A HISTOPATHOLOGICAL STUDY OF HEARTS AND SPLEENS OF HAMSTERS (*MESOCRICETUS AURATUS*) INFECTED WITH *LEPTOSPIRA INTERROGANS*, SEROVAR PYROGENES

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Abstract. The effects of *Leptospira interrogans* on the heart and spleen of hamsters were studied histopathologically. Infected hamsters were sacrificed at 1 hour, 6 hours and on days 1, 2, 3, 4, 5 and 6 after inoculation with *Leptospira interrogans* serovar pyrogenes. The heart and spleen of each of the sacrificed animals were removed and processed for routine conventional light microscopy. Infected hearts showed degenerative change of the cardiac muscle cells composed of cellular swelling, condensation of chromatin granules, pyknotic nuclei and acidophilic cytoplasm. Congestion of the cardiac blood vessels and hemorrhagic areas were found. Necrosis of the cardiac muscle cells was surrounded by numerous inflammatory cells. In the spleen, cellular necrosis was found scattered throughout the splenic cord. The splenic sinusoids were dilated and congested with many hemorrhagic areas. Inflammatory cell infiltration was also noted in the splenic parenchyma and the splenic sinusoids.

INTRODUCTION

In Thailand, two important studies of *Leptospira interrogans* serovar bataviae were reported by Sitprija and Evan (1970) and Sitprija *et al* (1980). The former report studied the renal pathological changes in leptospirosis, mainly in patients with impaired renal function, which varied from mild to severe requiring dialysis. The main findings were interstitial nephritis and acute tubular necrosis. The latter paper studied the pathogenesis of the renal lesion in leptospirosis in both humans and hamsters which had been experimentally infected with leptospires. In 2001, the microanatomical changes of many organs, including the kidneys, lungs, liver, gastrocnemius and hamstring muscles in hamsters infected with *Leptospira interrogans* serovar pyrogenes were studied by Pilakasiri *et al* (2001). They found that the infected kidneys showed degenerative changes of the renal tubular cells, glomerular damage, congestion of renal blood vessels, and hemorrhage of the interstitium and renal tubules. Interstitial nephritis and pyelonephritis were found. In the lung, interstitial and intra-alveolar hemorrhage, bronchopneumonitis, and interstitial pneumonitis were found. The infected liver showed hepatocellular necrosis, vascular congestion, prominent Kupffer cells, and inflammatory cell infiltration. In the gastrocnemius and hamstring muscles, congestion of blood vessels, inflammatory cell infiltration, and muscle cell necrosis were found. In this study, the histopatho-

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logical changes of the heart and spleen in hamsters infected with *Leptospira interrogans* serovar pyrogenes were studied in order to observe the progressive histological changes.

MATERIALS AND METHODS

Leptospira interrogans serovar pyrogenes was isolated from a febrile patient with clinical leptospirosis in Buri Ram Province in October 1999. These pathogens were cultured and used in this study.

Animal inoculation

Six control hamsters injected intraperitoneally with 0.5 ml PBS served as the control group. The other 24 animals were the experimental groups. They were injected intraperitoneally with 0.5 ml of PBS containing $1x10^8$ leptospires/ml.

Three of the control hamsters were sacrificed on day 2 and the last three were killed on day 5. Three infected hamsters were sacrificed at a time at 1 hour, 6 hours and on days 1, 2, 3, 4, 5 and 6 after inoculation. The heart and spleen of all the sacrificed animals were removed and processed for histopathological study.

Preparation of specimen for light microscopy

All the dissected organs were fixed with Bouin's solution for 2 days. The fixed organs were washed in 50% ethanol, followed by 70% ethanol until all the yellow coloration, due to picric acid, was extracted. The fixed organs were then cut into small pieces, dehydrated in a graded series of ethanol solutions and further processed by routine conventional techniques for light microscopy. After processing, the specimens were cut to a thickness of 4 to 6 µm with a rotary microtome, placed on the slides and dried at 60ºC. These sections were then transferred to xylene to remove the paraffin from the sections before being stained with hematoxylin and eosin.

Examination of the sections

All stained sections of the heart and spleen from both the control group and the infected groups were examined carefully under the light microscope. The microanatomical changes in each organ found in the infected group were compared with those of the control group, recorded and photographed.

