Immunohistochemical Localization of the Iron-Binding Protein Lactoferrin in Human Bronchial Glands

A specific iron-binding protein, different from serum transferrin, has been isolated from bovine[1] and human[2-5] milk, and has recently been reported also to occur in sputum from human subjects suffering from different respiratory diseases[6]. Among the various names given to this protein, e.g. red milk protein[1], lactoferrin[3], lactotransferrin[7], and lactosiderophilin[5], the two latter are particularly ill-chosen, because they suggest a non-existing[8] structural relationship with the iron-binding protein from serum. The name lactoferrin, initially proposed by BLANC and ISLIKER[3], will be used in the present publication.

Unpublished work from our laboratory has demonstrated the presence of lactoferrin in human saliva and semen, and confirmed its occurrence in bronchial secretions. The protein was readily identified on account of its red color, electrophoretic mobility, and iron-binding capacity, as well as by its reaction with a specific rabbit antiserum raised against a purified preparation of lactoferrin isolated from human milk (Figure 1), following the procedure of BLANC and ISLIKER[7].

In order to identify the cells from which this protein originates in the bronchial tree, cryostat sections of three biopsy specimens obtained during bronchoscopy and four specimens obtained during thoracic surgery were submitted to staining with fluorescein-labelled antiserum against lactoferrin. Prior to use, the absence of anti-blood-group activity in the antiserum was verified, both by agglutination tests on Rh^+ human erythrocytes of blood groups A, B and O, and by application of the fluorescent antiserum on cryostat section of intestinal biopsies. In no instance was erythrocyte-agglutinating activity or staining of blood-group-active intestinal mucus observed.

In all instances an intense fluorescence was observed in the acinar cells of the tracheal and bronchial glands (Figure 2). The specificity of the reaction was demonstrated by exposing adjoining sections of the biopsies to fluorescein-labelled antiserum which had been exhausted by means of human milk whey serum (Figure 2, below) or purified lactoferrin. No fluorescence was observed in the cells of the surface epithelium, nor in any other histological structure of the bronchial wall. In specimens of lung tissue obtained at surgery, the epithelial lining of the alveolae consistently failed to show any specific fluorescence.

Histological descriptions of the glandular structures of the bronchial mucosa usually refer to two main morphological types of cells, which may perhaps represent different stages of activity and differentiation of the same cell lineage: the so-called serous cell, having a clear, granular cytoplasm, and the mucous cell, which is distended by large mucin-containing secretory cisterns. In the present study, lactoferrin was consistently found to be associated with the serous cells (see Figure 3). The mucous cells (Mu in Figure 3) often displayed a certain amount of specific fluorescence in the basal part of their cytoplasm, i.e. in the flattened portion of the cell which was not occupied

