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Lactoferrin in benign hypertrophy and carcinomas of the prostatic gland

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Summary. Using immunoperoxidase procedures, the presence of lactoferrin was investigated in benign hypertrophy (40 cases), differentiated adenocarcinomas (20 cases) and undifferentiated carcinomas (10 cases) of the prostate. In benign hypertrophy, the glandular epithelium and the secretory product were consistently negative. Strong staining for lactoferrin was always observed in neoplastic cells of differentiated adenocarcinomas and in their endoluminal material; in contrast, a very slight positivity was noted in undifferentiated carcinomas.

These findings are discussed in relation to the different degree of neoplastic glandular differentiation and functional activity of prostatic carcinomas.

Key words: Lactoferrin – Benign prostatic hypertrophy – Prostatic carcinoma

Lactoferrin (Lf) is an iron-binding protein found in many external secretions (Masson and Hermans 1967). In recent years, since the introduction of suitable immunofluorescence and immunoperoxidase methods (Reitamo et al. 1980), Lf has been detected in human salivary glands (Reitamo et al. 1980; Korsrud and Brandtzaeg 1982), in mucus neck cells of the stomach, in epithelial cells of the duodenum and in the proximal tubule of the kidney (Mason and Taylor 1978; Isobe et al. 1979), in lactating mammary glands and in bronchial glands (Mason and Taylor 1978), in myoid cells (Mason 1977) and in polymorphonuclear leucocytes and monocytes (Parmley et al. 1982).

Studies concerning the presence of lactoferrin in prostatic tissue have only been done by radial-immunodiffusion in homogenates of prostatic cancer (Loisillier et al. 1967; Van Sande and Van Camp 1981) and benign hypertrophy (Van Sande and Van Camp 1981). Loisillier et al. (1967) esti-

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mated that the lactoferrin concentration was about 5 times higher in cancerous tissue than in normal; low lactoferrin levels, have been referred to in both prostatic benign hypertrophy and adenocarcinomas (Van Sande and Van Camp 1981).

In view of these findings, we have applied the immunoperoxidase technique to prostatic cancer and benign prostatic hypertrophy, in order to study the morphological distribution of lactoferrin which has not yet been investigated.

Materials and methods

Seventy prostate samples were obtained by transurethral resection. Using the criteria of Mostofi and Price (1979) the histopathological diagnosis was: benign hypertrophy (40 cases), differentiated adenocarcinomas (20 cases), undifferentiated carcinomas (10 cases). All patients (age range 62–80 years) were untreated with oestrogens and/or antiandrogens.

Normal prostatic tissue was not available. It is important to note that autopsy material is unsuitable for studying proteins, due to the proteolytic activity of prostatic tissue (Van Camp and Van Sande 1980).

All prostatic specimens, fixed in Bouin's solution for 3 h at room temperature, were embedded in paraffin at 55° C for 2 h. 4–5 μ thick sections were treated serially for 30 min each time: 1. with 0.1% H_2O_2 in methanol to block the intrinsic peroxidase activity; 2. with normal sheep serum 1–3% to prevent non-specific adherence of serum proteins; 3. with rabbit anti-human lactoferrin (purchased from Dakopatts, Copenhagen, Denmark) (dilution 1:200); 4. with sheep anti-rabbit globulin antiserum (Behring Institute) (dilution 1:20); 5. with rabbit anti-horseradish PAP complexes (Dakopatts) (dilution 1:25). For the demonstration of peroxidase activity, the sections were incubated in darkness (Weir et al. 1974) for 10 min with 3–3′ diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO USA). To test the specificity of the lactoferrin staining, the specific antiserum was replaced by either phosphate buffered saline, normal rabbit serum or rabbit anti-human lactoferrin absorbed with purified human lactoferrin (Sigma Chemical Co., St. Louis, MO USA): negative results were obtained.

Results

Benign prostatic hypertrophy. In the epithelium of prostatic glands, the reaction for lactoferrin was consistently negative (Fig. 1); the same finding was noted in enlarged acini with papillary processes (Fig. 1). The secretory products inside the lumina were unstained (Fig. 1); the surrounding fibromuscular tissue was also negative with the exception of some monocytes and granulocytes.

