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Pathologic Study of Mice Infected with *Rickettsia tsutsugamushi* R19 Strain

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Scrub typhus, an acute febrile infectious disease caused by R. tsutsugamushi, has been reported from various parts of the far east and pacific rim of Asia including Korea. It is well known that all human pathogenic rickettsia share an affinity to endothelial cells of the small blood vessels and evoke vascular inflammation variably associated with a rash, microthrombi, and hemorrhage. We infected the ICR mice by inoculating sublethal doses of R. tsutsugamushi R19 strain intraperitoneally and observed the pathologic changes by time sequence. The histopathologic features of experimentally induced scrub typhus in the mice were generally nonspecific interstitial inflammations characterized by interstitial pneumonitis, periportal inflammation, multifocal hepatic necrosis, interstitial nephritis, sinusoidal engorgement, and lymphohistiocytic cell infiltration in lymph nodes and spleen. Contrary to the general features of other rickettsial diseases, the pathologic process of scrub typhus experimentally induced by R. tsutsugamushi R19 strain mainly involved the interstitial connective tissue but not the blood vessels.

Key Words: *R. tsutsugamushi, scrub typhus, Boryong serotype*

INTRODUCTION

The histopathologic findings in rickettsial diseases are known to be characteristic. Multiplication of the organisms in the endothelial cells lining the small blood vessels causes endothelial proliferation and perivascular inflammatory cell infiltration, which results in hemorrhage and thrombosis. The result is a widespread infectious vasculitis (Kissane, 1985; Cotran et al., 1989).

The intraperitoneal inoculation of mice with the Karp strain of *R.tsutsugamushi*, the causative agent of tsutsugamushi disease, leads to peritonitis, hepatic granulomas,and splenomegaly (Catanzaro et al., 1976). *R.tsutsugamushi* strains vary considerably in antigenic compositions and in some cases in virulence and other biological properties. Three main antigenic types are generally recognized: Gilliam, Karp, and Kato (Weiss and Moulder, 1984). The predominant serotype of Korean strains of *R.tsutsugamushi* is different from the above three classical strains and the nature of the antigenic characteristics of Korean strain has been previously reported (Chang et al., 1990).

In this study, we observed the pathologic findings of ICR mice which were experimentally infected with *R.tsutsugamushi* R19 strain (Kang et al., 1991).

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MATERIALS AND METHODS

Animals

Male ICR mice grown in our laboratory were used throughout the study.Fourteen mice were infected by intraperitoneal injection of sublethal doses of Rl9 strain which were grown on L cell mono

layer in a volume of 0.5ml and two mice were intraperitonealy injected with 0.5ml of normal saline.

Rickettsial strains

The virulent RI9 strain (Kang et al., 1991) isolated from a patient in Inchon city, Korea was used in this study. The serotype of this strain was previously identified as a new serotype named 'Boryong' by a panel of monoclonal antibodies (Chang et al., 1990;Kang et al., 1991). To isolate the *R.tsutsugamushi* R19 strain, blood samples were injected intraperitoneally into ICR mice. Fourteen days after injection, the spleens were harvested from the mice and homogenized. The homogenates were used to inoculate L-cell culture.

Preparation of specimens

At various time intervals (3,7,10,14,15,16,17 days) after infection, two mice were killed at each time by cervical dislocation and subjected to gross and microscopic observation of thoracoabdominal contents. Representative samples of various abdominal organs as well as abdominal muscles, were promptly fixed in 10% buffered neutral formalin and handled in the usual manner for light microscopic examination. Sections were stained with hematoxylin and eosin.

To observe the rickettsial organisms on paraffin embedded sections, modified Giemsa (Walbach et al., 1922) staining was performed. Cell block of cultured *R. tsutsugamushi* organisms in L cells were used as a positive control.

RESULTS

Gross Examination

External examination did not reveal any specific pathologic changes. No abnormal skin lesions were found around the inoculation sites. All infected mice showed mild degree of splenomegaly and ascites. Other organs did not reveal any conspicuous pathologic changes.

Microscopic Examination

Lungs

Interstitial pneumonitis of varing intensity was found in all stages of infection. The lungs from infected mice sacrificed on day 3 and 7 showed marked dilatation and congestion of septal capillaries, extravasation of red blood cells into the alveoli, and patch conspicuous septal widening by the lymphocytes, histiocytes, and a few polymorphonuclear leukocytes (Fig. 1). The alveolar septa also showed edema especially around the perivascular areas. Although there was peri-bronchiolar or periarterial inflammatory cell infiltration, no active bronchiolitis or arteritis was present. There were a few arteries and veins showing inflammatory cells on their walls, however, neither fibrinoid necrosis nor fibrin thrombus was present (Fig. 2). The pleura was rather unremarkable. The lungs of the later stage (sacrificed on day 10) showed similar pathology although the degree of severity was increased. The lungs of mice sacrificed on days 14, 15, 16 and 17 after inoculation showed pronounced interstitial pneumonitis with prominent alveolar lining cells (Fig. 3).

Liver

Multifocal necrosis and periportal inflammation were the most significant findings observed in all stages of infection. The liver from infected mice sacrificed on days 3 and 7 showed Kupffer cell hyperplasia and focal necrosis with accompanying infiltration of lymphocytes, histiocytes, and polymorphonuclear leukocytes (Fig. 4). Sinusoids were dilated and the periportal areas showed marked infiltration of various types of inflammatory cells (Fig. 5). There was no significant bile stasis. The foci of necrosis became more pronounced the with formation of acidophilic bodies in the later stage(sacrificed on day 10) and inflammatory cells became mainly histiocytes, however, a few polymorphonuclear leukocytes were also found. The sections from days 14, 15, l6, and 17 showed evidence of liver cell degeneration and focal extramedullary hemopoiesis (Fig. 6).

Spleen

Sections of the spleen in all stages of infection revealed mainly sinusoidal engorgement. The follicles tended to be small and marginal zones were rather blurred (Fig.7). In the earlier stage (sacrificed before day 10), the prominent cells in the sinuses and cords were lymphocytes, histiocytes, and polymorphonuclear leukocytes, The polymorphonuclear leukocytes were largely replaced by histiocytes in the later stage.

Lymph nodes

Lymph nodes in all stages of infection showed hyperplasia mainly due to the distension of sinusoids by histiocytes (Fig. 8).

