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

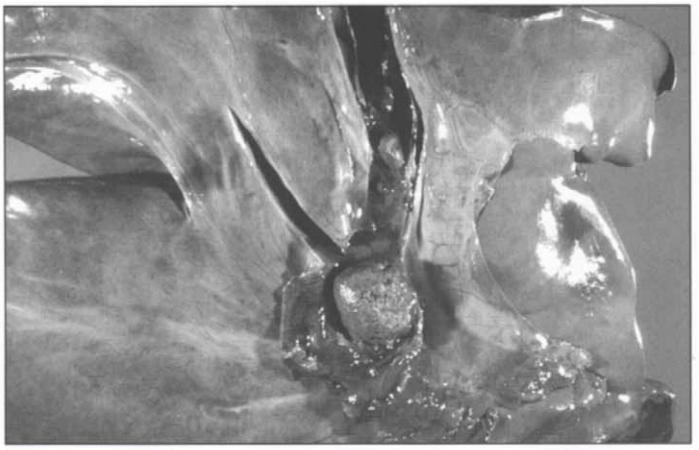

Figure 2.53 Cholelith obstructing the bile duct in a horse.

by masses of detritus and biliary constituents, parasites, or cicatricial stenosis of the ducts. Adult ascarids may cause mechanical obstruction. Inspissated bile-stained friable plugs are occasionally responsible for obstructions in segments of the liver in horses. Tumors of the pancreas and duodenum, and tumors and abscesses of the hilus of the liver and portal nodes, may cause compression stenosis of the ducts. Edematous swelling of the papilla in enteritis may also be of significance. Biliary obstruction by abnormal intraluminal mucoid secretion (*gallbladder mucocele*) has been reported in dogs (see section on Ectopic, metaplastic, and hyperplastic lesions, below).

The consequences of biliary obstruction depend on the site and duration of the obstruction. When the main duct is involved, there is jaundice. When one of the hepatic ducts is involved, there is no jaundice, and depending on the efficiency of biliary collaterals, there may be no pigmentation of the obstructed segments of liver. Increases in serum γ-glutamyltranspeptidase and alkaline phosphatase usually occur when a sufficiently large amount of the duct system is affected. The ducts undergo progressive cylindric dilation, which may be extreme. The smallest interlobular ducts and the cholangioles proliferate. There is inflammation in the walls of the ducts and the portal triads, and this is probably due in part to chemical irritation by bile acids but is largely due to secondary bacterial infections. These infections may be acute and purulent, or low-grade; in these cases, bacteria may not be easily cultured. The cholangiohepatitis that almost inevitably follows has been described earlier.

Inflammatory stenoses of larger ducts may recanalize via mucosal glands, which can proliferate and link to form a tortuous detour around the obstruction.

Rupture of the biliary tract or the gallbladder causes steady leakage of bile into the peritoneal cavity, the omentum being unable to seal even small defects. The bile salts are very irritating and may cause *acute chemical peritonitis*. The peritoneal effusion that follows may remain sterile; more often it is infected by enteric bacteria, and severe diffuse peritonitis ensues. This may be rapidly fatal, particularly if clostridia are involved. Most perforations of the biliary tract are traumatic in origin.

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INFECTIOUS DISORDERS OF THE LIVER

Viral infections

Various systemic viral diseases may affect the liver. Adenoviral infections of lambs, calves, and goat kids can cause multifocal hepatic necrosis and cholangitis, in addition to pneumonia. Lymphohistiocytic hepatitis with single-cell necrosis of hepatocytes and perilobular fibrosis is a frequent histologic lesion in cases of postweaning multisystemic wasting syndrome in pigs, associated with *Porcine circovirus* type 2 infection. Neonatal *Canid herpesvirus 1* infection in puppies causes disseminated focal necrosis and hemorrhages in parenchymal organs, including the liver, with formation of amphophilic intranuclear inclusion bodies in epithelial cells of kidney, lung, and liver. Similar microfoci of hepatic necrosis sometimes occur in aborted or newborn foals with congenital *Equid herpesvirus 1* infections (see Fig. 2.26). *Feline coronavirus* infection can cause granulomatous hepatitis in some infected cats, as part of feline infectious peritonitis.

The viral diseases included below are those in which the liver is the major target organ for viruses that cause substantial hepatic disease that sometimes culminates in hepatic failure. Unlike humans, in which several pathogenic hepatitis viruses from various families are well described, the major domestic mammals are vulnerable to few viruses that target hepatocytes. Some hepadnaviruses have been found in ducks, woodchucks, and herons. Closely related human and swine genotypes of *Hepatitis E virus* (HEV – Caliciviridae) can replicate in porcine hepatocytes wherein antigens and viral nucleic acids can be detected. There is evidence for fecal–oral transmission of HEV in pigs, and zoonotic transmission, but experimental HEV infection has not been reported as a primary cause of hepatitis in pigs.

Infectious canine hepatitis

Canine adenovirus 1 (CAdV-1) infection can cause infectious canine hepatitis, a severe liver disease in dogs and other canids. Vaccination has made the disease rare in many countries in which it was endemic.

Figure 2.54 Infectious canine hepatitis (*Canine adenovirus 1* infection). **A. Corneal edema** in convalescent stage. **B. Intranuclear inclusion body** in hepatocyte.

Deaths from infectious canine hepatitis are usually sporadic, although small outbreaks can occur among young dogs in kennels. Fatalities seldom occur among dogs more than 2 years of age. In areas where the disease is not controlled by vaccination, it is probable that most dogs in the general population contact CAdV-1 in the first 2 years of life and suffer either inapparent infection or mild febrile illness with pharyngitis and tonsillitis.

In more severe cases, there is vomiting, melena, high fever, and abdominal pain. There may be petechiae on the gums; the mucous membranes are blanched, and only occasionally are they slightly jaundiced. Nervous signs of nonspecific character occur in a few cases. There is also a peracute form of the disease in which the animal is found dead without signs of illness, or after an illness of only a few hours. In convalescence, there may be a unilateral or bilateral opacity of the cornea caused by edema, which disappears spontaneously (Fig. 2.54A).

Canine adenovirus 1 has special tropism for endothelium, mesothelium, and hepatic parenchyma, and it is injury to these that is responsible for the pathologic features of edema, hemorrhage (which is predominantly serosal), and hepatic necrosis. The histologic specificity of the lesions depends on the demonstration of *large, solid intranuclear inclusion bodies in endothelium or hepatic parenchyma* (Fig. 2.54B). Inclusions are occasionally observed in other differentiated cells but always have the same morphologic and tinctorial features, being deeply acidophilic with a blue tint.

The morbid picture of spontaneously fatal cases is usually distinct enough to allow a diagnosis to be made grossly at necropsy. Superficial lymph nodes are edematous, slightly congested, and often hemorrhagic. Blotchy hemorrhages may be present on the serous membranes (Fig. 2.55A), and there is usually a small quantity of fluid, clear or blood-stained, in the abdomen. Hemorrhages on the serosa of the cranial surface of the stomach are usually linear, the so-called *paintbrush* type. Jaundice, if present, is slight. The mesenteries are slightly moist, and the serosa of the small intestine has a ground-glass appearance. The liver is slightly enlarged, with sharp edges, and is turgid and friable, sometimes congested, with a fine, uniform, yellow mottling. Red strands of fibrin can be found on its capsule, especially between the lobes. In the majority of cases, the wall of the gallbladder is edematous (Fig. 2.55B); when edema is mild, it may be detected only in the attachments of the gallbladder. In cases in which the gallbladder is edematous, it may also be darkened by intramural hemorrhages.

Gross lesions in other organs are inconstant. Small hemorrhagic infarcts may be found in the renal cortices of young puppies. Hemorrhages may occur in the lungs, and occasionally there are irregular areas of hemorrhagic consolidation in the caudal lobes. Hemorrhages in the brain occur in a small percentage of cases. These are capillary and venular hemorrhages best appreciated when darkened by formalin, and then, depending on their concentration, the affected portions of brain appear gray or dark brown. Microscopic hemorrhages occur in any part of the brain, but when numerous enough to be grossly visible, they are confined to the midbrain and brainstem, avoiding the cerebral cortex and cerebellum. Hemorrhagic necrosis of medullary and endosteal elements occurs in the metaphyses of long bones in young dogs, and the hemorrhages are readily visible through the thin cortex of the distal ends of the ribs.

At low magnification, the histologic changes in the liver are quite reminiscent of the zonal necrosis of acute hepatotoxicities. *There is an as yet unexplained susceptibility of the periacinar parenchyma to necrosis in this disease.* Close to the portal triads, the hepatocytes may be near normal in appearance, except for loss of basophilia and the presence of a scattering of inclusion bodies. In spontaneously fatal cases, most of the parenchyma of the peripheral and central portions of the acini is dead, the hepatocytes having undergone granular acidophilic coagulative necrosis, and in some of these, ghosts of inclusion bodies may be detectable. The margin between necrotic parenchyma and viable tissue is usually quite sharp, although in the viable tissue there are many individual hepatocytes undergoing apoptosis, most of them without inclusion bodies. Fatty changes are common but not constant. The dead cells do not remain long, so the sinusoids become dilated and filled with blood. The reticulin framework remains intact, an observation in keeping with the fact that, *in recovered cases, restitution of the liver is complete.* Massive necrosis with collapse does not occur. As is typical of severe periacinar necrosis, the necrotic zones, initially eccentric areas about hepatic venules, extend and link up to isolate portal units. Intranuclear inclusions can be found in Kupffer cells in variable numbers. Many of the Kupffer cells are dead, others are proliferating, and others are actively phagocytic in the removal of debris. Leukocytic reactions in the liver are mild and are directed against the necrotic tissue; mononuclear cells are present, but neutrophils, many degenerating, predominate. There is some collection of bile pigment, but it is moderate, in keeping with the short course of the disease.

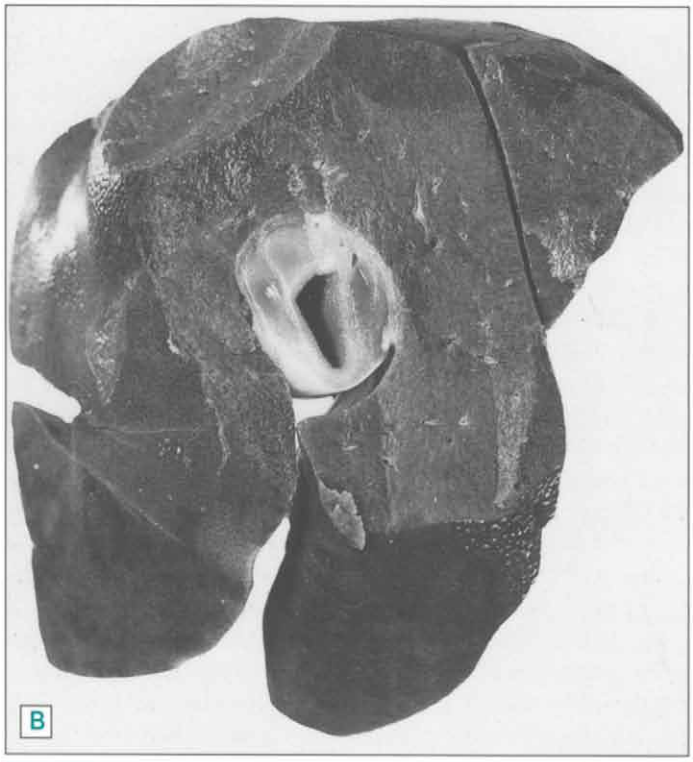

Figure 2.55 Infectious canine hepatitis. **A. Serosal hemorrhages** over the intestines. **B.** Severe **edema of the gallbladder wall**.

Microscopic lesions in other organs are largely due to injury to endothelium. Inclusion bodies in endothelial cells can be difficult to find and are looked for with most profit in renal glomeruli, where endothelium is concentrated. Occasionally, they are found in the epithelium of collecting tubules. When areas of hemorrhagic consolidation of the lungs are present, there is hemorrhage, edema, and fibrin formation in the alveoli, and in these consolidated areas, inclusions are often common in alveolar capillaries and even in dying cells of the bronchial epithelium. Changes in the brain are essentially secondary to vascular injury and may be absent. Hemorrhages, if present, are from capillaries and small venules, and inclusions in endothelial nuclei can usually be found in vessels that have bled. Other endothelial and adventitial cells are hyperplastic and mixed with a few lymphocytes. Small foci of softening or demyelination may be present in relation to the hemorrhages.

Lymphoreticular tissues are congested, and inclusions may be found in the primitive reticulum cells of follicles, in the red pulp of the spleen, and in macrophages anywhere.

