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Mousepox, Liver, Mouse

Robert O. Jacoby

Synonyms. Infectious ectromelia

Gross Appearance

The liver is a major site of viral replication in mousepox, but gross lesions, even during acute disease, are not readily apparent until shortly before death. Severely affected livers are usually swollen and friable and may occupy up to half the volume of the peritoneal cavity, whereas mildly affected livers may remain grossly normal or have sparse focal necrosis. Necrotic areas appear first as pinpoint yellow–white foci, but increase rapidly in size and number. Confluent areas of necrosis can produce a reticulated pattern of yellow–brown to pink discoloration on the surface and throughout the parenchyma. Areas of hemorrhage also may develop. The pale hue of severely affected livers is in part due to fatty change, and the fat content of such livers can be as much as four times normal. Livers from mice that survive acute infection usually have a normal gross appearance. A few small scars may be present, however, especially at

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the margins (Fenner 1948d, 1949b: Allen et al. 1981).

Microscopic Features

The major liver lesion is coagulation necrosis, which begins in a random fashion among individual or small groups of hepatocytes approximately 5 days after infection (Fig. 159). In highly susceptible mice, necrotic areas enlarge rapidly and in 2–4 days lead to extensive necrosis and variable degrees of hemorrhage.

Hepatocytes in early lesions or at the margins of advanced lesions can undergo ballooning degeneration or can shrink and develop intensely eosinophilic cytoplasm. Hepatocytes with clear cytoplasm can occur at some distance from necrotic foci and have reportedly undergone glycogen depletion (De Burgh 1950). Nuclei of infected cells may be transiently enlarged, but quickly become pyknotic or karyorrhectic.

Two types of intracytoplasmic inclusions occur in mousepox: the early or type B inclusion and the late or type A inclusion. Type B inclusions commonly develop in the liver, whereas type A inclusions are rare. Type B inclusions are basophilic to amphophilic and can occur singly or in groups (Fig. 160). They may be surrounded by a thin "halo", but this is often hard to see in formalin-fixed sections. Type B inclusions also are difficult to detect unless the hematoxylin staining time is at least doubled (Allen et al. 1981). Alternatively, because they are sites of viral replication (Cairns 1960; kameyama et al. 1959), they can be detected in formalin-fixed liver by immunohistochemical methods. Immunoperoxidase staining of infected hepatocytes will reveal intracytoplasmic antigen in particulate and diffuse distribution (Fig. 161). Viral antigen also can be found in cells lining vascular channels, especially during early phases of infection. Type A inclusions are large and intensely eosinophilic. They can more readily be detected in other tissues, particularly skin (Fig. 162), mucous membranes, and intestinal epithelium, to help confirm the etiology of hepatic lesions.

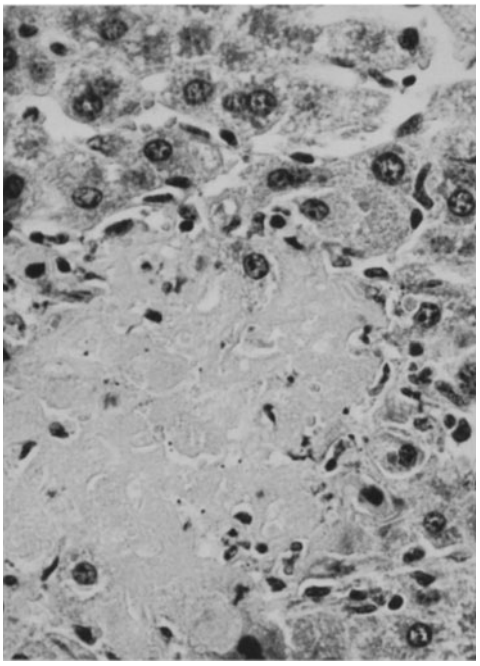
The inflammatory response during fulminating hepatic necrosis is minimal. Some mononuclear cells and polymorphonuclear leukocytes may infiltrate portal triads or the margin of necrotic lesions. In milder or more prolonged hepatic infection, such as occurs in genetically resistant mice, inflammatory infiltrates are more prominent. The mononuclear cell response is compatible with host defenses mediated by cellular immunity (Blanden 1974).

