antigen was seen in the brain, spinal cord, meninges, liver, pancreas, spleen, lymph nodes, and blood vessels.

By electron microscopy, Papadimitriou (1967) traced the evolution of the virus in the central nervous system of neonatal mice inoculated intracerebrally. The earliest appearance of virions was in lymphocytes within vessels. They were then found in capillary endothelial cells, and finally, about 1 week after inoculation and preceded by the appearance of perinuclear "viroplasm," both complete and coreless virions could be seen in neurons.

More recently, Weiner et al. (1980c) determined that the SI gene segment, which encodes the viral hemagglutinin, is responsible for binding the virion to lymphocytes as well as to neuronal cells (Weiner et al., 1977).

Following ingestion, reovirus 1 has been demonstrated to enter intestinal M cells, those specialized epithelial cells covering Peyer's patches, but not other intestinal epithelial cells of sucking mice. It would be of interest to know if the same holds true for reovirus 3 infection (Wolf et al., 1981).

Papadimitriou (1965, 1966, 1968) also studied viral replication by electron microscopy in the mucosa of the common bile duct and in the liver.

Reovirus 3 has been reported to be oncolytic for a mouse ascites tumor by Bennett (1960), Bennett et al. (1967,a,b), and Nelson and Tarnowski (1960).

5. Growth in Tissue Culture

Most of the studies dealing with replication of the reoviruses have been accomplished in tissue culture, frequently in plaque assays in L-cell monolayers. Other cells that have been successfully employed are primary kidney monolayers of rhesus, patas, and capuchin monkeys, as well as those of pigs, cats, and dogs. Continuous lines, such as FL human amnion, BS-C-1, and KB, have also been used (Hsiung, 1958; Cook, 1963; McClain et al., 1967; Rhim and Melnick, 1961; Harford et al., 1962). Harford et al. (1962) described large masses of
virus-like particles, often in crystalline array, in the cytoplasm of KB cells. Extracellular virus was seen in position, as if entering cells by pinocytosis or phagocytosis.

The effects of enzymes are of interest on theoretical as well as practical grounds. Pancreatin treatment was studied by Wally et al. (1966), who ascertained that virus yield was greatly enhanced, perhaps because the enzyme permits rapid transmission of virus from cell to cell. Other proteolytic enzymes that enhance reovirus infectivity are ficin, papain, pepsin, pronase, and trypsin. Enhancement was also achieved with 0.1 \( N \) HCl. Carboxypeptidase, collagenase, and leucine aminopeptidase were ineffective.

Spendlove et al. (1970), Nonoyama et al. (1970), and Joklik (1972) studied the enhancing effect of chymotrypsin on reovirus 3. The resulting product, termed a subviral particle (SVP), depends upon the virus concentration, enzyme concentration, and ionic strength of the suspending medium. It may consist of virions lacking a portion of the capsid proteins, or of cores only, which are noninfectious but do possess transcriptase activity.

Borsa et al. (1979) have detected two modes of entry of reovirus particles into cultures of L cells. The complete particle enters almost exclusively by viropexis involving phagocytic vacuoles, whereas the intermediate subviral particles apparently gain direct access without the aid of phagocytosis.

Early steps in virus replication in L cells were described by Silverstein et al. (1972). Soon after entering the cell, the virions are sequestered in lysosomes. The outer layer of the viral capsomers is digested by hydrolases, but the core proteins remain untouched. The resulting structure is similar in protein composition to SVP produced in vitro by chymotrypsin digestion of intact virions. Within the cells, 10 hr after infection, synthesis of the progeny progresses with conservation of the parental RNA, which retains its macromolecular state throughout the replicative cycle. Rhim et al. (1962) reported that in monkey kidney cells, virus protein antigen was first detectable 12 hr after infection, and RNA, as determined by acridine orange staining, was increased in the cytoplasm at 16 hr, with maximum staining at 54 hr after infection. The nucleus did not seem to play a role in virus synthesis. For additional details, see Borsa et al. (1973a,b,c, 1974, 1979).

6. Growth in Fertile Hens’ Eggs

Stanley et al. (1953, 1954) reported the development of pocks on the chorioallantois of 12-day-old chick embryos inoculated with infectious brain and liver. The embryos appeared unaffected, and with succeeding passages, the pocks could no longer be observed, although oral inoculation of suckling mice with chorioallantoic suspensions resulted in active disease. Essentially the same results were found following amniotic inoculation.

C. Pathogenesis

1. Clinical Disease, Morbidity, and Mortality

The experimental and natural diseases appear identical except for variations in intensity of signs, perhaps due to differences in infectious dose. Up to 16 days after intraperitoneal inoculation, the mice appear emaciated and uncoordinated. The hair is oily and matted—the so-called oily hair effect (OHE)—an effect that can be demonstrated in contact animals as well. However, in these mice it disappears as soon as the diseased animal is removed from the healthy ones. The effect was ultimately traced to a high proportion of fat in the intestinal contents (steatorrhea): 12.9% in infected animals as compared with 4.6% in normal mice. Feces may contain as much as 29% fat in infected mice (Stanley et al., 1953, 1954).

Branski et al. (1980b) have reported that reovirus 3 inoculated intraperitoneally into adult and suckling mice resulted in no histologic or pancreatic zymogen changes in the former, whereas in the latter, amylase and lipase activities were significantly decreased and trypsin and chymotrypsin were increased. Peptidase A and B remained unchanged in infected animals. Histologic change in the suckling mice was observed 6 days after infection and was confined to a mild mononuclear infiltrate. Branski et al. (1980a) extended their studies further with an examination of the brush border of intestinal epithelium in suckling mice, observing that no significant changes were found in intestinal morphology or activity of enzymes tested at 3 days after infection. By the sixth day, villi were shortened and there was a mild mononuclear infiltrate in the lamina propria. Lactase and enterokinase activities were significantly decreased and alkaline phosphatase remained unchanged, whereas maltase and isoleucine activities were increased in infected mice.

Jaundice may be apparent in the ears, feet, nose, and tail. Neurologic signs are observed in many mice: incoordination, with tremors and paralysis occurring just before death. Central nervous system tropism has been linked to the viral hemagglutinin by Weiner et al. (1980a).

One of the earliest signs in some mice is marked abdominal distention. In mice that are recovering and in which OHE and neurologic signs had been seen, alopecia may occur. The virus has been implicated in the “runting syndrome” as well (Stanley and Leak, 1963).

Stanley et al. (1964) studied the disease in survivors of the acute illness between 28 and 700 days after peroral inoculation with 1 \( ID_{50} \) of virus. Ascites, rather prevalent in parenterally infected animals, was not frequent among them, but all had previously shown OHE and stunting. Neurologic signs had been most common 2–20 days after infection. In 7 of 25 animals, jaundice had lasted for 3 weeks, and alopecia over the lumbar and occipital regions occurred in the same number.
Infectious virus could not be recovered from any tissue during this "chronic" stage. Of the 1528 mice exposed in that study, 639 (42%) developed clinical signs in the acute phase. Of these, 25 survived as runts.

Onodera et al. (1978) studied reovirus 3 infection in α and β pancreatic cells by viral and insulin immunofluorescent techniques using SJL/J mice and the Abney strain of reovirus that had been passaged at least seven times in cultures of pancreatic B cells from the same strain of mice. They found viral antigen only in insulin-containing β cells and none in glucagon-producing α cells. The virus destroyed β cells, resulting in decreased insulin production and an altered response to the glucose tolerance test. The implication was that this could serve as an animal model for juvenile onset diabetes in man.

