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MORPHOLOGICAL AND VIROLOGICAL STUDIES IN SIX AUTOPSIES OF CHILDREN WITH ADENOVIRUS PNEUMONIA

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Pulmonary lesions compatible with adenovirus infection were detected by gross and microscopic examination of autopsy tissues from children aged from 5 to 34 months. Hepatic lesions indicative of systemic infection were also found in four of the children. The viral etiology was confirmed in three cases by in-situ *hybridization, electron-microscopy and immunofluorescence performed in parafin-embedded tissues, and in one case by cell culture isolation of adenovirus type 2 from nasopharyngeal exudate. Routine testing by methods additional to conventional light microscopy would probably have revealed a larger number of adenovirus infections among the 1,103 autopsy records analyzed in this study.*

Key words: adenovirus infection – viral pneumonia – childhood pneumonia

Although adenovirus are best known for their role in acute respiratory tract infections they have been associated with an increasing spectrum of diseases.

Pneumonia is one of the main causes of child mortality in Rio de Janeiro and after respiratory syncytial virus, adenovirus are the second major virus to infect the upper and lower respiratory tract of children (Nascimento et al., 1991b). Despite the severity of those infections, reports of fatal cases in this city are rare (Barth et al., 1988). This report describes six paediatric autopsy cases with a diagnosis of adenoviral pneumonia. The material for review was obtained from the files of the "Departamento de Patologia, Instituto Fernandes Figueira, (IFF), Rio de Janeiro (Brasil)".

MATERIALS AND METHODS

The autopsy files at IFF include 1,103 complete autopsies of children (more than 5 days old). Of these, six exhibited morphological features compatible with acute adenoviral infection. In each instance, the clinical and pathological data were critically reviewed and are summarized in Table.

Morphological examination – Tissue samples from infants and children were collected during routine autopsies and studied with the light microscope using routine histologic techniques. Formalin-fixed tissue sections were embedded in paraffin, and sections of 5 μ were routinely stained with hematoxylin-eosin. The Shorr, Papanicolau, and Giemsa methods of staining were used for identification of viral inclusions.

In-situ *hybridization* – It was performed as described by Nascimento et al. (1991a). A recombinant plasmid A1 carrying a cloned insertion of Bam H1 fragments C and D of adenovirus type 2 in pAt153 was used as specific probe (Gomes et al., 1985). The DNA was labelled with biotin-11-dUTP (Enzo Biochem, Inc.) using a nick-translation kit (Gibco BRL) and following the recommended

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protocol. DNA from plasmid pBR328 was used as a negative control probe with comparable specific activity.

Immunofluorescence – An antihexon (Pereira et al., 1985) serum raised in guinea pig was used as specific serum in an indirect test, as described by Gardner & McQuillin (1980). Anti-guinea-pig IgG fluorescein conjugate was obtained from Sigma.

Virus isolation and identification – Nasopharyngeal aspirates were inoculated into Hep-2, LLC-MK2, MDCK cells, which were incubated at 34-35 °C and observed for cytopathic effect over a period of up to 21 days. the adenovirus isolated was identified by immunofluorescence and by the neutralization test as described by Grist et al. (1979) using rabbit antisera supplied by Central Public Health Laboratory (Colindale, London).

Electron microscopy – Six samples of lungs and one of liver were processed for electron-microscopy after deparaffination in xylene, followed by hydration, fixation in glutaraldehyde and osmium tetroxide, dehydration though a series of alcohols, and embedding in polylite resin. Thin sections were stained by uranyl acetate and lead citrate. The sections were observed at EM Philips 301, at 80 kv.

RESULTS

Significant pathologic findings were observed in the lungs and liver. All six cases had histological features of both lungs compatible with adenovirus infection. There was bronchoalveolar air-space necrosis secondary to a severe necrotizing bronchitis, bronchiolitis, and alveolitis (Fig. 1). Prominent hyaline membrane lined the air spaces. An intense mononuclear cell infiltrate was present in the interstitium and extensive cellular debris were seen in the air space. Alveolar and bronchiolar lining cells were enlarged, especially adjacent to the necrotic regions, and many contained large, amphophilic nuclear inclusions (Fig. 2). The infected cells were clustered in foci of infection centered about airways, sparing the intertitial cells (Fig. 3).

