ULTRASTRUCTURAL STUDIES OF BOVINE URINARY BLADDERS WITH SPECIAL REFERENCE TO ENZOOTIC BOVINE HEMATURIA

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Occurrence of enzootic bovine hematuria has been reported in cattle and water buffaloes from various districts in the world. The disease is usually associated with neoplastic changes on the cystic mucosa. Among the neoplastic lesions, hemangiendothelioma, adenocarcinoma, transitional cell carcinoma, papilloma, fibroma, leiomyoma, squamous cell carcinoma, and myxoma have been observed. Two or more different types of neoplasm have frequently been recognized on the cystic mucosa. Recently Muqera and Nderito reported cases in which the cystic lesion was composed of predominant transitional cell carcinoma in association with papilloma, and which showed no hematuria.

Regarding the etiology, the fern, some components of plants in the hematuria district, and Encephalartos hildebrandii were pointed out as carcinogenic factors. Brodie and Olson reported their success in producing a neoplastic lesion on the cystic mucosa by injecting bovine cutaneous papilloma virus experimentally. Satoh et al., Satoh and Yoshikawa, and Yoshikawa asserted their opinion from a morphological point of view that vasomotoric disorder due to degeneration of the peripheral nervous system in the urinary bladder should not be neglected. Recently, Ushijima et al. and Matsukawa et al. conducted very interesting histochemical studies on cystic tumors in the disease. They reported that an increase in the enzyme, β-glucuronidase, had been noticed in the neoplastic tissues in the urinary bladder.

No electron microscopic findings on the hematuria bladder have been published in the knowledge of the authors. Any ultrastructural change of such bladder is obscure. Ultrastructural studies were carried out on 12 neoplastic cases of the urinary bladder encountered in hematuria districts by using the histochemical technic.

MATERIALS AND METHODS

Twelve cases examined are shown in Table 1. Of them, eleven were obtained at the Morioka Abattoir, and one was surgically removed at the Veterinary Hospital, Iwate University. Specimens were collected from them, placed in a drop of fixative on the glass plate, and cut into slices by a double-edged razor within 30 minutes after slaughter or removal. For ultrastructural studies, specimens from some cases were fixed, for 90 minutes, in chilled 1% osmium tetroxide solution buffered with 0.2 M s-collidine containing 45 mg/ml of sucrose at pH 7.5. Others were fixed, for 2 hours, in chilled 0.067 M
phosphate buffer solution containing 2.5% glutaraldehyde at pH 7.3. Post-fixation was carried out in the osmium tetroxide solution. Some of the materials were fixed in chilled glutaraldehyde buffered with 0.067 M cacodylate buffer at pH 7.3 for histochemical studies. The fixed materials were sliced into more tiny pieces than usual. They were incubated in the substrate solution at 37°C for 90 minutes, and post-osmicated\(^{1,11,17,40,44}\).

The specimens fixed were rinsed with 0.2 M s-collidine buffer, and embedded in Epon 812 by Luft’s method\(^{18}\). They were sliced into sections one micron and 400 Å in thickness by using a Porter-Blum MT-I microtome. One-micron sections were stained with toluidine blue for light microscopy. The thinner sections were collected on copper grids and stained with uranyl acetate and lead citrate stain\(^{29}\), except some for use in cytochemical studies. A Hitachi HU 125E electron microscope was used for observation of the specimens.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Date collected</th>
<th>Breed</th>
<th>Age (years)</th>
<th>Histological diagnosis</th>
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<tr>
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<td>Nov. 2, '67</td>
<td>H</td>
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<td>Hemangioma</td>
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<tr>
<td>2(^{a})</td>
<td>Mar. 14, '68</td>
<td>H</td>
<td>9</td>
<td>Adenocarcinoma</td>
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<tr>
<td>3(^{a})</td>
<td>Mar. 11, '69</td>
<td>H</td>
<td>3</td>
<td>Hemangioma</td>
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<td>4(^{a})</td>
<td>Sept. 26, '69</td>
<td>H</td>
<td>7</td>
<td>Transitional cell carcinoma</td>
</tr>
<tr>
<td>5(^{a})</td>
<td>Dec. 24, '69</td>
<td>Jb</td>
<td>6</td>
<td>Hemangioma</td>
</tr>
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<td>6</td>
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<td>Transitional cell carcinoma</td>
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<td>7</td>
<td>Feb. 23, '70</td>
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<td>9</td>
<td>Apr. 16, '70</td>
<td>H</td>
<td>8</td>
<td>Fibroma</td>
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<tr>
<td>12(^{a})</td>
<td>June 6, '69</td>
<td>Jsh</td>
<td>8</td>
<td>Hemangioma</td>
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</tbody>
</table>

