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Occurrence of Intracytoplasmic Inclusion Bodies in the Digestive Epithelium of Fallow Deer (Dama dama L)

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Diaz R., C. Rehbinder and R. Feinstein: Occurrence of intracytoplasmic inclusion bodies in the digestive epithelium of fallow deer (Dama dama L). Acta vet. scand. 1989, 30, 301-305. – Intracytoplasmic inclusion bodies were observed in the digestive epithelium of fallow deer (*Dama dama L*) suffering from bovine virus diarrhea/mucosal disease. Similar inclusion bodies were also found in the ruminal epithelium of fallow deer subjected to overfeeding by supplementary food. Inclusion bodies were not found in the upper alimentary mucosa of clinically healthy deer but were frequently found when these tissues were subjected to autolysis. At electron microscopical studies the inclusion bodies were found to consist of granular protein-like material encircled by a single membrane. Such inclusion bodies may constitute a non-specific degenerative cell response which could be elicited by diverse factors including autolysis.

cervidae; electronmicroscopi; lesions; inclusions; autolysis.

Introduction

During recent years erosive, ulcerative, and necrotizing lesions associated with intracytoplasmic inclusion bodies were observed in the digestive epithelium of various cervidae such as reindeer (*Rangifer tarandus L*), roe deer (*Capreolus capreolus L*), moose (*Alces alces L*), and fallow deer (*Dama dama L*). Neither the cause of these changes nor the significance of the inclusion bodies was clarified. The type of lesions found as well as the occurrence of inclusion bodies were suggestive of a viral etiology. Electron microscopical studies, however, consistently failed to reveal virus particles (*Rehbinder et al.* 1985, *Feinstein et al.* 1987). Finally, the hypothesis was raised that irrespective of the etiology the inclusion bodies observed could constitute a non-specific degenerative cell response of the digestive epithelium (*Diaz et al.* 1988). To test this possibility, in the present study, we examined alimentary tissues from clinically healthy fallow deer and also from fallow deer suffering a disease resembling BVD/MD. In order to evaluate whether autolysis could play a role in the genesis of inclusion bodies, we allowed alimentary tissues from clinically healthy fallow deer to undergo from mild to advanced autolysis by delaying the start of fixation.

Material and methods

The present study compised 11 fallow deer. Out of them, a group of 4 fallow deer, of both sexes, aged between 6 months and 4 years, was involved in an outbreak of BVD/MD in Uppland (*Diaz et al.* 1988). These animals were found dead and were

submitted for routine necropsy at the National Veterinary Institute, Uppsala.

Seven clinically healthy, male fallow deer, approximately 1.5 year old, were deliberately killed as controls. Of these, 4 fallow deer from a farm in Uppland were shot in January 1987. The rest, 3 fallow deer from a farm in Sörmland, were shot in November 1987. During the wintertime both the diseased and the control fallow deer were supplementary fed with hay, chaff, and branches of trees and bushes.

Post mortem examination of the control fallow deer was performed immediately after killing. For histology, tissue samples from alimentary mucous membranes were fixed immediately by immersion in 10% buffered formaline, pH 7.4 (0 h sample) but, in 3 animals, the same tissues were also deliberately maintained during 24, 48, and 96 h at 20°C before the start of formaline fixation. The material was routinely processed, embedded in paraffin, and 4 µm thick sections were cut and stained with haematoxylin and eosin, van Gieson, PAS, and according to Ayoub Shklar method for keratin and prekeratin. In addition, samples from diseased fallow deer were also stained for DNA by using the ethidium bromide method, as described elsewhere (*Strang et al.* 1985). For DNA detection we employed a Zeiss Universal Microscope Fluorometer equipped with a HBO 100 light source, a PB 516 exiter filter, FT 580 beam splitter and LP 590 + BG 18 barrier filters. A uranyl glass standard was used for controlling equipment stability.

For electron microscopy selected areas of alimentary mucosa were processed as described elsewhere (*Rehbinder et al.* 1985).

