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Cytological Findings in Transrectal Aspiration Biopsy on Hormone- and Radio-Treated Carcinoma of the Prostate*

P. Spieler, F. Gloor, N. Egle, and K. Bandhauer
Departments of Pathology and Urology, Kantonsspital, CH-9007 St. Gallen, Switzerland

Summary. The study reports cytological findings in 63 cases of radio- and/or oestrogen-treated carcinoma of the prostate. The different effects of therapy on the tumor cells in the cytological smear are described and discussed.

An evaluation of the results, depending on the type of therapy, showed, that radio-treated tumors, due to the rapid and strong sclerosis of connective tissue, were less suitable for aspiration biopsy than hormone-treated tumors. Further, we were able to show that the effect of therapy in well-differentiated tumors is better than in poorly differentiated tumors.

Cytological examination of treated prostate carcinoma is a simple method, quickly carried out, repeatable several times, and of high diagnostic value.

Aspiration biopsy is most of all indicated for follow-up of the less well-differentiated and the hormone-treated carcinomas.

Key words: Cytodiagnosis – Aspiration biopsy – Carcinoma, prostate – Estrogen therapy – Radiotherapy.

Introduction

In 1960 Franzen et al. reported for the first time on the cytological primary diagnosis of carcinoma of the prostate with transrectal aspiration biopsy. The daily diagnostic usage of this method has increased (Esposti, 1966; Franzen et al., 1960) and in 1971 Esposti published a paper about the usage of the method for the follow-up of hormone-treated carcinomas of the prostate.

The histological findings of the therapeutic changes of the primary tumor have been long known through the research of Schenken et al. (1942) and Fergusson and Franks (1952).

The following work goes into the problem of aspiration biopsy of the treated carcinoma of the prostate with respect to the morphological and clinical applications.

* Herrn Prof. Chr. Hedinger, Zürich, zu seinem 60. Geburtstag gewidmet

Table 1. Morphological criteria for grading primary carcinomas of prostate in Papanicolaou-stained smears

	High differentiation	Moderate differentiation	Poor differentiation
A. *Structural criteria*	Mostly mono-layered sheets, regularly shaped	Often multi-layered clusters, regularly shaped	Multi-layered clusters, irregularly shaped, many isolated tumor-cells
B. *Cellular criteria*			
Nucleus	Unimorph, enlarged	Polymorph, greatly enlarged	Highly polymorph, great variations in size
Chromatin	Granular, dense	Granular, dense	Granular, dense, often irregularly distributed
Nucleolus	Obligatory, unimorph	Obligatory, large	Obligatory, polymorph, frequently multiple
Cytoplasm	Large, mostly clearly defined, vacuolated, rarely lipofuscinpigment	Large, mostly not clearly defined, vacuolated	Large, not clearly defined, vacuolated

Material and Methods

Since October 1972, all patients with carcinoma of the prostate under treatment in the Urological Department of the Kantonsspital St. Gallen, have undergone a check-up every 6 months. We have evaluated the results on 63 patients.

The primary diagnosis of carcinoma was based in 56% on a histological examination (transperineal biopsy or transurethral resection of the prostate), in 38% on a combined histological and cytological (transrectal aspiration biopsy) examination and in 6% on a cytological examination only.

The varied degrees of differentiation of the tumors were classified histologically and cytologically into three categories: high differentiation, moderate differentiation, poor differentiation.

In the many cases with mixed degrees of differentiation, we classified according to the most undifferentiated part of the tumor. The cytomorphological criteria used to specify the different grades are summarized in Table 1.

The following standard therapies were applied to our patients:

1. Radiotherapy. Cobalt-60 high-voltage irradiation with cross-fire technique: Irradiation from dorsal and ventral with the beam-line on the prostate. Dosage between 5,000 to 6,000 rad depending on the constitution of the patient and the clinical stage of the tumor. Standard hormone therapy always followed irradiation.