The detailed pathogenesis of infectious canine hepatitis has yet to be worked out. Many infections appear to be clinically silent, and other dogs recover after mild febrile disease with tonsillitis. The fulminating pattern of clinical disease and convalescent phenomena needs further examination. Some sudden deaths in this disease are associated with midbrain hemorrhage, and others occur with, at most, slight structural evidence of liver injury. Following oral exposure, which is probably the natural route of infection, viral multiplication occurs in the tonsils and leads to tonsillitis. The tonsillitis is sometimes quite severe and may be fatal with extensive clear edema of the throat and larynx. Fever accompanies the tonsillitis and apparently precedes the viremic phase, which is of short duration and accompanied by severe leukopenia. Hepatic necrosis develops at about day 7 of experimental infection. The sequence of developments in the liver is not clear, but it is possible that viral proliferation occurs first in Kupffer cells. In surviving animals, hepatic regeneration occurs rapidly, and there do not appear to be any significant residual lesions. Small foci of hepatocellular necrosis involving one to several liver cells may still be present at 2 weeks, and foci of proliferated Kupffer cells may be detectable for another week or two, but progressive hepatic injury does not occur in the natural disease. Progressive hepatic injury does not seem to follow the acute phase of the natural disease, despite the fact that adenoviral antigen may be demonstrated immunohistochemically in Kupffer cells in dogs with various forms of chronic hepatitis, and chronic hepatitis has been produced by experimental adenoviral infection in dogs after certain immunological manipulations. This is consistent with failure to demonstrate virus in the liver after about day 10 of experimental infection. A review of 45 dogs with chronic hepatitis or cirrhosis failed to reveal CAdV-1 by either polymerase chain reaction or immunohistochemistry, although the possibility that the virus initiates hepatic damage by provoking self-perpetuating hepatitis could not be excluded. Focal interstitial nephritis occurs commonly, and the cellular infiltrates are persistent but not functionally significant. They consist of interstitial lymphocytic accumulations, especially about the corticomedullary junction and in the loose stroma of the pelvis.

Corneal edema is a late development (see Fig. 2.54A). It may occur by day 7 of infection, but is usually delayed to between 14 and 21 days. Viral antigen can be detected in these eyes by fluorescent techniques, but not in the corneal structures. Inflammatory edema is

present in iris, ciliary apparatus, and corneal propria, and inflammatory cells are abundant in the filtration angle and iris. The infiltrates are principally plasma cells, and there is evidence that the ocular lesion is a hypersensitivity reaction to viral antigen.

Originally it was assumed that the widespread tendency to hemorrhage in this disease was due to leakage from damaged vascular endothelium, coupled with an inability on the part of the damaged liver to replace clotting factors. While these effects play a role, it is now known that *the exhaustion of clotting factors is in large part due to their accelerated consumption, as the widespread endothelial damage is a potent initiator of the clotting cascade.*

Wesselsbron disease

This disease is caused by *Wesselsbron virus*, an arthropod-borne flavivirus that, according to serological surveys, is widespread in Africa in various species of animals and birds. Various *Aedes* mosquitoes are the vectors. Humans are also susceptible to clinical and inapparent infection. *The virus produces outbreaks of abortion and perinatal death in sheep.* Susceptible adults rarely show clinical signs but may have a biphasic febrile response to infection; other clinical signs, when present, are of hepatitis and jaundice.

The lesions in lambs dying within 12 hours of birth consist mainly of widespread petechiae and gastrointestinal hemorrhage; longer survival allows jaundice to develop, and the liver becomes orange-yellow, enlarged, friable, and patchily congested. The bile in the gallbladder becomes thick and dark in some cases, but this may be due more to hemorrhage into the gallbladder than to hemolysis. Lymph nodes are rather constantly enlarged, congested, and edematous.

The most characteristic histologic changes are seen in the liver. There are randomly scattered foci of necrosis, with apoptosis and proliferation of sinusoidal lining cells (Fig. 2.56). Mononuclear cells and pigment-filled macrophages accumulate in the portal stroma as well as in the sinusoids. In a variable proportion of cases, *hepatocyte nuclei may contain eosinophilic, irregular inclusions.* These are not accompanied by as much margination of nuclear chromatin as that associated with conventional viral inclusions, and their significance is obscure. Wesselsbron viral antigen can be demonstrated in necrotic acidophilic and degenerating hepatocytes and rarely in the inclusions. In jaundiced animals there may be considerable canalicular cholestasis; whether or not this is the result of hemolysis does not appear to have been determined. Hepatocellular proliferation is apparent in the less acute cases. Lymphoid follicles in lymph nodes and spleen show pronounced lymphocyte necrosis and stimulation of lymphoblasts.

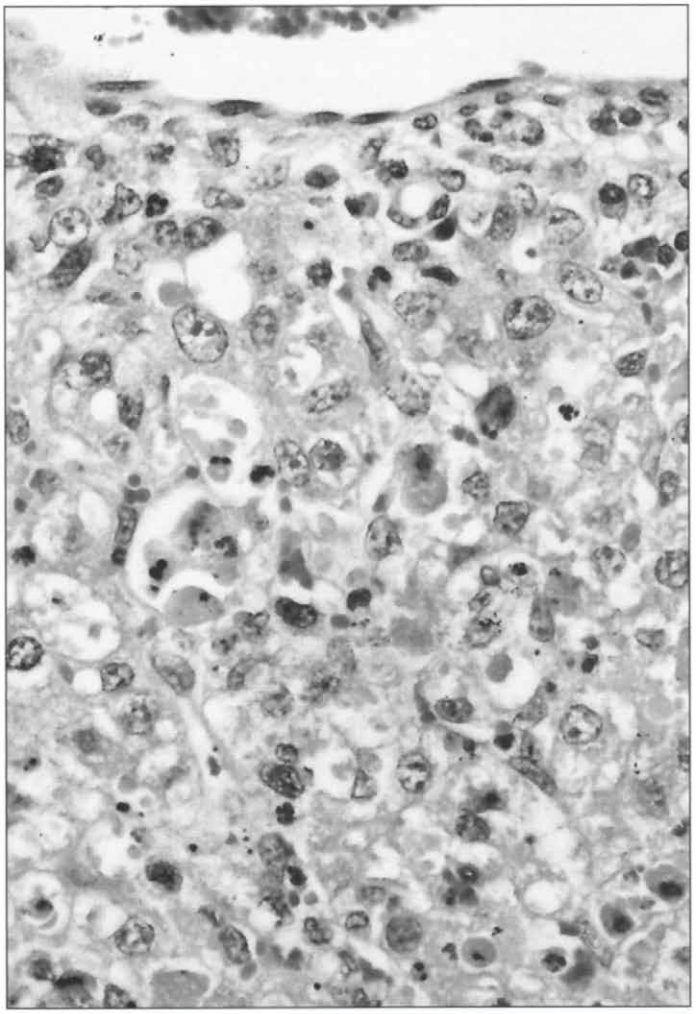

Figure 2.56 Focal necrosis and apoptosis in Wesselsbron disease in a lamb. (Courtesy of S Youssef.)

Rift Valley fever

This is an *arthropod-borne viral infection of ruminants and humans*, in many respects similar to Wesselsbron disease. *Rift Valley fever virus* (RVFV) is, however, responsible for greater losses than the former infection. Morbidity and mortality may occur in adult sheep; death sometimes occurs in adult cattle, but it is chiefly a disease of the young, causing *heavy mortality among lambs, kids, and calves and abortion in ewes, does, and cows.* The infection in enzootic form is widespread in eastern and southern Africa but it has, in plague-like proportions, extended to West Africa, Egypt, and the Arabian peninsula. RVFV is a member of the *Phlebovirus* genus, one of the five genera in the family Bunyaviridae. It is transmitted by many species of mosquito of the genera *Culex* and *Aedes,* in which transovarial passage can occur. Mosquitoes, once infected, remain so and in them the virus is not pathogenic. High levels of viremia occur in sheep and cattle and are maintained for up to 5 days. During epizootics, the virus may be spread by fomites, aerosols, and mechanically by other biting insects.

In endemic situations, the disease in adults is usually mild, but in epidemics in sheep and goats, severe illness occurs with fever, vomiting, mucopurulent nasal discharge, and dysentery. The mortality rate is then very high in lambs and up to 50% in adults. The disease in cattle is less severe but pregnant animals abort and the mortality rate in calves may reach 30%.

As in Wesselsbron disease, *the gross postmortem picture is dominated by widespread hemorrhage, ranging from serosal petechiae to severe gastrointestinal bleeding.* The liver in the acute cases in neonatal lambs is similar to that in cases of Wesselsbron disease, being yellow, swollen, soft, and patchily congested or hemorrhagic. In older animals and in less acute cases, however, the liver tends to be darker and show scattered pale foci of necrosis 1–2 mm in diameter. There may be fibrinous perihepatitis, edema of the gallbladder wall, and moderate, blood-tinged ascites. Experimental infection of calves produced encephalomyelitis in an animal that survived the initial viremic stage.

Within 12 hours of experimental infection of lambs, there are *randomly distributed foci of hepatocellular necrosis in the liver.* These foci include knots of inflammatory cells and prominent apoptotic bodies and initially involve about half a dozen hepatocytes (Fig. 2.57). Within a few hours, however, these primary foci enlarge and may become almost confluent. In the meantime, the remaining parenchyma may rapidly undergo necrosis that spares only a small rim of periportal hepatocytes. In naturally infected calves, the primary foci of necrosis undergo lysis more rapidly than the surrounding parenchyma; these foci thus have a striking "washed-out" appearance. Where the expanding foci of necrosis include portal triads, there may follow fibrinous vasculitis and thrombosis. Fibrin deposition in sinusoids is common, and so is mineralization of necrotic hepatocytes. Cholestasis is apparent in sections but is not a prominent feature.

Eosinophilic intranuclear inclusion bodies, often elongated, are sometimes seen in degenerate hepatocytes; there is associated nuclear vesiculation and chromatin margination. There is necrosis in germinal centers of lymphoid follicles in lymph nodes and spleen similar to that seen in Wesselsbron disease. Renal glomerular hypercellularity and necrosis have been described in the experimental disease. By immunohistochemistry, RVFV antigen can be detected in focal areas in cytoplasm of degenerating and necrotic hepatocytes but not in nonparenchymal cells.

Ultrastructural studies have shown condensation of degenerate hepatocytes, abundant apoptosis, and the presence of mummified, membrane-bound fragments of hepatocellular cytoplasm, but sinusoidal lining cells are not notably damaged; the Kupffer cells instead participate in the uptake of the dying hepatocytes. There is abundant fibrin in sinusoids in the vicinity of the primary foci of necrosis and also within hepatocytes and macrophages. *The intranuclear inclusions are composed not of recognizable viral particles but of filaments.* Virus is occasionally discernible in the cytoplasm, associated with tubular membranes.

It seems that the primary infection of the liver produces the primary necrotic foci, from which more virus spreads to damage neighboring parenchyma; the virus clearly has a marked preference for hepatocytes over other tissues. The hemorrhagic component of the syndrome is probably related to consumption of clotting factors; there is no direct evidence for the endothelial damage seen in infectious canine hepatitis.

There has been some confusion in early reports from Africa of liver diseases of ruminants. Some cases of phyto- and mycotoxicosis and of chronic copper poisoning have probably been ascribed to viral infection, and vice versa. It is also understandable that Rift Valley fever and Wesselsbron disease may have been mistaken for one another, but these two diseases may be distinguished by careful pathologic examination as well as by immunodetection or isolation of the viruses. *In summary, Rift Valley fever is characterized by obvious focal hepatic necrosis, on which is superimposed an almost massive periacinar and midzonal necrosis; cholestasis is not as prominent as it is in Wesselsbron disease. The liver lesions in the latter disease consist of smaller, randomly distributed foci of hepatocellular necrosis, more active reaction by the sinusoidal lining cells, and more obvious cholestasis.*

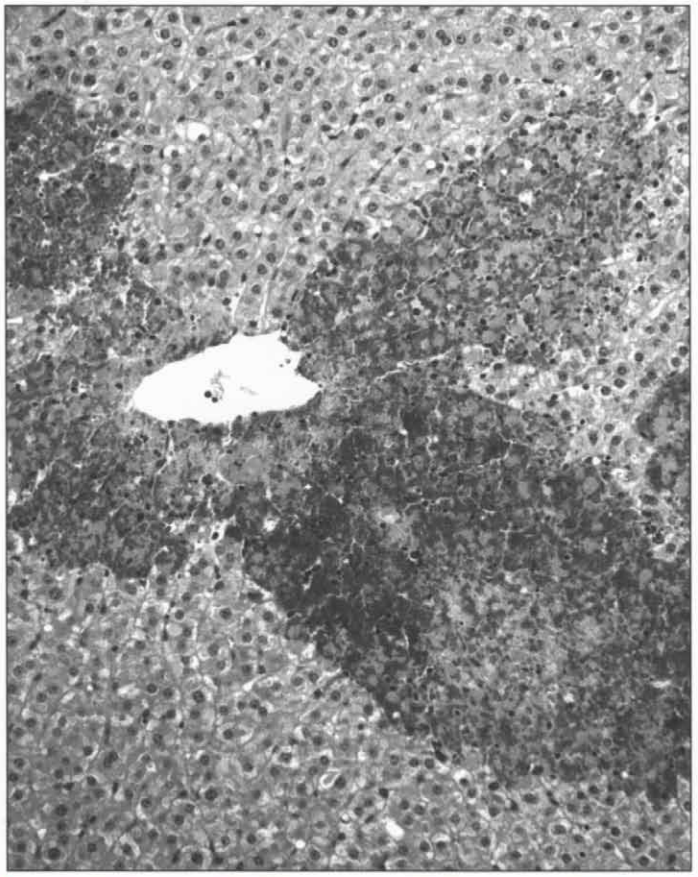

Figure 2.57 Focal necrosis with hemorrhage in Rift Valley fever in a lamb. (Courtesy of S Youssef.)