RESULTS

The hearts of the infected hamsters showed numerous histological changes compared with the normal controls (Fig 1A), including degenerative changes of the cardiac muscle cells, congestion of the cardiac blood vessels, hemorrhage and inflammatory cell infiltration.

The cardiac muscle cells showed vacuolar degeneration. They were enlarged and contained a lightly stained vacuolated cytoplasm. The nuclei were still intact with a vesicular appearance. The boundary of each cell was hardly distinguishable (Fig 1B-C). This pathologic change was first found in the group one hour post-infection. It was found in all infected groups from the first hour to six days after infection but was most clearly observed in the groups into earlier hours of infection. The groups later in the infection had many other pathologic changes which obscured this early finding.

The vessels were dilated and congested (Fig 2A). Some leukocytes were also found. Vascular congestion was first found in the group one hour post-infection. This appearance was found in all infected groups from one hour to six days post-infection.

There were many hemorrhagic areas and interstitial edema on the cardiac sections (Fig 2B). These features were seen in each of the infected groups from six hours to six days post-infection and were more prominent in the later groups.

Some cardiac muscle cells showed various degrees of necrosis. The necrotic process began with condensation of chromatin in the

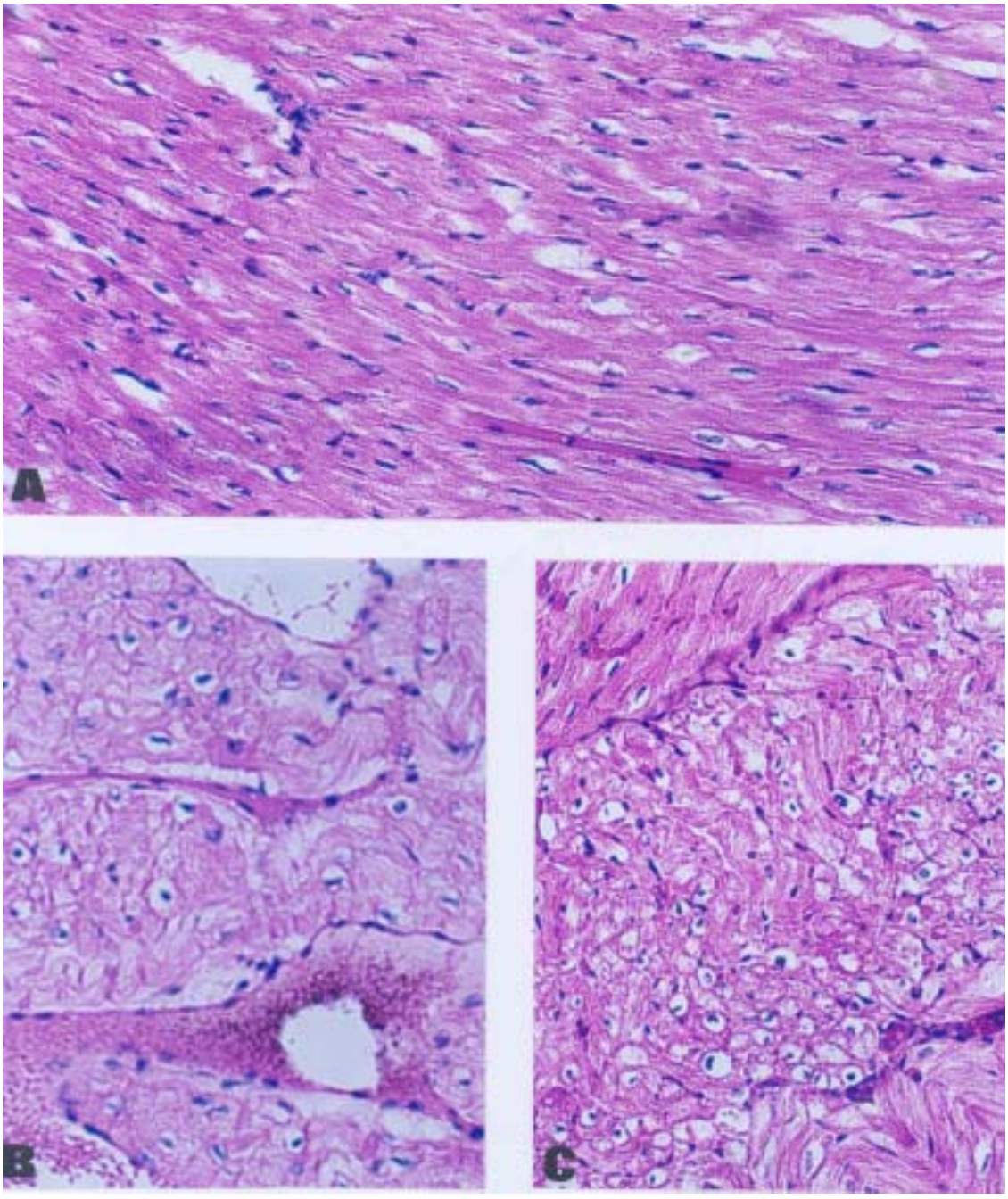

Fig 1–Photomicrographs of the heart illustrating cardiac muscle cells of a normal control hamster (A), one hour (B) and four days post-infection (C) with *Leptospira interrogans*.

A) Normal control hamster, the cardiac muscle cells contain one or two nuclei and abundant cytoplasm which branches to give the appearance of a continuous network (X20). B-C) The cardiac muscle cells of hamsters infected with *Leptospira interrogans* showing vacuolar degeneration. The nuclei are still intact with vesicular appearance. The boundary of each cell is hardly distinguished (X20).

