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Immunofluorescence Studies of Disseminated Hantaan Virus Infection of Suckling Mice

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Hantaan virus, the etiological agent of Korean hemorrhagic fever, was inoculated intracerebrally or intraperitoneally into suckling mice, and the course of the infection was followed by infectivity titration and immunofluorescence studies. Mice became ill and were moribund by 13 to 14 days postinfection. In mice inoculated either intracerebrally or intraperitoneally, virus antigen was present in brain, heart, lungs, liver, and kidney. Less consistently, specific fluorescence was observed in spleen, pituitary gland, thymus, lymph nodes, adrenal, pancreas, salivary glands, trigeminal ganglia, adipose tissue, intestine, and muscle. In all of these tissues, the primary target of infection was the capillary endothelium. In mice inoculated intracerebrally, virus antigen was present mainly in choroid plexus, hippocampal nuclei, and meninges, but in mice inoculated intraperitoneally, central nervous system infection was marked by antigen accumulation in cortical nuclei and thalamus.

The geographically disparate conditions of hemorrhagic fever with renal syndrome (HFRS) in Asia and nephropathia epidemica (NE) in Scandinavia were recognized by Gajdusek (2) in 1953 to be similar clinically and epidemiologically. His hypothesis that these diseases were etiologically related has been tested and confirmed by recent serological studies (12, 19) made possible by the isolation of Hantaan virus (HTN) (11) and its identification as the etiological agent of Korean hemorrhagic fever (8, 11). In recognition of the problems posed by the numerous synonyms for HFRS and nephropathia epidemica e.g., epidemic hemorrhagic fever, hemorrhagic nephroso-nephritis, and benign epidemic nephropathy, the World Health Organization proposed that these and related clinical entities be subsumed under the appellation HFRS (25).

From the first epidemiological studies of HFRS by Russian and Japanese virologists in the 1930s, the role of rodents as natural reservoir hosts has been widely suspected (15, 21). In 1978, Lee confirmed this impression when he isolated HTN from tissues of the rodent *Apodemus agrarius coreae* and established that it was the cause of Korean hemorrhagic fever (9, 11). Other rodents may be important reservoirs of HTN-related viruses elsewhere: infected laboratory rats have been the source of HFRS outbreaks in Japan (3, 24); *Clethrionomys glareolus* has been shown to be the reservoir of the related agent of nephropathia epidemica (1, 5); and *Rattus norvegicus* has been implicated in urban outbreaks of HFRS in Japan (20) and in HTN-related enzootics in the United States (7, 14, 23).

Despite considerable research into HTN infections of wild rodents (9, 10), a suitable laboratory animal model of HTN infection has only recently been described by Lee et al., who showed that Wistar rats could be infected with HTN (13). A detailed immunofluorescence (IF) study of the distribution of HTN antigen in an experimental animal model, however, has not yet been reported. We present the results of an IF study of suckling mice infected with HTN by intracerebral (i.c.) and intraperitoneal (i.p.) inoculation, a model that results in consistent, disseminated, and lethal infection.

MATERIALS AND METHODS

Mice. Outbred ICR mice were obtained from the Centers for Disease Control rodent colony. Monthly serological surveys for reovirus-3 antibody have failed to detect recent evidence of this infection in the colony. One hundred adult ICR mice and 121 Sherman rats from the colony were examined for HTN antibody by the indirect fluorescent-antibody (IFA) test. None had HTN antibody at a serum dilution of 1:4. For experimental work, animals were transferred to the P-4 maximum containment laboratory at the Centers for Disease Control and housed in Bioclean (Fieldstone

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TABLE 1. Hantaan virus infection of suckling ICR mice by i.c. inoculation[a]

Mouse passage level	Inoculum	Log_{10} infectious titer of inoculum[b]	Log_{10} mouse ID_{50}[c]	Log_{10} mouse LD_{50}[c]
SM1	Tissue culture fluid	6.7 ± 0.9	−3.2 ± 0.4	−1.8 ± 0.4
SM2	First passage SM brain	6.5 ± 0.9	−4.9 ± 0.7	−3.7 < < −4.7 ± 0.7
SM3	Second passage SM brain	7.2 ± 0.9	−5.5 ± 0.6	−5.2 ± 0.6
SM4	Third passage SM brain	8.5 ± 0.9	−5.7 ± 0.7	−5.6 ± 0.7
SM5	Fourth passage SM brain	7.9 ± 0.9	−5.7 ± 0.7	−5.6 ± 0.7

[a] SM, Suckling mouse.
[b] $TCID_{50}$ per 0.1 ml, determined in E-6 cells.
[c] Endpoint ± 2 standard deviations per 0.02 ml of inoculum.

Corporation) hoods. (Animals were cared for in accordance with the guide for care and use of laboratory animals of the Institute of Laboratory Animal Resources, National Research Council.)