Canine acidophil-cell hepatitis

This is a transmissible hepatitis of dogs described only in the UK and Ireland. It has been transmitted experimentally to normal dogs and rats by parenteral injections of liver and serum from affected animals. The naturally occurring disease takes the form of *chronic progressive liver damage with eventual liver failure*, attended clinically by ascites, signs of hepatic encephalopathy, and causing shrinkage and nodular distortion of the liver. *The most characteristic histologic feature of affected livers is the presence of shrunken, acidophilic hepatocytes that are concentrated initially in the limiting plates adjacent to the portal triads.* There is little or no inflammation associated with affected cells, but portal triads eventually become linked by fibrous trabeculae. Experimentally infected dogs usually remain clinically normal, but regular serum sampling reveals intermittent elevations in alanine aminotransferase levels. The characteristic acidophilic hepatocytes tend not to be so obvious in some experimentally transmitted cases, which raises the possibility that this virus may be involved in otherwise unexplained cases of chronic canine liver disease.

No infectious agent has been isolated from the original cases of canine acidophil cell hepatitis. Recent efforts to reproduce the condition by transmission of material from various spontaneous cases of canine hepatitis have had limited success. Transient hepatitis has been observed in one recipient. However, no agent has been found and polymerase chain reaction screening of livers has been negative for hepadnaviruses, *Canine adenovirus 1, Hepatitis E virus, Hepatitis C virus*, and *Canine parvovirus*.

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Bacterial infections

Bacterial hepatitis is common, but, with a few important exceptions, is usually focally distributed and of little clinical significance. Bacteria may gain entrance to the liver in various ways: by direct implantation, for example by foreign-body penetration from the reticulum; by invasion of the capsule from an adjacent focus of suppurative peritonitis; hematogenously via the hepatic artery or portal and umbilical veins; or via the bile ducts.

Excepting peracute septicemias, *there are few specific bacterial infections that have a sustained or repeated bacteremic phase without producing hepatic lesions*. There are, in addition, many cases of nonspecific bacteremia, especially portal in origin, in which focal hepatitis occurs. Because their differential diagnosis is of some importance, it is probably useful to list here those specific bacterial diseases in which focal hepatitis is expected or characteristic, but not constant. The specific infections may occur as fetal or perinatal infections. The list includes *Listeria monocytogenes* in fetal and neonatal lambs, calves, foals, and piglets; *Campylobacter fetus* in fetal and neonatal lambs; *Actinobacillus equuli* in foals; *A. suis* in pigs; *Yersinia pseudotuberculosis* in lambs and occasionally in dogs and cats; *Francisella* (*Yersinia*) *tularensis* in lambs and cats; *Mannheimia haemolytica* and *Histophilus somni* (*Haemophilus agni*) in lambs; *Salmonella* spp. in all hosts (see Fig. 2.25), *Clostridium piliforme* (Tyzzer's disease) in foals and dogs; *Nocardia asteroides* in dogs; and the mycobacteria in all hosts.

Hepatic abscess

Hepatic abscesses, quite apart from the lesions of the specific infections just given, *are common*, especially in cattle. They may arise by direct implantation of a foreign body from the reticulum or by direct invasion of the capsule from a suppurative lesion of traumatic reticulitis and may be single or multiple, but in either case they are often preferentially distributed to the left lobe. They may be hematogenous from portal emboli, or by direct extension of omphalophlebitis. Arteriogenic abscesses via the hepatic artery may occur in pyemias but are quite uncommon.

Omphalogenic abscesses are more common in calves than in other species but occur in all. The bacterial flora is frequently mixed, but *Arcanobacterium* (formerly *Actinomyces* or *Corynebacterium*) *pyogenes*, *Fusobacterium necrophorum*, streptococci, and staphylococci usually predominate. Hepatic abscesses are not inevitable sequelae to omphalitis or even to omphalophlebitis, but they do not develop from navel infections in the absence of omphalophlebitis. As there is no flow of blood in these vessels after birth, involvement of the liver is by direct growth along the physiologic thrombus. Omphalophlebitis can be quite severe without extension to the liver. Hepatic abscesses of omphalogenic origin are often restricted to the left lobe (Fig. 2.58), but they may be restricted to the right or be diffuse in their distribution.

Hepatic abscesses are also common and of much economic importance in feedlot cattle. They are usually found at slaughter but, when numerous, may be fatal after a few days of vague digestive illness. Their pathogenesis and character are discussed with rumenitis, to which they are a sequel (see Vol. 2, Alimentary system). Liver abscesses in feedlot sheep likely have a similar pathogenesis, with *F. necrophorum* as the primary isolate. A second category includes parasitic granulomas populated by various opportunistic bacteria.

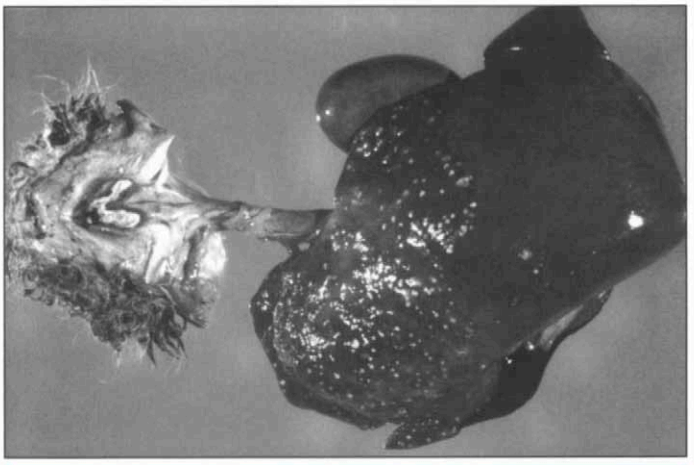

Figure 2.58 Omphalophlebitis with **miliary metastatic abscesses** in the left lobe of the liver in a calf.

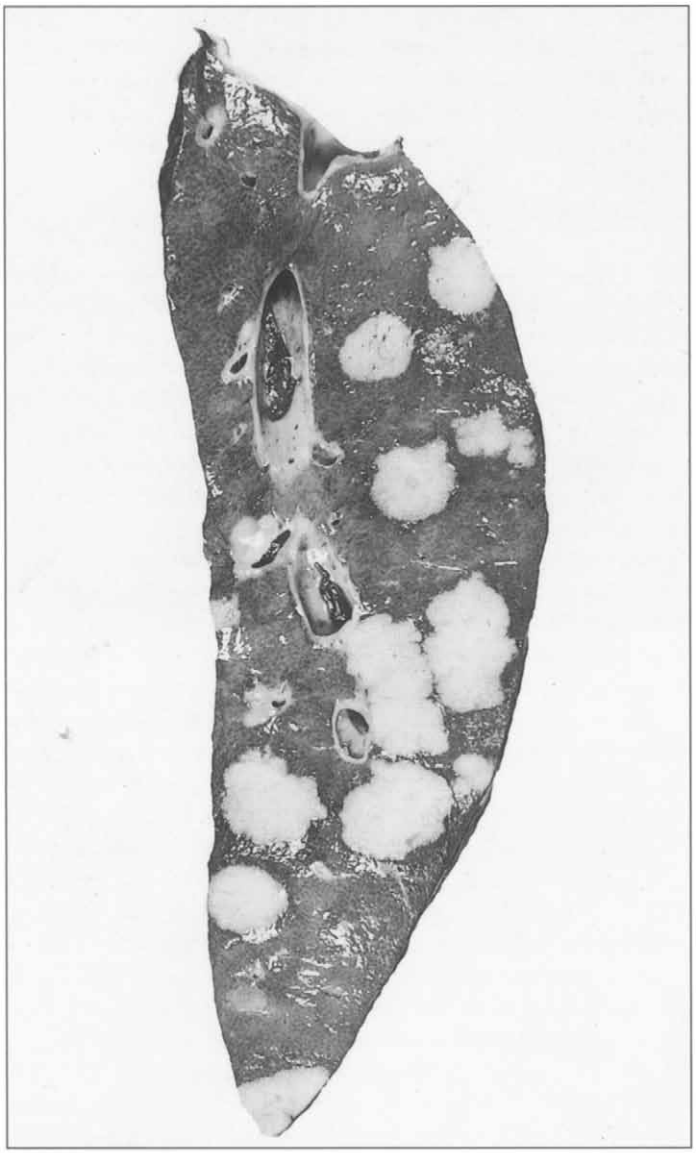

Figure 2.59 Early **hepatic necrobacillosis** in an ox (*Fusobacterium necrophorum* infection). Pale areas of coagulative necrosis are bordered by acute inflammation.

Hepatic abscesses of biliary origin occur in all animals. They are perhaps most frequent in pigs in which ascarids have migrated into the bile ducts. Cholangitic abscesses in horses, dogs, and cats are usually caused by enterobacteria as part of a fulminating ascending cholangiohepatitis that is fatal after a short course.

The *sequelae* of hepatic abscessation are variable. Usually, they are insignificant and asymptomatic. Sterilization of the focus with either resorption and complete healing or encapsulation is common. Those near the surface of the liver regularly produce fibrinous and then fibrous inflammation of the capsule and adhesion to adjacent viscera. They seldom perforate the capsule but do commonly break into hepatic veins to produce any one or a combination of thrombophlebitis of the vena cava, endocarditis, or pulmonary abscesses or embolism. Acute extension of a hepatic abscess into the major hepatic vein can lead to pulmonary embolism that can be acutely fatal. In adults, death may occur if the hepatic abscesses are multiple and fresh, and especially if they are necrobacillary in origin; death is probably the result of toxemia. Generalization is common, especially from omphalogenic abscesses of young animals.

Hepatic necrobacillosis

Occasionally, *F. necrophorum* infection of the liver is observed following *omphalophlebitis in lambs and calves*, or as a complication of *rumenitis in adult cattle*. In feedlot cattle, both *F. necrophorum* subsp. *necrophorum* (biotype A) and subsp. *funduliforme* (biotype B) have been isolated. The hepatic lesions are multiple and typical of necrobacillary infection, being slightly elevated, rounded, dry areas of coagulative necrosis, sometimes a few centimeters in diameter (Fig. 2.59) and surrounded by a zone of intense hyperemia. Affected neonates seldom live long enough for the necrotic foci to liquefy and assume the appearance of ordinary abscesses, but this may be seen in adult cattle. *The histologic appearance of the foci in the stage of coagulative necrosis is quite characteristic.* The necrotic amorphous central area is bordered by a zone of wholesale destruction of leukocytes, whose nuclear chromatin is dissipated in a finely divided form, and among which the filamentous fusobacteria are mostly concentrated. Outside this zone there is severe hyperemia and hemorrhage, and thrombosis of local vessels is common. The lesion in neonatal lambs is to be distinguished from that caused by *Campylobacter fetus*.

The pathogenic mechanisms of *F. necrophorum* involve various toxins, especially a high-molecular-weight leukotoxin specifically toxic to ruminant neutrophils. This unique toxin activates neutrophils and induces their apoptosis, consistent with the remarkable abscess-inducing propensity of *F. necrophorum* in ruminants. However, other toxins, including collagenolytic activity, are also implicated as virulence factors. Mixed infections are frequent, and synergism between *F. necrophorum* and other pathogens may also play a role in the pathogenesis of liver necrosis and abscessation.

Necrotic hepatitis (black disease)

Organisms of the genus *Clostridium* are notably circuitous in their means of producing disease. This is true of *C. novyi*; *type B strains produce potent exotoxins and are the cause of black disease (infectious necrotic hepatitis)*. The alpha toxin of *C. novyi* belongs with the large clostridial cytotoxins produced by *C. difficile* and *C. sordelli*. These toxins enter cells by receptor-mediated endocytosis and inhibit ras and rho guanosine triphosphatases by glycosylation. The beta toxin is a necrotizing and hemolytic phospholipase C (lecithinase). Black disease occurs in nonimmune animals when these exotoxins are released by *C. novyi* within an anaerobic focus in the liver. These anaerobic sites, which provide a suitable environment for germination of *C. novyi* spores, are most commonly a result of migrating helminths.