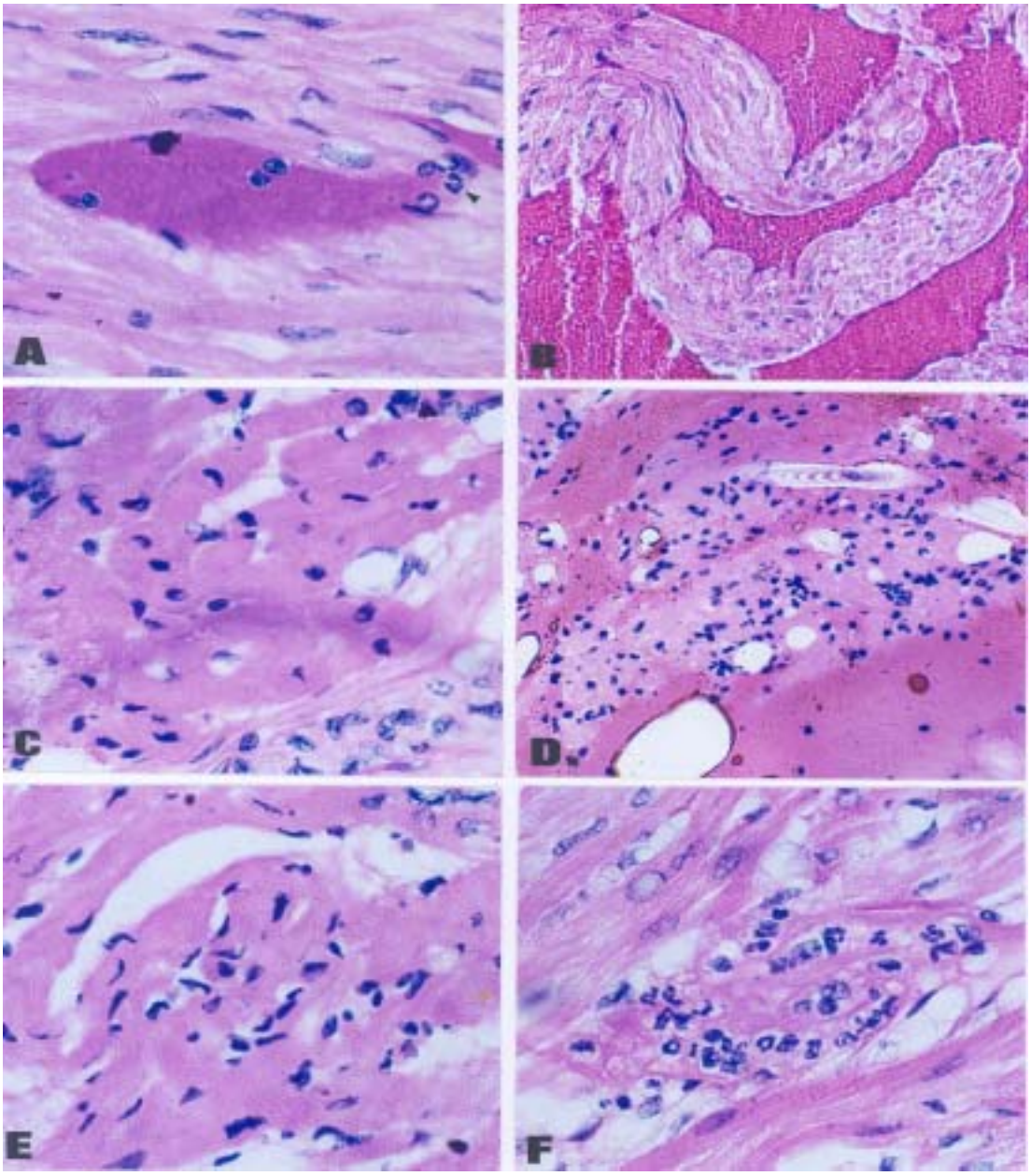

Fig 2–Photomicrographs illustrating the cardiac muscle cells of hamsters infected with *Leptospira interrogans* at one hour (A and C), one day (B), six hours (D and E) and six days (F).

A) The specimen obtained at one hour post-infection shows dilatation of blood vessels. These blood vessels are congested with red blood cells. Some leukocytes (arrow head) were also found (X40). B) The heart one day post-infection reveals areas of interstitial edema and hemorrhage (X10). C-F) Cardiac muscle cells show various degrees of necrosis. The necrotic process begins with condensation of chromatin in the nuclei of the one hour post-infection group (C). The nuclei of cardiac muscle cells are pyknotic and the cytoplasm are altered and appear as homogeneous acidophilic substance. The necrotic cells are surrounded by numerous inflammatory cells (X40, X20, X40 and X40, respectively).

