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HEPATIC LESIONS IN WOODCHUCKS (*MARMOTA MONAX*) SERONEGATIVE FOR WOODCHUCK HEPATITIS VIRUS

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ABSTRACT: Livers from 128 captive woodchucks (*Marmota monax*) that were negative for serological markers of woodchuck hepatitis virus (WHV) were examined grossly and histologically. Livers from 19 additional seronegative woodchucks were examined only grossly. The most common finding (61% of histological specimens) was mononuclear cells in portal areas. Moderate to severe portal infiltrates in association with similar scattered mononuclear cell aggregates and extramedullary hematopoiesis, were present in woodchucks that had extrahepatic inflammatory lesions. We concluded that these changes represent a response to a variety of chronic infections and are not specific for WHV infection. Other findings included parasitic granulomas, focal and diffuse lipidosis, bile duct proliferation, lesions secondary to cardiovascular compromise, and vasculitis. Two woodchucks had neoplastic lesions.

Key words: *Marmota monax*, woodchuck, liver, nonviral hepatitis, pathology.

INTRODUCTION

The woodchuck, *Marmota monax*, has gained importance as a laboratory animal (Young and Sims, 1979), particularly for the study of viral hepatitis and primary hepatocellular neoplasia (Summers et al., 1978; Snyder et al., 1982; Popper et al., 1987). Few commercial and research breeding colonies have been established. Most woodchucks now used for research were born in the wild, trapped, and permitted only brief periods of adaptation to laboratory conditions before being used in studies. A previous study (Fleming, 1972) reported the occurrence of lesions and diseases in a wild, apparently healthy woodchuck population located in Tompkins County, New York (USA). Parasitic diseases and infectious diseases of public health significance were emphasized. As the use of the woodchuck in the laboratory increases, the recognition of associated disease problems is necessary. A detailed survey, including histologic examination of apparently normal major organs, especially the liver, is essential to provide a baseline prior to laboratory manipulation. In this report, the spontaneous hepatic lesions of adult woodchucks, seronegative for markers of woodchuck hepatitis virus (WHV), are described. All woodchucks were trapped in central New York and maintained in captivity for a period that ranged from 1 day to >2 yr.

MATERIALS AND METHODS

One hundred and forty seven woodchucks, ≥1-yr-old, from Cornell University woodchuck colonies (Cornell University, Ithaca, New York 14853, USA) were used in this study. Following capture, woodchucks were transferred to facilities at Cornell University where they were anesthetized by intramuscular injection of xylazine (Rompum®, Haver-Lockhart, Bayvet Division, Miles Laboratory, Inc., Shawnee, Kansas 66201, USA) and ketamine (Ketaset®, Bristol Laboratories, Division of Bristol-Myers, Co., Syracuse, New York 13201, USA) and given a general physical examination. Blood samples were taken via saphenous or jugular venipunture and submitted for analysis of hepatic enzymes (Gastroenterology Unit, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA) and serologic markers (WHV surface antigen and antibodies to WHV core and surface antigen) of WHV infection (John L. Gerin, Division of Molecular Virology and Immunology, Georgetown University, Rockville, Maryland 20852, USA). Age was estimated based on previously described criteria (Young and Sims, 1979). All woodchucks were maintained in wire cages with enclosed wooden nest boxes supplied with woodshavings and/or straw bedding material. They were fed pelleted rabbit chow (Big Red Rabbit Chow®, Agway,

Inc., Syracuse, New York 13201, USA) and tap water, *ad libitum*. Animals were anesthetized every 3 to 6 months for general physical examination and blood sample collection, but invasive experimental procedures were not done.

Eighty five woodchucks died spontanteously. Forty even animals were killed because of obvious clinical disease or lesions. Fifteen apparently healthy woodchucks were killed. Complete necropsy examinations were performed on all woodchucks using standard techniques. Body weights and weights of major organs including liver and opened gall bladder were recorded. Tissues were examined grossly only in 19 woodchucks because advanced autolytic change had occurred. Tissue samples from the remaining 128 woodchucks were fixed by immersion in neutral buffered 10% formalin, embedded in paraffin, sectioned at 4 to 6 microns, and stained with hematoxylin and eosin for light microscopic examination. In some cases, periodic acid Schiff (PAS) and oil red O stains were used to help differentiate glycogen from fat; Masson's trichrome stain was used to determine the presence of connective tissue.