2. Pathology

a. Gross Pathology. The liver is enlarged, dark in color, with focal circular yellow regions of varying diameter up to 3 mm. The intestine may appear reddened and distended. In some cases, the heart shows small circular epicardial foci, and hemorrhagic areas are occasionally found in the lungs. The brain may be swollen and congested. In infants, the intestinal contents are often lemon yellow in color. Other viscera appear normal (Stanley et al., 1953; Walters et al., 1963). As noted above (Section III,B,1), Stanley (1974) regards the acute phase of the disease as limited to 28 days.

In the chronic phase, the mice are wasted, sometimes jaundiced, and may display OHE or areas of alopecia. The slightly enlarged liver is dark, and small subcapsular yellowish foci are seen. The peritoneum is congested; exudate is sometimes observed. Splenomegaly may be moderate late in the course of the disease (Stanley et al., 1964).

b. Microscopic Pathology. Walters et al. (1963) described the histopathology of the acute disease following oral inoculation of large or small doses of virus in suckling mice. The same picture was encountered in both instances, except that the lesions were less intense following small doses. The liver showed minimal change at 4 days. Beneath the capsule and near the centrilobular veins were aggregates of mononuclear cells and a few segmented neutrophils. Eosinophilic degeneration of hepatocytes could be seen at this time. Throughout the next few days, the foci of hepatocyte necrosis underwent cystic change due to lysis of the eosinophilic material. Dense eosinophilic structures, similar to Councilman bodies, could be seen lying free or within macrophages. Foci of proliferating mesenchymal cells occurred in all lobular zones. They varied from small ones, the result of recent necrosis, to very large regions that coalesced with other similar foci. At the same time, about the seventh day after infection, hepatocytes enlarged to three to four times the normal size. The nuclei were not affected, although they seemed slightly less basophilic than usual. Hyperplasia of Kupffer cells and sinusoidal collections of macrophages, lymphocytes, polymorphonuclear leukocytes, and debris were evident. By the fourteenth day, many necrotic cells had lysed, and organization of cells peripheral to the necrotic foci began. Nevertheless, new necrotic regions were still developing.

In the pancreas, changes progressed from cytoplasmic vacuolization and eosinophilic degeneration beginning on the third day following inoculation. Duct cells were prominent. By 10–14 days, total necrosis of the pancreas occurred. The islets remained normal throughout.

In the salivary glands, changes similar to those seen in the pancreas took place, whereas in the heart, necrotic lesions appeared during the second week after inoculation in the papillary muscles of the left ventricle. The degeneration was eosinophilic and associated with edema and lymphocytic and macrophagic infiltrates. Repair began on the twelfth day, and by 2 weeks, all hearts appeared normal.

Some degenerative changes also took place in the skeletal musculature, and in the lungs scattered hemorrhages and pulmonary edema with a leukocytic reaction were noted. The gastrointestinal tract showed only dilatation of the central villous lymphatics and submucosal lymph channels. In some animals the spleen manifested follicular hyperplasia.

The thymus and other organs appeared normal.

In the central nervous system, neuronal degeneration began about the ninth day and was most prominent in the brain stem and cerebral hemispheres. By the tenth day, perivascular cuffing as well as neuronal satellitosis was evident. The meninges were infiltrated with round cells and netrophilic leukocytes. By the fourteenth day encephalitis was severe and widespread, with small hemorrhages occurring in necrotic regions.

In suckling rats inoculated intracerebrally with reovirus types 1, 2, or 3, viral cytoplasmic inclusions and intranuclear bodies corresponding to Cowdry type B inclusions have been observed (Margolis et al., 1975). The latter were seen in cells that were free from intracytoplasmic inclusions; they were readily found in weanlings, were unassociated with inflammatory changes, and persisted for long periods without cytolysis. Electron microscopy of the choroid plexus showed no secondary virus particles present and demonstrated that the nuclear inclusions were composed of granular elements sometimes enclosed in lamellar membranes similar to "nuclear bodies." The authors believe that the Cowdry type B inclusion is identical to the nuclear body. In the chronic disease, Stanley et al. (1964) found that at 4 weeks after inoculation the liver still displayed small necrotic foci and areas of recent necrosis undergoing resolution. Hyaline bodies were numerous, and in the unaffected parenchyma the cells were large and often binucleate. Mitoses were rare. Recurrence of the acute picture was seen at 5 weeks: Large regions of necrosis with central
lys and suppuration were found. Thereafter the picture of bile duct occlusion developed, and the remaining parenchyma showed many mitoses. In some animals, it was evident that there were continuous cycles of necrosis and resolution throughout life.

Changes leading to acinar necrosis took place in the pancreas and salivary glands, whereas in the central nervous system lesions were variable and absent after 12 weeks. Lesions in the heart and skeletal muscles occurred about 5 weeks after infection. Interstitial pneumonia was seen from the fourth to the tenth week. The thymus remained normal, and the adrenals appeared unaffected. Some animals showed atrophy of the epidermis with loss of hair, subcutaneous edema, and infiltration with polymorphonuclear leukocytes.

In experimentally induced disease using large inocula intraperitoneally, chronic biliary obstruction may be brought about in weanlings (Phillips et al., 1969).

Ultrastructurally, Papadimitriou (1965) ascertained that the virus reaches the liver in membrane-bound inclusions in the cytoplasm of leukocytes, which then undergo degeneration. The resulting debris is engulfed by Kupffer cells, and by phagocytosis the virus then enters the hepatocytes, where it replicates. In the biliary tract, Papadimitriou (1968) determined that virus invades many of the lining cells of the common bile duct, which eventually die. Bile canaliculi are dilated and their microvilli swollen. The common bile duct is dilated, and the ampullary region becomes obstructed with debris. It is this obstruction that leads to both hepatic and pancreatic dysfunction, for after studying the pancreatic lesions further, Papadimitriou and Walters (1967) concluded that, even though virions were seen in pancreatic acinar cells, the principal cause of acinar degeneration is ductal obstruction.

3. Transmission

It is clear that the agent can be transmitted by the oral route as well as by parenteral inoculation. Further, L. Parker et al. (1965) isolated a strain of reovirus 3 from a pool of Aedes vigilax and another from a pool of Culex fatigans in Western Australia. A third strain was isolated from members of a litter of sentinel mice that had been exposed overnight in the same region. The authors believe that mosquito transmission may account for the ubiquity of reovirus antibodies in vertebrates throughout the world. Subsequently, McCrea et al. (1968) reported the maintenance of reovirus 3 through a number of the developmental stages of Culex pipiens fatigans.

4. Immune Response

Mice respond to the natural infection with neutralizing, hemagglutination-inhibiting, and complement-fixing antibodies. As indicated earlier (Section III,B,3,e), precipitating antibodies can also be demonstrated. Based on the neutralization test, Weiner and Fields (1977) determined that the S/ genome segment is linked to type specificity, and Finberg et al. (1979) found that the same genome segment is also responsible for the production of cytolytic T lymphocytes after reovirus infection.

Tytell et al. (1967), working with reovirus 3 RNA, found that it was highly active in inducing interferon in rabbits and tissue culture, and Lai and Joklik (1973) showed that coreless virions as well as those lacking the outer capsid shell induce no interferon. The question of the role of interferon in protection of mice from either the acute or chronic infection remains an intriguing problem.

In an effort to permit a precise definition of the host cellular immune response to viral antigens, Weiner et al. (1980b) and Greene and Weiner (1980) have examined delayed type hypersensitivity (DTH) in mice infected with reovirus 3 following foot pad inoculation with active virus. They found that DTH could be transferred by lymph node cells, that it was mediated by T cells and was type specific. By means of recombinant viral clones of types 1, 2, and 3, they showed that the S/ gene determined serotype specificity, whereas from adaptive transfer experiments, it was clear that certain structures of the major histocompatibility complex were needed for transfer of reactivity. Route of inoculation and viral infectivity (intact as opposed to uv-irradiated virus) determined the ability of mice to produce immunocompetent T cells that mediated the DTH reaction. DTH was not conferred by UV-irradiated virus. This tolerance was due to active suppression by means of generation of suppressor T cells. Serotype-specific tolerance by suppressor T cells was regarded as a property of the viral hemagglutinin, which also determined serotype-specific humoral (neutralizing) and cytolytic T-cell responses.