2. Hormone Therapy. Primary orchiectomy followed by a massive dose of stilboestrol-diphosphate-Na (Honvan®) from 6 to 12 g during a period of 10 days. After that, at regular intervals of 4 weeks, continuous therapy with 40 mg polyoestradiol-phosphate (Estradurin®) i.m. and 1 mg stilboestrol p.o.

Combined radio- and hormone therapy was applied to 24 patients, hormone therapy alone to 39 patients.

The interval between the beginning of the therapy and the appearance of the first therapy-induced changes of the tumor depends on the type of therapy chosen and the histological picture of the primary tumor. With oestrogen-treated carcinomas the interval can be to 4 months (Esposti, 1971).

We decided, therefore, to carry out the first morphological check-up examination after 6 months from beginning of therapy. Further check-ups took place every 6 months. Of the 63 patients, 37 are under control for 6 months, 11 for 1 year, 9 for $1^1/_2$ years, 5 for 2 years and 1 for 3 years.

Up to now we have examined 131 specimens. The aspiration biopsy was used on its own 49 times and twice the histological biopsy was carried out on its own. Cytological and histological material was examined 40 times.

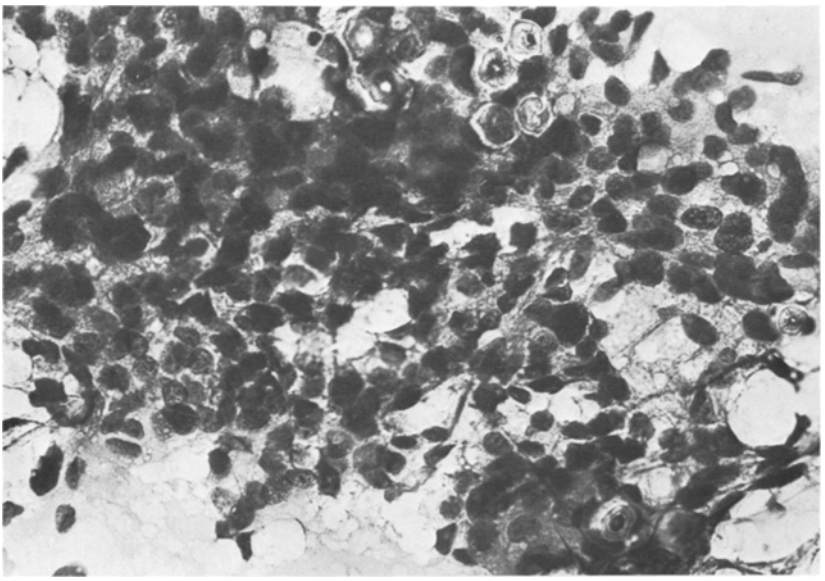

Fig. 1. Cytoplasmic changes (MB 13162/73): empty vacuoles pushing nuclei aside. Melting of vacuoles of neighbouring cells. Wet fixed smear, Papanicolaou-stained (× 500)

Cytological smears were fixed wet with Pro Fixx® and stained after Papanicolaou. The histological biopsies were fixed in 4% formol and then embedded in paraffin. The sections were stained with H & E, PAS and according to van Gieson.

Results

In the cytological smears, we were able to prove the following changes on the tumor cells, resulting from therapy:

1. Cytoplasm Changes

Whereas the cytoplasm of the primary carcinoma is a honeycomb structure, we find in the cytoplasm of treated carcinomas multiple, large, bubble-like, empty vacuoles, which push the nucleus aside. Vacuoles of neighbouring cells melt into one another and the appropriate nuclei are often conglomerated (Fig. 1).

In the histological section, the therapy-induced vacuoles are located basally. This is not recognizable in the cytological smear.

2. Regressive Changes

a) On the Cell Clusters. Three stages can be differentiated cytologically during the degenerative process (Fig. 2A–C). The first stage results in a diminution of the carcinoma cell clusters through shrinkage of the cytoplasm and the nuclei.

