Electron Microscopy for Rapid Identification of Animal Viruses in Hematoxylin-Eosin Sections

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ABSTRACT

Routine hematoxylin-eosin stained, paraffin sections were processed for electron microscopy, using a rapid method for localization of animal viruses. Formalin fixation was effective in preserving DNA as well as RNA viruses, however cellular fine structural details and organelles were not well preserved. The procedure is useful for morphological recognition of viral groups and as a rapid diagnostic aid for identifying viral disease.

RÉSUMÉ

Les auteurs ont préparé des coupes histologiques, enrobées dans la paraffine et colorées à l'hématoxyline et à l'éosine, pour la microscopie électronique, en se servant d'une méthode rapide qui permet de localiser les virus. La fixation à la formaline préserva bien les virus ADN et ARN, mais non les détails structuraux ou les organelles des cellcles. Ce procédé s'avéra utile comme moyen de reconnaître la morphologie des virus et de diagnostiquer rapidement certaines maladies virales.

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INTRODUCTION

Two main procedures are available for electron microscope (EM) characterization of viruses: (a) negative staining which is applied to the virus specimen obtained either as scrapings and fluids or as tissue culture isolates and (b) conventional thin sectioning of glutaraldehyde and osmium tetroxide (OSO_4) fixed tissue and subsequent positive staining. Direct samples for negative staining (7) and rapid processing and embedding procedures for thin sectioning (6, 8) provide quick morphological identification of animal viruses. The present report describes a retrospective rapid method by which a broad group of viruses can be identified by using routine formalin fixed paraffin sections stained with hematoxylin and eosin (H & E).

MATERIALS AND METHODS

The described procedure is modified from previously published methods (1, 10). Formalin-fixed, paraffin sections stained with H & E were used to localize the inclusion bodies or lesions. The appropriate area was marked at the bottom of the slide with a diamond pencil and the slides were placed in Coplin jars containing xylene at 60°C.

Most coverslips were removed easily in 15-20 minutes, and excess tissue around the lesions was trimmed with a sharp razor blade. The slides without coverslips were then immersed for five minutes each in (a) fresh xylene, (b) 50% xylene: 50% propylene oxide and (c) absolute propylene oxide at room temperature. The slides were transferred to a Petri dish and a few drops of 25:75 mixture of epon 812 and propylene oxide were placed on the sections. In five to seven minutes the propylene oxide evaporated leaving the epon residue on the slide. Slides were then cured in an oven for ten minutes at 60°C. After this initial curing, slides were removed from the oven and a 00 regular BEEM capsule[1] filled with complete epon 812 mixture was inverted over the lesions. The slides were cured overnight (12 to 16 hours) at 60°C. After this polymerization, the stained H & E lesions embedded in epon, were ready for removal from the slide. The removal was completed easily by holding the top of the capsule and pulling it laterally from the slide after cooling the slides in cold tap water.

The blocks were then trimmed and sectioned with an LKB Ultratome III using a diamond knife. Thin sections were stained with saturated aqueous uranyl acetate at room temperature for 30 minutes and lead citrate (9) for two minutes and examined in a Philips 201 EM at 60 kv.

Hematoxylin and eosin sections of avian hemorrhagic enteritis from a turkey, infectious laryngotracheitis in a chicken, coyote viral papillomatosis, pseudocowpox, paramyxovirus in canine distemper and transmissible gastroenteritis of swine were examined using the technique described.

RESULTS

Examination of H & E sections of a turkey spleen with suspected hemorrhagic enteritis indicated the presence of large basophilic inclusion bodies in swollen reticuloendothelial cells (2). These inclusion bodies, when removed from the slide using the present procedure and examined under the EM, showed numerous adenovirus particles, usually within the nucleus. Morphologically the virions were arranged in large crystalline arrays. Most particles had electron-dense centres and cores, measuring 60-70 nm in diameter (Fig. 1).

Epithelial cells of tracheal mucosa of chickens revealed the presence of intranuclear inclusion bodies in H & E sections, and infectious laryngotracheitis was suspected. The same area when observed with EM confirmed the presence of herpesvirus particles (Fig. 2) showing typical nucleocapsids within the nucleus (4). The virions were approximately 120-200 nm in diameter. Shrinkage of the DNA core and some distortion of the protein capsids was evident, nevertheless particles can be easily recognized.