C. novyi is widely distributed in soil, and the spores are continually ingested by grazing animals in areas where black disease occurs. Some spores cross the mucous membranes, probably in phagocytes, and remain as latent infections in histiocytic cells, mainly in the liver, spleen, and bone marrow. The duration of latency in tissue is not known, but it can be many months. In endemic areas, many healthy sheep, cattle, and dogs harbor latent infections in their livers. *Black disease is principally an acutely fatal disease of sheep in regions where the inciting helminths are endemic.* The disease is most commonly initiated by migrating larvae of the common liver fluke *Fasciola hepatica*.

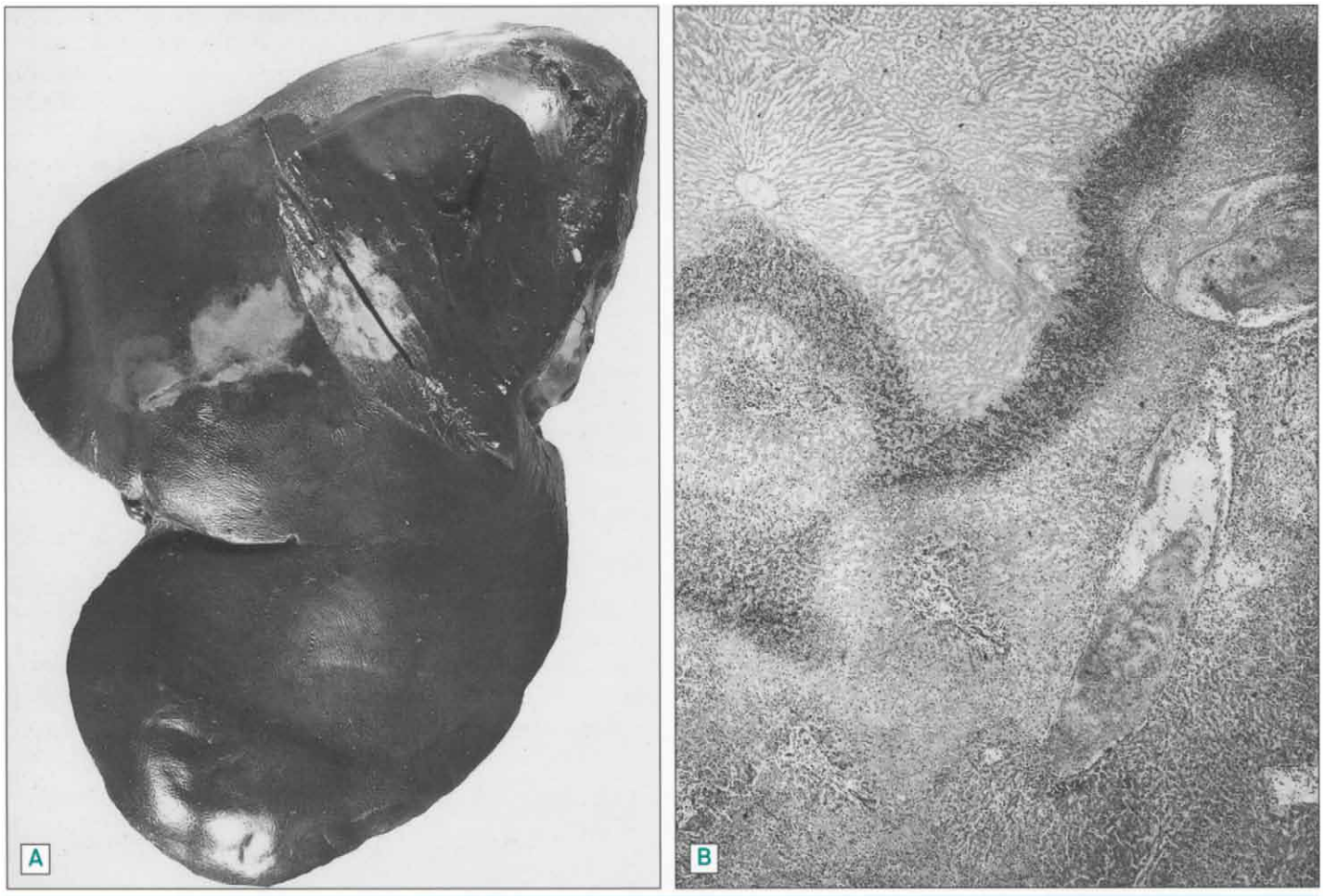

Figure 2.60 A. Infectious necrotic hepatitis ("black disease") in a sheep. Note irregular pale area of necrosis in right lobe. **B.** Section of (**A**). Vascular thrombosis and an area of **coagulative necrosis** isolated by a zone of acute inflammatory infiltrate.

In Bessarabia and France, it is endemically related to the distribution of *Dicrocoelium dendriticum*, the lancet liver fluke. Sporadic cases may be related to *Cysticercus tenuicollis* infection or may be idiopathic.

Deaths in sheep from black disease occur rapidly and usually without warning signs. Illness, if observed, is brief and characterized by reluctance to move, drowsiness, rapid respiration, and quiet subsidence. Affected animals are usually in good nutritional condition. Postmortem decomposition occurs rapidly. *The name of the disease is derived from the appearance of flayed skins, the dark coloration being caused by an unusual degree of subcutaneous venous congestion.* Frequently there is edema of the sternal subcutis, and airways contain stable foam. Serous cavities contain an abundance of fluid that clots on exposure to air. The fluid is usually straw-colored, but that in the abdomen may be tinged with blood. The volume of fluid in the abdomen and thorax may vary from about 50 mL to 1.5 L. The pericardial sac is distended with similar fluid in amounts up to ~300 mL. Subendocardial hemorrhages in the left ventricle are almost constant. Patchy areas of congestion and hemorrhage may be present in the pyloric part of the abomasum and in the small intestine.

The typical and diagnostic lesions occur in the liver and are always present. They are usually clearly evident on the capsular surface, the diaphragmatic surface especially, but the organ may have to be sliced carefully to find them. The liver will be the seat of either the acute traumatic hemorrhagic lesions of acute fascioliasis, or the cholangiohepatitis of the chronic disease, or both. *The lesion of black disease, and occasionally there are several, is a yellow-white area of necrosis 2–3 cm in diameter, surrounded by a broad zone of intense hyperemia, roughly circular in outline, and extending hemispherically into the substance of the organ* (Fig. 2.60). There may be a coagulum of fibrin on the capsular surface overlying the necrotic area. Occasionally, the essential lesions are rectilinear in shape or very irregular. The lesions appear homogeneous on the cut surface, but some contain poorly defined centers of soft or cheesy material.

The histologic evolution of the hepatic lesions begins with the necrotic and hemorrhagic tracts caused by wandering immature flukes. These are sinuous tunnels ~0.5 cm in diameter that contain blood, necrotic hepatic cells, and the leukocytes, chiefly eosinophils, attracted by the flukes. About the tunnels is a narrow zone of coagulative necrosis, also produced by the flukes. As usual, the necrotic tissue is demarcated by a thin zone of scavenger cells, chiefly neutrophils. If latent spores are present in the necrotic areas, they quickly vegetate and are visible in sections as large, gram-positive bacilli. In nonimmune animals, the vegetative organisms elaborate exotoxins that cause necrosis of the surrounding tissue, including the eosinophils of the fluke tunnel. As the area of necrosis expands, the bacterial proliferation keeps pace so that bacilli can be found in all parts of

the necrotic focus but not in the surrounding viable tissue. *Usually bacteria are concentrated at the advancing margin of the lesion,* just inside a zone of infiltrated neutrophils. At about the time of death and immediately afterward, the bacilli scatter in the liver and to other organs.

Bacillary hemoglobinuria

Bacillary hemoglobinuria is a counterpart of black disease. The cause is *Clostridium haemolyticum*, which is closely related to *C. novyi*. Both species produce the beta toxin, a necrotizing and hemolytic lecithinase (phospholipase C). The pathogenesis of the two diseases is comparable, as both depend on a focus of hepatic injury within which latent spores can germinate. *Bacillary hemoglobinuria as an endemic disease exists only in areas where Fasciola hepatica abounds, and it is probable that flukes are the primary cause of the initiating lesion.* The disease does occur sporadically where there are no flukes and may be prompted by other parasites or other diverse focal lesions, which are smudged out in the expanding areas of necrosis. There is scant information on the ecology of the organism, but it is clear that it has its own environmental requirements, and the disease will not persist in areas where these requirements are not met. The spores will remain in the livers of cattle for several months after removal from pastures where the disease is endemic. Spores may persist in the bones of cadavers for 2 years. Spores of this and other sporulating anaerobes can frequently be demonstrated in the liver, where they are probably retained in Kupffer cells.

Bacillary hemoglobinuria occurs in *cattle and sheep*. It is characterized clinically by *intravascular hemolysis with anemia and hemoglobinuria*, but, perhaps reflecting variety in exotoxins between strains of the organism, hemolysis may not be a feature. The essential lesion is hepatic and similar to that of black disease but is much larger and usually single. It has been described as an infarct secondary to portal thrombosis, and although this may occur in isolated cases, it is scarcely a creditable pathogenesis for a disease of endemic occurrence. Thrombosis does occur in the affected areas but can be a result rather than a cause of the initial lesion and is found more frequently in the hepatic venules than in branches of the portal vein. There is severe anemia, the kidneys are speckled red or brown by hemoglobin, and the urine is of port-wine color. Peritoneal vessels are injected, and in some cases there is severe, dry, fibrinohemorrhagic peritonitis.

Clostridium piliforme infection

Clostridium piliforme (formerly *Bacillus piliformis*) infection has been known for a long time as *Tyzzer's disease*, a cause of severe losses in laboratory rodents; however, it has also been reported in *foals, calves, dogs, and cats*. Although the disease is probably initiated by an intestinal infection, lesions in the gut are less specific and constant than those in the liver, which consist of *focal hepatitis and necrosis.*

Affected foals usually die between the ages of 1 and 4 weeks; often they are found dead after a short illness. The liver shows pale foci up to a few millimeters across; these are represented microscopically by randomly distributed foci of coagulative necrosis with moderate neutrophilic infiltrate. This lesion is not diagnostic in itself; its specificity depends on the presence of the causal organism in hepatocytes in the periphery of the necrotic zones. At present, *C. piliforme* can only be isolated with difficulty on artificial media, so diagnosis is usually based on demonstration of the large, long bacilli in the cytoplasm of degenerate and also otherwise apparently normal hepatocytes at the periphery of the necrotic zones. The organisms are gram-negative and are best delineated with silver impregnation techniques such as that of Warthin–Starry, but they may be seen with routine stains such as Giemsa, particularly when the material is fresh. Differentiation from other organisms including postmortem saprophytes can be achieved by immunohistochemistry or immunofluorescence. *The bacilli tend to lie in sheaves or bundles* (Fig. 2.61). There may also be colitis sufficiently severe to cause diarrhea, but not as severe as that seen in rabbits with this disease.

Only a few cases of Tyzzer's disease have been reported in dogs and cats. The liver lesions are essentially the same as those in foals and rodents, and there is also enteritis or enterocolitis. Immunodeficiency predisposes to the disease because it occurs sporadically in dogs that have undergone immunosuppressive or anticancer therapy. Such cases may be complicated by concurrent viral, mycotic, or protozoal infections.