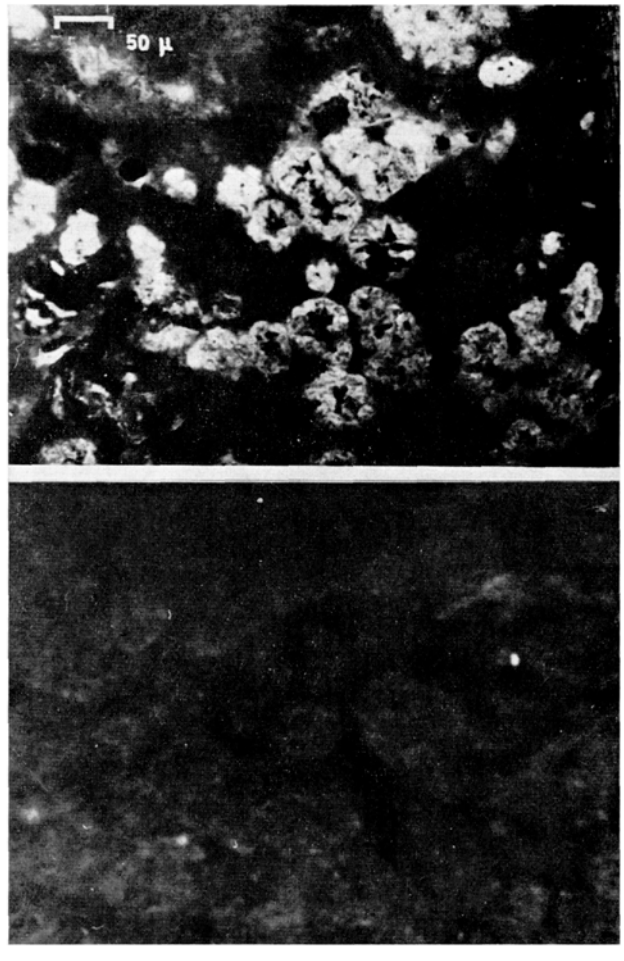


Fig. 2. Immunohistochemical demonstration of lactoferrin in human bronchial glands. Above: low-magnification view of bronchial biopsy section stained with fluorescein-labelled anti-lactoferrin. Below: same magnification of adjoining section stained with antiserum previously absorbed with lactoserum.

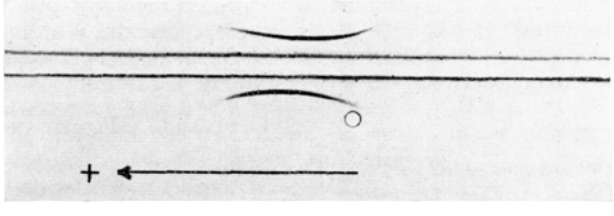
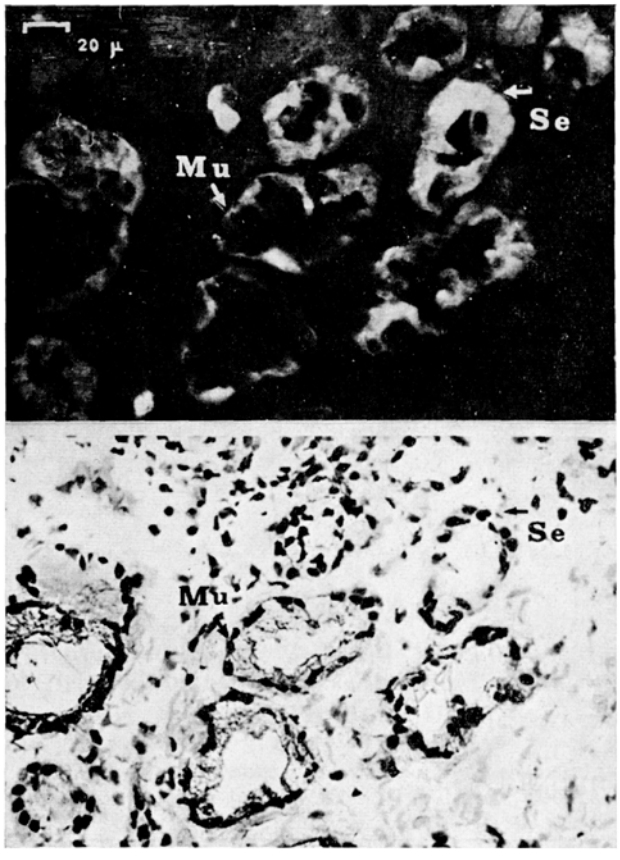


Fig. 1. Immunoelectrophoretic demonstration of lactoferrin in bronchial secretions. Upper well: bronchial aspirate obtained during bronchoscopy. Lower well: whey from human milk. Antibody slit: antiserum specifically directed against lactoferrin from human milk.