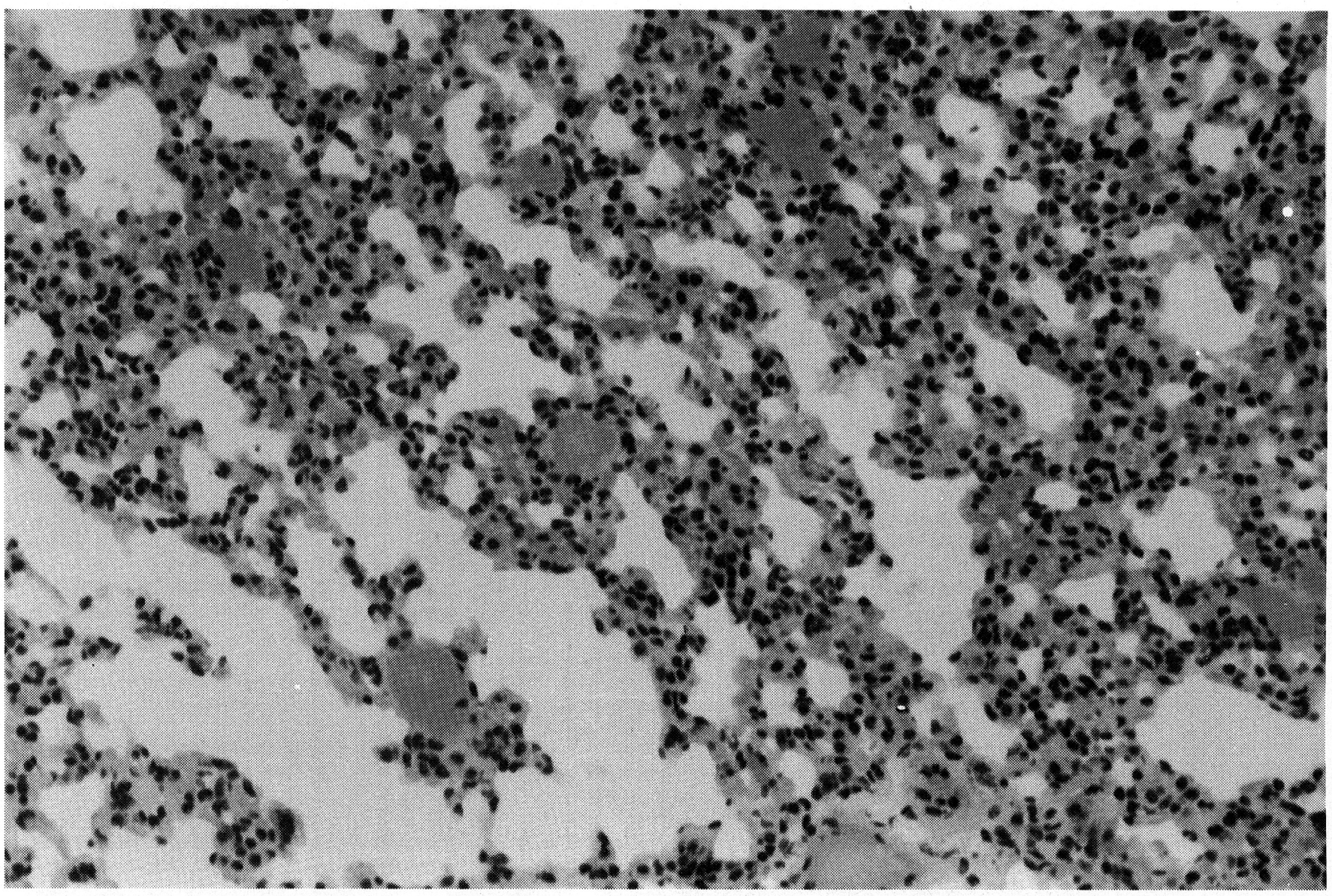

Fig. 1. Interstitial pneumonitis showing patch conspicuous septal wideing by inflammatory cell infiltrates and dilatation of septal capillaries.