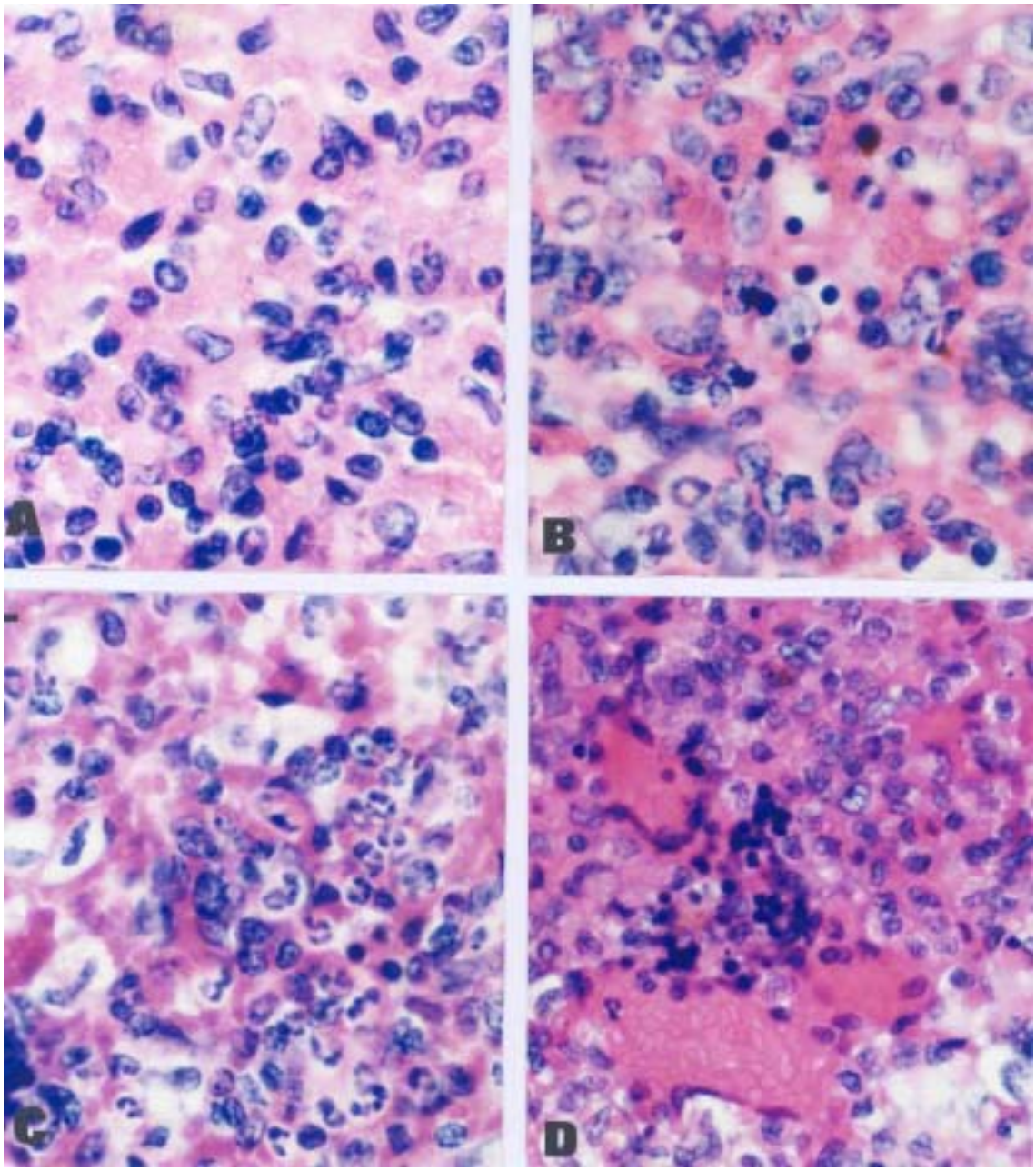

Fig 3–Photomicrographs of the spleen of a normal control hamster (A), at six hours, six days, and five days post-infection with *Leptospira interrogans* (B-D, respectively).

A) Photomicrograph illustrates the parenchyma of red pulp which is permeated by broad interconnected venous sinuses (X40). B) Small foci of cellular necrosis are found scattered throughout the splenic cord. Some nuclei are condensed and some have a pyknotic appearance. The splenic sinusoids are dilated and congested (X40). C) Inflammatory cell infiltration, mainly neutrophils and lymphocytes, are observed (X40). D) The specimen shows many hemorrhagic areas and inflammatory cell infiltration in the red pulp of the infected spleen (X20).