Results

Pathological investigations

Diseased animals: The clinical and necropsy findings are described elsewhere (*Díaz et al.* 1988). BVD-virus was isolated from 1 of the fallow deer. Histopathology of the mucous membranes of the digestive tract revealed marked inter- and intracellular oedema, congestion, erosions, and mild mononuclear cell infiltration of the lamina propria and submucosa. In the mucous membranes of the mouth, oesophagus and fore-stomachs numerous cells in stratum basale and spinosum contained intracytoplasmic basophilic inclusion bodies. In general, cells presented only 1 inclusion body but in occasional cells 2 could be seen. Inclusion bodies were round, 2 to 10 µm in diameter, and often compressed the nuclei into a crescent shape. Inclusion bodies were frequently surrounded by a clear halo. Inclusion bodies were consistently negative for DNA and they were also negative for keratin and prekeratin.

Control group: The animals were apparently healthy and in good nutritional state. Macroscopical lesions were not observed. Microscopical study: In 0 h samples the mucous membranes of the oral cavity and oesophagus appeared normal. The rumen presented degenerative changes in all epithelial layers and mild oedema of the lamina propia and submucosa. Intraepithelial vesicles were frequently observed, appearing as circumscribed areas filled with slightly eosinophilic fluid. Numerous cells in stratum basale and stratum spinosum exhibited intracytoplasmic inclusion bodies. Inclusion bodies were basophilic, round, 2 to 10 µm in diameter and compressed the nuclei into a crescent shape. Inclusion bodies were negative for DNA, keratin and prekeratin. In one rumen intraepithelial microabscesses with bacterial colonies were observed in stratum granulosum and corneum. Frequently, cells in stratum granulosum and corneum appeared markedly distended by fluid.