Remarks. All the cases are female. \(^{a}\) showing hematuria clinically. \(^{3}\) Biopsy case. H: Holstein-Friesian, Jb: Japanese Black, Jsh: Japanese Short Horn.

RESULTS

Hemangioma

Six cases presented the lesion of hemangioma. All of them showed hematuria clinically. Neoplastic endothelial cells got in touch with one another by tight junctions and desmosomes. They showed an irregular transformation, presenting many cytoplasmic processes which were frequently desquamated from the basement membrane. They also became thinner than usual. Among these cells were frequently formed intercellular apertures and fenestrations (Fig. 1). A fragmenary basement membrane was observed inconstantly and partially. Occasionally, the endothelial cells got directly in contact with such cells as smooth muscle cells, pericytes, and fibroblasts, and collagenous fibers in some portions of the epithelium without the basement membrane. Edematous
condition was frequently seen in perivascular areas. Various lysosomes, myelin-like substances, mitochondria, pinocytic vesicles, and rough-surfaced endoplasmic reticulum (ER) were observed in the cytoplasm of the endothelial cells typically. The ERs were dilated frequently. Lysosomes were numerous in the endothelial cells in hemorrhagic lesions (Fig. 2). Moreover, erythrocytes were seen passing through the loosened intercellular junctions and fragmentary basement membrane in the neoplastic lesions. Some erythrocytes were present in edematous connective tissues surrounding the blood vessels in the lesion (Fig. 3). There were neutrophils passing through the tight junction of endothelial cells and even through the endothelial cytoplasm itself. The blood corpuscles flowed into the cavity of the urinary bladder, passing through the basement membrane and intercellular spaces of endothelial cells (Fig. 4). The cytoplasm of some epithelial cells contained fine granules enclosed in a membrane. Frequently, many lysosomes were noted in some pericytes at the subendothelium of the neoplastic blood vessels.

Perivascular hemorrhage and edema were observed in non-neoplastic terminal blood vessels which were located near the lesions of hemangiomia (Fig. 5). No morbidity was recognized in the endothelial cells of any non-neoplastic blood vessel.

Epithelial cells covering the neoplastic lesions showed degenerative changes. They also contained lysosomes and myelin-like substances in the cytoplasm. Mucoid substances, as well as erythrocytes, were frequently recognized in the intercellular spaces of the epithelium.

Adenoma and adenocarcinoma

An acinus of the adenoma tissue was encircled with a regular basement membrane. The epithelial cells had microvilli projecting into the lumen of the acinus. The intercellular junctions were composed of intermediate junctions and desmosomes. Many ERs, ribosomes, secretory granules, and a few mitochondria were observed in the cytoplasm. The nuclei were pyknotic and located eccentrically. Those cells looked like a type of glandular cells or goblet cells, showing hypersecretosis (Fig. 6). There were two types of granules in the cells. Granules of one type were high in electron density and stained positively with alcian blue by the light-microscopic technic. Those of the other type were moderate in electron density and stainable by the PAS technic. Accordingly, the former might be regarded as granules of carbohydrate and the latter as those of mucoprotein.