1 M. L. GROVES, J. Am. chem. Soc. *82*, 3345 (1960).
2 B. JOHANSSON, Nature *181*, 996 (1958).
3 B. BLANC and H. ISLIKER, Helv. physiol. pharmac. Acta *19*, C13 (1961).
4 R. GRUETTNER, K. H. SCHAEFFER, and W. SCHROETER, Klin. Wschr. *38*, 1162 (1960).
5 J. MONTREUIL, J. TONNELAT, and S. MULLET, Biochim. biophys. Acta *45*, 413 (1960).
6 G. BISERTE, R. HAVEZ, and R. CUVELIER, Exposés. ann. Biochim. méd. *24*, 85 (1963).
7 B. BLANC and H. ISLIKER, Bull. Soc. Chim. biol. *43*, 929 (1961).
8 B. BLANC, E. BUJARD, and J. MAURON, Exper. *19*, 299 (1963).



by the mucin-containing vacuoles, and which surrounded the nucleus. It should be stressed, however, that the contents of the secretory vacuoles of the goblet cells were usually lost during the processing of the tissues, so that no conclusion can as yet be drawn regarding the absence or presence of lactoferrin in these structures[9].

Résumé. Les techniques immunohistochimiques ont permis de localiser la lactoferrine dans les cellules séreuses des acini des glandes bronchiques.

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Fig. 3. Comparative staining of serous and mucous acinar cells. Above: high-magnification view of bronchial biopsy section stained with fluorescein-labelled antilactoferrin. Below: same section after staining by the periodic acid-Schiff procedure. Se: serous cells. Mu: mucous cells.

Effect of Thiamine Analogs on the Electrical Activity of the Rabbit Vagus Nerve[1]

In an investigation of the role of thiamine in nervous tissue, Armett and Cooper[2] found that pyrithiamine, an antimetabolite of thiamine, when applied to the non-myelinated fibers of the rabbit vagus nerve produced an increase in the amplitude of the compound action potential that was irreversible over a 90 min period (Figure 1). These effects could be prevented by pretreatment of the nerve with thiamine. In subsequent biochemical investigations[3], no correlation could be obtained between the electrophysiological effect of pyrithiamine and an interference with the known enzymatic reactions that require thiamine pyrophosphate (TPP) as a coenzyme. In addition, no inhibition by pyrithiamine of the synthesis of TPP could be demonstrated. However, it could be shown that incubation of the vagus nerve with the antimetabolite caused a release of thiamine from the nerve.

In an effort to gain some information on the specificity of the electrical effect of pyrithiamine, a variety of other analogs of thiamine were tested. These experiments are the basis of this report.

The structures of the compounds that were examined are shown in Figure 2; these analogs were kindly supplied by Merck, Sharp and Dohme Research Laboratories. Pyrithiamine and thiamine were obtained from Calbiochem.

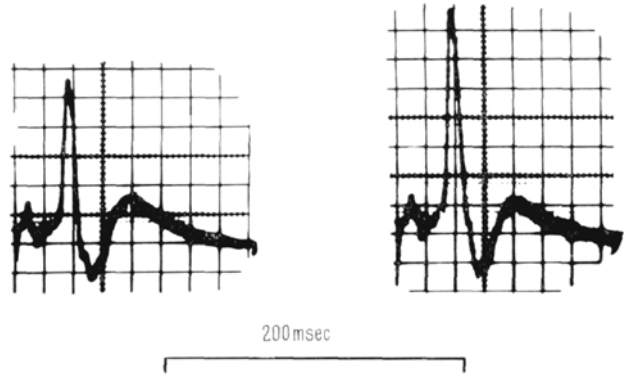

Fig. 1. The effect of pyrithiamine on the compound diphasic action potential of the non-myelinated nerve fibers of the rabbit vagus nerve. The left-hand record is a control action potential and the right-hand record is the action potential 10 min after the addition of pyrithiamine (5 m*M*) to the bathing solution.

[1] This work was supported by grants from the US Public Health Service, Merck, Sharp and Dohme, and The Burroughs Wellcome Company.

[2] C. J. Armett and J. R. Cooper, J. Pharmacol. exp. Therap. *148*, 137 (1965).

[3] J. H. Pincus and J. R. Cooper, in preparation.