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Lipofuscin in Malignant and Non-Malignant Human Prostatic Tissue

J. Müntzing and T. Nilsson

Research Laboratories, AB Leo, Helsingborg, Sweden and the Department of Urology, University of Lund, Lund, Sweden

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Summary. Fluorescence microscopy of untreated, human non-malignant and carcinomatous prostatic tissue revealed numerous lipofuscin pigment granules in the non-malignant epithelial cells, but only few or none in the carcinomatous cells. In patients treated with Estracyt® the carcinomatous cells often contained numerous lipofuscin granules, more numerous than what was generally seen after treatment with estrogen. It is tentatively suggested that this increase in lipofuscin might be secondary to cellular damage with a consequent catabolic process in the cell.

Zusammenfassung. Das Vorkommen von Lipofuscin-Granula in der menschlichen Prostata wurde mit einer fluorescenzmikroskopischen Methode untersucht. In nicht entarteten Epithelzellen fanden sich zahlreiche Granula. Dieses reichliche Vorkommen steht im Gegensatz zu den Verhältnissen bei Krebszellen, die nur wenige, wenn überhaupt, Lipofuscin-Granula enthielten. Bei Patienten, die mit Estracyt® behandelt worden waren, enthielten die Krebszellen oft zahlreiche Lipofuscin-Granula. Der hohe Gehalt an Lipofuscin nach der Behandlung könnte bedeuten, daß diese Zellschäden auslöst, die zu katabolen Vorgängen in der Zelle führen.

It is now generally accepted that the lipofuscin pigment granules, demonstrable in a variety of tissues (cf. Thompson, 1966), are of lysosomal origin. The granules are believed to be telolysosomes or residual bodies formed by a progressive accumulation of indigestible residues in secondary lysosomes (cf. Daems *et al.*, 1969; Ericsson, 1969). The lipofuscin content is increased in advanced age (cf. Thompson, 1966; Köhl, 1968; Koenig, 1969) and in the presence of certain diseases (Björkerud and Schelin, 1964; Thompson, 1966, and others).

Lipofuscin pigment has been demonstrated in non-malignant human prostatic tissue in advancing age (Hamperl, 1934; Brandes, 1966). It was therefore considered legitimate to find out whether this pigment occurs also in the carcinomatous prostate. In the present study malignant prostatic tissue was obtained from patients with untreated prostatic carcinoma as well as from patients subjected to estrogen treatment or treatment with Estracyt®, a compound, where a cytostatic agent is linked to estradiol (Jönsson and Högberg, 1971).

Materials and Methods

Non-malignant prostatic tissue was obtained from four patients aged 62 to 83, at prostatectomy because of benign nodular hyperplasia. Malignant prostatic tissue was obtained by perineal needle biopsy from 15 patients, aged 49 to 85, with untreated, moderately to well differentiated prostatic adenocarcinoma. Two of the 15 patients had metastases in the supraclavicular lymph nodes. Specimens of these secondaries were obtained by surgical biopsy.

After the tissue specimens had been obtained 9 of these patients were treated with polyestradiol phosphate and ethinylestradiol according to Jönsson (1971). The other 6 patients, who had advanced prostatic carcinoma, were treated with estradiol-3-N/bis(2-chloroethyl)/-carbamate-17-dihydrogenphosphate (Estracyt®) according to Jönsson and Högberg (1971). After treatment for 2 months prostatic biopsy specimens were again obtained from the 15 patients.

The tissue specimens were fixed in cold, 10% formalin, sectioned (10 μ) with a freezing microtome, mounted in glycerine and examined in a fluorescence microscope with dark-field illumination. With an activating filter with a peak between 390 and 410 mμ (Schott BG 12) and a secondary filter with high absorption below 490 mμ (Schott OG 4), the pigment granules in the prostata fluoresced golden yellow.

Results

In the benign prostatic tissue obtained from four patients the non-malignant epithelial cells contained yellow-fluorescent granules of various sizes (Fig. 1). The granules were often larger in the basal part of the cells than in the supranuclear part, where they were sometimes almost submicroscopic. The number of pigment granules varied widely from cell to cell. In cells with relatively few granules the large ones otherwise situated in the basal part of the cells were missing. Cells containing only the smaller-sized granules occurred almost exclusively in hyperplastic epithelium. The cells with the largest lipofuscin content were found in low epithelium, which showed no signs of hyperplasia. Clusters of pigment granules were occasionally seen in the fibromuscular stroma.