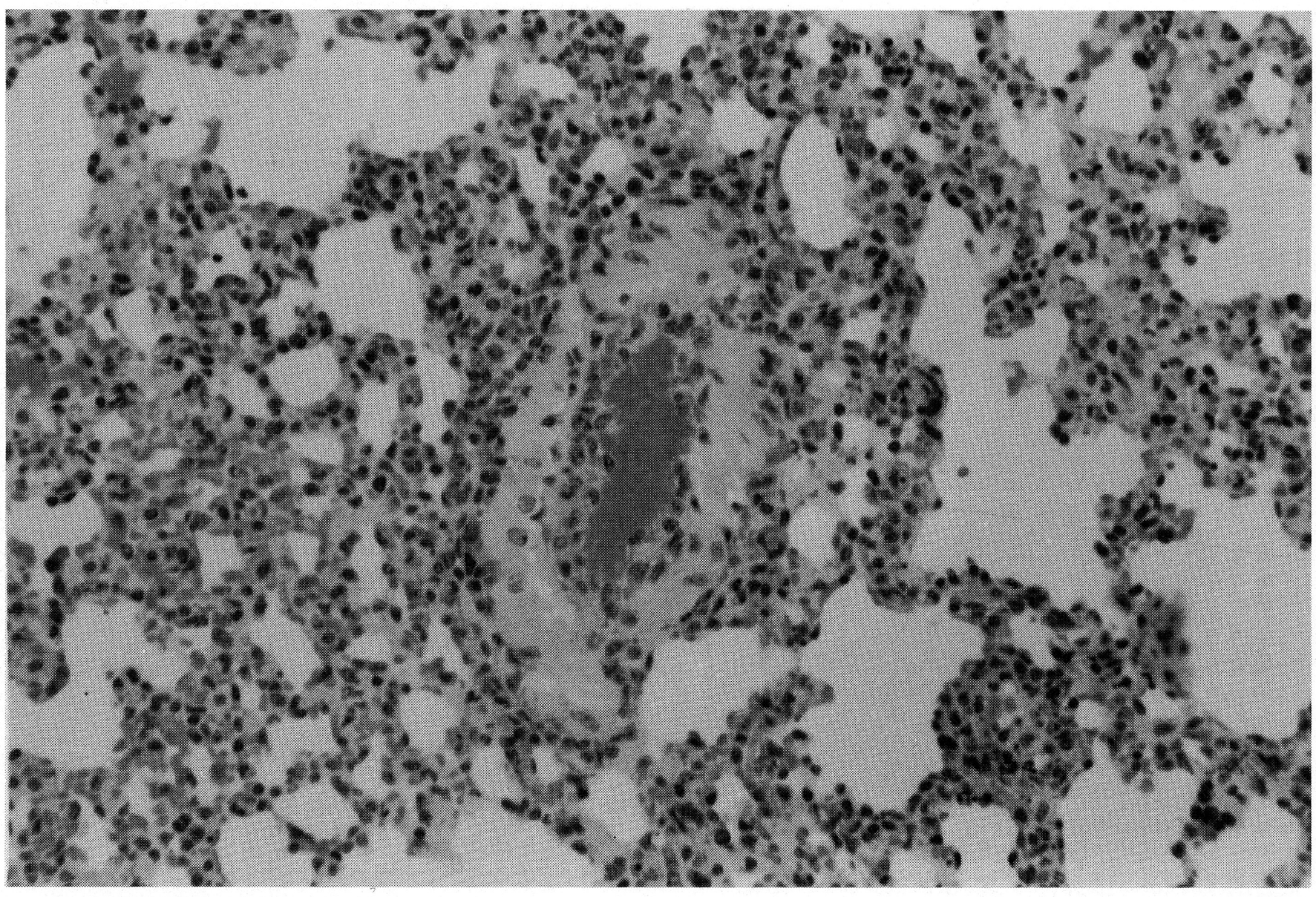

Fig. 2. Prominent periarterial inflammatory cell infiltration, but active arteritis is not present in a section of the lung.

Other organs

Sections of the other organs and tissues show nonspecific interstitial inflammation.

The heart in all stages of infection revealed mild interstitial myocarditis. The cells of the infiltrate consisted of lymphocytes, histiocytes, and a few polymorphonuclear leukocytes. The infiltrate was

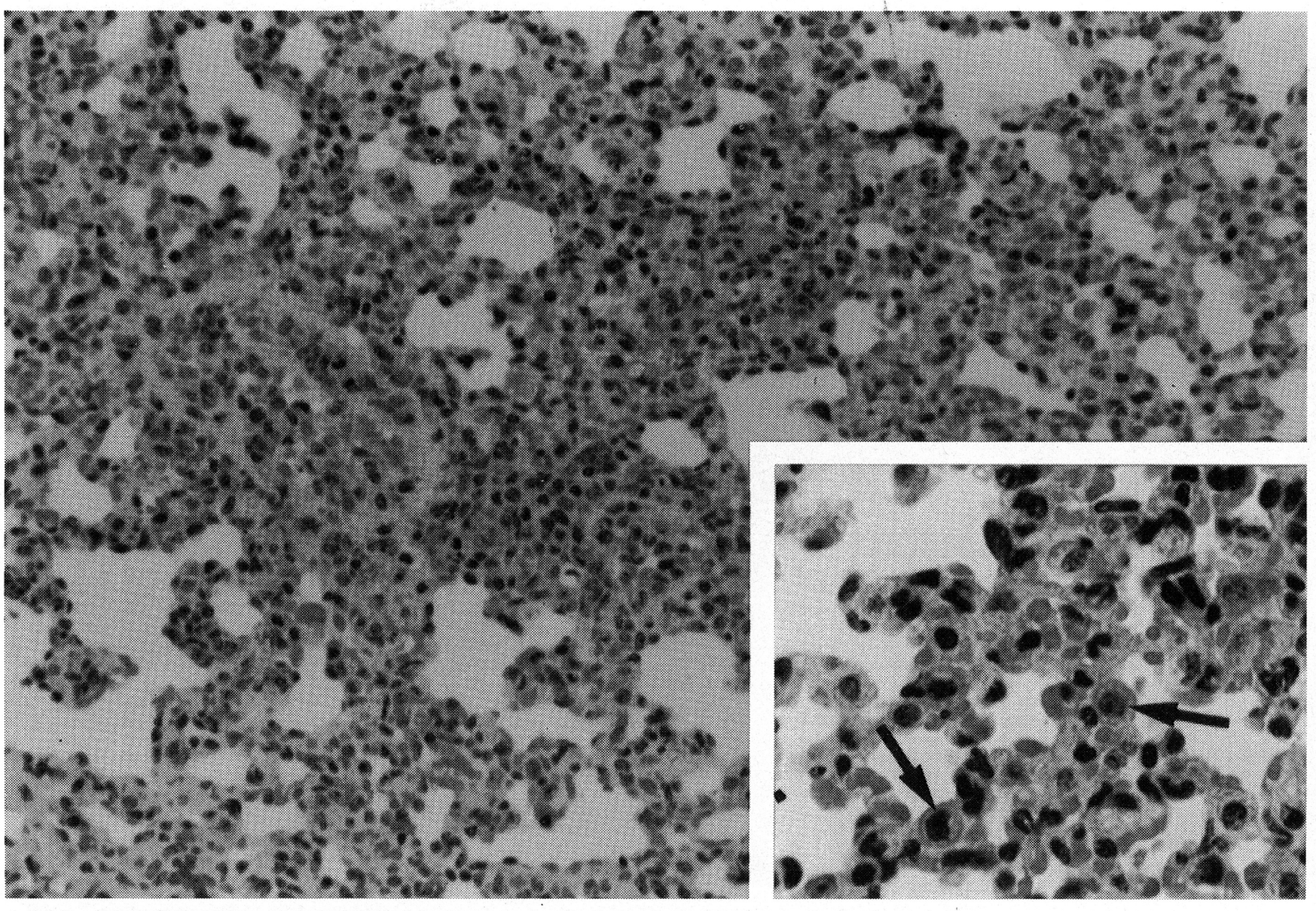

Fig. 3. Severe interstitial pneumonitis showing diffuse septal widening by lymphohistiocytic cell infiltration and prominent alveolar lining cells (arrows).