Light microscopy revealed frequently the presence of large cells with coarse eosinophilic granules in the intercellular spaces among the glandular cells in the lesions of adenoma, as well as adenocarcinoma. These cells were also recognized in the intercellular spaces of the epithelium covering the lesions. The smooth-surfaced endoplasmic reticulum (sER), Golgi complex, and mitochondria were noticed to have developed conspicuously in the above-mentioned granular cells. The eosinophilic granules measured up to 3 microns in diameter. Some of them were observed fusing with one another. Vacuolization occurred to some granules occasionally (Fig. 7).

Transitional cell carcinoma

Carcinoma was recognized in 4 cases. The neoplasm consisted of small cuboidal cells. The nuclei of these cells contained scarce chromatin and had an irregular notch of the nuclear envelope. Desmosomes and intermediate junctions were present among these cells, showing simple interdigitation. Lysosomes, filaments, and vesicles were recognized in the cytoplasm. Amorphous substances of high electron density were frequently contained in the intercellular spaces. Most of them were seen adhering to the cell membrane. Large lysosomes or secretary granules were observed in cells facing the lumina of the carcinoma nests. The neoplastic cells were divided into pale and dark cells (Fig. 8). Pale cells were small and low in electron density. Numerous mitochondria
and sERs and 2 or 3 Golgi complexes were observed in the cytoplasm. Filaments were scarce. Lysosomes were frequently seen in pale cells. Dark cells were high in electron density. Pyknotic chromatin and an undifferentiated nuclear envelope were recognized. Numerous mitochondria, lysosomes, and filaments and a few myelin-like substances were observed clearly in the cytoplasm.

There were peculiar organelles in both types of cells, they were 1,500 to 2,000 A or more in diameter and high in electron density. They had double membranes and contained amorphous substances in the central part of the organelles (Figs. 8 and 9). Acidphosphatase was demonstrated histochemically in some of the organelles (Figs 9 and 10). At the same time, the enzyme was distributed in lysosomes and phagosomes. Accordingly, the organelles may be regarded as types of lysosomes. They were distributed more densely in the pale cells than in the dark cells. Moreover, acidphosphatase was demonstrated in mucoprotein-like substances distributed in the intercellular spaces, especially predominantly in those distributed in the spaces among the dark cells.

Obvious hemorrhages were frequently recognized in the peripheral areas of terminal blood vessels in and around the neoplastic tissues. Additionally, various types of atypical leukemic cells were present in case No. 3. The perivascular hemorrhages seemed to have been caused by the mechanical effect of intense and expansive proliferation of leukemic cells.

Fibroma

Fibroma was recognized in 5 cases. In one of these cases, it was associated with transitional cell carcinoma. Lesions were situated in the propria layer. No neoplastic tissues invaded the muscular layer. The epithelial cells covering the neoplastic tissues were low in electron density, and contained numerous sERs and vesicles in the cytoplasm. Lysosomes and lipofuscin pigment (postlysosomes) increased in the cells. A moderate increase in lysosomes was observed also in the epithelial cells of cystitis lesions which were frequently associated with fibroma. A small number of lysosomes with double membranes, which were usually present in transitional-cell-carcinoma cells, appeared similarly in the epithelial cells of the cystitis lesions. No cases of fibroma were associated with hematuria clinically. Perivascular hemorrhages, however, were seen in some parts of the propria layer. No changes occurred in the endothelial cells of the hemorrhagic lesions. Numerous lysosomes were scattered in hemorrhagic foci. They had probably been oozed out of degenerated cells (Fig. 11). A large number of mast cells and a small number of plasma cells were contained in the propria layer, including neoplastic tissues.