Leptospirosis

Leptospirosis is associated with acute jaundice and cholestatic hepatic disease and renal failure in dogs, and is discussed in more detail in Vol. 2, Urinary system. The hepatic lesions described following acute experimental infection of dogs with *Leptospira kirschneri* serovar *grippotyphosa* include mixed perivascular periportal infiltrates of neutrophils,

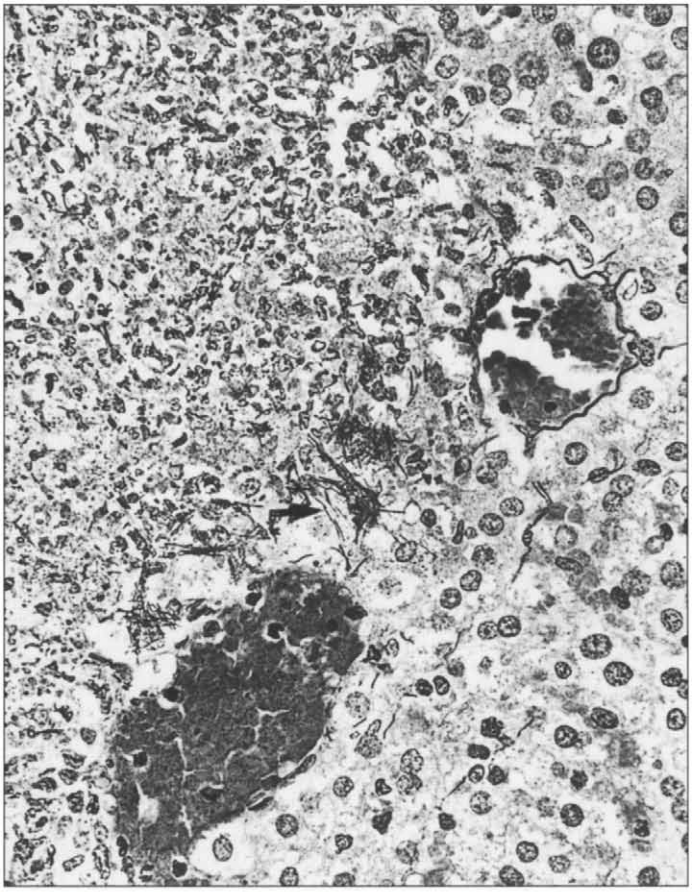

Figure 2.61 Focal hepatitis in *Clostridium piliforme* (*Bacillus piliformis*) infection (**Tyzzer's disease**). Bacilli in bundles in hepatocytes at margin of lesion (arrow), with Warthin–Starry stain.

lymphocytes, and plasma cells, with mild hepatic lipidosis, dissociation of hepatocytes and intracanalicular bile plugs evident by day 12 postinfection, along with increased hepatocellular mitotic activity. *Clinical icterus has been attributed to cholestasis due to dissociation of hepatocytes*. In a retrospective study of dogs with supportive clinical signs and microscopic agglutination test titers of ⩾320 for one or more serovars tested including *autumnalis*, *bratislava*, *canicola*, *grippotyphosa*, *icterohaemorrhagiae*, and *pomona*, histologic lesions in the liver were subtle, with sinusoidal neutrophil margination, Kupffer cell hypertrophy, and low levels of hepatocellular single-cell necrosis and mitoses. Some livers had diffuse interstitial lymphocytic hepatitis, with mitotic figures, anisokaryosis, binucleation, and some degree of lobular collapse. Chronic hepatitis has also been experimentally produced by leptospiral infection in dogs; however, clinical cases are rarely documented. Sixteen juvenile Beagle dogs from a single breeding colony vaccinated for *L. interrogans* serogroups *canicola* and *icterohaemorrhagiae* developed chronic hepatitis. Leptospires were identified within bile canaliculi by special stains, electron microscopy, and immunohistochemical techniques. Significant antibody titers were not identified in 6 dogs from which leptospires were isolated. However, serological survey of kennelmates demonstrated high titers to serogroup *australis*.

Other bacteria

A single case of clinical disease associated with ***Helicobacter canis*** has been reported in a 2-month-old puppy with peracute disease causing weakness and vomiting prior to death. Multiple coalescing yellow foci in the liver up to 1.5 cm in diameter consisted of hepatocellular coagulative necrosis with infiltrating mononuclear cells and neutrophils. Spiral bacteria were visualized by Warthin–Starry silver stains in area of necrosis, within bile canaliculi and occasionally in bile duct lumens. This organism has previously been identified in the blood of diarrheic children, and in the feces of 4% of dogs in an epidemiologic study examining the incidence of *Campylobacter*-like organisms in 1000 dogs.

Bartonella spp. infection has been associated with a wide variety of granulomatous syndromes and peliosis hepatis in humans, often in association with immunosuppression; however, the extent to which infection induces disease in dogs is currently unknown. *Bartonella henselae* has been identified in liver tissue by polymerase chain reaction from a single case of peliosis hepatis in a Golden Retriever and granulomatous hepatitis in a Basset Hound, while *B. clarridgeiae* DNA was identified in liver tissue from a Doberman Pinscher with histologic lesions of Doberman hepatopathy.

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Helminthic infections

Various helminths, cestodes, nematodes, trematodes, and even the degenerate arachnid *Linguatula serrata* produce inflammation of liver and bile ducts. Those parasites that have the biliary system as their final habitat will be discussed in detail below. The others produce hepatic lesions in the course of their natural or accidental migrations, and are discussed under the organ (for most of them, the gut) that is their final habitat. It is useful however to describe briefly here the lesions produced by larvae in transit.

The initial lesion produced by wandering larvae is traumatic. Sinuous tunnels permeate the parenchyma and often breach the capsule. In the tunnels there are free red cells, degenerating hepatocytes, and leukocytes, chiefly eosinophils, which react to the parasites. Bordering the tunnel is a narrow zone of coagulative necrosis of parenchyma with infiltrated neutrophils at its margin. Eosinophils also infiltrate the portal triads. The necrotic parasitic tracts heal by scarification, and the fibroblastic tissue, infiltrated with eosinophils, is eventually incorporated into the portal units (interstitial hepatitis). Most larvae escape from the liver but some eventually become encapsulated in the liver in abscesses containing numerous eosinophils. The abscesses may caseate and come to resemble tubercles, and eventually many are heavily mineralized to form permanent pearly nodules. In sheep, the most common cause of this type of hepatitis (aside from liver fluke) is ***Cysticercus tenuicollis*** in its wandering phase. Lambs may die of severe hemorrhagic hepatitis (Fig. 2.62) caused by very heavy infections of this parasite, and in pigs, an aberrant host, *C. tenuicollis*, can produce a very intense inflammatory reaction.

In pigs, larvae of ***Ascaris suum*** and ***Stephanurus dentatus*** produce similar but distinct patterns of focal interstitial hepatitis. The ascarids produce their distinctive accentuation of the stroma ("*milk spots*") (Fig. 2.63) when quite small larvae are immobilized by the host's inflammatory reaction; thus the foci are relatively small. The fibrotic lesion produced by *S. dentatus* larvae, on the other hand, is less focal and more in the nature of a track, and there are usually small, inflamed, capsular craters where the larvae have emerged from the liver to migrate to their preferred perirenal site. There will be obvious portal phlebitis at the hepatic hilus when infection by *S. dentatus*

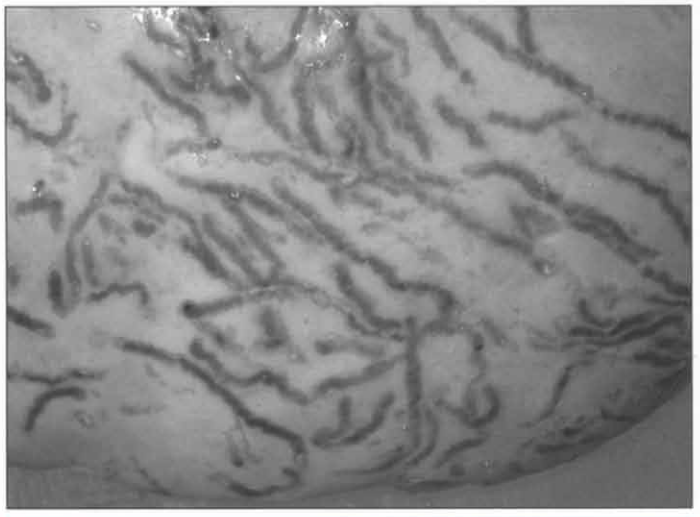

Figure 2.62 Hemorrhagic subcapsular migration tracks caused by *Cysticercus tenuicollis* in a sheep.

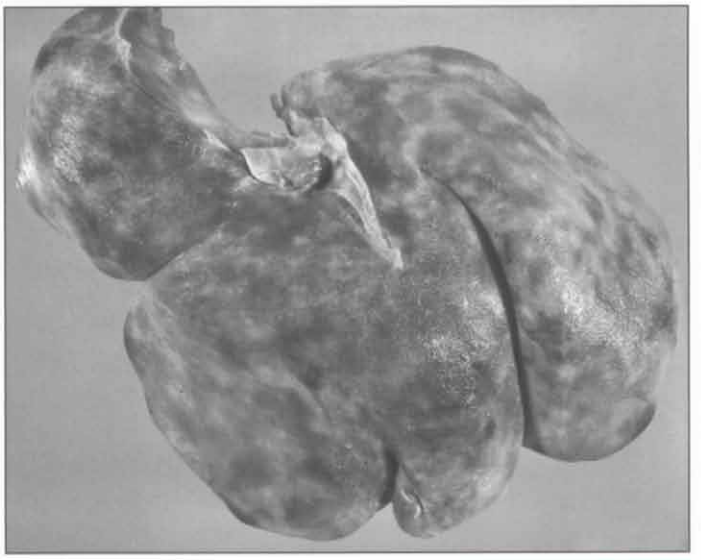

Figure 2.63 Multifocal interstitial hepatitis ("milk-spot liver") caused by *Ascaris suum* migration in a pig.

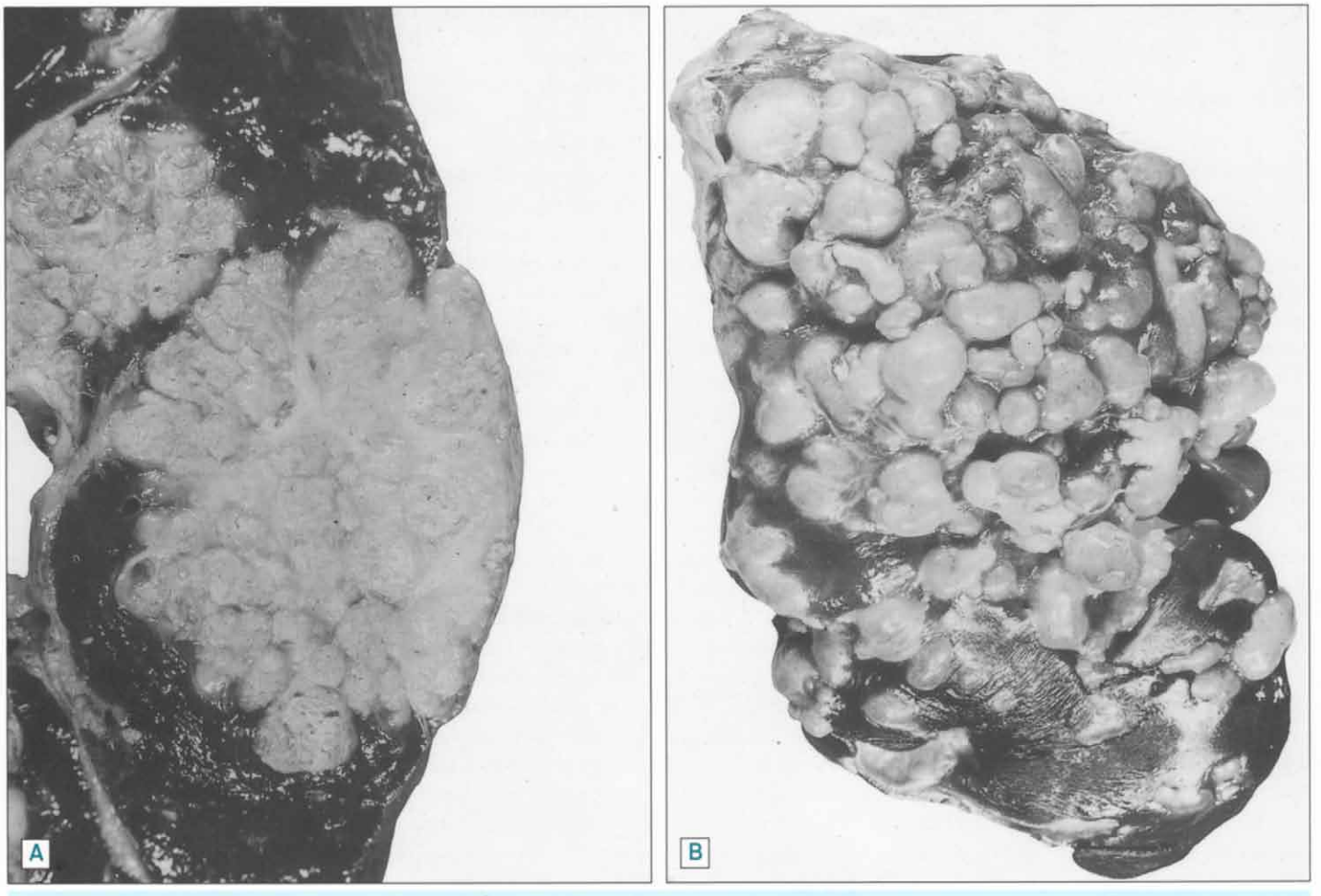

Figure 2.64 A. Degenerate *Echinococcus granulosus* resulting in mineralized membranous debris and fibrosis in an ox. **B.** Severe **hydatid liver disease** (*Echinococcus granulosus* infestation) in a sheep.

has been by the oral route, and in these livers the parenchymal lesion is more severe in this vicinity.