Virus. The 76-118 strain of HTN originally isolated by serial *Apodemus* passage from *Apodemus* lung (11) was passaged 4 times in A-549 cells and 18 times in E-6 cells (ATCC Vero clone CRL 1586). The virus stock (ATCC VR 938) was tested and found to be free of reoviruses 1, 2, and 3. Dilutions of virus for suckling mouse and cell culture inoculations were made in phosphate-buffered saline (pH 7.3) containing a final concentration of 0.75% bovine albumin, 400 μg of penicillin G per ml, 200 μg of streptomycin per ml, and 8 μg of amphotericin B per ml (BAPBS).

Cell culture titration. Titrations of the virus stock and infected suckling mouse brain suspensions were carried out in E-6 cells. Because HTN does not cause reliable cytopathic effect in this cell line, infection endpoints were determined by IF staining. Fourteen days post-inoculation (p.i.), cells were removed from tubes with glass beads, suspended in phosphate-buffered saline, and dropped into wells of Teflon-coated microscope slides for examination by the IFA technique (4). The primary antibody was human Korean hemorrhagic fever convalescent-phase serum (see below), and the second antibody was anti-human immunoglobulin G conjugated to fluorescein isothiocyanate (FITC) (Burroughs Wellcome Ltd.). The 50% tissue culture infectious dose ($TCID_{50}$) was calculated by the method of Reed and Muench (17).

Animal inoculation. Two- to 4-day-old mice were inoculated with 0.02 ml of virus i.c. or 0.03 ml i.p. Serial passages of virus in suckling mice were carried out by using dilutions of homogenized brain from moribund animals. Infected brains were homogenized as 20% suspensions (vol/vol) in BAPBS; suspensions were clarified by centrifugation (1,000 × *g*) at 4°C, and for titrations, after an initial twofold dilution, further 10-fold dilutions of the supernatants were made. Eight to 10 mice (one litter) were inoculated per dilution. One litter was inoculated with BAPBS alone and harvested 14 days p.i. as a control for the effects of gamma radiation on IF and histological studies.

Mouse 50% lethal doses and 50% infectious doses (LD_{50}s and ID_{50}s) were calculated by the method of Reed and Muench (17). Infection with HTN was defined by (i) death that occurred 7 or more days p.i., (ii) the presence of antibody to HTN 30 days p.i., or (iii) the presence of viral antigen in organs by IFA.

Seventeen animals inoculated i.p. were examined by IF and histological techniques: 10 from the first suckling mouse passage (SM1) and 7 from SM3. Thirty-eight animals inoculated i.c. were examined, 30 from SM1, 6 from SM2, and 2 from SM3. Animals were killed 12 days p.i., removed from the P-4 facility in sealed airtight bags, irradiated at 4°C with 2.6×10^6 rads from a ^{60}Co source, and necropsied under ordinary (P-2) laboratory conditions. Only brains were examined in 15 animals inoculated i.c. (SM1). From all others, brain, pituitary, lungs, thymus, heart, liver, spleen, pancreas, kidneys, muscle, intestines, and adrenal and salivary glands were divided for IF and histological studies.

IF studies. Frozen organs were embedded in OCT compound (Lab-Tek Products) and stored at −70°C until use. Sections (4 μm each) were cut in a cryostat, dried, and fixed in absolute acetone for 10 min. Sections were stained by using an indirect method (4). The primary antibody was human convalescent-phase serum from Korean War cases of epidemic hemorrhagic fever (sera were kindly supplied by W. Jellison). The sera had IFA titers to HTN of 1:>16,384 and were used at a 1:32 or 1:64 dilution. Anti-HTN rabbit serum that had an IFA titer of 1:320 (kindly supplied by G. French) and sera from rats naturally infected with HTN or a related virus (23) with an IFA titer of 1:8,192 were used in parallel with the human sera for confirmation of selected sections. Polyvalent goat antireovirus serum was used at a 1:32 dilution to stain representative sections from animals inoculated i.c.

Antisera were applied to sections which were then incubated for 1 h at room temperature in 100% humidity. After vigorous washing, sections were overlaid with antispecies IgG conjugated to FITC (anti-human IgG–FITC, Burroughs Wellcome, Ltd.; anti-rabbit IgG–FITC, Microbiological Associates; anti-rat IgG–FITC, and anti-goat IgG–FITC, Miles Laboratories, Inc.). After incubation for 1 h at room temperature, the sections were washed again and mounted for examination by fluorescence microscopy.

Histological studies. Tissues were fixed for 1 week in 10% buffered Formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin for examination by light microscopy.