Significant changes were seen in the multiple random irregular foci of hepatocellular necrosis varying in size from minute to confluent areas readily seen under the low power of the light microscope (Fig. 4). The necrotic foci consisted of eosinophilic masses were nuclear or cytoplasmic details could no longer be seen. In the viable border zones, the numerous cells exhibited intranuclear inclusions (Fig. 4). Most nuclei of the hepatocytes showed some degree of inclusion, with body formation

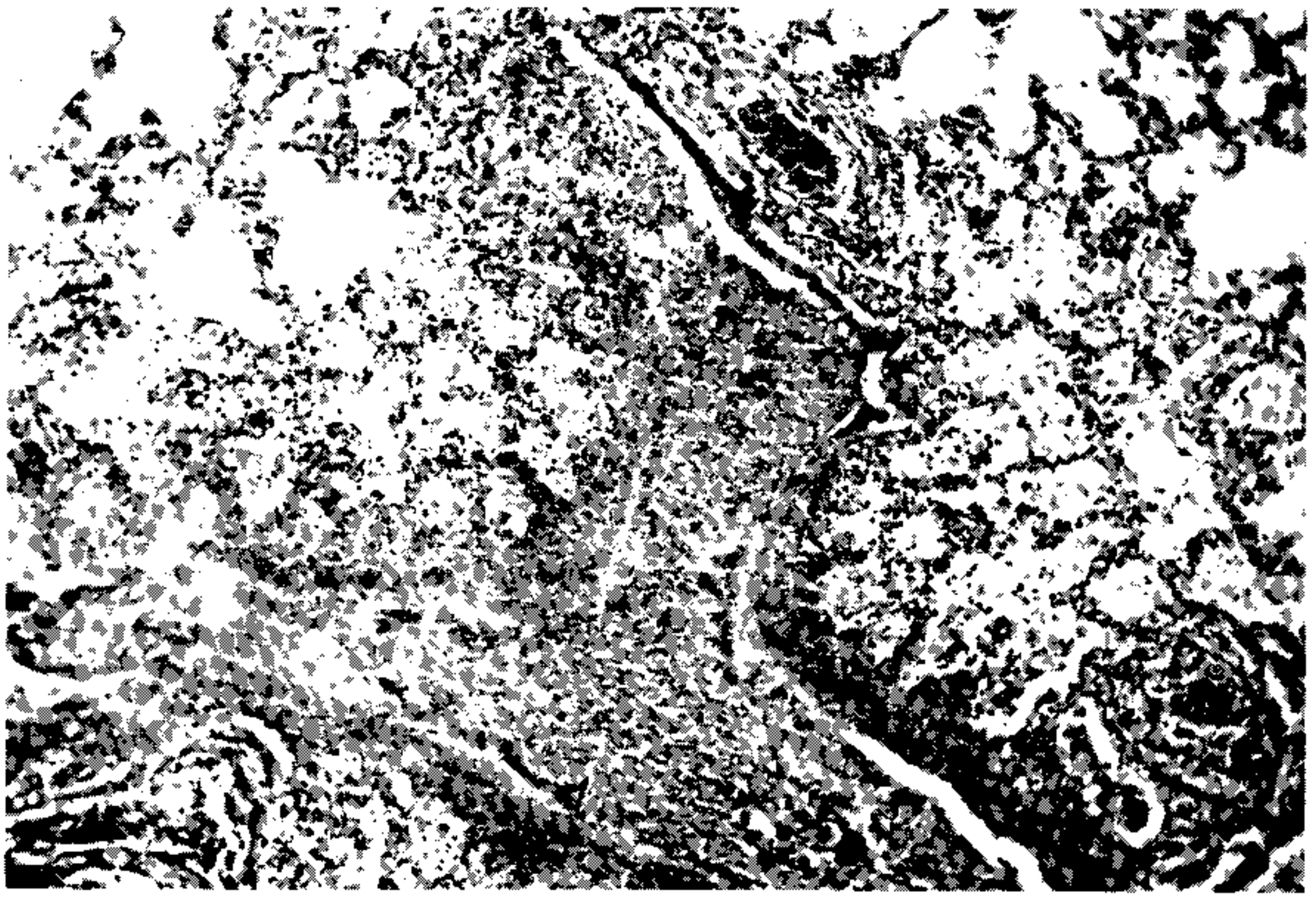

Fig. 1: necrotizing bronchiolitis; macrophagic and edematous alveolitis (Masson x 44).