In contrast with the benign prostatic epithelial cells, untreated cancerematous cells from all the 15 patients contained very little or no yellow-fluorescent granules (Fig. 2). In sections stained with haematoxylin and eosin cytoplasmic granules could not be detected (Fig. 5). Poorly differentiated soft tissue metastases found in two patients contained no pigment granules at all (Fig. 3).

In biopsy specimens obtained after treatment with polyestradiol phosphate and ethinylestradiol (9 patients) the number of pigment granules was as a rule as small as in the untreated carcinoma. The paucity of yellow-fluorescent granules in tumour cells after treatment with polyestradiol phosphate and ethinylestradiol contrasted with the high frequency of such cells containing abundant granules after treatment with Estracyt®. In the 6 patients treated with Estracyt® the malignant cells were sometimes loaded with yellow-fluorescent granules (Fig. 4). The content of granules varied, however, and in some tumour cells no yellow-fluorescent material could be observed at all. Basophile cytoplasmic granules with the same distribution as the fluorescent granules could be seen in sections stained with haematoxylin and eosin (Fig. 6).

Discussion

The fluorescent properties of lipofuscin granules are known from several investigations on various tissues (cf. Thompson, 1966). Lipofuscin pigment has been demonstrated in the form of yellow-fluorescent granules in the human epididymis (Köhl, 1968) and in human prostatic epithelial cells (Hamperl, 1934; Brandes, 1966). These observations support the presumption that the yellow fluorescence observed in the present study was emitted from lipofuscin granules.

It is generally accepted that lipofuscin granules are of lysosomal origin (Brandes, 1966; Köhl, 1968; Ericsson, 1969). This pigment occurs in increasing amounts