D. Epizootiology

1. Host Range

a. Species and Strains of Mice Affected. There is no evidence that any mouse strain is more or less susceptible to reovirus 3 infection than any other, provided the animals come from a colony that is free of the infection.

The host range is broad. Stanley (1974) cited at least 60 species that may be infected with reoviruses, and, as mentioned above, it is thought that the prevalence of antibodies in otherwise normal mammals is related to this fact and that mosquitoes or other insects may be operational in the spread of the infection.

b. Age and Sex Susceptibility. The acute disease affects mainly sucklings and weanlings, whereas the chronic disease is encountered in animals over 28 days of age. There is no indication that either sex is more or less susceptible than the other.
9. VIRAL DISEASES OF THE DIGESTIVE SYSTEM

c. Influence of Parity (Birth Order). In an epizootic described by Cook (1963), 130 of 800 first litters were affected. Later-parity litters were involved hardly at all.

2. Prevalence and Distribution

In view of the absent or low complement-fixing antibody titers in the presence of significant hemagglutination-inhibiting titers that follow natural infection, prevalence estimation by immunologic means may be difficult to assess. The data cited by Parker et al. (1966), 82% of 34 colonies positive, and by Descoteaux et al. (1977), 100% of five colonies positive, may be typical incidences for conventional mouse colonies.

As already indicated, reovirus 3 infection is regarded as worldwide in distribution.

3. Latent and Chronic Infection

This aspect of reovirus 3 infection has been covered above (Section III,B,1,2a,b).

4. Seasonal Periodicity

There is no evidence that epizootics occur preferentially at a particular time of the year.

E. Diagnosis

1. Serology

J. C. Parker et al. (1965, 1966) discussed the serologic diagnosis of reovirus 3 infection, concluding that for these purposes the hemagglutination inhibition test was the most reliable. In preparing type-specific antisera for standardization and controls, Behbehani et al. (1966) found that the ubiquity of inhibitory substances (antibodies included) in most mammalian species precluded accurate work. They therefore used domestic geese, in which virtually no hemagglutination inhibition or neutralizing antibodies could be detected prior to immunization. In any event, for routine surveillance, the hemagglutination inhibition test is currently utilized.

2. Virus Isolation and Visualization

Comments similar to those expressed for EDIM virus recovery and visualization apply. Although it is possible to perform these procedures, they are inefficient for routine diagnostic purposes. Stanley (1977) has outlined methods for this purpose. In brief, infectious material can be inoculated into tissue cultures (primary rhesus monkey or human kidney) or newborn mice (from reovirus 3 free colonies!), or the material may be subjected to immunofluorescent methods. An immunoperoxidase method (enzyme-labeled antibody) has been employed for reovirus 1 (Ubertini et al., 1971) and may find application for reovirus 3 diagnosis as well.

3. Observation of Clinical Signs; Necropsy of Animals

Although OHE may not be absolutely pathognomonic for reovirus 3 infection, it seems distinctive enough so that a provisional diagnosis may be made when it is seen. Stronger evidence is afforded if the animals are also jaundiced and wasted. Necropsy coupled with histopathologic examination is never a mistake and is to be encouraged.

4. Sentinel Mice

The placement of sentinel animals at strategic locations in an animal colony should be considered. Such animals may be regarded as expendable for sacrifice, necropsy, and virus isolation as well as for the acquisition of serum for antibody determinations.

F. Control and Prevention

Cesarean derivation and barrier maintenance are believed to be suitable techniques for control and prevention of reovirus 3 infection. Although no experimental evidence has been found, it is possible that the use of filter devices in conventional colonies might also be helpful in preventing the spread of infection. In the absence of information on the vertical transmission of the agent, it is impossible to evaluate the influence of that route on successful control of the endemic disease.

Although therapy is impractical from the standpoint of controlling epizootics of reovirus infection, it is nonetheless of considerable interest that Willey and Ushijima (1980) found that thymosin given intraperitoneally to 7-day-old mice that had been neonatally infected with reovirus 2 (2 LD50) significantly increased their mean survival time, provided it was administered at 2200 hr. When given at 0800 hr, significantly increased survival time was not observed, but when inoculated at 1200 hr, there was an apparent decrease in mean survival time.

IV. MURINE (MOUSE) HEPATITIS VIRUS INFECTION (MHV)

A. Historical Background

Knowledge of the murine hepatitis viruses dates from the isolation of a spontaneously occurring neurotropic murine virus, designated JHM, during the course of experiments on
the epidemiology of EDIM. The most distinctive feature of JHM virus infection was widespread destruction of myelin, regardless of the parenteral route of inoculation. Giant cells were noted in a number of tissues, and the liver manifested focal regions of necrosis. Young rats, cotton rats, and hamsters were also susceptible when inoculated intracerebrally (Cheever et al., 1949; Bailey et al., 1949).

Following those reports, a number of agents, differing from JHM virus and from each other mainly in pathogenicity, were isolated by others under a variety of circumstances. Even today, new strains may appear, resembling or differing from those already described with respect to virulence, tissue tropism, host range, and immunogenicity. The recovery of two coronavirus strains from cases of multiple sclerosis in man, one of which was isolated in suckling mice (Burks et al., 1980), draws attention to the great care that must be exercised in ascertaining that such agents do not originate in the mice, especially since, as is true in the present case, antigenic similarity with known murine coronaviruses may be demonstrated.

Because of the characteristic disease picture seen with each strain of MHV, several investigators are utilizing variants or mutants to elucidate the mechanisms of virus replication, viral nucleic acid and capsid protein structure, and virus-host interactions that result in different expressions of disease by very closely related agents (see, e.g., Haspel et al., 1978; Robb and Bond, 1979; Robb et al., 1979; Bond et al., 1979). Bang (1978) has addressed the genetics of resistance of mice to murine coronavirus infection.

Reviews on the subject include those by Piazza (1969), McIntosh (1977), Kapikian (1975), Andrewes et al. (1978), Holmes (1979), Garwes (1979), and Robb and Bond (1980).

**B. Properties of the Virus**

1. **Classification**

Based on their own electron microscopic studies and on those of David-Ferreira and Manaker (1965), Becker et al. (1967) considered that MHV might be an “IBV-like” (infectious bronchitis virus-like) agent. Virions of similar morphology seen in electron micrographs and also ether sensitive, as is IBV, were being isolated at that time from human cases of colds (Almeida and Tyrell, 1967). The following year, the term coronavirus was proposed for the group (Tyrell et al., 1968), and in 1975 the Coronaviridae became an official family with a single genus, *Coronavirus* (Tyrell et al., 1975). The name refers to coronalike surface projections (peplomers) which, when seen in negatively stained electron micrographs, resemble the sun’s corona.

Members of the group, their natural hosts, and the associated diseases are: human coronavirus (HCV) (common cold, perhaps pneumonia); infectious bronchitis virus of chickens (IBV) (infectious bronchitis, nephrosis, and uremia); transmissible gastroenteritis virus of pigs (TGEV) (gastroenteritis); hemagglutinating encephalomyelitis of pigs (HEV)* (encephalitis, vomiting, wasting); bluecomb disease virus of turkeys (TBDV) (infectious diarrhea, bluecomb disease); neonatal calf diarrhea coronavirus (NCDCV) (diarrhea); murine hepatitis virus (MHV) (hepatitis, encephalitis, wasting); rat coronavirus (RCV) (pneumonia of newborn rats); and rat sialodacryoadenitis virus (SDAV) (sialodacryoadenitis). Additional agents may be added to this group, e.g., “runde” virus (Traavik et al., 1977), a coronavirus causing cardiomyopathy in rabbits (Small et al., 1979).