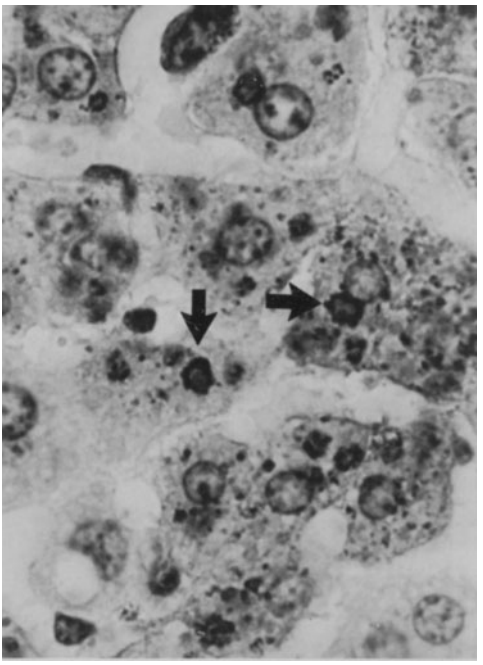
The histologic sequelae of mousepox in the liver have not been described in detail, but appear to be unremarkable. This is probably due to the fact that extensive hepatic involvement commonly leads to rapid death, whereas mild hepatic lesions are quickly repaired. Large or multinucleated hepatocytes can accumulate at the margin of necrotic lesions during repair. Chronic hepatitis has not been reported. "Hyalinized" areas have been found and may represent local postnecrotic scarring, but widespread fibrosis or cirrhosis does not occur.

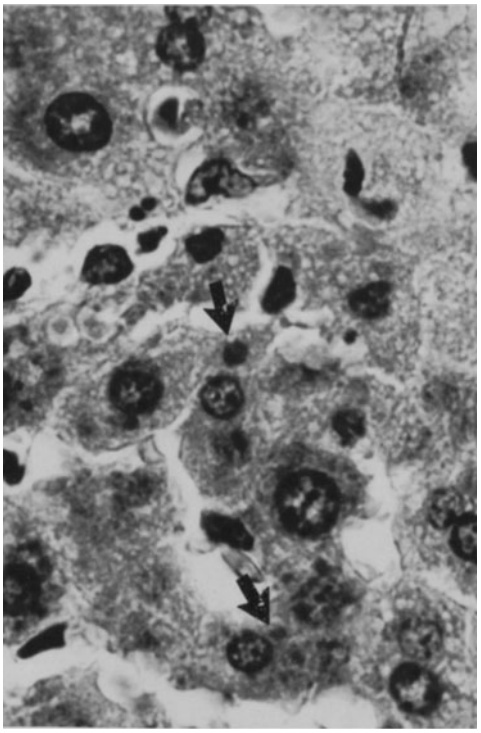
Ultrastructure

The most complete observations have been made by Leduc and Bernhard (1962) on livers of naturally infected mice. The earliest signs of infection are seen among periportal hepatocytes. Cytoplasmic changes are characterized by loss of glycogen, vesicular swelling of the endoplasmic reticulum, mitochondria, and Golgi apparatus and sparse lipid droplets (Fig. 163). Irregular dense bodies develop and are found occasionally in nucleoplasm. Nucleolar fragmentation is also observed. In moribund or necrotic cells, the cytoplasm is dense and, in addition to viral particles, contains membranous whorls, vesicles of various sizes, vacuoles with cell debris, and collapsed, distorted mitochondria. Nuclear chromatin is dense and marginated, and blisters form in the nuclear envelope. Although cells lining hepatic sinusoids support viral replication, they do not undergo necrosis.

Virus formation begins in granular to reticular cytoplasmic matrices of low electron density (Fig. 163). Matrix zones displace the cytoplasm and lack sharp boundaries. They are thought to be the ultrastructural correlates of type B inclusions. Three early forms of virus emerge from the matrix: (1) 220 mu, membrane-bound, oval particles with uniform viroplasm similar in appearance to matrix (Fig. 164 arrow), (2) membrane-bound oval particles with a dense nucleoid surrounded by a halo (Fig. 164), and (3) lobulated masses of matrix-like viroplasm partially enclosed by C-shaped membranes which probably represent incompletely developed viral particles (Fig. 164, arrow).







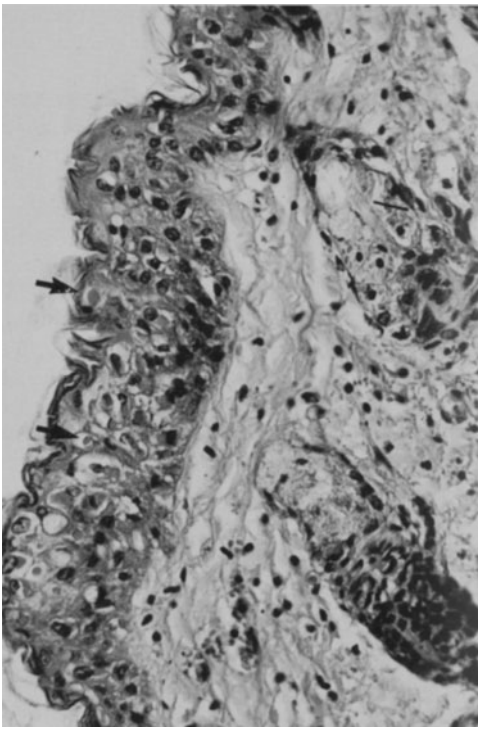


Fig. 159. (*upper left*) Focal coagulative necrosis in the liver of a mouse during the early stages of acute mousepox. H&E ×720