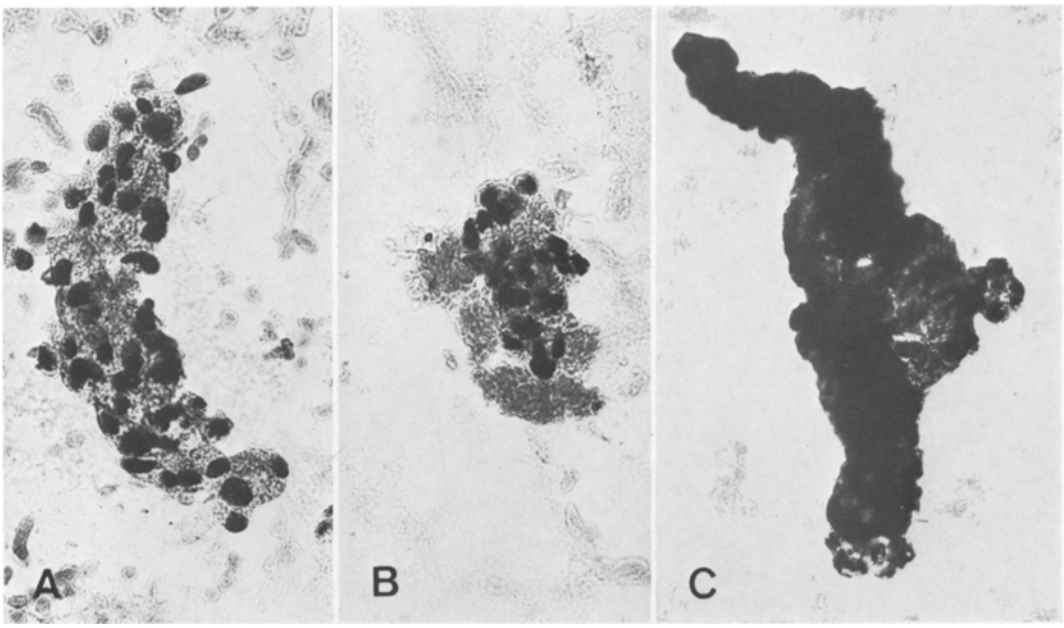

Fig. 2 A–C. Regressive changes on carcinoma-cell clusters (MB 2865/74): **A** First stage: shrinking of cytoplasm and nuclei. **B** Second stage: nuclei are packed together and coarsely structured. **C** Third stage: nuclei lie clustered together, homogeneously structured and strongly hyperchromatic. Cytoplasm has almost completely disappeared. Wet fixed smear, Papanicolaou-stained (× 400)