RESULTS

Clinical and gross findings. I.c. inoculation of suckling mice with HTN resulted in illness characterized by ruffled fur, hunched posture, hyperexcitability, and, terminally, by lethargy, coma, and, in some cases, convulsions. Ill animals commonly dragged their rear legs. Runting was a

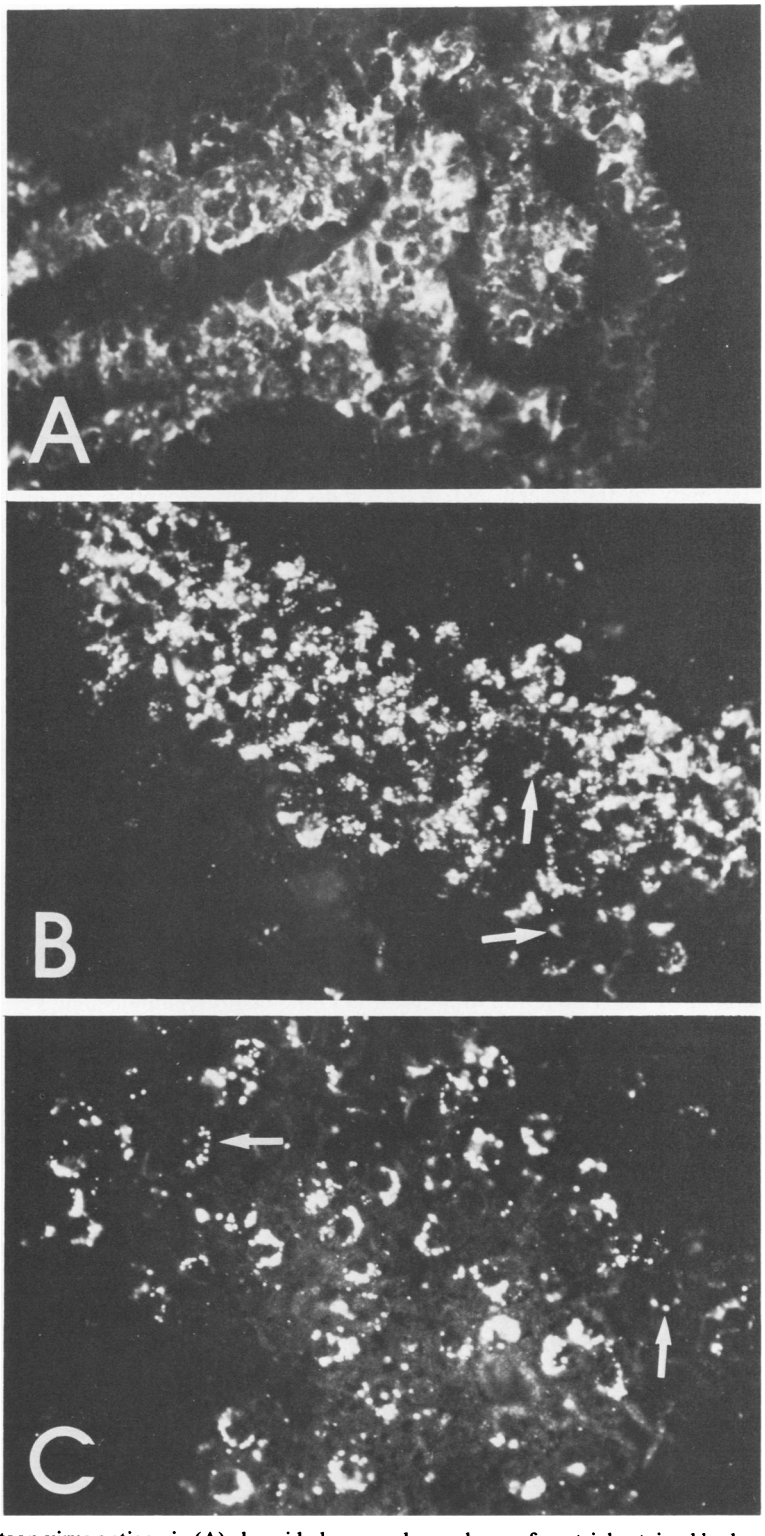

FIG. 1. Hantaan virus antigen in (A) choroid plexus and ependyma of ventricle stained by human antibody 13 days after i.c. inoculation, (B) hippocampal nuclei and capillary endothelium (arrows), and (C) nerve cells, glial cells, and capillary endothelium (arrows) of cerebral cortex after i.p. inoculation.

conspicuous feature of infection, especially in animals that received low virus dilutions and with progressive virus passage. In litters that received dilutions of virus near the ID_{50}, infected mice could often be distinguished from uninfected litter mates by their runted state. Although most infected animals had a progressive course of illness ending in death, three animals that were clinically ill recovered, albeit with significant growth retardation and paralysis and atrophy of hindquarters. Two animals that received a high dilution of virus and were never clinically ill were seropositive 30 days p.i. I.p. inoculation resulted in a similar clinical syndrome. In general, animals died within 1 to 4 days after signs of illness were first observed.

Gross findings at autopsy were unremarkable except for generalized runting and, in some cases, specific atrophy of the spleen and thymus. Evidence of hemorrhage was not observed. Organs appeared normal after gamma irradiation.