Prostatic carcinomas. In differentiated adenocarcinomas, an intense and diffuse cytoplasmatic positivity for lactoferrin was observed (Figs. 2 and 3); tumour cells arranged in acini or ducts were also strongly stained, although positive and negative tumour cells were found in direct contact (Fig. 4). Frequently, lactoferrin was noted in the secretory product inside the glandular lumina (Fig. 5). Areas of papillary, mucinous and cribriform carcinomas (Fig. 6) also showed a positive reaction for lactoferrin.

In case of undifferentiated carcinomas, tumour cells arranged in solid clusters or in little groups exhibited a very slight cytoplasmatic positivity (Fig. 7).

In all prostatic cancers, the occasional stromal inflammatory infiltrate showed a positivity only in monocytes and polymorphonuclear leucocytes.

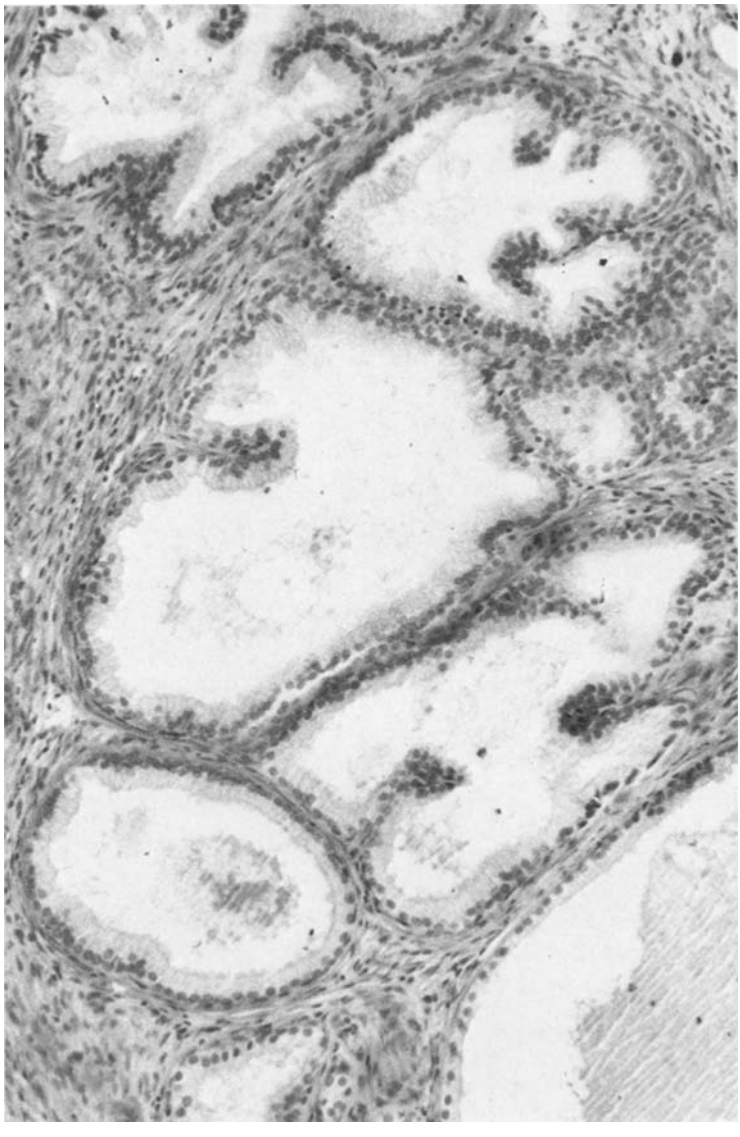


Fig. 1. Benign prostatic hypertrophy – The acinar epithelium and the secretory product are negatively stained. (Anti-lactoferrin immunoperoxidase, Mayer's Haemalum counterstain, ×100)

Discussion

Our morphological data have documented a different pattern of lactoferrin distribution in prostatic benign hypertrophy and cancer. In fact, the absence of this protein is a constant finding in all cases of benign hypertrophy, whereas a variously represented positivity is encountered in carcinomas. In particular a strongly positive reaction has been always observed in neo-