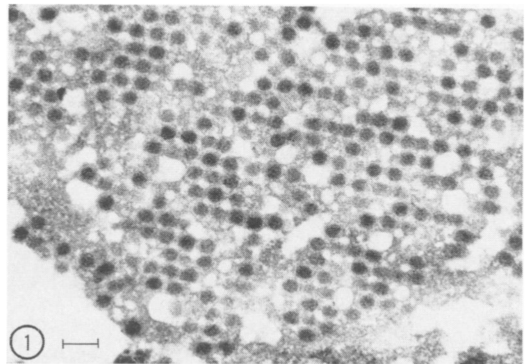

Fig. 1. Crystalline arrays of adenovirus particles in turkey spleen with hemorrhagic enteritis. Note: Predominant electron dense centres and a dense core. X45,985. Bar = 0.1 μ

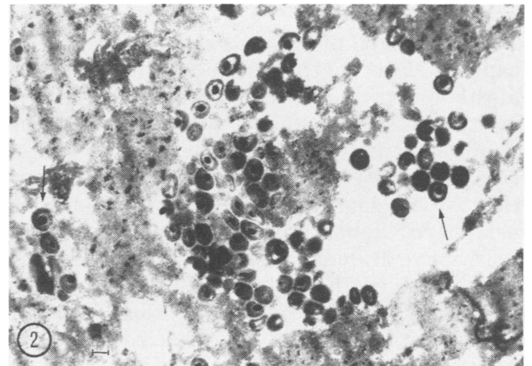

Fig. 2. Infectious laryngotracheitis in chicken, showing typical nucleocapsids of herpesvirus in the nucleus (arrows). X16,120. Bar = 0.1 μ

[1]Ladd Research Industries, P.O. Box 901, Burlington, Vermont.

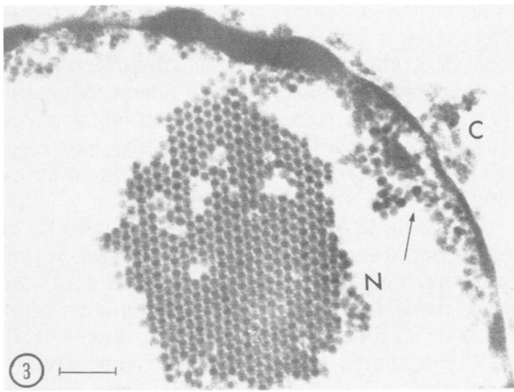

Fig. 3. Highly organized papilloma virus. Some particles are arranged at the nuclear (N) periphery (arrows). Fine structure of the nucleus and cytoplasm (C) can not be resolved. X66,280. Bar = 0.1 μ

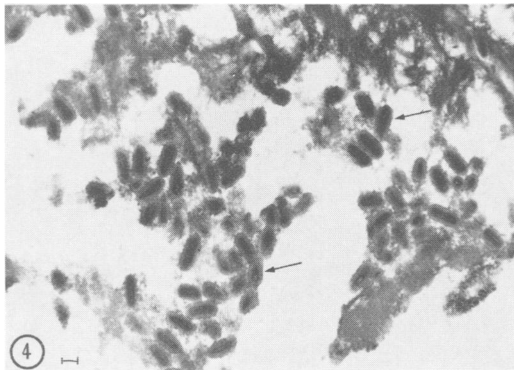

Fig. 4. Pseudocowpox virus showing electron dense DNA centres surrounded by protein layers (arrows). X16,120. Bar = 0.1 μ

Hematoxylin and eosin sections of coyote viral papilloma contained characteristic homogeneous nuclear inclusion bodies of roughly hexagonal to circular shape, forming "bird's eye" cells (1). When examined with the EM, crystalline arrays of virions roughly 40 nm in diameter were observed within the nucleus (Fig. 3). The viruses were arranged also at the nuclear periphery usually close to the inner membrane. The highly organized arrangement of crystalline arrays and the smaller size separated the papilloma virus from adenovirus. The viral fine structure was well preserved, however the cell nucleus showed empty areas with peripheral dense chromatin.

In oral lesions suspected with papular stomatitis, epithelial hydropic degeneration was observed. Affected cells, mostly in the stratum spinosum had pyknotic nuclei within vesiculated cells. Some vesiculated cells contained intracytoplasmic inclusions and cell debris. When this area was examined with EM, oval to cylindrical virions were found, with many circular forms appearing in cross sections. The centre of the particle was electron dense due to the presence of DNA (5) which was surrounded by membranous protein layers. Overall dimensions of the virion were approximately 150 x 350 nm (Fig. 4).