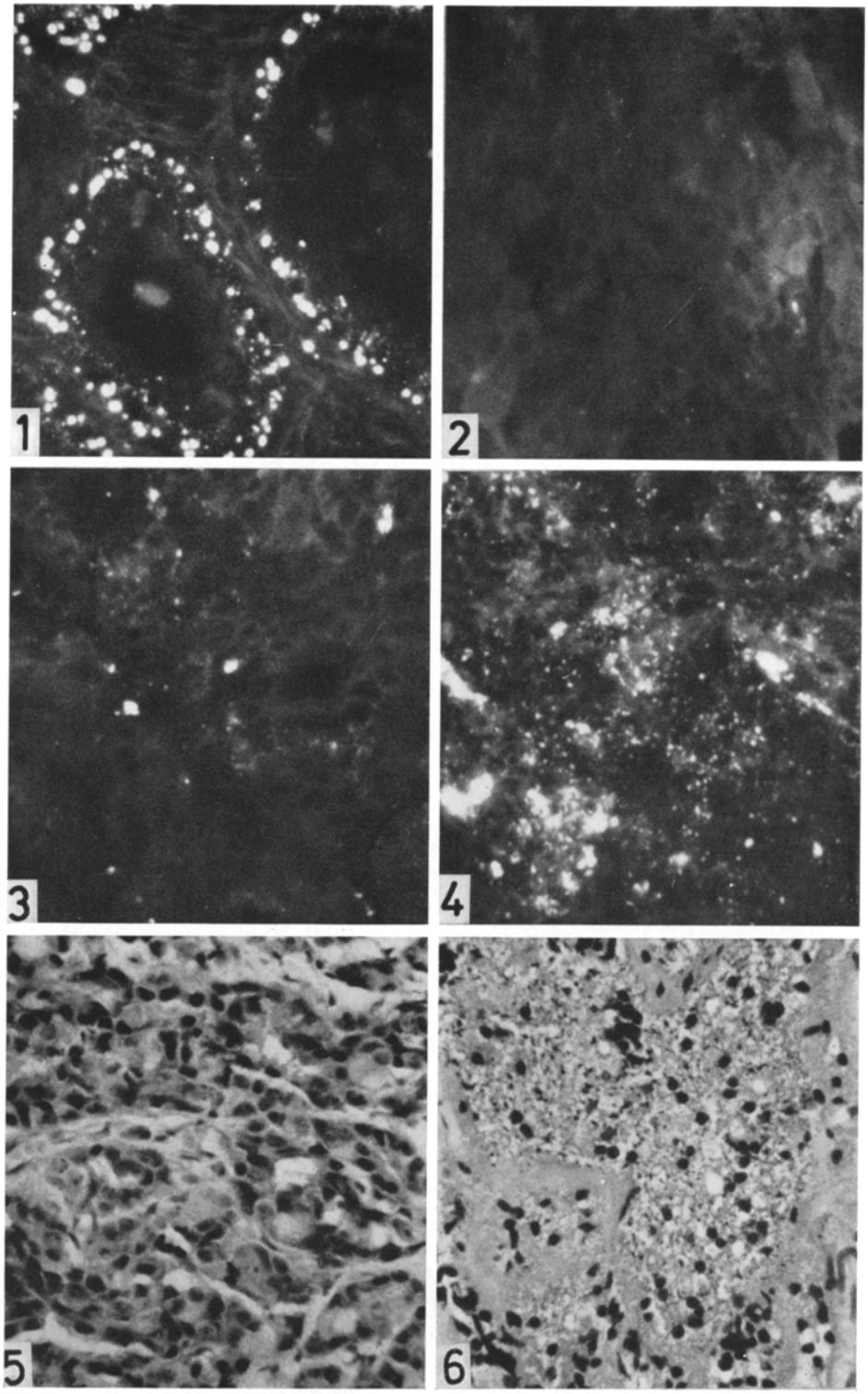

Fig. 1—6 (Legendes see p. 169)

in aging or degenerating cells (cf. Thompson, 1966). A high cellular content of lipofuscin might therefore be a sign of a catabolic process in the cell.

The finding in the present study of a high frequence of lipofuscin granules in histologically normal-looking prostatic epithelial cells is compatible with an earlier electron microscopic observation of Brandes (1966) who found a marked accumulation of dense structures resembling residual bodies in the prostate in elderly men. The observed lower content of lipofuscin in hyperplastic epithelial cells is in accordance with observations made in other tissues, i.e. that the content of lipofuscin is lower in younger cells than in aging ones (Strehler *et al.*, 1959). The difference in lipofuscin content between old and young cells was further demonstrated by the almost complete absence of lipofuscin in the malignant cells in the carcinomatous prostate.

The cellular content of lipofuscin may be increased not only with advancing cell age, but also in association with certain types of treatment that injure the cells (cf. Thompson, 1966). In the present study it was found that treatment with Estracyt® induced formation of lipofuscin in the malignant prostatic cells. As Estracyt® sometimes has a marked clinical effect on advanced prostatic carcinoma (Jönsson and Högberg, 1971; Nilsson and Müntzing, 1972) this formation of lipofuscin induced by Estracyt® may be regarded as a sign of a cellular injury followed by a catabolic process in the cell. The lipofuscin content was not increased after two months' treatment with polyestradiol phosphate and ethinylestradiol. However, it is possible that estrogen treatment for a longer period than two months might induce similar lipofuscin formation as induced by Estracyt®.

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Fig. 1. Benign human prostatic tissue. High incidence of lipofuscin granules in epithelial cells. × 360

Fig. 2. Carcinomatous human prostatic tissue. No lipofuscin granules in tumour cells. × 360

Fig. 3. Lymph node metastases of prostatic carcinoma. No lipofuscin granules in tumour cells. × 360

Fig. 4. Carcinomatous human prostatic tissue after treatment with Estracyt® for 2 months. Varying, often high, incidence of lipofuscin granules. × 360

Fig. 5. Carcinomatous human prostatic tissue. Haematoxylin and eosin. No cytoplasmic granulation. × 280

Fig. 6. Carcinomatous human prostatic tissue after treatment with Estracyt® for 2 months. Haematoxylin and eosin. Basophile cytoplasmic granules observable in the tumour cells. × 280

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Dr. J. Müntzing
Research Laboratories
AB Leo
Helsingborg, Sweden

Dr. T. Nilsson
Department of Urology
University of Lund
Lund, Sweden