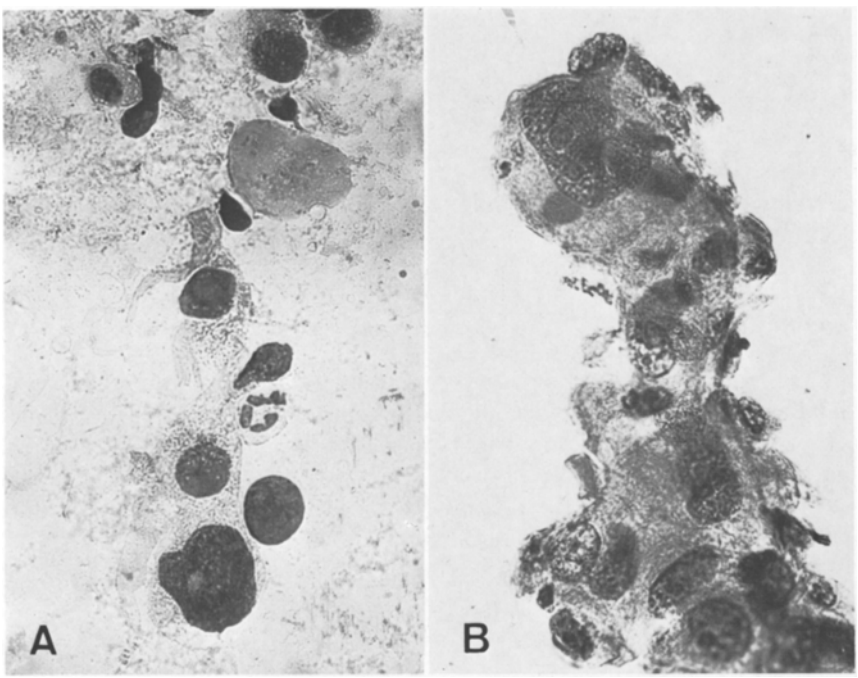

Fig. 3 A and B. Giant nuclei: **A** (MB 761/73) *At bottom*: a giant nucleus with lobulation and vacuolization. **B** (MB 4418/76) *At top*: a cell with two giant nuclei. Criteria of malignancy are well preserved. Wet fixed smears, Papanicolaou-stained (× 500)

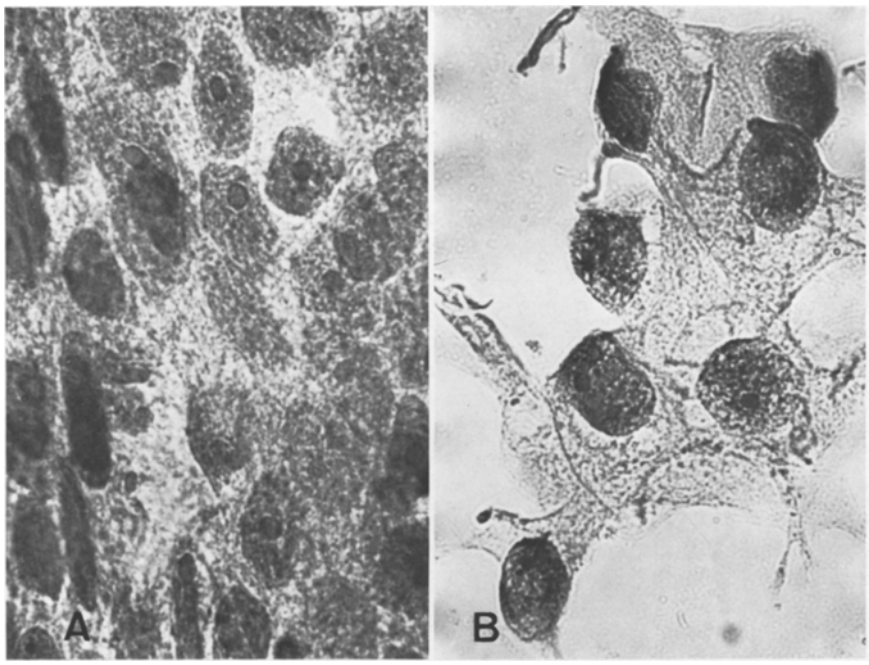

Fig. 4A and B. Wasting of nucleoli: **A** (MB 18762/72) Primary carcinoma before therapy. **B** (MB 13721/73) Same case after therapy, lasting 8 months: nucleoli are smaller and indistinct. Note also cytoplasmic vacuolization. Wet fixed smears, Papanicolaou-stained (× 1000)