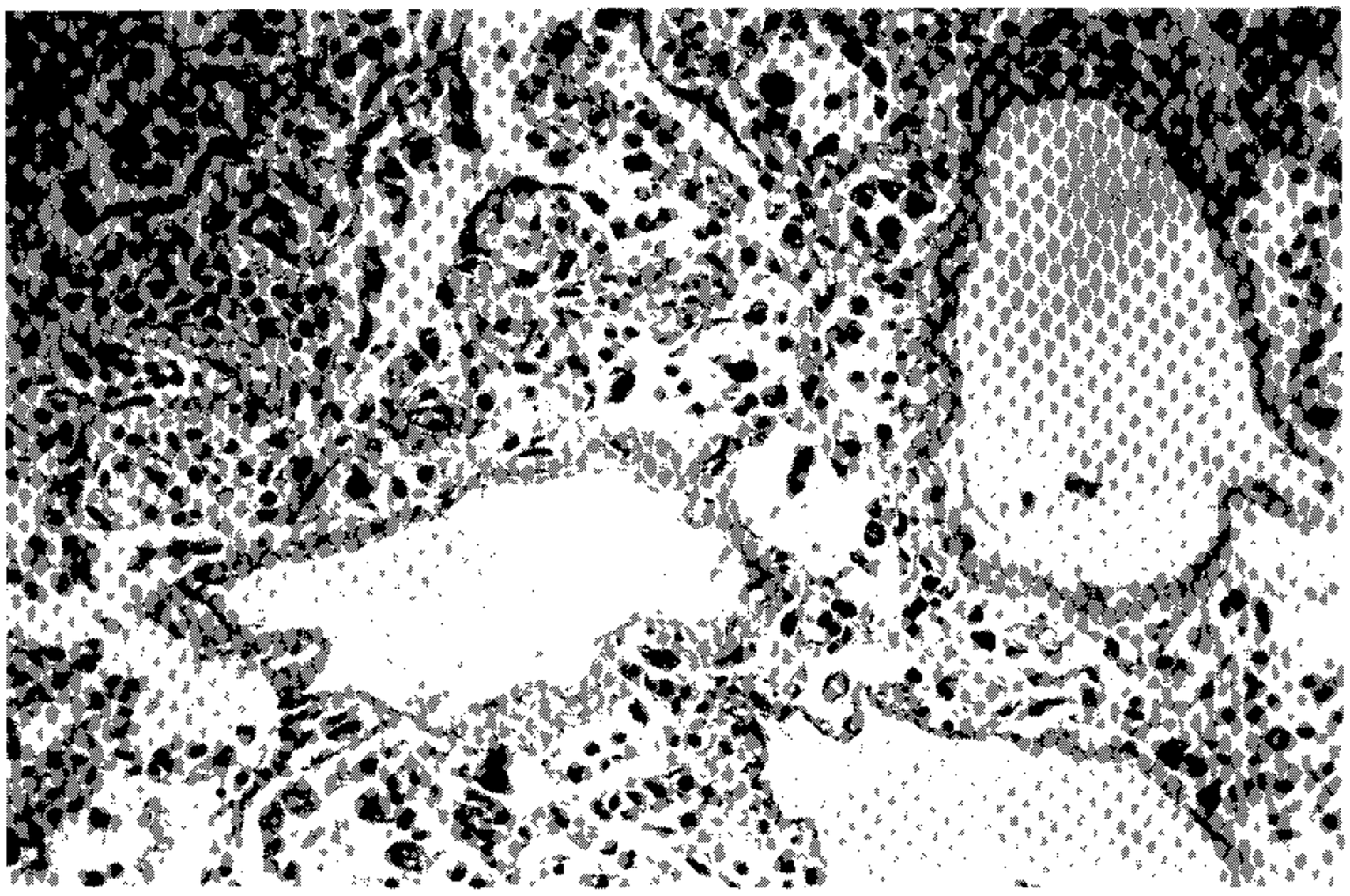

Fig. 2: hyaline membrane lining the air-spaces; enlarged alveolar lining cells containing nuclear inclusions; interstitial mononuclear cell infiltrate (Hematoxylin-eosin x 125).