Neoplastic lesions situated in the propria layer had an indistinct demarcation with normal connective tissues surrounding. They were composed of fibroblasts, fibrocytes, and fine collagenous fibers histologically. Fibroblasts were distributed scatteringly among collagenous fibers, to which they adhered. These fibers seemed to have been secreted from such cells as containing ERs, sERs, lysosomes, and pinocytotic vesicles abundantly (Fig. 12). Some of the fibroblasts had many lysosomes in their cytoplasm (Fig. 13). The secretion process of tropocollagen was not distinctly recognized within the cells, although some of the fibroblasts contained clumps of collagenous fibers. Perivascular hemorrhages were frequently recognized in terminal blood vessels and especially in the neoplastic tissues. The endothelial cells showed regular structures. Numerous lysosomes were present in the hemorrhagic lesions. They were possibly originated from fibroblasts or some other kind of cells containing many lysosomes.
DISCUSSION

Enzootic bovine hematuria was termed after its clinical symptom. Histopathologically, the disease seems to be associated primarily with neoplastic lesions on the cystic mucosa. On the other hand, the disease is described as "chronische vesikale Haematurie" in German literature\(^\text{10,34}\). The present cases examined were all obtained from hematuria districts. Seven of them showed hematuria, but the other five did not show such a clinical symptom.

The fine structures of the normal endothelium of the terminal vascular bed were studied by some investigators\(^\text{2,6,23}\). They are divided into two types, the structure of capillaries and that of postcapillaries. In the present cases, however, the endothelial cells in hemangioma lesions were morphologically differentiated from those of both types divided in the normal condition. Regarding the hemorrhages, there was an opinion that the degeneration of the peripheral nervous system might possibly be causative\(^\text{96-98,40}\). Miura et al.\(^\text{22}\) were not able to demonstrate any consistent morbidity of the peripheral nervous system of the urinary bladder in 21 cases of hematuria examined. Furthermore, Miura et al.\(^\text{21}\) recognized the frequent occurrence of edematous and degenerative lesions of peripheral nerve fibers distributed in the urinary bladder in their histopathologically examination of 121 cattle slaughtered. Recently, very interesting histochemical findings\(^\text{16,42}\) were published that an increase in activity of the enzyme, $\beta$-glucuronidase, had been noted in neoplastic tissues of the disease. This enzyme is one of the 55 enzymes, the presence of which has been demonstrated in the lysosome\(^\text{25}\). In the field of molecular pathology, it is a well known fact that the lysosome changes the permeability of blood vessels\(^\text{16,41}\). Increased lysosomes of endothelial cells in hemoangioma and numerous lysosomes in hemorrhagic lesions were expected to exert such influence as to elevate the permeability of the blood vessel. The present authors consider that hematuria may occur due to an atypical finding and that an increase in lysosome in the endothelium may be possibly resulted from the neoplastic change in the examined cases. In the hemorrhagic lesions of terminal blood vessels showing a normal structure, the expansive growth of neoplasm and the increase in lysosome in neoplastic cells near the blood vessels were considered to have an influence to cause hemorrhage on the blood vessels.

Some common changes were recognized in the epithelial cells covering the tumors and cystic area in some cases. These cells were richer in lysosomes and lipofuscin pigment (postlysosomes), as well as in secretory granules, than normal cells. In the hemorrhagic lesions, intercellular deposition of mucoprotein-like substances high in electron density and dilation of intercellular spaces were observed frequently. These changes may have resulted from the outbreak of unusual metabolic condition. The present authors consider that these morbid conditions of epithelial cells may have stimulated the occurrence of hematuria, in addition to hemorrhage from terminal blood vessels.

In the early stage of adenoma, the neoplastic cells resembled normal granular cells. An increase in ER and ribosomes, and a decrease in desmosome were recognized in the neoplasm as findings common to various types of neoplasm. Degenerative changes occurred to the nucleus in cells containing secretory granules and resembling goblet cells in structure (Fig. 6). These granules were identical with polysaccharide and mucoprotein.

Large eosinophilic granular cells were frequently observed in and around the adenoma lesion. They were not regarded as the cells of bone-marrow origin mentioned
in the previous report\textsuperscript{22}, nor morphologically identical with the mast cells which had been studied by Combs ultrastructurally (Fig. 7).

