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Histological and Histoenzymochemical Aspects of Ocular Tumours in Cattle

By

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The most frequent ocular tumours in cattle are those of the conjunctival mucosa and circumocular skin (conjunctival-palpebral tumours). They are also one of the most common forms of neoplasia in cattle (Willis, 1967; Morgan, 1969). In most countries, including Romania, these tumours, mainly malignant, also represent, except for leukosis, the most common form of malignant neoplasia in this species.

In the United States, Russell, Wynne and Loquvam (1956) estimated the incidence of these tumours as 0.8—1.6 %o. In our studies, on 420,203 cattle, conjunctival-palpebral tumours were found in 0.12 %o of the animals. The extensive study of Russell, Wynne and Loquvam (1956) emphasized that conjunctival-palpebral tumours comprise almost all the ocular tumours. In our own material, except for a malignant mixed tumour of the ciliary body (Ivascu, Simu and Onet, 1972), all the ocular tumours originated from the conjunctiva and the nictitating membrane, or from the eyelid and its annexes (lachrimal or sebaceous glands). Thus, the tumours originating from this structural complex, especially the squamous carcinoma which is by far the most common histological form, represent the pathological substratum of the so-called “cancer eye”.

This article discusses some peculiar clinical and pathological aspects concerning especially the histogenesis and histoenzymochemical profile of these tumours. Some similarities between bovine ocular tumours and human ones, suggesting possible aetiopathogenic similarities, add interest to this problem.
Material and Methods

Among 420,203 animals examined clinically, 504 ocular tumours were diagnosed. 163 of these tumours were examined histologically after fixation in 12% neutral formalin and Carnoy fixative, paraffin embedding and staining with hematoxylin-eosin, Gomori silver impregnation for reticulin fibres, Hotchkiss-MacManus PAS for neutral mucopolysaccharides and Masson trichrome for collagen fibres. In 50 of these cases, sections were also stained with Feulgen for desoxyribonucleic acid and with Brachet-Kurnick methyl green-pyronine for nucleic acids.

On 24 fresh blocks cut on a freezing microtome, the following histo-enzymatic methods were performed: Lillie for succinic dehydrogenase, Padykula-Herman for adenosine-triphosphatase, Gomori for acid phosphatase, Gomori-Takamatsu for alkaline phosphatase, Richterich-Martin for non-specific esterase and Richterich for lipase.

Results

All 163 ocular tumours examined histologically, except for a mixed melano-angiosarcoma of the ciliary body, originated from the conjunctival membrane or the eyelids and their annexes (Figs. 1 and 2, Table I).

Fig. 1. Carcinoma of the conjunctiva and lower lid (squamous carcinoma)

Fig. 2. Tumour originating from the nictitating membrane (squamous carcinoma)
Table 1
Anatomical origin of 162 ocular tumours

<table>
<thead>
<tr>
<th></th>
<th>DISPLASIA</th>
<th>PAPILLOMA</th>
<th>CINNOMA IN SITU</th>
<th>LEUKEMIC CHONDROMA</th>
<th>CARCINOMA</th>
<th>SEBACEOUS HYPERPLASIA</th>
<th>SEBACEOUS ADENOMA</th>
<th>SEBACEOUS CARCINOMA</th>
<th>Lymphangioma</th>
<th>FIBROMA</th>
<th>OTHER ECTODERMAL</th>
<th>ECTODERMAL UNCLASSIFIED</th>
</tr>
</thead>
<tbody>
<tr>
<td>EYELID AND PALPEBRAL CONJUNCTIVA</td>
<td>1</td>
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<td>6</td>
<td>25</td>
<td>25</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td></td>
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<tr>
<td>BULBAR CONJUNCTIVA</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>1</td>
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<tr>
<td>NICTITATING MEMBRANE</td>
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<td>2</td>
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<tr>
<td>SCLEROS-CORNEAL LIMBUS</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>7</td>
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<tr>
<td>LACRIMAL APPARATUS</td>
<td>---</td>
<td>---</td>
<td>4</td>
<td>1</td>
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<tr>
<td>INDEFINITE ORIGIN</td>
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<tr>
<td><strong>TOTAL 162</strong></td>
<td><strong>7</strong></td>
<td><strong>21</strong></td>
<td><strong>2</strong></td>
<td><strong>19</strong></td>
<td><strong>34</strong></td>
<td><strong>55</strong></td>
<td><strong>5</strong></td>
<td><strong>4</strong></td>
<td><strong>5</strong></td>
<td><strong>10</strong></td>
<td><strong>2</strong></td>
<td></td>
</tr>
</tbody>
</table>