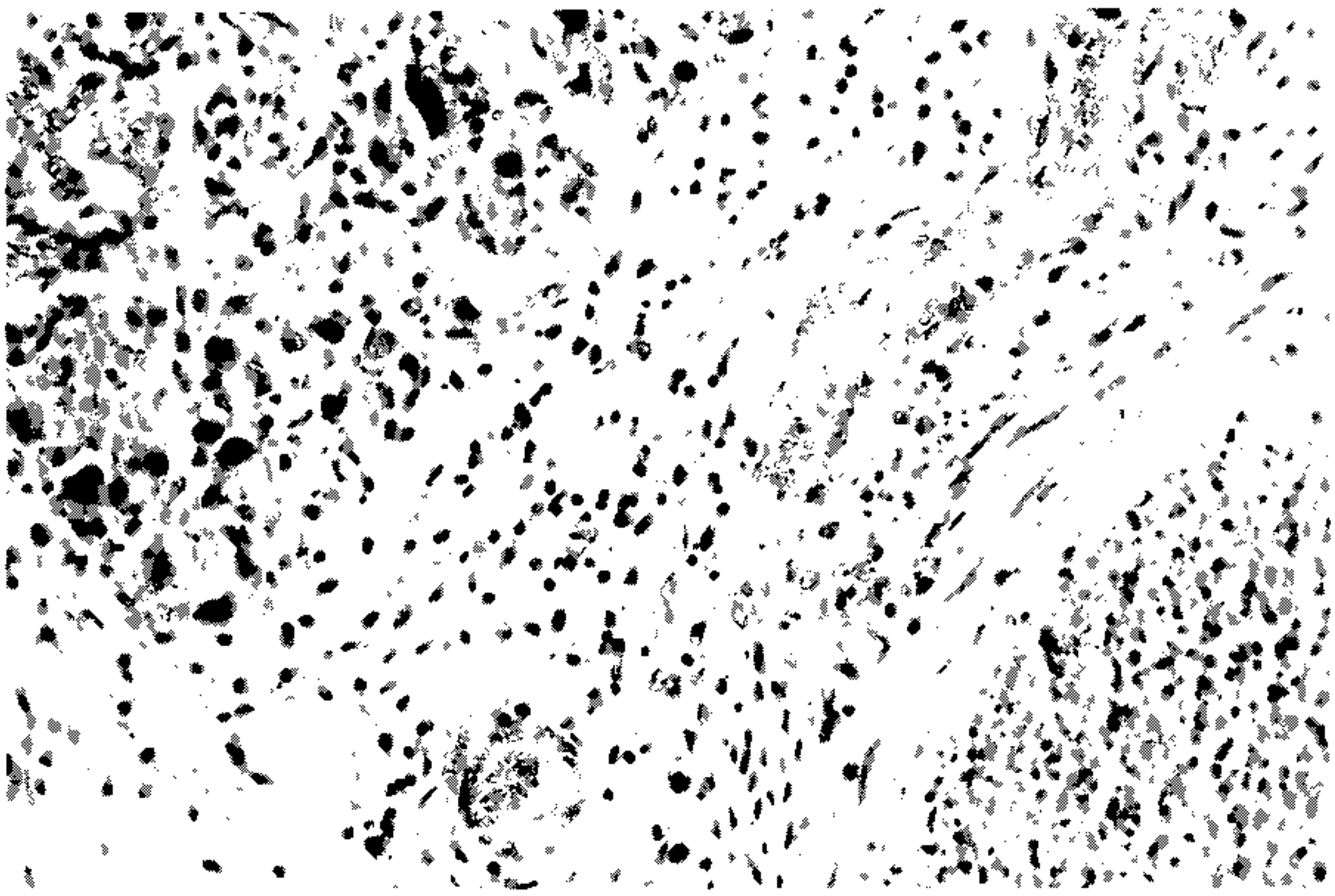

Fig. 3: interstitial septum exhibiting mononuclear infiltrate. Alveolar structure with nuclear inclusions. Part of a necrotic bronchiolus seen at right bottom (Hematoxylin-eosin x 125).