RESULTS

There was no evidence of WHV infection based on the absence of WHV surface antigen and antibodies to WHV core and surface antigens as determined by radioimmunoassay techniques in any serological sample obtained from the woodchucks included in this study. Serum levels of alanine aminotransferase, gamma glutamyl transferase, albumin, and protein initially occurred with the ranges determined to be normal for woodchucks (Hornbuckle et al., 1985).

Findings are summarized in Table 1. No lesions were observed in the livers of seven woodchucks.

The most common finding, observed in 61% of livers examined histologically, was the presence of lymphocytes and plasma cells in portal areas. This was not associated with gross lesions. The cellular infiltration was confined by the limiting plate hepatocytes and considered slight in 41 livers (Fig. 1), moderate in 35 livers (Fig. 2), and severe in two livers (Fig. 3). Larger cellular infiltrates still confined to portal areas were found in woodchucks that also had chronic inflammatory lesions in other tissues. The most common extra hepatic lesions were subcutaneous abscesses (32 animals). Nine woodchucks that had multifocal infiltrates of lymphocytes and plasma cells also had moderate cellular accumulations in portal areas.

TABLE 1. Findings in the livers of 147* woodchucks (*Marmota monax*).

Finding	Number affected
Lymphocytes, plasma cells—portal	
—slight	41
—modreate	35
—severe	2
Lymphocytes, plasma cells—multifocal	9
Extramedullary hematopoiesis	17
Acute multifocal necrosis	5
Lipidosis, diffuse	2
Lipidosis, focal	2
Anoxic degeneration	4
Passive congestion	2
Granuloma, parasitic (unidentified)	9
Granuloma, parasitic (*Capillaria* sp.)	1
Cyst, parasitic (*Taenia mustelae*)	6
Ackertia marmota (vessels)	6
Vasculitis	2
Portal vein thrombosis with infarction	1
Bile duct proliferation	14
Myeloproliferative disease	1
Hepatoma	1
No lesions	7

* 16 woodchucks had two lesions; two woodchucks had two lesions.

Extramedullary hematopoiesis was observed in 13% of livers examined histologically. Aggregates of megakaryocytes and immature erythrocytes and granulocytes caused small focal, irregular dilitation of sinusoids. Hepatomegaly was observed grossly in five of the most severely affected animals. In these cases the liver weighed between 4 to 5% of the woodchuck's body weight. In a survey of 69 normal woodchucks, liver weight was determined to be 4% of body weight (Roth, 1984). Generalized pallor was not detected. These woodchucks also had wide variety of chronic infections such as subcutaneous abscesses, osteomyelitis, and vegetative endocarditis.