Legend: 1. Stratum basale; 2. stratum spinosum profundum; 3. stratum spinosum superficiale; 4. connective tissue; 5. vessels

Histologically, the following forms were found:

- Epithelial dysplasia: 7 cases
- Papilloma: 21 cases
- In situ carcinoma: 2 cases
- Poorly differentiated squamous carcinoma: 19 cases
- Squamous carcinoma: 34 cases
- Keratinizing squamous carcinoma: 53 cases
- Sebaceous glands hyperplasias: 5 cases
- Sebaceous adenoma: 4 cases
- Adenocarcinoma: 5 cases
- Fibroma: 10 cases
- Lymphangioma: 2 cases

Fig. 3. Keratinizing squamous carcinoma. H. & E., × 160
The microscopical features of keratinizing squamous carcinoma, the most common histological form, are represented in Figure 3. The age distribution of the cattle bearing ocular tumours is shown in Figure 4. Most cattle with ocular lesions were of the Romanian spotted breed (95.5 %); a few affected cattle belonged to the Maramures brown or Transylvanian Pinzgau breeds. There is no evidence to suggest that the incidence of ocular cancer is higher in the Romanian spotted than in the other, since spotted breed animals represented about 90 % of all the animals examined.

The histochemical reactions reproduced the well known findings of an increased DNA and RNA content in malignant tumours, as well as a neutral

![Fig. 4. Age incidence of ocular tumours in cattle](image)

![Fig. 5. Succinic dehydrogenase activity in an epithelial plaque. Lillie, x 44](image)
mucopolysaccharide depolymerisation and a reticulin fibre lysis in the stroma of the invasive carcinomas.

The histoenzymatic reactions allowed the following conclusions to be drawn about the behaviour of the enzymes studied at the level of the dysplastic, benign or malignant neoplastic changes of the conjunctival-palpebral complex:

1. Succinic dehydrogenase is only moderately increased in dysplasia and papillomatous hyperplasia, but its activity is much increased at the periphery of the tumour cords (Fig. 5—7).

2. Adenosine-triphosphatase activity is also intensified in the peripheral areas of papillomas or carcinomas, but its activity is especially raised at the level of the endothelial cells of the vessels (Figs. 8 and 9).

Fig. 6. Succinic dehydrogenase activity in a papilloma. Lillie, × 44

Fig. 7. Succinic dehydrogenase activity in a squamous carcinoma. Lillie, × 44
3. Acid phosphatase is noticeably increased in the keratinizing areas of papillomas and carcinomas (Fig. 10).

Fig. 8. Adenosine-triphosphatase activity in a papilloma. Padykula-Herman, × 44

Fig. 9. Adenosine-triphosphatase activity in a squamous carcinoma. Padykula-Herman, × 64

4. Alkaline phosphatase activity, visible also in some tumour cells, is constantly increased in the stroma, especially in the walls of the vessels (Figs. 11 and 12).

5. Lipase activity, directly proportional to the keratinizing process, is increased in the respective areas of the papillomas and carcinomas, but generally is weaker than in normal epithelium.

6. Non-specific esterases have a similar behaviour, their activity being weaker in hyperplastic or neoplastic epithelia than in normal epithelium.
The comparative activity of these enzymes in normal, hyperplastic and neoplastic epithelia of the conjunctival membrane and eyelid may be seen in Table 2.

Fig. 10. Acid phosphatase activity in a squamous carcinoma. Gomori, × 64

Fig. 11. Alkaline phosphatase activity at the level of a papilloma. Gomori-Takamatsu, × 44

Discussion

Our results agree with those of other authors, especially Russell, Wynne and Loquvam (1956) who in their well documented work about bovine cancer eye showed that the most frequent tumour of the bovine eye is the squamous carcinoma (71.4 %). Considering only the conjunctival-palpebral complex, which is the preferential site of neoplastic changes in cattle, we found that
this squamous carcinoma had a incidence of 74.7% of all tumours of this complex.

![Image](image_url)

**Fig. 12.** Alkaline phosphatase activity at the level of a squamous carcinoma. Gomori-Takamatsu, × 64

<table>
<thead>
<tr>
<th>ENZYME</th>
<th>NORMAL</th>
<th>EPITHELIUM</th>
<th>DISPLASIA</th>
<th>PAPILLOMA</th>
<th>CARCINOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
<td>1 2 3 4 5</td>
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<tr>
<td>Succinic dehydrogenase</td>
<td>+ - - + +</td>
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<td>+ + + + +</td>
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<tr>
<td>Adenosin triphosphatase</td>
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<td>- - + + +</td>
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<tr>
<td>Acid phosphatase</td>
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<tr>
<td>Alkaline phosphatase</td>
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<tr>
<td>Lipase</td>
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<tr>
<td>Non-specific esterase</td>
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</tbody>
</table>