Migration tracks left by *larval strongyles* are common under the liver capsule in young horses and are probably related to the dense, discrete fibrous tags that are almost universally found on the diaphragmatic surface of the liver of mature horses. The range of strongyle species capable of causing these lesions has not been defined. There are other and more devious means by which parasites produce hepatic

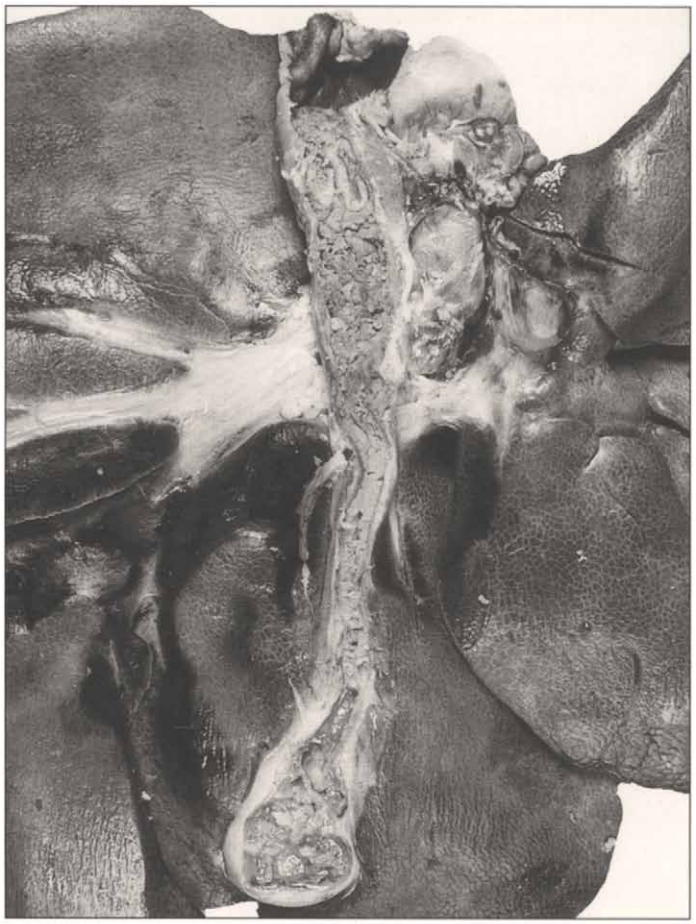

Figure 2.65 Cholangitis and cholecystitis secondary to *Ascaris suum* invasion in a pig.

lesions; the hydatid intermediate stages of *Echinococcus* encyst in the liver and may destroy much of it (Fig. 2.64); the larvae of *Ascaris suum* in cattle add to the usual insult by causing portal phlebitis and small areas of infarction; the adults of *Ascaris* in all species, but especially in pigs, may migrate into the bile ducts (Fig. 2.65); and the eggs of schistosomes enter in the portal blood to lodge in the intrahepatic portal vessels and provoke granulomatous inflammation.

Cestodes

Stilesia hepatica and ***Thysanosoma actinioides***, the "fringed tapeworm," are the only cestodes that inhabit the bile ducts. They are parasites of ruminants, *Stilesia* occurring in Africa, *Thysanosoma* in North and South America. The life cycles of the parasites are not completely known but probably involve oribatid mites as intermediate hosts. *T. actinioides* may also be found in the pancreatic ducts and small intestine. Usually, the infestations are light but, even when heavy, are not of much significance. Very heavy infestations by *S. hepatica* occur without signs of illness, though the bile ducts may be nearly occluded, slightly thickened, and dilated. Saccular dilations of the ducts may occur and be filled with worms. The fringed tapeworm is perhaps more pathogenic, and unthriftiness may accompany heavy infestations.

Echinococcus granulosus hydatid cysts occur most commonly in the liver of ruminants in endemic areas but have been reported as incidental necropsy findings in the liver of horses. The cysticerci and hydatids, which in the intermediate stages invade the liver, are discussed further in Vol. 2, Alimentary system.

Nematodes

Calodium hepaticum (Capillaria hepatica) is the one nematode that in the adult phase inhabits the liver. It is a slender worm, morphologically resembling the whipworms, and it lives in the parenchyma rather than in the bile ducts. The usual hosts of the adult stage are rodents, but sporadic infestations are observed in dogs. These worms are *not highly pathogenic*. The adults provoke some traumatic hepatitis, and the eggs, which are deposited in clusters, provoke the development of localized granulomas. The eggs are readily recognized by their ovoid shape and polar caps. The granulomas can be seen through the capsule or in the substance of the liver as yellow streaks or patches. The eggs cannot escape from the liver unless a predator eats them. Predators, however, act only as transport hosts, and the ingested eggs are passed in the feces. Larvae develop in the eggs only in the external environment, and the cycle is completed when a suitable host eats the mature larvae in the eggs.

Trematodes

Various trematodes (flukes) are parasitic in the livers of animals. They belong to the families Fasciolidae (*Fasciola hepatica*, *F. gigantica*, *Fascioloides magna*), Dicrocoeliidae (*Dicrocoelium dendriticum*, *D. hospes*, *Platynosomum concinnum*), and Opisthorchiidae (*Opisthorchis tenuicollis*, *O. sinensis*, *Pseudamphistomum truncatum*). The diseases produced are known collectively as **distomiasis**.

Fasciola hepatica, *the common liver fluke of sheep and cattle, is the most widespread and important of the group.* Patent infestations can develop in other wild and domestic animals and in humans. These flukes are leaf-shaped and ~2.5 cm long in sheep and slightly larger in cattle. *They are found in the bile ducts.* Being hermaphroditic, only one fluke is necessary to establish a patent infestation, and each adult may produce 20 000 eggs per day. The longevity of the adult flukes is amazing and is potentially as great as or greater than that of the host; they have been known to survive for 11 years, and it seems that they can produce eggs all this time. The eggs are eliminated in bile, and on pasture, in conditions of suitable warmth and moistness, hatch a larva (miracidium) in ~9 days. If the environmental temperature is low, the incubation period may be delayed for some months. The miracidium can survive only in moisture. It is actively motile and penetrates the tissues of the intermediate host, which is an aquatic snail. Different snails serve this purpose in different countries, but all of them belong to the genus *Lymnaea*.

Each *miracidium*, on penetrating a snail, develops into a mother *sporocyst* that reproduces, probably parthenogenetically, giving rise to a small number of the second generation, the *redia*. Each redia gives birth to either redia or *cercariae*, or to the two successively. Cercariae, the larval stage of the third (sexual) generation, first appear 1–2 months after the miracidium penetrates. Cercariae continue to escape daily for the life of the snail, but even so, total cercarial production is only 500–1000. They actively escape from the snail and are attracted to green plants, where they encyst and become infective metacercariae in 1 day. These can remain infective for 1 month in summer

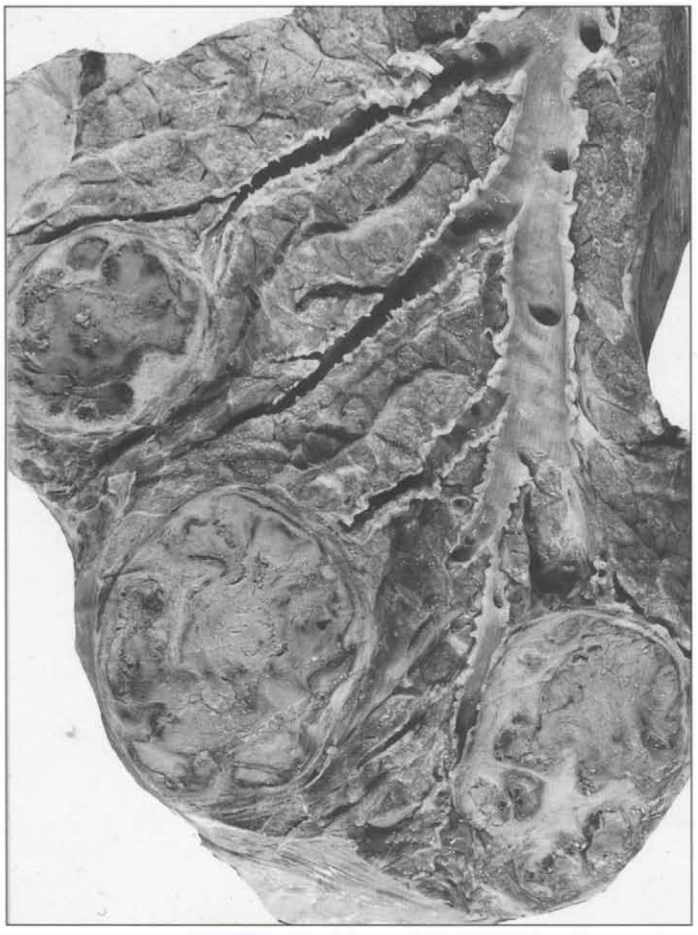

Figure 2.66 Three **caseous abscesses** at the ends of bronchi due to aberrant migration of flukes in an ox.

and up to 3 months in winter. The developmental events from egg to this stage take 1–2 months under favorable conditions.

Infestation occurs by ingestion. Excystment occurs in the duodenum. The young flukes penetrate the intestinal wall and cross the peritoneal cavity, attaching here and there to suck blood and penetrate the liver through its capsule; a few no doubt pass in the portal vessels or migrate up the bile duct. They wander in the liver for a month or more before settling down in the bile ducts to mature, which they do in 2–3 months. Some may, by accident, enter the hepatic veins and systemic circulation to lodge in unusual sites; intrauterine infestations are on record. *Lesions caused by aberrant flukes are quite common in bovine lung.* They consist of resilient nodules just under the pleura of the peripheral parts of the lung. They range in diameter from ~1 to many centimeters and consist of thinly encapsulated abscesses situated at the ends of bronchi (Fig. 2.66). The content is slightly mucoid, unevenly coagulated brown fluid; in some lesions the reaction is predominantly caseous. The location of the lesion suggests that it begins as a peripheral bronchiectasis that later becomes sealed off. The fluke persists in the debris, but is small and hard to find.

The essential lesions produced by *F. hepatica* occur in the liver and may be described, first, as those produced by the migratory larvae, and second as those produced by the mature flukes in the bile ducts; the two often intermingle. There is the further incidence of peritonitis, which is produced by the young flukes on their way to the liver and, perhaps also, by some that break out through the capsule.

Usually there is no obvious reaction to the passage of young flukes through the intestinal wall and across the peritoneal cavity, except for small hemorrhagic foci on the peritoneum, where the flukes have been temporarily attached. Few or many parasites may be found in any ascitic fluid and attached to the peritoneum of the diaphragm and the mesenteries. When the infestations are heavy and repeated, such as may be observed in sheep, cattle, and swine, *peritonitis* occurs. The young flukes at this stage are <1.0 mm long. The peritonitis may be acute and exudative or chronic and proliferative. It is usually concentrated on the hepatic capsule (Fig. 2.67), especially its visceral surface, but may be restricted to the parietal peritoneum or to the visceral peritoneum, including the mesenteries of the gut. In acute cases, there are fibrinohemorrhagic deposits on the serous surfaces, and in chronic cases there may be fibrous tags, with adhesions or a more or less diffuse thickening by connective tissue. Many young flukes can be found microscopically in the fibrinous deposits, and in the diffuse peritoneal thickenings there are tortuous migration tunnels containing blood, debris, and the young parasites (Fig. 2.68). In cases with involvement of the visceral peritoneum, young flukes can be found in enlarged mesenteric lymph nodes.

The acute lesions in the liver caused by the wandering flukes are basically traumatic, but there is an element of coagulative necrosis, which is possibly related to toxic excretions of the flukes. The migratory pathways are tortuous tunnels that appear on cross-section as hemorrhagic foci 2–3 mm in diameter. If a tunnel is followed, a young fluke less than 1.0 mm long can be found at the end. When the infestation is heavy, the liver may appear to be permeated by dark hemorrhagic streaks and foci. Older tunnels from which the debris has been cleared may appear as light yellow streaks due to infiltration of eosinophils (Fig. 2.67). Microscopically, fresh tunnels are filled with blood and degenerate hepatocytes and are soon infiltrated by eosinophils. Later, histiocytes and giant cells arrange themselves about the debris and remove it, and healing occurs by granulation tissue, which is rich in lymphocytes and eosinophils. In light infestations the scars may disappear, but in heavy infestations they may fuse with each other and with portal areas to produce moderate irregular fibrosis. There may, as yet, be no change in the bile ducts. Probably, *most of the young flukes reach the bile ducts, but some do not and become encysted in the parenchyma.* One or more flukes may be present in each cyst, which consists of a connective-tissue capsule and a dirty brown content of blood, detritus, and excrement from flukes. The cysts ultimately caseate and may mineralize or are obliterated by fibrous tissue. These cysts are most frequent on the visceral surface, where they cause bulging of the capsule.