ranging from one or several small nucleous-like bodies to a large, prominent, compact mass completely filling the nucleus. A distinct halo separated the inclusion body from the nuclear membrane, but on most instances no halo formation was noted.

Investigations of these formalin-fixed tissues by *in-situ* hybridization and immunofluorescence confirmed the viral etiology of the lesions in the lung in three cases (cases 4, 5, 6). In one case, adenovirus infection was found in both liver and lung tissues. Adenovirus type 2 was isolated from frozen nasopharyngeal secretion collected from this child before death (case 5). Virus isolation was not attempted for the remaining five cases due to lack of frozen specimens. Cells recorded as adenovirus DNA-

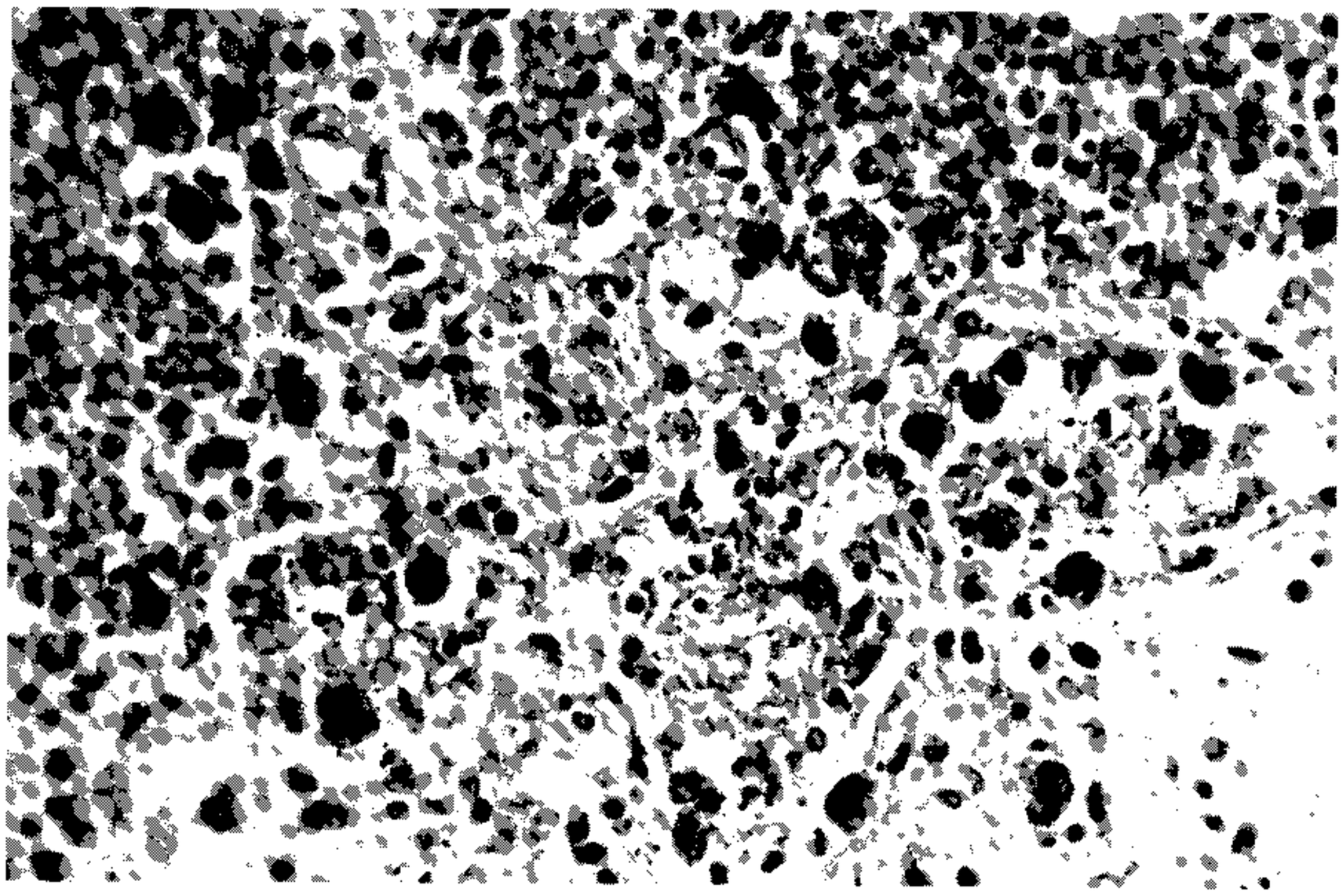

Fig. 4: presence of intranuclear inclusions in hepatocytes (Hematoxylin-eosin x 560).