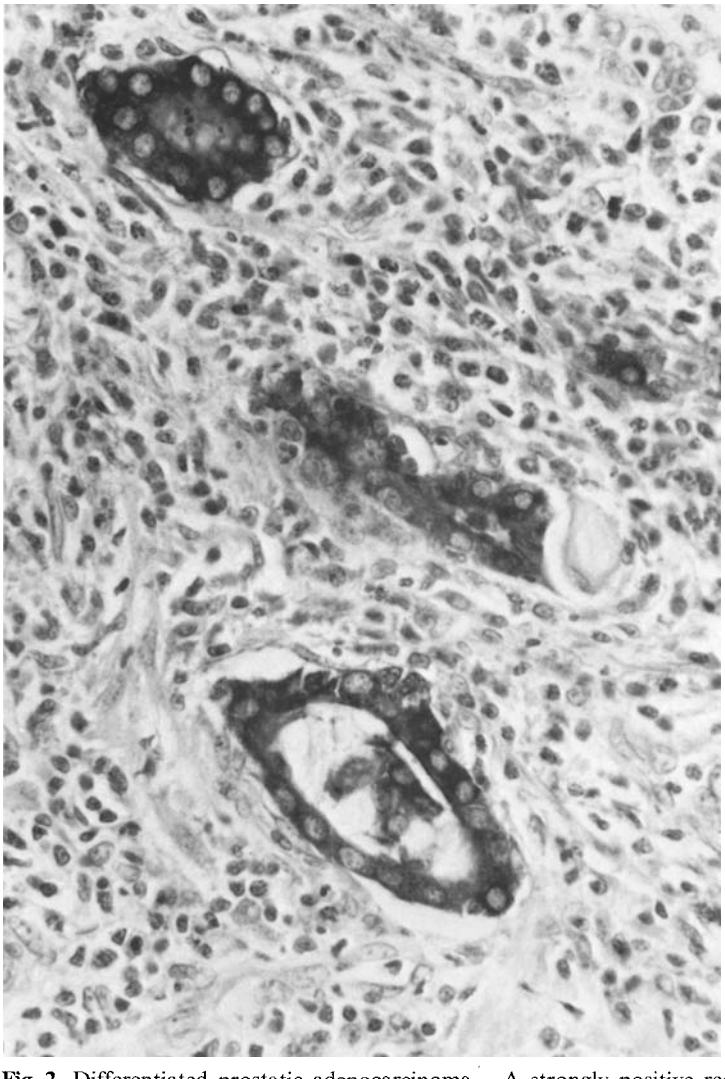


Fig. 2. Differentiated prostatic adenocarcinoma – A strongly positive reaction in neoplastic acinar-like structures is observed. (Anti-lactoferrin immunoperoxidase, Mayer's Haemalum counterstain, × 220)

plastic cells of differentiated adenocarcinomas as well as in their secretory products. In contrast only very slight staining has been noted in cells of undifferentiated prostatic carcinomas. The occasional presence of a chronic inflammatory infiltrate is not related to the intensity of the lactoferrin staining within neoplastic cells.

The demonstration of lactoferrin in our cases of prostatic carcinoma therefore suggests that this protein may be produced by the tumour tissue itself and that the variable positivity may be related to the different degree of glandular differentiation in carcinomas.