Acute, multifocal hepatic necrosis, char-

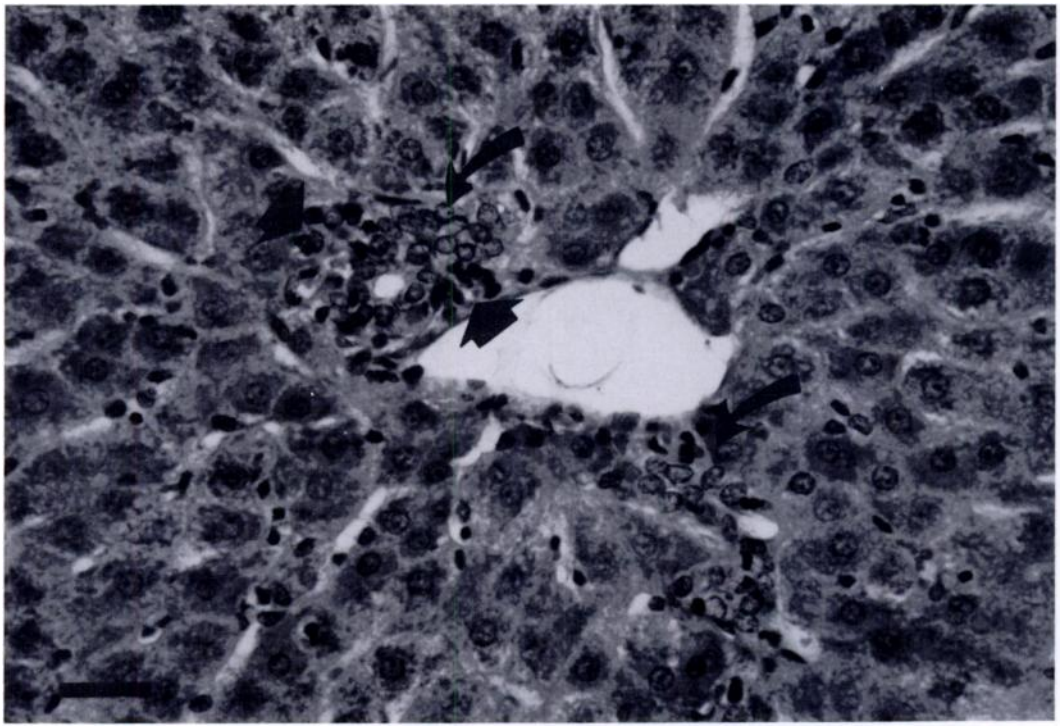

FIGURE 1. Photomicrograph of a portal area with a minimal number of lymphocytes and plasma cells (short arrows) associated with bile ductules (curved arrows). H&E. Bar = 50 μm.

acterized histologically by degeneration and fragmentation of hepatic cord cells with variable infiltrates of neutrophils, lymphocytes, plasma cells and erythrocytes was observed in five woodchucks. Grossly, the livers had finely roughened surfaces and were mottled bright red and brown, but were normal in size and consistency. A specific cause was not determined in any case. WHV infection was ruled out because of the lack of serological evidence of viral infection and the morphology of the lesions.

Diffuse lipidosis was observed in two obese animals. At necropsy the livers were slightly swollen and yellow tan, but a greasy texture was not noted. Histologically, almost all hepatic cord cells had large, discrete, usually single cytoplasmic vacuoles. Vacuolar contents usually stained positively with oil red O and did not stain with PAS. The cause for this lesion was not determined. Focal or "tension" lipidosis was observed in two woodchucks. These yellow, tan, straight edged lesions, present at the free edge of affected livers were associated with nearby connective tissue adhesions to the liver capsule. Histologically, these consisted of discrete foci of moderately to severely vacuolated hepatic cord cells.

Anoxic degeneration was recognized grossly as an accentuated lobular pattern with pallor of centrilobular zones in four woodchucks. The histological lesion consisted of centrilobular cord cell vacuolation. Affected cells contained small, multiple, clear to hazy cytoplasmic vacuoles. This lesion was associated with cardiovascular impairment. Two woodchucks with cardiomyopathy had lesions of passive congestion. Grossly, the livers had an accentuated lobular pattern with dark red centrilobular zones. The histological lesion consisted of accumulations of blood-filled sinusoids surrounding central veins and atrophy of hepatic cord cells in these areas.