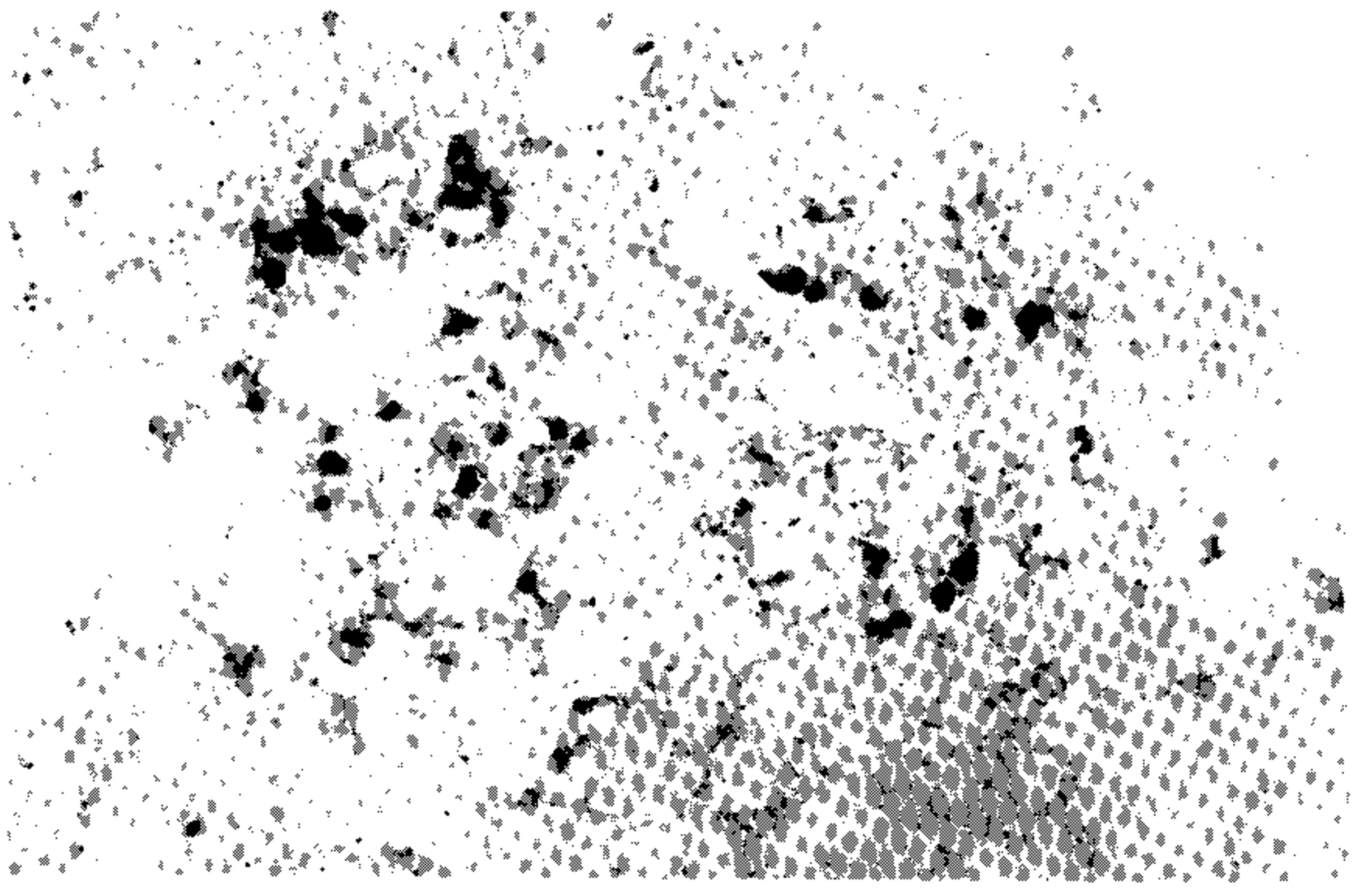

Fig. 5: *in-situ* hybridization using adenovirus-specific DNA probe. Hepatocyte containing adenovirus DNA stain darkly (x 125).

positive by *in-situ* hybridization appeared distinctly blue, in sharp contrast with the nostaining negative cells in tissue sections. In Fig. 5, the adenovirus DNA-positive cells stand out as darkly stained cells. Adenovirus hexon antigens were also found by immunofluorescence on cells showing the same distribution in the tissue as the adenovirus DNA-positive cells. From two cases (case 5, 6), other tissues, such as splen, adrenal, brain, pancreas (case 5) and kidney (case 6), were also examined by *in-situ* hybridization and immunofluorescence with negative results.

Adenovirus particles were found by electron microscopy in one liver (case 5) and both lungs of two cases (cases 5, 6). The virus particles were detected in the nucleus and in the cytoplasm, in a typical crystalline array (Fig. 6). Complete dearrangement and ruffling of the nuclear membrane, irregular marginalization of heterochromatin, deposit of fibrillar

Fig. 6: electron micrograph; nuclear region of hepatocyte showing ruffling of nuclear membrane, dearrangement of heterochromatin and viral particles (24.200 x; in set 55.00 x).

material, and presence of free virus particles in vacuoles or in the cytoplasm were noted.

DISCUSSION

This study attempted to determine the value of virological examination of autopsy material. The increasingly broad armamentarium used to check gross and histological diagnosis has been showing lack of specificity of numerous morphologic features previously considered attributable to a determinate etiology. Modern technique can improve diagnostic precision.