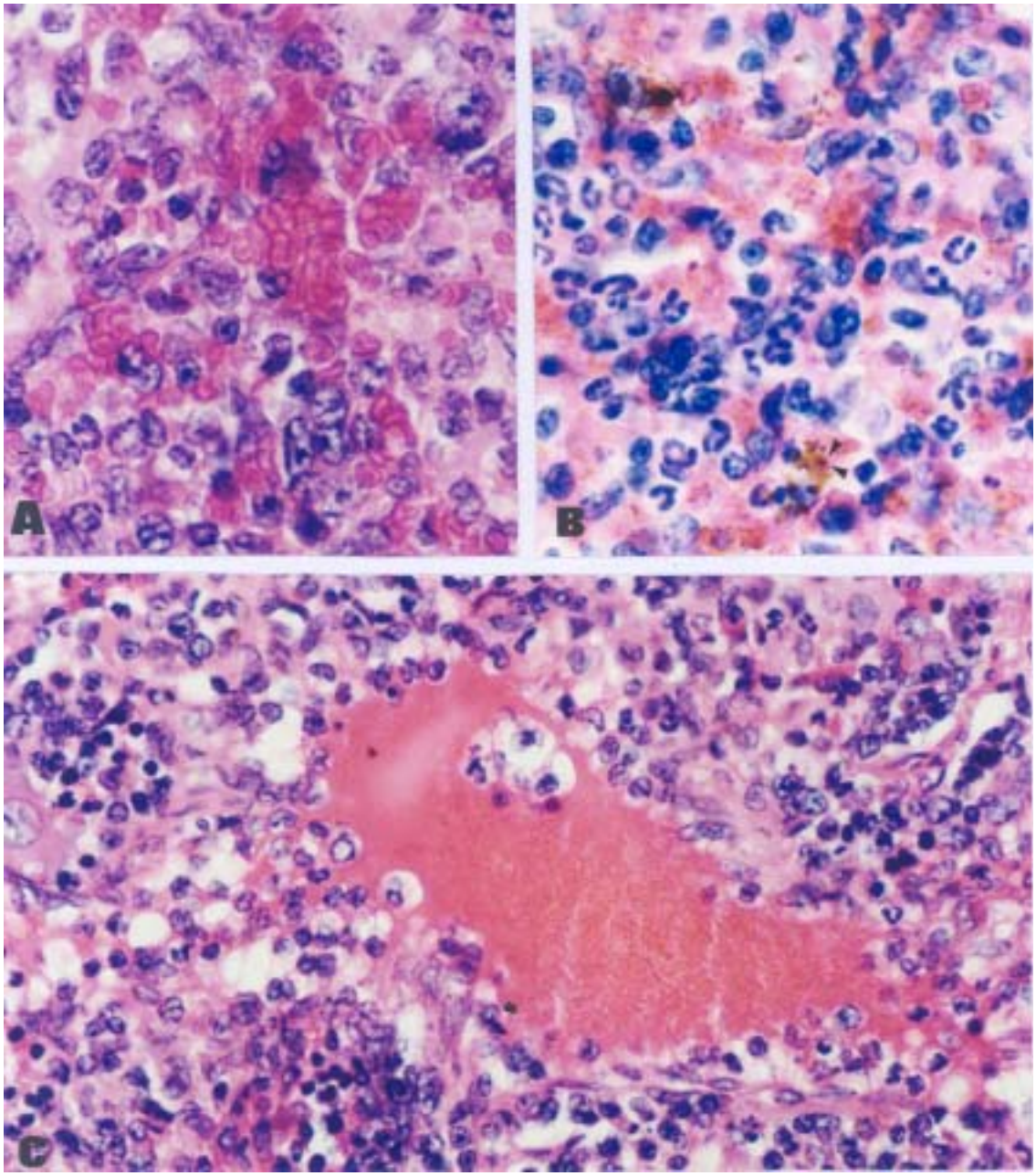

Fig 4–Photomicrographs of hamsters at six days (A and C), and six hours (B) post-infection with *Leptospira interrogans*.

A) The specimen shows dilatation of splenic sinusoids. These sinusoids are congested with red blood cells (X40). B) The spleen shows inflammatory cell infiltration in both the splenic parenchyma and splenic sinusoids. These inflammatory cells are composed of neutrophils and lymphocytes. The brown hemosiderin granules are seen in the dilated splenic sinusoids, and in the cytoplasm of macrophages (arrow head) (X40). C) Photomicrograph of spleen shows many hemorrhagic areas and inflammatory cell infiltration in red pulp (X40).

nuclei (Fig 2C), which then became pyknotic (Fig 2D-F). These features were first found in the group one hour post-infection. It was found in all the infected groups from the first hour of infection to six days after infection. In addition, some cardiac muscle cells showed necrotic cells with altered acidophilic cytoplasm (Fig 2C-F). These features were first found in the group one hour post-infection and found in all the subsequent infected groups.

The necrotic cardiac muscle cells were surrounded by numerous inflammtory cells (Fig 2F). This feature was first found in the group five days post-infection and found in all the infected groups thereafter.

The spleens of the infected hamsters showed numerous histological changes compared with the normal controls, including degenerative changes of the cells of the splenic cord, congestion of the splenic sinusoid, hemorrhage, and inflammatory cell infiltration.

Small foci of the cellular necrosis were found scatteringly throughout the splenic cord. Some nuclei were condensed and had a pyknotic appearance (Fig 3B-D). This pathologic change was first found in the group one hour post-infection. It was observed in all the infected group from the six hours to six days post-infection with increasing severity.

The splenic sinusoids were dilated and congested. Vascular congestion was first found in all the infected groups from the first hour to six days post-infection and more prominent in the later groups (Fig 3B, 4A). In the groups from six hours post-infection and onward, brown hemosiderin granules, the result of hemolysis of red blood cells, were seen in dilated splenic sinusoids, and in the cytoplasm of macrophages (Fig 4B).

There were multiple hemorrhagic areas scatteringly throughout the red pulp of the spleen (Fig 3D, 4C). This appearance was found in the group one day post-infection and in all later infected groups.

Inflammatory cells, mainly neutrophils and lymphocytes, were noted in the splenic parenchyma and sinusoids (Fig 3C-D and 4B-C). This change appeared first in the group one hour post-infection and persisted in all later groups.