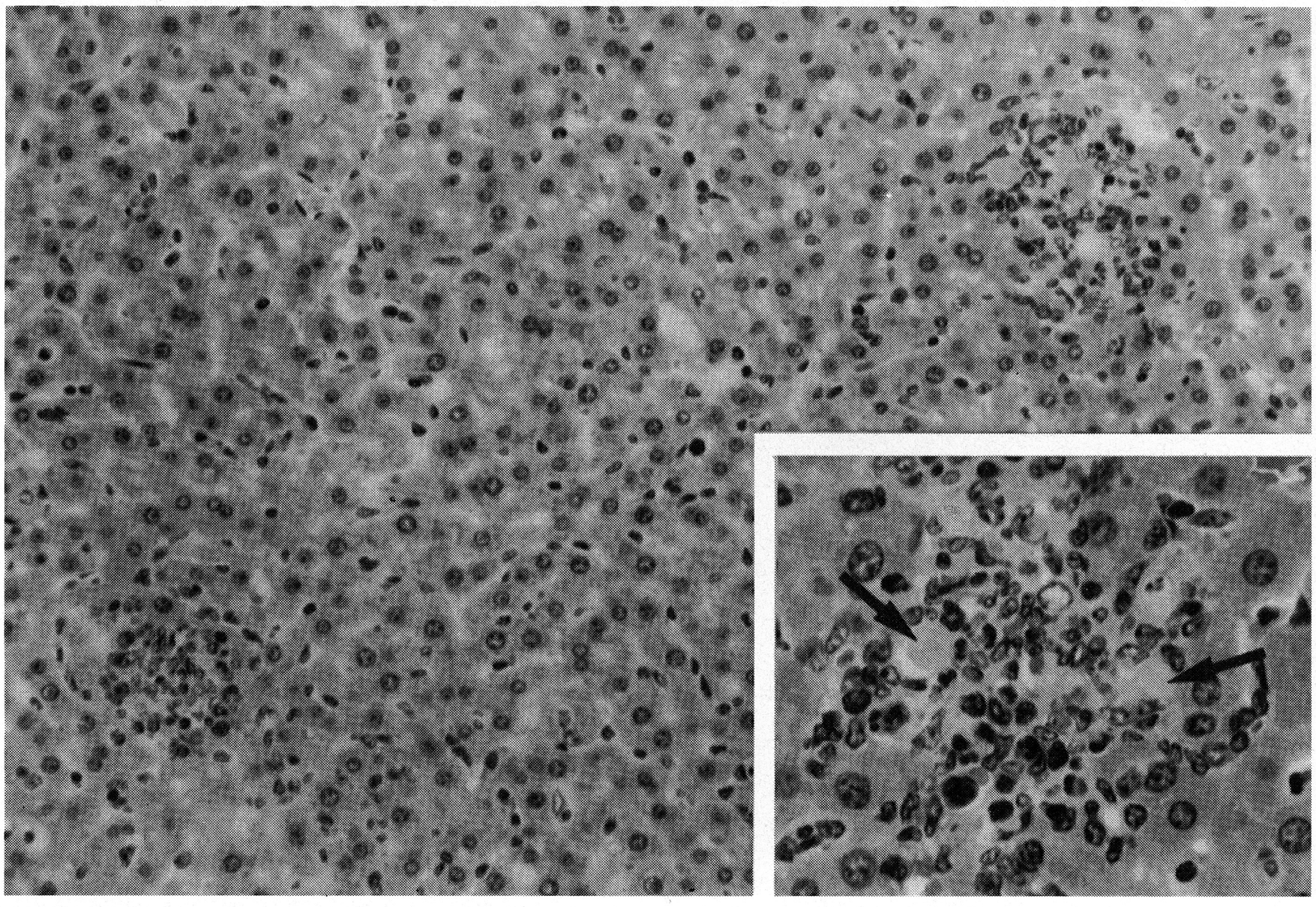

Fig. 4. Multifocal necrosis with Kupffer cell hyperplasia, mild sinusoidal dilatation, and acidophilic body formation (arrows).

located mainly between muscle fibers and some were in the perivascular connective tissues. The epicardium and the endocardium showed inflammatory cell infiltration of the same type as in the myocardium.

The kidneys in all stages of infection revealed interstitial nephritis characterized by mild swelling of the tubular epithelium, interstitial edema, and in-

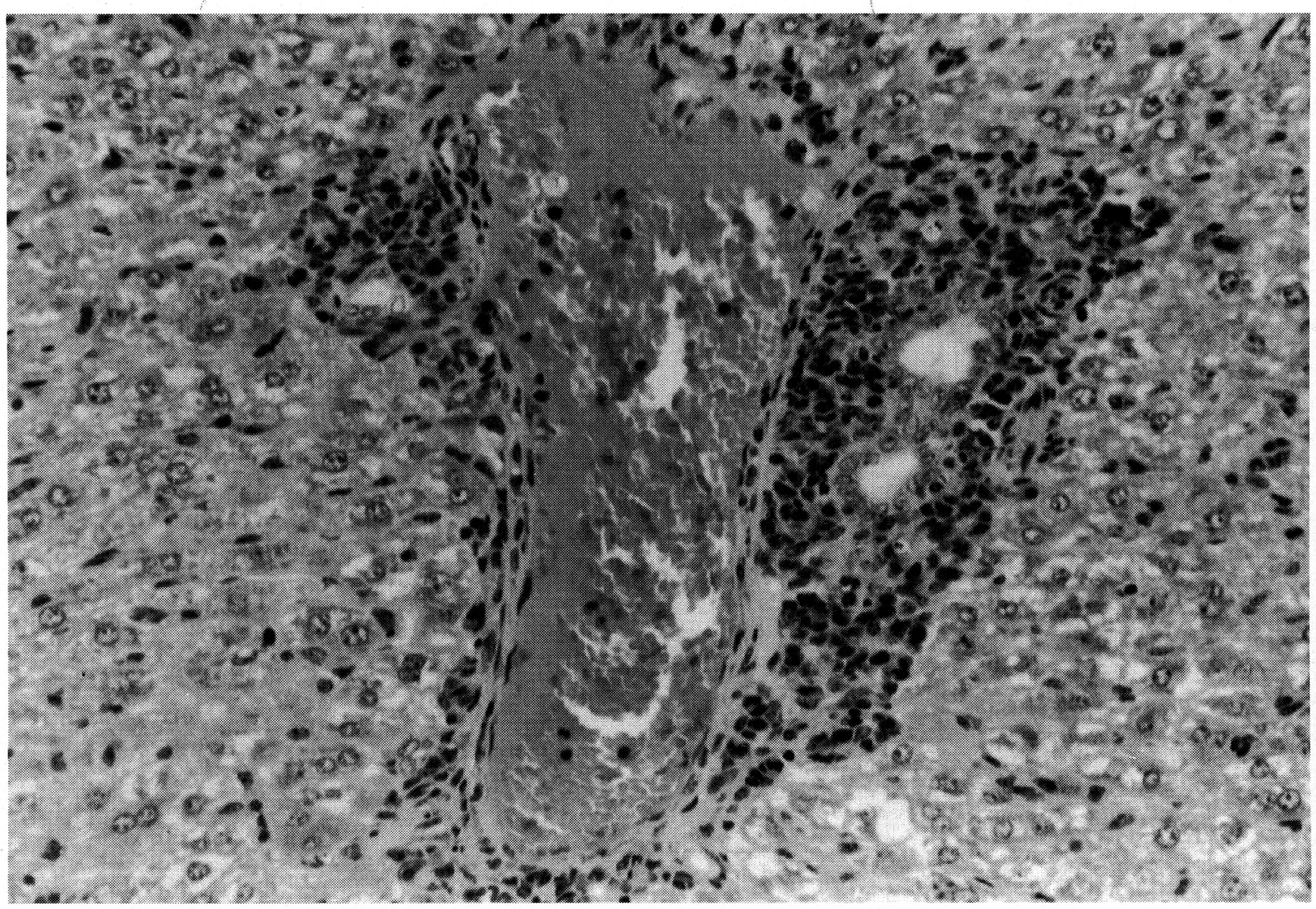

Fig. 5. Prominent periportal inflammation characterized by inflammatory cell infiltrates around the bile ductules and portal venules.