2. **Strains of Virus**

As noted, many strains of MHV have been recovered from mice under various circumstances. In addition to JHM, these include: MHV1, from “white mice” (P or Parkes strain), during attempts to adapt human hepatitis virus to animals (the strain was originally isolated as a dual agent consisting of the virus and a protozoan parasite, *Eperythrozoon coccoides* (Gledhill and Andrews, 1951); MHV2, from mice used to propagate murine leukemia virus (Nelson, 1952a,b); MHV3, also found during studies on adaptation of human hepatitis virus to mice (Dick et al., 1956); MHV-B (EHF-120), from mice used for human epidemic hemorrhagic fever (HEHF) adaptation attempts (Buescher, 1952); an unnamed strain from mice undergoing murine leukemia chemotherapy trials (Braunsteiner and Friend, 1954); H747, following intracerebral inoculation of suckling mice with HEHF materials (Morris, 1959); MHV-A59, during transfer of Molloney leukemia virus in mice (Manaker et al., 1961); MHV-S, from cesarean-derived mice that had been barrier-maintained before exposure to conventional mice (Rowe et al., 1963); MHV-C (MHVBALB/c), isolated during passage of spleens from leukemic mice (Nelson, 1955); four additional strains from spleens of leukemic mice or from natural outbreaks (MHV-SR1, -SR2, -SR3, -SR4) (Nelson, 1963, 1965); lethal intestinal virus of infant mice (LIVIM), from infant mice dying of a spontaneous infection (Kraft, 1962a), identified as an MHV strain by Broderson et al. (1976) and Hierholzer et al. (1979); and NuU, NuA, Nu66, from nude mice with hepatitis and wasting syndrome (Hirano et al., 1975). Other isolates have also been derived from nude mice (Sebesteny and Hill, 1974; Ward et al., 1977), and Fox et al. (1977) have described a strain that appeared during passage of an ascites myeloma cell line in BALB/c mice.

*HEV may also denote the hepatoencephalomyelitis virus of Stanley et al. (1954), which is identical to reovirus 3.
3. Physical Properties of the Virus

a. Morphology, Size, and Composition. As reviewed by McIntosh (1974), coronaviruses are pleomorphic, enveloped, and variable in size, measuring about 80–150 nm in diameter. Peplomers are 12–24 nm in length (Fig. 4).

Using various techniques, a number of workers have confirmed the diameter of MHV virions to fall within the range of the coronaviruses (Gledhill et al., 1955; Miyazaki et al., 1957; Kraft, 1962a; Starr et al., 1960). Svoboda et al. (1962) further described the virion as consisting of a nucleoid separated from an outer membrane by an electron-lucent space. David-Ferreira and Manaker (1965), working with MHV-A59 in tissue culture, found that the virions had a mean diameter of 75 nm with an electron-dense inner shell, 55 nm in diameter, separated from the outer double membrane by an electron-lucent space 8 nm wide.

Hirano et al. (1978) studied MHV2 and determined that the virus had a buoyant density of 1.183 gm/cm³ in sucrose, and that that fraction contained coronaviruslike particles measuring 70–130 nm in diameter. In negative contrast preparations, Davies and Macnaughton (1979) ascertained that the envelope diameter of MHV3 ranged from 76 to 121 nm (mean, 100 nm). Peplomers were 16.6–23.4 nm long. Not only was the virion significantly smaller than that of IBV, but the peplomers differed from those of both IBV and HCV 229E, being cone-rather than club-shaped.

Mallucci (1965) established that MHV3 is an RNA-containing virus with no DNA-dependent replicative phase. Structural proteins have been investigated by means of polyacrylamide gel electrophoresis. In purified preparations of JHM virus, Wege et al. (1979) identified six polypeptides, whereas Sturman (1977) found only four in MHV-A59. Also utilizing polyacrylamide gels, Macnaughton (1980) compared the polypeptide composition of two coronaviruses, HCV 229E and MHV3, and found similar patterns for both agents. He felt that the importance of this finding rests in the fact that not only were different hosts involved here, but different diseases as well, implying that factors other than those under study are instrumental in the outcome of a disease picture.

Fig. 4. Electron micrograph of an MHV particle prepared from the brain of a mouse inoculated intracerebrally. Pseudoreplica technique. Scale bar = 100 nm. (Courtesy of Harold S. Kaye, Center for Disease Control, Atlanta.)
b. Effect of Heat. In general, coronaviruses are inactivated at 56°C in 10–15 min, at 37°C in several days, and at 4°C in several months (Tyrell et al., 1968; Kapikian, 1975). Wild-type MHV isolates also fall into this range of sensitivity (Cheever et al., 1949; Gledhill and Andrews, 1951; Gledhill et al., 1955; Kraft, 1962a). Hirano et al. (1978), however, indicated that MHV2 is not completely destroyed at 56°C for 30 min and is stable at 50°C for 15 min in 1 M MgCl₂ or MgSO₄ but not in water. Freezing and thawing or sonication at 20 kc for 3 min does not affect the virus titer.

c. Effect of Chemicals. All coronaviruses are sensitive to ether when exposed overnight at 2°C-4°C. Chloroform also destroys or reduces infectivity (McIntosh, 1974). Fifty percent glycerol inactivates MHV1 after 6 weeks at 2°C (Gledhill and Andrews, 1951). Sodium deoxycholate reduces the titer of LIVIM significantly (Kraft, 1962a), but Calisher and Rowe (1966) regard MHV virus as moderately resistant. According to Hirano et al. (1978), MHV2 is completely inactivated by ether, chloroform, sodium deoxycholate, and β-propiolactone, but it is completely resistant to trypsin.

Mutagenesis has been reported by means of N-methyl-N’-nitroguanidine or 5-fluorouridine (Robb et al., 1979) and by 5-azacytidine or 5-fluorouracil (Haspel et al., 1978).

d. Effect of pH. The pH stability of all known murine hepatitis viruses has not been reported. For coronaviruses in general, acid sensitivity is regarded as variable (McIntosh, 1974). Hirano et al. (1978) found that MHV2 is stable between pH 3 and 9 at 37°C for 30 min.

e. Antigenic Determinants. Calisher and Rowe (1966) developed a soluble complement-fixing antigen for MHV. All strains tested, JHM, MHV1, MHV3, A59, and H747, as well as a number of field isolates, shared both a common complement-fixing and a neutralizing antigen in their tests. No two strains from different sources appeared to be identical, however.

Childs et al. (1980) examined the serologic relationships among five MHV strains by a plaque reduction test and confirmed some serologic relatedness among all of them, although two distinct groups emerged: one, MHV4 (JHM), and two, MHV1, MHV-S, MHV-A59, and MHV2.

Although they have been sought, hemagglutinins have not been demonstrated for MHV strains (see, e.g., Hirano et al., 1978; Kraft, 1962a; Bradburne, 1970; Miyazaki et al., 1957), but two human coronavirus strains do agglutinate human 0 erythrocytes (Kaye and Dowdle, 1969).

Precipitating antigens have been described. Bradburne (1970) found two precipitin arcs when MHV3 reacted with homologous hyperimmune serum.

4. Relationship to Other Viruses

Cheever et al. (1949) found JHM virus to be unrelated to other neurotropic viruses, including GD VII, pseudorabies, Lansing poliomyelitis, and Mengo virus. Kraft (1962b) found no relationship between LIVIM, EDIM, and reovirus 3.

With regard to other coronaviruses, the picture is somewhat different, for as a group, coronaviruses display complex serologic variability (Bradburne, 1970; McIntosh et al., 1969). MHV is serologically closely related to RCV and SDAV in complement fixation tests and distantly related to RCV in cross-neutralization tests (Parker et al., 1970; Bhatt et al., 1972). Several strains of MHV are closely related to human coronaviruses OC 38 and OC 43 (McIntosh et al., 1967), and MHV3 is related to HCV-229E (Bradburne, 1970). Antibody to MHV strains commonly found in human sera is probably present because of endemic human infection with related coronaviruses (Hartley et al., 1964).