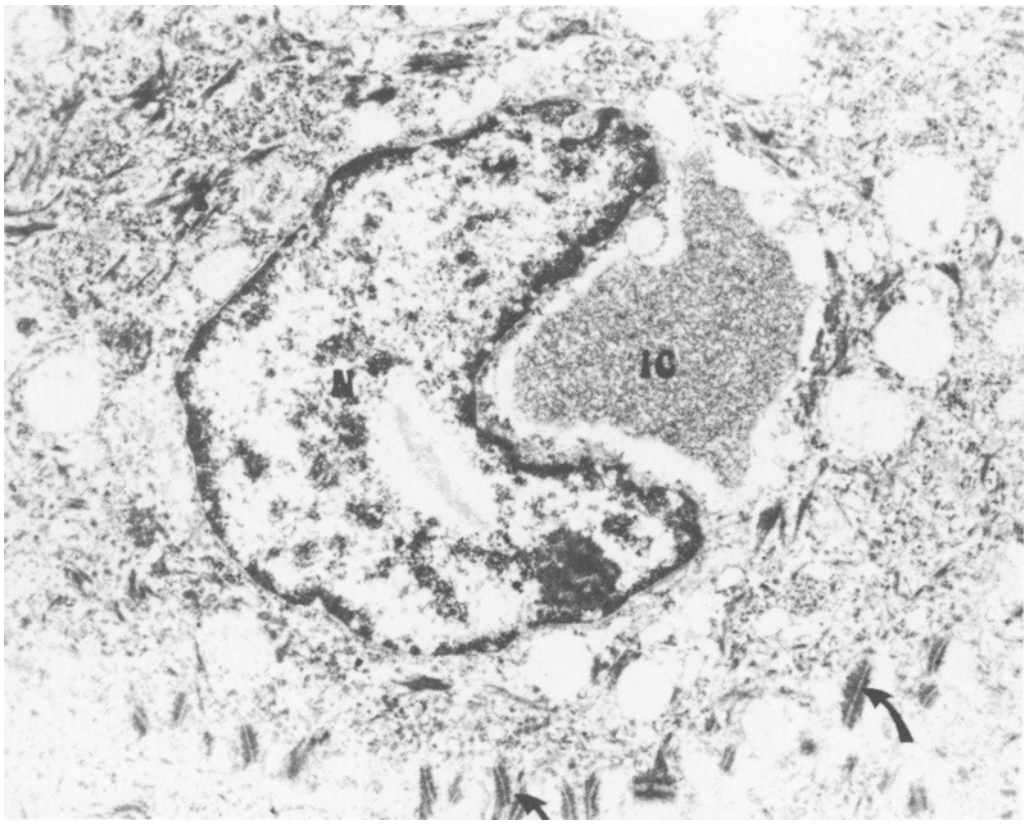

Figure 1. Healthy fallow deer (control group). Autolysis was allowed to proceed for 48 h before the start of fixation. Inclusion body (IC) compressing the nucleus (N) of a cell from the buccal epithelium. Clumping of tonofilaments (T) and desmosomes (arrows) are noticeable. x 11700.

In 24 h samples autolysis was moderate. In the oral cavity and oesophagus a few cells in stratum basale exhibited intracytoplasmic basophilic inclusion bodies. In the ruminal mucosa inclusion bodies seemed to be more numerous than in 0 h samples. In 48 h samples autolysis was advanced and numerous cells in stratum basale and spinosum of the mucous membranes of the mouth and oesophagus displayed basophilic inclusion bodies. In the ruminal mucosa the inclusion bodies had apparently increased in number. In 96 h samples autolysis was very pronounced, the histological details of the organs being hardly recognizable. However, rests of inclusion bodies were still discernible in a few cells of the buccal and ruminal epithelium.

Electron microscopy

Diseased animals: In spite of the advanced autolysis of the material studied the presence of intracytoplasmic inclusion bodies was confirmed. Some cells contained more than one inclusion body. Inclusion bodies appeared round or oval with a single membrane enclosing a granular protein-like content. Numerous inclusion bodies were partly filled or almost empty. Frequently, inclusions were located close to the nucleus which was compressed into a crescent shape. When compressed, nuclei showed a marked condensation of the chromatin and areas of low electron density in which varying patterns of a granular and fibrillar material was present. Cells either revealed dispersed organelles or a condensation of the cytoplasm with an increased electron density. A constant feature was clumping of tonofilaments and inter- and intracellular oedema. Viral particles were not observed.

Control group: In 0 h samples the mucous membranes of the mouth and oesophagus appeared normal except for mild inter- and intracellular edema. In cells of the ruminal epithelium a few inclusion bodies were observed which were as described for diseased deer. In 24 h samples we noticed a moderate inter- and intracellular edema. Inclusion bodies were observed in a few epithelial cells of the stratum basale of the mouth, oesophagus, and rumen. In general, they were as described above, although some of them displayed a looser granular content.

In 48 h samples autolysis was more advanced. Swelling of mitochondria and clumping of tonofilaments was clearly observed. Inter- and intracellular edema was pronounced and desmosomes were sharply demarcated. Numerous inclusion bodies appeared in cells of stratum basale and spinosum of the epithelium of the mouth, oesophagus and rumen. Inclusions were as described above (Fig. 1).

Electron microscopy of 96 h samples was not performed.

Discussion

In the present investigation we observed inclusion bodies in the epithelium of the mouth, oesophagus and rumen of diseased fallow deer. Electron microscopical studies revealed the inclusion bodies to consist of a granular protein-like material encircled by a single membrane. We noticed similar inclusion bodies in cells of stratum basale and spinosum of the ruminal epithelium of clinically healthy fallow deer, even when tissues were fixed immediately after excision. Comparable inclusion bodies were described in reindeer suffering from a disease exhibiting lesions similar to those of BVD/MD. (*Rehbinder et al.* 1985).