The characteristic histopathologic findings of adenovirus pneumonia were first described in children by Goodpasture et al. (1939). Although the exact etiologic agent had not been identified, the distinct morphological pattern of extensive exudates and necrosis of lung tissue, and the presence of unique haloed nuclear inclusions in respiratory tract epithelium pointed to a unique viral agent. Chany et al. (1958) considered necrosis of trachea and bronchial epithelium with destruction of the mucosa as the major pathologic alteration in adenoviral infection of the respiratory tract. The bronchial structures may be obstructed by a necrotic material and by a typical pattern of necrosis. Nuclear inclusions can strongly suggest the diagnosis of adenoviral infection. Involvement of the pulmonary parenchyma occurs as the infection progresses.

Similar pathologic findings have been observed by several authors in different geographical areas. Chih-Ch'Uan (1963) and Becroft (1967) showed identical lesions in the respiratory tract, especially involving the bronchial glands. Intranuclear inclusion are significant but not specific for infection by different adenovirus setotypes. Histopathological changes compatible with adenovirus infection were observed in postmortem lung tissue which on electron microscopy showed viral particles morphologically consistent with adenovirus group (Nahmias et al., 1967). Other authors including Landry et al. (1987) and Green & Williams (1989) considered that the light microscopic diagnosis of adenoviral infection lacks specificity and that adenovirus may be overlooked.