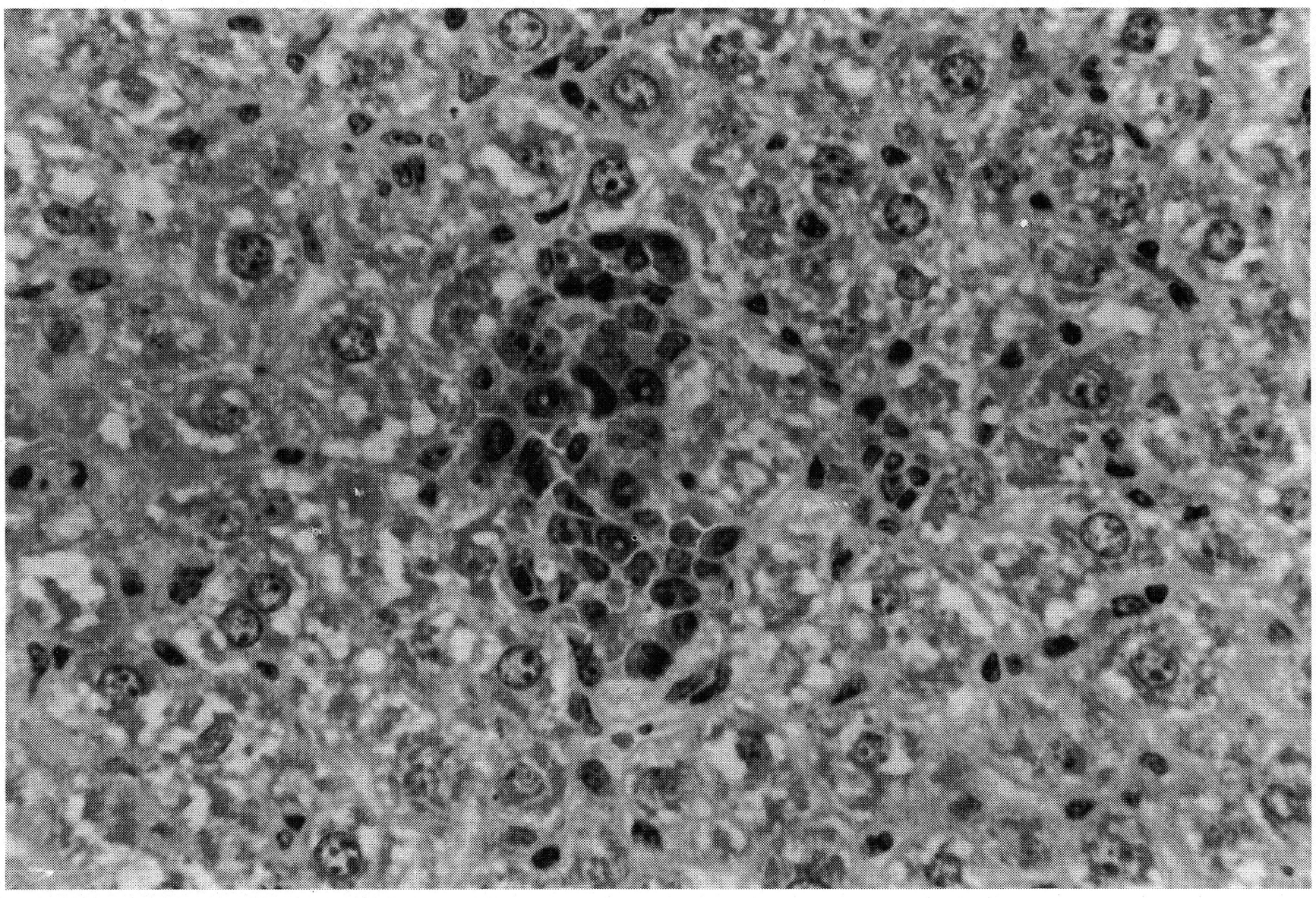

Fig. 6. Feathery degeneration of hepatocytes and focal extramedullary hemopoiesis.

flammatory cell infiltration. The inflammatory cells were almost entirely lymphocytes and histiocytes even in the early stage of infection. The glomeruli appeared unremarkable.

The adrenal glands in all stages of infection revealed interstitial inflammation which progressed with the stage of infection.