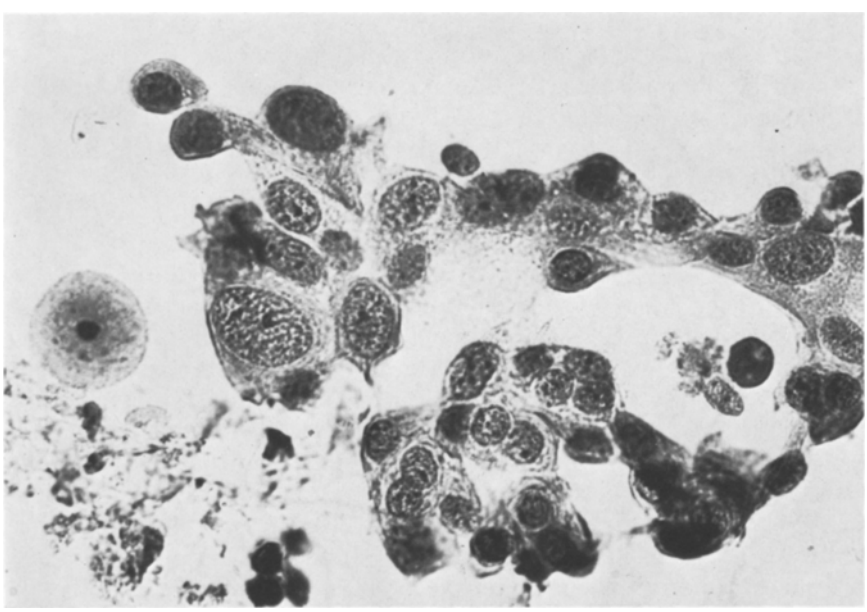

Fig. 5. Change of prostatic glandular epithelium into metaplastic squamous epithelium (MB 20556/75): Monolayered sheets, great differences in size of cells and nuclei, dense granular chromatin structure, well-defined nucleoli. Wet fixed smear, Papanicolaou-stained (× 500)

Table 2. Cytomorphological criteria for differentiation of carcinoma cells from metaplastic transformation stages

	Carcinoma	Metaplastic transformation stages
Cytoplasm	Vacuolized, mostly not clearly defined	Dense, cyanophilic, clearly defined
Chromatin	Very densly stored, coarse granular, irregularly distributed in nucleus	Loosely arranged, coarse granular, regularly distributed in nucleus
Nucleolus	Large, polymorph, may be difficult to recognize!	Small, round, centrally placed, can be absent
Nucleus membrane	Missing	Fine, but clear
Cell clusters	Irregularly shaped, multi-layered	Regularly shaped, always mono-layered

The cytoplasm tightens and the nucleoli reduce in volume (Fig. 2A). In the second stage the nuclei are tightly packed together, the chromatin is coarsly condensed and nucleoli can only rarely be found. The nuclei are hyperchromatic and enclosed by a homogeneous mass of cytoplasm (Fig. 2B). In the third stage the greatly shrunken, homogeneous, blue-violet-coloured nuclei lie clustered together. The cytoplasm has almost completely disappeared (Fig. 2C).

In the second and third stage the cytological criteria of malignancy are no longer recognizable (Fig. 2B, C).

b) Giant Nuclei. These are greatly enlarged nuclei which have conserved all criteria of malignancy. The nucleus may be lobulated and in the center vacuoles can often be observed (Fig. 3A, B).

c) Wasting of Nucleoli. Nuclear shrinkage and nuclear pyknosis take place simultaneously with the disappearence of the nucleolus. In some cases, changes on the nucleolus are a primary sign of nuclear degeneration. Compared to the findings on the primary tumor, the changes are characterized by diminution and bad coloration of the nucleolus (Fig. 4).

3. Benign Squamous-Cell Metaplasia

Squamous-cell metaplasia, as a result of oestrogen-therapy, is often found in cytological and histological specimens. In our material squamous-cell metaplasia could be found in 49 out of 106 cases. The metaplastic squamous cells show a large, mostly rounded cell body with a very prominent and dense cytoplasmic membrane. The cytoplasm stains cyanophilic or rarely, eosinophilic. Perinuclearly, there is usually glycogen embedded. The nuclei are round to oval with a dense, reticular chromatin structure or are sometimes pyknotic. The morphological stages of transformation of prostatic glandular cells into metaplastic squamous epithelium are of special interest to the cytologist. In the smear it is often difficult to differentiate them from malignant cells. The cells are arranged in monolayered sheets. Cells and nuclei are large and show great differences in size. The nuclei are hyperchromatic and the chromatin structure is granular. A round, well-defined nucleolus is easily detected in the center of the nucleus (Fig. 5 and Table 2).