Granulomas were most often associated

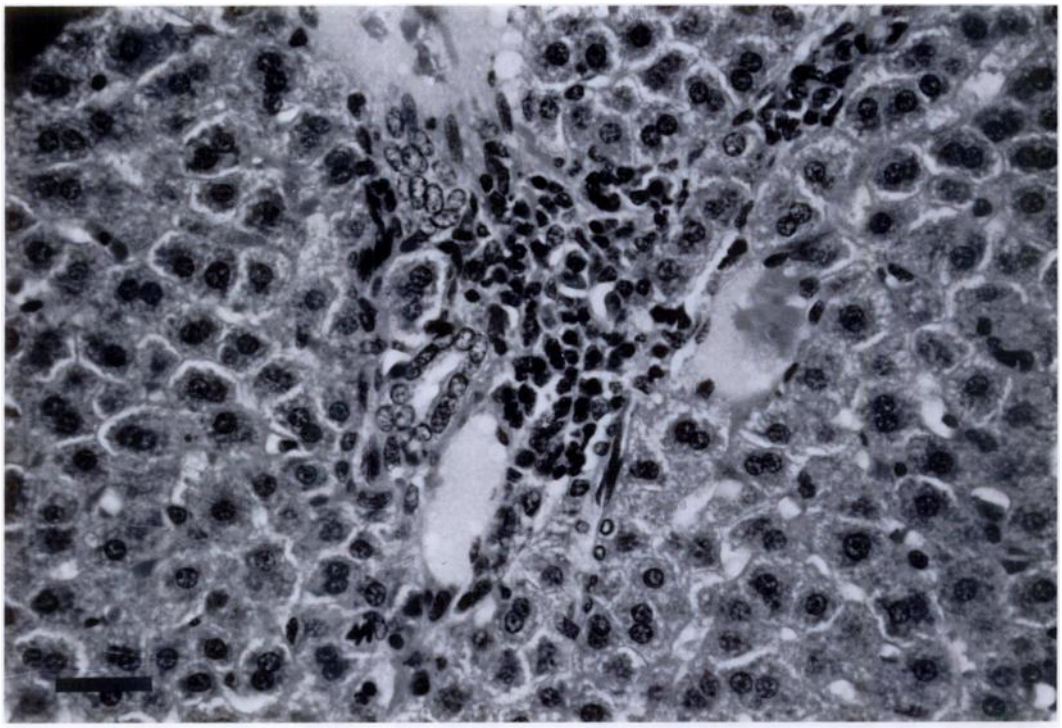

FIGURE 2. Photomicrograph of a portal area with a moderate infiltrate of lymphocytes and plasma cells. H&E. Bar = 50 μm.

with the migration of the intermediate stages of the cestode *Taenia mustelae*. In nine woodchucks, granulomas were observed but association with a specific parasite could not be demonstrated. Based on previous experience (Roth et al., 1982), these lesions were attributed to parasite migration. The filarid parasite, *Ackertia marmota*, was present in the lumens of parenchymal venous and lymphatic vessels in six woodchucks, but did not cause pathological changes.

Necrotizing vasculitis involving the small vessels in portal triads was found in two woodchucks. This was associated with grossly observable 1 to 2 mm irregular red foci scattered throughout diffusely pale parenchyma. In one woodchuck portal vein thrombosis with locally extensive infarction was also present. Histologically, affected vessels appeared densely pink and occasionally fragmented. Neutrophils, lymphocytes and erythrocytes infiltrated and surrounded vessel walls.

Bile duct proliferation was recognized histologically in the livers of 14 animals. All 14 animals were also emaciated. They had no body fat and the livers were slightly smaller and darker than expected. Histologically, hepatic cord cells were atrophic and densely stained.

Neoplastic lesions were observed in the livers of two animals. One woodchuck had a hepatoma (Roth et al., 1984) which was considered an incidental finding and the other had myeloproliferative disease. Grossly, the liver of the latter woodchuck was slightly enlarged, as determined by percent of body weight, and pale. A focal 2 × 5 mm tan nodule protruded slightly from the visceral surface of the right middle lobe. Histologically, the nodule consisted of immature granulocytes and few megakaryocytes. It caused compression of the immediately surrounding parenchyma. Similar cell populations caused irregular expansion of sinusoids. Additionally, there was slight splenomegaly and scat-