With successive passages of HTN in suckling mice, increasingly higher virus yields were obtained from harvested mouse brains (Table 1), as evidenced by increasing $TCID_{50}$s, mouse ID_{50}s, and LD_{50}s. The rate of change of infectivity (ID_{50}) and lethality (LD_{50}) declined by the fourth passage in suckling mice, so that only a fractional increase over the previous passage was noted. The virus ID_{50}/LD_{50} ratio was nearly 1; of 192 infected animals, 187 died (5 animals were alive 30 days p.i. and had IFA antibody to HTN).

Distribution of viral antigen by IF. To establish the distribution of viral antigen in various organs after infection, we examined by IF 38 suckling mice inoculated i.c. and 17 mice inoculated i.p. We found disseminated HTN infection in all 55 cases; this was evidenced by the presence of viral antigen in numerous organs and in capillary endothelium in every site studied. Without exception, HTN antigen was found in frozen sections of brain, heart, lungs, liver, and kidneys. HTN antigen also appeared, although not in every animal, in the spleen, pituitary gland, adipose tissue, thymus, salivary glands, pancreas, adrenal glands, lymph nodes, muscle, and intestines. No differences in the pattern of disseminated infection could be found between mice inoculated i.c. and i.p., except for the distribution of antigen within brain. Although the distribution of viral antigen in infected mice from SM1, SM2, and SM3 was similar, the quantity of antigen appeared greater in organs of animals that had received virus of higher mouse passage. Viral antigen was detected to a greater extent in parenchymal cells of infected organs in animals in the second and third mouse passages. Human, rabbit, and rat antibodies gave the same staining patterns in serial sections. No staining was observed with antireovirus antibody. Gamma irradiation did not result in nonspecific or unusual IF staining patterns in irradiated controls.

In mice inoculated i.c., viral antigen was concentrated mostly in the choroid plexus (Fig. 1A), ependyma of ventricles, hippocampal nuclei (Fig. 1B), meninges, cortical nuclei, and, less frequently, in the thalamic area, pons, cerebellum, trigeminal ganglia, and spinal cord dorsal root ganglia. Specific fluorescence appeared as an aggregation of small granules in the cytoplasm of neurons, glial cells, capillary or vascular endothelia, and adventitia of vessels. Dendrites of neurons also contained antigen.

I.p. inoculation resulted in a different distribution of antigen in the brain. HTN antigen was spread throughout the cortex (Fig. 1C) and thalamus without exception, but little antigen was seen in the choroid plexus or meninges. Schema of the typical distribution of HTN antigen after i.c. and i.p. inoculation are shown in Fig. 2A and B, respectively. Both the brightness of IF staining and the number of infected foci in the lungs, liver, and kidneys were greater in mice inoculated i.p. than in those inoculated i.c.

In lungs, antigen was detected as small foci in the interstitium, in some alveolar cells, capillary endothelia, and sometimes in the vascular adventitia (Fig. 3A). Specific fluorescence in the heart was located in the myocardium, endocardium, and, less frequently, in the epicardium (Fig. 3B). Viral antigen was detected in capillary endothelium of the heart in every animal examined.

IF examination of liver disclosed antigen in Kupffer cells, hepatocytes, capillary endothelium, and endothelium of small vessels, but not in infiltrated mononuclear cells in portal triads (Fig. 3C). In the kidneys, specific fluorescence was seen mainly in the capillaries of interstitial areas of the medulla (Fig. 3D) and less often in the cortex. In some cases, tubular epithelial cells in the medulla and glomerular capillaries contained antigen.

HTN antigen was detected less consistently in other organs. All spleens from mice inoculated i.p. contained viral antigen. In the spleen (Fig. 3E), capillary endothelium in the white pulp and marginal zones and histiocytes in red pulp were stained. There was specific fluorescence in epithelial cells and in the capillary endothelium of the pituitary gland. Viral antigen was also recognized in the adrenal medulla and cortex. The capillary endothelium of the pancreas, submandibular salivary glands (Fig. 3F), and pancreatic islet cells contained viral antigen. In most cases, after either i.c. or i.p. inoculation, brown fat from the neck and abdominal cavity exhibited positive staining (Fig. 3G). Antigen was also

detected in many animals in capillaries of subcapsular areas of the thymus and lymph nodes (Fig. 3H) and in the subserosa of the intestines in smooth muscle and connective tissue.

Histological findings. Tissue architecture and tissue staining characteristics were not affected by gamma irradiation. Microscopic observation of specimens stained with hematoxylin and eosin revealed passive hyperemia of all tissues. In the brain, small foci of necrotic neurons accompanied by glial cell proliferation and occasional hemorrhage were diffusely distributed. Mononuclear cell infiltration of the meninges or perivascular cuffs or both was observed in mice inoculated both i.c. and i.p.

In the heart, myocarditis or pericarditis was characterized by infiltration of mononuclear cells into the myocardium or epicardium or both. Inflammation was most marked in the atria. In the lungs, prominent features were congestion, edema, and interstitial pneumonitis. Focal areas of hemorrhage were found occasionally. The liver showed mononuclear cell infiltration in the portal triad and various degrees of liver cell degeneration. In kidneys, the cortex was usually unremarkable; however, marked medullary congestion with hemorrhage into the interstitium was a striking and consistent observation.