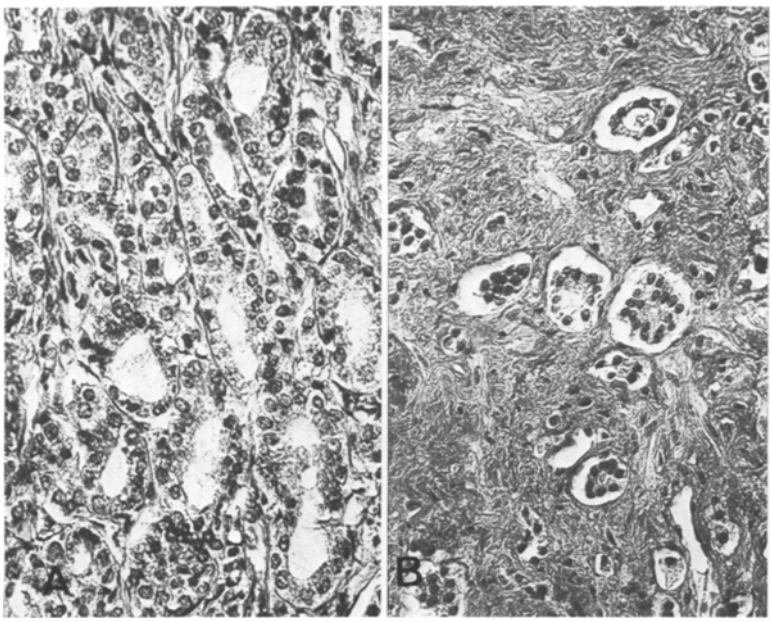

Fig. 6 A and B. Stromal changes: **A** (MB 16608/73) Primary carcinoma before therapy. **B** (MB 7713/74) Same case after therapy lasting 7 months. Paraffin-embedded histological sections, H & E-stained (×250)

4. Stromal Changes

The therapy-induced changes in the connective tissue can only be seen in the histological biopsy or transurethral resected material of the prostate, never in the cytological smear (Fig. 6B).

The indication for and application of aspiration biopsy are influenced by stromal changes. This will be discussed later.

Discussion

Several authors have reported on the qualitative cell changes in prostatic carcinomas caused by oestrogen- and/or radiotherapy (Alken et al., 1972, 1973, 1975; Böhni, 1956; Esposti, 1971; Fergusson and Franks, 1952; Franks, 1960; Kastendieck et al., 1975a and b; Kastendiek et al., 1976; Schenken et al., 1942). Kastendieck proved by ultrastructural examination, that the therapeutic effects on tumor cells are qualitatively independent of any applied therapy. Our findings with light microscopy correspond to those of Kastendieck. Only squamous-cell metaplasia could not be detected after irradiation. Stromal fibrosis, giant nuclei and nuclear degeneration are more prominent after irradiation than after hormonal therapy. We judge the effect of therapy according to the qualitative changes on the single cells and their frequency on the smears. With these criteria we

Table 3. Classification of therapeutic effect, based on qualitative and quantitative changes of tumor cells

Therapy-induced changes on primary tumor	Therapeutic effect		
	Good	Moderate	Poor
Cytoplasm vacuoles	+++	++	+
Degenerative nuclear changes: pyknosis, karyolysis, karyorhexis, giant nuclei, wasting of nucleolus	++	+	0
Unaltered carcinoma cells and tissue	+	++	+++
Fibrosis and sclerosis (for histology)	++	+	0
Squamous-cell metaplasia	Not taken into consideration		

have been able to classify the effect of therapy into three categories: good, moderate, poor (Table 3).

Vacuolization of cytoplasm under therapy is not identical with embeddment of glycogen. A series of examinations which we have carried out on alcohol-fixed perineal biopsies has shown glycogen embedment in approximately one-third of the primary and of the treated carcinomas. Glycogen in the cytoplasm of tumor cells was found independent of the primary tumor grading, of the quality and quantity of the therapy applied, and of the size of the vacuoles.

Cytological diagnosis of *regressive changes of carcinoma cell clusters* on smears is only permitted when, next to the degenerated tumor parts, intact carcinoma cells are still found. Morphological changes according to regression stage 2 can also be seen cytologically in epithelial clusters which originate from inflamed prostatic glands.