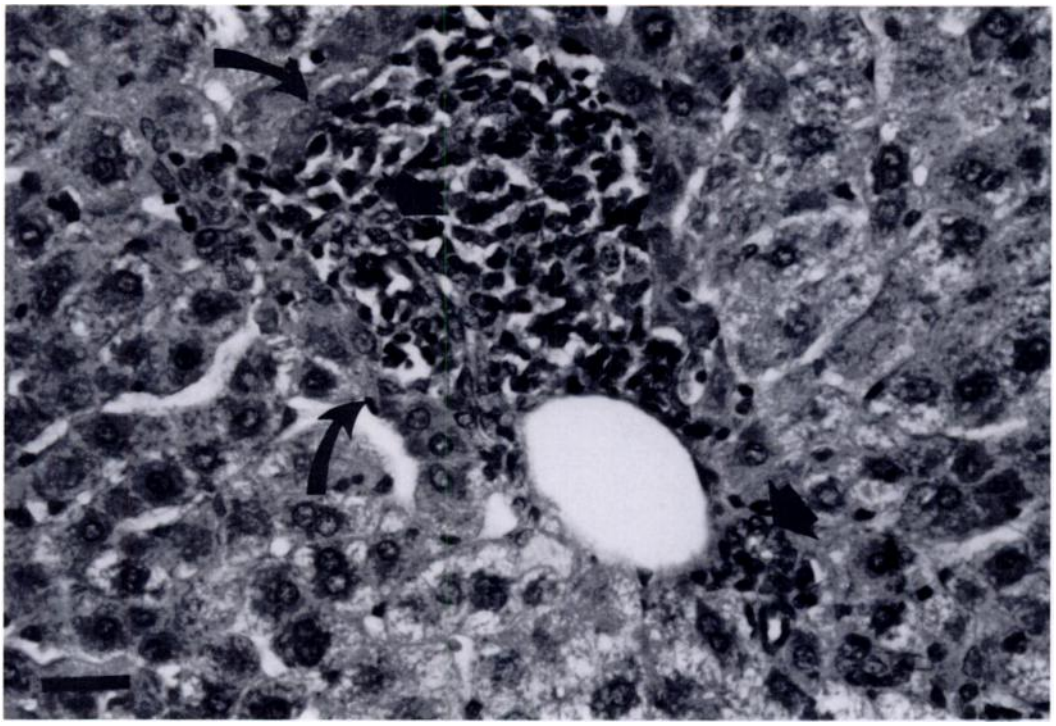

FIGURE 3. Photomicrograph of a portal area with a severe infiltrate of lymphocytes and plasma cells. The infiltrate is confined by limiting plate hepatocytes (short arrows). Small bile ductules are present (curved arrows). H&E. Bar = 50 μm.

tered lymph nodes were enlarged due to multifocal infiltration by neoplastic cells.

DISCUSSION

Portal and sinusoidal accumulations of lymphocytes and plasma cells and scattered foci of extramedullary hematopoiesis have been primarily associated with chronic WHV infection (Popper et al., 1981). The cellular aggregates in the woodchucks in this series were usually not extensive. The wide range of chronic, nonhepatic inflammatory lesions present in the animals with the most severe cellular aggregates or extramedullary hematopoiesis suggests that this liver lesion represents a reaction to any persistent infection. A similar lesion, referred to as hepatic leukocytosis, has been reported in association with chronic nonhepatic inflammatory lesions in domestic animal species (Jubb et al., 1985).

Diffuse hepatic lipidosis can be caused by toxins such as aflatoxin or carbon tetrachloride (Chopra et al., 1972; Edds, 1973) or metabolic upsets due to pancreatitis, diabetes mellitus, pregnancy, or acute fasting (Morrow et al., 1979; Gerloff and Hedt, 1984; Alpers and Isselbacher, 1975). Idiopathic hepatic lipidosis has been described as a fatal condition in cats (Cornelius and Jacobs, 1989). In the two woodchucks in this series lipidosis was suspected to have resulted from failure to adapt to laboratory conditions and diet, as both woodchucks died in the early fall, within 2 wk of having been trapped. In preparation for hibernation, woodchucks gain a substantial amount of weight during the summer (Young and Sims, 1979). Failure to eat the laboratory diet would cause excessive mobilization of fat from body stores. As in other animal species, this would interfere with the hepatic balance of deposition and secretion of triglycerides and fatty acids (Robbins and Kumar, 1987).

Focal lipidosis has been called tension lipidosis (Roth and King, 1982). These are foci of hepatocytes that have degenerated due to vascular compromise caused by adhesions to the liver capsule. The mechanism for the development of this lesion is believed to be the same in woodchucks.

Anoxic change and passive congestion of the liver occur as sequella to cardiovascular impairment (Jubb et al., 1985). Since one woodchuck had valvular endocarditis and the other five had cardiomyopathy, the development of these lesions in woodchucks was also considered to be secondary to cardiovascular compromise.

Parasitic granulomas are common in the liver in many animal species (Georgi, 1985). In occasional gross or histological specimens, as in seven of ours, the causative parasite may be actually seen. In many specimens, however, the discrete accumulation of eosinophils with fewer macrophages, giant cells and other inflammatory cells strongly suggests a parasitic etiology, even if a specific parasite is not found.

Causes of vaculitis and thrombosis include immune complex deposition and specific viral or bacterial infections in other species. Vascular degeneration has been reported in a woodchuck with a hepatoma (Bond, 1970). Immune complex deposition was not investigated in the two woodchucks that had vasculitis. They were negative for serologic markers of WHV infection and did not have hepatic necrosis or mononuclear cell infiltrates that characterize chronic persistent or chronic active WHV infection.

Bile duct proliferation may result from hepatotoxic damage, bile duct obstruction, and has been associated with starvation (Jubb et al., 1985; Roth, 1987) as it was in the woodchucks in this series. The specific mechanism for bile duct proliferation in emaciation has not been determined.

None of the lesions observed in this study were considered unique to the woodchuck. Most of the hepatic lesions observed were not directly associated with captivity. One notable exception is hepatic lipidosis which was associated with the failure to adapt to the laboratory environment.

As facilities for breeding and raising woodchucks for laboratory use expands there will be increasing uniformity among the animals used for studies. Until breeding colonies are well established, the spontaneous lesions in wild caught woodchuck populations needs to be documented.

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