Spleen and thymus follicles were slightly atrophic. Salivary, adrenal, and pituitary glands, pancreas, intestines, adipose tissue, lymph nodes, and bone marrow showed no characteristic changes.

DISCUSSION

In a preliminary communication, we reported that Hantaan virus causes lethal, disseminated infection of suckling mice (22). In this paper, we describe the distribution of viral antigen in mice infected i.c. and i.p. Although Hantaan virus infection caused few deaths in the first mouse passage from cell culture, viral antigen was widely distributed. With further passage of virus in mice, LD_{50}s increased but antigen remained in the same organs and cell types, although it appeared in greater quantity. Few differences in antigen distribution were detected in mice inoculated i.c. or i.p.

Five organs (brain, lungs, heart, liver, and kidneys) were infected in all animals, irrespective of the route of inoculation. In these organs, viral antigen was consistently detected in parenchymal cells as well as capillary endothelium. In endocrine glands such as pituitary and adrenal glands and pancreas, viral antigen was less often found in parenchymal cells. In other tissues such as brown fat, lymph nodes, and intestines, viral antigen, when it could be found, was localized only in capillary endothelium. In the spleen,

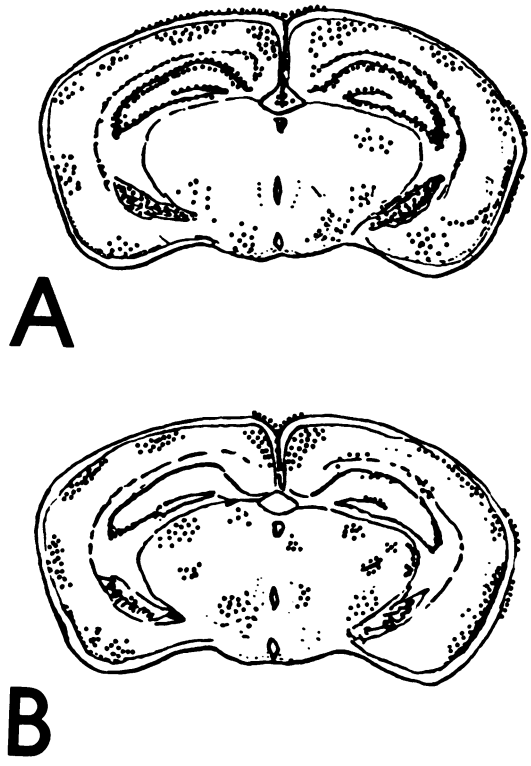

FIG. 2. Distribution of viral antigen in the brain after i.c. inoculation (A) and after i.p. inoculation (B).

Hantaan virus antigen was present simultaneously in red pulp and capillary endothelium in most cases, but in some instances, antigen was detected only in capillary endothelium. The widespread distribution of viral antigen in capillary endothelium suggests that it is the target cell of Hantaan infection in suckling mice.

The distribution of antigen in mice infected i.p. and i.c. did not differ, except for greater involvement of the meninges and choroid plexus in mice inoculated i.c. In unpublished work (T. Kurata and T. Tsai), we have found that suckling mice inoculated subcutaneously, intramuscularly, and intranasally have lethal disseminated infection, with an antigen distribution indistinguishable from that found in mice inoculated i.p. The regular and extensive infection of brain, regardless of the route of infection, suggests that HTN is highly neuroinvasive and neurotropic in suckling mice.

The fidelity of the suckling mouse model of HTN infection to human HFRS cannot be fully evaluated because few human cases have been examined for HTN antigen by IF. We have examined Formalin-fixed materials from six Korean War cases by IF (tissues kindly provided by D. Wear, Armed Forces Institute of Pathology). Sections were treated with trypsin and stained