Fig. 160. (*lower left*) Type B inclusions (*arrows*) in hepatocytes of a mouse infected with ectromelia virus. Double stained with Harris' hematoxylin and counterstained with eosin, ×1800

Fig. 161. (*upper right*) Ectromelia viral antigen in hepatocytes. Intracytoplasmic bodies of various size correspond to type B inclusions (*arrows*). Avidin-biotin-conjugate, immunoperoxidase method, ×1800

Fig. 162. (*lower right*) Type A inclusions of ectromelia virus (*arrows*) in the epidermis of a mouse. H&E, ×450

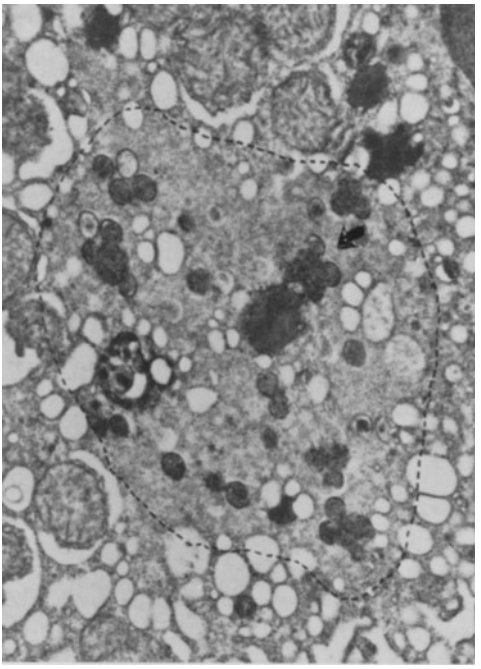


Fig. 163. Hepatocyte infected with mousepox. Matrix zone (encircled by *dotted line*) contains developing virus particles. Incomplete C-shaped particles presumably represent immature virions (*arrow*). (From Leduc and Bernhard 1962, with permission of *Journal of Ultrastructural Research.*) TEM, ×17000

Mature virus particles are seen only in necrotic hepatocytes, but they also occur in viable Kupffer's cells and sinusoidal endothelium.

Differential Diagnosis

Several naturally occurring murine viruses cause necrotizing hepatitis in mice. Hepatotropic strains of mouse coronaviruses (mouse hepatitis viruses) may induce acute multifocal hepatic necrosis, but lesions, especially in adult mice, are seldom as severe as those of lethal mousepox (see p. 194). Because the course of coronaviral hepatitis is often longer than that of mousepox, signs of repair (mitotic activity, large or binucleated hepatocytes) can be found more commonly at the margins of necrotic lesions. Inflammation also may be more prominent. Syncytia, which are common in mouse coronavirus infection, are not typical of mousepox, whereas viral inclusions are not found in coronaviral hepatitis. Extrahepatic manifestations, especially necrosis of spleen and other tissues, are also more severe in mousepox. Several strains of mouse coronavirus are neurotropic, whereas mousepox does not cause significant lesions of the central nervous system unless virus is inoculated intracerebrally.

Reovirus-3 can cause necrotizin hepatitis in infant mice, but not in adults (see p. 196) Necrosis tends to begin in centrilobular zones and to spread peripherally (Walters et al. 1963). Mice that recover may develop chronic active hepatitis that has immunopathologic overtones expressed by a significant, persistent inflammatory response (Stanley 1974). The disease in infant mice is frequently accompanied by necrosis in other organs; most frequently among these is brain, a finding that is not compatible with mousepox. Mouse cytomegalovirus and lymphocytic choriomeningitis virus can cause hepatic necrosis after experimental inoculation, but infection with these agents is not prevalent among well-managed mouse colonies.