Transitional cell carcinoma of the urinary bladder in man was classified into 4 grades according to malignancy\textsuperscript{39}. The cells forming it were studied electron microscopically and divided into two groups, pale and dark cells\textsuperscript{130}. Both pale and dark cells, as well as intermediate cells, were present in the present cases of carcinoma (Fig. 8). The unique lysosomes observed in the present cases, however, had not been described in any previous paper dealing with human cases of carcinoma. These lysosomes were amorphous and high in electron density at their centers (Fig. 9). They were 1,500 to 2,000 Å in diameter and circumscribed by double membranes. They were recognized also in similar figures in degenerated cells. In guinea pigs, particles resembling the lysosomes were reported to be seen as cytoplasmic inclusions\textsuperscript{20,35}. Ultrahistochemically, the lysosome contained acidphosphatase-positive substances (Fig. 10). It would be placed under the category of autolysosome or terolysosome. Acidphosphatase was distributed abundantly in the intercellular spaces of the dark cells. It may have been originated from the lysosome.

A number of papers have been published recently on ultrastructural studies of fibrogenesis\textsuperscript{4,12,13,43}. There are 2 theories concerning the fibrogenesis of fibroma. In one opinion, such fibrogenesis as close to the normal conditions may be regarded as fibroma conditions from observations on tissue culture\textsuperscript{40}. In the other opinion, a heterogenous synthesis of trophocollagen may occur at first and collagenous fibers may appear in sER and the residual bodies of the cells\textsuperscript{43}. In the present cases, no synthesis of trophocollagen was recognized. However, collagenous fibers, as well as filaments, were present in the cytoplasm (Fig. 12). The existence of collagenous fibers in the cells may indicate that such fibrogenesis as different from the normal one took place in neoplastic fibroblasts.

Concerning the etiology of the neoplastic lesion in the present cases, no essential findings were noted, as mentioned elsewhere. It can be stressed, however, that neoplastic growth and increased lysosomes, which may probably result from metabolic disorders of neoplastic cells, would be causative to a phenomenon of hematuria.

**SUMMARY**

Electron microscopic and ultrahistochemical surveys were conducted on twelve cases of neoplastic lesions in the urinary bladder encountered among cattle in a hematuria district.

Seven of the twelve cases showed hematuria, but the remainder were subclinical cases. Histological examination of the neoplastic lesions revealed hemangioma, adenoma, transitional cell carcinoma, fibroma and lymphosarcoma associated with transitional cell carcinoma. Two or three of the different types of neoplastic lesions were recognized on the same bladder in some cases.

Atypical endothelial cells were observed in the hemangioma. Formation of intercellular apertures, and fenestration and increase of lysosomes were evident. Numerous lysosomes and dilated intercellular junctions were seen in the cells of the hemorrhagic lesions. Blood corpuscles were emigrated to perivascular tissues through the junctions loosened. They oozed out into the cystic cavity through the intercellular spaces of the epithelium.

Numerous secretory types of cells were noted in adenoma lesions. Secretory granules were divided into two types, carbohydrate and mucoprotein. Unique cells with gross eosinophilic granules were present in these cases.
There were two types of cells, pale and dark, among the cells of transitional-cell-carcinoma. Increased unique lysosomes were recognized ultrahistochemically in both types of neoplastic cells.

Unusual fibrogenesis was noted in many fibroblasts in the fibroma tissue. Numerous lysosomes were contained in the neoplastic cells.

Hemorrhages in hemangioma may be attributed to morphological changes, as well as metabolic disorders, of neoplastic endothelial cells in the lesions. In the neoplastic lesions, except those of hemangioma, expansive growth and metabolic disorders of neoplastic cells would be an important factor causing hemorrhages through the wall of terminal blood vessels.