**Our cases also confirm the suggestion of the American authors that a progression of the invasive cancer histogenesis can be traced, starting from simple acanthotic-dyskeratotic hyperplasia of a limited area of the conjunctival epithelium ("plaque" of the American authors) and passing via papilloma and in situ carcinoma to invasive carcinoma.**

These observations of RUSSELL, WYNNE and LOQUVAM (1956) on a cancer with high incidence originating from squamous epithelium in cattle were followed in the next decade by studies of several groups of authors on a
cancer of high incidence in woman originating also from squamous epithelium, cervico-uterine carcinoma. These authors showed that in most cases the invasive neoplastic lesions are preceded by dysplastic or intraepithelial neoplastic changes (Nieburgs, 1963; Koss, Stewart, Foote, Jordan, Bader and Day, 1963). The phenomenon has been studied in Romania especially by Kiricuta, Munteanu, Risca and Simu (1972).

This possible progression of the conjunctival-palpebral changes in cattle calls for a similar conclusion on the prophylactic importance of the diagnosis of these pathological conditions in the early stage of plaque or papilloma, when adequate treatment may preserve the animal's eye.

Proceeding from human pathology, we have classified squamous carcinomas of the bovine eye into three morphological variants. Some cases exhibiting a predominance of uniformly small cells with poorly defined cytoplasmic outlines and without epithelial pearl formation or isolated cell keratinization were termed "poorly differentiated squamous carcinoma". The majority of the carcinomas can, however, be classified as "squamous" or "keratinizing squamous carcinomas". The former is characterized by large squamous cells, often rich in vacuoles, without keratinizing phenomena. The constant appearance of keratin or the presence of epithelial pearls is the characteristic feature of the keratinizing forms; the keratinizing process is often very marked.

The histochecmical study of these tumours confirms the previous data about an increase of nucleic acids in the tumour cells as well as disintegration of the mucopolysaccharides in the stroma of the tumours. The histochemical profile does not show any consistent differences in comparison with other malignant tumours arising from an epidermoid epithelium, i.e. cervico-uterine epidermoid epithelium in women (Thiery and Willighagen, 1966). Essential histochemical differences between the normal and neoplastic epithelium are not apparent. Only a complete examination of the quantitative histo-enzymology, as recommended by Wegmann (1970), would allow of a histochemical differential diagnosis.

The relatively low incidence of cancer eye in Romania, in comparison with the data of the American authors could be explained by racial and especially climatic differences: in Romania, cattle are less exposed to the action of carcinogenic factors, especially solar radiation.

The interesting analogy between two forms of cancer originating in two different species, concerning the progression of the changes, emphasizes the importance of the early detection of pre-neoplastic changes in veterinary as in human pathology.

Based upon some epidemiological conclusions, we have suggested (Simu and Kiricuta, 1972) that the high incidence of cervico-uterine cancer in women may be the result of overstimulation imposed on an organ insufficiently adapted phylogenetically to the conditions of a permanent sexual life; in most cases of cervico-uterine cancer an early sexual life, sexual excesses or deficient hygiene of the two partners may be traced (Rotkin, 1966). Similarly, a high incidence of bovine ocular carcinoma might be related to husbandry conditions, and especially to prolonged exposure to solar radiations.

Summary

Clinical examination of 420,203 cattle in Romania revealed 504 ocular tumours (0.12 \%/o), 163 of which were examined histologically. Except for a mixed melanomasarcomatous tumour of the ciliary body, all these tumours originated from the conjunctival membrane or the lids and their annexes and exhibited the microscopical features of epithelial dysplasia, papilloma, intra-
epithelial carcinoma and especially more or less differentiated forms of squamous carcinoma.

Histoenzymochemical examinations showed an increase in DNA and RNA content in the malignant cells, as well as neutral mucopolysaccharide depolymerization and reticulin fibre lysis in the stroma of the invasive carcinomas. Succinic dehydrogenase activity was moderately increased in preneoplastic changes, and appreciably increased at the periphery of the tumour cords. Acid phosphatase activity was appreciably increased in the keratinizing areas of papillomas and carcinomas, as was lipase activity. Non-specific esterase has a weaker activity in hyperplastic or neoplastic epithelia than in normal epithelia. The activity of alkaline phosphatase and adenosin-triphosphatase was increased in the stroma of the carcinomas.

The results confirmed that, in Romania also, squamous carcinoma is by far the most frequent tumour of the cancer eye and that a progression of the benign hyperplasias, by way of intraepithelial malignant lesions, to invasive malignant forms may occur. A discussion of the comparative pathology of the tumours with those of the human cervix follows.