Karyolysis and karyorhexis are degenerative phenomena which can only be observed after irradiation. They practically always appear together with nuclear pyknosis and intact tumor cells. According to some authors *giant nuclei* can only be seen after irradiation (Esposti, 1966). In our material we have had two cases with giant-nucleus formation, which appeared in the histological and in the cytological specimens. Both cases had been treated with hormones only.

Benign squamous-cell metaplasia always develops out of normal epithelium of the prostatic gland. Metaplastic change of malignant tumor epithelium into squamous epithelium, like the type found in carcinomas of the endometrium, could not be detected in our specimens. Malignancy of metaplastic tissue is unknown (Kastendieck et al., 1975a).

Kastendieck has proven by ultrastructural studies that the changed cells are genuine squamous cells. Classification of the cells in the cytological smear is without doubt also possible. Sometimes their cytoplasm in the Papanicolaou-stained smear is yellow or orange; this is due to the content of keratin and its precursors. In the cytoplasm of hormone-induced metaplastic squamous cells, glycogen is always seen in large quantities.

The metaplastic change from glandular epithelium to squamous epithelium is a characteristic indication of previous oestrogen influence. It is, however, nonspecific, as squamous-cell metaplasia can also be observed together with infarction of the prostate and after cryosurgical operations in this organ. In our view, the occurrence of squamous-cell metaplasia does not tell us anything about the response of the carcinoma to the therapy applied. Squamous-cell metaplasia has been found in tumors with good response to therapy as well as in tumors resistant to therapy. We have therefore not taken this into consideration when judging the effect of therapy.

Stromal changes can only be seen in histological material. We recognize two stages: Initially, after beginning therapy, there is a strong slackening, myxoid degeneration and vacuolisation of the stroma, and a fragmentation of the fibres is observed.

After radiotherapy this stage is mostly fleeting, and within a few days a strong sclerosis takes place. On the other hand, after oestrogen therapy only, we could observe primary stromal changes lasting up to 18 months after the beginning of therapy.

Whether the early stromal changes are a result of cellular degeneration, or whether they have appeared through direct hormonal effect on the intercellular substance is controversial (Fergusson and Franks, 1952; Franks, 1960). In the second stage, a strong increase of connective tissue is observed. The epithelial tumor tissue shows degenerative changes and turns into small fragmented tubules and bands (Fig. 6).

All therapy-induced changes, exept for stromal fibrosis, are recognizable in the cytological smear. In advanced stages, as a result of the increase and sclerosis of connective tissue, it is impossible to obtain a useful specimen with the fine needle. In these cases the organ is firm and the specimen is "empty," i.e., in the stained smear we find only some erythrocytes and a few scattered mesenchymal cells. Small stromal cylinders cannot be smeared and float away during the staining process.

Since the fibrosis is rather prominent after irradiation, satisfactory samples for examination can rarely be obtained, even at the first check-up. Therefore, in these cases, we use the perineal biopsy for the morphological diagnosis of the effectiveness of the therapy. In hormone-treated carcinoma of the prostate, the situation is different. Therapy-induced changes, as a rule, take place very slowly, so that we are enabled to obtain a good specimen with aspiration biopsy even after a long duration of therapy. The results of 21 radiological and 38 hormonal treated carcinomas are shown in Table 4.

Looking at the results of the cytological control biopsies, with regard to the grade of differentiation of the primary tumor, one observes that highly differentiated carcinomas show better a response to therapy than less well-differentiated ones, independent of the type of therapy. Furthermore, the "empty" aspiration biopsies of the well-differentiated tumors are far more frequent which, indirectly and with some reservations, points to the good therapy-sensitivity of the tumors.

Table 4. Effectiveness of aspiration biopsy after hormone and irradiation in comparison with effectiveness after hormonal therapy only, at the first and second checkups (independent of stage of tumor and primary degree of differentiation): 59 cases

Combined radiation and hormone therapy		Hormone therapy only	
First check-up ($^1/_2$ year) 21 cases	Second check-up (1 year) 9 cases	First check-up ($^1/_2$ year) 38 cases	Second check-up (1 year) 7 cases
9	6	9	7
12	3	29	


= Not satisfactory for examination.