Hematoxylin and eosin sections of lung from a skunk with bronchopneumonia contained eosinophilic inclusion bodies in bronchial epithelium and syncytial giant cells. When these areas were examined with EM, typical particles of paramyxovirus (canine distemper) were found. The morphology of the virus was well preserved with predominant circular profiles of approximately 80 nm diameter. Long filaments also were present with characteristic spikes (4) on the outer surface (Fig. 5, arrows).

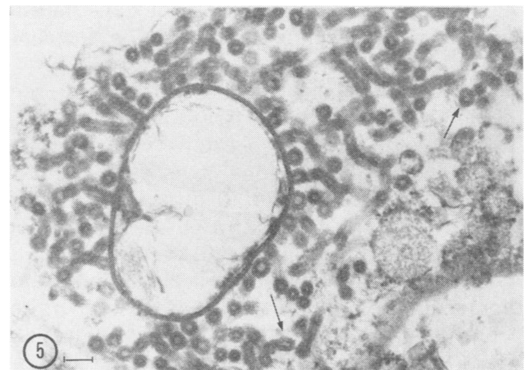

Fig. 5. Circular and filamentous paromyxo particles in canine distemper. Note fine spikes on the outer surface (arrows). X31,715. Bar = 0.1 μ

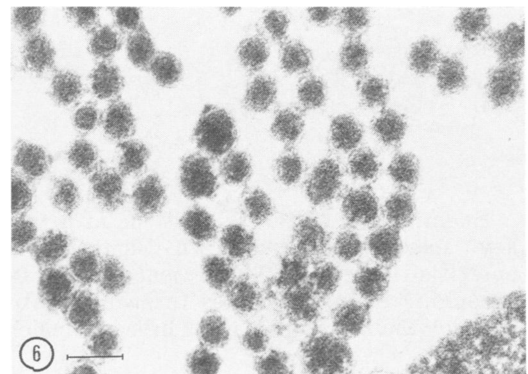

Fig. 6. Coronavirus demonstrated in transmissible gastroenteritis of swine. Particles are spherical with electron dense centre. X70,300. Bar = 0.1 μ

Hematoxylin and eosin sections of jejunum contained lesions of villous atrophy characteristic of transmissible gastroentereritis (TGE). Blocks were taken from the epithelial surface including microvilli. Electron microscope examinations of this area revealed the presence of coronavirus (3). The particles were spherical, ranging in size from 70 to 100 nm and were made up of a double membrane around a electron dense core (Fig. 6).

DISCUSSION

In our laboratory, preparation of H & E slides takes approximately 12 hours, a procedure which facilitates rapid examination of viral lesions with the light microscope. Precisely marked lesions then are processed for EM in a further 12 to 16 hours. This procedure provided rapid presumptive confirmation of diagnoses of hemorrhagic enteritis, infectious laryngotracheitis, papillomatosis, papular stomatitis, canine distemper and TGE, thus saving a minimum of two to three weeks over routine tissue culture and serum neutralization methods.

In conventional electron microscopy, several days are required to process as well as to isolate a normal or pathological area utilizing toluidine blue stained, 2 μ epon sections. The present procedure uses precut and stained sections allowing rapid precision trimming of the epon blocks. Pathologists can mark the routine slide and send it to the EM laboratory for one day viral identification work. Additionally retrospective identification studies are also possible by this procedure.

When compared to the method of Takeda (10) the present procedure eliminates several processing steps. The polyethylene capsules are not glued to the glass slides for 24 hours. No postfixation in OSO_4, restaining with H & E, dehydration and slow curing is required. As slides are not postfixed in OSO_4, no destaining occurs and lesions are ready for observation in the dissecting microscope as soon as polymerization is completed. Further, this procedure does not involve complicated embedding of the tissues on top of a small dish of epon and cutting a wedge shaped area to be glued to an old epon block as described in the procedure of Blank *et al* (1). Because tissues are always treated with epon mixture, no external agent is introduced and thus artifacts are avoided.

From this investigation, it is clear that 10% buffered formalin is adequate to preserve fine structural morphology of some DNA as well as RNA viruses. Cellular fine structural details and organelles are not well preserved. However, membranes and overall cell structure can be easily visualized with EM permitting localization of virus particles within the cell. Since cellular morphology is not well preserved, the sequence of viral synthesis, its effect within the cell and the adjacent fine structure can not be studied. Thus this procedure is restricted to the identification of viral groups in selected diagnostic cases.

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