5. Growth in Mice

Electron micrographs and studies using fluorochrome stains indicate that coronaviruses develop exclusively in the cytoplasm of infected cells, that the virions collect in cytoplasmic vesicles of diverse size, that particles may also be seen in the matrix outside of the endoplasmic reticulum as well as in the Golgi apparatus, and that they are not observed in the nucleus. In the main, replication involves budding into cytoplasmic cisternae, but tubular structures have also been seen within the cytoplasm during virus formation (Ruebner et al., 1967; Starr et al., 1960; Watanabe, 1969a,b).

Wilsnack (1971), confirming the work of Boss and Jones (1963), elicited immunofluorescent antigen staining in sinusoidal lining cells in necrotic liver foci of weanling mice within 24 hr after intraperitoneal inoculation of the A59 strain. Stainable antigen in intestinal impression smears of mice infected by cage contact was also demonstrated.

Piazza et al. (1967) examined the fate of MHV3 after intravenous inoculation. The agent was not demonstrable between 40 min and 3.5 hr, when it appeared first in the spleen, then in the liver (4 hr) and blood (4.5 hr). At 5 hr it was recoverable from brain and kidney. High titers were then reached in all organs. Barinsky and Dementiev (1968) studied bone marrow involvement in MHV3 infection and found that the marrow yielded high virus titers long before the appearance of clinical signs, persisting almost until death. Chromosomal aberrations were frequently noted in the infected marrow.

Watanabe (1969a,b) described MHV2 replication in the liver by means of electron micrographs. Budding occurred in hepatocytes but never in Kupffer cells, although virions could be seen in these. In hepatocytes, particles were undetectable 64 hr after intraperitoneal inoculation, but they were subsequently...
observed 8 hr later. They were most numerous within perisinusoidal spaces. Viral multiplication was demonstrated in the pancreas by Fujiwara et al. (1975). Virions were present in both the matrix and the endoplasmic cisternae of secretory pancreatic cells.

Concerning the neurotropic variants of MHV, Lampert et al. (1973) saw particles of the JHM strain in cells identified as oligodendrocytes in the brain stem and spinal cord in weanling mice, and Weiner (1973) demonstrated immunofluorescent staining in cells of the white matter (glia) of the spinal cord.

Taguchi et al. (1979b) have shown that the low-virulence strain, MHV-S, multiplies first in the nasal mucosa after intranasal inoculation, antigen being found in both the sensory olfactory cells and supporting (sustentacular) cells. Thereafter, the cells of the olfactory bulb and other brain regions are affected.

6. Growth in Tissue Culture

A number of cell systems have been successfully employed for in vitro growth of mouse hepatitis viruses: MHV-C in mouse embryo explants (Mosley, 1961); MHV1 in newborn mouse kidney explants (Starr and Pollard, 1959); MHV-S in mouse embryo explants (Gompels, 1953) and in liver (Gallily et al., 1964); MHV-B in liver cell monolayers (Paradisi and Piccinino, 1968); MHV3 in liver explants (Vainio, 1961); MHV-B in liver cells (Miyazaki et al., 1957); MHV2 and MHV3 in DBT cells (Hirano et al., 1978; Takayama and Kirn, 1978); and various strains in NCTC 1469 cells (David-Ferreira and Manaker, 1965; Wilsnack et al., 1971; Hartley and Rowe, 1963).

Mallucci (1965), Seamer (1965), and Lewis and Starr (1972) described syncytium formation by MHV in mouse macrophage cultures, and a plaque assay for MHV2 in primary peritoneal macrophage cultures was described by Shif and Bang (1966). Laufs (1967) also described multinucleated giant cells with as many as 200 nuclei per cell in macrophage cultures infected with MHV3. Using autoradiography, he ascertained that there was no DNA synthesis in them and that they originated from cell fusion.

Macrophages derived from either liver or peritoneal washings are of enormous interest for the question of host cell–virus interactions. Bang and Warwick (1959, 1960) showed that virulent MHV2 had a selective destructive effect on macrophages cultured from the liver of newborn mice without affecting fibroblasts or epithelial cells. They concluded that such tissue susceptibility is a property of the reticuloendothelial system. Macrophage cultures from resistant mouse strains were not destroyed, whereas those from susceptible strains were killed. Those obtained from F1 animals of susceptible × resistant crosses demonstrated that in vitro as well as in vivo susceptibility is inherited and that genetic segregation of susceptibility and resistance occurs in the F2 generation and in backcrosses. They proposed that susceptibility was a dominant trait and unifactorial. Shif and Bang (1970) enlarged on those findings, showing that adsorption of virus to cells was not impaired.

Gallily et al. (1967) determined that susceptibility of macrophages in culture reflects alterations in the host. For example, C3H infant mice are susceptible to and die from infection with MHV2, whereas weanlings and adults are more resistant. Macrophages from the mice mirror these changes in resistance as the animals age. Kantoch et al. (1963) determined that temporary susceptibility could be induced in resistant cells in culture if they were exposed to homogenates of susceptible cells, and Gallily et al. (1964) showed that macrophages from genetically resistant mice treated with cortisone to enhance susceptibility behave in culture as if they were from susceptible animals.

Macrophages from mice susceptible to MHV2 virus can be converted to resistance by the intraperitoneal inoculation of concanavalin A in the donor mice. This enhanced resistance is also expressed in vivo (Weiser and Bang, 1977).

7. Growth in Fertile Hens’ Eggs

Cheever et al. (1949), Nelson (1952b), and Kraft (1962a) all reported failure to propagate JHM, MHV2, and LIVIM, respectively, in embryonated hens’ eggs. Evidence of positive results concerning other strains has not been found in the literature.

C. Pathogenesis

1. Clinical Signs, Morbidity, and Mortality

The acute and chronic clinical signs of MHV infection in sucklings, weanlings, and adults vary with the virus strain and its tissue tropism, the mouse strain, the age of the mice, and the presence or absence of enhancing or inhibitory factors in the mice or their environment. Signs of the acute disease are distinct but not pathognomonic: ruffled hair, depression or listlessness, inanition, dehydration, weight loss, huddling with cagemates, muscle tremors, deeply colored urine, and reluctance to move when prodded. These signs are seen in various combinations. In older animals, ascites and wasting tend to occur. In infections with neurotropic variants, such as JHM, the principal sign in weanlings and adults is a flaccid paralysis of the hindlimbs that may be preceded by ruffled hair and depression. Conjunctivitis may occur. Convulsions and hyperirritability are infrequent, as are tremors. The righting ability may be compromised, and circling is occasionally seen.

In suckling mice, rapid wasting with or without neurologic
signs may take place, accompanied in some cases by diarrhea, inanition, and dehydration (Fig. 2).

Mortality and morbidity are variable, ranging between almost 0 and 100% depending on factors like those affecting clinical signs.

Of importance to users and breeders of mice alike is the fact that a number of agents and procedures are known to modify the reactivity (and therefore the clinical signs) of mice to both spontaneous and experimental infection. Examples of these, together with pertinent references, are presented in Table I.

LePrévost et al. (1975a,b) have taken the view that there are three types of sensitivity to MHV3 infection in mice: resistance, full susceptibility, and semisusceptibility. These are reflected in the susceptibility of their macrophages (Virelizier and Allison, 1976). Further, they consider two distinct phases of the disease, acute and chronic, as virtually distinct entities. C3H mice, regarded by others as resistant, are considered by them to be semisusceptible, since approximately 50% of adult infected animals resist the acute disease, and young animals remain susceptible up to 8 weeks of age. In most of the survivors of the acute disease, chronic illness with wasting and occasional paralysis results. In this period, virus can regularly be recovered from brain, liver, spleen, and lymph nodes.