DISCUSSION

Almost all the organs studied showed histopathological differences between the hamsters infected with *Leptospira interrogans* and the normal control hamsters. The lesions found in leptospiral infection were not confined only to the kidney and liver, but involved other organs and tissues as well. A report by Pilakasiri *et al* (2001) stated the kidney, lung, liver, gastrocnemius and hamstring muscles of hamsters infected with *Leptospira interrogans* showed various degrees of histopathological changes.

The various stages of change affecting the cardiac muscle cells were observed in this study. In the earliest infected group at one hour, cardiac muscle cells were enlarged and contained the lightly stained vacuolated cytoplasm. This has been described as the cloudy swelling (Damjanov, 1996). This may be related to the swelling of organelles; the cells became waterlogged and vacuoles appeared in the cytoplasm (Burkitt *et al* 1996). Faine *et al* (1999) noted that cardiac muscle fibers may be edematous, flagmented, vacuolated and degenerated in leptospiral infection.

In this study, most cardiac muscle cells showed various degrees of degenerative and necrotic change of their nuclei, including condensation of the nuclear chromatin and pyknosis. These histological observations were mentioned as a sign of myocardial necrosis (Thomas, 1989). According to Arean (1962), the hearts of fatal human leptospirosis cases shows foci of necrosis. Park *et al* (1989) reported that focal myocardial cell degeneration was found in five fatal cases with leptospiral infection.

The cytoplasm of cardiac muscle cells stained bright red with eosin and had an acidophilic homogenous substance. This phenomenon may be related to hyalin (Thomas, 1989). Arean (1962) found granular degeneration with intense acidophilia and clumping of sarcoplasma in the heart of fatal human leptospirosis cases. Park *et al* (1989) reported focal hyalinization of sarcoplasma in five cases infected with leptospirosis.

In this study, necrotic cardiac muscle cells were surrounded by numerous inflammatory cells. This phenomenon is a sign of myocarditis. Sutliff *et al* (1953) reported that acute myocarditis was found in a case infected with *Leptospira pomona*. Faine *et al* (1999) suggested that some cases of leptospiral infection died from toxic myocarditis with localized foci of inflammatory cells infiltration.

Numerous red blood cells were also observed in blood vessels in this study. The above feature is a sign of vascular congestion (Damjanov, 1996). Park *et al* (1989) reported that congestion was also found in the hearts of fatal cases with leptospiral infection. The interstitium was filled with red blood cells and homogenous acidophilic fluid. These features were mentioned as signs of interstitial hemorrhage and interstitial edema, respectively. Hemorrhage is the escape of the blood from the blood vessels. Edema is either the excessive accumulation of fluids inside cells, in extracellular interstitial spaces or in body cavities (Damjanov, 1996). These features may be the result of either endothelial damage caused by leptospires or an inflammatory response provoked by leptospiral toxin. The primary lesion in all forms of leptospirosis in all animals is the damage to the endothelial cell membranes of small blood vessels caused by leptospiral toxin. The immediate effect is to loosen the junctions between cells, allowing fluid and leptospires to migrate into extravascular spaces, followed by erythrocytes, if the damage is severe and prolonged (De Brito *et al*, 1979). According to Arean (1957), hemorrhages were found focally between myocardial fibers. Interstitial edema was pronounced in most studied cases. Park *et al* (1989) noted microscopic hemorrhage in the hearts of fatal cases.

Spleens of leptospirotic hamsters were studied for histopathological changes. It was found that the severity of the lesions increased with the longer the period of infection. Starting with the group one hour post-infection, small foci of cellular necrosis was noted to be scattered throughout the splenic cords. After longer periods of infection, progressive changes in vasculature and congestion of the splenic sinusoids with hemorrhagic areas scattered throughout the red pulp were noted. Later, hemosiderin granules, the result of the hemolysis of the red blood cells, were seen. These changes in the spleens of experimental hamsters are similar to those found in 33 fatal cases of human leptospirosis reported by Arean (1962). They found all cases showed large zones of hemorrhage which resulted from disruption of splenic vasculature. Seven cases showed gross splenomegaly and the histopathology showed congestion with scattered hemorrhagic areas. Faine *et al* (1999) reported that the spleen of infected humans could be either normal or enlarged. Histopathology revealed diffuse or focal hemorrhagic areas with inflammatory cell infiltration, phagocytic cells containing erythrocytes or pigment were also noted. These findings were similar to those found in animal models.