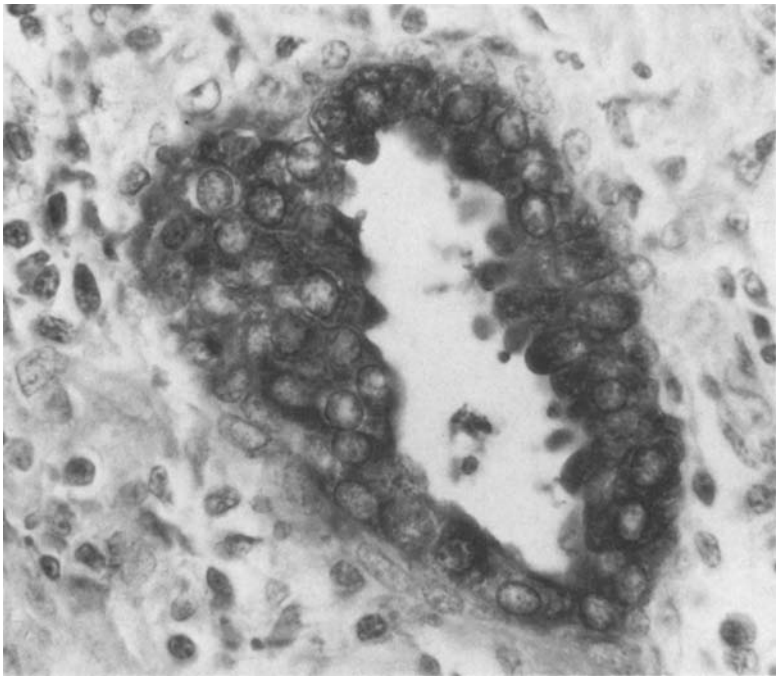


Fig. 3. Differentiated prostatic adenocarcinoma – Intensely stained tumour cells (Anti-lactoferrin immunoperoxidase, Mayer's Haemalum counterstain, × 330)

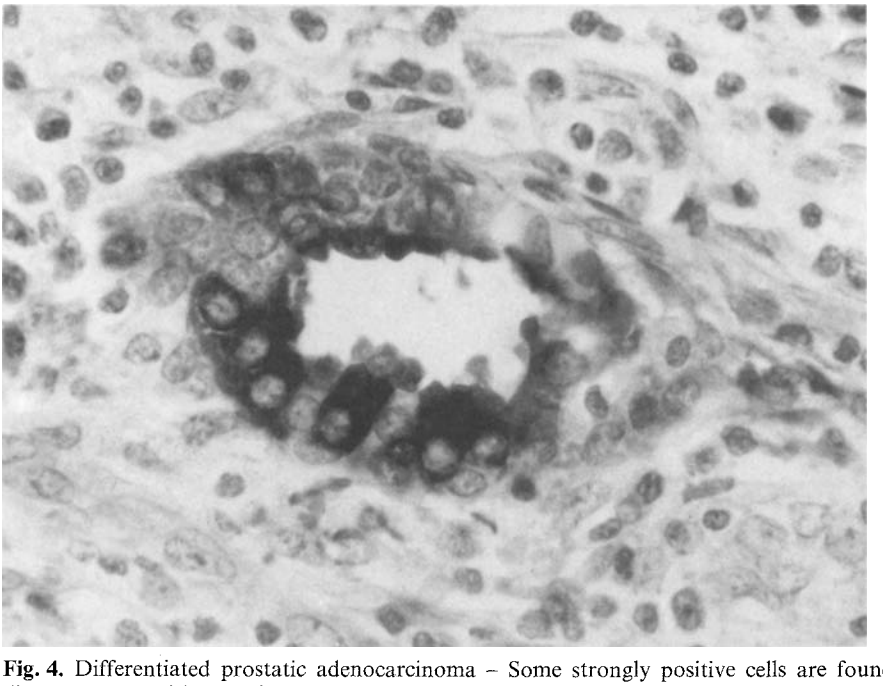


Fig. 4. Differentiated prostatic adenocarcinoma – Some strongly positive cells are found in direct contact with negative ones (Anti-lactoferrin immunoperoxidase, Mayer's Haemalum counterstain, × 330)