Table I

| Factor                              | Reference                          |
|-------------------------------------|------------------------------------|
| MHV enhanced by:                    |                                    |
| Eperythrozoon coccoides             | Gledhill and Andrewes (1951)        |
| Eperythrozoon coccoides             | Nelson (1952b)                     |
| Eperythrozoon coccoides             | Niven et al. (1952)                |
| Eperythrozoon coccoides             | Lavelle and Bang (1973)            |
| Moloney and Friend leukemia         | Gledhill (1961)                    |
| Urethane, methylformamide           | Braunsteiner and Friend (1954)      |
| X irradiation                       | Vella and Starr (1965)             |
| X irradiation                       | Dupuy et al. (1975)                |
| Cortisone                           | Lavelle and Starr (1969)           |
| Cortisone                           | Ruebner et al. (1967)              |
| Cortisone                           | Datta and Isselbacher (1969)        |
| Cyclophosphamide                    | Willenborg et al. (1973)           |
| Cyclophosphamide                    | Weiner (1973)                      |
| Neonatal thymectomy                 | East et al. (1963)                 |
| Neonatal thymectomy                 | Sheets et al. (1978)               |
| Neonatal thymectomy                 | Dupuy et al. (1975)                |
| Antilymphocyte serum                | Dupuy et al., (1975)               |
| K virus (papovavirus)               | Tisdale (1963)                     |
| Splenectomy                         | Stone et al. (1967)                |
| MHV suppressed by:                  |                                    |
| Triolein (triglycerol oleate)       | Lavelle and Starr (1969)           |
| S. typhosa endotoxin                | Vella and Starr (1965)             |
| MHV infection enhances:             |                                    |
| Response of nu/nu mice to sheep erythrocytes | Tamura and Fujiwara (1979)       |

DBA/2 mice, on the other hand, are regarded as fully susceptible since deaths begin 4-6 days after infection, even when the mice are 90 days old, whereas the A/J strain is resistant, being susceptible to the acute disease only up to 3-4 weeks of age. Although C3H mice are partially susceptible to the acute phase of the disease, they are fully susceptible to the chronic stage.

Virelizier et al. (1975) have described the neuropathologic effects of chronic MHV3 infection in C3H mice. Chronic illness lasted up to 12 months, with eventual paralysis of the limbs. In contrast to the susceptible strains, C57BL and DBA2, which die within 1 week after infection, most of the C3H mice appeared normal until 2-12 weeks after inoculation. Ruffled hair and loss of condition and activity then appeared, followed by neurologic signs: incoordination and paresis of one or more limbs, especially the hindlimbs. Circling when suspended by the tail was also seen. The signs were somewhat variable from one animal to the next and tended to be progressively more severe up to the time of death, 2-12 months after infection.

In neonatally thymectomized mice, East et al. (1963) described a wasting syndrome: gradual deterioration in physical condition, progressive loss of weight, and a "curious, high-stepping but coordinated gait" [p. 1069]. Depletion of lymphocytes was found in the lymph nodes, spleen, and peripheral blood.

In nude (nu/nu) mice, MHV takes on special significance. Indeed, perhaps the best description of the clinical signs may be found in the original report describing this mutant mouse (Flanagan, 1966), published before runt disease, as the wasting syndrome was called, came to be recognized as something other than a genetic effect.

MHV-infected nude mice lose weight slowly or rapidly. They move stiffly with a stilted gait, and their faces assume a pointed, anxious appearance. Partial paralysis may develop first in the hindlimbs and then in the forelimbs, resulting in almost total immobility. Nu/+ heterozygotes are not affected in this way. Flanagan (1966) found that at weaning, the nude animals were much smaller than controls (heterozygotes), that 55% died within 2 weeks of birth, and that 100% were dead by 25 weeks of age, whereas only 6% of controls died in the same period.

It was not until Sebesteny and Hill (1974) described a spontaneous MHV infection in nude mice that the agent was considered as a cause of runt disease. Hirano et al. (1975) confirmed these findings when he isolated the NuU strain from nude mice, and Tamura et al. (1977) then established persistent infection with wasting in nu/nu mice after intraperitoneal inoculation of MHV-NuU (Tamura et al., 1976) as further proof.

Tamura et al. (1980) demonstrated increased phagocytic activity of macrophages in MHV-NuU infected nu/nu mice. UV-inactivated virus did not elicit the effect. The authors believe that fixed macrophages in athymic mice may be acti-
vated, as was indicated by Nickol and Bonventre (1977) and by Cheers and Waller (1975) in certain bacterial infections in nude mice. Tamura et al. (1979) further found that inoculation of nu/nu mice with silica, which is toxic for macrophages, simultaneously with MHV-NuU caused death within 2 weeks, whereas those mice receiving no silica survived longer than 3 weeks. Silica given 4 days after the virus had no effect, indicating the importance of the intact macrophage in early resistance to MHV in these athymic mice.

2. Pathology

a. Gross Pathology. In weanling and adult mice, the most striking and constant feature, regardless of virus strain, appears in the liver, which shows sparse to extensive mottling during the acute disease. The incubation period in susceptible mice is generally short. For example, liver lesions occur as early as 41 hr after infection with MHV3 (Jones and Cohen, 1962).

Other gross findings include occasional ascites and exudate on the surface of the liver in MHV3-infected weanlings, with ascites a more constant finding in adults (Dick et al., 1956). Dark brown material suggesting decomposed blood can be seen in the intestine in JHM infection (Bailey et al., 1949) and in MHV2 infection (Nelson, 1952b). Kidney pallor, deep yellow urine, occasional jaundice, and hemorrhagic peritoneal exudate are sometimes evident.

In suckling mice, the liver may not be grossly affected, but Rowe et al. (1963) indicated that the only gross findings in MHV-S infection were "small yellow or whitish spots on the liver" [p. 162]. Kraft (1962a) and Biggers et al. (1964) found no gross abnormalities in the liver even after repeated oral passage of LIVIM virus, nor did Broderson et al. (1976) mention them in their report. Dick et al. (1956), on the other hand, found that sucklings showed extensive liver change within 72 hr of inoculation with MHV3, occasionally accompanied by jaundice and peritoneal hemorrhagic exudate. Ishida et al. (1978), too, saw numerous necrotic foci in the liver of nurslings dying of MHV infection during an epizootic.

It is probably indicative of the protean nature of MHV infection that neither Rowe et al. (1963) nor Dick et al. (1956) mentioned the gross appearance of the gastrointestinal tract in infected infant mice, whereas Kraft (1962a), Ishida et al. (1978), Broderson et al. (1976), and Hierholzer et al. (1979) encountered shrunken, empty stomachs, intestines filled with watery to mucoid yellowish, sometimes gaseous, contents, and an intestinal wall so thin that it sometimes ruptured during life.

b. Microscopic Pathology. In the liver, focal necrosis is found in a high proportion of mice during the acute phase of the disease. It may be assumed that lesions are present in all animals but that sectioning techniques cause some of them to be missed. The periphery of the lesions displays a sharp boundary between normal hepatocytes and cells undergoing hyaline degeneration. The hyaline material is intensely eosinophilic and has been compared to Councilman bodies in human hepatitis (Svoboda et al., 1962) (Fig. 5). Early in the course of infection, basophilic cytoplasmic inclusions may be seen. Hepatocyte nuclei degenerate after the cytoplasm. In the center of the necrotic focus, hepatocytes have disappeared, leaving only collapsed reticulum and fat-laden phagocytes. Polymorphonuclear leukocytes appear early but then die and are phagocytosed along with the necrotic debris. Multinucleated giant cells are seen, and in animals surviving for some time, necrotic cells may become calcified. Aside from the focal lesions, the remaining liver parenchyma appears unaffected.