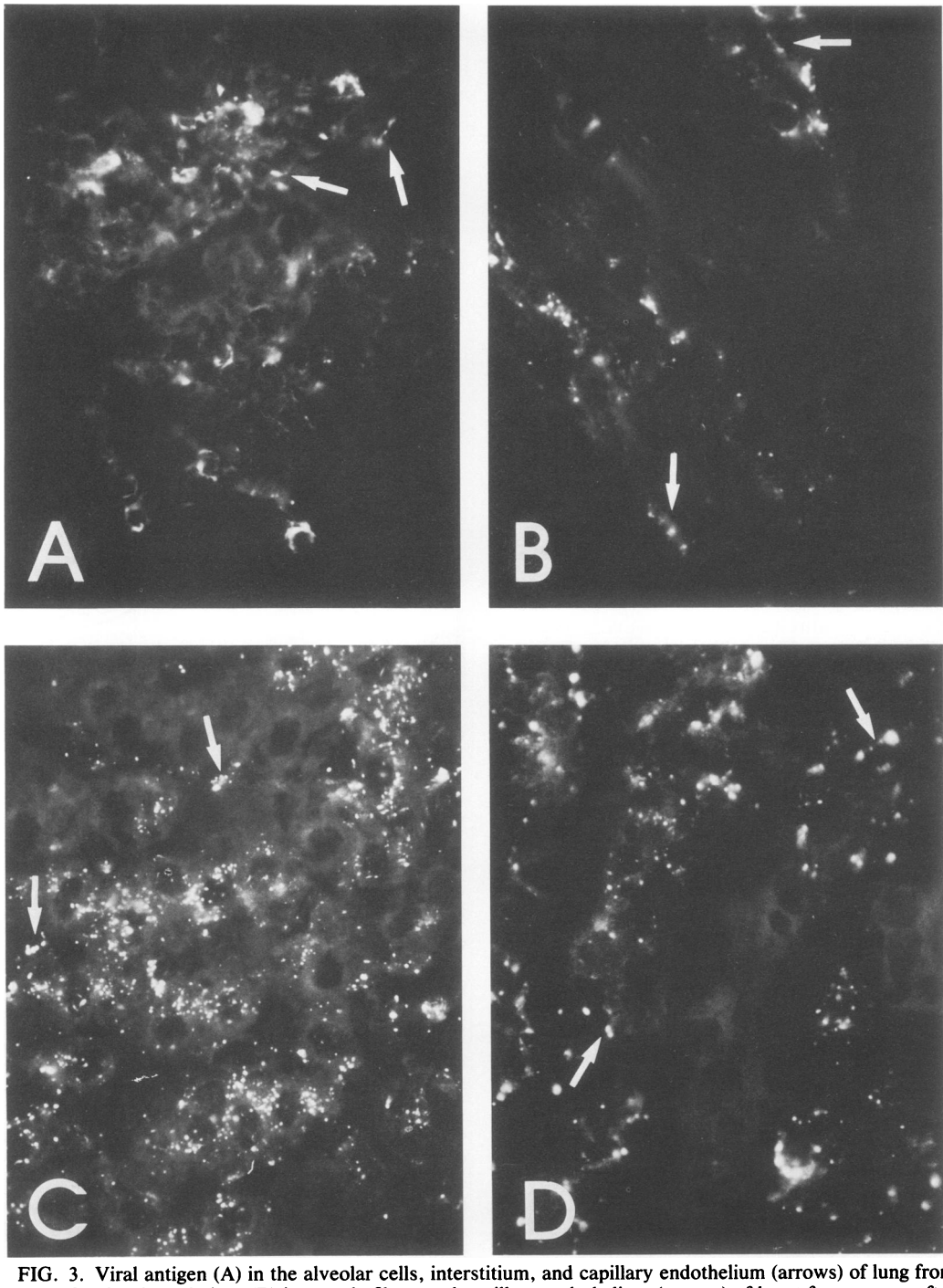

FIG. 3. Viral antigen (A) in the alveolar cells, interstitium, and capillary endothelium (arrows) of lung from mouse after i.c. inoculation, (B) in muscle fibers and capillary endothelium (arrows) of heart from mouse after i.p. inoculation, (C) in liver cells, Kupffer cells, and capillary endothelium (arrows) of the liver after i.p. inoculation, (D) in interstitial cells and capillary endothelium (arrows) of renal medulla from i.p. inoculated mouse, (E) in histiocytes and capillary endothelium (arrows) of the spleen from i.p. inoculated mouse, (F) in glandular cells and capillary endothelium (arrows) in submandibular gland from i.p. inoculated mouse, (G) in adipose tissue from abdominal cavity in i.c. inoculated mouse, with examples of antigen in capillary endothelium indicated by arrows, and (H) in capillary endothelium (arrow) in subcapsular area of cervical lymph node after i.c. inoculation.