The gastrointestinal tract revealed a mild degree

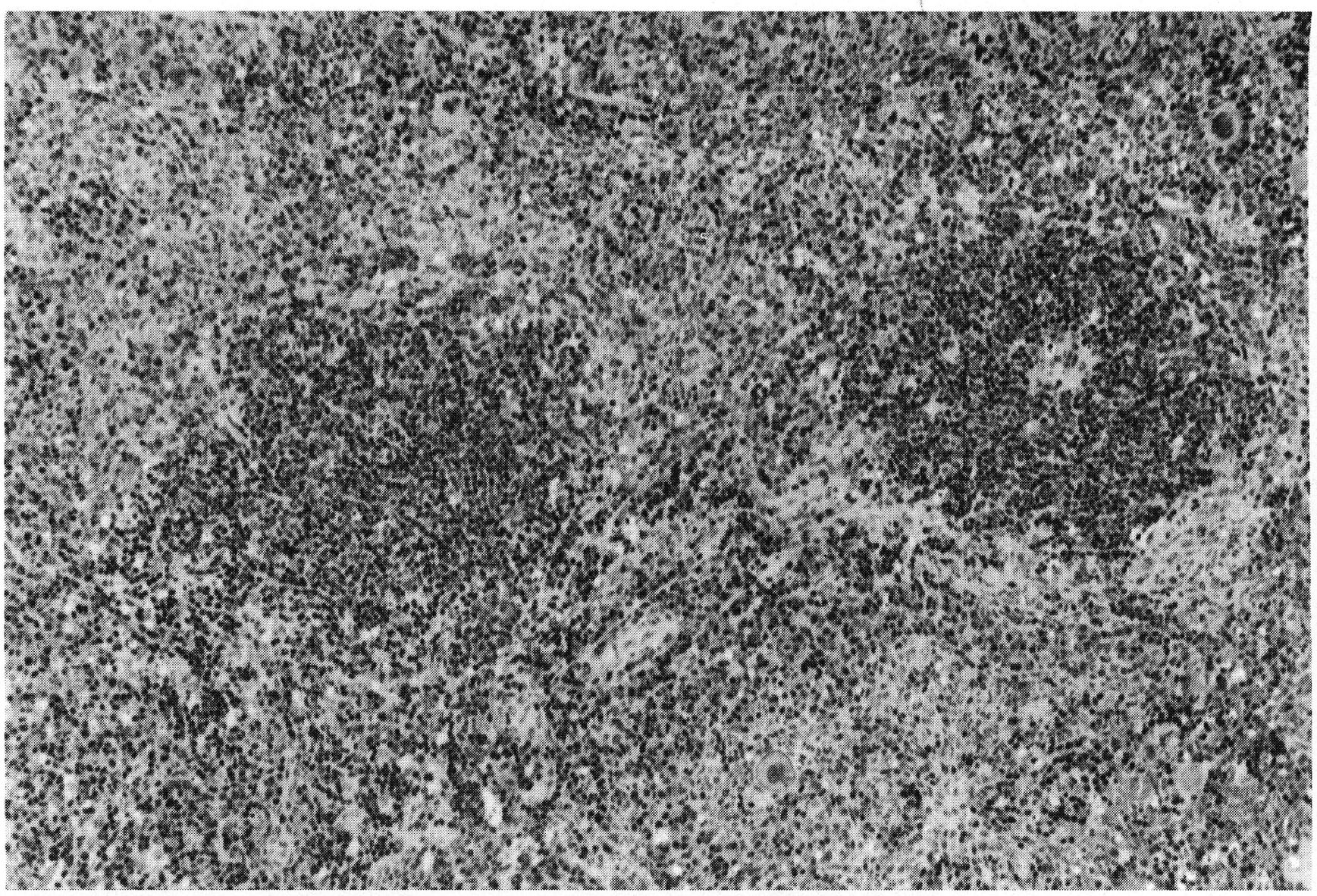

Fig. 7. Spleen shows small sized lymphoid follicles with blurring of the marginal zone (arrow). Sinusoids are dilated by lymphohistiocytic cells.

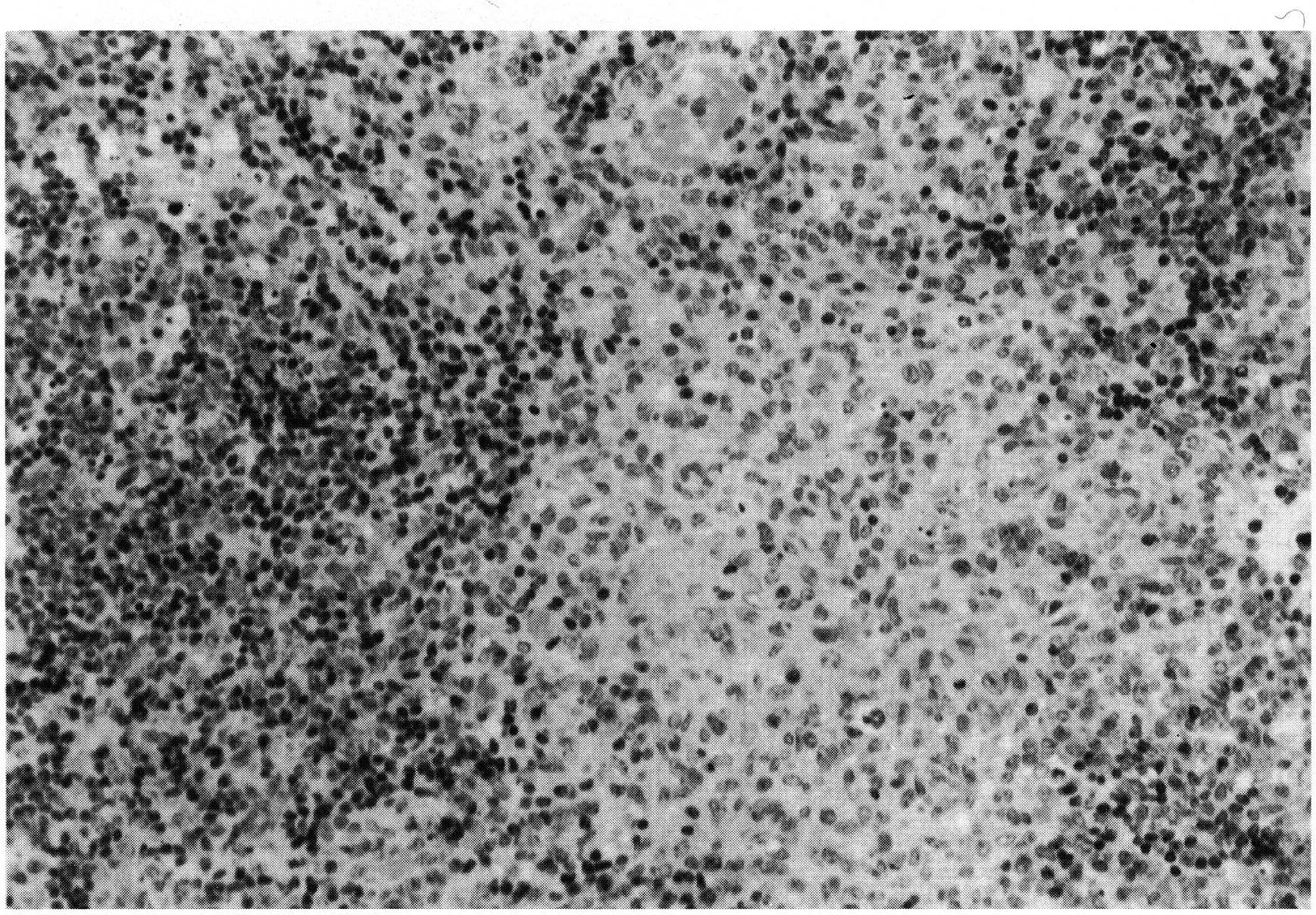

Fig. 8. Lymph node shows distension of the sinusoidal spaces by histiocytic cells.

of lymphohistiocytic cell infiltration of the wall.

The pancreas in the later stages(sacrificed after day 10) showed focal interstitial inflammatory cell infiltrate composed mainly of lymphohistiocytic cells and a few polymorphonuclear leukocytes. The acini and Langerhans islets were unremarkable.