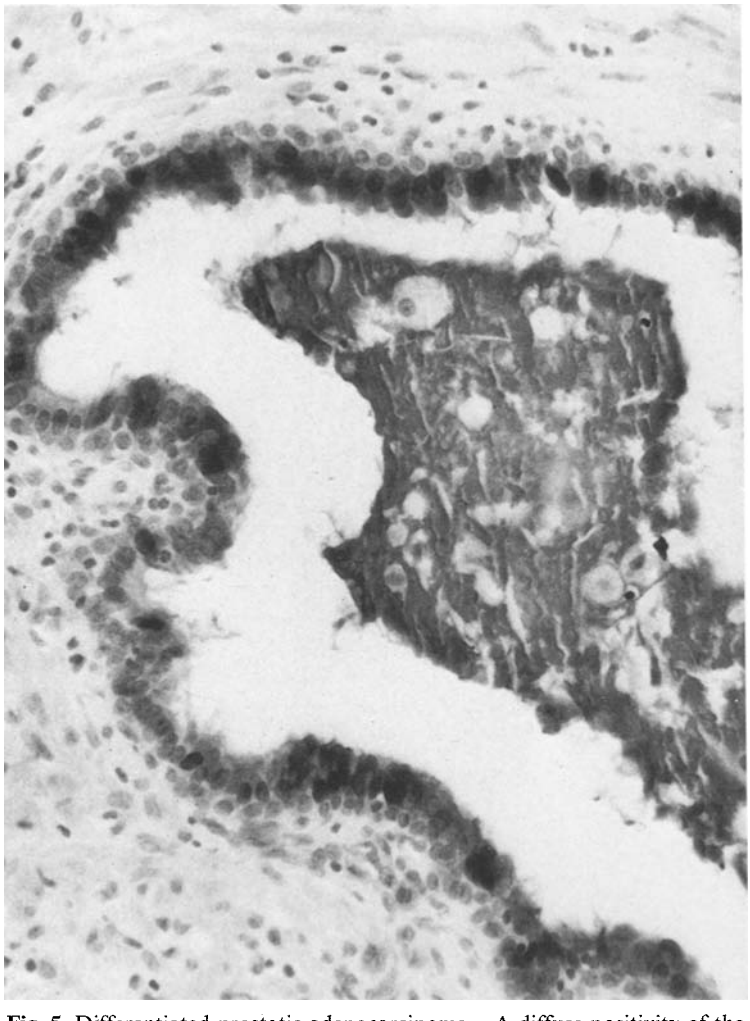
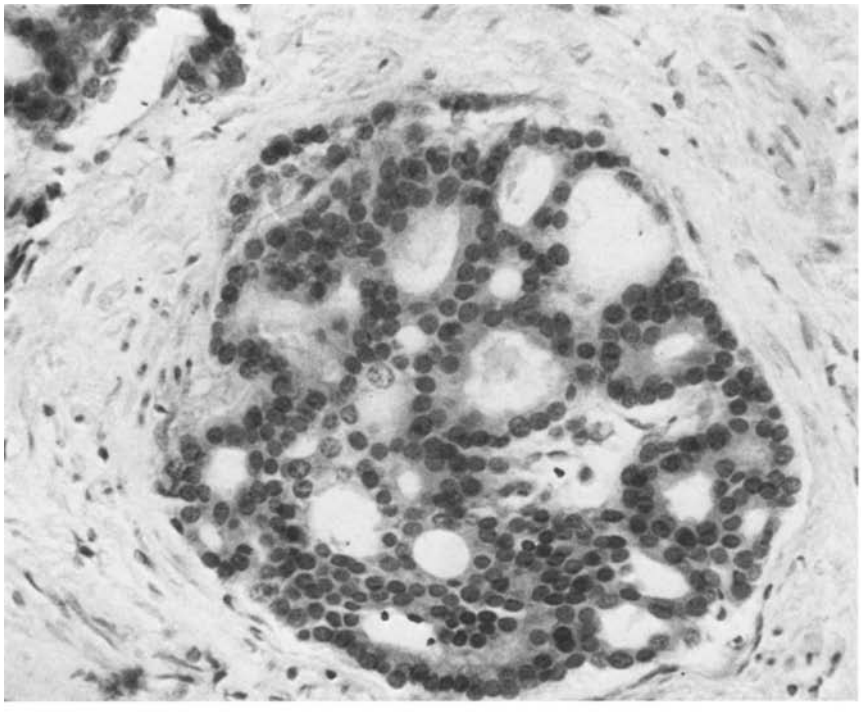