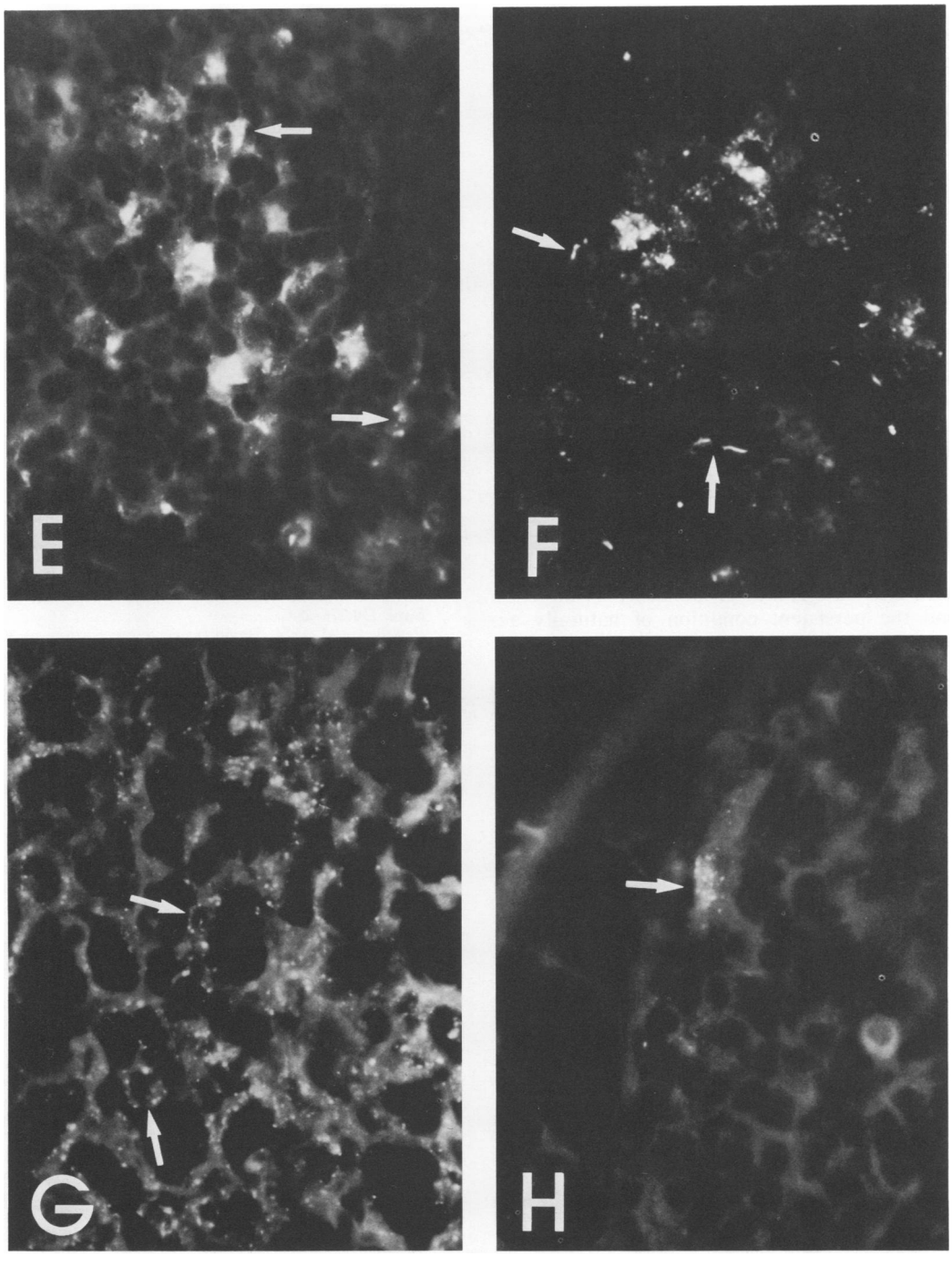

FIG. 3—*Continued*

by IF for HTN antigen; specific fluorescence was found in capillary endothelium of spleen and liver, in Kupffer cells, in epithelial cells of the pituitary gland, and in the adrenal medulla (Kurata and Tsai, unpublished data). These preliminary observations suggest that there may be similarities in the distribution of viral antigen in human and suckling mouse infection. Histopathologically, the reported myocarditis, interstitial pneumonitis, and nephritis, and focal hemorrhages of brain and other organs seen in human HFRS (16, 18), are similar to the lesions we

observed in suckling mice. In humans, hemorrhage and mononuclear cell infiltration in the atria have been distinctive features of myocardial pathology (16), as was the case in infected mice. The observations of congestion and exudation of erythrocytes in the renal medulla of experimental animals are congruent with the renal pathology reported in human infection (16, 18).

Because persistently infected rodents are the reservoir hosts of HTN and related viruses (6, 8, 10), laboratory mice may prove useful in elucidating the mechanisms of viral persistence. In six wild *Rattus norvegicus* caught in U.S. cities, HTN antigen was found by IF in spleen, kidney, and lung. Viral antigen was found in the capillary endothelium of spleen and in interstitial areas of lung and renal medulla (T. Kurata and T. Tsai, unpublished data). We have similarly observed persistence of antigen in spleen, kidney, lung, and heart of mice examined 3 months after inoculation and in brain 5 months after i.c. inoculation (Kurata and Tsai, unpublished data). Thus, the mouse may prove a useful model for both the acute HFRS of human HTN infection and the persistent condition of naturally acquired rodent infection.