Differentiation of the various forms of viral hepatitis in mice can be difficult. Therefore, in the absence of specific morphological changes, ancillary data should be collected to confirm the diagnosis. These include one or more of the following:

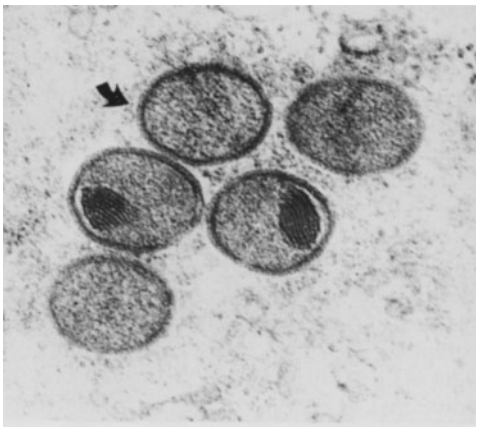


Fig. 164. Two types of mousepox viral particles. Oval particles with uniform viroplasm (*arrow*) and particles with nucleoids of precisely aligned filaments. (From Leduc and Bernhard 1962, with permission of *Journal of Ultrastructural Research.*) TEM, ×53000

viral serology, immunohistochemical demonstration of viral antigen in tissue, electron microscopy for viral particles, and, when feasible, viral isolation.

Tyzzer's disease should be considered whenever hepatic necrosis is found in mice. The causative organism, *Bacillus piliformis*, is difficult to find in hematoxylin and eosin (H&E)-stained sections, but can be demonstrated with special stains (Giemsa, periodic acid-Schiff, Warthin-Starry; see p. 201). Organisms are generally found in the cytoplasm of hepatocytes bordering necrotic areas. They may be present in small numbers, making it necessary to examine several sections to locate them. Although the acute form of other bacterial infections such as salmonellosis may cause necrotizing hepatitis, these organisms are rarely found in modern vivariums. Nevertheless, it is advisable to obtain bacterial cultures from mice with hepatic necrosis.

Biologic Features

The clinical expression of mousepox can vary and is influenced strongly by genotype (Briody et al. 1956; Briody 1966; O'Neill and Blanden 1983; Bhatt and Jacoby 1985). Broadly speaking, three clinical courses are recognized. *Highly susceptible mice* develop acute, fatal disease wherein apparently healthy mice may die within several hours from the onset of illness. This form of mousepox is closely associated with severe hepatic necrosis. Clinical signs are relatively nonspecific and include hunched posture, rough haircoat, and diarrhea. If infection has occurred through skin abrasion, careful examination of the skin may reveal a "primary lesion," which indicates the initial site of viral replication (Fenner 1947). *Moderately susceptible mice* often develop a chronic form of mousepox with variable mortality. It is characterized by erosive or ulcerative dermatitis which is most easily observed on the ears, tail, and feet, but which also may occur as a general exanthema. These mice can also have edema of the face and extremities and conjunctivitis. Amputation of part or all of one or more limbs or the tail can occur, a lesion that gave rise to the term "infectious ectromelia." Skin lesions among survivors of chronic mousepox heal as hairless scars. *Resistant mice* commonly have asymptomatic infection (Fenner 1982).

From an epizootiologic aspect, acute, lethal mousepox is a major hazard to a mouse colony, but is self-limiting provided that additional susceptible animals are not introduced during an outbreak. Chronic or asymptomatically infected mice are a relatively greater hazard in the long run, because they can perpetuate enzootic infection (Fenner 1984a,b). Introduction of highly susceptible mice to an enzootically infected colony may initiate a clinically explosive and devastating outbreak.

The prevalence and biologic significance of latent, persistent mousepox infection (carrier state) is unsettled. Evidence for a carrier state is sparse (Gledhill 1962; Horzinek and Höpken 1965), but has not been thoroughly or systematically discounted. If a carrier state occurs, its expression is likely to be influenced by mouse genotype. Briody and coworkers (Briody et al. 1956) and Briody (1959), for example, studied responses of mice to natural epizootics of mousepox and found that some inbred strains such as DBA/1, A and C3H were highly susceptible to lethal infection, whereas other strains such as C57BL/6, AKR, and BALB/c were highly resistant. Although these findings underscore the significance of genotype for the outcome of infection, they should be extrapolated cautiously, because recent evidence suggests that susceptibility to lethal mousepox can vary even among sublines of a given inbred strain (Bhatt and Jacoby 1985).