Fig. 5. Differentiated prostatic adenocarcinoma – A diffuse positivity of the secretory product is observed in the lumen of a duct-like structure. The staining of neoplastic cells is also evident (Anti-lactoferrin immunoperoxidase, Mayer's Haemalum counterstain, × 250)

Interestingly, in other neoplastic conditions such as adenocarcinomas of the parotid gland, an intense cytoplasmatic positivity for lactoferrin has been described (Caselitz et al. 1981); in this study, squamous cell carcinomas and anaplastic carcinomas were constantly negative. It has thus been suggested that lactoferrin may be interpreted as a "marker" of glandular or acinar differentiation of parotid carcinomas (Caselitz et al. 1981).

Fig. 6. An area of cribriform carcinoma shows a positive reaction (Anti-lactoferrin immunoperoxidase, Mayer's Haemalum counterstain, × 220).

Fig. 7. Undifferentiated prostatic carcinoma – A very slight cytoplasmatic staining is observed in neoplastic clusters (Anti-lactoferrin immunoperoxidase, Mayer's Haemalum, × 330)



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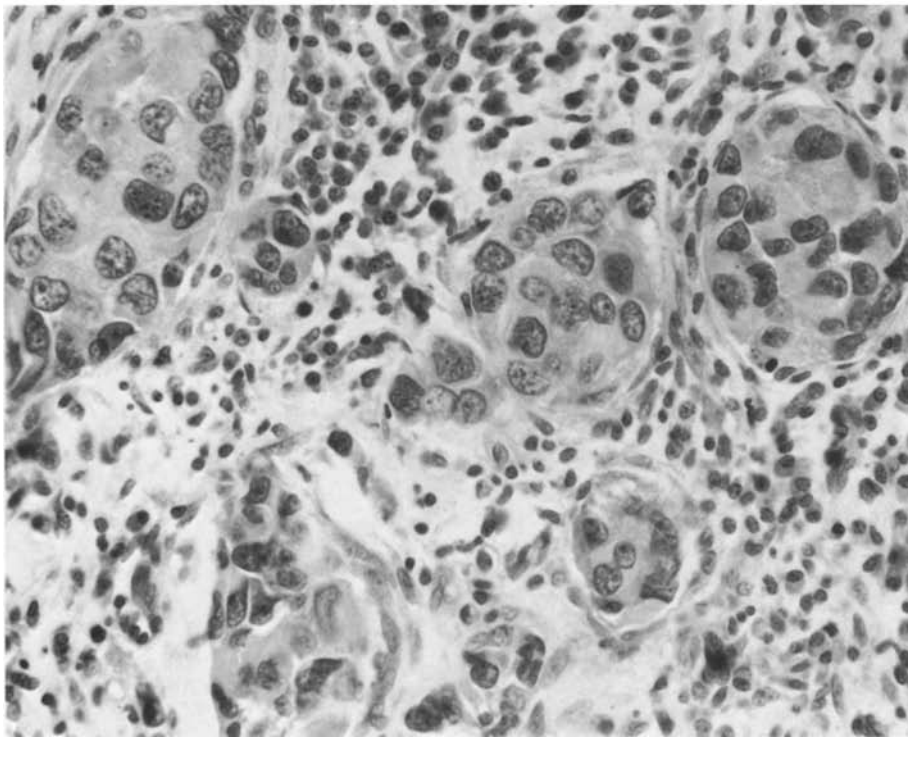

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Other substances have been studied in relation to the prostatic cancer differentiation. Specifically, different amounts of acid prostatic phosphatase have been found to be directly proportional to the number of acini and inversely related to the degree of anaplasia (Parkin et al. 1964). More recently, the surface distribution of specific carbohydrate binding-sites for lectins (Concanavalia ensiformis and Triticum vulgare) has been demonstrated with an intense staining in well differentiated prostatic carcinomas and a decrease or absence in staining in poorly differentiated ones (Ucci et al. 1983).

The present observation, that lactoferrin is mostly produced by differentiated prostatic carcinomas, may be a link between morphological differentiation and the functional neoplastic activity. We intend to continue our studies by examining metastases.

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