= Satisfactory for examination.

In a former study, including 76 cases of hormone- and radio-treated carcinomas of the prostate, we demonstrated that the morphological control results correspond well with the clinical course of the tumor (Egle and Spieler, 1976).

References

Alken, C.E., Dhom, G., Hohbach, M., Sachse, D., Schröder, F.H., Straube, W.: Therapie des Prostatakarzinoms und Verlaufskontrolle (I). Urologe A (Berl.) **11**, 216–220 (1972)

Alken, C.E., Dhom, G., Straube, W., Braun, J.S., Kopper, B., Rehker, H.: Therapie des Prostatakarzinoms und Verlaufskontrolle (III). Urologe A (Berl.) **14**, 112–116 (1975)

Alken, C.E., Dhom, G., Straube, W., Schmidt-Hermes, H.-J., Moormann, J.G., Moeller, J.F.: Therapie des Prostatakarzinoms und Verlaufskontrolle (II). Urologe A (Berl.) **12**, 191–197 (1973)

Böhni, H.P.: Heilungsvorgänge in osteoplastischen Knochenmetastasen eines mit Oestrogenen behandelten Prostatakarzinoms. Z. Urol. **49**, 30–47 (1956)

Egle, N., Spieler, P.: Zytologische und histologische Verlaufskontrollen beim hochvoltbestrahlten Prostatakarzinom. Helv. chir. Acta, **43**, 337 (1976)

Esposti, P.L.: Cytologic diagnosis of prostatic tumors with the aid of transrectal aspiration biopsy. Acta cytol. (Philad.) **10**, 182–186 (1966)

Esposti, P.L.: Cytologic malignancy grading of prostatic carcinoma by transrectal aspiration biopsy. Scand. J. Urol. Nephrol. **5**, 199–205 (1971)

Fergusson, J.D., Franks, L.M.: The response of prostatic carcinoma to oestrogen treatment. Brit. J. Surg. **40**, 422–428 (1952)

Franks, L.M.: Estrogen-treated prostatic cancer. Cancer (Philad.) **13**, 490–501 (1960)

Franzén, S., Gierth, G., Zajicek, J.: Cytological diagnosis of prostatic tumours by transrectal aspiration biopsy: a preliminary report. Brit. J. Urol. **32**, 193–196 (1960)

Gaynor, E.P.: Zur Frage des Prostatakrebses. Virchows Arch. path. Anat. **301**, 602–652 (1938)

Kastendieck, H., Altenähr, E.: Morphogenese und Bedeutung von Epithelmetaplasien in der menschlichen Prostata. Eine elektronen-mikroskopische Studie. Virchows Arch. Abt. A Path. Anat. and Histol. **365**, 137–150 (1975a)

Kastendieck, H., Altenähr, E., Burchardt, P.: Zur Ultrastruktur des behandelten Prostatakarzinoms. Virchows Arch. Abt. A Path. Anat. and Histol. **366**, 287–304 (1975 b)

Kastendieck, H., Altenähr, E., Burchardt, P., Becker, H., Franke, H.D., Klosterhalfen, H.: Morphologische und klinische Behandlungsergebnisse nach kombinierter Hormon- und Strahlentherapie des Prostatakarzinoms. Dtsch. med. Wschr. **101**, 571–576 (1976)

McMahon, M.J., Butler, A.V.J., Thomas, G.H.: Morphological responses of prostatic carcinoma to testosterone in organ culture. Brit. J. Cancer **26**, 388–394 (1972)

Pellet, A., Grétillat, P.A.: Diagnostic cytologique des tumeurs prostatiques par ponction transrectale à l'aiguille fine de Franzén. Ann. Urol. **5**, 247–252 (1971)

Schenken, J.R., Burns, E.L., Kahle, P.J.: The effect of diethylstilbestrol and diethylstilbestrol-dipropionate on carcinoma of the prostate gland. II. Cytologic changes following treatment. J. Urol. (Baltimore) **48**, 99–112 (1942)

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