

Cytomorphologic Findings in Tracheobronchial Secretions from Horses with Acute or Chronic Pulmonary Disease

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Cytological examination of tracheobronchial secretions was, until a short time ago, used mainly to differentiate the various forms of pneumonopathy into allergic, non-allergic and parasitic categories. Various authors, using diverse methods to collect pulmonary secretions, have used this classification (Fischer, 1980; Raidt, 1980; and Petzoldt, 1981). After the introduction of the flexible fibroscope for the examination of horses with pulmonary diseases, the collection of tracheobronchial secretions under visual control became easier. Collection of these secretions, which originate from the lower respiratory tree, allows a precise cytomorphologic examination. The results can then be used as diagnostic aids in the examination of horses with pulmonary diseases.

We undertook this study to see if it was possible to expand the amount of diagnostic information which could be gained by examining tracheobronchial secretions from horses with COPD. This concern became even more acute when we realized that bacteriologic examination proved to be of little diagnostic significance in horses with chronic pulmonary diseases (Hajer and Sasse, 1980).

Besides performing a quantitative cytologic examination, our main interest was to assess the morphologic properties of the sample. These findings and their possible significance will be presented in this paper.

The cytomorphologic alterations are most interesting when they are not analysed alone, but when the dynamics of pulmonary clearance are taken into consideration at the same time.

By evaluating morphologic alterations and the appearance of structures not commonly found in tracheobronchial secretions from animals without pulmonary diseases, it was possible to assess pulmonary clearance.

Pulmonary clearance, or mucociliary clearance, is understood to be a mechanism of cleansing the airways. This mechanism requires the coordinated activity of the cellular cilia and the production of different secretions which form the bronchial mucus.

In healthy horses, inhaled particles that reach the deeper parts of the respiratory tree are eliminated in less than one hour by the mucociliary system. Cells naturally exfoliated from the lung should be eliminated with the same velocity. Specific elements in the secretions reflect the functional state of the mucociliary system with respect to the clearance of inhaled and endogenous elements.

Our cytomorphologic examinations were performed on secretions from horses with pulmonary diseases at various stages. The secretions were collected from the tracheal bifurcation and deeper regions of the bronchial tree with the aid of an endoscope. The examination was conducted immediately after samples were collected. The secretions were examined natively and after staining according to the Papanheim, Papanicolaou and Hansel methods.

Epithelial Cells

Epithelial cells are easily recognized when they maintain their cilia or when the cilia are lost they can be identified by their cuticular membranes. They may take any shape from long and cylindrical, to a shorter, almost cuboidal shape, depending on their origin in the respiratory mucous membrane. Damage to the epithelial membrane, such as exfoliation of cilia from the epithelial cells or morphologic cellular alterations, has been described in human medicine by Papanicolaou in 1956 and was referred to as ciliocytophthoria. We found alterations similar to those in horses with pulmonary diseases. They were seen mainly in horses presented with fever and a cough 24 hours before the cytologic examination was performed. Such cellular alterations eventually provoke disturbances in the mucociliary system. The so-called „creola bodies“ are exfoliations of the mucosa in the form of clumps of cells. These abnormalities were described in humans after an asthmatic crisis. In horses with pulmonary diseases, the „creola bodies“ are found after a rise in temperature and following bouts of coughing. Once these exfoliations occur, metaplasia of the basal cells develops in these areas and the mucociliary transport system is damaged (Naylor, 1962).

Basal cells found in the secretions provide evidence that some areas of the pulmonary mucosa have undergone metaplasia (Morgenroth, 1982; Weller, 1953).

In horses with chronic obstructive pulmonary disease, the occurrence of hyperplasia of goblet cells is common, even in the small bronchi and bronchioles where these cells are not normally present.

Sometimes it is possible to find mucus-producing cells in varying quantities. They are distinguished from goblet cells by the high number of nucleoli. When stained using the Papanheim method the cytoplasm of such cells presents a basophilic coloration. In these cells the muco-polysaccharide acids also stain positively with Alcian blue (Deconto, 1983). Recent work with the electron microscope has shown the granules in the cytoplasm of these cells to be very similar to goblet cell granules. We believe that the appearance of such cells in secretions indicates the presence of an early stage of hyperplasia of mucus-producing cells.

Macrophages

The vast majority of macrophages are derived from the alveoli. They are always present in tracheobronchial secretions, unless the material examined is aspirated saliva. Presence of large particles in the cytoplasm of these cells, such as hyphae or fungal spores, indicates aberrant deposi-

tion of particles in the respiratory tract. Normally, large particles, that is above 10 micrometers, should remain in the upper regions of the respiratory tree (Rühle, 1978; Köhler et al. 1980). When the macrophages are retained in the smaller bronchioles and alveoli, due to bronchoconstriction or obstruction of the bronchi by the accumulation of secretions and edema of the mucosa, they phagocytize excess surfactant and become so-called foamy macrophages (Kissler, 1980; Cohen and Gold, 1975).

An increase in the number of foamy macrophages in the tracheobronchial secretions after administration of bronchodilators should be interpreted as evidence that there has been obstruction of the small bronchi and bronchioles.

Foreign body giant cells originate from the fusion of various macrophages or monocytes (Kraus, 1982).

They form when inhaled particles of foreign material appear in quantities too great to be phagocytized by only one cell. Their presence is always an indication of the depression of pulmonary clearance or prolonged bronchial obstruction. When these cells originate from small bronchi or bronchioles, they can present vacuoles like the foamy macrophages.

Granulocytes

(Neutrophils, Eosinophils and Basophils)

The number of neutrophils in tracheobronchial secretions varies according to the stage of pulmonary disease. In acute infections of the respiratory tract, unless they are parasitic in origin, the number of granulocytes is relatively small. In chronic stages, their number increases as the case tends to worsen. Signs of disturbed clearance appear when the cytologic examination shows a large number of degenerative cells, mainly neutrophils. Massive numbers of eosinophils are invariably found in infections due to *Dictyocaulus arnfieldi* (Fischer, 1980; Raidt, 1980). Mast cells are also found in the secretions from allergic animals. Sometimes small eosinophilic particles are found in the secretions. They are

granules which are freed by the disruption of eosinophils. The so-called Charcot-Leyden crystals are formed from the electrodense region of these granules. It is still not clear if their appearance is an indication of allergy in the horse. The presence of these crystals in tracheobronchial secretions of patients with bronchial asthma has been known in human medicine for a long time (Leyden, 1872).

Spirals of Curschmann

Spirals of Curschmann are formations which appear in the secretions from animals with a chronic disease. They are often seen in the secretion of horses which have areas of increased resonance during pulmonary percussion. The spirals are also found in patients following inhalation of harmful gases. The presence of Curschmann's spirals indicates temporary obstruction and collapse of mucociliary transport in the small airways. In chronic bronchitis without clinical signs, the presence of these spirals indicates small airway disease (Deconto, 1983).

Activity of Neutrophils

In some secretions, especially from animals in the advanced stage of chronic pulmonary disease, clumps of neutrophils appear around inhaled particles. These formations are very similar to those of macrophages or monocytes forming giant cells. In these cases, the neutrophils and macrophages are probably still active. We believe that these cellular formations are probably signs of immunologic reactions in the mucus. In recent research it has been found that the number of macrophages decreased in the secretions from horses with advanced COPD. There was also a significant increase in the levels of immunoglobulins A, G and M.

Therefore, we assume there is some immunologic alteration in horses with advanced COPD since macrophages are cells related to immunologic function.

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