Slight variations in liver pathology may be seen with different routes of infection, virus strains, age of animals, etc. (see, e.g., Rubeber and Bramhall, 1960; Gledhill et al., 1952; Nelson, 1953).

In sucklings infected with the LIVIM strain, the liver is minimally affected. Necrotic foci are very small and inconspicuous (D. C. Biggers, personal communication), whereas in sucklings infected with other strains, necrotic foci are obvious and distinct and not difficult to locate in histologic preparations.

Liver regeneration may take place as early as 10-14 days after infection (Ruebner and Bramhall, 1960), ranging from complete healing to chronic scarring with intermediate gradations.

Kupffer cells were examined by Ruebner and Miyai (1962) and Ruebner et al. (1967). They may undergo nuclear pyknosis and karyorrhexis 24 hr after intravenous inoculation of MHV3.

![Fig. 5. Focal necrotic liver lesion in MHV infection. Note the sharp demarcation between normal hepatocytes and the periphery of the lesion. Intensely eosinophilic material (Councilman body) can be seen within a hepatocyte (arrow). Scale bar = 20 μm.](image-url)
On the ultrastructural level, Svoboda et al. (1962) found virions of MHV2 or MHV3 to be more numerous in the space of Disse (between hepatocytes and endothelial lining cells) than in the neighboring cells themselves.

In infection with neurotropic variants, such as JHM, the principal lesions appear in the central nervous system (Bailey et al., 1949). Meningitis is present but varies in degree and location. In the brain, lesions may be found in all regions, but the hippocampus and its connections, the olfactory lobes, the periependymal tissues, and the brain stem seem to be affected most often. Necrotizing lesions predominate in the olfactory lobes and hippocampal regions, whereas demyelination is the major change in the brain stem. Some exudate may be found around blood vessels associated with lesions, and at about 5 days after infection, proliferating pericytes and scant lymphocytic cuffing can be seen. Peripheral nerves show no change.

In sucklings, JHM virus produces extensive lesions in the brain and cord at 6–8 days. Meningitis is present, and large regions of necrosis with many giant cells occur throughout the brain. In the cord, the lesions consist of spongy necrosis of the central gray matter. Ganglion cells appear unaffected.

Powell and Lampert (1975) described the ultrastructural changes taking place in the oligodendroglia. Infected cells undergo hypertrophy before degenerating. The hypertrophic cells show abundant microtubules, filaments, and mitochondria, aggregates of electron-dense particles, and numerous unusual plasma membrane connections to myelin lamellae. Vacuolar and hydropic changes are prominent in the degenerating cells, and postinfection recurrence of the demyelination develops subsequent to the acute disease. The question of recurrent demyelination has been addressed by Herndon et al. (1975), who found evidence of renewed demyelination in mice 16 months after infection, at a time when the acute lesions had resolved into small foci of fibrillary gliosis with an increased size and number of astrocytic processes. The importance of this finding for investigations into the cause of multiple sclerosis and other demyelinating diseases in man is obvious and has been addressed by, among others, Lucas et al. (1977) and Lampert (1978).

In sucklings infected with enterotropic strains (Kraft, 1962a; Broderson et al., 1976; Ishida et al., 1978; Ishida and Fujiwara, 1979; Rowe et al., 1963; Hierholzer et al., 1979), the entire intestinal tract may be involved. Multinucleated giant cells, regarded as altered or fused enterocytes, may be numerous. Some are pinched off and appear free in the lumen. Villi are stunted or disappear altogether (Fig. 6). There is no inflammatory reaction early in the infection. Basophilic intracytoplasmic material may also be seen at this time. Eosinophilic hyaline degeneration of enterocytes and giant cells is noted.

Necrosis was not seen in the spleen in JHM infection (Bailey et al., 1949), but others have observed it with hepatotropic strains (Dick et al., 1956; Ruebner and Bramhall, 1960; Hirano and Ruebner, 1966), and Biggart and Ruebner (1970) attribute the change to virus replication in lymphocytes.

In other organs, minute superficial necrotic foci may be found in the stomach. No changes are seen in the heart, lungs, pancreas, kidney, adrenals, voluntary muscle, femoral or vertebral bone marrow, or pituitary gland, although virus may be isolated from some of those organs. Occasional giant cells are found in peripancreatic lymph nodes and in Peyer's patches.

Flanagan (1966) described the histopathology of the hepatitis seen in the original nude mice. With time, progressively larger necrotic foci may be found in the stomach. No changes are seen in the heart, lungs, pancreas, kidney, adrenals, voluntary muscle, femoral or vertebral bone marrow, or pituitary gland, although virus may be isolated from some of those organs. Occasional giant cells are found in peripancreatic lymph nodes and in Peyer's patches.

Flanagan (1966) described the histopathology of the hepatitis seen in the original nude mice. With time, progressively larger regions of liver tissue became necrotic until the foci joined, sometimes consuming an entire lobe. Giant cells, called balloon cells† by Flanagan, contained basophilic bodies. Necrotic foci were infiltrated with neutrophilic leukocytes and phagocytes. Even where necrosis reached an advanced stage, a few

†Kraft (1962b) independently had applied the same name to giant cells in the intestinal epithelium of LIVIM-infected infant mice.
islands of hepatocytes, markedly hypertrophied, remained. Changes in other organs were not described.

The liver lesions observed in other nude mice infected with MHV were similar. Sebesteny and Hill (1974) noted central nervous system lesions in their nude mice. Ward et al. (1977) also encountered central nervous system lesions in addition to vascular changes, giant cell peritonitis, ascites, and giant cells in the villous epithelium of the intestines.

3. Transmission

Since virus can be transmitted perorally and intranasally, it may be assumed that these are the principal routes of natural infection. Transmission may be mediated by both the airborne and contact modes. Feces, nasopharyngeal exudates, and perhaps urine would be sources of infection.

Evidence of vertical transmission is at hand, but reports are conflicting. Piccinino et al. (1966) stated that, although virus (MHV3) antigen can be stained in the placenta by immunofluorescence, infectious virus could not be isolated from fetuses or newborn animals. On the other hand, Katami et al. (1978), using JHM and slightly different techniques and timing of virus inoculation in pregnant females, demonstrated virus-specific antigen in the placentas, as well as in the visceral yolk sac and fetal livers. Furthermore, they visualized characteristic coronavirus particles in these locations by electron microscopy. Histopathologically, degenerative and necrotic changes were seen in all those tissues and also in the fetal bone marrow.

In commenting on these findings, it is probably important to consider that different virus strains were utilized, and in addition, Piccinino et al. (1966) inoculated the females late in pregnancy, whereas Katami et al. (1978) took advantage of the early and midgestational periods. It will be of great importance for the epizootiology of MHV to ascertain if strains other than JHM are capable of being transmitted vertically.

Mosquitoes (A. aegypti and C. pipiens fatigans) have been incriminated as potential mechanical carriers of MHV (Ishii et al., 1974).

4. Immune Response

The dynamics of the rise in neutralizing and complement-fixing antibodies following natural infection are not readily apparent from the literature. In general, however, titers appear to be low or absent in infected colonies. Thus, Fujiwara et al. (1976b), for example, described an epizootic that suddenly appeared in a colony in which retired breeders had been monitored for complement-fixing antibodies with consistently negative results. After the epizootic was underway, only mice 6 weeks of age or older demonstrated antibodies, but the percent positive was low.

In order to increase the sensitivity of the complement fixation test, Fujiwara (1971) had devised a method utilizing a booster dose of inactivated MHV polyvalent antigen to elicit an anamnestic response. Without the booster, for example, only 2.4% of 40 retired breeder females had demonstrable antibodies, whereas 32.4% of 41 were positive after the booster dose was given. On the other hand, 4-week-old male weanlings, with or without booster, showed no antibody in the same colony. In a conventional colony of DDD mice, furthermore, seropositivity increased from 0 to 12% and in ddY mice from 6.3 to 45% as a result of the booster technique. In barrier-maintained animals, the booster did not elicit antibodies where there had been none before. Presumably, these animals were free from MHV infection.