Heavy infestations by immature flukes may cause death in the stage of acute hepatitis. Such an outcome is not common, but occurs in sheep. It is estimated that 10 000 metacercariae ingested over a short period are necessary to produce acute death in sheep. Death may occur suddenly or after a few days of fever, lassitude, inappetance, and abdominal tenderness. This is also the stage of the parasitism in which *black disease* occurs (see above).

Mature flukes are present in the larger bile ducts and cause cholangiohepatitis. The relative importance of different factors in their pathogenicity is not known, but they cause mechanical irritation by the action of their suckers and scales, cause obstruction of the ducts with some degree of biliary retention, predispose to bacterial infections,

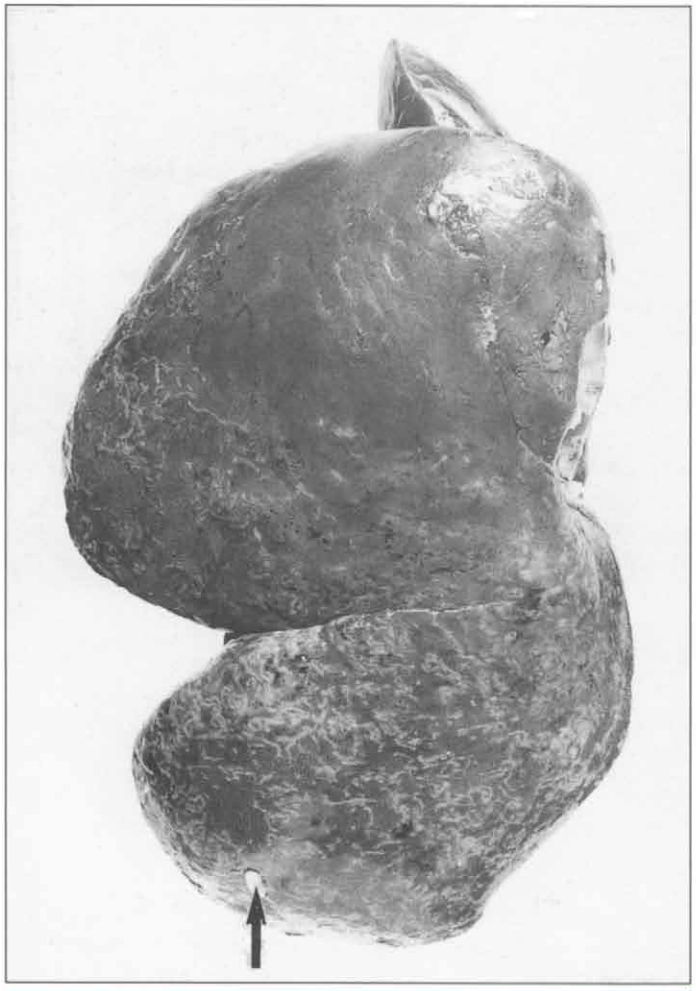

Figure 2.67 Tracks of immature ***Fasciola hepatica*** **flukes** in acute infestation, concentrated in left lobe of a sheep. Degenerate cysticercus (arrow).

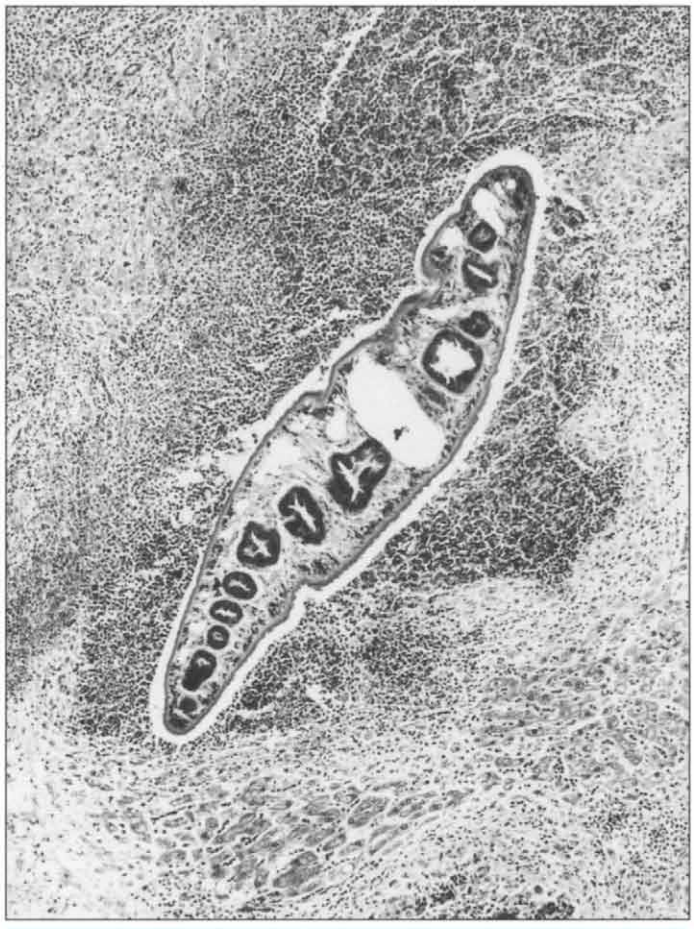

Figure 2.68 Immature fluke, accompanied by acute inflammation in a sheep.

suck blood, and probably produce toxic and irritative metabolic excretions.

The *biliary changes* occur in all lobes but are usually most severe in the left, and the right may be moderately hypertrophied (Fig. 2.69A). From the hilus, *the bile ducts on the visceral surface stand out as white, firm, branching cords* that in extreme instances may be 2 cm in diameter and allow detectable fluctuation over extended segments or in localized areas of ectasia. This dilation of the ducts in sheep, swine, and horses is largely mechanical and is due to *distension by masses of flukes and bile*. It is permitted by the relative paucity of new connective-tissue formation in the walls of the ducts in these species; this in turn is probably related to the rather mild catarrhal type of inflammation in the lumina of the ducts. In *cattle*, desquamative and ulcerative lesions in the large bile ducts are more severe than in other species, and there is a correspondingly greater proliferation of granulation tissue in and about the walls of the ducts. The walls of the ducts in cattle are, in consequence, much thickened, and the lumen is irregularly stenotic and dilated and lined largely by granulation tissue. This contributes the typical "*pipe stem*" appearance to the ducts in cattle; the connective tissue may be, in addition, mineralized, sometimes so heavily that it cannot be cut with a knife. The bile ducts contain dirty dark-brown fluid of a mucinous or tough consistency, formed from degenerate floccular bile, pus, desquamated cells and detritus, clumps of flukes, and small masses of eggs in dark-brown granular aggregates.

Although the lesions are most obvious in ducts large enough to contain the flukes, there is, with time and severe or repeated infestations, *progressive inflammation in the smaller portal units* due to direct irritation by the flukes, superimposed infections, and biliary stasis. The course of events is as described earlier for subacute and chronic cholangiohepatitis. The proliferating connective tissue and bile ductules in individual portal areas extend to join each other and the scars left over from the migratory phase, so that inflammatory fibrosis may obliterate parenchyma in many foci. In such livers, the left lobe, which is the one most severely affected, may be atrophied, indurated, and irregular (Fig. 2.69B).

The development of cholangiohepatitis of the degree described depends on long-standing or heavy infestations. Lesions of lesser severity, or those less fully developed, are associated with light infestations of short duration. They may then be recognized only by local dilations of the ducts, or even these may not be readily apparent. In such mild infestations, the fact of past or present parasitism may only be suggested by the detection of characteristic black iron-porphyrin pigments, grossly visible in the hilar nodes. It also contributes to the character of the biliary contents.

Chronic debility with vague digestive disturbances is common in chronic fascioliasis, and deaths are common among sheep. Clinically and at postmortem there are, in addition to the essential lesions,

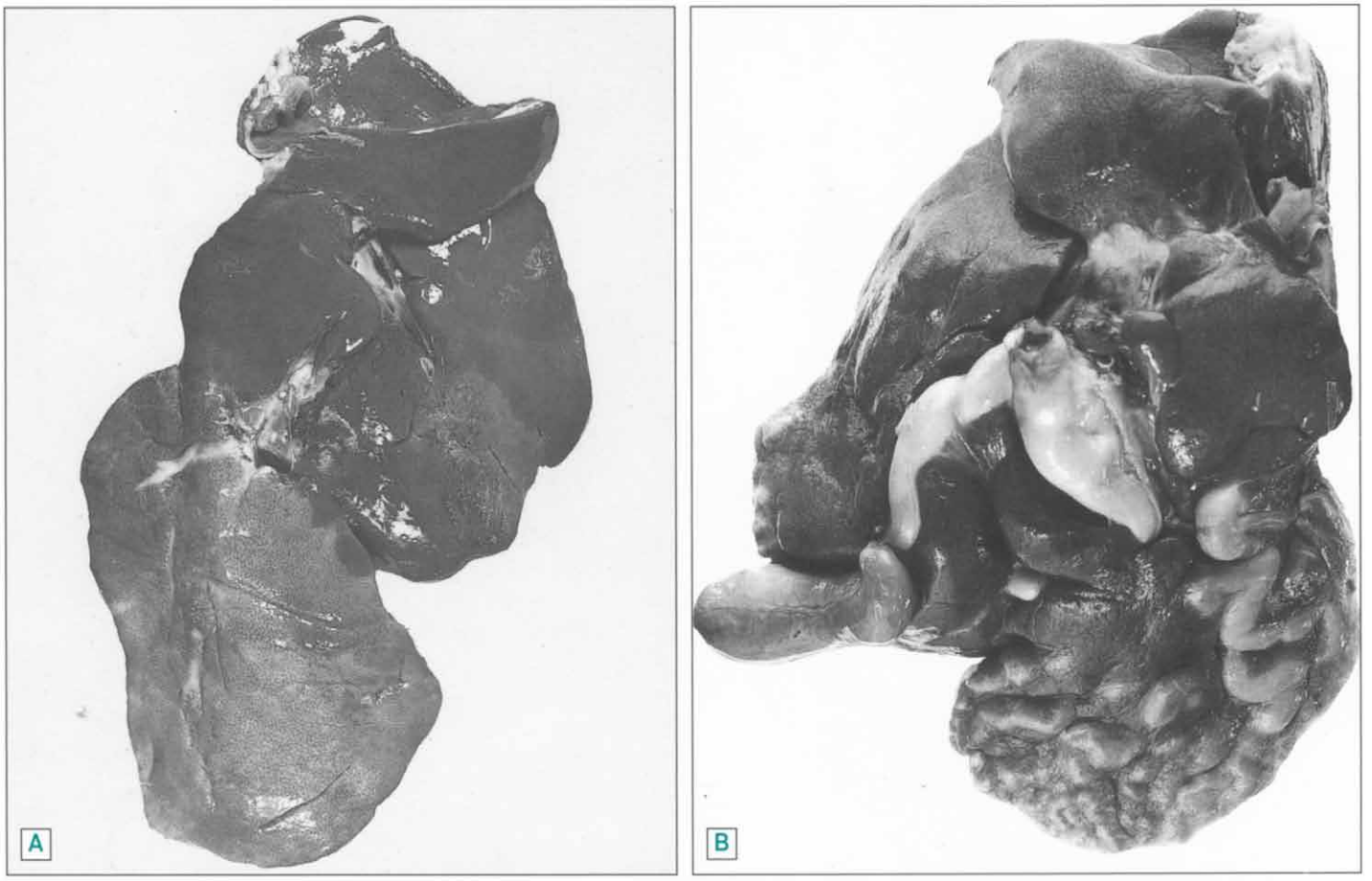

Figure 2.69 Fascioliasis in sheep. A. Subacute *Fasciola hepatica* infestation, causing early **cholangiohepatitis and cholestasis** in left lobe. **B.** Bile duct ectasia, atrophy of left lobe, hypertrophy of right lobe resulting from **chronic fascioliasis**.

more or less severe anemia, moderate anasarca, and cachexia. Jaundice is seldom seen.

Fasciola gigantica displaces *F. hepatica* as the common liver fluke in many parts of Africa and in nearby countries, southeast Asia, and the Hawaiian islands. It is two or three times as large as *F. hepatica*, but its life cycle and pathogenicity are comparable.

Fascioloides magna *is the large liver fluke of North America.* It is a parasite of ruminants and lives in the hepatic parenchyma, not in the bile ducts, although in tolerant hosts, Cervidae, the cysts in which it localizes communicate with the bile ducts to provide an exit for ova and excrement. The life cycle of this parasite generally parallels that of *Fasciola hepatica*. The young flukes are very destructive as they wander in the liver. In cattle, they wander briefly, producing large necrotic tunnels before becoming encysted. The cysts, enclosed by connective tissue, do not communicate with bile ducts but form permanent enclosures for the flukes, their excreta, and ova. *The cysts, which may be 2–5 cm in diameter, are remarkable for the large deposits of jet black, sooty iron-porphyrin pigment they contain* (Fig. 2.70), and except for the flukes and soft contents, they superficially resemble heavily pigmented melanotic tumors. Commonly, these flukes pass from the liver to the lungs of cattle, to produce lesions of similar character. In sheep, this parasite wanders continuously in the liver, producing black, tortuous tracts, which may be 2 cm in diameter, and extensive parenchymal destruction. Even a few flukes may kill a sheep.