REFERENCES

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牛の血尿症膀胱に関する電子顕微鏡的研究

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岩手大学農学部家畜病理学教室
(昭和45年8月26日受付)

血尿症地帯の牛膀胱腫瘍12例について、電子顕微鏡的研究および電顕レベルにおける組織化学的検索を行ない、これらの結果から、血尿症の病理発生に検討を加えた。

12例中7例に臨床上血尿を認め、血管腔腫瘍、移行上皮腫および線維腫が、単独あるいは2ないし3種共存して認められた。また1例では、移行上皮腫とリンパ肉腫が同時に出認められた。

血管腫の内皮は異形性を示し、Lysosomeに富む。細胞間狭帯またはfenestrationの形成がみられ、内皮細胞間は平開し、ここの血球の流出を認め、さらに上皮細胞間隙を絹で肺膿内に排出される所見を得た。

腎臓では、分泌形の細胞が多く、分泌顆粒は2種類。すなわち多糖類と粘液蛋白とに分けられ、またこの症例では、特殊な粗大化粧顆粒細胞の出現を見た。

移行上皮腫では、腫瘍細胞は明および暗細胞にわけられ、組織化学的に特殊なLysosomeの増加が観察された。

線維腫腫病変にみられる線維芽細胞細胞は、正常なfibrogenesisと異なる現象が観察された。

以上の所見より、血管腫における出血は、腫瘍性血管内皮の形態的な変化、および細胞の代謝異常によるものであることがうかがえた。他の腫瘍病変における出血は、圧排性を示す腫瘍増生およ
び腫瘍細胞の代謝異常が、隣接末梢血管から出血を起こさせる重要な原因になるものと考えられ

EXPLANATION of PLATES

PLATE I

Fig. 1. An atypical endothelial cell (End) in the hemangioma lesion. It contains several lysosomes (Lys) and dilated endoplasmic reticulum. Arrow indicates the formation of intercellular aperture. Case 1, ×6,200.

Fig. 2. Perivascular lesion in hemangioma. Note numerous lysosomes (Lys) in endothelial cells (End) and fragmentary basement membrane (Bm). Case 4, ×5,600.

Fig. 3. Hemorrhagic lesion in hemangioma. Erythrocytes (Rc) have been emigrated in perivascular tissues through loosened intercellular junctions of endothelial cells. Case 4, ×3,000.

PLATE II

Fig. 4. Erythrocytes in intercellular spaces (Is) of the epithelium. Case 5, ×5,200.

Fig. 5. Emigration of erythrocytes (Rc) from a terminal blood vessel near the hemangioma lesion. An endothelial cell (End) and pericytes (Pc) show normal structures. Case 2, ×5,000.

Fig. 6. Goblet cell-like adenocarcinoma cells. Numerous microvilli (Mv) are observed. Case 1, ×8,000.

PLATE III

Fig. 7. A large cell with gross eosinophilic granules in intercellular spaces of epithelial cells. Case 4, ×6,400.

Fig. 8. Pale cells (Pc) and dark cells (Dc) in transitional-cell-carcinoma. Numerous unique lysosomes (Lys) are present. Case 5, ×11,800.

Fig. 9. Unique lysosomes in a pale cell. Case 5, ×48,000.

Fig. 10. Unique lysosomes with acidphosphatase-positive substances at the center. Case 10, ×37,500.

PLATE IV

Fig. 11. Perivascular hemorrhage from a terminal blood vessel in the propria layer of a fibroma case. Endothelial cells (End) have a normal structure Numerous lysosomes (Lys) and erythrocytes (Rc) are observed in the perivascular area. Case 9, ×4,900.

Fig. 12. Collagenous fibers (Cf) attached to fibroblasts are shown in the fibroma lesion. These cells contain filaments and collagenous fibers. Case 9, ×5,100.

Fig. 13. Numerous lysosomes (Lys) are observed in a fibroblast in the fibroma lesion. Case 9, ×5,100.