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LITERATURE CITED

1. **Brummer-Korvenkontio, M., A. Vaheri, T. Hovi, C. H. von Bonsdorff, J. Vuorimies, T. Manni, K. Penttinen, N. Oker-Blom, and J. Lähdevirta.** 1980. Nephropathia epidemica: detection of antigen in bank voles and serologic diagnosis of human infection. J. Infect. Dis. **141**:131–134.
2. **Gajdusek, D. C.** 1953. Acute infectious hemorrhagic fevers and mycotoxicoses in the USSR. Medical science publication no. 2. Walter Reed Army Medical Center, Washington, D.C.
3. **Kawamata, J.** 1982. Laboratory cases of epidemic haemorrhagic fever and their control in Japan. W.H.O. working group on haemorrhagic fevers with renal syndrome, Tokyo, Japan.
4. **Kawamura, A., Jr.** 1977. Staining methods, p. 77–94. *In* A. Kawamura (ed.), Fluorescent antibody techniques and their applications. University Park Press, Baltimore.
5. **Korpela, H., and J. Lähdevirta.** 1978. The role of small rodents and patterns of living in the epidemiology of nephropathia epidemica. Scand. J. Infect. Dis. **10**:303–305.
6. **Lähdevirta, J.** 1971. Nephropathia epidemica in Finland: a clinical, histological, and epidemiological study. Ann. Clin. Res. **3**(Suppl. 8):1–154.
7. **LeDuc, J. W., G. A. Smith, L. R. Bagley, S. E. Hasty, and K. M. Johnson.** 1982. Preliminary evidence that Hantaan or a closely related virus is enzootic in domestic rodents. N. Engl. J. Med. **307**:623.
8. **Lee, H. W.** 1982. Korean hemorrhagic fever. Prog. Med. Virol. **28**:96–113.
9. **Lee, H. W., G. R. French, P. W. Lee, L. J. Baek, K. Tuschiya, and R. S. Foulke.** 1981. Observations on natural and laboratory infection of rodents with the etiologic agent of Korean hemorrhagic fever. Am. J. Trop. Med. Hyg. **30**:477–482.
10. **Lee, H. W., P. W. Lee, L. J. Baek, C. K. Song, and I. W. Seong.** 1981. Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent *Apodemus agrarius*. Am. J. Trop. Med. Hyg. **30**:1106–1112.
11. **Lee, H. W., P. W. Lee, and K. M. Johnson.** 1978. Isolation of the etiologic agent of Korean hemorrhagic fever. J. Infect Dis. **137**:298–308.
12. **Lee, H. W., P. W. Lee, J. Lähdevirta, and M. Brummer-Korvenkontio.** 1979. Aetiologic relation between Korean hemorrhagic fever and nephropathia epidemica. Lancet **i**:186–187.
13. **Lee, P. W., H. L. Amyx, C. J. Gibbs, Jr., D. C. Gajdusek, and H. W. Lee.** 1981. Propagation of Korean hemorrhagic fever virus in laboratory rats. Infect. Immun. **31**:334–338.
14. **Lee, P. W., R. Yanagihara, M. C. Franko, H. L. Amyx, C. J. Gibbs, Jr., D. C. Gajdusek, and R. Traub.** 1982. Preliminary evidence that Hantaan or a closely related virus is enzootic in domestic rodents. N. Engl. J. Med. **307**:624–625.
15. **Mayer, C. F.** 1952. Epidemic hemorrhagic fever of the Far East, or endemic hemorrhagic nephroso-nephritis. Mil. Surg. **110**:276–284.
16. **Powell, G. M.** 1954. Hemorrhagic fever: a study of 300 cases. Medicine (Baltimore) **33**:97–153.
17. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27**:493–497.
18. **Smorodintsev, A. A., L. I. Kazbintsev, and V. G. Chudakov (ed.).** 1963. Virus hemorrhagic fevers. S. Sivian Press, Jerusalem, Israel.
19. **Svedmyr, A., H. W. Lee, A. Berglund, B. Hoorn, K. Nyström, and D. C. Gajdusek.** 1979. Epidemic nephropathy in Scandinavia is related to Korean hemorrhagic fever. Lancet **i**:100.
20. **Tamura, M.** 1964. Occurrence of epidemic hemorrhagic fever in Osaka City: first cases found in Japan with characteristic feature of marked proteinuria. Biken J. **7**:79–94.
21. **Traub, R., M. Hertig, W. H. Lawrence, and T. T. Harriss.** 1954. Potential vectors and reservoirs for hemorrhagic fever in Korea. Am. J. Hyg. **59**:291–305.
22. **Tsai, T. F., S. P. Bauer, J. B. McCormick, and T. Kurata.** 1982. Intracerebral inoculation of suckling mice with Hantaan virus. Lancet **i**:503–504.
23. **Tsai, T. F., S. P. Bauer, D. R. Sasso, J. B. McCormick, H. Bradford, C. T. Caraway, L. M. McFarland, O. Medrano, and G. Soulie.** 1982. Preliminary evidence that Hantaan or a closely related virus is enzootic in domestic rodents. N. Engl. J. Med. **307**:623–624.
24. **Umenai, T., H. W. Lee, P. W. Lee, T. Saito, T. Toyoda, M. Hongo, K. Yoshinaga, T. Nobunaga, T. Horiuchi, and N. Ishida.** 1979. Korean haemorrhagic fever in staff in an animal laboratory. Lancet **i**:1314–1316.
25. **World Health Organization.** Report of the working group on hemorrhagic fever with renal syndrome, Tokyo, Japan, 22 to 24 February, 1982.