From the foregoing, it is evident that the classic humoral immune response to MHV seems to be weak, and that a colony in which serologic tests are consistently negative is not necessarily free from MHV infection. Perhaps of even greater importance to an understanding of resistance mechanisms in this infectious disease are studies concerning immunopathology. Using MHV3 as a model, LePrévost et al. (1975b) examined both humoral and cell-mediated immunity and the effect of immunosuppression by antilymphocyte serum. Levy-LeBlond and Dupuy (1977) extended these studies to conclude that at least two types of mature cells are required for transfer of MHV resistance to newborn mice of the A/J (resistant) strain: T lymphocytes and an adherent spleen cell population. Transfer of isologous cells consisting of purified lymphocytes and adherent spleen cells did not, however, confer resistance on newborn mice (A/J strain) (Tardieu et al., 1980). Addition of nonadherent spleen cells (or bone marrow or peritoneal exudate cells) to T lymphocytes and adherent spleen cells resulted in 88% protection. Clearly, cell-mediated immune functions play a significant role in resistance of mice to MHV3 infection.

In contrast to the findings of Bang and Warwick (1960), who concluded that one gene (or factor) was responsible for host susceptibility in MHV2 infection, Stohlmann and Frelinger (1978) showed that two genes are required for resistance of the central nervous system in SJL mice to fatal disease due to MHV/JHM. Further, Stohlmann et al. (1980) reported that there is an age-related change in resistant mice that protects them from acute central nervous system disease. They identified this change as due to a maturing adherent spleen or peritoneal exudate cell population.

In extensive genetic studies, Lévy-LeBlond et al. (1979) found no correlation between the H-2 locus and either the acute or chronic disease in C57BL (susceptible) or A/J (resistant) animals. Using congenic C3H lines, however, they were able to show that the H-2 allele enables both heterozygous and homozygous animals to resist the development of the chronic disease. They believe, therefore, that MHV sensitivity appears to be influenced by at least two major genes: one for the acute disease, and the other, linked to the H-2 gene complex, for the chronic disease.

Mice also produce interferon as a result of MHV infection.
(Virelizier et al., 1976; Virelizier and Gresser, 1978), and Taguchi et al. (1979a) ascribe the greater susceptibility of suckling C3H/HeJms mice to serum levels of interferon that are considerably lower than in weanling and adult mice, as well as to greater macrophage sensitivity in the neonates. MHV-induced interferon may be regarded as the principal mechanism by which the virus modifies the immune responsiveness of mice to sheep red blood cells, for example. (See also Tamura and Fujiwara, 1979.) Further, of practical importance to users and breeders of mice, MHV3 infection interferes with the secretion of lymphocyte interferon that is normally induced by Sendai virus infection, thus perhaps operating to increase the susceptibility of the MHV-infected mice to Sendai virus infection.

D. Epizootiology

1. Host Range

a. Species Affected. A number of attempts have been made to infect other species. Cheever et al. (1949) infected cotton rats, rats, and hamsters intracerebrally, but rabbits and guinea pigs failed to respond. Sebesteny and Hill (1974) attempted to infect infant Wistar rats and hamsters using virus recovered from nude mice. All survived at least 21 days without signs of illness.

Of considerable importance is a report by Taguchi et al. (1979c) concerning asymptomatic MHV-S infection in suckling rats following intranasal inoculation. The agent multiplied mainly in the nasal epithelium without any clinical signs. Neutralizing antibodies were produced, however, and could also be demonstrated in adult rats following infection. Necrotic changes took place in the nasal mucosa, and cytoplasmic immunofluorescence was demonstrated in the nasal epithelium 2 days after intranasal inoculation of 10-day-old rats.

b. Age and Sex Susceptibility. Age susceptibility has been amply addressed above in the consideration of virus growth in mice and tissue culture and in the discussion of the clinical picture. There appears to be no difference in susceptibility between the sexes (Taguchi et al., 1976). Parker et al. (1966) found that the incidence of complement-fixing antibodies was greater in females than in males under colony conditions, ascribing this difference to continual exposure to virus-infected litters.

c. Influence of Parity (Birth Order). There appears to be no evidence that first litters are significantly more susceptible than later ones.

d. Strains of Mice. As noted above (Section IV.B, 2 and C,1), the susceptibility of various mouse strains depends on the age of the host at the time of infection, the virus strain, and the host genotype. A completely resistant mouse strain has not been reported.

2. Prevalence and Distribution

Prevalence of MHV infection in an animal colony is often difficult to ascertain. In addition to examples given earlier, Descoteaux et al. (1977) found a low prevalence of hepatitis antibodies in three of five colonies studied in Canada. Complement-fixing antibodies ranged in titer from 8 to 32, 8 being considered positive. Fewer than 20% of the animals, which were 6–9 months old at the time of testing, were positive.

In distribution, MHV is regarded as occurring worldwide.

3. Latent and Chronic Infections

These have been defined as occurring more than 2 months after intracerebral, intraperitoneal, intranasal, or intravenous inoculation (as reviewed in Robb and Bond, 1980). The chronic clinical manifestations range from none to porencephaly, paralysis, hepatitis, immunodeficiency manifestations, encephalitis, lymph node adenopathy, and vasculitis. Virus may or may not be isolated. Cells stainable by immunofluorescence may be found. Demyelination with or without remyelination may occur. Occasional scattered mononuclear cell infiltrates may be evident.

4. Seasonal Periodicity

There is no evidence that seasonal changes influence the occurrence of epizootics of MHV infection.

E. Diagnosis

1. Serology

As indicated above, serologic testing is routinely carried out by means of the complement fixation test. Cross-reactions with other coronaviruses must be taken into account when interpreting the results, however. Neutralization tests performed in tissue culture systems are also possible.

Employing virus strain A59 as antigen in the ELISA, Peters et al. (1979) found a high prevalence of MHV antibodies in colonies with a low incidence of both complement-fixing and neutralizing antibodies.

Hierholzer and Tannock (1977) have used the single radial hemolysis test for human coronavirus serodiagnosis. They then applied it to some MHV strains (Hierholzer et al., 1979).
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2. Virus Isolation and Visualization

These techniques are helpful under certain circumstances, e.g., experimental investigations, but they are inefficient for field conditions.

3. Necropsy; Sentinel Animals

Necropsy of dead or sick animals is always useful, although a definitive diagnosis in the absence of serologic evidence cannot be made. Nevertheless, wherever possible, gross and microscopic pathologic examination should be undertaken. Sentinel animals, especially gnotobiotics, could be incorporated into a program of colony health surveillance. These animals can then be checked at predetermined intervals for clinical, serologic, and histopathologic evidence of endemic disease in the colony.

F. Control and Prevention

Control of MHV is difficult in mouse colonies unless caesarian derivation coupled with barrier maintenance is undertaken. With the finding that vertical transmission is possible, however, barrier maintenance alone may not be adequate if, for example, such transmission is frequent.

Using small numbers of animals for experimental purposes, Kraft (1962a), Fox et al. (1977), and Rowe et al., 1963 found that filters on the animal cages were efficacious in separating healthy from diseased animals.

In nude mice Fujiwara et al. (1976a) attempted vaccination. Antibody levels could be enhanced by transfer of spleen cells from vaccinated heterozygotes (nu/+ ) to the homozygotes. Tamura et al. (1976) found that nu/nu mice could resist MHV infection when they previously received thymocytes from weanling nu/+ littermates. They were then not only able to produce antibody but survived a challenge infection as well.

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