The **dicrocoelid flukes** inhabit both biliary and pancreatic ducts. *Eurytrema pancreaticum* prefers the pancreas (and is described with that organ) but in heavy infestations can be found in the bile ducts. *Dicrocoelium* and *Platynosomum* prefer the bile ducts. These are small, narrow flukes 0.5–1.0 cm long and may easily be mistaken for small masses of inspissated bile pigment. They are not highly pathogenic, and even in heavy infestations there may be no signs of the toxemia observed in infestations by *F. hepatica*. These flukes may occur as mixed infestations.

Platynosomum fastosum is a parasite of cats in North America and the Amazonian regions of South America. The life cycle involves snails and lizards and presumably an arthropod. The infested livers are enlarged, friable, and may be bile-stained. There is catarrhal inflammation of biliary passages, but it is not severe, and the walls may not be much thickened. The ducts are dilated and easily visible. There are vague digestive disturbances, and heavy infestations may cause complete anorexia and death.

Dicrocoelium hospes is found in cattle in countries south of the Sahara. Little is known of it, but it is presumed to be comparable in all respects to the better-known *D. dendriticum* (the lancet fluke or small liver fluke), which is common in Europe and Asia and sparsely distributed in the Americas and North Africa. *Dicrocoelium* spp. are found in dry lowland or mountain pastures, whereas *Fasciola* spp. occur in wetter habitats, so the prevalence of *Dicrocoelium* is increasing

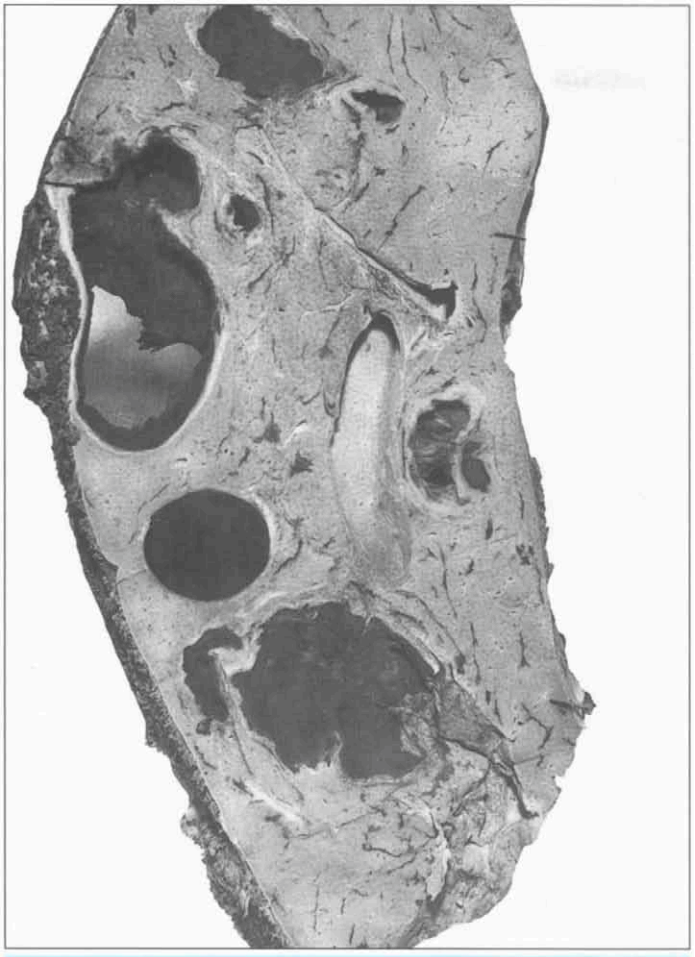

Figure 2.70 Destructive pigmented lesions produced by *Fascioloides magna* in an ox.

with desertification. This fluke is no more fastidious in its choice of final hosts than many other species of fluke, and, depending on opportunity, it can infest all domestic species, with the possible exception of cats. It is, however, of most importance as a parasite of sheep and cattle, in which it inhabits the bile ducts. Other domestic species and rodents are important as reservoirs.

The life cycle of ***Dicrocoelium dendriticum*** differs in some details from that of *F. hepatica*. The eggs are embryonated when laid and do not hatch until swallowed by one of the many genera of land snails that are the first intermediate hosts. In the snails, the mother sporocyst produces a second generation of daughter sporocysts, which in turn produce cercariae. The cercariae leave the snail in damp weather and are expelled from the snail's lung, clumped together in slime balls. The slime balls are not infective until the cercariae are swallowed by, and encyst in, the ant *Formica fusca*; other ants may be involved in different countries. The cycle is completed when the definitive hosts swallow the ants. The route of migration of the larvae from the gut to the liver is probably via the bile ducts from the duodenum.

The pathologic changes in the liver produced by *D. dendriticum* are those of cholangiohepatitis that is less severe than that produced by *Fasciola hepatica*. The severity and diffuseness of the hepatic lesion are determined by the number of lancet flukes present, and they may be in the thousands. The flukes and their eggs darken the dilated ducts. Even in early infestations, there may be some scarring of the organ at its periphery. In heavy infestations of long standing, there is extensive biliary fibrosis, producing an organ that is indurated, scarred, and lumpy and that at the margins may bear areas that are shrunken and completely sclerotic. The histologic changes are the same as those in fascioliasis, with perhaps more remarkable hyperplasia of the mucous glands of the large ducts.

The **opisthorchid flukes** are parasites in the bile ducts of carnivores. They may also occur in swine and humans, and one species, *Opisthorchis sinensis* (*Clonorchis sinensis*), is an important human parasite. There is some uncertainty regarding the proper classification of these flukes, and some of those given below may not be valid species. The life cycles, where known, include mollusks as the first intermediate hosts and freshwater fish as the second.

Metorchis conjunctus *is the common liver fluke of cats and dogs in North America* and is important as a parasite of sled dogs in the Canadian Northwest Territories. The first intermediate host is the snail *Amnicola limosa porosa*, and the second is the common suckerfish *Catostomus commersonii*. The cercariae actively burrow into the musculature of the fish to encyst and become infective. The immature flukes crawl into the bile ducts from the duodenum and mature in ~28 days. Infestations may persist for more than 5 years. *Metorchis albidis* has been described in a dog from Alaska, *Parametorchis complexus* in cats in the USA, and *Amphimerus pseudofelineus* in cats and coyotes in the USA and Panama; the life cycles are not known but are presumed to include fish. *Metorchis bilis* has been reported in red foxes and occasionally in cats from Germany.

Opisthorchis felineus *is the lanceolate fluke of the bile ducts of cats, dogs, and foxes in Europe and Russia.* It is particularly common in eastern Europe and Siberia and is more sparse in other areas. *Metorchis bilis* infects red foxes. *Opisthorchis sinensis* is well documented as the Oriental or Chinese liver fluke; it is endemic in Japan, Korea, southern China, and southeast Asia. There are additional species of *Opisthorchis* in humans and animals, but they are less well known than the species cited. The first intermediate hosts for the miracidia of *O. tenuicollis* and *O. sinensis* are snails of the genus *Bithynia*, and several genera of cyprinid fishes can act as second intermediate hosts.

Pseudamphistomum truncatum occurs in carnivores and humans sporadically in Europe and Asia. Its life cycle is as for *Opisthorchis*.

The opisthorchid flukes, so far as known, resemble *Dicrocoelium* in migrating up the bile ducts to their habitat. This may be the reason that they are more numerous in the left than in the right lobes of the liver. They can probably live in the liver for as long as the host lives. The pathologic effects are comparable to those of *D. dendriticum*. Light infestations may be asymptomatic, and heavy infestations may cause jaundice, chronic cholangiohepatitis, and severe biliary fibrosis. Both in humans and animals, adenomatous and carcinomatous changes of the biliary glands have occurred in association with these parasites; the association is probably more than coincidental.

Protozoal infections

Protozoal hepatitis is due mainly to infection with *Toxoplasma*, *Neospora*, and *Leishmania*, described elsewhere. Granulomatous hepatitis has been described in dogs associated with systemic infections by *Hepatozoon canis*.

Hepatic coccidiosis, expressed as acute cholangiohepatitis similar to that in mink and rabbits, has been observed in isolated cases in the goat, calf, and dog. These infections are usually considered aberrant,

and often coincide with intestinal coccidiosis. Coccidial meronts and in some cases gamonts are present within the cytoplasm of biliary epithelium. The organisms are presently unclassified.

Fungal infections

Fungal infections of the forestomachs with hematogenous dissemination to the liver occur occasionally in cattle and sheep, usually as a complication of rumenitis. Lesions in the liver are typically hemorrhagic infarcts initially or granulomatous with chronicity, and are associated with infection by *Aspergillus fumigatus* and various members of the class Zygomycetes.

Granulomatous hepatitis associated with disseminated fungal or algal infections has also been reported in dogs. The species involved include *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Sporothrix schenckii*, *Aspergillus* sp., and *Prototheca* sp.

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TOXIC HEPATIC DISEASE

Role of hepatic biotransformation

An understanding of hepatic biotransformation is essential for an appreciation of the hepatotoxic potential of foreign compounds. Lipid-soluble compounds, including endogenous metabolites as well as nonpolar foreign compounds (known as **xenobiotics**, and *including plant or fungal-derived secondary metabolites consumed in food, environmental chemicals, and drugs*) must be converted to water-soluble products to permit elimination from the body in urine and bile. The enzymatic machinery responsible for this biotransformation is most active in the liver. The liver is strategically located to intercept ingested foreign compounds, and it is likely that *hepatic biotransformation enzymes* co-evolved in part to deal with naturally occurring toxic and lipid-soluble metabolites produced by plants that formed part of the diet. While these enzyme systems were formerly referred to as drug-metabolizing enzymes, or liver detoxification systems, recent research has underscored the importance of these systems in metabolism of endogenous compounds, including arachidonic acid, eicosanoids, cholesterol, biosynthesis of bile acids, and synthesis and metabolism of steroids and vitamin D.

Enzymatic biotransformation in the liver consists of two phases. *Phase I reactions* typically involve the addition of reactive polar groups through *oxidation* and, less frequently, reduction and hydrolysis reactions. In *phase II*, the metabolic product of phase I (or the original compound if it possesses a polar group that permits metabolism) undergoes *conjugation*, typically with water, glutathione, sulfate, glucuronate, or other groups. The conjugate is typically less toxic, and more water-soluble than the parent compound.

Phase I reactions are mainly performed by members of the *cytochrome P450 superfamily*, the so-called *mixed-function oxidase system*, which are integral parts of the smooth endoplasmic reticulum. The oxidative reactions of phase I are a double-edged sword, and while they are important in providing reactive sites for further conjugative detoxification reactions, in the process they can yield transient reactive intermediates, including epoxides and free radicals, which can potentially bind to and damage adjacent macromolecules, resulting in hepatotoxicity. *Toxic hepatic injury depends on the balance between production of reactive metabolites and their detoxification by conjugation and other protective reactions.* The cytochrome P450s can be found in all parts of the hepatic acinus, but the hepatocytes of zone 3 have a higher content than those of zone 1, which apparently accounts for the *zone 3 predominance of injury produced by compounds metabolized by this system*, including carbon tetrachloride and acetaminophen. In contrast, *hepatocytes in zone 1 are more susceptible to direct-acting toxicants such as metal salts*, due to their proximity to incoming portal and arterial vascular flow.

Phase II reactions are generally *detoxification reactions*, although there are exceptions. The enzymes of phase II are predominantly located in the cytosol, and include various transferases capable of conjugating molecules containing suitable polar groups with endogenous substances. One important conjugation pathway is with reduced glutathione, mediated by the glutathione *S*-transferases. Active metabolites of acetaminophen and various other agents are detoxified by conjugation with glutathione, and depletion of glutathione reserves plays a critical role in the development of toxicity. Hepatic glutathione is also important in the removal of free radicals and reactive oxygen species through the action of glutathione peroxidase.

Efforts to elucidate mechanisms for hepatic injury are complicated by the presence of multiple alternative pathways for metabolism of compounds, the presence of many different isoforms of various enzyme families, particularly the cytochromes P450, and the increasing numbers of genetic polymorphisms identified within individual alleles coding for these isoforms. Many genes for cytochromes P450 and various phase II conjugation enzymes have regulatory sequences that include various transcriptional response elements that respond to nuclear receptors. These receptors resemble steroid receptors and include the arylhydrocarbon hydroxylase receptor, peroxisome proliferator-activated receptors, the constitutive androstane receptor, and the pregnane X receptor. These receptors are activated by various xenobiotics and endogenous ligands so the overall