In this autopsy series, the morphological pulmonary features were identical to those described above in adenovirus infections, although the degree of necrosis of bronchi and

TABLE

Autopsies of children with adenovirus pneumonia diagnosed by histopathology from 1953 to 1989

Case No.	Age	Clinical data	Autopsy finding and diagnosis	Typical adenovirus inclusions	DNA *in situ* hybridization		Virology IFA (Hexon)		Electron microscopy	
					lung	liver	lung	liver	lung	liver
1	1y2m	Enterocolitis. Pneumonia.	Viral pneumonia. Necrotic bronchitis bronchiolitis. Viral systemic diseases.	pos.	neg.	neg.	neg.	neg.	neg.	ND
2	1y5m	Fever. Gastro-enterocolitis. Bronchopneumo-nia. Coma.	Lung-idem. Immunodeficiency.	pos.	neg.	neg.	neg.	neg.	neg.	ND
3	2y1m	Fever. Pleural effusion. Pneumonia.	Lung-idem. Chronic visceral infection. Falcemia.	pos.	neg.	neg.	neg.	neg.	neg.	ND
4	8m	Fever. Laryngo-stridor.	Lung-idem. Streptococcal pneumonia. Necrotic tracheitis. Viral systemic disease.	pos.	pos.	neg.	pos.	neg.	neg.	ND
5	1y4m	Fever. Cough. Viral pneumonia.	Lung-idem. Viral systemic disease. Immunol. dificiency.	pos.	pos.	pos.	pos.	pos.	pos.	pos.
6	5m	Fever. Cough. Pneumonia. Coma.	Lung-idem. Reye's syndrome. Viral systemic disease.	pos.	pos.	neg.	pos.	neg.	pos.	ND

IFA: immunofluyorescence assay; ND: not done; Neg.: negative; Pos.: positive.
Adenovirus type 2 isolated from naso-pharynx before death.

bronchioli, and parenchymal areas, and the numerous typical nuclear inclusions in epithelial cells enables experimental pathologists to favor the diagnosis of adenovirus.

As Aterman et al. emphasize (1973), an association between adenovirus and liver cell damage has not frequently been demonstrated in humans. Benyesch-Melnick & Rosenberg (1964) reported the isolation of adenovirus type 7 from an infant who died of viral pneumonia presenting widespread dissemination of adenovirus. Two years later, Wigger & Blanc (1966) presented a similar case in which widespread necrotizing bronchial lesions were also accompanied by focal areas of necrosis with hepatic intranuclear inclusions. Similar foci were present in other organs. In 1979, Carmichael et al. demonstrated adenovirus by electron microscopic examination of liver tissue and confirmed the diagnosis by isolation of adenovirus type 2 from several organs.

In the present series, microscopic sections of the liver in all cases revealed focal necrosis distributed at random inside the lobules, as well as amphophilic or basophilic nuclear inclusions within hepatocytes. Fat degeneration was present in all six cases. This was predominantly periportal and macrovacuolar and may be attributed to malnutrition. In case 6, with the probable diagnosis of Reye' syndrome, a microvacuolar and disseminated type of fat degeneration was also observed.

The recombinant plasmid A1 used as specific probe is suitable for the detection of adenovirus belonging to at least subgroups B, C and E (Gomes et al., 1985). Antihexon serum is able to detect all the viruses belonging to the Mastadenovirus group (Pereira, 1989). For cases 1, 2 and 3, where evidence of adenovirus DNA, antigens, or particles could not be found, the presence of intranuclear inclusions

of the pathological changes in tissues could be due to another etiologic agent. Although light microscopic lesions in the liver were found in four cases, the presence of adenovirus as found by *in-situ* hybridization or immunofluorescence in liver cells was detected in only one case (case 5). The ultraestructural aspects of the virus, including its size and localization and configuration in the cell, is typical of adenovirus. The appearance of individual particles and their crystalline arrangement closely resemble the standard ultraestructural description of adenovirus (Horne, 1973).

The histological features found in other tissues were probably not correlated with adenovirus multiplication in theses tissues. It should be noted that some tissues were tested after 25 years of storage, and the three negative cases are the oldest tissues. The reported cases demonstrate the usefulness of DNA probe analysis, electron microscopy, and immunofluorescence for retrospective diagnosis in autopsy material embedded in paraffin.

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