Most lesions caused by leptospiral infection were generally believed to be confined to the kidney. However, this study indicates damage to the spleen as well. It has been proposed that pathogenic leptospires multiply in the blood stream of infected animals and invade many organs of the body (Hartley, 1952; De Brito *et al*, 1965, 1967; Marchall, 1974). In the spleens of early infection there was cellular necrosis, thought to be caused by leptospi-

ral toxin. Leptospiral toxin has been suggested as the main cause of cellular damage in leptospirosis (Arean, 1962; Arean *et al*, 1964). Later, the toxin may also cause damage to the vascular walls by altering the permeability of sinusoidal capillaries allowing the leakage of red blood cells, progressing to hemorrhagic areas scattered throughout the red pulp. Densely packed red blood cells and features of vascular congestion demonstrated in infected spleens were also seen in other organs. Hemosiderin, the result of hemolysis of red blood cells was found later in animal models has also been seen in human leptospiral infection (Faine *et al*, 1999). Hemolysis of red blood cells results in a release of hemoglobin, which is then phagocytosed by macrophages and stored in the cytoplasm in the form of hemosiderin (Damjanov, 1996).

REFERENCES

Arean VM. Leptospira myocarditis. *Lab Invest* 1957; 6: 462-71.

Arean VM. The pathologic anatomy and pathogenesis of fatal human leptospirosis (Weil's disease). *Am J Pathol* 1962; 40: 393-414.

Arean VM. Studied on the pathogenesis of leptospirosis II a clinico-pathologic of hepatic and renal function in experimental leptospiral infection. *Lab Invest* 1962; 11: 273-87.

Arean VM, Sarasin G, Green JH. The pathogenesis of leptospirosis: toxin production by *Leptospira icterohaemorrhagiae*. *Am J Vet Res* 1964; 25: 836-42.

Burkitt HG, Stevens AS, Lowe JS, Young B. Wheater's basic histopathology. 3rd ed. London: Churchill Livingstone, 1996.

Damjanov I, ed. Histopathology: a color atlas and textbook. 1st ed. Baltimore, USA: Williams & Wilkins, 1996.

De Brito T, Bohm GM, Yasuda PH. Vascular damage in acute experimental leptospirosis of the guinea-pig. *J Pathol* 1979; 128: 177-82.

De Brito T, Freymuller E, Penna DO, Santos HS, De Almeida SS, Ayroza Galvao PA. Electron microscopy of biopsied kidney in human leptospirosis. *Am J Trop Med Hyg* 1965; 14: 397-403.

De Brito T, Penna DO, Pereira VG, Hoshino S. Kidney biopsies in human leptospirosis: a biochemical and electron microscopy study. *Virchows Arch Path Anat Pathol* 1967; 343: 124-35.

Faine S, Adler B, Borin C, Perolat P. Leptospira and leptospirosis. 2nd ed. Melbourne: MediSci 1999.

Hartley WJ. Ovine leptospirosis. *Aust Vet J* 1952; 28: 169.

Marchall RB. Ultrastructural changes in renal tubules of sheep following experimental infection with *Leptospira interrogans* serotype pomona. *J Med Microbiol* 1974; 7: 505-8.

Pilakasiri K, Phulsuksombati D, Muensoongnoen J, *et al*. Microanatomical study of the organs of hamsters (*Mesocricetus auratus*) infected with *Leptospira interrogans* serovar pyrogenes which caused an outbreak in Thailand 1999. *Siriraj Hosp Gaz* 2001; 53: 887-905.

Park SK, Lee SH, Rhee YK, *et al*. Leptospirosis in Chonbuk province of Korean in 1987: a study of 93 patients. *Am J Trop Med Hyg* 1989; 41: 345-51.

Sitprija V, Evans H. The kidney in human leptospirosis. *Am J Med* 1970; 49: 780-88.

Sitprija V, Pipatanagul V, Mertowidjojo K, Boonpucknavig V, Boonpucknavig S. Pathogenesis of renal disease in leptospirosis: clinical and experimental studied. *Kidney Int* 1980; 17: 827-36.

Sutliff WD, Shepard R, Dunham WB. Acute Leptospira pomona arthritis and myocarditis. *Ann Intern Med* 1953; 39: 134-46.

Thomas C, ed. Histopathology, textbook of color atlas. 8th ed. Toronto: BC Decker, 1989.