**Zusammenfassung**

**Histologische und enzymhistochemische Aspekte bei Augenblastomen der Rinder**

Von 420 203 rumänischen Rindern wiesen 504 (0,12 %) Augenblastome auf, wovon 163 histologisch untersucht wurden. Abgesehen von einem Melanosarkom des Ziliarkörpers gingen alle Tumoren von der Konjunktiva oder den Lidern bzw. deren Adnexe aus. Es handelt sich um epitheliale Dysplasien, Papillome, Karzinome und speziell mehr oder weniger stark differenzierte Plattenepipither-Karzinome.


Die Ergebnisse bestätigen, daß auch in Rumänien das Plattenepipither-Karzinom das weitaus häufigste Blastom des Auges darstellt, und daß gutartige Hyperplasien durch intraepitheliale maligne Läsionen in infiltrierende bösartige Tumormformen übergehen können. Die Atiologie der beschriebenen Augentumoren wird mit der der menschlichen Zervixtumoren verglichen.

**Résumé**

**Aspects histologiques et d'histochimie enzymatique de blastomes oculaires chez le bovin**

Sur 420 203 bovins roumains, 504 (0,12 %) ont présenté des blastomes oculaires; on a examiné 163 cas histologiquement. Abstraction faite d'un mélanosarcome du corps ciliaire, toutes les tumeurs provenaient de la conjonctive ou des paupières, respectivement de leurs annexes. Il s'agissait de
dysplasies épithéliales, de papillomes, de carcinomes et plus spécialement de carcinomes plus ou moins différenciés de l'épithélium pavimenteux.

Les cellules cancéreuses ont montré à l'histochimie enzymatique une augmentation en ADN et en ARN ainsi qu’une dépolymérisation des mucopolisaccharides neutres et une lyse des fibres de la réticuline dans le stroma de l’infiltration carcinomateuse.

Il y a une légère élévation de l’activité de la succinate-déhydrogénase dans les parties prénéoplastiques et une élévation marquée dans les cordons périphériques des blastomes. Il y a eu nette augmentation de l’activité des phosphatases acides ainsi que de lipases dans les pourtours cornés des papillomes et carcinomes. Les estérases non spécifiques se sont montrées moins actives dans les épithèles hyperplasiques et néoplasiques que dans les cellules épithéliales normales. L’activité des phosphatases alcalines et de l’ATPase était augmentée dans le stroma des carcinomes. Les résultats ont montré qu’en Roumanie le carcinome de l'épithélium pavimenteux représentait le blastome oculaire le plus fréquent et que des hyperplasies bénignes pouvaient conduire à des formes de tumeurs infiltrantes et malignes avec des lésions intraépithéliales. On a comparé l’étiologie des tumeurs oculaires décrites avec les tumeurs du cervix chez l’être humain.

Resumen
Aspectos histológicos y enzimohistoquímicos en los blastomas oculares de reses vacunas

Entre 420.203 reses bovinas examinadas clínicamente en Rumanía, 504 (0,12 %) revelaron blastomas oculares, entre los cuales se examinaron histológicamente 163. Exceptuando un melanosarcoma del cuerpo ciliar, todos los tumores se originaban desde la membrana conjuntiva o desde los párpados y sus anejos. Se trataba de displasias epiteliales, papilomas, carcinomas y sobre todo formas más o menos diferenciadas de carcinoma escamoso.

Los exámenes histoenzimquímicos mostraron un aumento en el contenido de ADN y ARN en las células malignas, así como una depolimerización de los mucopolisacáridos neutros y lisis de las fibras de reticulina en el estroma de los carcinomas invasores. La actividad de la dehidrogenasa succínica se hallaba aumentada ligeramente en las partes preneoplásicas v de forma considerable en la periferia de los cordones tumorales. Las actividades de la fosfatasa ácida y de la lipasa se hallaban aumentadas de manera clara en los areales queratinizantes de los papilomas y carcinomas. La actividad de la esterasa no específica mostraba en los epitelios hiperplásicos o neoplásicos una actividad más reducida que en las células epiteliales normales. La actividad de la fosfatasa alcalina y de la ATPasa estaban aumentadas en el estroma de los carcinomas. Los resultados confirman que también en Rumanía es el carcinoma escamoso el blastoma más frecuente con mucho en el ojo y que las hiperplasias benignas pueden convertirse en formas tumorales malignas infiltrantes mediante lesiones intraepiteliales malignas. La etiología de los tumores oculares descritos se compara con la de los tumores en el cuello uterino de la mujer.

References


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