The skeletal muscle in all stages of infection

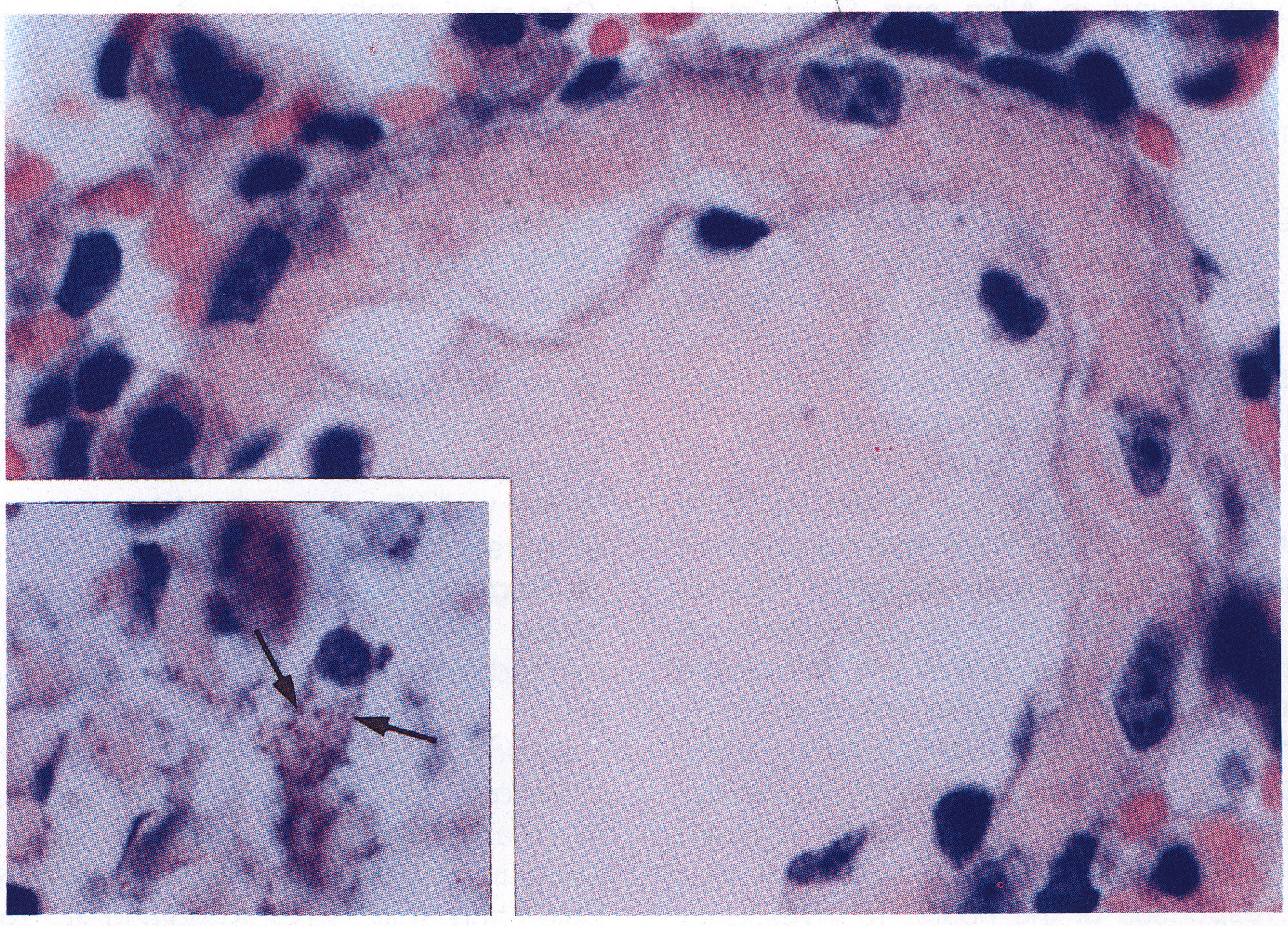

Fig. 9. *R. tsutsugamushi* was not observed in the vessel wall. *R. tsutsugamushi* in cultured L cells (arrows). Modified Giemsa.

showed interstitial inflammation.

Observation for Rickettsial Organism

All sections obtained from infected miced were stained, however, no definite rickettsial organism could be observed (Fig. 9).

DISCUSSION

Scrub typhus, caused by *R.tsutsugamushi,* is an acute febrile mite-borne rickettsial disease endemic in countries of the far east and pacific rim of Asia including China, Indonesia, Australia, the Soviet Union, and India. Sporadic cases have also been reported in the U.S. military persons stationed in Korea since the 1950's and this disease in Korean residents was first reported in 1986 and has been found to be prevalent in all parts of Korea (Munro-Faure et al., 1951; Jackson et al., 1957; Lee et al., 1986; Chang, 1988; kim et al., 1988).

Although there were few experiments describing the morphologic changes of *R.tustusgamushi* infection in mice, (Catanzaro et al., 1976; Ewing et al., 1978) those were limited only to the liver, spleen and the peritoneum. Therefore we can not compare the pathologic changes of the mice infected with *R.tsutsugamushi* R19 strain with the other classical strains of *R.tsutsugamushi.*

The main histologic features of the scrub typhus in mice inoculate with *R.tsutsugamushi* R19 strain were nonspecific inflammation of the interstitium in various organs ; the heart, lungs, kidneys, adrenals, pancreas, and gastrointestinal tract. All fourteen mice showed interstitial pneumonitis, multifocal hepatic necrosis and periportal inflammation, and sinusoidal infiltration of lymphocytes and histiocytes of the lymph nodes and spleen. Similar findings have been described in human autopsy study (Allen and Spitz, 1945). In the present study, the liver showed multifocal necrosis and periportal inflammation similar to the findings described in human (Allen and Spitz, 1945; Choi et al., 1989). Several small granulomas composed of mononuclear cells were also observed in the present study and similar findings were observed in another experimental study (Catanzaro et al., 1976), too The histologic features of the spleen in the present study were different from those of Catanzaro et al. (1976) which showed enlargement of white pulp with prominent germinal center formation. In our study, the white pulp appeared to be small partly due to sinusoidal engorgement and mononuclear cell infiltration. The same features

were also observed in Allen and Spitz' study (1945). Although Allen and Spitz reported observing acute diffuse glomerulonephritis in 30 percent of the human autopsy cases (19 of 64 cases), we were not able to find any light microscopic evidence of glomerular damage. In the present study, interstitial nephritis was continuously present in all stages of infection but glomerular injury was not. In Choi et al's report (1989) of the case of tsutsugamushi disease associated with acute renal failure and hepatitis, the kidney biopsy showed diffuse interstitial edema, multifocal patch interstitial infiltrates in the deep cortex but no evidence of glomerular damage. Since there was no previous report regarding the pathologic chage of the kidney in the mice infected with *R. tsutsugamushi*, we are not sure whether this is due to the differences in the species of the host or in the strains of the infectious organism. Two mice sacrified on the same day appeared to show similar changes but the degree of inflammation was not the same. It might well be true that there would be difference between the responses of the hosts. To investigate the differences between the hosts, more mice with same infection period should be examined.