Fenner (1947, 1948a–d, 1949a,b) is largely responsible for deciphering the pathogenesis of mousepox. Although ectromelia virus can enter the body by several routes, including the respiratory tract, the most common mode of entry is thought to be through abraded skin. Virus replicates at the site of entry and infects the draining lymph node. It reenters the lymphatics and finally reaches the blood to produce a primary viremia. During primary viremia, virus invades parenchymal organs, including the liver. Immunofluorescent studies by Mims (1964) disclosed that blood-borne virus first infects littoral cells lining vascular channels and that infected cells seed virus to hepatocytes. Mice that survive initial infection of parenchymatous tissues develop a secondary viremia during which virus is widely distributed to skin and causes a typical pox rash. Recovery from mousepox and resistance to lethal infection depends on intact cellular immunity (Blanden 1974). Evidence that humoral responses

are critical to host survival are less compelling (Schell 1960a,b).

Ectromelia virus is an orthopoxvirus that is morphologically identical and antigenically similar to vaccinia virus, a relationship which enables vaccinia virus to elicit protective immunity to mousepox (Briody 1959). The virus grows in a number of continuous cell lines, including L cells and Vero cells, and classic pox lesions are produced if virus is grown on the chorioallantoic membrane of embryonated hen eggs (Fenner 1982). A line of kidney cells (BS-C-1), derived from African green monkeys, is highly susceptible to ectromelia virus (Bhatt and Jacoby 1985).

Several strains of ectromelia virus have been studied intensively. The Moscow strain is highly virulent, whereas the Hampstead strain is less so (Fenner 1949b). Avirulent strains of Hampstead virus replicate poorly in hepatic Kupffer's cells, but readily invade hepatocytes. It has been proposed from this that virulence may depend on the ability of virus to infect Kupffer's cells and that survival of the host may depend on the ability of Kupffer's cells to protect hepatocytes from viral invasion (Roberts 1964). A strain of ectromelia virus (NIH-79) was isolated from a recent mousepox epizootic in the United States (Allen et al. 1981). Its behavior in mice is under scrutiny, but is seems to be moderately to highly virulent (Bhatt and Jacoby 1985).

All strains of ectromelia virus studied thus far express an immunogenic hemagglutinin. Infected animals develop hemagglutinin-inhibiting antibody, which can be detected by routine serologic methods (Briody 1966). The sensitivity and specificity of hemagglutination inhibition has been the center of some controversy, and current evidence suggests that the hemagglutination inhibition (HAI) test, although still widely used, may elicit occasional false-positive results (Collins et al. 1981; Wallace et al. 1981). Other serologic tests used to detect infection include complement fixation (relatively insensitive), immunofluorescence (Christensen et al. 1966) virus neutralization. and enzyme-linked immunoadsorbent assay (ELISA). ELISA is highly sensitive, but it must be refined before it can be used to discriminate ectromelia-infected from vaccinated mice (Buller et al. 1983; Collins et al. 1981). HAI is still the test of choice for this purpose. The strain of vaccinia virus used to immunize mice (IHD-T) is hemagglutinin deficient (Briody 1959). Thus vaccinia-immune mice should remain free of HAI antibody to either vaccinia virus or ectromelia virus.

Naturally occurring mousepox is limited to mice. Outbreaks in laboratory mice have occurred in Europe, Asia, Australia, and the United States. The risk of introducing infection in a susceptible population is increased by extensive exchange of mice and mouse tissues among laboratories. The spread of mousepox in a newly infected colony depends on a combination of factors such as mouse genotype, husbandry, viral virulence, and experimental manipulation. Although mousepox is an infectious disease, it can spread slowly even among mice housed in the same room (Wallace et al. 1981). *In utero* infection has been produced experimentally (Schwanzer et al. 1975; Wylekshanin 1935), but its prevalence and significance in naturally occurring disease have not been established.

Comparison with Other Species

The skin form of mousepox has been studied extensively as a model of a viral exanthem because it resembles human smallpox (Fenner 1948c). Hepatic lesions, however, are not characteristic of smallpox or of poxvirus diseases of domestic mammals. A notable exception is rabbit pox, which tends to be severe and generalized and is frequently accompanied by hepatic necrosis (Greene 1934).

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Reovirus Type 3 Infection, Liver, Mouse

Stephen W. Barthold

Synonyms. Reo-3 virus infection, hepatoencephalomyelitis virus (HEV) infection, ECHO 10 virus infection

Gross Appearance

Natural infection of adult mice with reovirus type 3 is asymptomatic. Infection of neonatal mice can result in runting, emaciation, jaundice, bilirubinuria, conjunctivitis, incoordination, tremor, paralysis, and oily hair effect. Survivors are runted with transient alopecia, which is most marked along the dorsum of the head, neck, and rear legs. Yellow or blood-tinged peritoneal exudate can be present. Livers are enlarged and dark, with multiple sharply demarcated yellow foci up to 3 mm in diameter on all surfaces. Gallbladders