The fallow deer included in the present investigation was supplementary fed. In deer the rumen mucosa seems to follow an annual rhythm, with a less absorbing surface during

winter time. Furthermore, a concurrent adaptation of the ruminal flora to the harsher fodder also takes place. If, during severe winters when the animals are normally restricted to browsing, deer is offered food of high nutritional value, there is an evident risk of dysfunction or inability of the rumen flora to cope with this fodder, resulting in indigestion, rumenitis and even death (*Carhart* 1945, *Rehbinder & Ciszuk* 1985). It is thus likely that the lesions of the ruminal mucosa observed in control deer of the present study were due to supplementary feeding. Similar changes, albeit less pronounced, were also described in cattle under intensive management (*Jensen et al.* 1954, *Rowland et al.* 1969, *Landsverk* 1978).

In the present investigation the epithelium of the mouth and oesophagus of the control deer did not display inclusion bodies when tissues were fixed immediately after excision. When the start of fixation was delayed for 24 h, a few inclusion bodies were observed and electron microscopy revealed a single-layer membrane encircling a rather disperse granular material. When the start of fixation was delayed for 48 h inclusion bodies were more numerous and they frequently appeared partly filled or empty, which correlated with the observations on the diseased animals in which, depending on the degree of autolysis, the inclusion bodies contained a looser granular material or appeared empty. The nature and significance of inclusion bodies have been the subject of discussions. Frequently, inclusion bodies are sites of viral synthesis and maturation (*Watrach* 1972). However, some of the prominent cytoplasmic eosinophilic inclusions of pox-virus infected cells represent a late cellular response rather than a site of viral replication (*Okada & Fujimoto* 1975).

Cytosegresome formations (Councilman bodies, acidophilic bodies), are round, refractile, eosinophilic structures observed in liver cells sublethally injured by a variety of insults, such as hypoxia, diverse intoxications, mulnutrition, specific deficiencies and some viral infections (*Kelly* 1985). They consist of masses of organelles gathered and condensed, sequestered from remaining cytoplasm by membranes that fuse with lysosomes (autolysosomes). Intracytoplasmic eosinophilic inclusion bodies also represent an aberrant formation of the rough endoplasmic reticulum in Purkinje cells and neurons of several nuclei, described in association with neoplastic reticulosis of the central nervous system (*Cameron & Conroy* 1974). In diverse animal species, such as rats and mice, inclusion bodies develop in the cytoplasm of hepatocytes from both normal animals and also during the course of certain intoxications. Under light microscope, these inclusion bodies appear as a large eosinophilic mass which displaces the nuclei and other organelles to the cell periphery. Electronmicroscopy has revealed that these inclusions form as a result of dilatation and develoment of cisternae in the rough endoplasmic reticulum, with accumulation of granular protein material in the dilated cisternae (*Toth & Sugár* 1985, *van Zwieten & Hollander* 1985). Summarizing, we observed intracytoplasmic inclusion bodies which under electronmicroscopy dit not reveal any virus particles but contained a granular protein like material. The inclusion bodies observed by us differed morphologically from these described above. In addition, we were able to produce inclusion bodies by delaying the start of fixation of tissues (thus allowing autolysis to proceed). Hence, it appears likely that the inclusion bodies noticed in the present study represented a non-specific degenerative response which could be elicited by diverse factors producing cell injury including autolysis.

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Sammanfattning

Förekomst av intracytoplasmatiska inklusionskroppar i digestionsapparatens slemhinneepitel hos dovhjort (Dama dama L).

Intracytoplasmatiska inklusionskroppar påvisades i digestionsapparatens slemhinneepitel hos dovhjort (Dama dama L) lidande från bovin virus diarré/mucosal disease. Liknande inklusionskroppar förelåg också i vämepitelet hos dovhjortar utsatta för en överutfodring med tillskottsfoder. Inklusionskroppar kunde inte påvisas i övre digestionsapparatens epitel hos kliniskt friska djur men var ofta förekommande när ifrågavarande vävnader utsattes för autolys. Elektronmikroskopiskt befanns inklusionskropparna bestå av ett granulärt proteinliknande material omgivet av ett enkelt membran. Dessa inklusionskroppar kan utgöra ett oespecifikt cellulärt svar på degenerativa processer som kan utlösas av flera olika faktorer inklusive autolys.

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