It is well known that all human pathogenic rickettsia share an affinity for small blood vessel endothelium and cause widespread vasculitis and thrombosis. Although there were perivascular inflammatory cell cuffings, we were not able to find any evidence of active vasculitis in any of the above mentioned organs. We have tried to identify the rickettsial organisms on paraffin embedded sections, but failed to observe definite rickettsial organisms in any of the sections obtained from infected mice although a positive control slide showed characteristic rickettsial organisms. Since this staining method is not specific for *R. tsutsugamushi* R19 strain only, immunohistochemical staining using specific monoclonal antibody should be performed to confirm the organisms. In Allen and Spitz' study of the human autopsy cases(1945), they were able to find vasculitis in macular rash areas of the autopsy patients, but vasculitis in thoracoabdominal organs was not prominent. Since we inoculated the organisms into the peritoneal cavity, it is not surprising that we could not find the skin lesion in any mice.

From the pathologic point of view, rickettsioses have long been regarded as a form of diffuse vascular disease. This concept may well be true in other rickettsiosis, however, the histopathology of the scrub typhus in human(Allen and Spits, 1945; Choi et al., 1989) and mice may warrant a change in this regard. Although focal thrombophlebitis in scrub typhus is not uncommon, actual arteritis can occur rarely. In our study, a few vessels show scant inflammatory cell infiltrate on their walls, however, this does not seem to be a primary lesion but rather appears to be a secondary infiltrate from periarterial inflammatory cell cuffings. Another investigation using fluorescent antibody technique in experimentally infected mice revealed that *R.tsutsugamushi* antigens were deposited mainly in the mesenchymal connective tissue but not in the vessel wall (Kundin et al., 1964). Since there is another report indicating that the site of growth of *R. tsutsugamushi* in mice varies with inoculating routes (Murata et al., 1985), further investigation using different routes of inoculation is recommended.

Besides the well known vasculitis theory, immunologic reaction to rickettsial organisms or the cumulative effect of rickettsial toxin have also been introduced as a pathogenetic mechanism (Allen and Spitz, 1945; Kundin et al., 1964; Cotran et al., 1989). Further study should be performed to define the actual mechanism of tissue damage by this organism.

CONCLUSION

1. The histopathologic features of mice infected with *R. tsutsugamushi* R19 strain were general non specific interstitial inflammations.
2. Interstitial pneumonitis of marked degree was a prominent feature in scrub typhus.
3. Hepatic injury was characterized by multifocal necrosis and periportal inflammation.
4. The lymph nodes and spleen showed hyperplasia mainly due to sinusoidal engorgement and lymphohistiocytic cell infiltration.
5. Contrary to the general concept of rickettsial disease, there was a sparsity of histologically evident vasculitis in scrub typhus.

REFERENCES

Allen AC, Spitz S. : *A comparative study of the pathology of scrub typhus (Tsutsugamushi disease) and other rickettsial diseases. Am J Pathol 21:603-681, 1945.*

Catanzaro PJ, Shirai A, Hilder brandt PK, Osterman JV. : *Host defenses in experimental scrub typhus; Histological correlates. Infect Immune 13: 861-875, 1976.*

Chang WH. : *Occurence of tsutsugamushi disease and prototypes of R. tsutsugamushi in Korea. J Kor Med*

Assoc 36:601-607, 1988.

Chang WH, Kang JS, Lee WK, Choi MS, Lee JH. : *Serological classification by monoclonal antibodies of Rickettsia tsutsugamushi isolated in Korea. J Clin Microbiol 28:685-688, 1990.*

Choi DS, Lee KH, Park JH, Kwon S0. : *A case of Rickettsia tsutsugamushi associated with acute renal failure and hepatitis. Infect 21:117-122, 1989.*

Cotran RS, Kumar V, Robbins SL. : *Robbins pathologic basis of disease. 4th ed., W.B. Saunders Co., Philadelphia, London, Toronto, Montreal, Sydney, Tokyo. 328-333, 1989.*

Ewing EP,JR., Takeuchi A, Shirai A. Osterman JV. : *Experimental infection of mouse peritoneal mesothelium with scrub typhus rickettsiae: An ultrastructural study. Infect Immune 19:1069-1075, 1978.*

Jackson EB, Danauskas JK, Smadel JE ,Fuller HS ,Coale MC ,Bozenam FM. : *Occurrence of Rickettsia tsutsugamushi in Korean rodents and chiggers. Am J Hyg 6: 309-320, 1957.*

Kang JS, Lim BU, Chang WH. : *Characterization of a species-specific antigen of Rickettsia tsutsugamushi isolated in Korea. J Kor Soc Microbiol 26:443-450, 1991.*

Kim MH, Kim SK, Park IS, Oh DY, Pio SJ, Hyun CO, Kim SJ, Hong SY. : *The 50 cases of tsutsugamushi disease occurs in Choong-Chung-Nam-Do on Autumn, 1987. J Kor Med Assoc 31:969-976, 1988.*

Kissane JM. : *Anderson's pathology. 8th ed., The C.V. Mosby Co., St. Louis. 335-341, 1985.*

Kundin WD, Liu C, Harmon P,Rodina P. : *Pathogenesis of scrub typhus infection (Rickettsia tsutsugamushi) as studied by immunofluorescence. J Immunol 93:772-781, 1964.*

Lee JS, Ahn C, Kim YK, Lee M. : *Thirteen cases of rickettsial infection including nine cases of tsutsugamushi disease first confirmed in Korea. J Kor Med Assoc 29:430-438, 1986.*

Munro-Faure AD, Andrew R, Missen GAK, Mackay-Dick J. : *Scrub typhus in Korea. J Royal Army Med Corps 97: 227, 1951.*

Murata M, Sudo K, Suzuki K.Aoyama Y,Nogami S,Tanaka H, Kawamura A. : *Proliferating sites of Rickettsia tsutsugamushi in mice by different routes of inoculation evidenced with immunofluorescence. Japan J Exp Med 55:193-199,1985.*

Walbach SB, Todd JL, Palfrey FW. : *The etiology of pathologic Typhus. 1st ed., Harvard University Press, Cambridge. 13-14.*

Weiss E, Moulder JW. : *Bergey's mannual of systemic bacteriology. Williams & Wilkins Co., Baltimore. 687-698, 1984.*